GLUTAMINE SUPPLEMENTATION

IN ONCOLOGY:

A SYSTEMATIC REVIEW

by
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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

Background

Glutamine (GLN), the most abundant amino acid in plasma and preferred fuel for enterocytes and lymphocytes, is recommended for use in various clinical settings (including critical illness). In cancer patients a state of "GLN depletion" has been reported. GLN is not widely supplemented, because the tumour is believed to utilize large amounts of GLN, and results of clinical benefits are inconsistent and conflicting.

Objectives

This systematic review assessed the efficacy and safety of GLN supplementation in patients with cancer and the effect on tumour growth in tumour-bearing animals. Primary objectives included clinical outcomes (mortality, survival, body weight change, infectious complications and length of hospital stay), duration and severity of mucositis and diarrhoea and tumour growth (tumour -weight, -volume, -weight/volume change). Secondary outcomes included description of practice issues such as GLN status, route as well as dose and timing of GLN intervention.

Search strategy

Electronic search of bibliographic databases from 1800 until 24 March 2010. Hand-searching of bibliographies of retrieved articles and personal files.

Selection criteria

Randomized controlled trials (RCTs) of GLN supplementation (by any route) in humans and animals with cancer.

Data collection and analysis

Two authors independently assessed trial quality and extracted data. Study authors were contacted for additional information. The random-effects model was used to estimate overall risk ratio/mean difference in effect size. $P<0.05$ was considered to be statistically significant.

Results

Forty-two RCTs involving 2 687 humans (mostly adults) and eighteen experimental studies involving 441 animals (exclusively rats) were included. The quality of evidence was low, hence the high risk of bias, inconsistency, unexplained heterogeneity and imprecision of results. Based on nine RCTs involving 727 humans, $\geq$ grade 2 mucositis were significantly less common with GLN supplementation compared to controls (risk ratio (RR) 0.76, 95% confidence interval (CI) 0.60-0.75, P=0.03). Four RCTs involving 130 humans indicate that, compared to controls, GLN supplementation significantly shortened the duration of diarrhoea by 1.26 days (95% CI -2.28 to -0.24, P=0.02). The information available for mortality, survival, body weight change, infectious complications, duration of mucositis and severity of diarrhoea did not indicate that the efficacy

and/or safety of GLN supplementation compared to controls was different. Twelve RCTs involving 269 rats indicated that, compared to controls, GLN supplementation resulted in a significantly smaller tumour weight of 0.77 grams (95% CI -1.47 to -0.07, P=0.03). Nine RCTs involving 178 rats indicated that, compared to controls, GLN supplementation significantly reduced tumour volume by 1.16 cm^3 (95% CI -2.19 to -0.12, P=0.03). Based on nine RCTs, involving 104 rats, GLN supplementation reached borderline significance with an increased tumour volume loss of 2.46 cm^3 (95% CI -4.97-0.05, P=0.05) compared to controls.

Conclusions

GLN supplementation appears to be safe for use in patients with cancer. GLN might be more effective than controls in preventing clinically significant mucositis and in reducing the duration of diarrhoea. Further research is warranted regarding the effect of routine GLN supplementation on clinical outcomes in oncology.

OPSOMMING

Agtergrond

Glutamien (GLN), die oorvloedigste aminosuur in plasma, en voorkeur-energiebron vir enterosiete en limfosiete, word aanbeveel vir gebruik in verskeie kliniese situasies (insluitend kritieke siekte). "GLN-uitputting" is in kankerpasiënte gerapporteer. GLN-supplementasie word nie algemeen gebruik nie weens die tumor se hoë GLN-verbruik asook die inkonsekwente en teenstrydige resultate oor voorgestelde kliniese voordele.

Doelwitte

Hierdie sistematiese literatuuroorsig assesseer die effektiwiteit en veiligheid van GLN-supplementasie in kankerpasiënte asook die effek op tumorgroei in tumordraende diere. Primêre doelwitte sluit in kliniese uitkomste (mortaliteit, oorlewing, liggaamsmassaverandering, infektiewe komplikasies en lengte van hospitaalverblyf), duur en erns van mukositis en diarree asook tumorgroei (tumorgewig, -volume, -gewig/volumeverandering). Sekondêre uitkomste sluit beskrywing van praktyke soos die GLN-status, -roete, -dosis en tydsbeplanning van GLN-intervensie in.

Soektogstrategie

Elektroniese soektog van bibliografiese databasisse vanaf 1800 tot 24 Maart 2010. Hersiening van bibliografieë van artikels en persoonlike lêers.

Seleksiekriteria

Ewekansige gekontroleerde proewe (RCT's) van GLN-supplementasie (via enige roete) in mense en diere met kanker.

Dataversameling en -analise

Twee outeurs het onafhanklik die proefgehalte geassesseer en data-ekstraksie onderneem. Outeurs van artikels is geraadpleeg vir addisionele inligting. Die stogastiese-effektemodel is gebruik om die algehele risikoverhouding of gemiddelde verskil in effekgrootte te skat. $P<0.05$ is as statisties beduidend geag.

Hoofresultate

Twee-en-veertig RCT's met 2 687 mense (hoofsaaklik volwassenes) en agtien eksperimentele RCT's met 441 diere (uitsluitlik rotte) is ingesluit. Die gehalte van navorsing is laag, weens die hoë risiko vir sistematiese foute, asook die teenstrydigheid, onverklaarde heterogeniteit en onakkuraatheid van die resultate. Gebaseer op nege RCT's met 727 mense, was $\geq$ graad 2 mukositis beduidend minder algemeen met GLN supplementasie in vergelyking met kontroles (relatiewe risikio (RR) 0.76, 95% vertrouensinterval (VI) 0.060-0.75, $P=0.03$). Vier RCT's met 130 mense dui aan dat GLN-supplementasie, in vergelyking met kontroles, die duur van diarree beduidend met 1.26 dae

(95% VI -2.28 tot -0.24, P=0.02) verkort het. Die inligting beskikbaar vir mortaliteit, oorlewing, liggaamsmassaverandering, infektiewe komplikasies, duur van mukositis en erns van diarree het geen verskil in effektiwiteit en/of veiligheid van GLN-supplementasie teenoor kontroles aangedui nie. Twaalf RCT's met 296 rotte het aangedui dat GLN-supplementasie, in vergelyking met kontroles, 'n beduidend kleiner tumorgewig van 0.77 gram (95% VI, -1.47 tot -0.07, P=0.03) tot gevolg het. Nege RCT's met 178 rotte het aangedui dat GLN-supplementasie, in vergelyking met kontroles, tumorvolume beduidend verlaag het met 1.16 cm^3 (95% VI -2.19 tot -0.12, P=0.03). Gebaseer op nege RCT's, met 104 rotte, het GLN-supplementasie grenslyn-beduidenis gehaal met 'n tumorvolume verlies van 2.46 cm^3 (95% VI -4.97-0.05, P=0.05) in vergelyking met kontroles.

Gevolgtrekkings

GLN-supplementasie blyk veilig te wees vir gebruik in kankerpasiënte. GLN mag potensieel meer effektief wees as kontroles in die verkoming van klinies betekenisvolle mukositis en in die verkorting van die duur van diarree. Verdere navorsing is nodig oor die effek van roetine GLN-supplementasie op kliniese uitkomste in onkologie.

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LIST OF ABBREVIATIONS

5-FU	5-fluorouracil
AA(s)	Amino acid(s)
AC	Doxorubicin and cyclophasphamide.
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
ANC	Absolute neutrophil count
ANLL	Acute non-lymphocytic leukaemia
ANLL/MDS	Acute non-lymphocytic leukaemia and myelodysplastic syndrome
aPBST	Autologous peripheral blood stem cell transplantation
Ara-C	Cytarabine
Arg	Arginine
Asp	Aspartate
ASPEN	The American Society of Enteral and Parenteral Nutrition
ATP	Adenosine triphosphate
BCAA(s)	Branched-chain amino acid(s)
BCKA	Branched-chain alpha ketoacids
BEAM	BCNU 300 mg/m^2, etoposide 200 mg/m^2, cytarabine 200 mg/m^2, melphalan 140 mg/m^2
BER	Basal energy requirements
Beta-hCG	Serum beta human chorionic gonadotrophin
BIA	Bio-electrical impedance analyses
BMI	Body mass index
BMT	Bone marrow transplant
BrdU	Bromo-deoxyuridine labelling index
BSC	Best supportive care
BUCY	Busulphan 14 or 16 mg/kg, cyclophosphamide 120 mg/kg
CAD	, doxorubicin, and dacarbazine
CAF	*Cyclophosphamide, doxorubicin, and 5-flourouracil*
CCPG	The Canadian Critical Care Clinical Practice Guidelines Committee
CCOP	Community Clinical Oncology Program
CDDPAdr	Cisplatin and doxorubicin
CF	Citrovorum factor
CFO	Calcium-folinate
Chi^2	Chi-squared test
CI(s)	Confidence interval(s)
Cit	Citrulline
CLL	Chronic lymphocytic leukaemia
CML	Chronic myelogenous leukaemia
CNS	Central nervous system
COLD	Chronic obstructive lung disease
CP	Carbamoylphosphate
CPT-11	7-ethyl-10-[4-(1-piperidinol)-1-piperidno]carbonyloxy-camptothecin
CR	Complete remission
CRP	C-reactive protein
CRT	Chemo-radiation therapy or radiochemotherapy
Hsp	Heat shock protein
HSV	Herpes simplex virus
IA	Ifosfamide and doxorubicin
IBD	Inflammatory bowel disease
IBW	Ideal body weight
ICU	Intensive care unit
Ida	Idarubicin
IE	Infectious event
IL	Interleukin
IP	Intestinal permeability
ITT	Intention-to-treat
LCF	Levo citrovorum factor
L/M	Lactulose/mannitol ration
LEV	Levamisole
LOS	Length of hospital stay or Length of stay
LPS	Lipopolysaccharide
LV	Leucovorin
MAC	Mid-arm circumference
MAMC	Mid-arm muscle circumference
MBI	Mucosal barrier injury
MD	Mean difference
MDS	Myelodysplastic syndrome
MECOG	Modified Eastern Cooperative Oncology Group grading system
MM	Multiple myeloma
M-MNC	Mesenteric blood mononuclear cells
Mo	Month
mTOR	Mammalian target of rapamycin
MPD	Myeloproliferative disorder
MTX	High dose methotrexate
MUAC	Mid-upper-arm circumference
NAD^+	Nicotinamide adenine dinucleotide
NADPH/ $NADP^+$	Nicotinamide adenine dinucleotide phosphate
NCCTG	North Central Cancer Treatment Group
NCI	National Cancer Institute Bethesda, Maryland
NCOG	Northern California Oncology Group
NEAA	Non-essential amino acids
NHL	Non-Hodgkin's lymphoma
NIE	Non-infectious event
NK	Natural killer
NKCC	Natural killer cell cytotoxicity
NO	Nitric oxide
NPE	Non-protein energy (calories)
NS	Not significant
NRI	Nutritional risk index

CTCAE	Common Terminology Criteria for Adverse Events, version 3.0	OMS	Objective mucositis score
CVC	Central venous catheter	OR	Odds ratio
CY	Cyclophosphamide	PAP	Plasmin - alpha2 -antiplasmin
CYTBI	TBI 14.4 Gy over 8 fractions, cyclophosphamide 120 mg/kg	PBPC	Peripheral blood prognitor cell
DA	Dark Agouti	PBSCT	Periphheral blood stem cell transplantation
DAMA	Dark Agouti mammary adenocarcinoma	PD	Progressive disease
df	Degrees of freedom	PD	Pancreaticoduodenectomy
DGS	Daily gut score	PGE2	Prostaglandin E2
DM	Diabetes mellitus	PN	Parenteral nutrition
DMBA	7,12-dimethylbenz[a] anthracene	PNI	Prognostic nutritional index
DMS	Daily mucositis score	POMS	Profile of Mood States
DSH	Delayed skin hypersensitivity	PR	Partial remission
DXM	Dextrinomaltose	PSP	Phenolsulfonphthalein
E	Energy	Pts	Patients
EAA	Essential amino acids	PUFA	Polyunsaturated fatty acids
ECOG	Eastern Cooperative Oncologic Group	RBP	Retinol-binding protein
ESPEN	The European Society for Clinical Nutrition and Metabolism	RCT(s)	Randomized controlled trial(s)/ Verewekansigde gekontroleerde proef(we)
FA	Freamine	RevMan5	Review Manager version 5
FAC	5-Flourouracil, doxorubicin, cyclophosphamide	RR	Risk ratio
FAcid	Folinic acid	RRR	Relative risk reduction
FAM	5-FU 600 mg/m^2, IV, day 1-5; doxorubicin 30 mg/m^2, IV, day 1; mitomycin 10 mg/m^2, IV, day 1	RT	Radiation therapy
FFM	Fat-free mass	RTOG	Radiation Therapy Oncology Group
FOLFOX	Oxaliplatin 85mg/m^2, day 1; folinic acid 200 mg/m^2, day 1, 2; 5-FU 400 mg/m^2 bolus + 600 mg/m^2 infusion over 22 hrs, day 1, 2	SAG	Superoxide anion generation
FSR	Fractional synthesis rate	SCT	Stem cell transplant
FTBI	Full total body irradiation	SFA	Saturated fatty acids
FU	Fluorouracil	SGA	SGA- Subjective global assessment
G	Gram	SMD	Standardized mean difference
GABA	γ-aminobutyrate	STPN	Standard TPN
G-CSF	Granulocyte-colony stimulating factor	TAT	Thrombin-antithrombin
GI	Gastrointestinal	TaxCh	Taxane-based chemotherapy
GLN	Glutamine	TBI	Total body irradiation
Glu	Glucose	TCA	Tricarboxylic acid
Glut	Glutamate	TLC	Total lymphocyte count
GMD	Geometric mean diameter	TNF-α	Tumour necrosis factor-alpha
GS	Glutamine synthetase	TPN	Total parenteral nutrition
GSH	Glutathione	TST	Triceps skinfold thickness
GTPN	Glutamine supplemented TPN	VAdrC	Vincristine, doxorubicin, and cyclophosphamide
GVDH	Graft-versus-host-disease	VI	Vertrouensinterval
HD	Hodgkin's disease	VOD	Veno-occlusive disease
HL	Hodgkin's lymphoma	WBC	White blood cell
HMB	Beta-hydroxy-beta-methylbutyrate	WHO	World Health Organization
HSC	Human stem cell	WP	Whole protein

LIST OF DEFINITIONS

Bias:[1] A bias is a systematic error, or deviation from the truth, in results or inferences. Biases can operate in either direction - leading to underestimation or overestimation of the true intervention effect. Biases can vary in magnitude - some are small (and trivial compared to the observed effect) and some are more substantial (so that an apparent finding may be entirely due to bias). Because the results of a study may in fact be unbiased despite a methodological flaw, it is more appropriate to consider the risk of bias. The reliability of the results of a randomized trial depends on the extent to which potential sources of bias have been avoided. Potential sources of bias include sequence generation (selection bias); allocation concealment (selection bias); blinding (performance bias, detection bias, attrition bias); incomplete outcome data (attrition bias); selective reporting (reporting bias); other sources of bias and publication bias.

Blinding:[1] Blinding (also referred to as masking) is the process by which study participants and personnel, including outcome assessors and care providers, are kept unaware of the intervention allocations after inclusion of participants into the study. Blinding may reduce the risk that knowledge of which intervention was received, rather than the intervention itself, affects outcomes and assessments of outcomes. Blinding seeks to prevent performance and detection bias by protecting the sequence after assignment and cannot always be implemented.

Chi-squared (Chi^2) test:[1] The Chi-squared test is a formal test for heterogeneity and is included in the forest plots in Cochrane reviews. It assesses whether observed differences in results are comparable with chance alone. A low P-value (or a large Chi^2 statistic relative to its degree of freedom) provides evidence of heterogeneity of intervention effects (variation in effect estimates beyond chance). Care must be taken in the interpretation of the Chi-^{2} test, since it has low power in the situation of meta-analysis when studies have a small sample size or are few in number. This means that while a statistically significant result may indicate a problem with heterogeneity, a non-significant result must not be taken as evidence of no heterogeneity. This is why a P-value of 0.10, rather than the conventional level of 0.05, is sometimes used to determine statistical significance.

Concealment of allocation:[1] Allocation concealment seeks to prevent selection bias in intervention assignment by protecting the allocation sequence before and until assignment, and can always be successfully implemented regardless of the study topic.

Confidence interval (CI):[1] The results for both individual studies and meta-analyses are reported with a point estimated together with an associated CI. The CI describes the uncertainty inherent in this estimate and describes a range of values within which we can be reasonably sure that the true

effect actually lies. If the CI is relatively narrow (e.g. 0.70 to 0.80), the effect size is known precisely. If the CI is wider (e.g. 0.60 to 0.93), the uncertainty is greater, although there may still be enough precision to make decisions about the utility of the intervention. Intervals that are very wide (e.g. 0.50 to 1.10) indicate that we have little knowledge about the effect and that further information is needed. A 95% CI is often interpreted as indicating a range within which we can be 95% certain that the true effect lies. The width of the CI for an individual study depends to a large extent on the sample size. Larger studies tend to give more precise estimates of effects (and hence have narrower CIs) than smaller studies. For continuous outcomes precision depends also on the variability in the outcome measurements (the standard deviation of measurements across individuals); for dichotomous outcomes it depends on the risk of the event, and for time-to-event outcomes it depends on the number of events observed. All these quantities are used in computation of the standard errors of effect estimates from which the CI is derived.

Continuous data:[1] Continuous data is where each individual's outcome data is a measurement of a numerical quantity that can take any value in a specified range (e.g. weight, area, volume).

Degrees of freedom (df):[1] The total number of studies included in a meta-analysis minus one equals the degrees of freedom (df) in a RevMan5 forest plot.

Dichotomous data:[1] Dichotomous (or binary) data is where each individual's outcome is one of only two possible categorical responses (e.g. dead or alive).

Fixed-effect meta-analysis:[1] If it is assumed that each study is estimating exactly the same quantity, a fixed-effect meta-analysis is used.

Forest Plot:[1] A forest plot displays effect measures and CIs for both individual studies and meta-analyses. Each study is represented by a block at the point estimate of intervention effect with a horizontal line extending either side of the block. The area of the block indicates the weight assigned to that study in the meta-analysis while the horizontal line depicts the CI (usually with a 95% level of confidence). The CI depicts the range of intervention effects compatible with the study's result and indicates whether each was individually statistically significant. The size of the block draws the eye towards the studies with larger weight (usually those with narrower CIs), which dominate the calculation of the pooled result.

Funnel plot:[1] A funnel plot is a simple scatter plot of the intervention effect estimates from individual studies against some measure of each study's size or precision. In common with forest plots, it is most common to plot the effect estimates on the horizontal scale and thus the measure of study size on the vertical axis. This is the opposite of conventional graphical displays for scatter plots, in which the outcome (e.g. intervention effect) is plotted on the vertical axis and the covariate (e.g. study size) is plotted on the horizontal axis. The name "funnel plot" arises from the

fact that precision of the estimated intervention effect increases as the size of the study increases. Effect estimates from small studies will therefore scatter more widely at the bottom of the graph, with the spread narrowing among larger studies. In the absence of bias the plot should approximately resemble a symmetrical (inverted) funnel. The more pronounced the asymmetry, the more likely it is that the amount of bias will be substantial. As a rule of thumb, tests for funnel plot asymmetry should be used only when there are at least 10 studies included in the meta-analysis, because when there are fewer studies, the power of the tests is too low to distinguish chance from real symmetry.

Gavage:[2] Introduction of nutritive material into the stomach by means of a tube.

Heterogeneity:[1] Any kind of variability among studies in a systematic review may be termed heterogeneity. Variability in the participants, interventions and outcomes of studies may be described as clinical diversity (or clinical heterogeneity), and variability in study design and risk of bias may be described as methodological diversity (or methodological heterogeneity). Variability in the intervention effects being evaluated in the different studies is known as statistical heterogeneity and is a consequence of clinical or methodological diversity, or both, among studies. Statistical heterogeneity manifests itself in the observed intervention effects being more different from each other than one would expect due to random error (chance) alone. It is important to consider to what extent the results of studies are consistent. If CIs for the results of individual studies (generally depicted graphically, using horizontal lines) have poor overlap, this generally indicates the presence of statistical heterogeneity.

I^2:[1] I^2 is a useful statistic that has been developed for quantifying inconsistency across studies that move the focus away from testing whether heterogeneity is present to assessing its impact on the meta-analysis. $I^2 = (Q\text{-}df)/Q \times 100\%$ where Q is the Chi^2 statistic and df is its degrees of freedom. This describes the percentage of the variability in effect estimates that is due to heterogeneity rather than sampling error (chance). The importance of the observed value for I^2 depends on (i) magnitude and direction of effects, and (ii) strength of evidence for heterogeneity (e.g. P-value from the chi-squared tests), or a CI for I^2. A rough guide to interpretation is as follows: 0% to 40% might not be important; 30% to 60% may represent moderate heterogeneity; 50% to 90% may represent substantial heterogeneity and 75% to 100% considerable heterogeneity.

Incomplete outcome data:[1] Missing outcome data due to attrition (dropout and withdrawal) during the study or exclusions from the analysis raise the possibility that the observed effect estimate is biased. The term incomplete outcome data refers to both attrition and exclusions. When an individual participant's outcome data is not available, it is referred to as missing. Attrition may

occur for the following reasons: Participants withdraw or are withdrawn from the study; participants do not attend an appointment at which outcomes should have been measured; participants attend an appointment but do not provide relevant data; participants fail to complete diaries or questionnaires; participants cannot be located (loss to follow-up); the study investigators decide, usually inappropriately, to cease follow-up; data or records are lost, or are unavailable for other reasons. In addition, some participants may become excluded from analysis for the following reasons: Some enrolled patients were later found to be ineligible; an 'as-treated' (or per-protocol) analysis is performed or the study analysis excluded some participants for other reasons. Some exclusions of participants may be justifiable, in which case they need not be considered as leading to missing outcome data. The intention to exclude such participants should be specified before the outcome data is seen.

Intention-to-treat analysis (ITT):[1] An ITT analysis is often recommended as the least biased way to estimate intervention effects in randomized trials. The principles of ITT analyses are: (i) Keep participants in the intervention groups to which they were randomized, regardless of the intervention they actually received; (ii) Measure outcome data on all participants; and (iii) Include all randomized participants in the analyses. The first principle can always be applied. However, the second is often impossible due to attrition beyond the control of the trialists. Consequently, the third principle of conducting an analysis that include all participants can only be followed by making assumptions about the missing values. Thus very few trials can perform a true ITT analysis without making imputations, especially when there is extended follow-up.

Mean Difference (MD):[1] The MD, or more correctly, the "difference in means" is a standard statistic that measures the absolute difference between the mean values in two groups in a clinical trial. It estimates the amount by which the experimental intervention changes the outcomes on average compared to the control. It can be used as a summary statistic in meta-analysis when outcome measurements in all studies are made on the same scale.

Meta-analysis:[1] Meta-analysis is the use of statistical methods to summarize (combine) the results of independent studies (two or more). By combining information from all relevant studies, meta-analyses can provide more precise estimates of the effects of health care than those derived from the individual studies included within a review. They also facilitate investigations of the consistency of evidence across studies and the exploration of differences across studies. Meta-analyses focus on pair-wise comparisons of interventions, such as an experimental intervention versus a control intervention, or the comparison of two experimental interventions. The outcomes of two groups treated differently are known as the effect, the treatment effect or the intervention effect. Whether analysis of included studies is narrative or quantitive, a general framework for synthesis

may be provided by considering four questions: (i) What is the direction of the effect? (ii) What is the size of the effect? (iii) Is the effect consistent across studies? and (iv) What is the strength of evidence for the effect? Meta-analyses provide a statistical method for questions (i) to (iii). Assessment of question (iv) relies additionally on judgments based on assessments of study design and risk of bias, as well as statistical measures of uncertainty.

Narrative review:[1] Narrative review (synthesis) uses subjective (rather than statistical) methods to follow through questions (i) to (iv) (as stated under meta-analysis definition) for reviews where meta-analyses are either not feasible or not sensible. In a narrative synthesis the method used for each stage should be pre-specified, justified and followed systematically. Bias may be introduced if the results of one study are inappropriately stressed over those of another.

Odds ratio (OR):[1] This is the ratio of the probability that a particular event will occur to the probability that it will not occur and can be any number between zero and infinity. In health care it is the ratio of the number of people with the event to the number without. It is commonly expressed as a ratio of two integers. For example, an OR of 0.01 is often written as 1:100, OR of 0.33 as 1:3 and OR of 3 as 3:1. OR is difficult to interpret. OR describes the multiplication of the odds of the outcome that occur with use of the intervention. To understand what an OR means in terms of changes in numbers of events, it is simplest to first convert it into a risk ratio, and then interpret the risk ratio in context of a typical control group risk.

Pair feeding:[3, 4, 5] Pair feeding is necessary to balance the chow intake among the animal groups to ensure an isonitrogenous and isocaloric diet, because the tumour and/or the various treatments may depress food intake. Pair feeding can be conducted by balancing chow intake among animals. This follows the idea that more chow is given to the animal that ate less, whereas less chow is given to the animal that ate more.

Point estimate:[1] The results for both individual studies and meta-analyses are reported with a point estimated together with an associated CI. The point estimate is the best guess of the magnitude and direction of the experimental intervention's effect compared to the control intervention.

Random effects meta-analysis:[1] The combination of intervention effect estimates across studies may optionally incorporate an assumption that the studies are not all estimating the same intervention effect, but estimate intervention effect that follows a distribution across studies.

Randomization:[1] Randomization allows for the sequence of allocation to interventions to be unpredictable. Randomization with no constraints to generate an allocation sequence is called simple randomization or unrestricted randomization. In principle, this could be achieved by allocating interventions using methods such as repeated coin-tossing, throwing dice or dealing

previously shuffled cards. More commonly it is achieved by referring to a published list of random numbers, or to a list of random assignments generated by a computer. Sometimes restricted randomization is used to generate a sequence to ensure particular allocation ratios in the intervention groups (e.g. 1:1). Blocked randomization (random permuted blocks) is a common form of restricted randomization. Blocking ensures that the numbers of participants to be assigned to each of the comparison groups will be balanced within blocks of, for example, five in one group and five in the other for every 10 consecutively entered patients. The block size may be randomly varied to reduce the likelihood of foreknowledge of intervention assignment. Also common is stratified randomization, in which restricted randomization is performed separately within strata. This generates separate randomization schedules for subsets of participants defined by potentially important prognostic factors, such as disease severity and study centres.

Randomized controlled trials (RCTs):[1] Work consisting of a clinical trial that involves at least one test and one control treatment, concurrent enrolment and follow-up of the test- and control-treated groups, and in which the treatments to be administered are selected by a random process. The individuals (or other units) followed in the trial were definitely or possibly assigned prospectively to one of two (or more) alternate forms of health care, using random allocation or some quasi-random method of allocation.

Review Manager version 5 (RevMan5):[1] The Cochrane Information Management System (IMS) consists of two main components, the Cochrane review writing software, Review Manager (RevMan5), which can perform a variety of meta-analyses, and a central server for managing documents and contact details, Archie. RevMan5 can be freely used by authors preparing a Cochrane review and by academic institutions.

Risk Ratio (RR):[1] Risk describes the probability with which a health outcome (usually an adverse event) will occur. In research risk is commonly expressed as a decimal number between 0 and 1, although it is occasionally converted into a percentage. In "summary of findings" tables it is often expressed as a number of individuals per 1 000. It is simple to grasp the relationship between risk and the likely occurrence of events: in a sample of 100 people the number of events observed will on average be the risk multiplied by 100. RR describes the multiplication of the risk that occurs with the use of the experimental intervention. For example, a RR of 3 for a treatment implies that events with treatment are three times more likely than events without treatment. Alternatively it can be said that treatment increases the risk of events by 100 x (RR -1) % = 200%. Similarly a RR of 0.25 is interpreted as the probability of an event with treatment being one quarter of that without treatment. This may be expressed alternatively by saying that treatment decreases risk of events by 100 x (1-RR) % = 75%. This is known as the relative risk reduction (RRR).

Selective outcome reporting:[1] Selective outcome reporting has been defined as the selection of a subset of the original variables recorded, on the basis of the results, for inclusion in publication of trials. The particular concern is that statistically non-significant results might be selectively withheld from publication.

Sensitivity analysis:[1] A sensitivity analysis is a repeat of the primary analysis or meta-analysis, substituting alternative decisions or ranges of values for decisions that were arbitrary or unclear. There are many decision nodes within the systematic review process which can generate a need for a sensitivity analysis. Examples include: searching for studies, eligibility criteria, what data should be analyzed and analyses methods. Some sensitivity analyses can be pre-specified in the study protocol, but many issues suitable for sensitivity analyses are only identified during the review process where the individual peculiarities of the studies under investigation are identified. When sensitivity analyses show that the overall result and conclusions are not affected by the different decisions that could be made during the review process, the results of the review can be regarded with a high degree of certainty.

Sequence generation:[1] The starting point for an unbiased intervention study is the use of a mechanism that ensures that the same kinds of participants receive each intervention. Several interrelated processes need to be considered. Firstly an allocation sequence must be used that, if perfectly implemented, would balance prognostic factors, on average, evenly across intervention groups. Secondly the most important among the practical aspects is concealment of the allocation sequence, which is the use of mechanisms to prevent foreknowledge of the next assignment.

Standardized mean difference (SMD):[1] The SMD is used as a summary statistic in meta-analysis when the studies all assess the same outcome, but measure it in a variety of ways (different scales). In this circumstance it is necessary to standardize the results of the studies to a uniform scale before they can be combined. The SMD expresses the size of the intervention effect in each study relative to the variability observed in that study. (Again in reality the intervention effect is a difference in means and not a mean of differences). Thus studies for which the difference in means is the same proportion of the standard deviation will have the same SMD, regardless of the actual scales used to make the measurements. However, the method assumes that the differences in standard deviations among studies reflect differences in measurement scales and not real differences in variability among study populations. This assumption may be problematic in some circumstances where real differences in variability are expected between the participants in different studies. The overall intervention effect can be difficult to interpret as the SMD expresses it in units of standard deviation rather than the original units of any of the measurement scales used in the review. The SMD is the difference in mean effects in the experimental and control

groups divided by the pooled standard deviation of participants' outcomes. The value of an SMD thus depends on both the size of the effect (the difference between means) and the standard deviation of the outcomes (the inherent variability among participants). Rules of thumb exist for interpreting SMD's, which have arisen mainly from researchers in the social sciences (for example, <0.41 = small, 0.40 to 0.70 = moderate, >0.70 = large).

Subgroup analysis:[1] Subgroup analyses involve splitting all the participant data into subgroups, often so as to make comparisons between them. Subgroup analyses may be done for subsets of participants (such as males or females), or subsets of studies (such as different geographical locations). Subgroup analyses may be done as a means of investigating heterogeneous results, or to answer specific questions about particular patients groups, types of interventions or types of study. When there are only two subgroups, the overlap of the confidence intervals of the summary estimates in the two groups can be considered. Non-overlap of the confidence intervals indicates statistical significance, but the confidence intervals can overlap to a small degree and the difference may still be statistically significant. Subgroup analyses are entirely observational in their nature. Even if individuals are randomized to one group or another within a clinical trial, they are not randomized to go in one trial or another. Hence subgroup analyses suffer the limitations of any observational investigation, including possible bias through confounding by other study-level characteristics. Furthermore, even a genuine difference between subgroups is not necessarily due to the classification of the subgroups.

Systematic review:[1] A systematic review attempts to collate all empirical evidence that fits pre-specified eligibility criteria in order to answer a specific research question. It uses explicit, systematic methods that are selected with a view to minimize bias, thus providing more reliable findings from which conclusions can be drawn and decisions made. The key characteristics of a systematic review are: a clearly stated set of objectives with pre-defined eligibility criteria for studies; an explicit, reproducible methodology; a systematic search that attempts to identify all studies that would meet eligibility criteria; an assessment of the validity of the findings of the included studies, for example through assessment of risk of bias; and a systematic presentation and synthesis of the characteristics and findings of the included studies.

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CHAPTER 1: BACKGROUND AND MOTIVATION FOR THE STUDY

1.1 INTRODUCTION

Glutamine (GLN) is the most abundant amino acid in plasma and the preferred fuel for fast proliferating cells, including enterocytes, lymphocytes and tumour cells. GLN was originally classified as a nonessential amino acid and is synthesized *de novo* with the contribution from the diet being dispensable. However, under conditions of metabolic stress induced by trauma, sepsis, burn injury and cancer the rate of glutamine utilization exceeds the body's ability to endogenously synthesize GLN and as a result GLN has come to be classified as conditionally essential under these circumstances. Recommendations for GLN supplementation have been made in various clinical settings (including critical illness). In patients with cancer a state of "GLN depletion" has been defined as part of the cancer cachexia syndrome marked by massive depletion of skeletal muscle GLN. The tumour has been described as a "glutamine trap", utilizing large amounts of GLN and reducing the availability of GLN to host tissues. Emerging evidence from preclinical studies indicates a reduced tumour growth in rat models after GLN supplementation, possibly via an immunological route, based on an increased natural killer (NK) cell activity. In addition GLN supplementation improves the efficacy of anti-tumour treatments in tumour-bearing animal models. In humans there is promising evidence supporting the experimental evidence. The side effects of cancer therapies, including bone marrow transplantation (BMT), surgery, radiation and/or chemotherapy are extensive. The gastro-intestinal side effects have a considerable impact on the cancer patients' ability to consume enough nutrients to maintain their nutritional status and quality of life. An additional role is suggested for GLN in reducing the toxicity of cancer therapies (including incidence of side effects such as mucositis, diarrhoea, neuropathy, veno-occlusive disease (VOD) and cardiotoxicity). The safety of GLN supplementation in oncology has been suggested by several authors, but not all are convinced that GLN has any role whatsoever and some are still concerned about "feeding" the tumour with an exogenous GLN supply. As yet no recommendations for clinical practice have been made, even though it is clear that some patients may well derive benefit from such supplementation.

1.2 GLUTAMINE AND ITS METABOLISM IN HEALTHY PERSONS

Glutamine is one of 20 amino acids (AA) encoded by the standard genetic code.[6] Amino acids are important substrates for numerous functions of which protein synthesis and growth are primary and others include gluconeogenesis for energy sourcing, ureagenesis for detoxification and regulation of cell metabolism in general.[7] Plasma concentrations of AA are maintained relatively constant in

the post-absorptive state of healthy adults, but during the neonatal period, under catabolic conditions and in disease most AA levels are changed (Figure 1.1 and Figure 1. 2).[7,8,9]

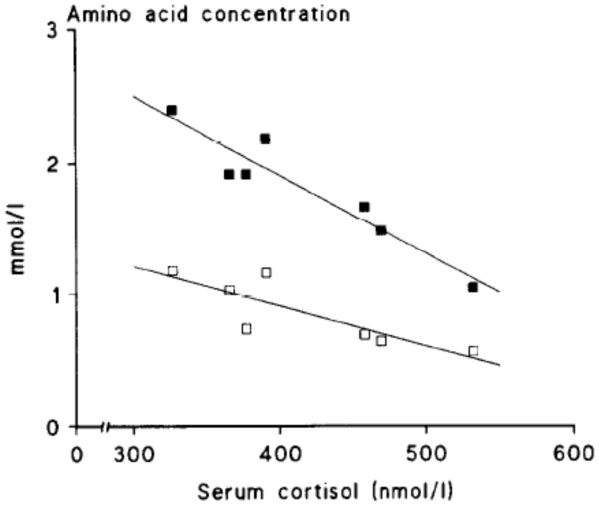
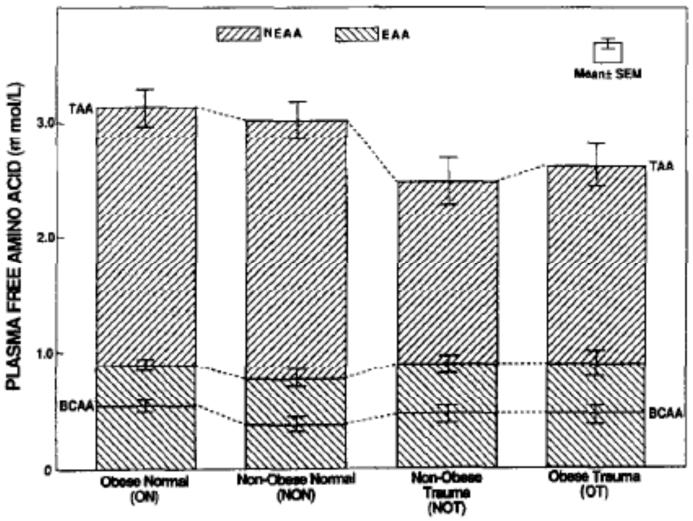


Figure 1.1: The relationship between serum cortisol concentration (normal range: 240-740 nmol/L), and the sums of non-essential (■, r= -0.95, P<0.01) and essential (□, r= -0.84, P= 0.02) plasma amino acid concentrations in surgical patients. Source:[8] Soop M, 1990:9.



NEAA: Non-essential amino acids (alanine, glycine, serine, glutamine, proline, arginine, histidine, taurine, glutamic acid, tyrosine, ornithine, citrulline, asparagine and cysteine), EAA: Essential amino acids (BCAA along with phenylalanine, tryptophan, methionine, threonine and lysine), SEM: Standard error of the mean, TAA: Total amino acids (sum of 8 EAA and 14 NEEA), BCAA: Branched-chain amino acids (valine, leucine and isoleucine)

Figure 1.2: Plasma-free AA levels in obese normal, non-obese normal, non-obese trauma, and obese trauma subjects (*N*=10 in each group). Source:[9] Jeevanandam M, 1991:40.

Based on the required dietary (exogenous) intake to maintain nitrogen balance or growth, AA's can be classified as either:[7,10]

- nutritionally essential (indispensable); or
- non-essential (dispensable) in the diet; or
- conditionally essential in certain conditions, requiring supplementation of the diet.

Essential amino acids (EAA)[7,10,11] are defined as those AA (Table 1.1) whose carbon skeletons cannot be synthesized or those that are inadequately synthesized *de novo* (endogenous) by the body relative to needs and which must be provided by the diet to meet optimal requirements. Non-essential AA (NEAA)[7,10,11] are those AA (Table 1.1) which can be synthesized *de novo* in adequate amounts by the body to meet optimal requirements. Conditionally essential AA[7,10,11] are those that normally can be synthesized in adequate amounts by the body, but which must be provided from the diet to meet optimal needs under conditions where rates of utilization are greater than rates of synthesis. Arginine, cysteine, GLN, leucine, proline and tryptophan are further classified as functional AA,[7] because of their function as regulators of key metabolic pathways that are necessary for maintenance, growth, reproduction and immunity in organisms, and also in maximizing efficiency of food utilization, enhancing protein accretion, reducing adiposity and improving the general health of humans.

Table 1.1: Amino acid classification and plasma, urine and cerebrospinal fluid concentrations in healthy adults *(Determined by Ion Exchange Chromatography)*

ESSENTIAL AMINO ACIDS (EAA)				**NON-ESSENTIAL AMINO ACIDS (NEAA)**			
Amino acids	**Plasma †**	**Urine ‡**	**CSF †**	**Amino acids**	**Plasma †**	**Urine ‡**	**CSF †**
Arginine[a]	80 (20)	<5	18.3 (3.2)	Alanine	333 (74)	16-68 (30)	23.2 (5.1)
Histidine	82 (10)	26-153 (79)	11.9 (1.7)	Asparagine	41 (10)		5.4 (1.4)
Isoleucine	62 (14)	<4	3.9 (1.0)	Aspartate	3 (1)	2-7 (4)	0.6 (0.3)
Leucine	123 (25)	2-11 (5)	10.1 (2.1)	Citrulline	38 (8)	<4	1.5 (0.5)
Lysine	188 (32)	7-58 (17)	21.7 (3.7)	Cysteine	52 (11)	6-34 (13)	0.1 (0.1)
Methionine	25 (4)	2-1 (6)	1.9 (0.7)	Glutamate	24 (15)	<12	11.3 (6.4)
Phenylalanine	57 (9)	2-19 (7)	6.5 (1.2)	**Glutamine**	586 (84)	20-76 (36)	444 (84)
Threonine	140 (33)	7-29 (13)	27.7 (4.7)	Glycine	230 (52)	43-173 (107)	4.7 (1.5)
Tryptophan	-	-	-	Proline	168 (60)	<9	-
Valine	233 (43)	3-13 (5)	15 (2.8)	Ornithine	55 (16)	<5	3.7 (1.0)
				Serine	114 (19)	21-50 (30)	24.5 (4.4)
				Taurine	55 (13)	16-180 (72)	6.8 (1.7)
				Tyrosine	59 (12)	2-23 (10)	6.4 (1.5)
				3-Methyl-histidine	3 (2)	19-47 (32)	-

† CSF: Cerebrospinal fluid, mean (standard deviation), umol/L; ‡ Range (mean), umol/mol of creatinine

[a]Arginine is an EAA for young mammals on the basis of functional needs for vascular homeostasis, spermatogenesis and fetal growth, although it may not be required in the diet to maintain nitrogen balance in adults of most species.

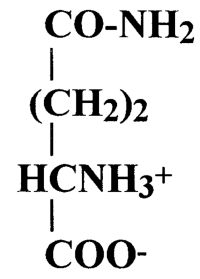
Source:[7,11-12] Wu G, 2009:39; Cynober LA, 2004:2nd Ed; Decker DM, 2002:6.

Ritthausen was the first to record the discovery of GLN in 1866.[13] GLN became known as the most abundant free proteic amino acid in the human plasma and its importance for optimal growth of cells in culture has been documented since the 1950's.[10,13-15] Fifty percent (50%)[16] of the whole body pool of free AAs comprises of GLN. In mammals the physiological level of GLN is approximately 650 µmol/L plasma[11,17] and exceeds that of other amino acids (AAs) by far (Table 1.1). GLN constitutes approximately 25%[13] in extracellular fluid and 60-75%[13,16] in skeletal muscle (20050(514 µmol/L intracellular water)[11] with most of the remains stored in the liver.[16]

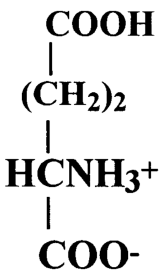
Skeletal muscle serves as a major source[18-19] of GLN during periods of metabolic stress. Plasma contains only a small proportion of the free GLN pool; therefore the plasma concentration is not necessarily a true reflection of intracellular concentrations, and is not representative of possible changes occurring in the free amino acid pool as a whole under conditions of stress.[13] An extensive exchange of GLN exists between GLN-producing and GLN-utilizing (consuming) tissues. The plasma GLN level is determined by the balance of GLN production and GLN utilization. GLN was at first classified as a NEAA in healthy adults in 1938 because of the *de novo* synthesis by many tissues according to need.[19] Healthy adults in the basal state have an endogenous GLN production rate of 40-80 g/24 hours,[20-21] providing 60-80% of the plasma flux of GLN through *de novo* synthesis; therefore the contribution by the diet (5-40%)[11,20] is dispensable. It is contained in most dietary proteins (including fish, red meat, beans, dairy products and others), which is considered an adequate exogenous source of GLN for healthy individuals, providing 5-20 g of GLN per day (if the protein intake is between 100-200 g).[12,20]

GLN and its main substrate for endogenous synthesis, glutamate (together with proline, histidine, arginine and ornithine), comprise 25% of the dietary amino acid intake and constitute the "glutamate family" (Figure 1.3) of AAs, of which all is broken down to glutamate for disposal in the body.[14]

The GLN molecule ($C_5H_{10}N_2O_3$, Figure 1.3) contains 5 carbon atoms and bears a 5-carboxamide in its R-group, which can readily be deaminated for nitrogen release. The C_5 structure makes GLN compatible with the C_5-dicarboxylic acids of the tricarboxylic acid (TCA) cycle and its metabolites. Therefore, GLN plays a central role in nitrogen and carbohydrate metabolism in cells.[11]

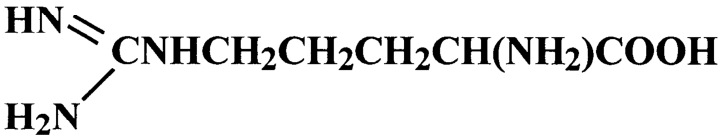


Glutamine

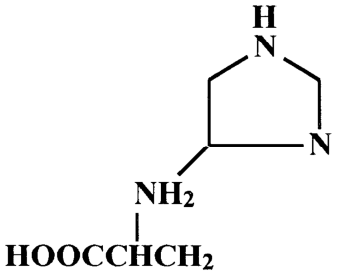


Glutamic acid

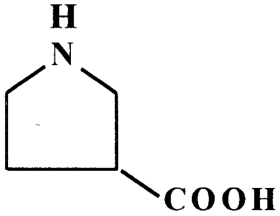
Ornithine



Arginine



Histidine



Proline

Figure 1.3: Glutamate family of amino acids. Source:[14] Tapiero H, 2002:56.

It has been well documented that conditions of metabolic stress induce a state of GLN deficiency characterized by depleted muscle GLN pools followed by reduced plasma concentrations, especially in critical illness, particularly surgery, trauma/injury, burns, sepsis and cancer.[8,22-23] Low plasma GLN concentrations have been associated with higher age,[23] mortality[21-24] and morbidity[21-22,24] in intensive care unit (ICU) patients. Many authors have debated the non-essential

status of GLN over the past two decades in stressful situations,[10,18,22] since data suggests that there is some benefit to be derived from exogenous supplementation of GLN[10] under certain conditions. Current data acknowledges that GLN fulfills the requirements for a conditionally essential amino acid in critically ill patients, and it has been classified as such.[7,19,25]

1.2.1 Endogenous GLN Synthesis

GLN is synthesized from glutamate, which itself is readily convertible to α-ketoglutarate, feeding the TCA cycle.[11] GLN is formed by enzymatically replacing a hydroxyl side chain of glutamate with an amine functional group (NH_4^+) by the action of GLN synthetase (GS).[6, 11] GS is an enzyme that can be found in the cytosol of most cell types (including brain atrocities,[10, 25] adipose tissue, heart and placenta), but net synthesis of GLN occurs predominantly in skeletal muscle and the lungs.[11,19,21] It promotes the reaction:[11]

Adenosine triphosphate (ATP) + NH_4^+ + glutamate → ADP + P_1 + GLN + H^+

The activity of GS is regulated by its substrates and products. It has been shown that the GLN formation rate decreases when plasma GLN is restored in patients receiving exogenous GLN administration. This indicated that GLN synthetases' activity in skeletal muscle is regulated to maintain plasma GLN at a defined level.[11]

1.2.2 GLN Oxidation

GLN is consumed rapidly by especially fast proliferating cells. GLN catabolism/breakdown/oxidation takes place in the mitochondria, where GLN is converted to glutamate and ammonia by the mitochondrial enzyme glutaminase in the nonreversible reaction:[11]

GLN + H_2O → glutamate + NH_4^+

Glutaminase activity is found only in a small number of tissues, including liver, kidney, enterocytes (gut), and immune cells (leucocytes), which are the main consumers of GLN.[11]

1.2.3 GLN Transport

The GLN formed by GS is an uncharged water-soluble compound that can readily pass through cell membranes, whereas the precursor glutamate, which bears a net charge, needs facilitation by specific transport systems.[11] However, GLN does not freely cross the plasma membrane. Plasma GLN uptake and exchange are mediated by a number of transporters (Na^+-dependent and Na^+-independent transport systems) that show a tissue-specific expression pattern.[11] Humans hold GLN free in solution in skeletal muscle at a gradient of 32:1 over plasma levels by these active transport mechanisms.[22]

1.2.4 Inter-organ Exchanges

Skeletal muscle (Figure 1.4) takes up branched-chain amino acids (BCAAs) from the arterial blood as the major source of nitrogen and carbon to synthesize GLN. The GLN formation rate in skeletal muscle increases in response to enhanced GLN withdrawal from the plasma by GLN-utilizing cells under stress conditions.[11] Skeletal muscle can also utilize α-ketoglutarate (derived primarily from glucose) for GLN synthesis.[11] Thus, the availability of α-ketoglutarate as secondary substrate for GLN synthesis can help to spare muscle GLN pools in patients during conditions of stress, demonstrating that synthesis of GLN in skeletal muscle can keep pace with GLN consumption if ample precursors are provided.[11,26-27] Excess GLN is released into blood circulation and transported to GLN-consuming tissues.[11] The activity of GS in skeletal muscle is regulated to maintain plasma GLN at a defined level.[11]

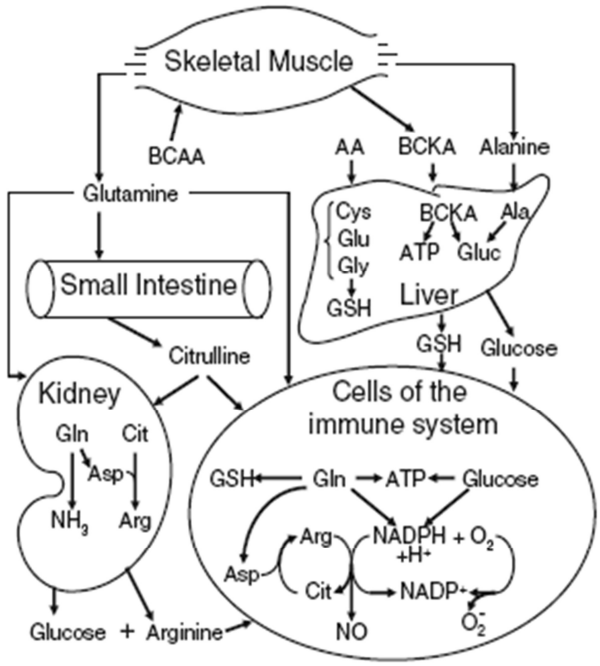
Like skeletal muscle, the lungs (Figure 1.4) act as a GLN donor in the post-absorptive state.[11] The lungs obtain the required substrates for GLN synthesis from circulation.[19] Eighty percent of the total GLN released from the lungs comes from uptake of precursor nitrogen in the form of ammonia and glutamate from circulation.[19] Although the lung vasculature has a lower GLN concentration compared to skeletal muscle, the lungs have a higher blood flow and therefore are able to release similar amounts of GLN into circulation.[19]

The liver (Figure 1.4) has an elegant mechanism to detoxify ammonia and supply GLN. The periportal region contains hepatic isozymes of the enzyme glutaminase and is the site for urea biosynthesis from high incoming concentrations of ammonia from the splanchnic bed while at the same time ammonia and glutamate are generated from incoming GLN.[11] On the other hand high

levels of GS are present in the specialized cells around the hepatic outflow in the perivenous portion of the liver that act as scavengers of excess ammonia, converting glutamate into GLN.[28] This distribution aids to detoxify the blood from portal and systemic circulation of toxic ammonia while contributing GLN to the systemic supply.[11] The liver is the primary organ for the synthesis of glutathione (from glutamate, glycine and cysteine) and of glucose (from alanine) for use by extra hepatic cells (including immunocytes) and other tissues.[7,10]

The kidney (Figure 1.4) uses a kidney-type isozyme of glutaminase to hydrolyze GLN to produce ammonia, which is excreted in the urine to dispose of excess nitrogen.[18-19,28]

In contrast, gut enterocytes (Figure 1.4) and leucocytes (Figure 1.4) utilize GLN as a main respiratory fuel for ATP production.[11] GLN is the preferred oxidative energy substrate for these rapidly proliferating cells, even over glucose.[10,20] In the healthy individual GLN and other NEAA (including glutamate and aspartate) are extensively oxidized by absorptive epithelial cells (enterocytes) of the small intestine, such that nearly all of them in a conventional diet do not enter the portal vein.[7] The small intestine utilizes GLN from both the arterial circulation and intestinal lumen, but takes up glutamate and aspartate only from the intestinal lumen.[7] The catabolism of GLN in the intestine results in the production of CO_2, alanine, pyruvate and lactate from the carbon skeleton and of ammonia and alanine from the carboxamide and amino groups. Complete oxidation of GLN via glutamate, α-ketoglutarate and the TCA cycle results in 27 ATP equivalents (compared to 36 ATP produced from glucose),[10] which is one of the highest rates of all NEAAs.[11] In addition, the small intestine (Figure 1.4) utilizes GLN to synthesize citrulline, which is converted into arginine in the kidneys, cells of the immune system and other cell types.[11] Lymphoid tissues, neutrophils, monocytes and macrophages also have a high rate of GLN utilization.[11] Lymphocytes require GLN in order to proliferate in response to an antigenic challenge.[11] Macrophages may consume GLN as a source of energy and as a precursor for nucleotides.[19] The preference for GLN over glucose as energetic fuels becomes more pronounced when enterocytes and leucocytes are stressed, as demonstrated by the increase in the relative amount of GLN oxidation in GLN-utilizing cells as glucose levels fall.[18,28] For example the rate of GLN utilization of human neutrophils was recently found to depend on the extracellular glucose concentration.[7,20,22] GLN metabolism may therefore be particularly important as a source of fuel for neutrophils and other rapidly proliferating cells in situations where the blood glucose concentration is low, which is often the case in critical illness.



Skeletal muscle takes up branched-chain amino acids (BCAA) from the arterial blood, synthesizes both alanine and glutamine from BCAA and alpha-ketoglutarate, and releases those two amino acids into the circulation. The small intestine utilizes glutamine to synthesize citrulline, which is converted into arginine in kidneys, cells of immune system, and other cell types.

AA: Amino acids, Arg: Arginine, Asp: Aspartate, ATP: Adenosine triphosphate, BCKA: Branched-chain alpha-ketoacids, Cit: Citrulline, Cys: Cysteine, Gln: Glutamine, Glu: Glutamate, Gluc: Glucose, Gly: Glycine, GSH: Glutathione, NADPH: Nicotinamide adenine dinucleotide phosphate, NO: Nitric oxide

Figure 1.4: Inter-organ metabolism of branched-chain amino acids, glutamine and arginine and its role in immune function. Source:[7] Wu G, 2009:37.

1.2.5 Functions of GLN

"Most amino acids have multiple functions, but GLN appears to be the most versatile...", according to Sir Hans Krebs, pioneer of biochemical research.[11]

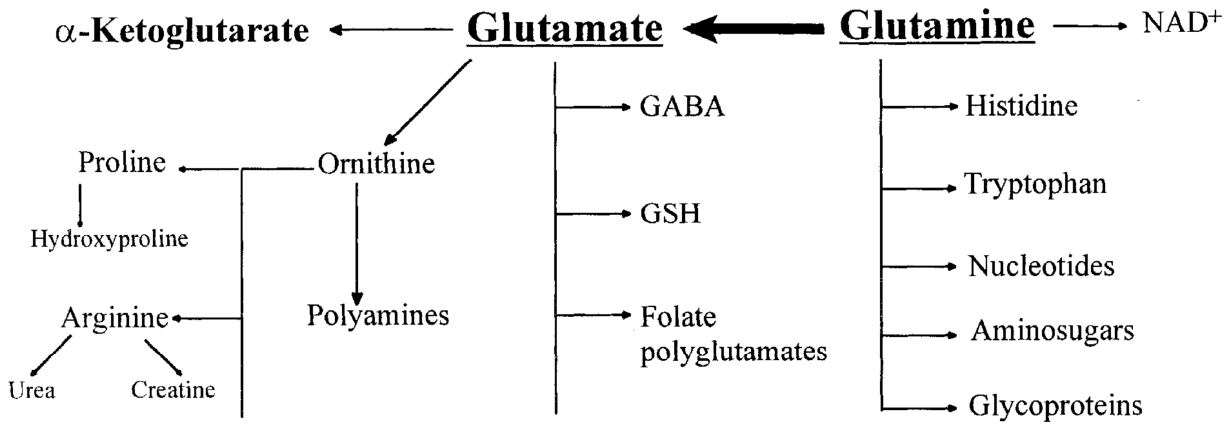
The functions of GLN and its major metabolites (Figure 1.5, Table 1.2) within cells can generally be separated into the following four categories:

(1) Its role in nitrogen transport;

(2) Its importance in maintaining the cellular redox state;

(3) Its position as a metabolic intermediate; and

(4) Its role as an energy source.[7,10,11,14,18,28]



NAD+: Nicotinamide adenine dinucleotide;GABA: γ-minobutyrate; GSH: Glutathione

Figure 1.5: Metabolic products derived from GLN. Source:[14] Tapiero H, 2002:56.

In the first place GLN is responsible for preserving the concentration of GLN in plasma. When plasma GLN is insufficient to satisfy the demand, GLN synthesis occurs from skeletal muscle and the liver with a GLN flux to plasma to maintain physiological levels of 650 μmol/l.[14]

Glutamine is an essential precursor for protein and amino acid synthesis and an important regulator of protein turnover through cellular mammalian target of rapamycin (mTOR) signalling, gene expression and immune function.[11,14,28]

GLN is the major respiratory fuel for rapidly proliferating cells (including enterocytes, colonocytes, lymphocytes, macrophages and tumours).[7] The oxidation of GLN by these cells generates ATP for cellular respiration and they strictly depend on the availability of GLN as an energy source.[13]

The release of GLN from skeletal muscle, lungs, adipose tissue, brain and liver serves to transport nitrogen and carbon to GLN-utilizing cells for metabolic intermediates and macromolecular synthesis.[11] GLN functions as a key link within carbon metabolism of carbohydrates and proteins and also plays an important role in the growth of GLN-utilizing cells, namely fibroblasts, lymphocytes and enterocytes.[14] In addition GLN has a function in the synthesis of purine, pyrimidine nucleotide, ornitine, citrulline, arginine, proline, asparagines and in particular nicotinamide adenine dinucleotide phosphate (NADPH/NADP+).[11]

GLN acts as a nitrogen reservoir and thus improves nitrogen balance, being the most important nitrogen carrier in the body, and consequently serves as a nitrogen transporter between various tissues/organs. In fact, GLN accounts for 30-35% of all amino acid-derived nitrogen transported by blood.[11,18-19] It donates nitrogen for the synthesis of purines, pyrimidines, nucleotides and amino sugars (Figure 1.5).[14]

As a source of intracellular glutamate, GLN provides one of the components of the major intracellular antioxidant glutathione (GSH) - protecting the cell against oxidative injury in the course of supporting GSH metabolism and homeostasis.[11] Availability of GLN for GSH synthesis may have profound effects on cellular redox control and its depletion is associated with severe clinical symptoms.[18] There is evidence that exogenous provision of GLN dipeptide attenuates posttraumatic glutathione depletion in human muscle.[13]

In addition GLN plays a role in whole-body glucose homeostasis. The gluconeogenesis from GLN occurs primarily in the kidney and the liver during periods of starvation.[11,28] Renal gluconeogenesis contributes to 20-25% of whole-body glucose production.[11] Overall GLN gluconeogenesis is responsible for about 5% of systemic glucose appearance, and renal production of glucose from GLN accounts for nearly 75% of all glucose derived from GLN.[14]

Other functions of GLN include the direct interference with apoptosis and hepatic ureagenesis.[7,11] GLN is the major source of nitrogen used in the liver for ureagenesis.[11]

Glutamine metabolites (Figure 1.5, Table 1.2) are further involved in several important functions.

Table 1.2: Functions of GLN metabolites

Functions of GLN metabolites – *glutamate (Glut) and aspartate (Asp)*
• Excitatory neurotransmitters. Deamination of GLN via glutaminase produces glutamate, a precursor of gamma-amino butyric acid, a neurotransmission inhibitor. L-glutamate is the most abundant free amino acid in the brain and it is the major excitatory neurotransmitter of the vertebrate central nervous system (CNS). Most free L-glutamic acid in the brain is derived from local synthesis from L-GLN and Krebs cycle intermediates. It clearly plays an important role in neuronal differentiation, migration and survival in the developing brain via facilitated Ca^{++} transport.
• Components of the malate shuttle.
• Cell energetics and metabolism.
• Glutamate is converted to α-ketoglutarate and ammonia by the enzyme glutamate dehydrogenase. α-ketogluterate is an integral component of the citric acid cycle.
• Ammonia detoxification. The interconversion of charged glutamate to uncharged GLN includes the toxic compound ammonia. The formed GLN is nontoxic and membrane permeable. Thus GLN formation from glutamate assists with detoxifying ammonia and removing it from the cell. This ammonia detoxification is particularly important in brain astrocytes. GLN serves as a "nitrogen shuttle", which provides a vehicle for transportation of ammonia in a nontoxic form from peripheral tissues to visceral organs where it can be excreted as ammonium by the kidneys or converted to urea by the liver.
• Major fuels for enterocytes.
Functions of GLN metabolite – *glucosamine-6-P*
• Synthesis of amino sugars and glycoprotein.
• Inhibition of nitric oxide (NO) synthesis.
Functions of GLN metabolite – *ammonia*
• Renal regulation of acid-base balance. GLN is one of the most important substrates for ammoniagenesis in the gut and kidney. The kidney disposes of excess nitrogen by consuming GLN and excreting the ammonia produced.
• Synthesis of glutamate and carbamoylphosphate (CP). In the liver, ammonia can combine with CO_2 to form carbamoylphosphate, which subsequently enters the urea cycle.
Functions of GLN metabolite – *foly-polyglutamates:*
• GLN is a component of polyglutamated folic acid - intracellular substrates and regulators of one carbon metabolism. Foly-polyglutamates synthesis is required for normal folate retention by cells.

Source:[7,10-11,14,18,25,28] Wu G, 2009:37; Lacey JM, 1990:48; Cynober LA, 2004; Tapiero H,2002:56; Labow BI, 2000:24; Mates JM, 2009:in press; Abcouver SF, 1999.

1.3 GLUTAMINE METABOLISM IN CRITICAL ILLNESS

Critical illness induces a catabolic state as a result of metabolic stress induced by surgery, trauma or burn injury and can be characterized by a loss of nitrogen from the body, breakdown of skeletal muscle protein,[22] profound glutathione and GLN depletion[13,22-23,29] (Figure 1.6), depressed immune function,[30] the translocation of the amino acids to visceral organs and the wound site from skeletal muscle and increased excretion of creatinine and 3'-methyl-histidine, both found mainly in muscle tissue.[14] In humans, plasma and muscle GLN levels are lowered by up to 50% by sepsis, major injury/trauma, burn injury[11,13] and following surgery.[8]

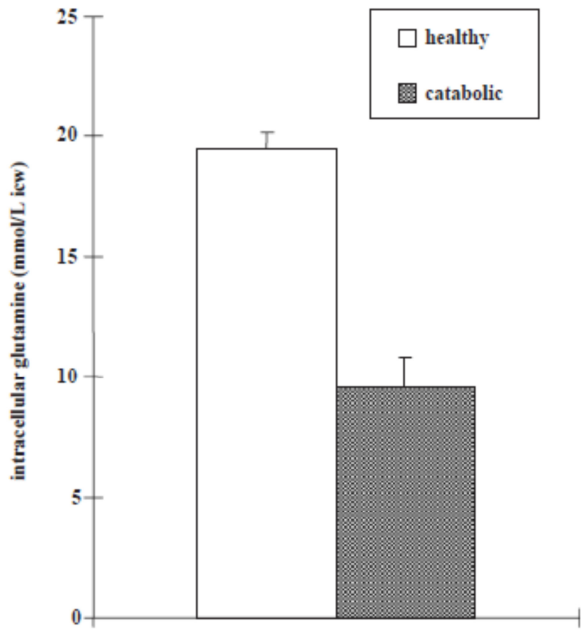
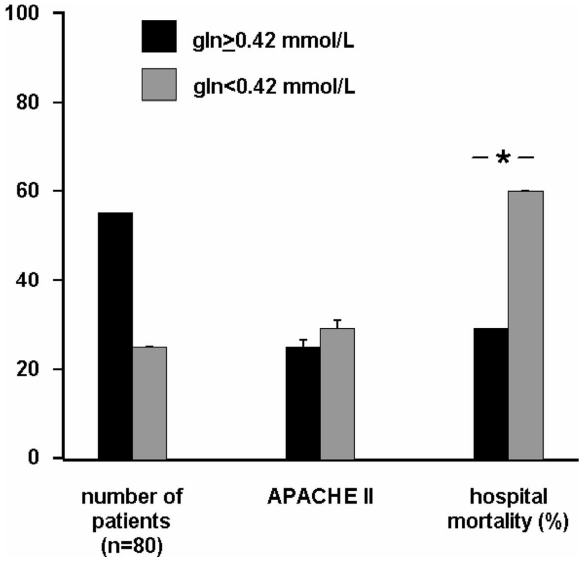


Figure 1.6: Depletion of intracellular muscle free glutamine from studies in Stockholm, New York, and Manchester (*N*=126). Mean (SEM). Source:[13] Furst P, 2004:1.

Plasma GLN depletion at admission has been deemed a predictor of poor outcome in ICU patients with sepsis[20] (Figure 1.7) and several other critical injuries and illness.[23]



Although the Acute Physiology and Chronic Health Evaluation (*APACHE*) II scores were comparable at admission, mortality was doubled in patients with a plasma glutamine level <0.42 mmol/L.

Figure 1.7: Plasma GLN depletion at admission is a predictor of a poor outcome in intensive care unit patients with sepsis. Source:[20] Tjader I, 2007:35.

A major benefit of nutritional support supplemented with GLN or GLN precursors (such as α-ketoglutarate) during critical illness is its muscle-sparing effect.[26] In the absence of nutritional support (which is often the case in ICU), the precursors for enhanced GLN production by skeletal muscle are derived mainly from proteolysis of muscle protein.[20] In addition there is evidence that GLN supplemented total parenteral nutrition (TPN), increased the total appearance rate of GLN ($P<0,05$), but did not inhibit or increase the endogenous appearance rate.[31]

The initial response to septic stress involves the export of GLN to the splanchnic bed and immune system from the free amino acid pool in skeletal muscle.[22] The liver becomes the major organ of GLN uptake and the negative nitrogen balance observed after injury is primarily a consequence of the increased excretion of urea in the urine.[22] Increased GLN formation in the liver is mediated by tumour necrosis factor-alpha (TNF-α) glucocorticoids and prostaglandins.[22] In skeletal muscle and lungs, GS gene expression and activity increases whereas no change in glutaminase activity is observed during sepsis.[20] Despite an increase in GS expression and activity, the rate of release exceeds that of synthesis resulting in intracellular GLN pool depletion.[22] In the inflammatory state, GLN utilization is tenfold greater in proliferating lymphocytes than in resting cells.[14] This leads to an increased rate of *de novo* GLN synthesis in skeletal muscle and lungs in response to enhanced GLN withdrawal from the plasma by GLN-utilizing cells under stress conditions.[11] However, recent evidence suggests that the endogenous GLN synthesis rate in critically ill patients remains similar to

that in the basal state of healthy adults and the efflux of GLN from muscle appears to remain constant or is only slightly elevated[21], probably because ICU patients are constantly fed enterally or parenterally over time.

During catabolic states the balance that exists between GLN production and GLN oxidation is significantly altered towards increased oxidation resulting in GLN depletion.[18] A combination of increased GLN oxidation by GLN-consuming cells and enhanced gluconeogenesis that occur in injured patients,[14] together with a decrease in nutrient intake and uptake (malabsorption), creates a higher GLN demand than normal.[11] When its consumption by GLN-utilizing cells exceeds the body's ability to synthesize it endogenously,[12,28,32] GLN becomes a conditionally essential AA.[10] Muscle remains a major producer of GLN in such catabolic states and although plasma GLN levels are usually maintained in injured patients, lung and muscle stores can rapidly be depleted to concentrations of 25% of normal levels after severe injury or infection and 50% after surgery (Figure 1.6).[18-19,28] Muscle maintains a rapid efflux of GLN in part by increasing the rate of muscle proteolysis while decreasing the rate of protein synthesis.[28] This increases the intracellular pool of amino acids for production of GLN, which can be derived from all AAs through their conversion to glutamate, either directly or through α-ketoglutarate.[26-28] Muscle GLN stores and GLN supplies by muscle proteolysis do not suffice to meet demand for GLN, because of either the magnitude of GLN use or depletion of muscle mass. The shortage of GLN is eventually reflected in decreased plasma GLN levels of up to 30%,[19] and GLN-utilizing cells therefore suffer from GLN starvation under these conditions.[10-11,21] Decreased intramuscular GLN levels have been associated with a negative protein balance in skeletal muscle, suggesting a possible link with protein turnover in catabolic conditions.[7]

Gut enterocytes and leucocyte immune cells exhibit the highest susceptibility to this state of "GLN depletion" under catabolic conditions, since GLN is used as the major substrate for ATP formation by these cells.[11] Both cell types play important roles in the defense against microorganisms. Enterocytes are essential for maintenance of the integrity of the gut mucosa, which will prevent the translocation of bacteria from the lumen into the abdominal cavity. On the other hand leucocytes must detect and eliminate invading microorganisms in the course of the immune response.[11] Both capabilities are essential during critical illness. However, reduced availability of GLN under these conditions has adverse effects on the energy metabolism, protein synthesis, cell-protective mechanisms, viability, immunosupression and function in general of GLN-consuming cells.[11] However, some authors[15,33] do not believe that the "GLN deficiency state" in critical illness has been validated. Narrative reviews by these authors[15,33] of evidence published from 1985 to

2002 highlighted that the decreased plasma GLN levels during critical illness are not specific to this amino acid. They both reported that there is not enough evidence that a so-called "GLN deficiency" predicts a worse outcome. Alpers[15] concluded that the evidence reviewed at that time did not necessarily predict a special need or role for GLN in critical illness.

GLN starvation will lead to a reduction of available intracellular ATP for energy metabolism. This cellular energy depletion leads to activation of the activated protein kinase (AMPK) pathway, which regulates enzyme activity and gene expression. The AMPK functions as a metabolic switch that stimulates catabolic pathways and inhibits anabolic pathways and protein synthesis, thereby preserving ATP.[11] In the course of inflammation cells are confronted with a number of cytotoxic mediators, including endotoxin, cytokines and reactive oxygen species (ROS).[11]

Cells express a group of proteins, the stress or heat shock proteins (Hsp) (especially Hsp70, a 70-kDA heat shock protein highly inducible in leucocytes), that is essential to cellular protection and survival under stressful conditions in critical illness.[11,22] GLN starving cells are unable to express normal amounts of Hsp70. Since plasma GLN depletion occurs during systemic inflammation, the impaired Hsp70 expression under these conditions is likely to have deleterious effects on the survival and function of leucocytes and may contribute to the immunosuppression observed in these patients.[11]

In addition, GLN starvation leads to reduced intracellular glutathione (GSH) levels. The oxidation of GSH to glutathione disulphide (GSSG) plays a central role in the protection against genotoxic agents and oxidants, as well as in the control of cellular thiol/disulfide redox state, which is essential for normal redox signalling. A decrease in the GSH:GSSG ratio, which determines intracellular redox state, affects the activity of redox-sensitive kinases which is an important cell protective mechanism.[11] Oxidizing conditions activate pathways that reduce cytokine production and proliferation of cells but increase apoptosis.[11] GLN starvation is characterized by cell shrinkage, which affects the osmo-signalling pathways and increases susceptibility of the cell to apoptosis triggers.[11]

Reduced availability of amino acids in general (but especially GLN) reduces the activity of mTOR, which is a central regulatory factor in the control of cell translation.[11] The available GLN is also detected by glutaminyl-tRNA synthetase, which acts as a regulatory cofactor in the FAS-mediated apoptosis pathway.[11] All these mechanisms result in a net reduction of anabolic pathways and an increase in catabolic pathways in order to spare resources.[11] In addition, several of these mechanisms lead to an increased susceptibility of the cell to apoptosis triggers.[11]

However, protein catabolism is a generalized response to trauma and does not reflect the simple loss of protein from injured tissue. In particular the hormonal and inflammatory environment is a major regulator of this catabolic response.[14] Cortisol (Figure 1.1) has a pronounced effect in stimulating GLN synthesis in skeletal muscle and glucagon appears to be essential in enhancing hepatic uptake of GLN to facilitate ureagenesis.[14] Inflammatory factors such as pro-inflammatory cytokines, leucotrienes and other factors such as catecholamines contribute to the catabolic response in general.[14] The kidney assists in neutralizing the large acid load that is generated after injury. The major pathway used is the increased uptake of GLN by the kidney, contributing to ammonia and ammonium ion production, which are excreted in the urine.[14] After thermal injury or elective surgery GLN concentrations in lymphocytes can fall to a low level, believed to contribute to impaired immunological function occurring after these injuries.[14] GLN supplementation has been reported to improve immunological function in immunosuppressed patients after elective surgery and burn injury.[14] In patients undergoing elective surgery, GLN supplementation can attenuate the negative post-operative nitrogen balance, diminishing the fall in intracellular concentrations of GLN in the skeletal muscle free amino acid pool and supporting muscle protein synthesis.[14] In addition GLN can enhance immunological responses and reduce bowel permeability in these patients.[14] Supplemented GLN is primarily utilized by tissues other than skeletal muscle, presumably in the splanchnic area.[24] GLN concentrations in muscle tissues cannot be restored to normal by exogenous supplementation, at least not during the ICU stay.[24]

In summary GLN seems to affect the immune system, antioxidant status, glucose metabolism and heat shock protein response in critically ill patients.[22] The proposed molecular mechanisms by which GLN may improve outcome in critical illness are summarized as follow:

- Tissue protection[11,34]
- Enhanced Hsp expression[11,34]
- Attenuated gut barrier dysfunction[10]
- Decreased cellular apoptosis[11,34]
- Anti-inflammatory/immune regulation: attenuation of cytokine release,[35] and Nuclear Factor-$_{\kappa}$B/stress kinase activation[34]
- Preservation of tissue metabolic function in stress states[34]
- Preservation of ATP levels following sepsis and injury[34]
- Attenuation of insulin resistance[34]
- Antioxidant/attenuation of inducible nitric oxide synthesase activation after sepsis and injury[34]

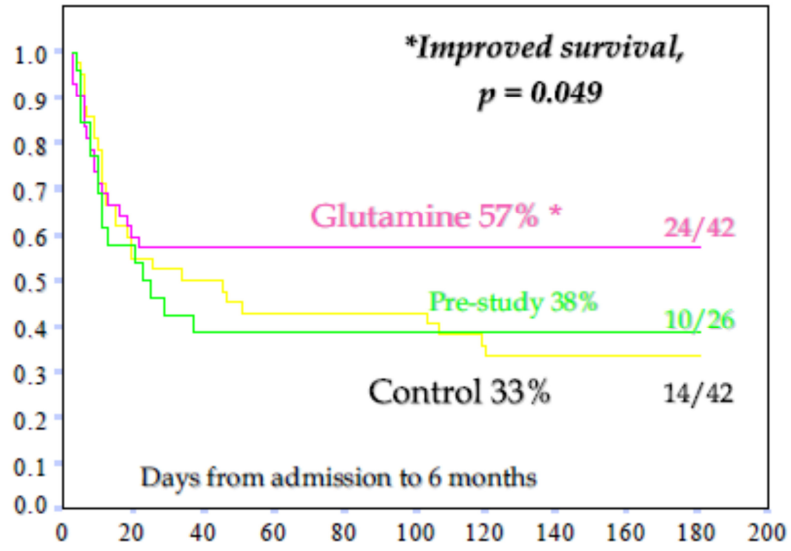
Many animal and human studies have focused on the role of GLN-supplemented nutrition in supporting the structural integrity and biochemical function of the gut, including:

- increasing jejunal mucosa weight and DNA content and attenuation of villous atrophy associated with standard TPN[18,28]
- increasing villous height and mucosal nitrogen content with GLN-supplemented parenteral nutrition (PN) or enteral nutrition[18,28,36]
- stimulating intestinal mucosal growth after starvation[18]
- the ability of GLN-supplemented PN or enteral nutrition to protect against aspirin-induced gastric ulcerations, peptic ulcer disease and severe enteritis following chemotherapy and radiation therapy (RT)[18]
- TPN supplemented with GLN increases gut glutaminase activity and stimulates gut GLN utilization[18]
- enhanced transport of GLN and AA across jejunal brush border[18]
- reducing intestinal permeability (IP) and bacterial translocation[18-19,28,36-37]
- normalizing secretory immunoglobin A levels, thus supporting immune function of small intestine[18,28]

1.3.1 Current Recommendations (Critical illness)

Clinical trials have reported mortality (Figure 1.8) and morbidity advantages in critically ill patients receiving supplemental GLN. In addition low plasma GLN levels on the day of admission have been described as a prognostic factor predicting an unfavourable outcome.[21,23-24,38] In 1990 Lacey and Wilmore[10] concluded that GLN "may be a conditionally essential amino acid for the critically ill". Since then recommendations for GLN supplementation in serious illness have been concluded in several systematic reviews of available evidence.[39-40]

Meta-analysis of 4 RCT in critical illness and 6 RCT in surgical patients revealed that seriously ill patients with gastrointestinal failure receiving parenteral nutrition should probably receive GLN supplementation for at least 6 days to derive maximum benefit with regard to clinical outcomes like the length of hospital stay, mortality (only critical illness) and infectious complications (only surgical patients).[39] The greatest effect on mortality was in patients receiving > 0.2 g/kg body weight per day of parenteral GLN.[39]

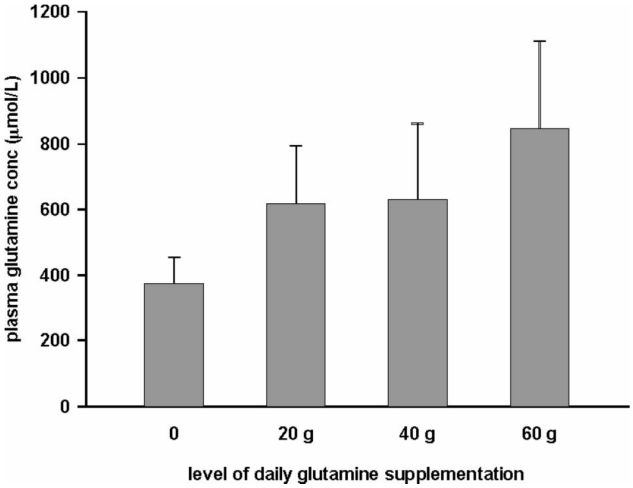


Survival is similar for the first 20 days but then significantly decreases in the control parenteral nutrition group compared with the glutamine parenteral nutrition group. The survival curve from similar matched patients requiring TPN before the study commenced is shown for comparison.

Figure 1.8: Survival curves for intensive care patients with multiple organ failure from admission to 6 months. Source:[38] Griffiths RD, 2004:1.

A systematic review of enteral nutritional support supplemented with GLN reported good overall tolerance, some improvement of immunological aspects in multiple trauma patients and cost reduction in critically ill patients based on some scientific evidence from non-randomized studies (Grade B recommendation).[40] It was recommended that early initiation of diet with enteral GLN administration of 20 - 30 g/day for at least 5 days should be considered (Grade C recommendation) in trauma and critical illness, including burns.[40]

From 2004 it was recommended that an intravenous GLN-containing dipeptide solution should be included whenever parenteral nutrition is given to critically ill patients.[24,41] Later in 2007 it was concluded that the advantages of GLN supplementation appear to be greater the more GLN is given and the advantage is greater when GLN is given parenterally.[22] When GLN is administered intravenously, there is a dose-response effect, in the sense that the higher the dose, the higher the plasma GLN concentration (Figure 1.9).[20-21,24]



The plasma glutamine level responds to exogenous intravenous supplementation in a dose-dependent manner.

conc: concentration

Figure 1.9: Plasma glutamine concentration can be normalized in all glutamine-depleted intensive care unit patients. Source:[20] Tjader I, 2007:35.

The dose response of exogenous GLN given via the enteral route is less well characterized, probably since there is an immediate uptake in the upper part of the gastrointestinal tract and a major portion of the administered GLN is utilized by the enterocytes and immunocompetent cells in the gut itself.[21] In critical illness, an additional problem to consider is the absorption and utilization of any enterally administered nutrient in general.[21] Recently a randomized, controlled pilot study including 44 medical and surgical patients studied the metabolic effects of alanyl-GLN dipeptide (0.5 g/kg/day goal) supplementation via either route on plasma GLN concentration and other markers.[42] Critically ill individuals receiving 0.5 g alanyl-GLN dipeptide/kg/day parenterally had ~30% higher plasma GLN concentrations compared to baseline than those receiving 0.32-0.02 g alanyl-GLN dipeptide/kg/day enterally of the original goal (Figure 1.10). Again, it was speculated that splanchnic bed GLN metabolism was responsible for the unchanged plasma GLN concentration, or it might be that a higher enteral dose is needed to increase plasma GLN levels in certain ICU patients.[42] It is important to note that the same study concluded that the route of administration otherwise did not appear to differentially effect other outcomes such as antioxidant capacity, oxidative stress markers, T-lymphocyte subsets, IP, IGF-1 levels or nitrogen balance.[42]

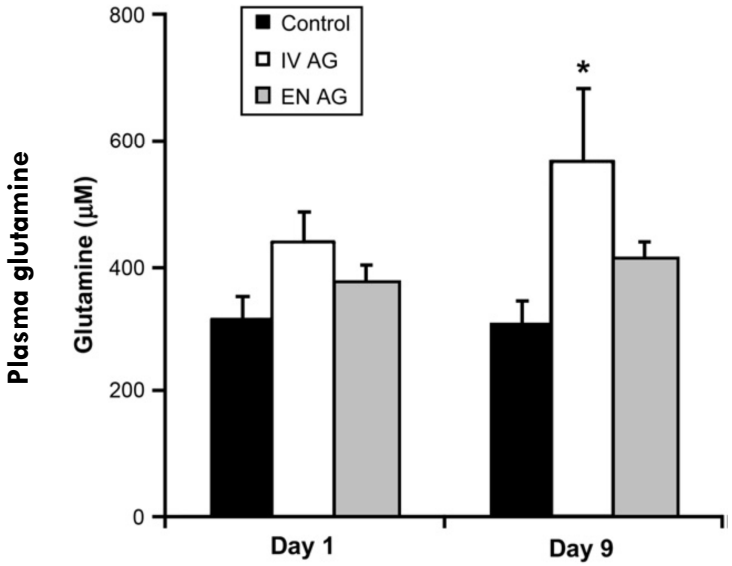


Figure 1.10: Plasma GLN concentrations were higher in the intravenous (IV) alanyl-GLN dipeptide (AG) group versus the control and enteral (EN) AG groups after 8 days of treatment. *P=0.039 versus baseline values. Source:[42] Luo M, 2008:27.

The parenteral route sustains a uniform distribution of the GLN to all parts of the body, in particular the gut enterocytes and the immune system.[24] Intravenous GLN may be administered in a central or peripheral vein.[21] There seems to be no contraindications to GLN supplementation in critically ill patients and it is regarded as safe even in patients with acute hepatic insufficiency who tend to have high plasma GLN levels,[10,21] although some commercial GLN–containing products do list hepatic insufficiency as a contraindication. There are concerns about elevated glutamate levels in the brain of patients with head injuries, secondary to exogenous GLN supplementation. However, there is currently no contraindications for using GLN in head trauma patients,[43] but further studies regarding outcome in these patients are encouraged.[21,43] A review by Wernerman[21] in 2008 recommends that for normalized plasma GLN concentrations in critically ill patients in ICU, 20 - 25 g GLN per day must be added to parenteral nutrition as standard care practice. For patients on continuous renal replacement therapy 25 - 35 g GLN per day is recommended.[21] Current recommendations based on clinical practice guidelines of major nutritional societies (The American Society of Enteral and Parenteral Nutrition (ASPEN), the European Society for Clinical Nutrition and Metabolism (ESPEN) and the Canadian Critical Care Clinical Practice Guidelines Committee (CCPG)) are in agreement that when PN is indicated in ICU patients, the amino acid solution should contain 0.2-0.4 g/kg/day of L-glutamine (e.g. 0.3-0.6 g/kg/day alanyl-GLN dipeptide).[44] For enteral recommendations more evidence is needed, but the addition of enteral glutamine to an enteral nutrition regimen (not already containing

supplemental glutamine) should be considered in thermally injured, trauma, and mixed ICU patients.[44]. To guide administration of GLN, measurement of plasma GLN concentration is advocated. To date there seems to be no reported adverse or negative effects attributable to GLN supplementation in the clinical setting.[21,24,43] In contrast to these recommendations Andrews[45] questions the evidence base of the recommendations from ESPEN and CCPG. Based on the first presentation of results of the Intensive Care Glutamine or Selenium Evaluation Trial (SIGNET), which studied 502 critical care patients using GLN supplementation in the currently recommended ranges, Andrews[45] strongly suggests that these recommendations will require urgent review.

1.3.2 Current Recommendations (Burn injury)

Patients with severe burns or inhalation injury are a unique subgroup of the critically injured. Burn patients are at an increased risk of infectious complications due to the loss of the skin barrier.[22] They may have a prolonged critical illness phase compared to other trauma and surgery patients.[22] Large burns are an extreme catabolic state; inflammation and hypermetabolism may persist well beyond 4 weeks of initial injury.[41] Caution is advised in adopting GLN therapy into routine clinical practice, since the justification and safety of long-term GLN supplementation is yet to be established.[41,43] However, the outlook for GLN therapy in burn injury is promising. Severe GLN depletion occurs in major thermal injury.[41] If supplementation is not provided, adverse effects may include an increased risk of mortality and infection, sustained inflammatory response, negative nitrogen balance, muscle wasting, and reduced gastrointestinal integrity.[41]

Mark Windle[41] published a review in 2006, focusing on GLN supplementation in critical illness regarding the evidence, recommendations and implications for clinical practice in burn care. The results of 9 randomized controlled trials as well as recommendations of 3 systematic reviews were considered.[41] The review concluded that GLN supplementation does appear to yield significant positive clinical outcomes and as a result confers certain cost advantages in critical illness.[41] GLN supplementation appears to be relatively safe up to a level of 0.57g/kg body weight per day.[41] There may be a role for supplementing GLN for 2 - 3 weeks after burn injury.[41] Monitoring plasma GLN concentration may help rationalize the initiation and duration of GLN therapy, although there may be limits to usefulness.[41] GLN flux, particularly in large burns, after the first few weeks of injury is also not fully understood.[41] The need for, and safety of, long-term GLN supplementation require further investigation.[41,43] GLN-supplemented parenteral nutrition is under-represented in reported studies involving burn patients.[41]

1.4 GLUTAMINE AND MALIGNANCY

Malignancy is an ensemble of disease states varying in biology, epidemiology, treatment regimes and prognosis.[11] No single animal or human tumour model exists to represent cancer, since cancer is not a single entity.[11] The current data on protein and amino acid metabolism in oncology emerged from a spectrum of animal models (mainly rat/mouse models) and a few highly defined patient groups.[11] The available evidence has to be considered with care within this context.

1.4.1 Glutamine Metabolism in the Tumour-bearing Host

Depletion of intramuscular and plasma GLN levels in patients and animals with cancer is well documented and acknowledged due to an induced state of physiological stress from the disease process itself, which is aggravated by the catabolic effects of antineoplastic therapies (surgery, BMT, chemotherapy and/or RT).[12,29,46-49] It is speculated that GLN depletion may lead to immune dysfunction, intestinal malfunction and a loss of protein and muscle wasting in oncology patients.[49-51]

The host responds to the presence of the tumour.[11] Tumour-specific catabolic mediators may alter the host metabolism of amino acids in various tissues, further stimulating the mobilization of amino acids from muscle stores.[11] In addition the host response to the tumour may also be influenced by endocrine and immunological changes that also impact on metabolism.[11] The overall impact of a tumour on whole-body amino acid metabolism will inevitably depend upon the type, size and location of the tumour.[11] Malignancy itself or its treatment often results in a poor dietary intake of amino acids, in addition to the increased requirements for both essential and non-essential AA. This drives the catabolism of endogenous protein reserves in skeletal muscle and eventually depletes intramuscular GLN levels and reduces plasma GLN levels in patients with cancer, resulting in a GLN deficiency.[11] Since inadequate dietary intake often accompanies these cancers, it is not clear to which extent tumour GLN metabolism is responsible for alterations in plasma concentrations.[11]

Evidence suggests strongly that the total and relative amounts of essential amino acids required are altered in patients with cancer.[11] Amino acids (GLN, arginine and cysteine) normally considered as nonessential for humans become conditionally essential in the diet under these catabolic circumstances.[11] Oncology patients frequently present with elevated protein catabolism, which is one of several manifestations of a hyper-metabolic state, and is also associated with

muscle wasting and cancer cachexia.[28] The tumour burden creates an exaggerated GLN demand that is met by increased GLN production by the host tissues.[28] There is a change in the relative amounts of different amino acids utilized, in association with a shift from peripheral protein synthesis toward the viscera.[11] The amino acid metabolism of the host and tumour interacts. In malignant cells transport of GLN across the plasma membrane occurs at a faster rate than in their non-malignant counterparts.[49] Moreover, mitochondrial glutaminase was reported to be more active in tumour cells than in normal cells. Tumour cells in culture require at least 10 times as much GLN as any other amino acid.[25,52] Through transamination and a truncated Krebs cycle, GLN is a preferred fuel source for many types of cancer cells,[53-54] second only to glucose[52] in most cell types. Animal and *in vitro* studies have demonstrated the dependency of experimental/implanted tumours on GLN for growth.[28,53,55-56] GLN's importance in tumour cell metabolism derives from the characteristics it shares with glucose.[52] Both nutrients help to satisfy two important needs for proliferating tumour cells: bioenergetics (ATP production) and the provision of intermediates for macromolecular synthesis.[52] High GLN levels in cell cultures of *in vitro* colon cancer cells and normal enterocytes have been shown to increase growth rates, decrease differentiation and decrease adhesion properties.[53,56] Metabolic reactions that use GLN for its α-nitrogen or the carbon skeleton require glutamate and not GLN as the substrate.[52] Tumour cells tend to have large intracellular pools of glutamate, but maintaining these pools depends on the ability to convert abundant extracellular GLN into glutamate, through the activity of phosphate-dependent glutaminase (a mitochondrial enzyme highly expressed in tumours and tumour cell lines).[52] A linear correlation between glutaminase activity and tumour growth is evident in several tumour models.[49,52-53] Not all metabolic functions of GLN require glutaminase activity, but this enzyme is essential to the metabolic phenotype of growing tumours.[52] The more rapidly growing and aggressive, the more GLN is metabolized by the tumour.[57] The malignant tumour acts as a "GLN trap" and may capture amino acids, competing with host tissues for circulating GLN, and this may be especially problematic in a situation where total dietary intake of amino acids is reduced.[12,49,54,58] Uptake and utilization of GLN by the tumour for APT production and nucleotide synthesis is a possible mechanism for low plasma GLN levels and compromized availability of GLN to host tissues in tumour-bearing animals. Tumour cells obtain a relatively high proportion of fuel for energy metabolism from complete and partial GLN oxidation.[49] However, tumour cells do not have a high capacity to synthesize GLN and rely on systemic GLN from the host.[49] This probably explains why intramuscular GLN formation and release are increased in tumour-bearing animals. This is attributed to enhanced rates of muscle protein breakdown and subsequent nitrogen donation from elevated catabolism of intramuscular BCAA.[49] Elevated turnover of GLN in muscle

from tumour-bearing rats is associated with a reduction in intramuscular GLN concentration.[59] Intracellular GLN is an important regulator of muscle protein synthesis, and low concentrations may contribute to muscle loss. As a result the skeletal muscle protein mass is progressively depleted. It is clear that GLN metabolism must be tied to cancer cachexia, since more than 90% of the body's GLN stores are in the muscle, and GLN is the major amino acid exported from the muscle during catabolic stress.[52] It is suggested that the demand for GLN by rapidly proliferating cells like lymphocytes or tumour cells might be the trigger for cachexia.[49,52] The observation that implanted tumour tissue in rodents rapidly triggers increased muscle GLN output and a drop in muscle GLN stores supports this hypothesis.[52]

Parenteral GLN dipeptide supplementation increased the total appearance rate of GLN ($P<0.05$)[31] and improved nitrogen balance postoperatively in colon cancer patients[30,59]-and BMT patients.[60] The liver can either produce or consume GLN, depending on the physiological state.[49] However, the influence of tumours on hepatic GLN metabolism is unclear. Both hepatic GLN uptake and release have been reported to be increased in rats bearing large tumours, without an increase in hepatic GLN oxidation; therefore hepatic protein mass is maintained or may even be increased in tumour-bearing animals.[11]

The effect that tumours have on other tissues that are high consumers of GLN, such as enterocytes and leukocyte immune cells, is unclear.[11] However, there is evidence that GLN-supplemented enteral nutrition modulated postsurgical immunosuppressive and inflammatory responses[29] and prevented a reduction in the lymphocyte count during radio/chemotherapy in patients with cancer.[37] Colorectal cancer induces a state of profound cellular and humeral immune suppression in human subjects.[30] An RCT by Jing-Xiang[30] showed that parenteral Ala-GLN dipeptide (~21-28 g/day for 7days) improves cellular and humeral immune function in patients with colorectal cancer postoperatively, yielding benefits to the patient with regard to bacterial translocation following infectious insults.[35] Evidence is emerging in this field, indicating faster total lymphocyte recovery in the postoperative period with GLN.[61]

1.4.2 Glutamine and Tumour Growth

In vitro data

A substantial body of *in vitro* evidence exists, indicating that GLN is the major respiratory fuel for tumour cells in culture.[28,49,52] There are concerns that tumours originating from mucosal crypts may be especially advantaged if supplemented, since GLN is the preferred fuel for gut mucosal cells[62] and human colon cancer cell lines[25,56] and as such may promote more aggressive tumour growth in patients with gastrointestinal and colon cancer.

Some authors are convinced that GLN supplements could be harmful to humans as long as the neoplasm is present and caution against the use of supplements until the neoplasm has been cured.[15,56,63-64]

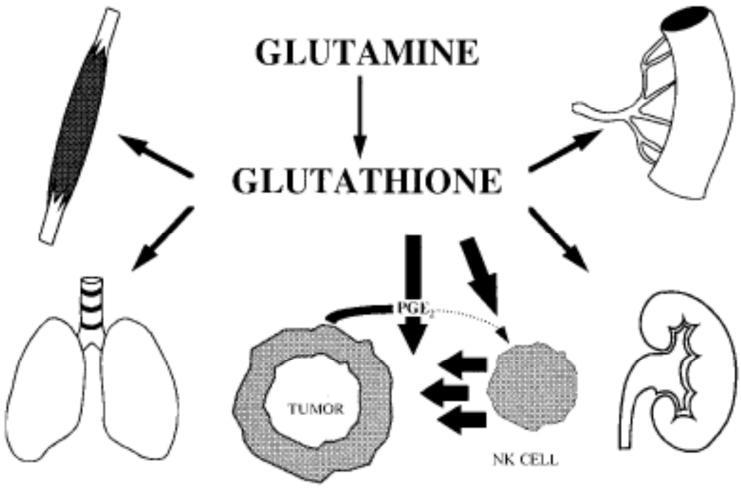
Experimental data

The dependence of tumour cell lines on GLN for growth has led to the investigation of amino acid deprivation to reduce tumour growth *in vivo*. An early nutritional approach involved formulation of diets lacking amino acids essential for tumour growth. Diets deficient in arginine or methionine slowed tumour growth in animal models[11] and antimetabolites blocking GLN metabolism (GLN analogues: Acivicin, Don) have been investigated as chemotherapeutic agents and have been successful in reducing tumour growth in rats.[65-69] These approaches were abandoned because of significant toxicity in the case of antimetabolites and of inducing amino acid deficiency in the host as well as the tumour.[11,33,49]

Kuhn[50] reviewed evidence in this regard and concluded that substantial experimental evidence is available to support the hypothesis that tumour cells have the ability to manipulate host metabolism to endogenously cover the needs if GLN is not available from exogenous sources. "Any measures to 'artificially' establish a situation of glutamine depletion, thus, cannot stop or even retard tumour growth. On the other hand, the host can develop serious glutamine depletion which is associated with impaired physiological functions like disturbed mucosal integrity and diminished immune competence. Following this line of reasoning, any nutritional efforts to provide sufficient exogenous glutamine might improve the general metabolic situation of the patient and, thus, life quality."[50]

It is rather clear that tumours depend on GLN for survival.[54] However, several authors[16,47,49,50-51,70] have over the past two decades reviewed the experimental data and hypothesized that supplemental GLN may benefit the tumour-bearing animals without enhancing the growth of the tumour. Even recently, it has been suggested that dietary GLN has an anti-cancer action,[50,70] based on several significant outcomes in animal models.[3-5,53,70-85] A significant reduction (of up to 50%) and delay (of 1-3 weeks) in tumour genesis ($P=0.03$),[3,5] tumour volume ($P<0.05$),[5,73,79] tumour weight ($P<0.05$)[4-5] and a decreased tumour growth rate,[12,72-73,86] of up to 40%[79,86] have been reported. Axillary metastasis were also decreased ($P<0.1$)[79] in GLN-supplemented rodents. This decrease in tumour growth was associated with a 30% (2.5 fold, $P<0.05$) increase in NK cell activity.[3,79,81,86] Evidence from another animal study demonstrates that glutamate derived from GLN promotes the inhibition of glutathione transport into tumour mitochondria, which may render tumour cells more susceptible to oxidative stress-induced mediators like cancer therapy.[72] A linear correlation between glutaminase activity and tumour growth rate is evident in several tumour types.[49,53] Certain types of tumours show high rates of glutaminolysis which create a metabolic condition in the mitochondria that stimulates mitochondrial production of ROS upon TNF-a, which in contrast can become a selective advantage when targeting cancer cells with cytotoxic drugs.[25] Oral GLN supplementation seems to boost the host immune reponse[49,79] (Figure 1.11) to implanted tumours in rodents, decreasing its growth, through the following mechanisms:

- Support of host GLN stores.[4-5]
- An increase in NK cell activity[5,72,81,86] of 2.5-fold ($P<0.05$).[79] GLN is the preferred fuel for NK cells, the body's tumour-killing cells.[57]
- Upregulation of rat (including gut and colon) GSH metabolism[4-5,72,82,87] and increased arterial GSH levels (25%, $p<0.05$).[79] Decreased tumour GSH in rats receiving MTX.[82,87] Depressed GSH levels in tumour cells increase susceptibility to chemotherapy, correlating with enhanced tumour volume loss.[87]
- Proportional (2.5-fold) GSH-mediated decrease in prostaglandin E2 (PGE2) synthesis.[79]

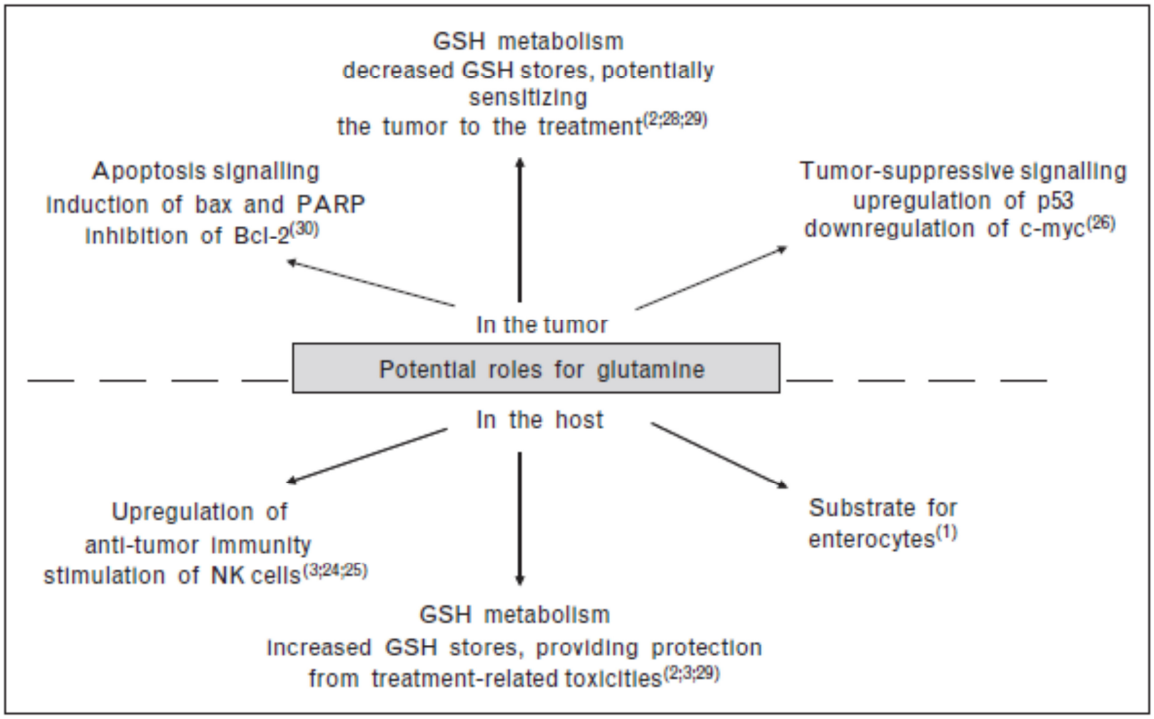


Glutamine through upregulation of Glutathione in host tissues blocks PGE2 synthesis, results in increased NK cytotoxicity, and decreased tumour growth.

PGE2: Prostaglandin E2, NK: Natural killer

Figure 1.11: Suggested mechanism of action of GLN supplementation. Source:[79] Klimberg VS,1996:16.

These findings have sustained interest in investigations exploring reasons for this emerging phenomena of a rather dichotomic action (Figure 1.12) of GLN by actually decreasing instead of stimulating tumour growth *in vivo*.[52,70]



GLN: Glutamine; GSH: Glutathione; NK: Natural killer; PARP: poly(ADP-ribose)polymerase, c-myc: a pro-proliferation factor, p53: signaling molecule, BcL-2: proapoptopic Bcl family.

Figure 1.12: Potential roles for GLN in the host (animal/human) versus in the tumour. Source:[70] Belabed L, 2009:12.

Clinical data

Published human trials showed that supplemented GLN either had no effect on tumour response (advanced breast cancer) to chemotherapy[88] or local control of cervical cancer.[89]

1.4.3 Effect of Glutamine on Cancer Treatment Efficacy and Toxicity

Experimental data

The effect of oral GLN on the efficacy and toxicity of anti-tumour drugs (chemotherapy) was examined by several researchers using *in vivo* animal models (rats).[71-73,77-78,87,90] An animal model (rats) by Klimberg[77] demonstrated in 1992 already that GLN supplementation significantly increased the tumour volume loss 24 and 48 hours after methotrexate chemotherapy. This observation was associated with a threefold[90] increase in tumour methotrexate concentrations and

correlated significantly with suppressed tumour glutaminase activity.[77,87] Other researchers followed, showing the same trend with longer periods (1-2 weeks) of follow-up after methotrexate administration using similar animal models. In some cases tumour volume loss was nearly doubled (45%) when GLN was added to the diet, compared to control groups (25% reduction, $P<0.05$).[78,90] The same trends of enhanced tumour chemo-sensitivity were shown with other chemotherapy regimens (such as 7-ethyl-10-[4-(1-piperidinol)-1-piperidno]carbonyloxy-camptothecin (CPT-11) and 5-fluorouracil (5-FU)).[73] In addition there is evidence that GLN-enriched diets decrease tumour GSH ($P<0.05$), while maintaining host GSH tissue stores. Decreased GSH levels in tumour cells increase susceptibility to chemotherapy. Decreased GSH content in tumour cells correlated with enhanced tumour volume loss after MTX chemotherapy.[87] Doxorubicon (DOX), another effective anti-neoplastic agent, is known for its cardiotoxicity in humans.[91] It was recently indicated that GLN supplementation is able to reduce DOX-induced cardiac damage in a tumour-bearing host (rat), by reducing DOX accumulation in the heart without affecting it in the tumours, enhancing the DOX therapeutic effectiveness.[91]

Data regarding other outcomes suggested that GLN supplementation enhances the selectivity of anti-tumour drugs by protecting normal tissues (especially the gut) from and possibly sensitizing tumour cells to chemotherapy treatment–related injury.[72,78,87,90] In addition, the toxicity of cancer therapy to the host is ameliorated,[72,78] specifically cardiotoxicity.[91-92] Decreased bacteraemia, "sepsis" and mortality were demonstrated,[78] as well as normalization of changes in peripheral leukocyte counts associated with the tumour-bearing state.[73]

Xue[72] demonstrated a reduced incidence and severity of late-onset diarrhoea following CPT11 treatment ($p<0.05$) with GLN supplementation in an animal model with implanted Ward colon tumour. In this study GLN supplementation was associated with potentially beneficial and protective responses in the rat colon including the following:[72]

- A 3.1-7.2 fold increase in Hsp25, -70, and-90a ($P<0,05$).
- Amplified reduced GSH (RGSH): GSSG ratio ($P<0.05$).
- Prevention of upregulated activity of a key bacterial enzyme (ß-glucoronidase) in the fecal content that mediates CPT-11 intestinal toxicity ($P<0.05$).
- Increased proportions of CD3+CD8+ lymphocytes and memory CD8+ subset in mesenteric lymph nodes following CPT-11 therapy.

However, GLN treatment did not alter CPT-11's anti-tumour activity, the amino acid concentrations (in plasma, colonic mucosa and tumour), Hsp expression, or the ratio of rGSH:GSSG in the tumour.[72]

One author[55] investigated the ability of GLN to increase radioresistance in squamous cell carcinoma of the cervix and concluded that although HeLa and Caski cells require GLN in the medium, supraphysiological GLN concentration does not increase tumour growth or radioresistance and it was recommended that GLN should be investigated further as a potential bowel radioprotector.

Many experimental studies using non-tumour-bearing animal models (including animals such as rats and dogs) have focused on the potential role of supplemental GLN in prevention or alleviation of other side effects and toxicities of chemo and/or RT regimes (such as mucositis, radiation enteritis, enterocolitis and diarrhoea), but will not be discussed for the purpose of this review.

Clinical data

It is important to appreciate the metabolic changes in cancer patients due to treatment factors.[11] Apart from a very small number of cancers deemed untreatable or too advanced except for palliative intervention, the vast majority of cancer patients receive aggressive therapy within the limits of tolerance.[11] Various specific amino acids may additionally be required for healing after surgery, tissue injury in the gut, or bone marrow after systemic therapy.[11] Surgery, RT and chemotherapy are associated with large metabolic changes in substrate utilization.[11]

A relative GLN deficiency due to increased requirements and reduced availability in the tumour-bearing host may reduce the tolerance of normal tissues to cancer treatment, necessitating reduced treatment doses and possibly diminishing the effects of treatment.[46] In patients with malignant tumours the IP may increase during prolonged parenteral nutrition after surgery, BMT and high-dose chemotherapy, thereby weakening the mucosal barrier function and predisposing patients to septic complications.[30] In addition systemic chemotherapy and RT generates reactive oxygen species that produce changes in the structure of the intestinal mucosa which are associated with increased IP and the incidence of oral mucositis.[93] There is evidence to suggest that GLN, as precursor for glutathione, plays a pivotal role in regulating the intracellular redox potential. Therefore supplementation may help protect gut mucosa from chemo-[48] and radiation[37] therapy-

induced damage by reducing the mucosal permeability and maintaining normal mucosal configuration and gut function via the reduced production of pro-inflammatory cytokines and cytokine-related apoptosis,[37,48,94] while sensitizing tumour cells to these agents via an immunomodelatory role.[46]

There are speculations in the literature that intestinal morphological and functional changes related to enteral fasting and PN are less significant in humans than in animal models and in fact may not be clinically significant at all.[33] Extrapolating that it is unclear whether GLN is necessary for the preservation of normal intestinal morphology and function during PN, it is questioned by some whether clinically relevant bacterial translocation even occurs in humans, much less whether there is any value in the prevention of such occurrences.[33]

Clinical trials are limited to investigating the dose-limiting toxicity of therapies and some measure of tumour response. [37,88,95-98] The results are inconsistent and conflicting, but confirm safety of GLN supplementation in clinical trials. The long-term effects of GLN supplementation is yet to be established.

There is a large body of evidence focusing on the effect of GLN supplementation on several clinically important outcomes in various oncology settings. Patients receiving treatment for cancer may experience severe toxicity and side effects of which mucositis/stomatitis must be the one drawing the greatest interest from investigators,[46,48,60,93-94,96-117] probably because it is very painful and it limits the patient's ability to eat and enjoy food and hence reduces the quality of life. Oral mucositis has emerged as an imported dose-limiting toxicity in patients receiving mucotoxic cancer therapy and is anticipated in 5-40% of patients receiving standard-dose chemotherapy. This affects more than 75% of those receiving either high-dose chemotherapy with stem cell transplantation (SCT) or RT for head and neck cancer.[94] The next important clinically significant side effect of cancer therapy is diarrhoea. Several RCTs [29,46,48,84,88-89,95-98,102,104,106,112-115,118-122] have investigated a possible role of GLN supplementation in reducing the duration and/or severity of this common complication. Some studies [37,48,51,93,98,102,109,115,120,123-125] test gut-barrier function/IP as an objective test for mucositis and bacterial translocation. Other outcomes related to toxicity of treatment include enteritis,[126] dysgeusia/taste alterations,[122] esophagitis,[127-129] ulcerations of the gastric/duodenal mucosa,[48] nausea/vomiting[118,120,122] and mental health (using several indexes for measurement including mood, anorexia and performance status).[106,121-122,130-131]

Accumulating evidence is suggesting that supplemental GLN may decrease tumour growth by upregulating certain aspects of the immune system.[70] Several studies [29-30,33,37,60-61,96,98,101,106,110,113,118,121,123-124,132-137] have investigated various immunological parameters to test this hypothesis in the clinical setting.

Clinical outcomes related to morbidity is represented to a lesser extent, but some inconclusive data has been reported regarding prevalence of Graft versus host disease (GVHD),[111-112] peripheral neuropathy,[122,138-139] veno-occlusive disease (VOD)/hepatic dysfunction,[60-61,92,106,140] renal function (urea, creatinine),[61,106] atrhralgia/myalgia[122] and surgical complications[61,141] in general.

Several studies have reported data on clinical outcomes including the length of hospital stay,[60-61,96-98,106,108-113,123,133,141-142] clinical infections,[60-61,98,106,108-109,111-113,123,136,141,143] indicators of nutritional status (body weight/change,[48,98,101,103,106,111-113,122,130,143-144] body mass index (BMI),[133] anthropometric measurements other than weight,[111,130,142-143] nitrogen balance,[30,59-60,136] albumin/pre-albumin/total protein[61,98,113,121,130] and the subjective global assessment (SGA)[141]), mortality,[60,97,104,106,108-109,111-112,122,130,133,140-142,144-145] survival at follow-up[60,97-99,106,112,123,145] and economical aspects.[98,110,113,146]

The study populations are extremely heterogenic and findings are inconsistent and even conflicting. Several systematic reviews[12,15-16,45-47,49-51,57,63,70,116,117,147-148] of the evidence have been done, but authors remain inconclusive about the optimal GLN dose, route, and timing of such supplementation in the various settings. In general there seems to be no warning of harm or any indication of toxicity caused by GLN, and supplementation is therefore deemed safe for use in pediatric [108,124,149] and adult [29,61,111,127,129,150] patients by several authors. Only a few urge a more prudent approach towards routine GLN supplementation in oncology patients[15,33,63-64,142,151] and especially in children with solid tumours.[152]

GLN-supplemented nutrition for patients with haematological malignancy and solid tumours receiving BMT has received the most attention in RCTs.[60,96-98,109-112,123,,140,145,153] Several reviews[57,148,154] of RCTs including BMT patients (with cancer and other diseases) have demonstrated that GLN-supplemented nutrition is beneficial in adult and pediatric patients undergoing BMT protocols with or without high-dose chemotherapy and/or RT.[108] BMT treatment regimes cause gastrointestinal complications, including nausea, vomiting, inflammation of the oral and esophageal mucosa (mucositis), abdominal pain and diarrhoea. After high-dose chemotherapy for BMT, patients may develop a potentially lethal VOD, which is due to

subendothelial swelling and narrowing of the central hepatic veins.[140] The VOD seems to be related at least in part to oxygen-free radical-mediated liver injury and depletion of glutathione and other antioxidants.[140] Falling protein C levels are predictive of severe VOD. Administration of intravenous and oral GLN alone[140] or in combination with oral vitamin E[14] significantly preserved protein C and albumin levels and diminished symptoms of veno-occlusive disease respectively, suggesting a role for GLN in the protection of hepatic function after BMT.[140] Some postulate that oral GLN supplementation should be considered as metabolic support in patients undergoing BMT and other adjuvant treatment for cancer.[108] Evidence is presented that GLN attenuates glutathione depletion in plasma, liver and gut after chemotherapy and upregulates systemic and tissue immune function during catabolic stress as a possible mechanism for the observed benefits.[14] In addition parenteral GLN may support lymphocyte recovery after BMT.[135] In contrast to these positive reports, Arfons and Lazarus[154] believe that GLN supplementation cannot be recommended to all BMT recipients as it has been shown to increase morbidity and mortality rates in autologous transplant patients (Figure 1.13).[98] Further warning is issued by a recently updated review by Murray and Pindoria,[155] reporting that routine use of PN and GLN in BMT patients predicted to have prolonged gastrointestinal failure.

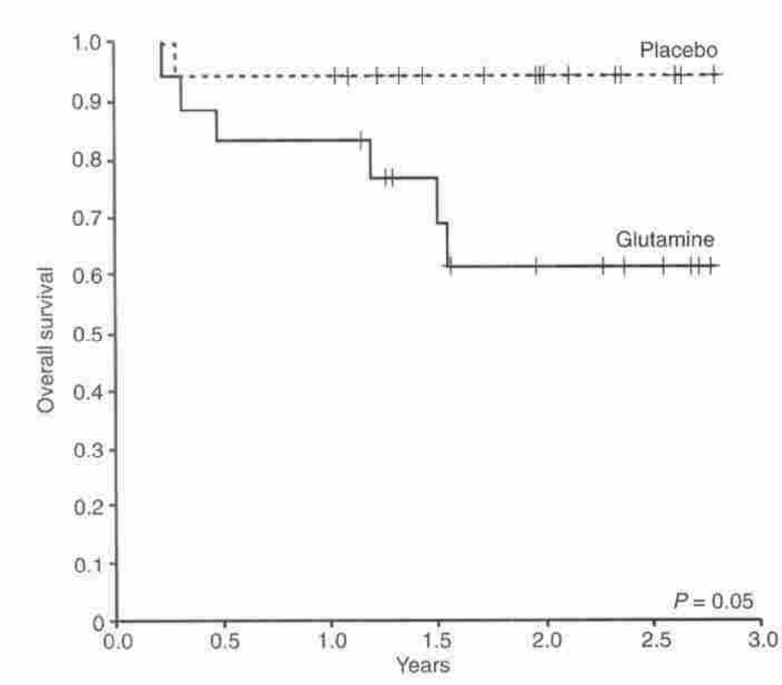


Figure 1.13: Overall survival in autologous transplant patients receiving 30 g alanyl-GLN dipeptide/day via the parenteral route from day+1 to+14 or discharge. (*N=40*)
Source:[98] Pytlik R, 2002:30.

1.4.4 Current Practices and Recommendations (Oncology)

GLN, as a dietary supplement, may be categorized as a complementary and alternative medical (CAM) therapy, which is presently not considered to be part of conventional medicine.[156] The National Center for Complementary and Alternative Medicine (NCCAM) at the US National Institute of Health was established in 1998, commissioned to investigate CAM therapies in the context of rigorous science and disseminating reputable information on CAM therapies to the public and professional communities.[156] The average prevalence rate of CAM use among cancer patients is 31% (data from 13 countries) with dietary supplement use of 35-50% among children with cancer in the US.[156] Less is known about the use of dietary supplements in developing countries. Many cancer patients and their families choose to use dietary supplements because of their ease of access without a prescription and the perception that they may reduce the side effects of anti-cancer treatment or even have a direct effect on the tumour.[156]

The safety of long-term/chronic use of GLN supplements in healthy or diseased individuals of all ages have not been established.[43] This is especially problematic since long-term/chronic use will most probably occur without any medical supervision. In the clinical setting supplemental GLN can be administered safely via the oral/enteral and/or parenteral route in healthy human volunteers[157] and patients.[43,50] No adverse effects have been demonstrated with GLN doses of 50-60 g/day over short-term periods in hospital patients.[43] Ward[149] conducted a GLN dose-finding study in pediatric oncology patients, investigating patient acceptability, plasma GLN and ammonia levels. It was concluded that doses of 0.35, 0.5 and 0.65 g/kg via the oral route was well tolerated with no untoward effect on plasma GLN and ammonia levels and is safe to use in a clinical study in pediatric oncology patients.[149]

To date no firm recommendations have been made for GLN supplementation in oncology. Results of studies including patients with cancer are inconsistent and high levels of heterogeneity exist with regard to study population/setting, route, dose and timing of GLN administration. A recent review of clinical and experimental evidence in oncology by Kuhn, Muscaritoli, Wischmeyer and Stehle[50] made no specific recommendation with regard to route, dose, timing or duration of GLN supplementation, although it was concluded that "appropriate exogenous GLN supply can beneficially contribute to diminish risks of high-dose chemotherapy and radiation" in various clinical situations, but also especially in BMT patients, possibly effecting outcome and improving quality of life in some.[50]

GLN is relatively insoluble, with a solubility of about 3.5 g per 100 ml, at 23°C.[10,13,96]. Suspensions are necessary to achieve concentrated doses. A 16 g dose requires about 600 ml liquid for administration in solution.[13] This represents a large proportion of the daily oral fluid intake of cancer patients and frequently presents a considerable burden to these patients, and especially children,[156] who are reluctant to eat or drink because of the painful mouth caused by mucositis and other side effects related to lack of appetite caused by cancer cachexia or antineoplastic treatment. Cancer patients in clinical trials were unable to consume all of the prescribed GLN dose (4 g/150 ml x 4/day) orally.[96] Mean consumption was 69(15% in the study group, which was not significantly different from the control group.[96] Dispension of GLN doses higher than 0.75 g/kg was found to be unpalatable in children, since the GLN did not disperse adequately at this dose.[149] It may be possible to increase intake of supplemental GLN further if it was mixed with yogurt, ice-cream or other soft food.[96] In addition L-GLN powder is highly unstable in aqueous solution at room temperature and must be refrigerated.[119]

Intravenous L-GLN may be given alone or as part of TPN,[50] by adding the crystalline amino acid to a commercially available amino acid solution. However, this preparation must be done daily at +4°C, under strict aseptic conditions in a local pharmacy, followed by laborious sterilization through membrane filtration.[13] In addition, to diminish the risk of precipitation, glutamine concentrations in such solutions should not exceed 1-2%.[13] Consequently the provision of adequate amounts of GLN to patients is challenging, especially in volume-restricted situations. Two stable and highly soluble synthetic dipeptides (Table 1.3), Ala-GLN and Gly-GLN, showed great potential as a way of providing GLN that is otherwise difficult to deliver.[13] The dipeptides with a GLN residue at the C-terminal position show high solubility in water (568 and 154g/L, respectively, Table 1.3).[13] They are sufficiently stable during heat sterilization and prolonged storage.[13]

Table 1.3: Chemical/physical characteristics of free GLN and synthetic GLN containing dipeptides.

	Solubility ($g/L\ H_2O$ at 20 °C)	**Stability**	**Share of GLN (g/20 g dipeptide)**
L-glutamine	36.0	No	-
L-Alanyl-L-glutamine	568.0	Yes	13.5
Glycyl-L-glutamine	154.0	Yes	14.4

Source:[13] Furst P, 2004:1.

Parenteral GLN/GLN-dipeptides have been described as being more effective than oral/enteral applications based on reports from 12 studies (including all designs).[50] It is hypothesized that parenteral application ensures 100% availability of the substrate, and that immune competent cells of the host can use GLN directly for protein synthesis, formation of GSH, or for energy production, therefore supporting host metabolism more effectively.[50]

Whether oral/enteral supplementation is as effective as intravenous supplementation is unclear, although animal and human studies show similar benefits for GLN administration via either route.[96,158] Although GLN is metabolized similarly whether it enters the gut mucosal cells across the brush border from the intestinal lumen, or across the basolateral cell membrane from arterial blood, it is thought that enteral administration provides an enhanced gut protective effect.[46] Ready-to-use enteral supplements are not routinely supplemented with glutamine because of the instability of glutamine in solution.[46] Powdered GLN is the supplement of choice.[46] It is virtually tasteless and can be mixed into any beverage or soft/moist food or dissolved in water and flushed into a feeding tube. Daily oral glutamine doses are best divided throughout the day to increase enterocyte contact and it is often advised that patients swish the solution like a mouthwash before swallowing.[46]

A recent systematic review by Crowther[57] indicated that for BMT patients there may be a benefit for oral glutamine in reducing mucositis and GVHD and for intravenous GLN in reducing infections, but the reviewer cautioned that this outcome may be at the expense of an increased relapse. Another review urged that one should consider the evidence that routine use of PN and GLN for BMT patients predicted to have prolonged gastrointestinal failure.[155]

A systematic review, published in 2008, by Clarkson and Worthington[117] investigated interventions for treating oral mucositis in patients with cancer receiving treatment and concluded that there was not enough evidence to support that GLN has an effect on mucositis.

There seems to be an agreement that further study of GLN supplementation in oncology is warranted, especially for large, multi-centre, RCTs.

On the other hand cancer biologists have recognized the importance of GLN as a tumour nutrient since the 1930's.[52] GLN contributes to essentially every core metabolic task of proliferating tumour cells. Recent findings[159] that c-myc (a pro-proliferation factor) controls GLN uptake and degradation and that GLN itself exerts influence over a number of signalling pathways that contribute to tumour growth, stimulate a renewed effort to understand the regulation of GLN

metabolism in tumours and to develop strategies to target GLN metabolism in cancer.[52] Future interest in tumour metabolism will likely focus on cellular GLN handling, revisiting the question of whether the appetite for glutamine displayed by tumours can be used against them.[52]

1.5 SUMMARY AND MOTIVATION FOR THE STUDY

The results of GLN studies in malignancy are inconsistent and inconclusive and no recommendations have been made with regard to optimal dose, route and timing of administration in various settings, although it is conceivable that some patients may possibly benefit from such supplementation, at least without stimulating more aggressive tumour growth based on available evidence from animal studies. The nutritional support of patients with cancer has not been a very successful area of research. It remains a major clinical problem because of cachexia, which most patients develop before death. The presence of cancer cachexia significantly limits the therapeutic alternatives such as more radical surgical operations, more intensive chemotherapy and/or RT. It is still not clear whether GLN supplementation has a role to play in improving the clinical outcome and quality of life in this diverse group of patients by limiting the toxicity of treatment modalities. A systematic review of the literature is needed to provide the best current evidence regarding possible benefits, risks and recommendations for GLN supplementation in cancer patients upon which decisions can be based in clinical settings.

CHAPTER 2: METHODOLOGY

2.1 OBJECTIVES

2.1.1 Purpose of the Study

To investigate the current evidence regarding the efficacy and safety of GLN supplementation* in oncology.

2.1.2 Specific Objectives

Primary objectives

To systematically review (with a meta-analysis where possible):

The safety and efficacy of GLN supplementation in humans with cancer in terms of

- Mortality
- Survival at follow-up
- Change in body weight (as a measure of nutritional status)
- Length of hospital stay
- Prevalence of clinical infections

The efficacy of GLN supplementation in humans with cancer in terms of

- Severity and duration of mucositis
- Severity and duration of diarrhoea

The effect of GLN supplementation on tumour growth in tumour-bearing animals and humans with cancer in terms of

- Tumour weight
- Tumour volume
- Tumour volume/weight change

Secondary objectives

To briefly summarize and describe (narrative review) in relation to GLN supplementation:

- The effect of GLN supplementation on the GLN status of humans with cancer.
- Practice issues of GLN supplementation in humans with cancer with regard to timing (duration, criteria for start/end and relation to treatment regimes), route of administration, doses administered and any other practice issues reported.

* For the purpose of this systematic review "GLN supplementation" refers to the administration of GLN enterally or parenterally.

2.2 CRITERIA FOR CONSIDERING STUDIES FOR THIS REVIEW

2.2.1 Types of Studies

Only randomized controlled trials were included in the final selection of studies, irrespective of method of randomization, extent of blinding or treatment of control group. Cross-over and parallel group designs were included. *(Original search for studies included all study types, since only a limited number of randomized controlled trials were expected in this area of research.)*

2.2.2 Types of Participants

Human subjects of all ages with any type of malignancy receiving no or any form of treatment for cancer (chemotherapy, RT, BMT, surgery or a combination).

Additionally for effect on tumour growth only - all tumour-bearing animals receiving no or any type of treatment for cancer (chemotherapy, RT or both).

2.2.3 Types of Interventions

RCTs comparing GLN supplementation (oral, enteral or parenteral) with control (placebo or standard care) intervention.

2.2.4 Types of Outcome Measures

Outcome measures considered as most important are listed below.

Primary outcomes (Humans only)

- Mortality during intervention – number of deaths during intervention
- Survival beyond 100 days – actual numbers of survivors at follow-up beyond 100 days or 1 year, 2 year or 3 year survival
- Nutritional status – difference in mean change in body weight (kg) from baseline to end of study between groups
- Length of hospital stay – mean duration of hospital stay
- Clinical infection – number of patients who developed clinical infections/infectious complications from start to end of study
- Mucositis – mean number of days patients had $\geq$ grade 1 mucositis and number of patients with mucositis ($\geq$ grade 2, $\geq$ grade 3) from start to end of study
- Diarrhoea – mean number of days patients had $\geq$ grade 1 diarrhoea and number of patients with diarrhoea ($\geq$ grade 1, $\geq$ grade 2, $\geq$ grade 3) from start to end of study

Primary outcomes (Humans and animals)

- Tumour growth – difference in mean tumour weight, tumour volume and tumour volume/weight change between groups

Secondary outcomes (Humans only)

- GLN status – effect of GLN supplementation on plasma GLN levels
- Timing of intervention – duration, start/end criteria, relation to treatment regime
- Route of administration – oral, oral swish and swallow, oral swish and expectorate, TPN, enteral
- Doses administered – total dose per day, grams per kg body weight per day
- Practice issues – any other practice issues reported

2.3 SEARCH METHODS FOR IDENTIFICATION OF STUDIES

2.3.1 Electronic Search

A search strategy with no language or RCT filter was used for initial citations from the electronic databases listed below.

Search strategy

1 Supplement* AND Glutamine AND (Cancer OR Oncology)

2 (Bone marrow transplant) OR (Radiation therapy) OR (Chemotherapy) AND 1

Databases

- PUBMED/MEDLINE – 1966 to 2009
- Science Citation Index – 1990 to 2009 (ISI Web of Science)
- EBSCO HOST – 1966 to 2009 (Academic Search Premier)
- Cochrane Library – 1800 to 2009 including:
    - CENTRAL (The Cochrane Central Register of Controlled Trials)
    - CDSR (Cochrane Database of Systematic Reviews)
- Proquest Medical Library – 1986 to 2009

2.3.2 Hand Search

Hand-searching of personal files and the reference lists of potentially eligible studies was done.

* All terms beginning with this root was included in the search (e.g. searching with the root *supplement* includes terms such as supplementary, supplemented and supplementation)

2.4 DATA COLLECTION AND ANALYSIS

2.4.1 Selection of Studies

During phase I of the study selection predetermined selection criteria (Table 2.1) were applied by the principle investigator to identify all potentially relevant studies. The initial study selection criteria were resolutely designed to include as many potential trials as possible.

Table 2.1: Phase I study selection criteria

Participants	Humans with cancer Animals with cancer
Intervention	GLN supplementation
Outcomes	Any
Study type	All
Language	All
Other	Insufficient information in title/abstract to make decision

Two independent reviewers,[160] Jeane-Marie Kruger (JMK) and Ingrid Davis (ID), each independently reviewed 10% of studies excluded by the principle investigator in phase I. Both reviewers are qualified dieticians experienced in meta-analysis and were trained by the principle in terms of systematic review processes, methodological quality assessment of trials and data extraction.

Full text papers were obtained for all abstracts appearing to meet the inclusion criteria or for those which there was insufficient information in the title/abstract to make a decision.

During the next selection phase (phase II) a second set of selection criteria (Table 2.2) was applied to the full report of all potentially eligible studies identified in phase I.

In addition to the principle investigator, both reviewers (JMK and ID) independently applied the same set of criteria to the full report of all potentially relevant studies, except for obvious exclusions (reviews, foreign languages, abstracts only, letters and correspondence). Any disagreements of opinion regarding the inclusion/exclusion of studies were resolved by discussion until consensus was reached.

Table 2.2: Phase II selection criteria

	INCLUSION CRITERIA		***EXCLUSION CRITERIA***
Participants	Humans with cancer Animals with cancer		*Humans without cancer* *Animals without cancer*
Interventions	GLN supplementation (enterally or parenterally)		*No GLN supplementation*
Outcomes	All **human studies** reporting on one or more of the following outcomes:		*Outcomes not considered in the objectives*
	GLN status Length of hospital stay Overall nutritional status Mortality rate Mucositis Stomatitis Ulcerations of the gastric and duodenal mucosa Diarrhoea Nausea	Gut barrier function Immunological parameters Cardiotoxicity Hepatic dysfunction Peripheral neuropathy Response of tumour cells to anti-cancer therapy Tumour growth	
	All **animal studies** reporting on one or more of the following:		
	Response of tumour cells to anti-cancer therapy	Tumour growth (Tumour weight/volume)	
Study design	Any study design		*Review articles, letters, correspondence*
Language	English language studies		*Foreign language studies*
Other	-		*Unpublished studies, abstracts only*

After completion of phase II many studies (of any design) were included. A third and final selection phase was done during which all studies other than RCTs were excluded. In addition to study design criteria, the final set of selection criteria (Phase III, Table 2.3) excluded studies not reporting on the outcomes included in the objectives of the current review.

All studies that initially appeared to be relevant, but which were subsequently excluded during phases II and III, were listed in a table of excluded studies along with reasons for their exclusion.

If two or more studies presented the same data from a single participant population, the data from the primary publication was included only once in the analysis. Duplicate study reports was identified by evaluating multiple publications by the same authors and traced to the primary publication.

Phase III was completed autonomously by the principle investigator and one independent reviewer, JMK. Any disagreement was resolved through discussion until consensus regarding inclusion of studies was reached. If consensus could not be reached, a second independent reviewer (ID) was consulted.

Table 2.3: Phase III study selection criteria

STUDY TYPE	RCT
Primary outcomes	HUMANS ONLY: • Mortality during intervention – number of deaths during intervention • Survival to day 100 –actual numbers of survivors at day 100 follow-up • Survival beyond 100 days – actual numbers of survivors at follow-up beyond 100 days or 1 year, 2 year or 3 year survival • Nutritional status – difference in mean change in body weight (kg) from baseline to end of study between groups • Length of hospital stay – mean duration of hospital stay from admission/day 0 to discharge • Clinical infection – number of patients who developed clinical infections from start to end of study • Mucositis – mean number of days patients had ≥ grade 1 mucositis and number of patients with mucositis (≥ grade 2, ≥ grade 3) from start to end of study • Diarrhoea – mean number of days patients had ≥ grade 1 diarrhoea and number of patients with diarrhoea (≥ grade 1, ≥ grade 2, ≥ grade 3) from start to end of study HUMANS AND ANIMALS: • Tumour growth – difference in mean tumourweight, volume and volume loss between groups
Secondary outcomes	HUMANS ONLY: • GLN status – effect of GLN supplementation on plasma GLN levels • Timing of intervention – duration, start/end criteria, relation to treatment regime • Route of administration – oral, oral swish and swallow, oral swish and expectorate, TPN, enteral • Doses administered – total dose per day, grams per kg body weight per day • Practice issues – any other practice issues reported

2.4.2 Data Extraction and Management

Data extraction forms were designed for collection of data on study design and methodology (Risk of bias tables), study setting, characteristics of participants, details of interventions, outcomes assessed and other (funding) information from included trials (characteristics of included studies tables). The principle investigator extracted all the data. Extracted data was reviewed by JMK for accuracy. Disagreements were resolved through discussion and consultation with ID.

Many of the published reports of included trials did not provide sufficient information for data analysis or quality assessment purposes. Attempts were made to contact all authors via e-mail to request or clarify the relevant details. Authors were not contacted if no e-mail address was published for correspondence. Information gathered in this way was included in brackets and in italic print in the "Characteristics of included studies" tables and "Risk of bias" tables.

The principle investigator prepared all data for statistical analysis using Review Manager Version 5 (RevMan5) software. Expert opinion and input was obtained regarding statistical analyses of the data.

2.4.3 Assessment of Risk of Bias in Included Studies

The recommended tool for assessing the risk of bias as described in the C*ochrane Handbook for Systematic Reviews of Interventions*[1] was used to evaluate potential sources of bias in methodology of all included trials (humans and animals). Good laboratory practice for preclinical/experimental study design, based on the original Stroke Therapy Academic Industry Roundtable (STAIR) guidelines, calls for the same methodological quality domains as the Cochrane Collaboration's tool for asssessing the risk of bias in clinical trials.[161]

It is a two-part tool addressing 6 methodological quality domains (Table 2.4). The first part of the tool involves describing what was reported to have happened in the study. The second part of the tool involves assigning a judgment relating to the risk of bias for that entry. This is achieved by answering a pre-specified question about the adequacy of the study in relation to the entry, such that a judgment of "Yes" indicative of low risk of bias, "No" indicative of high risk of bias, and "Unclear" indicative of unclear or unknown risk of bias can be made.

Table 2.4: The Cochrane Collaboration's tool for assessing risk of bias

DOMAIN	DESCRIPTION	REVIEW AUTHORS JUDGEMENT
Sequence generation	Describe the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups.	Was the allocation sequence adequately generated?
Allocation concealment	Describe the method used to conceal the allocation sequence in sufficient detail to determine whether intervention allocations could have been foreseen in advance of or during enrolment.	Was allocation adequately concealed?
Blinding of participants, personnel and outcome assessors	Describe all measures used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective. For preclinical studies it is required that outcome assessors/investigators are blinded.	Was knowledge of the allocated intervention adequately prevented during the study?
Incomplete outcome data	Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attrition and exclusions were reported, the numbers in each intervention group (compared to total randomized participants), reasons for attrition/exclusions where reported, and any re-inclusions in analyses performed by the review authors. For preclinical studies all randomized animals should be accounted for in the analyses. Criteria for exclusion and numbers and reasons for exclusions should be clear.	Were incomplete outcome data adequately addressed?
Selective outcome reporting	State how the possibility of selective outcome reporting was examined by the review authors, and what was found.	Are reports of the study free of suggestion of selective outcome reporting?
Other sources of bias	State any important concerns about bias not addressed in the other domains of the tool.	Was the study apparently free of other problems that could put it at a high risk of bias?

Source:[1,161] Higgins JP, 2009; Macleod MR, 2009:40.

The published aspects of methodological design and procedures of included studies were "quoted" within the "Risk of bias" table for each study representative of the first part of the tool. The principle investigator commented and assigned a judgment of either Yes, No or Unclear risk of bias for each of the six methodological quality domains.

Summarizing the risk of bias for an outcome within a study (across domains)

The risk of bias was summarized for each outcome (Table 2.5, 3rd column) within every study across domains by applying the criteria for Low (L), Moderate (M) or High (H) risk of bias.

Table 2.5: Summarizing risk of bias for a study across outcomes

RISK OF BIAS	INTERPRETATION	WITHIN A STUDY	ACROSS STUDIES
L = Low risk of bias	Plausible bias unlikely to seriously alter the results	**Low** risk of bias for all key domains	Most information is from studies at low risk of bias.
M = Moderate risk of bias	Plausible bias that raises some doubt about the results	**Unclear** risk of bias for one or more key domain(s)	Most information is from studies at low or unclear risk of bias.
H = High risk of bias	Plausible bias that seriously weakens confidence in results	**High** risk of bias for one or more key domain(s)	The proportion of information from studies at high risk of bias is sufficient to affect the interpretation of results.

Source:[1] Higgins JP, 2009.

The risk assessment was applied independently by one reviewer (JMK) in addition to the principle investigator. Disagreements were resolved through discussion with the third reviewer (ID).

2.4.4 Assessing the Quality of a Body of Evidence, the GRADE Approach[1]

GRADE is a system for grading the quality of evidence, developed by the Grades of Recommendation, Assessment, Development and Evaluation Working Group (GRADE Working Group). The GRADE approach defines the quality of a body of evidence for each individual outcome reported in a systematic review as the extent to which one can be confident that an estimate of effect or association is close to the quantity of specific interest. Quality of a body of evidence involves consideration of within-study risk of bias (methodological quality), directness of evidence, heterogeneity, precision of effect estimates and risk of publication bias. The Cochrane collaboration has adopted the principles of the GRADE system.[1]

The GRADE approach specifies four levels of quality (Table 2.6). The uppermost quality rating (High) is for evidence from randomized controlled trials.

Table 2.6: Levels of quality of a body of evidence in the GRADE approach

UNDERLYING METHODOLOGY	QUALITY RATING
Randomized trials; or double-upgraded observational studies	High
Downgraded randomized trials; or upgraded observational studies	Moderate
Double-downgraded randomized trials; or observational studies	Low
Triple-downgraded randomized trials; or downgraded observational studies; or case series/case reports	Very low

Source:[1] Higgins JP, 2009.

The body of evidence from randomized controlled trials can, however, be downgraded to moderate, low or even very low quality of evidence, depending on the presence of five factors (Table 2.7).[1] The rating may fall by one level for each factor present, up to a maximum of three levels for all factors.[1]

Table 2.7: Factors that may decrease the quality level of body of evidence

1. Limitation in the design and implementation of available studies suggesting likelihood of bias.
2. Indirectness of evidence (indirect population, intervention, control, outcomes).
3. Unexplained heterogeneity or inconsistency in results (including problems with subgroup analyses).
4. Imprecision of results (wide confidence intervals).
5. High probability of publication bias.

Source:1 Higgins JP, 2009.

Only evidence from randomized controlled trials was included in this review. Guidelines followed for moving from the assessment of the risk of bias of individual studies to GRADE judgments of study limitations for main outcomes are summarized in Table 2.8.

The final GRADE assessment of the quality of the body of evidence for each individual outcome investigated in this review is indicated in the appended summary of findings table, along with the reasons for downgrading of the evidence for each outcome.

Table 2.8: Guidelines for GRADE assessment: Going from assessments of risk of bias to judgments about study limitations for main outcomes

RISK OF BIAS	ACROSS STUDIES	INTERPRETATION	CONSIDERATIONS	GRADE ASSESSMENT OF STUDY LIMITATIONS
L=Low risk of bias	Most information is from studies at low risk of bias.	Plausible bias unlikely to seriously alter the results.	No apparent limitations.	No serious limitations, do not downgrade.
M=Moderate risk of bias	Most information is from studies at low or unclear risk of bias.	Plausible bias that raises some doubt about the results.	Potential limitations are unlikely to lower confidence in the estimate of effect. Potential limitations are likely to lower confidence in the estimate of effect.	No serious limitations, do not downgrade. Serious limitations, downgrade one level.
H=High risk of bias	The proportion of information from studies at high risk of bias is sufficient to affect the interpretation of results.	Plausible bias that seriously weakens confidence in results.	Crucial limitation for one criterion, or some limitations for multiple criteria, sufficient to lower confidence in the estimate of effect. Crucial limitation for one or more criterion(a) sufficient to substantially lower confidence in the estimate of effect.	Serious limitations, downgrade one level. Very serious limitations, downgrade two levels.

Source:1 Higgins JP, 2009.

2.4.5. Measures of Treatment Effect

Dichotomous data

- Mortality: Number of deaths during intervention (N)
- Survival: Number of survivors at follow-up (100 days, 1 year, 2 years, 3 years) (N)
- Clinical infection: Number of patients who developed clinical infection from start to end of study (N)
- Mucositis: Number of patients with ≥ grade 2, ≥ grade 3 mucositis (N)
- Diarrhoea: Number of patients with ≥ grade 1, ≥ grade 2, ≥ grade 3 diarrhoea (N)

For dichotomous outcomes, the estimates of effect of GLN supplementation were expressed as risk ratios (RR) and reported with 95% confidence intervals (CIs) using Mantel-Haenszel (MH) statistical method in RevMan 5.

Continuous data

- Nutritional status: Mean (SD) of change in body weight (kg) from baseline to end of study
- Length of hospital stay: Mean (SD) of length of hospital stay (days)
- Mucositis: Mean (SD) of duration of ≥ grade 1mucositis from start to end of study (days)

- Diarrhoea: Mean (SD) of duration of $\geq$ grade 1 diarrhoea from start to end of study (days)
- Tumour growth: Mean (SD) of tumour weight at end of study (g), Mean (SD) of tumour volume at end of study (cm^3), Mean (SD) of tumour weight loss from baseline to end of study (cm^3)

For continuous outcomes, summary effect estimates were expressed as mean difference (MD) with 95% CIs usinf inverse variance statistical method in RevMan 5.

Standard deviations were obtained from the standard error of a mean (SEM) by using the following formula:

Formula 2.1: $SD = SEM \times \sqrt{N}$

2.4.6 Unit of Analysis Issues

Cross-over trials

Cross-over trials were included in analysis for this review, since it is possible for patients to be receiving several chemotherapy cycles, healing completely from mucositis/diarrhoea in-between treatment sessions.[117] The treatment effects from cross-over trials and parallel design trials were combined when appropriate, using paired data from both periods of the cross-over studies.

2.4.7 Studies with Multiple Treatment Groups

In studies where participants were randomized to multiple treatment groups, only the data from the study group of interest and the control/placebo group were analyzed. Data of groups stratified according to disease were combined into one study group and one control group, using appropriate statistics:[1]

Formula 2.2: S = Square root of: $\sum\chi^2 - (\sum\chi)^2/n/n\text{-}1.$

2.4.8 Dealing with Missing Data

In studies where all results/statistics for outcomes of interest are not reported, the author was contacted to retrieve the missing data if an e-mail address was available. If not or in cases of no response, the statistics or level of significance reported was explored in the narrative review/discussion. The data was extracted for analysis as reported. In cases where data was reported as mean (SEM), it was converted to mean (SD) using appropriate formulae.[1] Issues relating to missing data due to drop-out/withdrawal and if this was addressed with intention-to-treat (ITT) analysis were part of the assessment of risk of bias. Studies that did not address missing data or did not report on ITT issues was scored as either High risk (No) or Moderate (Unclear) risk of bias.

2.4.9 Assessment of Heterogeneity

Statistical heterogeneity was identified using a Chi-square test and quantified by I^2 statistics as calculated within RevMan5 forest plots. Where the P-value was less than or equal to 0.01, this was interpreted as indicative of significant heterogeneity, and in this case a random effects model was used to derive a summary statistic with 95% CIs. I^2 statistics quantify inconsistency between 0-100%. Insignificant or moderate heterogeneity might present between 0-50%, while closer to 100% may represent substantial or considerable heterogeneity.[1]

Heterogeneity was investigated by performing an analysis on several subgroups within RevMan5:

Route of supplementation

- Oral (Swallow, Swish and swallow, Swish and expectorate)
- TPN
- Enteral (Sip feed/Tube feed)

Dose

- < 20 g per day (<0.28 g/kg/day)
- 21-30 g per day (0.30-0.42 g/kg/day)
- 31-40 g per day (0.44-0.57 g/kg/day)
- >40 g per day (>0.57 g/kg/day)

Duration of intervention

- <7 days
- 7-14 days
- 14-21 days
- >21 days

Cancer diagnoses

- Haematological malignancy
- Gastrointestinal malignancy
- Breast cancer
- Head and neck cancer
- Solid tumours
- Mixed types

Treatment

- Chemotherapy
- RT
- BMT with or without chemotherapy and/or RT
- Surgery
- None

Risk of bias (Summarized across domains, per outcome within each study)

- Low
- Moderate
- High

Criteria used for mucositis/diarrhoea

- Objective
- Subjective

Outcome assessment

- Patient reported
- Physician assessment

Animals: Type of tumour

Animals: Type of animal

2.4.10 Assessment of Reporting Biases

Publication bias was explored using a test for funnel plot asymmetry only when there were at least 10 studies included in the meta-analysis. When there are less studies, the power of the test is too low to distinguish chance from real asymmetry.[1]

2.4.11 Data Synthesis

When meta-analyses were possible, the random effects model was used for combining data of primary outcomes, due to the existence of a relatively high level of heterogeneity in-between studies. Sensitivity analyses were done, using the fixed effect model and standardized mean

difference. Studies in the meta-analysis were weighed according to study group size. A P-value smaller than 0.05 was considered to be indicative of a statistically significant effect, justifying rejection of the null hypothesis (GLN intervention has no effect on outcome in question, or there is no difference in the effect of GLN supplementation versus placebo/control).

Secondary outcomes or data unsuitable for meta-analysis were summarized and briefly described in a narrative review format, namely the effect of GLN supplementation on plasma GLN levels, the route of GLN administration, timing and dose of GLN intervention and other practice issues reported.

2.5 ETHICS AND LEGAL ASPECTS

The study protocol was approved (N08/01/012) by the Committee for Human Research of the Faculty of Health Sciences, Stellenbosch University (APPENDIX 6.1).

CHAPTER 3: RESULTS

3.1 DESCRIPTION OF STUDIES

3.1.1 Results of the Search

Initial search

The initial electronic search of databases (Table 3.1) identified a total of **731** citations after correction for duplicate hits across databases (-772).

Phase I selection

Phase I selection (Table 3.1) identified **276** potentially eligible studies. Two independent reviewers each reviewed 10% of studies excluded by the principle investigator. Twenty-two additional citations were added from hand-searching of personal files and reference lists of cited studies resulting in a total of **298 potentially eligible studies** plus **2 additional studies at citation update** of which the full text reports was to be retrieved for application of Phase II selection criteria.

Table 3.1: Summary of initial citations per database and outcome of Phase I selection

ELECTRONIC DATABASE	PERIOD SEARCHED	Citations from INITIAL SEARCH	Potentially eligible (PHASE I SELECTION)
PUBMED/MEDLINE	1966 to 18 Aug 2008	481	228
Science Citation Index (ISI Web of Science)	1990 to 18 Aug 2008	505	258
EBSCO HOST	1966 to 18 Aug 2008	337	256
Cochrane Library	1800 to 18 Aug 2008	127	69
Proquest Medical Library	1986 to 18 Aug 2008	53	37
SUB TOTAL		**1503**	**843**
Correction for duplication		*-(772)*	*-(572)*
SUBTOTAL		**731**	**276**
Citations from personal files and reference lists			+ 22
Citation update: 24 March 2010			+ 2
TOTAL POTENTIALLY ELIGIBLE CITATIONS (PHASE I):			**300**

Full text reports were mostly retrieved from on-line intranet sources (*N*=188) and subsequently paper copies (*N* =26) from open shelves within the JS Gericke and Health Sciences Libraries of Stellenbosch University, Cape Town. The full texts of foreign language reports were not retrieved (*N* =25). The unavailable reports (*N*=61) were retrieved with the help of the library assistant,

Wilhelmine Pool, from other South African libraries. Some of these articles were not available in South Africa or online (*N*=31) due to embargo. These reports were requested from overseas libraries for RCTs only (*N*=5).

Phase II selection

Phase II selection criteria were applied to full reports by the principle investigator. One hundred and forty studies were obvious exclusions (reviews, abstracts, foreign language reports, editor's letters and correspondence/discussions). The remaining 160 full reports were, in addition, reviewed by two independent reviewers. Ninety (90) studies, of any study design, remained included after completion of phase II selection.

Phase III selection

Only RCTs were included in the third and final selection phase. Twenty-three studies of other designs were excluded by the principle investigator and two independent reviewers. Seven additional studies were excluded, based on the outcomes of interest (Phase III selection criteria). Sixty trials were included for analysis in the current review.

The outcome of the methodical application of inclusion/exclusion criteria from phase II – III as described in section 2.4.1 to the 300 full text reports is systematically summarized in APPENDIX 6.2 and illustrated in Figure 3.1 below, indicating actions of the principle investigator and independent reviewers and the quantitive outcome of selection at each phase.

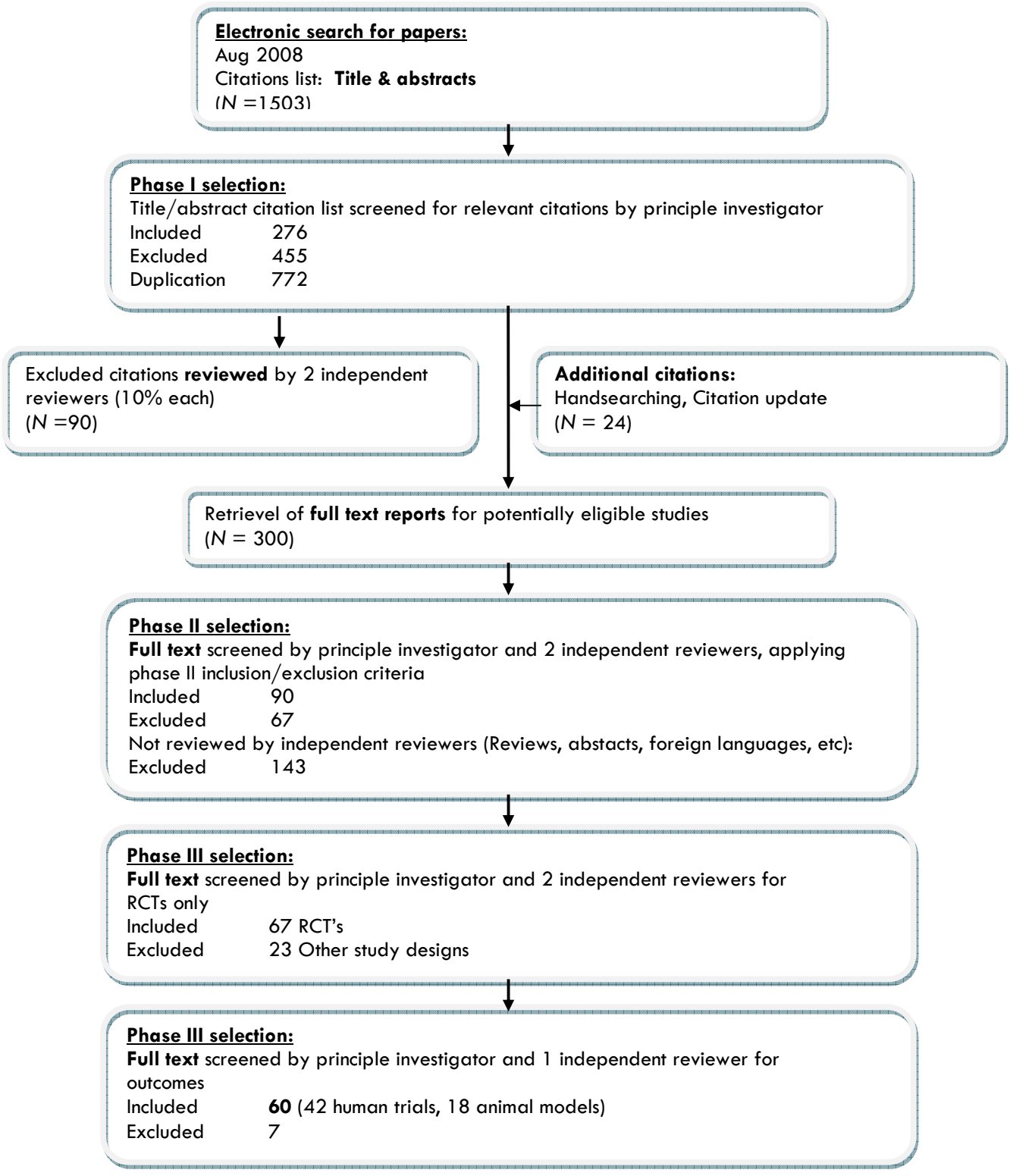


Figure 3.1: Phases I to III of study selection

3.1.2 Excluded studies

The principal reason for exclusion of full text reports (*N*=240) was review articles (*N* =90), then studies on subjects without cancer (humans *N* =16, animals *N* =28), no full text published – only abstracts, correspondence or letters (*N* =28), foreign languages (*N* =25), subjects receiving no GLN supplementation (humans *N* =4, animals *N* =11), outcomes not considered in objectives (*N* =15), study designs other than RCTs (*N* =23). APPENDIX 6.3 documents detailed information regarding the foremost reasons for exclusion, specified per study. A complete reference list of excluded studies is also tabulated in APPENDIX 6.3. Studies excluded due to reporting in languages other than English are further summarized in APPENDIX 6.4, indicating possible eligible studies recommended for future assessment. Based on information available in English abstract/title, six of these studies might possibly be eligible for inclusion in this review.

3.1.3 Included studies

Sixty RCTs have been included (*N* =60) in this review (Table 3.2), of which 42 are human trials and 18 experimental animal models. Of the 42 human trials and 18 animal studies, 35 and 16 respectively reported sufficient data for synthesis in meta-analysis. The outcomes of the remaining 7 human trials[37,59,100,110,120,134,153] and 2 animal studies[72,73] are discussed supplementary in the narrative review. Bibliographic information and sources of included studies are detailed in APPENDIX 6.5 and the characteristics of included studies are summarized in APPENDIX 6.6.

Table 3.2: Study ID and reference index of included studies (*N* =60)

Anderson 1998 (100)	Daniele 2001 (102)	Kozelsky 2003 (119)	Scheid 2004 (134)	Yoshida 1998 (37)	Klimberg 1992a (78)
Berk 2008 (162)	Decker Baumann 1999 (48)	Li 2009 (120)	Scheltinga 1991 (143)	Ziegler 1992 (60)	Klimberg 1996a (79)
Blijlevens 2005 (109)	Erdem 2002 (144)	Marton 2010 (164)	Schloerb 1993 (111)	Austgen 1992 (74)	Robinson 1999 (80)
Bozzetti 1997 (95)	Gianotti 2009 (163)	May 2002 (130)	Schloerb 1999 (112)	Bartlett 1995 (75)	Rouse 1995 (87)
Brown 1998 (140)	Hallay 2002 (133)	O'Riordian 1994 (136)	Sornsuvit 2008 (113)	Fahr 1994 (86)	Rubio 1998(90)
Canovas 2000 (153)	Huang 2000 (103)	Oguz 2007 (141)	Stehle 1989 (59)	Kaibara 1994 (76)	Shewchuk 1997 (81)
Cerchietti 2006 (101)	Jebb 1994 (104)	Okuno 1999 (105)	Strasser 2008 (122)	Kaufmann 2003 (3)	Xue 2007 (71)
Choi 2007 (93)	Jebb 1995 (96)	Peterson 2007 (94)	Sykorova 2005 (145)	Kaufmann 2007 (4)	Xue 2008 (72)
Coghlin Dickson 2000 (97)	Jo 2006 (142)	Piccirillo 2003 (110)	Van Zaanen 1994 (106)	Kaufmann 2008a (5)	Xue 2009(73)
Da Gama Torres 2008 (123)	Klek 2005 (61)	Pytlik 2002a (98)	Wu 2001 (29)	Klimberg 1992 (77)	Yoshida 1995 (82)

3.1.3.1 Human studies (*N* =42)

Characteristics of study setting

Of the 42 included trials 38 were designed as parallel group studies [29,37,48,59-61,93,95-98,101-103,105-106,109-113,119,122-123,130,133-134,136,140-145,153,162-164] and only 4 had a cross-over design.[94,100,104,120] Most of the paired data was published in an inappropriate form for inclusion in the meta-analysis and will be discussed in the narrative review.

Thirty-seven (88%) of the 42 included trials were conducted at a single site of which 6 were conducted in the USA,[60,100,105,111-112,143] 6 in the UK,[96,104,109,134,136,140] 3 in Italy,[95,102,110] 2 in Turkey,[141,144] the Czech Republic,[98,145] Germany,[59,106] Korea,[93,142] Hungary,[133,164] and China[29,120] and 1 each in Spain,[153] Argentina,[101] Taiwan,[103] California,[97] Brazil,[123] Berlin,[48] Cracow,[61] Thailand,[113] Switzerland,[122] and Japan.[37] Only five trials were conducted in multiple centres, of which three took place in the USA [119,130,162] and one each in Russia[94] and Italy.[163]

Of the 42 included studies, the first human trial was published in 1989,[59] followed by annual publications of at least one trial ranging from 1-4 publications per year up to March 2010.

Funding

Twenty-six (61.9%) trials received external funding of which 13 received funds from either nongovernmental organizations[60,97,100,110,113,120,130,143,163] or government grants.[98,102,119,145] The other 13 trials disclosed that they received some form of support from the pharmaceutical industry. [37,48,59,94,101,106,109,111-112,122,123,136,162] The remaining 16 (38%) trials made no revelation of funding received.[29,61,93,95-96,103-105,133-134,140-142,144,153,164]

Characteristics of Participants

A total of 2 687 participants were included in the respective studies. The median number of participants per study was 44, ranging from 12 to 428 (Figure 3.2). Twenty-five (59.5%) of the trials included less than 50 participants,[29,48,60,96,98,100-101,106,109-110,112,120,122,130,133-134,136,140,143-145] of which 4 were less than 20.[37,59,103,113] In only one study[153] the number of participants included was unclear.

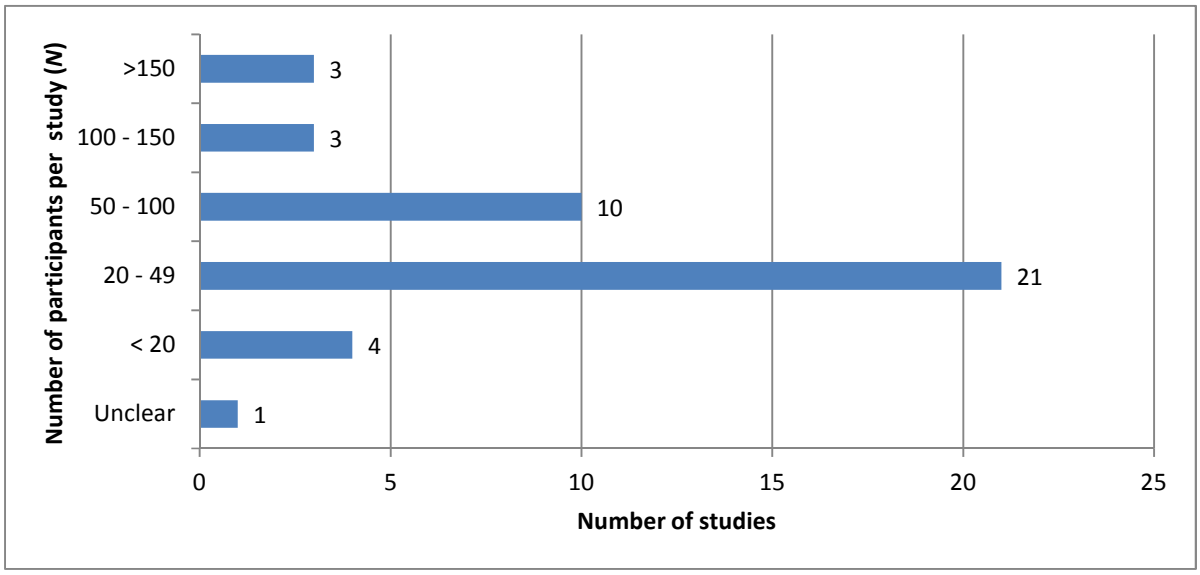


Figure 3.2: Number of studies by number of included participants

Thirty-six (85.7%) of the included trials recruited only adult patients [29,37,48,59-61,93-95,98,101-103,105-106,109-113,119-120,122-123,130,133-134,136,141,142-145,162-164] with an age range of 18-91 years and 3 included both children and adults (difference in age as large as 17-59,[97] 4-43 years[100] and 16-62 years).[140] None included solely children/adolescent patients and the age group was unclear in 3 trials.[96,104,153] All studies included male and female subjects.

The cancer diagnoses of study participants (Figure 3.3) was mostly gastrointestinal malignancies (35%), of which 5 studies included a combination of gastrointestinal malignancies ranging from esophagus, stomach, colon, rectum, pancreas, gall bladder, liver and ileal to unknown sites.[29,104,120,144,163] Five studies included only colorectal and/or metastatic colorectal cancer,[48,59,102,136,141] 3 studies only esophageal cancer[37,133,164] and 1 study each periampullary tumours[142] and gastric cancer.[61] Haematological malignancies were exclusively included in 12 studies (28.5%) [60,96-97,106,109-110,113,123,134,140,143,145] and haematological malignancy mixed with multiple sclerosis, breast cancer, seminoma and unspecified solid tumours in another 3 studies.[98,111-112] Squamous head and neck cancer was included in 2 studies[101,103], breast cancer in 2 studies[94-95] and Ewing's sarcoma, osteosarcoma, rhabdomysarcoma and neuroblastoma in another.[100] The remaining 5 studies [93,119,122130,162] included a wide range of cancer diagnoses, among which solid tumours/adenocarcinoma/squamous cell carcinoma of the esophagus, colorectal, periampulla, biliary, head and neck, uterine, gall bladder, breast, lung, ovarian, pancreatic, prostate, liver, rectal, gynaecological and other unspecified types, with the cancer diagnoses being unclear in 2.[105,153]

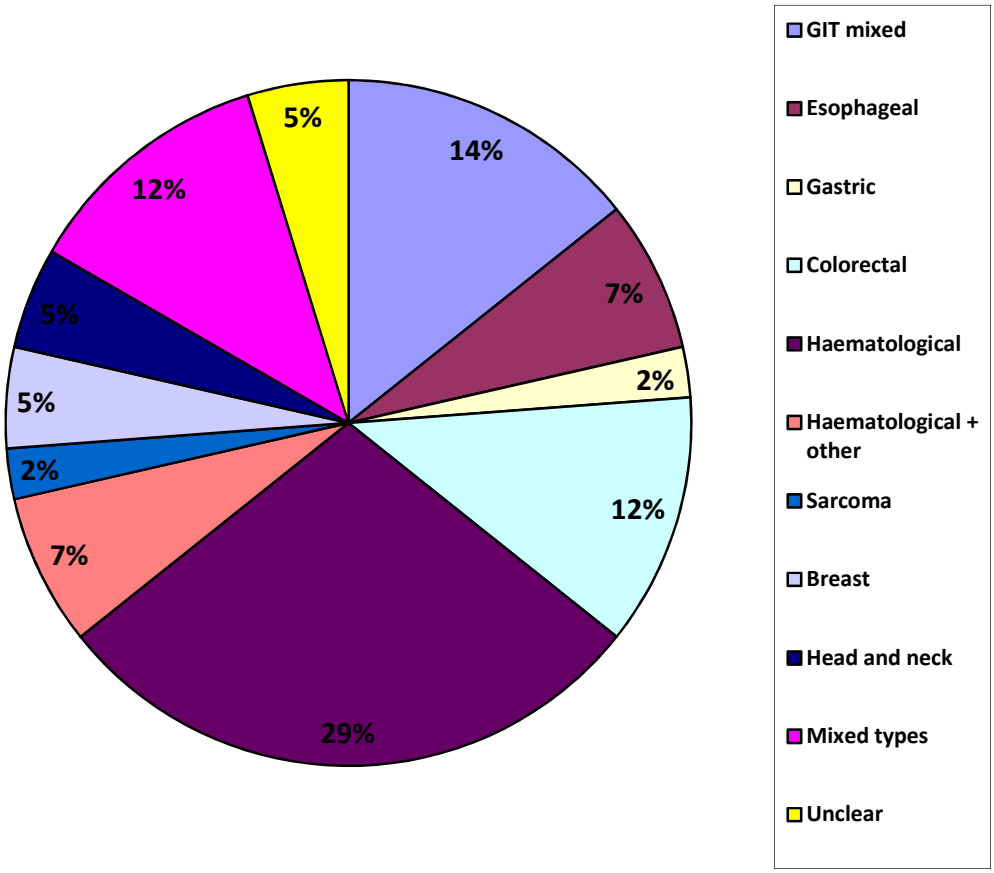


Figure 3.3 Number of studies per cancer diagnoses

The patients in thirteen (30.9%) of the trials received BMT[60,96-98,109-112,123,140,143,145,153] as primary treatment modality, with preparative or conditioning regimes of either chemotherapy,[96,98,153] RT[97] or a combination (CRT)[60,109,111-112,134,140,143,145] of both. Thirteen (30.9%) trials reported treatment with chemotherapy[48,93-95,100,102,104-106,113,120,122,134] only, and concurrent with RT (CRT) in 4 trials,[37,101,119,133] of which 9 included 5-FU.[37,48,93-94,101-102,104-105,120] The chemotherapy regimen was well described in the majority of trials. The total RT dose reported in 8 trials was between 14 and 70 Gy.[37,101,103,111-112,119,140,143,145] Eleven (26.1%) studies included patients receiving surgery for GIT malignancies.[29,59,61,102,133,136,141-142,144,163-164] Only one (2.3%) trial[130] reported no treatment besides GLN intervention and another two required no treatment for inclusion, but allowed chemotherapy[130,162] and/or RT.[130]

The percentage withdrawal was clear in all trials ranging from 0% to 81%, with a median of 8%. Fifteen (35%) of the published reports indicated no apparent

withdrawals (0%).[29,59,93,98,101,103,105,110,113,119-120,123,143,153] The reporting of reasons for withdrawal/dropout from respective study groups was unclear in only 1 trial.[94]

Characteristics of the interventions

Everyone of the trials provided a clear description of the study intervention (GLN) and placebo/control used, as well as the dose administered and the route of administration. L-GLN powder[29,37,60,93,95-97,100,102-105,111-112,119,122,130,133,143-144,153,162] was used in 22 (52.3%) of the included trials; Saforis[94] (composed of GLN in a novel, proprietary drug-delivery system (UpTec)) in one (2.3%), L-alanyl-L-GLN dipeptide,[59,61,98,101,106,109-110,113,120,123,141,145,163-164] in 14 (33.3%) and glycyl-L-GLN dipeptide[48,134,136,140,142] in 5 (11.9%). Thirty-eight (90.4%) studies used a placebo (Table 3.2) versus 4 that used a non-treatment control ("Best Supportive Care (BSC)"[93] and no supplementation[48,120,141]).

Table 3.3: Placebo/treatment controls used

versus ORAL GLN	versus PARENTERAL GLN	versus ENTERAL GLN
Glycine[100,112119] Maltodextrin[95,122] Dextrinomaltose[153] Maltodestrins[102] Powdered sugar[97] Normal saline[103] Polycal[96,104] Isonitrogenous, isocaloric mixture[162] Standard amino acid solution intravenously[37] Identical appearing placebo[105,94]	Standard amino mix[109,113,123] Isonitrogenous mixture of non-essential amino acids[106,130,140] Parenteral isonitrogenous amino acid solution[98] Corresponding amounts of free alanine and glycine nitrogen[59] Commercially available isonitrogenous and isocaloric TPN formula[111,142-143] GLN free standard TPN solution [60-61,134,145] Conventional TPN[136] Freamine[110] Normal saline[101] Vehicle only[163] Placebo (unclear)[164]	GLN free polymeric enteral formula (ENSURE)[144] GLN poor Nutrison Multi-fibre diet[29,133]

Co-interventions was used in 6 studies, namely arginine,[133,144] ß-hydroxy-ß-methylbutyrate (HMB)/arginine,[130,162] arginine/omega 3[29] and glycyl-L-tyrosine.[59] Two studies had an interruption of protocol, resulting in change-over to a new GLN product as a result of interruption in funding[112] or availability of a new product.[110] GLN was supplemented via the oral route in 17 (40.4%) studies of which 10 were apparently swallowed,[37,93,95,97,102,112,119,122,130,153] 5 swished and swallowed,[94,96,100,104-105] and 1 swished and expectorated.[103] In twenty-two (52.3%) studies GLN was administered intravenously as either part of parenteral (PN) or total parenteral nutrition (TPN).[48,59-61,98,101,106,109-111,113,120,123,134,136,140-143,145,163-164] Three studies (7.1%) used enteral supplementation.[29,133,144.] Intervention dose and duration of intervention (Table 3.3) were well described in all trials.

Table 3.4: Dose of GLN supplementation in humans (Specified per route and GLN type)

ROUTE		< 20 g per day (<0.28 g/kg/day) (*N=12*)	20-30 g per day (0.28-0.42 g/kg/day) (*N=19*)	31-40 g per day (0.44-0.57 g/kg/day) (*N=8*)	>40 g per day (>0.57 g/kg/day) (*N=3*)
SWISH & EXPECTORATE L-GLN (*N*=1)		8 g/day[103]			
ORAL L-GLN (*N=16*)		4 g/day[100] 7.5 g/day[94] 8 g/day[105,119] 14 g/day[130] 16 g/day[96,104] 18 g/day[102]	20 g/day[153] 28 g/day[162] 30 g/day[37,93,95,97,112,122]		
ENTERAL L-GLN (*N*=3)		14.2 g/Liter[144]	1.3 g/100 mL/day (2000 mL/day[133]	1.3 g/100 mL/day (2400 mL/day)[29]	
TPN	**L-GLN (*N*=3)**			0.57 g/kg/day[60,111,143]	
	L-ALANYL-L-GLN DIPEPTIDE (*N*=14)	13.46 g/day[110]	0.28 g/day[59] 0.3-0.4 g/kg/day[123] 0.4 g/kg/day[101,163] 20 g/day[120] 30 g/day[98,113]	0.5 g/day[145,164] 0.57 g/kg/day[109] 40 g/day[106]	1 g/kg/day[141] 2 ml/kg/day[61]
	GLYCYL-L-GLN DIPEPTIDE (*N =5*)	0.18 g/kg/day[136] 0.2 g/kg/day[142]	0.4 g/kg/day[48] 30.27 g/day[134]		50 g/day[140]

Table 3.5: Duration of GLN administration in humans (Specified per route and GLN type)

ROUTE		<7days (*N=3*)	7-14 days (*N=15*)	15-21 days (*N=11*)	>21 days (*N=13*)
SWISH & EXPECTORATE L-GLN (*N*=1)					32 days[103]
ORAL L-GLN (*N*=16			8 days[96] ≥ 14 days[100,105] ±14 days[153]	15 days[93,102] **20.5(15.4) days*[112] 21 days[94,119] ‡21(4-41) days*[97]	**25.6(2.2) days*[96] 28 days[37] 8 weeks[162] 74 days[122] ±80 days[95] 4-24 weeks[130]
ENTERAL (*N*=3)			7 days[29] 10 days[133]	17 days[144]	
TPN	**L-GLN (*N*=3)**				**26(2) days*[60] **30(5) days*[111] **27(1) days[143]
	L-ALANYL-L-GLN DIPEPTIDE (*N*=14)	5 days[59,120]	7 days[123,142] **7.1(1.8) days*[163] ‡8.5(7-10) days*[61] ±10 days[101141,164] **13.8(3.1) days*[98]	**16.5(13.5) days*[113] †19 days[109] ‡18(13-25) days*[106]	22 days[145] ‡28(23-70)days*[110]
	GLYCYL-L-GLN DIPEPTIDE (*N =5*)	5 days[136]	7 days[142] 13(5-34) days*[134]	18 days[48]	±25 days[140]

**Mean (SD), ‡ median (range), † median, *in GLN group.

Characteristics of outcome measures

Mucositis

Seventeen scales (Table 3.6) were used to access the presence and severity of mucositis in the respective studies, of which 4 was subjective and patient-reported and 13 more objective and investigator-reported (physician, nurse or endoscopist). The criteria for assessment of severity was published for all but 5 of the scales (APPENDIX 6.8).

Table 3.6: Mucositis grading scales used in included studies

PATIENT-REPORTED (SUBJECTIVE)	**STUDY ID**	**GRADES**
Modified Eastern Cooperative Oncology Group (MECOG) grading criteria (Mouth sore? Yes/No. How soreness affected eating)	Anderson 1998,[100] Huang 2000,[103] Sornsuvit 2008[113]	0-4
Scoring system for patient-reported symptoms. (Mouth comfort/ease of eating scored from 1-5)	Jebb 1994,[104] Sornsuvit 2008[113]	0-4
Patient mucositis assessment (How does your mouth feel today?)	Jebb 1995[96]	0-4
The North Central Cancer Treatment Group (NCCTG) criteria for reporting mucositis	Okuno 1999[105]	Unclear
PHYSICIAN/NURSE ASSESSMENT (OBJECTIVE)		**GRADES**
Daily mucositis score (DMS) (Lesions, erythema, oedema, pain, bleeding, dryness and production of viscous mucus. Score for each item of 0(normal) to 3(severe))	Blijlevens 2005,[109] Piccirillo 2003[110]	0-3
World Health Organization (WHO) scale (Symptoms (pain), functions (ability to drink and eat), presence of lesions (ulcers, erythema))	Peterson 2006,[94] Cerchietti 2006,[101] Decker-Baumann 1999,[48] Jebb 1994,[104] van Zaanen 1994[106]	0-4
Objective mucositis score (OMS) (Grading of oral erythema, and ulceration size in nine oral sites. OMS < 1.5 = not severe; OMS $\geq$ 1.5 = severe)	Cerchietti 2006[101]	0-3
Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0)	Choi 2007[93]	0-5 (Death)
Mucositis grading criteria	Okuno 1999[105]	0-4
Toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organisation for Research and Treatment of Cancer (EORTC) (Objective)	Huang 2000[103]	0-4
Observer Mucositis Score	Jebb 1995[96]	0-4
Common toxicity criteria of the National Cancer Institute Bethesda, Maryland (NCI)	Daniele 2001,[102] Bozzetti 1997[95]	0-4
Nebraska Oral Assesment Score ("Individual item scores (Grade 1 to 3) were summed to obtain an overall mucositis score. The following indexes were recorded: MUCPEAK – the peak oral mucositis score reached in each individual patient. MUC9, MUC10, MUC11, MUC12, MUC13, MUC14 – the number of days with mucositis of at least the particular score")	Pytlik 2002a[98]	0-3
Gastrointestinal toxicity classified according to Northern California Oncology Group (NCOG)	Canovas 2000[153]	Unclear
Stanford University Hospital BMT toxicity scale. ("The number of days of mucositis included days of grades 2-4 mucositis. Grades 0-1 were not considered significant mucositis.")	Coghlin-Dickson 2000[97]	Unclear
"Incidence and severity of mucositis was based on review of each patient chart by one individual: Diagnoses, treatment and frequent mention of mucositis in progress notes were accepted as presence of mucositis."	Schloerb 1999[112]	Unclear
"Oral mucosa was examined for the presence and severity of mucositis and graded as a function of the degree of inflammation."	Schloerb 1993,[111] Ziegler 1992[60]	Unclear

Diarrhoea

Eight different sets of criteria (Table 3.5) were used to define the presence and/or severity of diarrhoea. Two authors were unclear about how the presence and/or severity of diarrhoea were defined and graded.[29,112] Criteria for grades of severity (grade 0 to 4/5) were published in only 3 trials.[95,104,119] Details regarding the various scales, with the published criteria and grades, are summarized in APPENDIX 6.9.

Table 3.7: Diarrhoea grading scales used in included studies

DIARRHOEA PRESENCE	STUDY ID	GRADES
Scoring system for **patient-reported** symptoms. Stool consistency (1-5)	Jebb 1994[104]	0-4
Common toxicity criteria of the National Cancer Institute Bethesda, Maryland (NCI)	Bozzetti 1997,[95] Daniele 2001,[102] Kozelsky 2003,[119] Sornsuvit 2008[113]	0-5 (Death)
Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0)	Strasser 2008[122]	0-5
World Health Organization (WHO) classification (grade 0-4)	Decker-Baumann 1999,[48] Li 2009,[120] Van Zaanen 1994[106]	0-4
Gastrointestinal toxicity classified according to Northern California Oncology Group (NCOG)	Canovas 2000[153]	Unclear
Diarrhoea, > 3 loose stools per day	Pytlik 2002[98]	Unclear
Diarrhoea; > 4 loose stools per day	Jebb 1995[96]	Unclear
Stool output of volume > 500 ml in 24-hour period was notable diarrhoea	Coghlin Dickson 2000[97]	Unclear
Unclear	Schloerb 1999,[112] Wu 2001[29]	Unclear

3.1.3.2 Animal studies

Characteristics of study setting

All 18 animal studies had a parallel group design. Nine (50%) of the studies were conducted in the USA,[3-5,74-75,77-79,86] 4 in Canada,[71-73,81] 2 each in Japan[76,82] and Arkansas[87,90] and one in France.[80]

Three of the included studies were published during 1992.[74,77-78] A further 9 studies were published between 1994 and 2003.[3,75-76,79,81-82,86-87,90] The next two included studies were only published in 2007,[4,71] with another 4 publications up to 2009.[5,72-73,80]

Funding

Fourteen experimental studies reported receiving external funding, of which 13 were grants from nongovernmental associations[4-5,71-75,78-81,86-87] and 1 from the pharmaceutical industry.[82] Four of the included studies made no mention of funding.[3,76-77,90]

Characteristics of animal models

All included animal models made use of rats for experiments. A total number of 441 rats of different species and tumour models (Table 3.8) was included in the respective studies, with a median of 21 rats per study, ranging from 14[80] to 59[81] (Figure 3.4). Seventeen (94.4%) of the studies included less than 50 rats,[3,71-75,77,81-82,86] of which 8 studies were less than 20. [4-5,76,78-80,87,90]

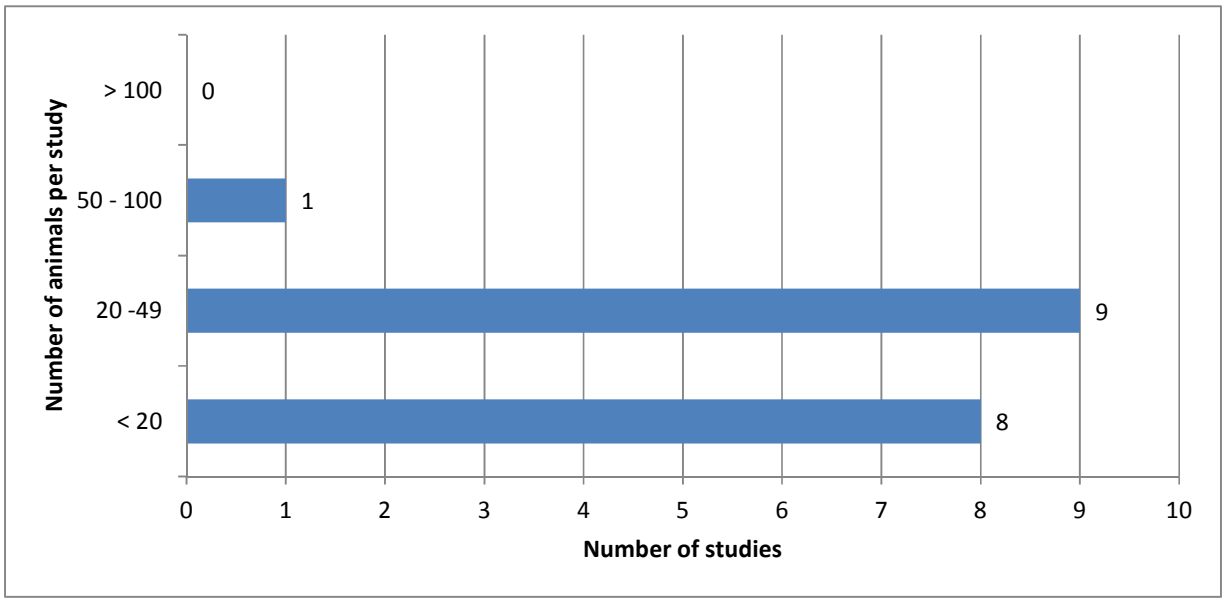


Figure 3.4: Number of studies per number of animals (rats)

Seven studies included only male rats, 10 studies only female rats and the sex was unclear in one study (Table 3.8).

Table 3.8: Characteristics of experimental animal models

RAT SPECIES	**STUDIES**
Male Donryu rats (*N=2*)	Kaibara 1994;[76] Yoshida 1995[82]
Male Fischer 344 rats (*N=5*)	Austgen 1992[74]; Fahr 1994;[86] Klimberg 1992a;[78] Rouse 1995;[87] Rubio 1998[90]
Female Fischer rats (*N=6*)	Kaufmann 2007;[4] Klimberg 1992;[77] Klimberg 1996a;[79] Xue 2007;[71] Xue 2008;[72] Xue 2009[73]
Female Sprague Dawley rats (*N=3*)	Kaufmann 2003;[3] Kaufmann 2008a;[5] Shewchuk 1997[81]
Female Lewis/Wistar rats (*N=1*)	Bartlett 1995[75]
Buffalo rats (*N=1*)	Robinson 1999[80]
Implanted tumour models	**Studies**
Viable 3 -methylcholanthrene-induced fibrosarcoma (*N=6*)	Austgen 1992;[74] Fahr 1994;[86] Klimberg 1992;[77] Klimberg 1992a;[78] Rouse 1995;[87] Rubio 1998[90]
Ward colorectal tumour (*N=3*)	Xue 2007;[71] Xue 2008;[72] Xue 2009[73]
MTF – mammary tumour (*N=2*)	Kaufmann 2007;[4] Klimberg 1996a[79]
Morris hepatoma 7777 (*N=2*)	Robinson 1999;[80] Shewchuk 1997[81]
MAC-33 (mammary adenocarcinoma cells) (*N=1*)	Bartlett 1995[75]
AH109A rat ascites hepatoma (*N=2*)	Kaibara 1994;[76] Yoshida 1995[82]
Induced tumours	**Studies**
Mammary tumours with oral DMBA (*N=2*)	Kaufmann 2003;[3] Kaufmann 2008a[5]

Fourteen (77.8%) of the included studies had no apparent dropouts. Four studies reported dropouts of 8.3%,[72] 31.8%[73] and 56%[78] respectively due to mortality and one study had a dropout of 16%[74] for which no reason was given.

Characteristics of intervention

All studies used either an isocaloric and/or isonitrogenous placebo mixture/diet via the same route as the GLN supplementation (Table 3.9). Nine studies reported that the animals received pair-fed[3-5,77-79,86-87,90] rat chow. The remaining half of the studies allowed free access to food. It was common practice to allow water *ad libitum*.

Table 3.9: Placebo mixture/diet used

Versus TPN	Versus GAVAGE	Versus DIET
Equimolar mixture of three other nonessential amino acids (serine, proline, glycine)[74] Isonitrogenous amino acid solution[82] Isonitrogenous and isocaloric standard TPN[76]	Isonitrogenous amount of glycine [77,86-87,90] Isonitrogenous amount of Freamine[3-5,79] Isovolemic sterile water[72]	Isocaloric diet containing isonitrogenous amount of glycine[75,78,81] Isoenergetic and isonitrogenous diet with control mixture of amino acids[80] Isoenergetic and isonitrogenous diet with control mixture of amino acids alanine, serine, glycine and histidine[73,71]

Two studies used alanyl-GLN[76,82] via the parenteral route as opposed to L-GLN in the rest. Nine (50%) of the studies supplemented L-GLN via gavage,[3-5,72,77,79,86-87,90] 6 (33.3%) used diet[71,73,75,78,80-81] as vehicle for L-GLN and 3 (16.7%) TPN[74,76,82] of which one used L-GLN.[74]

The GLN dose unit was specified according to the route used and was not easily comparable between studies (Table 3.10). Eight (44%) studies administered 1g GLN/kg body weight/day via Gavage,[3-5,77,79,86-87,90] 2 studies administered 0.75 g GLN/kg body weight/day of which one was via diet[71] and one via gavage,[72] and one 0.5 g GLN/kg body weight/day via diet.[78] Seven studies reported dose as g GLN/kg diet ranging from 1.1 g alanyl-GLN/100 ml TPN diet (25% of total TPN nitrogen of diet),[82] 1.5 g alanyl-GLN/100 ml TPN diet,[76] 20 g GLN/kg diet,[73,81] 30 g GLN/kg diet[75] to 40 g GLN/kg diet[80] and one study reported a dose of 222 ml of 3% L-GLN per liter (20% of total TPN nitrogen of diet).[74] It is unclear how much diet was consumed per day.

Table 3.10: Dose of GLN supplementation in animals (Specified per route and GLN type)

ROUTE		Lowest				Highest
DIET L-GLN (N=6)		0.5 g GLN/kg body weight/day.[78]	0.75 g GLN/kg body weight/day[71]	20 g GLN/kg diet[73,81]	30 g GLN/kg diet[75]	40 g GLN/kg diet[80]
GAVAGE L-GLN (*N* =9)			0.75 g GLN/kg body weight/day[72]	1g GLN/kg body weight/day[3-5,77,79,86-87,90]		
TPN (*N*=3)	L-GLN	222 ml of 3% L-GLN per liter (20% of total TPN nitrogen of diet)[74]				
	alanyl-GLN		1.1 g alanyl-GLN/100 ml TPN diet (25% of total TPN nitrogen of diet)[82]	1.5 g alanyl-GLN/100 ml TPN diet[76]		

The duration of GLN supplementation was clearly described in all studies (Table 3.11). The 3 studies using TPN as route of administration all had a short intervention period of less than 7 days, compared to the oral route of which the duration of intervention ranged from 4 days to 17 weeks.

Table 3.11: Duration of GLN administration in animals (Specified per route and GLN type)

ROUTE		<7 DAYS (*N=6*)	7-14 DAYS (*N=5*)	15-21 DAYS (*N=1*)	>21 DAYS (*N=6*)
DIET L-GLN (N=6)			9 days[71] 2 weeks[78,80]	21 days[81]	25 days[75] 34 days[73]
GAVAGE L-GLN (N =9)		4 days[87,90] 4-5 days[77]	9 days[72] 2 weeks[4]		23 days[86] 7 weeks[79] 11 weeks[5] 16–17 weeks[3]
TPN (*N*=3)	**L-GLN**	5-6 days[74]			
	alanyl-GLN	5 days[76] 6 days[82]			

3.2 RISK OF BIAS IN INCLUDED STUDIES

3.2.1 Human studies

Details of the judgment of risk of bias across six methodological quality domains for each included human study are presented in APPENDIX 6.7.

Adequate sequence generation

The method of randomization was adequate (Yes, ☑) in 27 (64.2%, Figure 3.5, Table 3.12) studies. Fourteen (33.3%) studies did not provide details regarding sequence generation (Unclear, (?)). Only one study[106] was judged to have introduced a high risk of bias (No, ☒) through unacceptable randomization practices.

Allocation concealment

The concealment of allocation (Figure 3.5, Table 3.12) was adequate (Yes, ☑) for 22 (52.4%) of the 42 studies and it was unclear (Unclear, (?)) in the remaining 20 (47.6%); in no studies it was considered as inadequate (No, ☒).

Blinding

In summary, 32 (76.1%) (Figure 3.5, Table 3.12) studies followed a double-blinded study design (Yes, ☑). Two of the remaining 10 studies[37,141] were open-label trials (no blinding after randomization, (No, ☒)) and the remaining eight did not publish enough details regarding blinding of participants, care providers and outcome assessors (Unclear, (?)).

Blinding of participants, care providers and outcome assessors was contemplated to be a possible source of bias in particularly the assessment of mucositis. Twenty-one studies reported outcome data for either mucositis presence, severity or duration, of which 17 described a double-blinded design and in the remaining 4 studies [48,93,103,113] it was unclear whether patients, care providers and outcome assessors were blinded to treatment allocation.

Incomplete outcome data

Fifteen (35.7%) (Figure 3.5, Table 3.12) of the 42 studies revealed no apparent loss to follow-up (Yes, ☑); the rest of the trials all reported the percentage dropout/withdrawal and specified the reasons for this (Yes, ☑), except for one,[94] who did not specify reasons, but in this case it was indicated that ITT analyses was done. Only 3 studies [104,111,133] did not indicate how loss to follow-

up was addressed in the analysis of data (Unclear, (?)). Incomplete outcome data in the long term did not introduce bias in any of the studies and was only assessed in studies reporting on long-term outcomes as such.

Selective reporting

The original protocol was not available for any of the included studies. However, no selective reporting (Figure 3.5, Table 3.12) was apparent for any of the included studies (Yes, ☑).

Other potential sources of bias

Other sources of bias (Figure 3.5, Table 3.12) were identified in 11 (26.2%) studies. Co-interventions introduced a high risk of bias in 6 studies.[29,59,130,133,144,162] In one cross-over design study[106] some of the patients were randomized more than once, which is considered to introduce substantial bias. Unclear risk of bias was introduced by data collection from medical charts after discharge with regard to prevelance of mucositis[97], discontinuing study due to unavailability of GLN supplement[142] and change in GLN product due to interruption of funding[112] and availability of new product.[110]

The reviewer acknowledges the fact that 13 trials[37,48,59,94,101,106,109,111-112,122,123,136,162] received some form of support from the pharmaceutical industry, which may represent a conflict of interest and as such introduce bias. Such funding was noted in the risk of bias tables (APPENDIX 6.7).

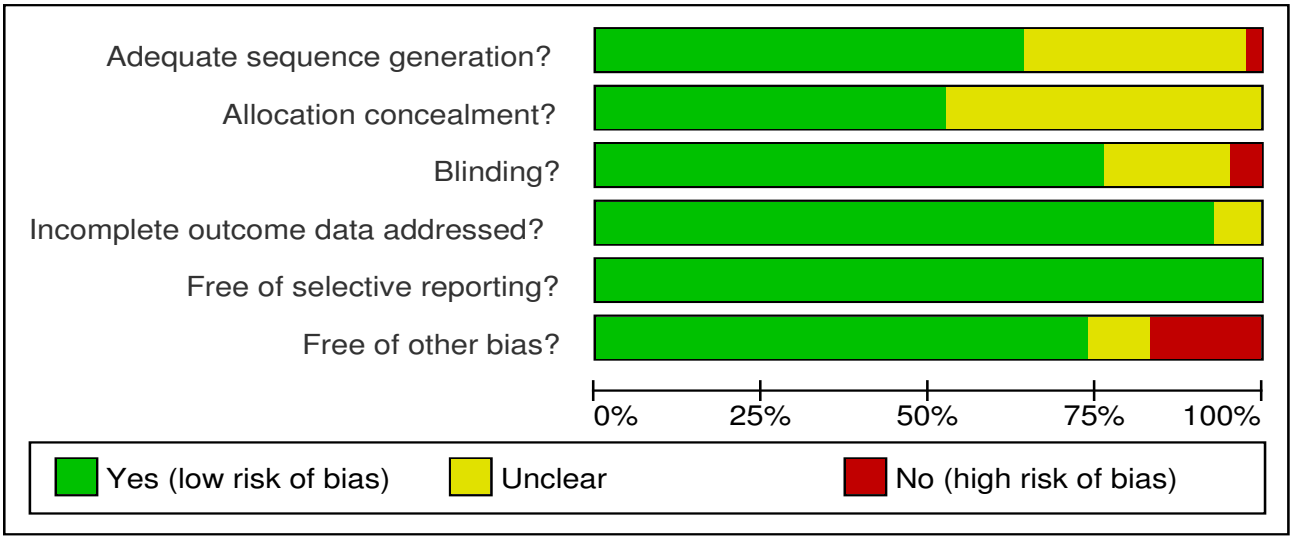


Figure 3.5: Methodological quality graph: Human studies (*N* =42). Source: RevMan5

Table 3.12: Methodological quality summary: Judgment of 6 methodological quality domains for each included human study

☑ Yes (Low risk of bias) (?) Unclear (Uncertain risk of bias) ☒ No (High risk of bias)	Adequate sequence generation?	Allocation concealment?	Blinding?	Incomplete outcome data addressed?	Free of selective reporting?	Free of other bias?	☑ Yes (Low risk of bias) (?) Unclear (Uncertain risk of bias) ☒ No (High risk of bias)	Adequate sequence generation?	Allocation concealment?	Blinding?	Incomplete outcome data addressed?	Free of selective reporting?	Free of other bias?
Anderson 1998 (100)	☑	(?)	☑	☑	☑	☑	Li 2009 (120)	☑	(?)	☑	☑	☑	☑
Berk 2008 (162)	☑	☑	☑	☑	☑	☒	Marton 2010 (164)	☑	☑	☑	☑	☑	☑
Blijlevens 2005 (109)	☑	☑	☑	☑	☑	☑	May 2002 (130)	☑	☑	☑	☑	☑	☒
Bozzetti 1997 (95)	☑	☑	☑	☑	☑	☑	O'Riordian 1994 (136)	(?)	(?)	☑	☑	☑	☑
Brown 1998 (140)	(?)	(?)	☑	☑	☑	☑	Oguz 2007 (141)	(?)	(?)	☒	☑	☑	☑
Canovas 2000 (153)	(?)	(?)	☑	☑	☑	☑	Okuno 1999 (105)	(?)	(?)	☑	☑	☑	☑
Cerchietti 2006 (101)	☑	☑	☑	☑	☑	☑	Peterson 2007 (94)	(?)	(?)	☑	☑	☑	☑
Choi 2007 (93)	☑	☑	(?)	☑	☑	☑	Piccirillo 2003 (110)	☑	☑	☑	☑	☑	(?)
Coghlin Dickson 2000 (97)	☑	☑	☑	☑	☑	(?)	Pytlik 2002a (98)	☑	☑	☑	☑	☑	☑
Da Gama Torres 2008 (123)	☑	☑	☑	☑	☑	☑	Scheid 2004 (134)	☑	☑	☑	☑	☑	☑
Daniele 2001 (102)	☑	☑	☑	☑	☑	☑	Scheltinga 1991 (143)	☑	☑	☑	☑	☑	☑
Decker Baumann 1999 (48)	(?)	(?)	(?)	☑	☑	☑	Schloerb 1993 (111)	☑	☑	☑	(?)	☑	☑
Erdem 2002 (144)	(?)	(?)	(?)	☑	☑	☒	Schloerb 1999 (112)	☑	☑	☑	☑	☑	(?)
Gianotti 2009 (163)	☑	☑	(?)	☑	☑	☑	Sornsuvit 2008 (113)	(?)	(?)	(?)	☑	☑	☑
Hallay 2002 (133)	☑	☑	☑	(?)	☑	☒	Stehle 1989 (59)	(?)	(?)	(?)	☑	☑	☒
Huang 2000 (103)	☑	(?)	(?)	☑	☑	☑	Strasser 2008 (122)	☑	☑	☑	☑	☑	☑
Jebb 1994 (104)	(?)	(?)	☑	(?)	☑	☑	Sykorova 2005 (145)	☑	☑	☑	☑	☑	☑
Jebb 1995 (96)	☑	☑	☑	☑	☑	☑	Van Zaanen 1994 (106)	☒	(?)	☑	☑	☑	☒
Jo 2006 (142)	☑	(?)	☑	☑	☑	(?)	Wu 2001 (29)	(?)	(?)	☑	☑	☑	☒
Klek 2005 (61)	(?)	(?)	(?)	☑	☑	☑	Yoshida 1998 (37)	(?)	(?)	☒	☑	☑	☑
Kozelsky 2003 (119)	☑	(?)	☑	☑	☑	☑	Ziegler 1992 (60)	☑	☑	☑	☑	☑	☑

Unpublished correspondence

Letters were sent via e-mail to request additional information on methodology, group sizes and missing data. Seven authors replied, providing additional information not published in the original reports. In 5 of these studies [60,101,133,110,145] their responses changed the judgment from "Unclear" to "Yes".

3.2.2 Animal studies

Details of the judgment of risk of bias across six methodological quality domains for each included animal study are presented in APPENDIX 6.7.

Allocation concealment

Not one of the animal studies made mention of the concealment of allocation (Figure 3.6, Table 3.13). Allocation is considered as concealed if the investigator responsible for the tumour implantation and treatment and decisions regarding the care of the experimental animals has no foreknowledge of the experimental group to which an animal belongs. Allocation concealment might be achieved by having the experimental intervention administered by an independent investigator, or by having an independent investigator prepare the drug individually and label it for each animal according to the randomization schedule.[161] A 100% judgment of "Unclear" risk of bias ((?)) has been made for all animal studies in this regard.

Sequence generation

All but 2 (11.1%) studies[71,72] mentioned that animals were randomly allocated or randomly divided into groups. The method of randomization was not described in any of the studies. Acceptable methods of randomization of animals to study groups include coin tossing and computer-generated randomization schedules. Picking animals 'at random' from a cage is unlikely to provide adequate randomization.[161] However, a judgment of "Yes"(☑), was passed for the 16 studies (88.9%) which explicitly stated the 'randomization' of animals in the published report or through correspondence. It was also noted that most good preclinical investigators[165] publish details of their animal models and procedures in an initial article and only refer to these references in subsequent publications for an extensive explanation of their methods. These earlier references, which were retrieved to aid in the judgment of risk of bias in included studies, were applicable and were indicated as such in the risk of bias tables.[3-4,77-79]

Blinding

The rats will probably be blinded to what they are receiving (Suzanne Klimberg, Klimbergsuzanne@ uams.edu, 11 May 2009).[3] The Collaborative Approach to Meta-analysis and Review of Animal Data from Experimental Stroke (CAMARADES) checklist was designed to identify flaws in individual preclinical studies in a different field of animal research,[165] although it is criticized by some as an "unproven gold standard for assessing predictive usefulness of preclinical investigations".[165] The CAMARADES list requires 10 items, including 2 items concerning proper blinding (caregiver and outcome assessor).[165] In addition the Stroke Therapy Academic Industry Roundtable (STAIR) participants established guidelines for good laboratory practice, which require that at least the assessment of outcome, tumour weight/volume in this case is blinded.[161]

Correspondence from Vickie Baracos (vickieb@cancerboard.ab.ca)[71] explains in detail how the care providers were blinded in one study[71]. However, another author, Suzanne Klimberg (Klimbergsuzanne@uams.edu, 11 May 2009),[3] questioned whether it is possible for investigators to be blinded to what they are gavaging the rats with. Yihong Kaufmann (KaufmannYihong@ uams.edu, 8 Aug 2009)[5] communicated that all groups were usually identified after randomization. The studies from the latter two authors were judged as no-blinding (☒).[3-5,77-79]

Nine studies (50%) indicated that the animals were pair-fed,[3-5,77-79,86-87,90] but only one author[4] published the details of how pair feeding were achieved ("Pair feeding is necessary to ensure similar chow intake among animals, which followed the idea: more chow was given to the animal that ate less, whereas less chow was given to the animal that ate more").[4] It is unclear ((?)) how pair feeding will affect blinding of the care provider or the introduction of bias.

In summary none of the animal studies was double-blinded. Six (33.3%) were rated as no-blinding (No, ☒)[3-5,77-79] and the remaining 12 (66.6%) unclear ((?)), since none of the studies reported whether assessment of tumour weight/volume was blinded.

Incomplete outcome data

Fourteen (77.8%) (Figure 3.6, Table 3.13) of the animal studies had no apparent dropouts (Yes, ☑). Three studies[72-73,78] reported some percentage of mortality and explored possible reasons for this, but none of them indicated how this loss to follow-up was addressed in the analysis of outcome measures (Unclear, (?)). In one study[74] the number of rats has dropped from time of randomization to reporting of outcomes, but no reasons are given for this – the numbers of rats

per group are just smaller (No, ☒). Good laboratory practice guidelines require that all randomized animals should be accounted for in the data presented.[161] Some animals may, for very good reasons, be excluded from analysis, but the circumstances under which these exclusions will occur should be determined in advance, and any exclusion should occur without knowledge of the experimental group to which the animal belongs. The criteria for exclusion and the number of animals excluded should be reported.[161]

Selective reporting

The original protocol was not available for any of the included studies. However, no selective reporting (Figure 3.6, Table 3.13) was apparent for any of the included studies (Yes, ☑).

Other potential sources of bias

High risk of bias (No, ☒) was introduced (Figure 3.6, Table 3.13) by non-specific groups sizes (n=≥7) in one study,[80] since the formula used for calculation of standard deviation (SD)) from standard error of the mean (SEM) requires the actual group size.

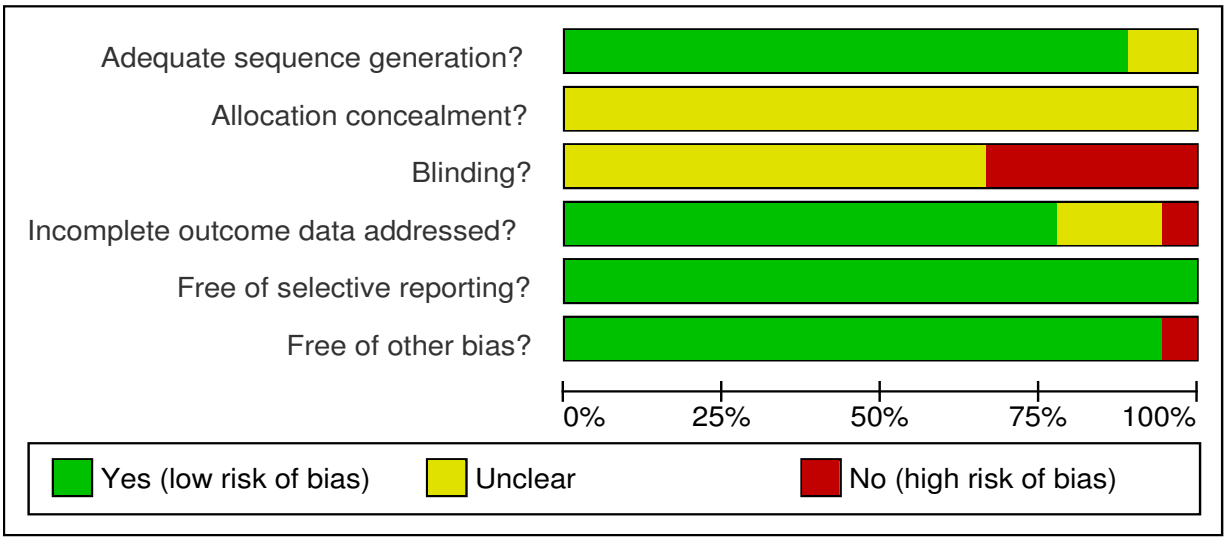


Figure 3.6: Methodological quality graph: Animal studies (*N*=18). *Source: RevMan5*

Table 3.13: Methodological quality summary: Judgment of 6 methodological quality domains for each individual included animal study

☑ Yes (Low risk of bias) (?)Unclear (Uncertain risk of bias) ☒ No (High risk of bias) **PF** (Pair-fed)	Adequate sequence generation?	Allocation concealment?	Blinding?	Incomplete outcome data addressed?	Free of selective reporting?	Free of other bias?
Austgen 1992 (74)	☑	(?)	(?)	☒	☑	☑
Bartlett 1995 (75)	☑	(?)	(?)	☑	☑	☑
Fahr 1994 (86)	☑	(?)	(?)PF	☑	☑	☑
Kaibara 1994 (76)	☑	(?)	(?)	☑	☑	☑
Kaufmann 2003 (3)	☑	(?)	☒PF	☑	☑	☑
Kaufmann 2007 (4)	☑	(?)	☒PF	☑	☑	☑
Kaufmann 2008a (5)	☑	(?)	☒PF	☑	☑	☑
Klimberg 1992 (77)	☑	(?)	☒PF	☑	☑	☑
Klimberg 1992a (78)	☑	(?)	☒PF	(?)	☑	☑
Klimberg 1996a (79)	☑	(?)	☒PF	☑	☑	☑
Robinson 1999 (80)	☑	(?)	(?)	☑	☑	☒
Rouse 1995 (87)	☑	(?)	(?)PF	☑	☑	☑
Rubio 1998(90)	☑	(?)	(?)PF	☑	☑	☑
Shewchuk 1997 (81)	☑	(?)	(?)	☑	☑	☑
Xue 2007 (71)	(?)	(?)	(?)	☑	☑	☑
Xue 2008 (72)	(?)	(?)	(?)	(?)	☑	☑
Xue 2009(73)	☑	(?)	(?)	(?)	☑	☑
Yoshida 1995 (82)	☑	(?)	(?)	☑	☑	☑

Unpublished correspondence

Letters were sent via e-mail to request additional information regarding methodology, group sizes and missing data. Three authors replied.[3,5,71] Unpublished data changed the judgment from "Unclear" to "No" in 6 studies of the corresponding authors,[3-5,77-79] but "Unclear" remained as such for one, since the corresponding author did not clarify whether assessment of tumour weight/volume was blinded.[71]

3.3 EFFECTS OF INTERVENTIONS

3.3.1 Primary Outcomes (Comparison 1 Humans: GLN-supplemented vs. Control)

3.3.1.1 Mortality

RevMan5 Outcome 1.1: Mortality during intervention – Number of deaths during intervention

Sixteen studies,[60,104,106,109,111-112,122,130,133,140-142,144,162-164] including 1521 participants, reported dichotomous data on mortality during intervention suitable for meta-analysis (Figure 3.7).

Four of these studies were judged to have a low risk of bias[60,109,122,164] as summarized across all 6 methodological quality domains (Table 3.12). A moderate risk of bias was introduced in 6 studies[104,111-112,140,142,163] due to several unclear aspects of methodological design. A high risk of bias were introduced in the remaining 6 studies, mainly due to the use of co-interventions [130,133,144,162] in four studies and no-blinding in one.[141] One study had a problematic cross-over design, where some of the patients was randomized more than twice, it was unclear how this was addressed in the analysis of mortality.[106]

The cancer diagnoses of participants included in respective studies varied from haematological malignancies[60,106,109,140] to gastrointestinal malignancies as previously defined,[104,144,163] of which some included specifically colorectal cancer,[141] periampullary tumours[142] and esophageal cancer[133,164] and the rest included mixed types as previously defined.[111-112,122,130,162]

None of the individual studies reported a significant difference ($P<0.05$) in mortality outcome between the GLN-supplemented and control groups during the intervention. Across all studies the mortality rate in the GLN group was 54/773 (6.9%) and 54/748 (7.2 %) in the control group.

Combination of the results, using a random effects meta-analysis model, for mortality data of 1521 participants in the 16 studies synthesized a Risk Ratio (RR) value of 0.96 (95% Confidence Interval (CI), 0.67 to 1.37, $P=0.81$; Figure 3.7). The 95% CI is imprecise and the P-value ($P=0.81$) indicates that at this stage the result obtained is not significant (NS) and that there is not enough knowledge about the effect of GLN supplementation on mortality.

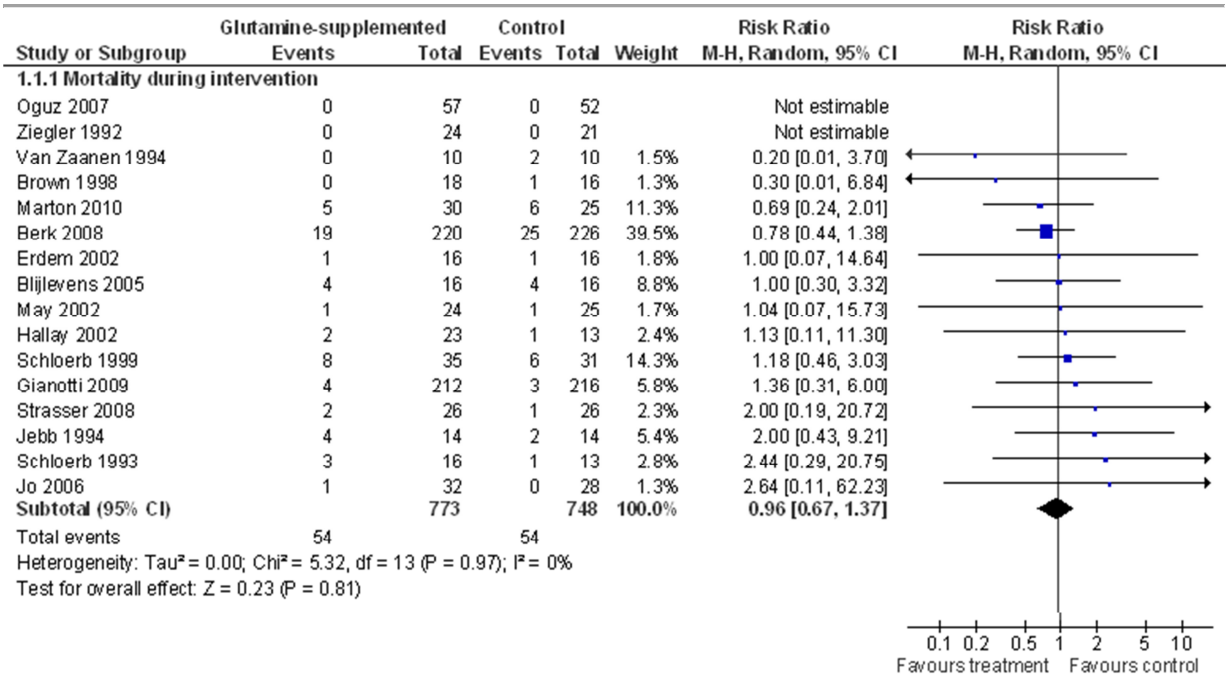


Figure 3.7: Forest plot of the effect of GLN supplementation *versus* controls on mortality during intervention

A sensitivity analysis excluding the 5 studies with a high risk of bias [106,130,133,144,162] did not change the overall result and conclusions (1.15 RR, 95% CI 0.71 to 1.88, P=0.57). Changing the method of analysis to a fixed-effect model also did not alter the meta-analysis result significantly (0.95 RR, 0.67 to 1.35, P=0.79). Sensitivity was further explored by changing the effect measure to Odds Ratio (OR). Neither did these results (0.95 OR, 95% CI, 0.63 to 1.43, P=0.81) change the overall results and therefore the conclusions remain and should be regarded with a high degree of certainty based on the available evidence.

The Chi-squared (Chi^2) test result (Chi^2 = 5.32, df=13, P=0.97, Figure 3.7) and I^2=0% indicate no evidence of statistical heterogeneity beyond chance. The funnel plot (Figure 3.8) of the effect estimates from the individual studies against a measure of each study's size/precision presents an asymmetrical appearance with the funnel itself almost in proportion with a concentration on the right side. This appearance may suggest the presence of bias and can probably be explained by small-study effects (selection bias, poor methodological quality, true heterogeneity, artefactual or chance)[1] rather than publication bias, possibly overestimating the effects of GLN supplementation and the results should be interpreted with caution.

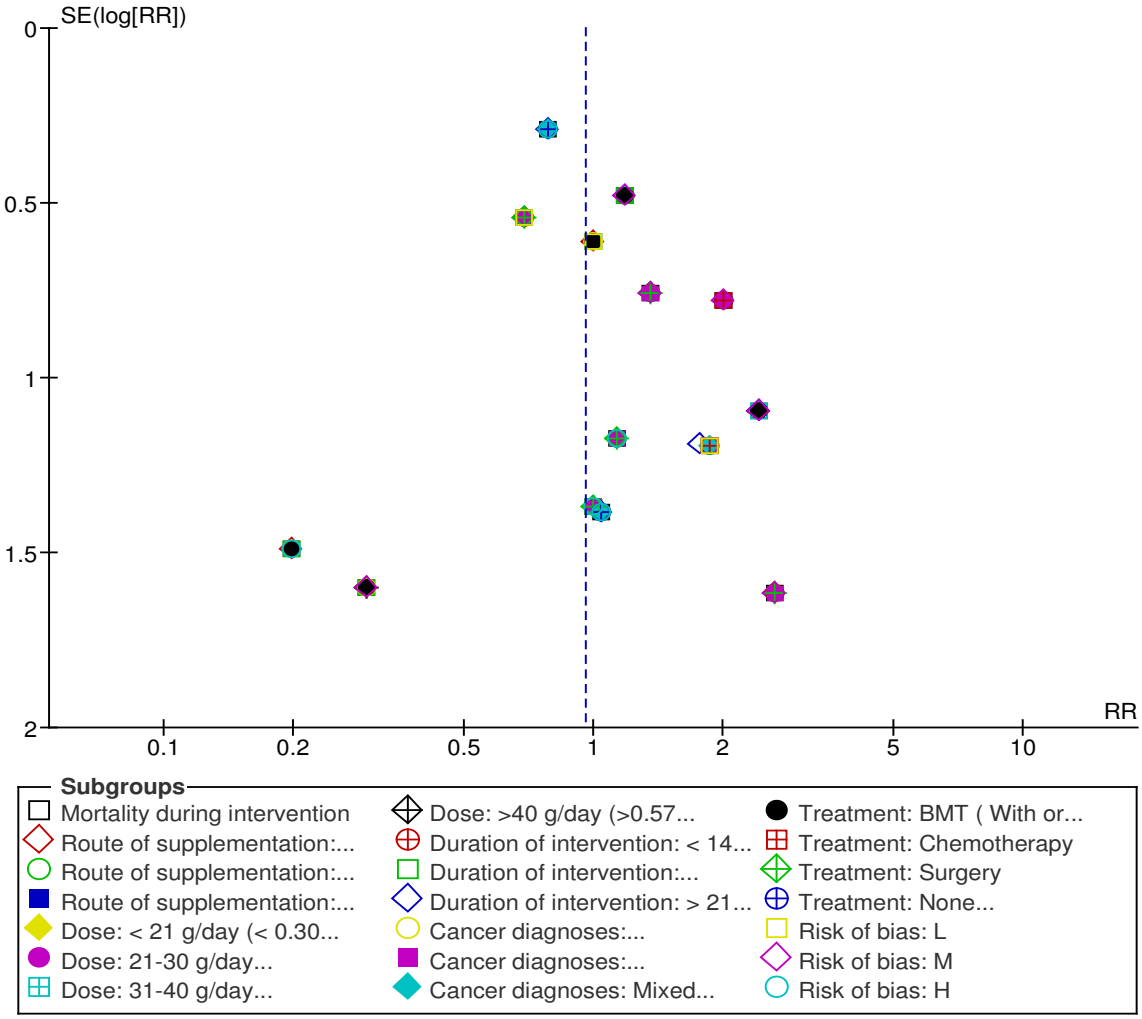


Figure 3.8: Funnel plot of studies using GLN supplementation (Mortality)

Subgroup analysis (APPENDIX 6.10) was undertaken to investigate possible sources of clinical and methodological heterogeneity and its influence on the RR effect estimate of GLN supplementation (as pre-defined in section 2.4.8). On visual inspection, it is clear that the 95% confidence intervals of all 16 individual studies for mortality overlap to a large degree (Figure 3.7). Consequently, as expected, the subgroup analysis could not identify groups with a significant difference in effect, confirming that GLN supplementation in these 16 studies does not present strong evidence that GLN supplementation has an effect on mortality during intervention, irrespective of route, dose, and duration of intervention, cancer diagnoses and treatment or risk of bias.

3.3.1.2 Survival at Follow-up

RevMan5 Outcome 1.2 Survival at follow-up

Seven studies reported on survival at follow-up from 3 months to 3 years, of which 6 studies (Figure 3.9), including 260 participants, were suitable for meta-analysis. Three studies reported survival up to day 100[60,123,] and three beyond 100 days (1 year,[106] 2 year[97-98] or 3 year[145] survival).

One study was judged to introduce a high risk of bias[106] due to a problematic cross-over design and unclear aspects of sequence generation and allocation concealment. The remaining 5 were judged to be of low risk of bias (Table 3.12).[60,97-98,123,145]

Cancer diagnoses of participants were haematological malignancies in all but one study,[110] which also included multiple sclerosis and solid tumours in addition to several haematological malignancies.

Da Gama Torres 2008[123] reported a significantly higher **survival** rate for the GLN group:

- Day 100: (23/27 (85%) in GLN vs. 16/26 (62%) in Control, P=0.05)[123]
- Day 180: (20/27 (74%) in GLN vs. 12/26 (46%) in Control, P=0.03)[123]

In contrast, Pytlik 2002a[98] reported a significantly worse outcome for the GLN group:

- 1 Year **mortality**: 6/21 (28.5%) in GLN vs. 1/19 (5%) in Control, P=0.05)[98]

One study did not present any actual data, but reported a "suggestion of improved long-term survival associated with GLN supplementation (P=0.0572)"[112] in a study population with haematological malignancies and solid tumours. This study was judged to be of moderate risk primarily because there was a change in the TPN GLN product used during the study due to interruption of funding. Data of four studies (Table 3.14) indicated no association with improved survival in either GLN-supplemented or control groups.

Table 3.14: Summary of results of four studies reporting no significant difference between GLN-supplemented groups *versus* their controls regarding survival at follow-up

STUDY ID (Reference number)	DETAIL	GLN GROUP	CONTROL GROUP	COMMENTS
Coghlin Dickson 2000 (97)	2 year Kaplan Meier estimates	19/29 (70%) (95% CI, 48 to 92)	16/29 (45%) (95% CI, 27 to 63)	P=0.31
Sykorova 2005 (145)	3 year survival	15/24 (63%)	17/20 (85 %)	P=0.17
Van Zaanen 1994 (106)	1 year survival	6/10 (60%)	6/10 (60%)	-
Ziegler 1992 (60)	1 year survival	20/24 (83%)	18/21 (86%)	-

The summary survival rate in the GLN-supplemented group is 98/135 (72.5%) vs. 91/125 (72.8%) in the controls.

Pooling the results from all 6 studies with a random effects meta-analysis model revealed an RR value of 0.97 (95% CI, 0.78 to 1.19, P=0.75, Figure 3.9), which was not significant. The 95% CI is not well defined, indicating some degree of uncertainty as to the precise effect of GLN supplementation. At this point there is not enough evidence to either support or refute that GLN supplementation has an effect on survival beyond 100 days up to 3 years compared to controls.

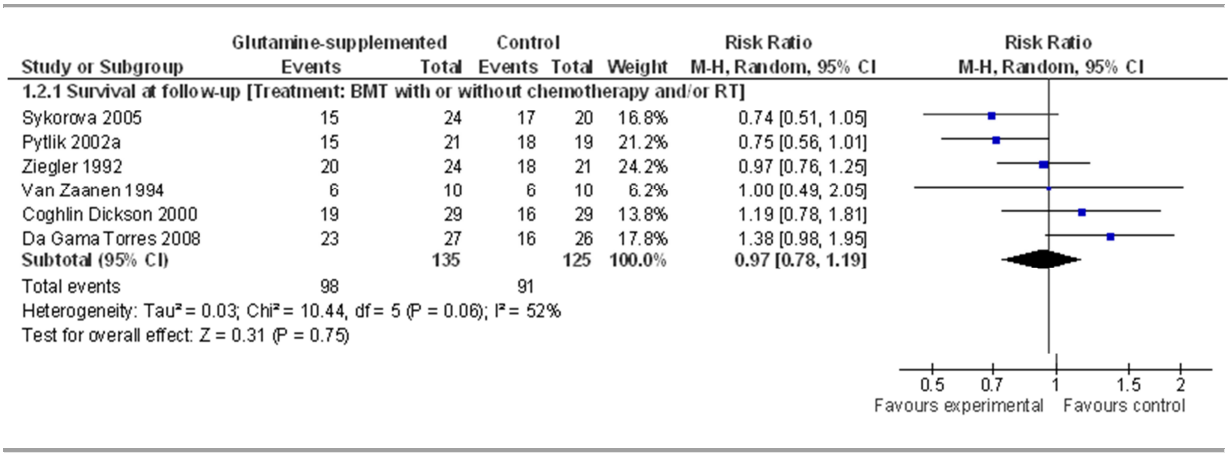


Figure 3.9: Forest plot of the effect of GLN supplementation *versus* controls on survival at follow-up

Sensitivity analysis excluding the smallest study,[106] also of high risk of bias in this case, did not alter the results (0.97 RR, 95% CI, 0.76 to 1.22 P=0.77). Changing the method of analysis to a fixed model did not alter overall conclusion (0.99 RR, 95% CI, 0.86 to 1.15, P=0.91), although it did narrow the CI. Changing the effect measure to OR (0.90, 95% CI, 0.38 to 2.16, P=0.81) also did not alter results or conclusions. The initial results can therefore be regarded with a high degree of certainty.

The Chi^2 test results (Chi^2=10.44, df=5, P=0.06, Figure 3.9) indicate significant statistical (P<0.1) heterogeneity. In addition the I^2 of 52% suggests substantial inconsistency[1] in effect estimates. Visual inspection of the forest plot (Figure 3.9) indicates that there is poor overlap between the 95% CI of individual studies, in particular with the complete opposite direction of effect in 2 studies.[98,145] All 6 trials recruited patients with mainly haematological malignancies receiving BMT

with/without chemotherapy and/or RT. Results of subsequent subgroup analysis (APPENDIX 6.10) could not explain the heterogeneity observed in effect of GLN on survival between different studies. Neither route, dose, duration of intervention, period of follow-up or risk of bias subgroup analysis changed the overall results and conclusions and therefore the result remains inconclusive.

3.3.1.3 Length of Stay (LOS)

RevMan5 Outcome 1.3: Length of hospital stay - Mean duration of hospital stay (days) from admission/day 0 to discharge

Seventeen studies reported on length of hospital stay, but only 10 (*N* =816, Figure 3.10) were suitable for meta-analysis.[60,96,98,109,111-113,123,141,163] The data of the remaining 7 studies were reported as mean/median (range) days LOS and is discussed.[61,97,106,110,133-134,142]

Six of the studies included in the meta-analyses were judged to introduce a low risk of bias [60,96,98,109,123,134] across all methodological quality domains (Table 3.12). Only one study in the meta-analysis[141] and two in the narrative review[106,133] were judged to introduce a high risk of bias, mainly due to the use of co-interventions[133] and problematic cross-over design[106] and no blinding.[141] The remaining 8 studies introduced a moderate risk of bias due to unclear aspects of sequence generation,[113,119] allocation concealment[61,113,119,142] and/or blinding,[61,113,119,163] unclear analyses of incomplete outcome data,[111,133] change in GLN product,[110,112] spurious data collection[97] and the discontinuation of one study.[142]

Cancer diagnoses of participants in respective studies varied from haematological malignancies[60,96,109,113,123] to gastrointestinal cancer,[163] colorectal cancer[141] and 3 studies included mainly haematological malignancies mixed with other cancer types as previously defined.[98,111-112]

Two studies reported a significantly decreased <u>postoperative LOS in GLN groups compared to controls</u>:

- Mean (SEM) days: (GLN: 6.0 (1.2) vs. Control: 8.3 (1.1), P<0.001)[141] and
- Mean(range) days: (GLN: 14.8 (8-41) vs. Control: 16.4 (9-45), no P-value).[61]

In addition two studies reported a significantly reduced <u>LOS (from BMT until discharge) in GLN groups as compared to controls:</u>

- Mean (SEM) days: (GLN: 26.9 (1.3) vs. Control: 32.7 (2.1), P<0.05)[111] and
- Mean (SEM) days: (GLN: 29 (1) vs. Control: 36 (2), P=0.017).[60]

Twelve studies (Table 3.15) reported similar LOS results for both GLN-supplemented and control groups. Only one study reported a significant increase in the LOS after BMT of the GLN-supplemented group, which drastically skewed the results and probably introduced high levels of heterogeneity:

- Mean (SD) days: (GLN: 13.8 (3.1) vs Control: 11.8 (2.2), P=0.04)[98]

Table 3.15: Summary of results of twelve studies reporting similar results for GLN-supplemented groups *versus* their controls regarding length of stay

STUDY ID (Reference number)	DETAIL	GLN GROUP	CONTROL GROUP	COMMENTS
Blijlevens 2005 (109) Unpublished data (Blijlevens NMA e-mail correspondence, 14 Dec 2009)	LOS Mean (SD) days	36.19 (7.89)	41.19 (12.36)	P=0.2
Da Gama Torres 2008 (123) Unpublished data (Torres H e-mail correspondence 11 Oct 2009)	LOS Mean (SD) days	39.4 (12.02)	38.35 (11.59)	P=0.736
Coghlin Dickson 2000 (97)	LOS Median (range) days	21 (4-41)	19 (5-53)	P=0.97
Gianotti 2009 (163)	Length of postoperative stay Mean (SD) days	10.2 (4.8)	9.9 (3.9)	P=0.90
Jo 2006 (142)	Postoperative hospital stay Median (range) days	14.0 (9-54)	14.5 (9-41)	P=0.197
Piccirillo 2003 (110)	Days of hospitalization Median (range) days	28 (23-70)	27 (23-39)	P=0.15
Scheid 2004 (134)	LOS Median(range) days	30.5 (15-570)	34.5 (20-47)	P=0.58
Jebb 1995 (96)	Hospital stay after BMT Mean (SD) days	25.6 (2.2)	28.3 (5.5)	no P-value
Schloerb 1999 (112)	Hospital days Mean (SD)	20.5 (15.4)	26.7 (19.3)	no P-value
Sornsuvit 2008 (113)	LOS Mean (SD) days	36 (13.0)	30.8 (3.6)	no P-value
Hallay 2002 (133)	Clinical stay Mean (no SD) days	25.2	22.8	no P-value
Van Zaanen 1994 (106)	LOS Median (range) days	39 (24-81)	26 (24-36)	no P-value

Overall the mean difference (MD) for LOS was -1.68 days (95% CI, -3.67 to 0.31, P=0.10, Figure 3.10), using a random effects meta-analysis to pool the results of the 10 suitable studies. The 95% CI is not precise and furthermore the null value is included and therefore the possibility that GLN supplementation has no effect on LOS whatsoever compared to the control group cannot be excluded. More evidence is needed to clarify this.

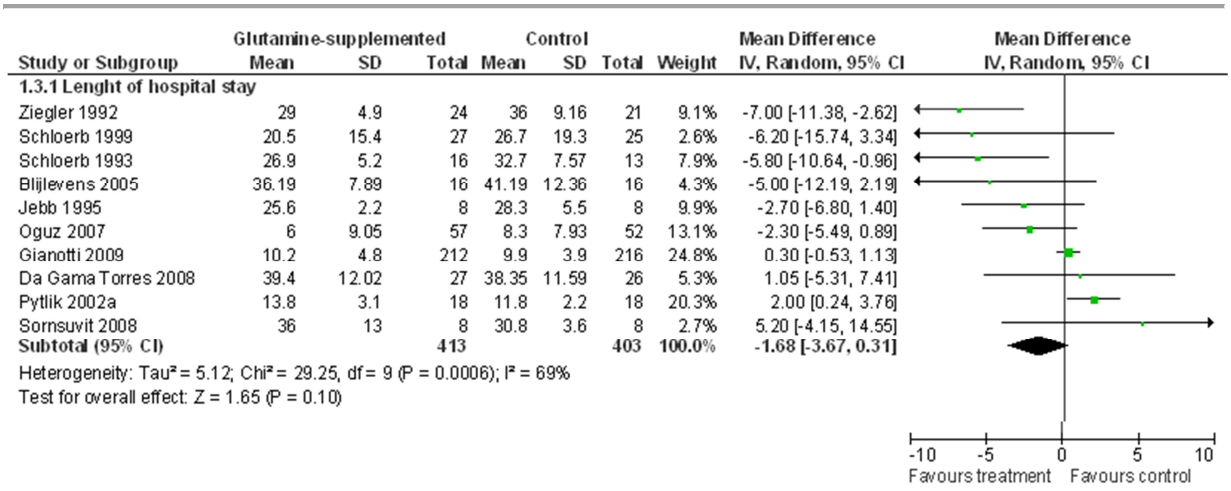


Figure 3.10: Forest plot of effect of GLN supplementation *versus* controls on length of stay

Sensitivity analysis revealed that changing the random analysis model to a fixed analysis model diminished the trend (-1.68 days, P=0.10) towards shortened LOS with a more precise 95% CI (0.00 MD, 95% CI, -0.69 to 0.69, P=0.99), still inclusive of the null value. Changing the effect measure from MD to Standardized Mean Difference (Std MD) (Std MD -0.20, 95% CI, -0.49 to 0.09, P=0.18) gave the same overall impression, indicating only a small effect (Std MD <0.20) as a rule of thumb.[1] The sensitivity analysis of especially a fixed model of analysis challenges the initial results, and must therefore be interpreted with caution.

The Chi^2 test results (Chi^2 =29.25, df=9, P=0.0006, Figure 3.13) provide evidence of statistical heterogeneity in intervention effects beyond chance. The variability in results can be quantified as considerable based on the I^2 test (I^2 =69%). In addition visual inspection of the funnel plot (Figure 3.11) portrays an almost inverted funnel, but with a higher concentration of studies on the left side of the plot. This may be explained by presence of publication bias (since only 10 of the 17 RCTs reporting on LOS published data suitable for meta-analysis) or other bias (such as study size effects and poor methodological design of small studies), which could account for the apparent trend observed towards reduced LOS. However, the appearance of the forest plot (Figure 3.10) necessitates a subgroup analysis to investigate the observed heterogeneity and inconsistency in results.

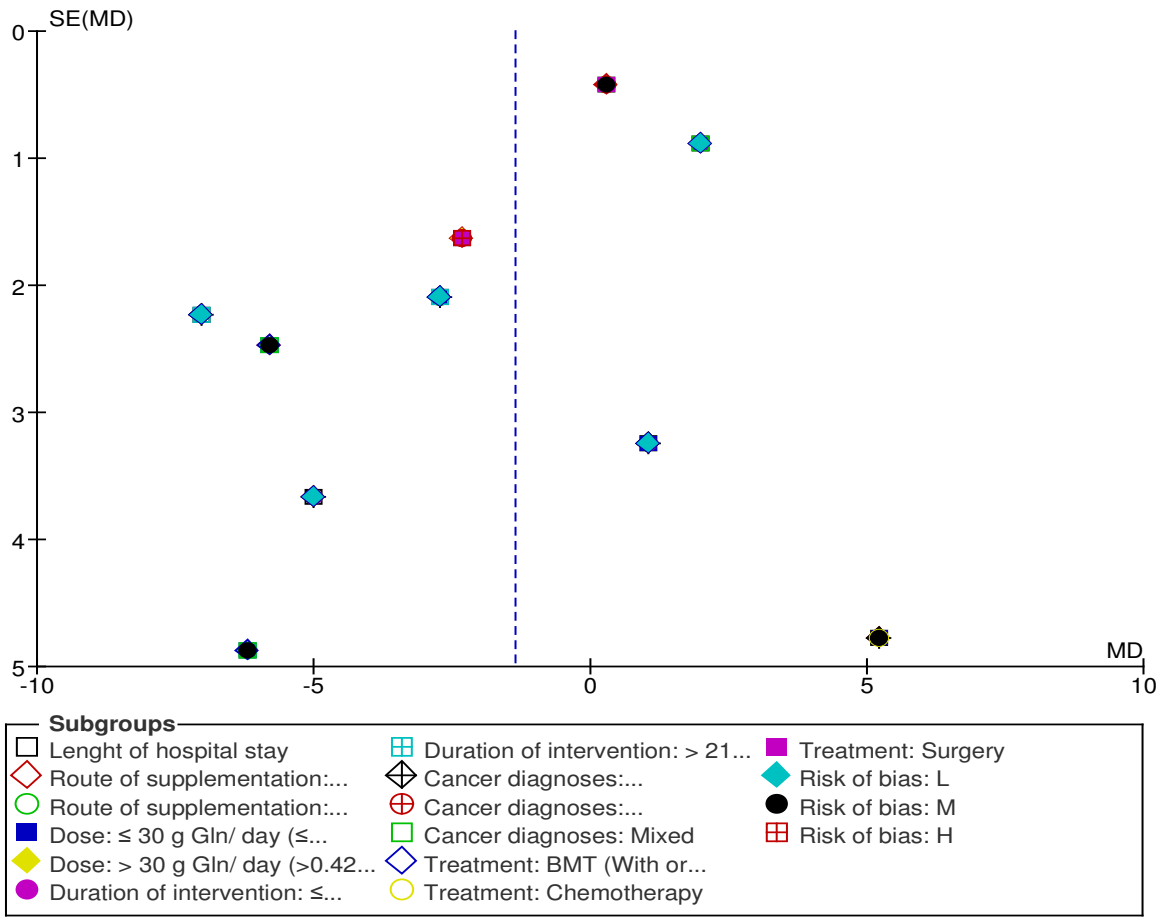


Figure 3.11: Funnel plot of studies using GLN supplementation (Length of stay)

Analysis of predefined subgroups (APPENDIX 6.10) did provide additional information regarding the effect of GLN supplementation in certain circumstances and might explain some of the heterogeneity observed in effects of individual studies. The 4 studies[60,109,111,141] which used a higher GLN dose (>30 g/day) and the 4 studies[60,96,111-112] with a longer duration of intervention (>21 days) had a statistically significant P-value, rejecting the null hypothesis for LOS (-4.51 MD, 95% CI -6.85 to -2.16, P =0.0002 and -5.09 MD, 95% CI -7.55 to -2.63, P<0.0001 respectively) compared to the control groups. However, there still remains a high degree of overlap between the 95% CI of the subgroups, indicating that the change in effect between subgroups might not be significant. The subgroup analysis for route of supplementation, cancer diagnoses and treatment or risk of bias did not challenge the original conclusions.

3.3.1.4 Body Weight Change

RevMan5 Outcome 1.4: Nutritional status: Body weight change – Difference in mean change in body weight (kg) from baseline to end of study between groups

Fourteen studies reported on body weight change of which continuous data was available or estimated for 8 (*N*=224).[48,101,111-113,130,143-144] Data from 6 studies was not suitable for meta-analysis, but will be discussed.[98,103,106,122,140,162]

Four of the studies were judged to be of low risk of bias (Table 3.12).[98,101,122,143] Six studies introduced a moderate risk of bias due to vague aspects of sequence generation,[48,113,140] allocation concealment,[48,103,106,113,140] blinding,[48,103,113] addressing of incomplete outcomes in analyses[111] or a change in the GLN product used.[112] Four studies either used co-interventions [130,144,162] or problematic cross-over design,[106] introducing a high risk of bias.

The cancer diagnoses of patients were very variable for this outcome, including head and neck cancer,[101,103] colorectal cancer[48] and other gastrointestinal malignancies,[144] solid tumours of mixed sites,[130,162] haematological malignancies[104,106,113,143,] as previously defined, also mixed with solid cancers of mixed sites[98,111-112] and mixed types in general as previously defined.[122]

Three studies reported significantly less weight loss or more weight gain in the GLN group as compared to controls:

- <u>Weight gain at 4 weeks of supplementation</u>
  Mean (SEM) kg
  (GLN 0.95 (0.66) vs. Control -0.26 (0.78), P<0.05)[130]
  <u>Weight gain at 24 weeks of supplementation</u>
  Mean (SEM) kg
  (GLN 2.27 (1.17), P=0.24, "Significantly different from zero, P=0.06" and vs. Control 0.27 (1.39), P=0.84)[130]
- <u>Weight change from baseline to hospital discharge</u>
  Mean (SD) kg
  (GLN -0.42 (2.9) vs. Control -3.3 (2.0), P <0.05)[113]
- <u>Gain in weight per treatment cycle</u>
  Median(range) kg
  (GLN 4.4 (1.5-8.2) vs. Control 1.3 (-2 to 7.5), P=0.05)[106]

In one study the control group had significantly less weight loss compared to the GLN-supplemented group:

- Difference between post-TPN and initial body weight measurements

  Mean (SEM) kg

  (GLN -0.7 (1.0) vs. Control +1.8 (0.9), P<0.05)[143]

  Change in body weight over 8 days preceding the post-TPN determination,

  Mean (SEM) kg

  (GLN-5.0 (1.1) vs. Control -2.8 (0.9), no P-value)[143]

The remaining 10 studies (Table 3.16) indicated no significant difference between the change in body weight between the study and control groups.

Table 3.16: Summary of results of ten studies reporting no significan difference between GLN-supplemented groups *versus* their controls regarding body weight change

STUDY ID (Reference number)	DETAIL	GLN GROUP	CONTROL GROUP	COMMENTS
Cerchietti 2006 [101]	Body weight change from baseline to after chemo-radiation therapy (CRT) Mean (SD) kg	-3.3 (2.6)	-5.77 (2.2)	P=NS
Decker-Baumann 1999 (48)	Body weight change calculated from weight before and after final chemotherapy cycle Mean (SD) kg	0.2 (3.567), P=0.9553	0.3 (6.478), P=0.9631	-
Erdem 2002 (144)	Body weight change calculated from preoperative day -1 to postoperative day +10 Mean (SD) kg	-0.3 (4.115), P=0.9419	0.39 (4.474), P=0.9305	-
Schloerb 1993 (111)	Change in weight Mean (SEM) kg	0.2 (1.0)	1.4 (0.8)	no P-value
Schloerb 1999 (112)	Weight change combined for all sub-groups Mean (SD) kg	1.2 (16.5)	-4.8 (10.7)	no P-value
Berk 2008 (162)	Change in weight Mean (SEM) %	2.23 (0.48)	2.47 (0.56)	P=0.78
Brown 1998 (140)	Weight loss over the transplant period Median (range) %	6.7 (4.3-8.3)	7.4 (4-10),	P=0.72
Huang 2000 (103)	Body weight change Mean kg	-1.7	-1.3	P=0.8070
Pytlik 2002a (98)	"There were no differences among the GLN and placebo groups in terms of changes in body weight…"			
Strasser 2008 (122)	"There was no difference in body weight changes during the study period between GLN and placebo patients."			

A random effects meta-analysis model resulted in a MD of 0.63 (95%CI, -0.99 to 2.25, P=0.45, Figure 3.12). The result is not significant and in addition the 95% CI is imprecise, also including the null value, indicating uncertainty regarding the effect of GLN supplementation on body weight of subjects, and the possibility of no effect whatsoever cannot be excluded. There is insufficient

evidence to either support or refute GLN as more or less effective than control in preventing a significant weight loss in cancer patients.

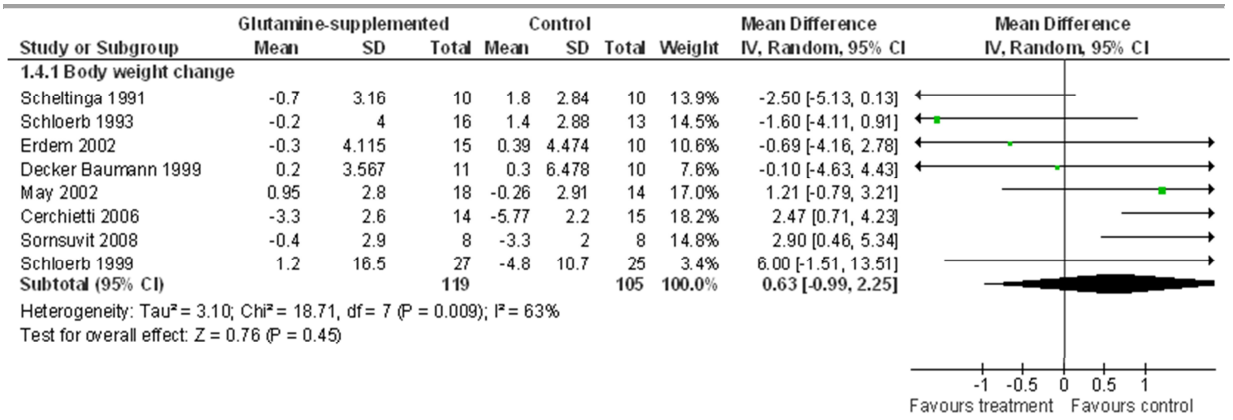


Figure 3.12: Forest plot of effect of GLN supplementation *versus* controls on body weight change

Sensitivity analysis was undertaken by changing the analysis model to fixed (0.85 MD, 95% CI, -0.06 to 1.77, P=0.07). The result remains not significant, but the 95% CI narrowed to some extent. However, it still includes the null value and the possibility of no effect. Changing the effect measure to Standardized MD (0.19, 95% CI, -0.23 to 0.61, P=0.38) did not change the overall result and conclusions. In addition the Std MD value achieved indicates a small effect (<0.20) as a rule of thumb.[1]

Considerable (I^2 =63%) and significant heterogeneity (Chi^2 =18.71, df=7, P=0.009, Figure 3.12) exists. This heterogeneity is evident on visual inspection of 95% CIs of individual studies on the forest plot (Figure 3.12). In order to explore this heterogeneity a subgroup analysis (APPENDIX 6.10) was carried out as predefined. The subgroup of studies which used moderate doses between 21-30 g GLN/day (0.30-0.42 g/kg/day), reached statistical significance in favor of the GLN group (2.49 MD, 95% CI, 1.15 to 3.83, P=0.0003, APPENDIX 6.10).[48,101,112-113] In contrast the subgroup with the highest dose of 31-40 g GLN/day (0.44–0.57 g/kg/day) significantly favored the control group (-2.03 MD, 95% CI, -3.84 to -0.21, P=0.03, APPENDIX 6.10).[111,143] Studies with a shorter duration of intervention (≤21 days) followed the same trend, reaching a significant result in favor of the GLN-supplemented group (1.80 MD, 95% CI, 0.25 to 3.35, P=0.02, APPENDIX 6.10)[48,101,113,144] as opposed to studies with a longer duration of supplementation >21 days (-0.26 MD, 95% CI, -2.75 to 2.23, P=0.84, APPENDIX 6.10).[111-

[112,130,143] There are not enough studies per subgroup to provide meaningful data with regard to cancer diagnoses and most treatment regime subgroups. However, the 3 studies in the subgroup receiving chemotherapy and/or RT also reached a significant result in favor of the GLN-supplemented group as opposed to controls (2.37 MD, 95% CI, 1.01 to 3.73, P=0.0006, APPENDIX 6.10).[48,101,113] Subgroups with regard to route of supplementation and risk of bias could not provide additional information.

3.3.1.5 Clinical Infection

RevMan5 Outcome 1.5: Number of patients who developed clinical infection during intervention

Twelve studies (*N*=906, Figure 3.13) reported dichotomous data on the number of patients who developed clinical infection,[60-61,98,109,111-113,123,136,141,143,163] another 4 reported on clinical infection as number of events[106] or published comments[101,133,164] suitable for descriptive analysis only.

Seven studies were judged to be of low risk of bias [60,98,101,109,123,143,164] and 6 had unclear risk of bias, mainly due to vague description of methodology regarding sequence generation,[61,113,136] allocation concealment,[61,113,136] blinding,[61,113,163] analysis of incomplete outcome[111] and change in GLN product[112] (Table 3.12). Three studies,[141] of which two studies[106,133] were not suitable for meta-analysis, were judged to have a high risk of bias, based on no-blinding,[141] use of co-interventions[133] and a problematic cross-over study design.[106]

The cancer diagnoses of participants varied across studies, of which haematological malignancy was the most prevalent,[60,109,113,123,143] followed by haematological malignancy combined with other types of cancer as previously defined[98,111-112] The remaining 4 studies included participants with gastrointestinal tract malignancies of various sites,[163-164] also specifically gastric cancer[61] and colorectal cancer.[136,141]

The number of patients developing clinical infections was not reported consistently as such throughout the included studies. Some authors reported only specific types of infectious complications (such as pneumonia or wound infection) per group. In some cases it was necessary to select a set of data to represent clinical infections to be able to import some of the data for meta-analysis. The true number of people affected by all types of infections is therefore not reflected in these cases, and could bias the outcome of this meta-analysis.

Three studies reported a significant decrease in the number of patients with clinical infection in the GLN-supplemented group as opposed to controls:

- Patients with wound infection
  (GLN 1/57 (2%) vs. Control 6/52 (12%), P=0.038)[141]
- Patients with clinical infection
  (GLN 0/10 (0%) vs. Control 5/10 (50%), P=0.033)[143]
- Patients with clinical infection
  (GLN 3/24 (12.5%) vs. Control 9/21 (42.8%), P=0.041)[60]

Three authors reported a non-significant reduced infection rate in the GLN-supplemented group:

- Clinical infection rate
  (GLN 21/27 (78%) vs. Control 24/26 (92%), P=0.25)[123]
- Patients with infectious complications
  GLN 14/212 (6.6%) vs. Control 37/216 (17.1%), P=0.55)[163]
- Total clinically defined infections
  (GLN 7 events vs. Control 11 events, no P-value)[109]

Unpublished data received from Blijlevens NMA (e-mail correspondence, 14 Dec 2009) also had a lower infection rate in the GLN group, although not significant:

- Number affected
  (GLN 14/16 (87.5%) vs. Control 16/16 (100%), no P-value)

The remaining 10 studies reported similar results for both groups (Table 3.17).

Table 3.17: Summary of results of ten studies reporting similar results for GLN-supplemented groups *versus* their controls regarding clinical infection

STUDY ID (Reference number)	DETAIL	GLN GROUP	CONTROL GROUP	COMMENTS
Klek 2005 (61)	**Pneumonia cases**	5/30 (16.6%)	8/30 (26.6%)	no P-value
Marton 2010 (164)	**Infectious complications** no data presented	"Following the surgical procedure, no significant difference was identified in the postoperative period."		no P-value
O'Riordian 1994 (136)	Patients with **clinical signs of infections** that required antibiotic therapy	1/11 (9%)	2/11 (18%)	no P-value
Pytlik 2002a (98)	Patients with **clinically proven infections**	**3**/21 (14%)	1/19 (5%)	no P-value
Schloerb 1993 (111)	Patients with **clinical infections**	6/16 (37.5%)	5/13 (38.4%)	no P-value
Schloerb 1999 (112)	Patients with **sepsis**	5/27 (18.5%)	4/25 (16%)	no P-value
Sornsuvit 2008 (113)	**Frequency of infection** Mean (SD)	3.3 (2.7)	2.7 (1.7)	-
	Patients **free from infection**	2/8 (25%)	2/8 (25%)	no P-value
Cerchietti 2006 (101)	Incidence of documented or **highly suspected infections,** no data presented	"no significant difference"		-
Hallay2002 (133)	**Pneumonia,** no data presented	"no differences between the two groups"		-
Van Zaanen 1994 (106)	**Microbiologically documented infections**	**5** blood, **3** CVC, 1 bronchial lavage	3 blood, 2 CVC, 1 bronchial lavage	no P-value

CVC: Central venous catheter

The collective clinical infection rate for the 12 studies providing dichotomous data is 106/459 (23.0%) in the GLN group vs. 123/447 (27.5%) in the control group.

Combining the results of the 12 studies which reported dichotomous data for the number of patients with some form of infection, using a random effects analysis model, synthesized an RR value of 0.87 (95% CI, 0.73 to 1.04 P=0.13, Figure 3.13). This result is not significant; the 95% CI is imprecise. More evidence is needed to either support or refute that GLN has an effect on the number of patients developing clinical infections. One should also consider here that 3 of the studies which did not publish any data (Table 3.17), also concluded that there was no difference between the two groups. It would be interesting to see what the effect of these missing data sets will be on the outcome of the meta-analysis result.

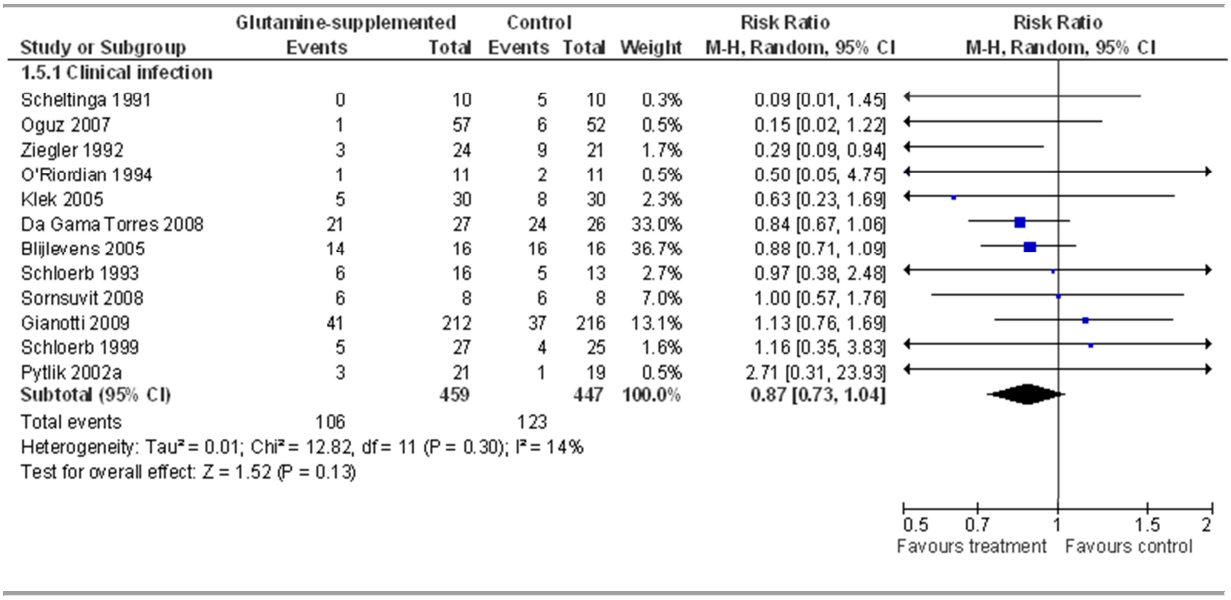


Figure 3.13: Forest plot of effect of GLN supplementation *versus* controls on clinical infection

A sensitivity analysis, changing the analysis model from random to fixed, produces a similar RR value of 0.84 (95% CI, 0.69 to 1.02, P=0.08, Figure 3.14) and, in addition, changing the effect measure from RR to OR (with random model), follows the same trend (0.60 OR, 95% CI, 0.34 to 1.05, P=0.07). The sensitivity analyses did not challenge the overall results and conclusions of the random effects analysis model and therefore these results must be regarded with a great degree of certainty based on the evidence presented here.

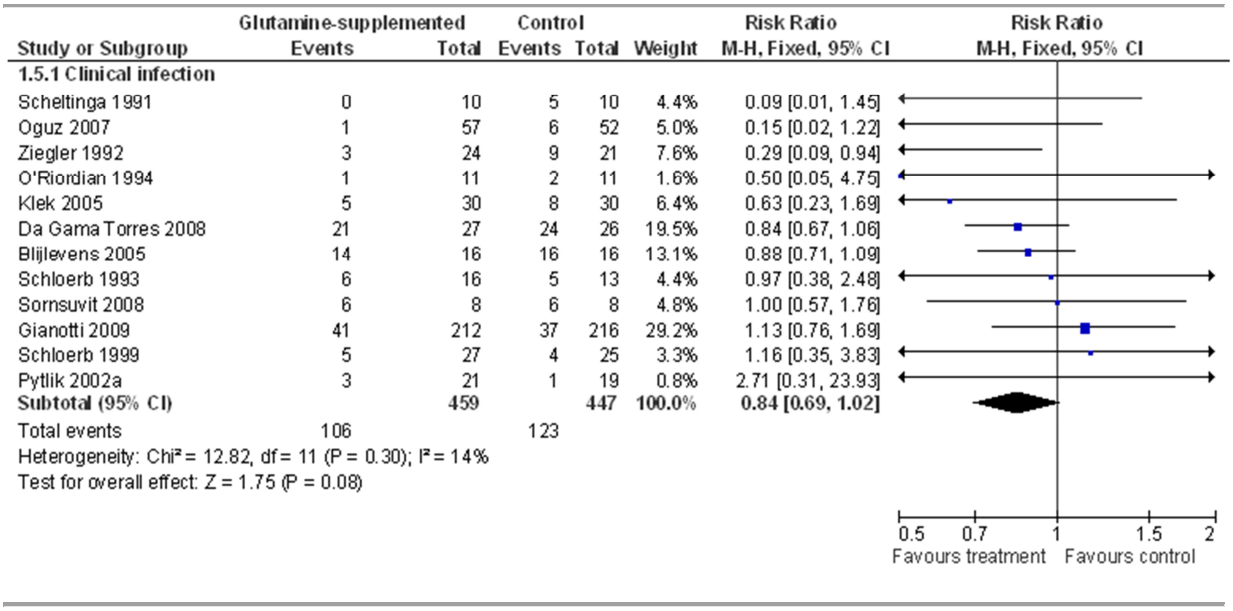


Figure 3.14: Forest plot of effect of GLN supplementation *versus* controls on clinical infection with a fixed model analysis

Some heterogeneity is present (Chi^2 =12.82, df=11, P=0.30, Figure 3.13), although not significant (P>0.1). The inconsistency in results is quantified by I^2 (I^2 = 14%, Figure 3.14) as mild heterogeneity, which might not be important.[1] On inspection of the funnel plot (Figure 3.15), an asymmetrical distribution is observed, with no studies at the bottom of the plot, indicating possible publication or other bias. Studies with results in both directions have been published and included in this review, but at least three authors did not publish their non-significant results; consequently publication bias might play some role. However, most of the studies were small in size and the heterogeneity might possibly be explained by small-study effects or poor methodological design.

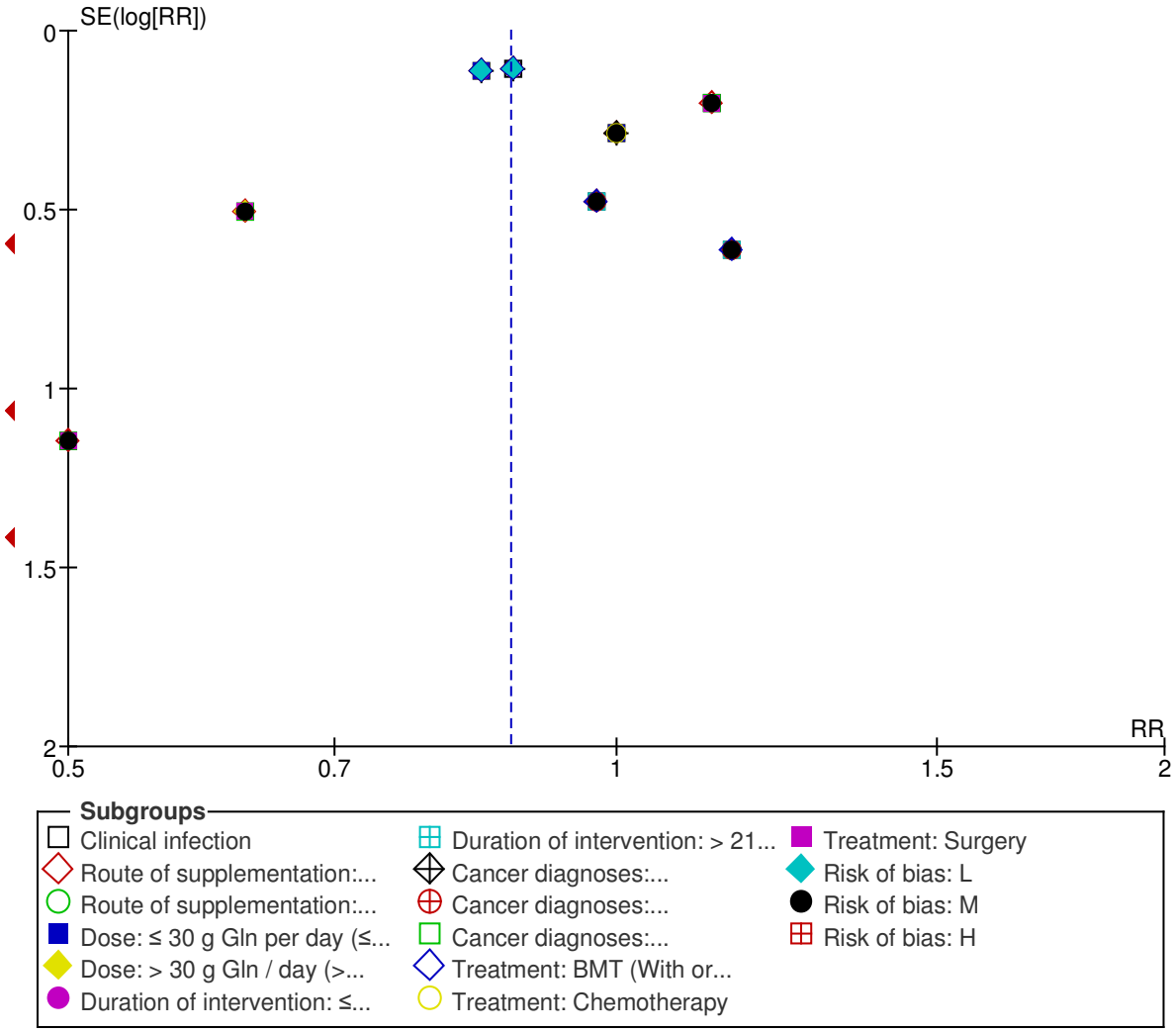


Figure 3.15: Funnel plot of studies using GLN supplementation (Clinical infection)

To further explore the inconsistency in result of GLN supplementation on clinical infection outcomes, a subgroup analysis (APPENDIX 6.10) was done as predefined. The clinical and methodological variation amongst studies could not explain the inconsistency in results as expected by the observed overlap of 95% CIs (Figure 3.13).

3.3.1.6 Mucositis Duration

RevMan5 Outcome 1.6. Mucositis: Duration (days) Mean and SD of days with ≥ grade 1 mucositis

Ten studies reported on the duration of different grades of mucositis: 3 (*N*=68) reported continuous data suitable for meta-analysis;[96,98,113] the other 7 is described.[97,100,102-103,105,110,153]

Seven of the studies were unclear about certain aspects of its methodological design, which introduced a moderate risk of bias (Table 3.12).[97,100,103,105,110,153] One of these studies was included in the meta-analysis and were unclear with regard to aspects of allocation concealment and blinding.[113] The remaining three studies were judged to be of low risk of bias,[102] of which two were included in meta-analysis.[96,98]

The cancer diagnoses of participants were primarily haematological malignancy[96,97,110113] and haematological malignancy mixed with solid tumours.[98] One study each included head and neck cancers,[103] colorectal cancer[102] and sarcomas,[100] as previously defined. The cancer diagnoses were unclear in two studies.[105,153]

The duration of oral mucositis or stomatitis was reported as days in all but one study, which reported mucositis duration as per the number of RT fractions.[103] Mucositis duration was reported for mucositis of $\geq$ grade 1 (including all grades/scores) and/or the duration for each different grade of severity by means of different assessment criteria as reported by either the patient (usually more subjective) and/or physician/nurse (usually more objective criteria). When available, the duration data for clinically significant mucositis[166] ($\geq$ grade 2 as defined by CTCAE v3.0: painful erythema and beginning of ulcers and inflammation or pseudomembranes, characterized by pain with chewing and swallowing, but the patient can still eat a modified solid/soft diet, APPENDIX 6.8)[167] were chosen for inclusion in the meta-analysis for mucositis duration.

Two authors (both not suitable for meta-analysis) reported a significant reduction in the duration of mucositis in the GLN-supplemented group:

- Patient-reported, Patient questionnaire/calendar

  "*Overall duration of oral mucositis* was 4.5 days less with GLN", data presented in figure only, P=0.0005[100]

  "*Days of oral mucositis* $\geq$ *Grade 2* was 4 days less with GLN", data presented in figure only, P=0.002)[100]

- Duration > Grade 2

  Number of fractions (mean)

  Subjective patient complaint (grade 0-4 scale)

  (9.5 GLN vs. 13.0 Control, P=0.1008)[103]

  Objective physician evaluation (RTOG/EORTC criteria)

  (5.8 GLN vs. 12.3 Control, P=0.0232)[103]

In contrast, Pytlik 2002a[98] reported a significantly worse outcome for the intervention group:

- Nurse assessment, Nebraska Oral Assessment Score
  Days of MUC13 score, mean (SD)
  (4.6 (4.8) GLN vs. 1.5 (2.4) Control, P=0.02)[98]

The other 7 studies (of which only three was suitable for meta-analysis, Figure 3.18) reported no significant reduction in mucositis duration with GLN supplementation as compared to controls, which represent a large body of evidence, but unfortunately not suitable for meta-analysis.

Table 3.18: Summary of results of seven studies reporting no significant difference between GLN-supplemented groups *versus* their controls regarding mucositis duration

STUDY ID (Reference number)	DETAIL	GLN GROUP	CONTROL GROUP	COMMENTS
Jebb 1995 (96)	Patient-reported: Patient mucositis assessment scale Days of **mucositis score ≥ 3** mean (SD)	1.4 (2.1)	2.1 (4.2)	no P-value NS
	Nurse assessment: Observer mucositis assessment criteria, Days of mucositis score ≥3, mean (SD)	5.9 (5.1)	7.0 (6.1)	NS, no P-value
Sornsuvit 2008 (113)	Subjective assessment, Criteria modified from Anderson 1998 & Jebb 1994, Days duration, mean (SD)	3.2 (6.0)	6.3 (11.2)	P=NS
Canovas 2000 (153)	NCOG criteria, Stomatitis duration Days, median (range)	4 (0-18)	4.5 (0-14)	NS
Coghlin Dickson 2000 (97)	Stanford University Hospital BMT Toxicity scale Days mucositis (grade 2-4), median (range)	13 (0-37)	13 (0-31)	P=0.83
Daniele 2001 (102)	Common toxicity criteria of the NCI Duration of stomatitis, mean	4.2	3.4	no P-value
Okuno 1999 (105)	Patient reported, The North Central Treatment Group, Duration of mucositis (days), median	9	9	P=0.58
Piccirillo 2003 (110)	Daily mucositis score Median (range)	11 (6-21)	13 (10-22)	P=0.17

The pooled results of the three studies suitable for meta-analysis synthesized an SMD value of 0.17 (95% CI, -59 to 0.93, P=0.66, Figure 3.16) indicative of a small effect (Rule of thumb; < 0.41).[1] The 95% CI includes the null value; therefore the possibility that GLN has no effect whatsoever cannot be excluded. These results are inconclusive and these three studies provide little knowledge about the effect, and further information is needed for meta-analysis.

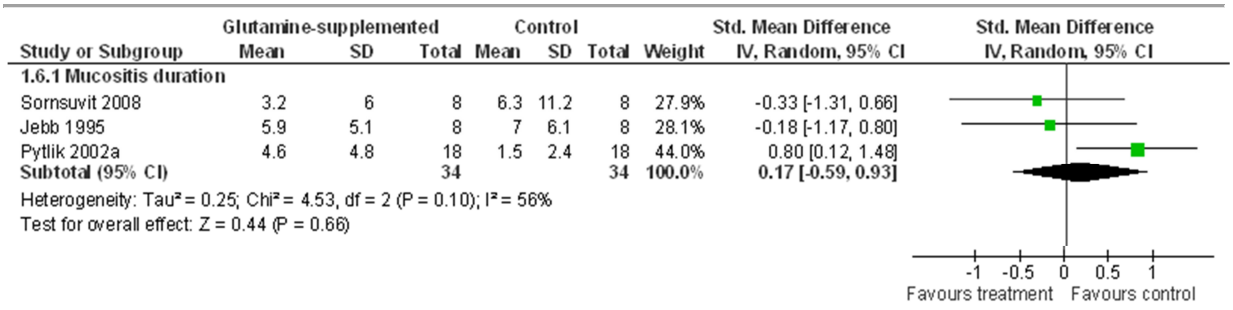


Figure 3.16: Forest plot of effect of GLN supplementation *versus* controls on mucositis duration

Sensitivity analysis with a fixed analysis model (0.28 Std MD, 95% CI, -0.20 to 0.77, P=0.25) or an MD effect measure (1.05 MD, 95% CI, -2.58 to 4.68, P=0.57) did not alter the overall result and conclusions.

Statistically significant heterogeneity exists (Chi^2 =4.53, df=2, P=0.10, Figure 3.17). The I^2 =52% indicates a moderate degree of heterogeneity.[1] Visual inspection of the 95% CI (Figure 3.16) shows this heterogeneity, mainly caused by the results from 1 study[98] leaning into the opposite direction. Further investigation of a funnel plot and predetermined subgroup analysis (data not shown) could not provide meaningful information (too few studies) and therefore more information is needed to draw meaningful conclusions.

3.3.1.7 Mucositis ≥ Grade 2

RevMan5 Outcome 1.7. Mucositis: Number of patients with ≥ grade 2

Grade 2 mucositis is classified as clinically significant[166] and is characterized subjectively by painful/sore mouth with pain when chewing and swallowing, but the patient can still eat a modified solid/soft diet. Objective indicators involve painful erythema, edema, patched ulceration and inflammation or pseudomembranes of the oral mucosa (CTCAE v3.0 criteria,[167] APPENDIX 6.8). Severe mucositis may directly have an effect on the clinical outcome of cancer patient treatment regimes and often results in increased pain, difficulty in swallowing, compromised nutritional intake and an increased risk of infection. Eventually these clinical sequelae increase morbidity, mortality and cost of care associated with cytotoxic therapy.[94,166]

Nine studies (*N*=727) published dichotomous data on the number of patients with at least grade 2 mucositis, suitable for meta-analysis;[93-94,97,101-104,113,141] an additional two studies[95,100] reported comments, but no data.

Three studies were judged to be of low risk of bias (Table 3.12).[95,101-102] The remaining studies were vague with regard to some aspects of their methodological design, introducing a moderate risk of bias. Six studies were unclear about allocation concealment,[94,100,103-105,113] three studies did not provide details regarding blinding of all parties involved,[93,103,113] one study failed to indicate how incomplete outcomes were addressed in analyses,[104] 4 studies was unclear about method of sequence generation[94,104-405,113] and one study collected data regarding presence of mucositis from notes in medical charts of patients.[97]

The cancer diagnoses of included patients ranged from head and neck cancer,[101,103] gastrointestinal malignancy as previously defined,[104] colorectal cancer,[102] breast cancer,[94-95] sarcomas[100] and mixed solid tumours as previously defined[93] to haematological malignancy.[97,113] The cancer diagnoses were unclear in one study.[105]

Four of the individual studies claimed a significantly reduced incidence of $\geq$ grade 2 mucositisin the GLN *versus* control group:

- Physician assessment, Objective Mucositis Score, WHO grading system
  Patients with severe objective mucositis (OMS>1.49)
  (GLN 2/14 (14%) vs. Control 10/15 (67%), P=0.007)[101]
- Physician assessment, CTCAE v 3.0
  Patients with mucositis/stomatitis grade 2-4
  (GLN 2/22 (9%) vs. Control 11/29 (38%, P<0.001)[93]
  Subjective patient complaint (Grade 0-4 criteria)
  Patients complaining of $\geq$ Grade 2 mucositis
  (GLN 7/8 (87.5%) vs. Control 8/9 (88.9%), P=0.1073)[103]
- Objective physician evaluation (RTOG/EORTC criteria)
  Patients with $\geq$ Grade 2 mucositis
  (GLN 5/8 (62.5%) vs. Control 9/9 (100%), P=0.0060)[103]
- Investigator assessment, WHO mucositis score
  Patients with $\geq$ grade 2 oral mucositis
  (GLN 63/163 (38.7%) vs. Control 81/163 (49.7%), P=0.026)[94]

- Patient-reported, Patient questionnaire/calendar

  Stomatitis ≥ Grade 2

  "GLN was associated with significantly less severe mouth pain", figure presentation of data only, P=0.002)[100]

The remaining 6 studies reported similar data for both GLN and control groups (Table 3.19).

Table 3.19: Summary of results for nine studies reporting similar data for GLN-supplemented groups *versus* their controls regarding number of patients with ≥ grade 2 mucositis

STUDY ID (Reference number)	DETAIL	GLN GROUP	CONTROL GROUP	COMMENTS
Coghlin Dickson 2000 (97)	Stanford University Hospital BMT Toxicity scale Patients with mucositis grades 2-4	19/29 (66%)	18/29 (62%)	P=0.79
Daniele 2001 (102)	Common toxicity criteria of the NCI Patients with ≥ grade 2 stomatitis	5/29 (17%)	7/33 (21%)	no P-value
Jebb 1994 (104)	Scoring system for patient-reported systems (Score 1 -5): Mouth comfort, mean (SD)	1.56 (0.66)	1.52 (0.62)	-
	Ease of eating, mean (SD)	1.40 (0.57)	1.36 (0.48)	-
	Observer assessment, WHO classification Patients with ≥ grade 2 mucositis	7/17 (41%)	10/17 (58.8%)	no P-value
Okuno 1999 (105)	Patient-reported, The North Central Treatment Group criteria for reporting mucositis Patients with ≥ grade 2 mucositis	22/63 (34.9%)	17/61 (27.8%)	no P-value
	Physician assessment, Mucositis grading criteria (grade 0-4) Patients with ≥ grade 2 mucositis	19/66 (28.8%)	20/68 (29.4%)	no P-value
Sornsuvit 2008 (113)	Subjective assessment, Criteria modified from Anderson 1998 & Jebb 1994 Patients with severity grade ≥ 2 mucositis	1/8 (12.5%)	3/8 (37.5%)	P>0.05
Bozzetti 1997 (95)	Common toxicity criteria of the NCI	No data presented: "Grade 1-2 mucositis had a similar prevalence in the two groups."		

The collective number of patients presenting with clinically significant mucositis from the dichotomous data of nine studies (Figure 3.17) is 123/356 (34.5%) in the GLN-supplemented group and 169/371 (45.5%) in the controls.

Combining the data with a random analysis model, concludes an RR value of 0.76 (95% CI, 0.60 to 0.97, P=0.03, Figure 3.17), indicative of a significantly reduced incidence of ≥ grade 2 mucositis in the group receiving GLN supplementation with a relative risk reduction (RRR) of 24%. These results must, however, be interpreted with caution, since the 95% CI is not precise (large number of small studies), signifying a certain degree of doubt with regard to the true effect of GLN supplementation.

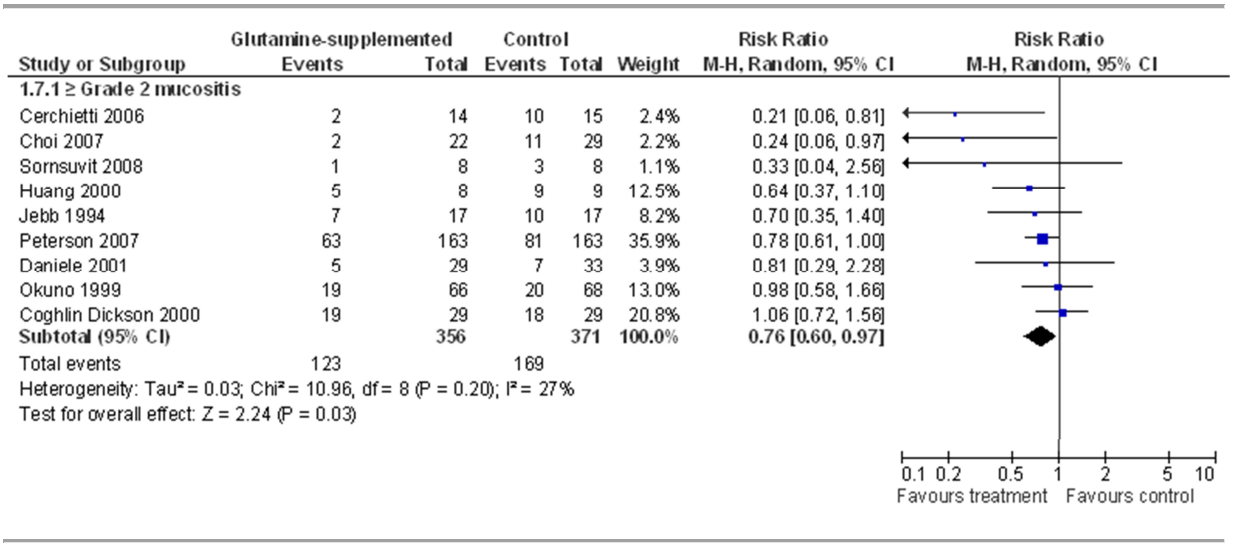


Figure 3.17: Forest plot of effect of GLN supplementation *versus* controls on number of patients with ≥ grade 2 mucositis

Sensitivity analysis, changing the random model to fixed, resulted in an RR value of 0.75 (95% CI, 0.63 to 0.89, P=0.001). Furthermore changing the effect measure from RR to Odds ratio (OR) revealed a point estimate of 0.56 (95% CI, 0.34 to 0.90, P=0.02). The sensitivity analyses produced a more precise 95% CI, upholding the significance of effect. The initial results obtained and conclusions drawn can be regarded with a high degree of certainty.

A minor degree of heterogeneity exists (I^2 =27%, Figure 3.17), but this may perhaps not be significant (Chi^2 = 10.96, df=8, P=0.20), suggesting true heterogeneity in the results of individual studies, most likely introduced by small study effects or poor methodological design in some cases. A subgroup analysis was undertaken to explore the inconsistency in results amongst studies. After subgroup analysis both the parenteral route (2 studies) of GLN supplementation with an RR value of 0.24 (95% CI of 0.08 to 0.75, P=0.01, APPENDIX 6.10)[101,113] and the oral route (7 studies) with RR value of 0.81 (95% CI 0.68 to 0.97, P=0.02, APPENDIX 6.10)[93-94,97,102-105] provided evidence of a significant effect in favour of GLN supplementation. The subgroup of studies supplementing GLN at a lower dose of <21 g/day (<0.3 g/kg/day) maintained a significantly reduced incidence of ≥ grade 2 mucositis (0.78 RR, 95% CI, 0.64 to 0.94, P=0.01, APPENDIX 6.10)[94,102-105] as opposed to a higher dose of 21 – 30 g/day (0.30 – 0.42 g/kg/day) (0.41 RR, 95% CI, 0.13 to 1.34, P=0.14, APPENDIX 6.10).[93,97,101,113] The same observation was made with regard to studies with a moderate risk of bias (0.80 RR, 95% CI, 0.65 to 0.99, P=0.04, APPENDIX 6.10),[93-94,97,103-105,113] as opposed to studies with a low risk of bias (0.45 RR, 95% CI,

0.12 to 1.65, P=0.23, APPENDIX 6.10).[101-102] The 95% CI of the subgroups overlaps to a large extent; therefore the difference between the relevant subgroups may possibly not be significant.

There were too few studies per subgroup for cancer diagnoses and treatment to provide additional information regarding the effect of GLN supplementation on the incidence of ≥ grade 2 mucositis in this population.

3.3.1.8 Mucositis ≥ Grade 3

RevMan5 Outcome 1.8. Mucositis: Number of patients with maximum grade (≥ 3/4)

Grade 3 mucositis is subjectively characterized by considerable mouth pain with redness, ulcers and inflammation and the patient may only be able to tolerate liquids or very soft food. Objective criteria include extensive erythema, confluent ulceration or pseudomembranes and bleeding with minor trauma (CTCAE v3.0 criteria,[167] APPENDIX 6.8). Grade 4 mucositis is subjectively characterized by severe mouth pain with ulcers and inflammation and the patient is unable to eat or drink. Objective criteria include overall inflammation/ulceration of the mouth ± throat, tissue necrosis, significant spontaneous bleeding, and thick stringy saliva requiring manual removal from the mouth ± saline nebulizers and enteral or parenteral nutrition is required (CTCAE v3.0 criteria,[167] APPENDIX 6.8). Data as reported for the maximum grade of mucositis (Grade 3 and/or 4) was included in the meta-analysis, as a measure of the maximum grade of mucositis.

Seven studies [93-94,101,104-105,113,133] with 607 participants reported dichotomous data suitable for meta-analysis.

Only one study affirmed all design aspects and was considered to be of low risk of bias.[101] The other 5 studies were judged to introduce a moderate risk of bias due to certain vague aspects of their methodological design (Table 3.12), including unclear allocation concealment,[94,103-105,113] unclear aspects of blinding,[93,103,113] unclear sequence generation[94,104-105,113] and failure to report if and how loss to follow-up was addressed in analyses.[104]

The cancer diagnoses of included patients were extremely heterogenic, ranging from head and neck cancer,[101,103] gastrointestinal malignancy as previously defined,[104] mixed solid tumours as previously defined[93] and breast cancer[94] to haematological malignancy.[113] The cancer diagnoses was unclear in one study.[105]

Three studies reported a significant reduction in the incidence of maximum grade mucositis in the GLN *versus* control groups:

- Physician assessment, Objective Mucositis Score, WHO grading system

  Patients with mucositis WHO Grade 4

  (GLN 0/14 (0%) vs. Control 5/15 (33%), P=0.042)[101]

- Subjective patient complaint (Grade 0-4 criteria)

  ≥ *Grade 3*

  (GLN 0/8 (0%) vs. Control 4/9 (44.4%), P=0.1073)[103]

- Objective physician evaluation (RTOG/EORTC criteria)

  ≥ *Grade 3*

  (GLN 0/8 (0%) vs. Control 5/9 (55.5%), P=0.0060)[103]

- Investigator assessment, WHO mucositis score

  Patients with ≥ grade 3 oral mucositis

  (GLN 2/163 (1.2%) vs. Control 11/163 (6.7%), P=0.05)[94]

The remaining 4 studies reported no significant difference in the GLN-supplemented and control groups (Table 3.20). It was noted that in 2 of these studies more cases of grade 3/4 mucositis was reported in the GLN-supplemented group, compared to controls.[104-105]

Table 3.20: Summary of results of four studies reporting no significant difference between GLN-supplemented groups *versus* their controls regarding number of patients with maximum grade (3/4) mucositis

STUDY ID (Reference number)	DETAIL	GLN GROUP	CONTROL GROUP	COMMENTS
Choi 2007 (93)	Physician assessment CTCAE v3.0 **Grade 4**	0/22 (0%)	1/29 (3.4%)	no P-value
Okuno 1999 (105)	Patient-reported The North Central Treatment Group criteria **Grade 4**	**3**/63 (4.7%)	0/61 (0%)	no P-value
	Physician assessment Mucositis grading criteria (Grade 0-4) **Grade 4**	**2**/66 (3%)	0/68 (0%)	no P-value
Sornsuvit 2008 (113)	Subjective assessment Criteria modified from Anderson 1998 & Jebb 1994 ≥ **Grade 3**	0/8 (0%)	2/8 (25%)	P>0.05
Jebb 1994 (104)	Observer assessment WHO classification ≥ **Grade 3**	**5**/17 (29.4%)	4/17 (23.5%)	no P-value

The collective (*N*=607) incidence of maximum grade of mucositis was 9/298 (3.0%) for the GLN-supplemented group and 28/309 (9.0%) for the controls.

A random effects meta-analysis model indicated that GLN supplementation did not have a significant effect on the incidence of maximum grade mucositis (0.41 RR, 95% CI, 0.14 to 1.19, P=0.10, Figure 3.18). The 95% CI interval is inconclusive and further information is needed to either support or refute that GLN supplementation has an effect on the number of patients presenting with ≥ grade 3 mucositis.

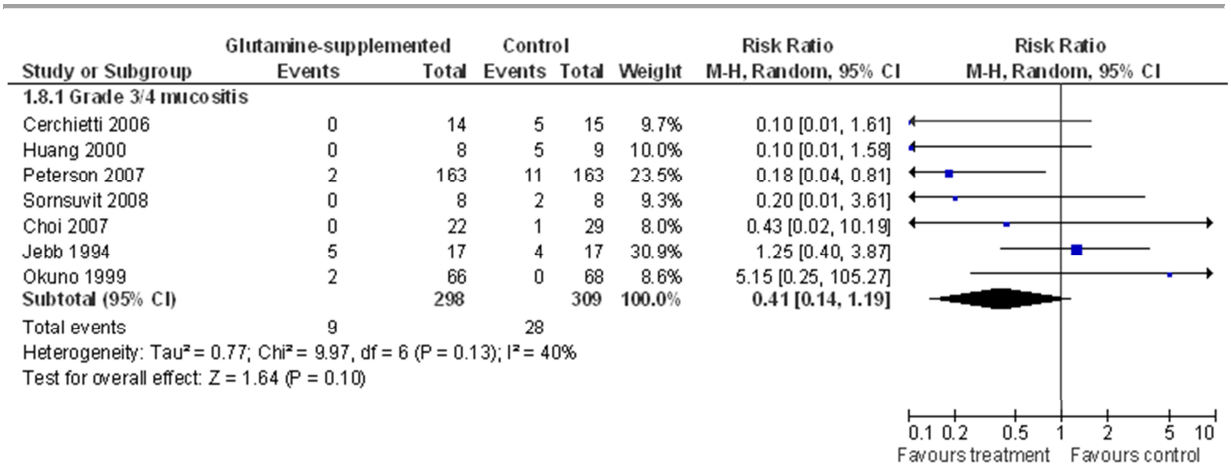


Figure 3.18: Forest plot of effect of GLN supplementation *versus* controls on *n*umber of patients with maximum grade (3/4) mucositis

Sensitivity analysis with a fixed effect model of analysis resulted in an RR value of 0.39 (95% CI, 0.20 to 0.75, P=0.004). On comparison the 95% CI remains imprecise; however, the P-value indicates a significantly reduced number of affected patients after GLN supplementation as compared to controls. Changing the effect measure to OR resulted in a point estimate of 0.34 (95% CI, 0.10 to 1.13 P=0.08), achieving the same overall result and conclusion. Further research is warranted to investigate the effect of GLN supplementation on the incidence of maximum grade mucositis. Although the fixed model of analyses challenges the initial result, a random effects model is used because of the variation in the scales and/or criteria used to assess the presence of grade 3/4 mucositis.

Visual inspection of the 95% CI (Figure 3.18) indicates a large degree of overlap between studies. In addition the Chi-squared test results (Chi^2 =9.97, df=6, P=0.13, Figure 3.18) are not

significant and the I^2 statistic (I^2 =40%) represents moderate heterogeneity in results which may possibly not be important.[1]

To further explore this heterogeneity a subgroup analysis was undertaken to investigate whether GLN supplementation has a significant effect in the predefined subgroups. There were too few studies per subgroup to make meaningful analysis for the cancer diagnoses, treatment, mucositis assessment tool and risk of bias subgroups (data not shown).

Meta-analysis of the results of the two studies supplementing GLN via the parenteral route reached borderline significance (0.14 RR, 95% CI, 0.02 to 1.03, P=0.05, APPENDIX 6.10)[101,113] in favour of GLN supplementation as opposed to the oral route (0.54 RR, 95% CI, 0.15 to 1.96, P=0.35, APPENDIX 6.10), which was used in the remaining 5 studies. The results from the 4 studies with a longer intervention period (>14 days) indicated a significantly reduced number of maximum grade mucositis in the GLN-supplemented group (0.19 RR, 95% CI, 0.06 to 0.57, P=0.003, APPENDIX 6.10)[93-94,103,113] as opposed to a shorter intervention of 7-14 days in the other 3 studies (0.90 RR, 95% CI, 0.14 to 5.82, P=0.92, APPENDIX 6.10). The overlap of the 95% CI of the respective summary estimates of subgroups suggests that the difference observed between subgroups might possibly not be statistically significant.

3.3.1.9 Diarrhoea Duration

RevMan5 Outcome 1.09. Diarrhoea: Duration (days) – mean and SD of number of days with ≥ grade 1 diarrhoea

Four studies reported complete continuous data suitable for meta-analysis;[96,98,102,113] another 3 reported results as mean (range)[95] or median (range)[97,153] and will be discussed only.

Four studies were judged to be of low risk of bias (Table 3.12) across all domains of methodological design.[95,96,98,102] Moderate risk of bias is introduced by three studies due to unclear concealment of allocation[113,153] and blinding,[113,153] unclear sequence allocation[113] and data collection from medical charts after discharge of patients.[97]

The cancer diagnoses of participants were mainly haematological malignancies[96-97,113] and haematological malignancy mixed with solid tumours.[98] One study each included participants with colorectal cancer,[102] breast cancer[95] and one was unclear about cancer diagnoses.[153]

The criteria used to define diarrhoea (APPENDIX 6.9) are not the same for all included studies. Presence of diarrhoea was defined as more than three[98] or more than four[96] loose stools per day or an increase of 2-3 (or < 4) stools/day compared to pre-therapy as per the common toxicity criteria of the NCI.[95,102,113] One author defined notable diarrhoea as a stool output of >500 ml in volume over a 24 hour period.[97]

None of the individual studies reported a significant reduction in the duration of diarrhoea subsequent to GLN supplementation. Two authors did report a trend at the 10% level (P<0.1) in favour of GLN supplementation:

- Common toxicity criteria of the NCI

  Duration in all patients, mean (SD) days (dataset included in meta-analysis)

  (1.5 (2.4) GLN (*N*=29) vs. 2.8 (3.0) Control (*N*=33), P=0.07)[102]

  Duration in patients with diarrhoea only, mean (SD) days

  (3.7 (2.5) GLN (*N*=12) vs. 4.9 (2.3) Control(*N*=19), P=0.09)[102]

- More than 3 loose stools per day

  Mean (SD) (days)

  (2.4 (2.3) GLN vs. 4.2 (3.4) Control, P=0.06)[98]

The other five studies showed similar data for both groups (Table 3.21).

Table 3.21: Summary of results of five studies reporting similar data for GLN-supplemented groups *versus* their controls regarding diarrhoea duration

STUDY ID (Reference number)	DETAIL	GLN GROUP	CONTROL GROUP	COMMENTS
Jebb 1995 (96)	More than 4 loose stools per day Mean (SD) days	3.1 (3.5)	3.3 (3.7)	no P-value
Sornsuvit 2008 (113)	NCI criteria Mean (SD) days	5.0 (3.7)	4.3 (5.7)	no P-value
Bozzetti 1997 (95)	NCI criteria Mean (range) days	2 (1-12)	**3** (1-12)	no P-value
Canovas 2000 (153)	NCOG criteria Median (range) days	4 (0-**22**)	4 (0-11)	no P-value
Coghlin Dickson 2000 (97)	> 500 ml stool output in 24 hours Median (range) days	**3** (0-9)	2 (0-**14**)	P=0.79

A random effects meta-analysis model, using Standardized Mean Difference (SMD) as an effect measure (due to different scales used to assess presence of diarrhoea), calculated a point measure of -0.38 SMD (95% CI, -0.73 to -0.03, P=0.03, Figure 3.19) in favour of the GLN-supplemented group. As a rule of thumb the apparent effect size is very small (<0.41).[1] In

addition the 95% CI is fairly imprecise, indicating uncertainty with regard to where the true effect of GLN lies. However, the result obtained shows a significant reduction (P=0.03) in the duration of diarrhoea in the group receiving GLN supplementation.

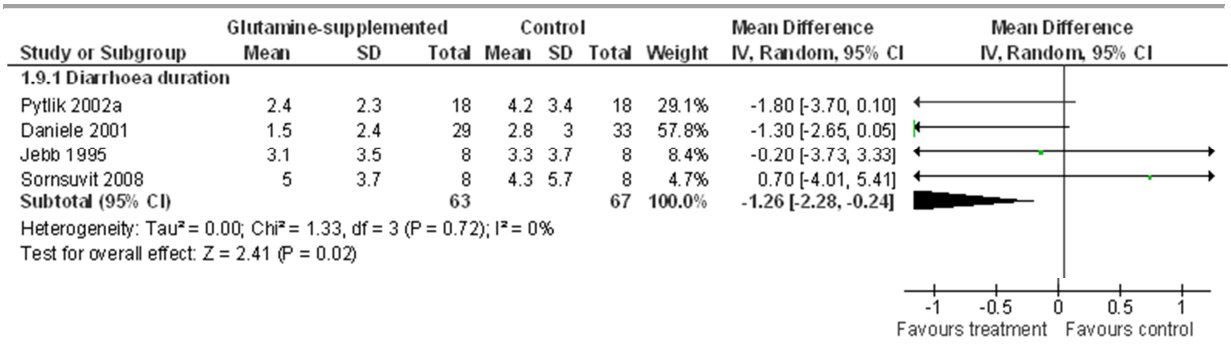


Figure 3.19a

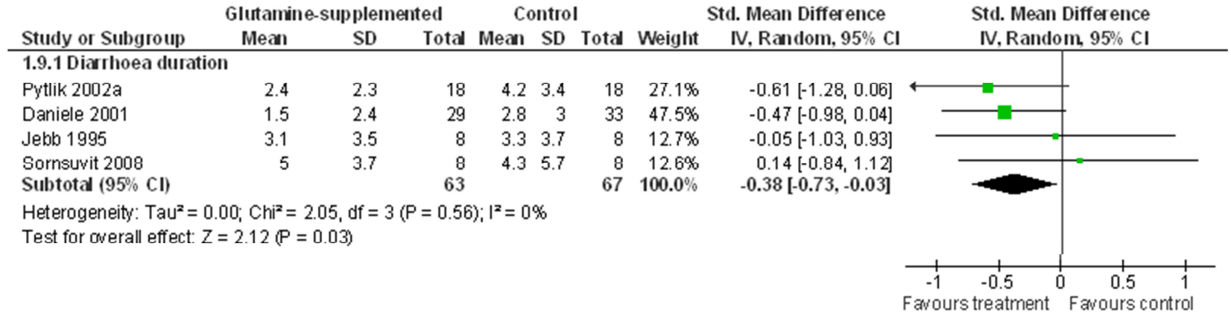


Figure 3.19b

Figure 3.19: Forest plot of effect of GLN supplementation *versus* controls on diarrhoea duration. Figure 3.19a (Mean difference), Figure 3.19b (Standardized mean difference)

Changing the effect measure to Mean difference (MD) in a sensitivity analysis did not affect the overall result (-1.26 MD, 95%CI, -2.28 to -0.24, P=0.02) and the same for a fixed effects model of analysis (-0.38 SMD, 95%CI -0.73 to -0.03, P=0.03). Therefore the overall result and conclusions remain.

There is no evidence of statistical heterogeneity (Chi ^{2} =2.05, df=3 (P=0.56), I^2 =0%, Figure 3.20).

There were too few studies per predefined subgroup to provide notable information or highlight differences in effect between these predefined subgroups (Data not shown).

3.3.1.10 Diarrhoea ≥ Grade 1

RevMan5 Outcome 1.10. Diarrhoea: Patients with ≥ grade 1 diarrhoea

Nine studies reported the number of patients with ≥ grade 1 diarrhoea. The reported data of 6 (*N*=397) was dichotomous[29,95,102,119,112,122] and 3 was not suitable for meta-analysis.[106,120,153]

Three studies were judged to be of low risk of bias across all six methodological quality domains (Table 3.12).[95,102,122] Four studies were unclear about either allocation concealment[29,119-120,153] and/or method of sequence generation[29,153] and one study had a change in the GLN product used,[112] introducing a moderate risk of bias. Two studies introduced a high risk of bias due to problematic cross-over design where some patients were randomized more than once[106] and one which used co-interventions.[29]

The cancer diagnoses of participants included gastrointestinal cancer,[29,120] of which one study included only colorectal cancer.[102] One study included advanced metastatic breast cancer.[95] Two studies included haematological malignancy[106,153] only and combined with solid tumours[112] in another. The remaining two studies included mixed types of cancer[119,122] as previously defined.

Again the criteria used to define diarrhoea are not the same for all included studies (APPENDIX 6.9). Grade 1 diarrhoea was most often defined as an increase of 2-3 (or < 4) stools/day compared to pretreatment as per the common toxicity criteria of the NCI.[95,102,119] Other authors did proclaim the criteria used (WHO[106,120], CTCAE V3.0,[145] and NCOG criteria[153]), but the details of these scales were not published within the studies. In two cases the criteria/definitions used are not reported.[29,112] When the number of participants with/without diarrhoea was reported per group, it was included as such. When the incidence of diarrhoea was reported per grade, the sum of participants presenting with grade 1 to 4 diarrhoea was calculated for this outcome.

One study reported a statistically significant reduction in the toxicity score for diarrhoea in the group receiving GLN supplementation as compared to controls.

- WHO classification (grade 0-4)
  Diarrhoea score mean (SD)
  (1.31 (0.25) GLN vs. 2.82 (0.34) Control, P<0.05)[120]

The remaining 8 studies presented similar data for both groups (Table 3.22).

Table 3.22: Summary of results of eight studies reporting similar results for GLN-supplemented groups *versus* their controls regarding the number of patients with ≥ grade 1 diarrhoea

STUDY ID (Reference number)	DETAIL	GLN GROUP	CONTROL GROUP	COMMENTS
Bozzetti 1997 (95)	Common toxicity criteria of the NCI Number of affected patients	16/33 (48%)	16/32 (5%)	no P-value
Daniele 2001 (102)	Common toxicity criteria of the NCI Number of patients with ≥ grade 1 diarrhoea	12/29 (4.1%)	**19**/33 (58%)	no P-value
Kozelsky 2003 (119)	Common toxicity criteria of the NCI Number of patients with any grade diarrhoea	51/64 (80%)	51/65 (79%)	P=0.99
Schloerb 1999 (112)	Criteria unclear Number with diarrhoea	13/27 (48.1%)	13/25 (52%)	no P-value
Strasser 2008 (122)	CTCAE v3.0 Diarrhoea ≥ grade 2	3/21 (14.2%)	**4**/20 (20%)	no P-value
Wu 2001 (29)	Criteria unclear Patients with diarrhoea	**3**/25 (12%)	2/23 (8.6%)	no P-value
Canovas 2000 (153)	NCOG criteria Patients with diarrhoea	70.6%	45.5%	no- P-value
van Zaanen 1994 (106)	Toxicity scores according to WHO classification Median (range)	0 (0-1.2)	0.3 (0-1.4)	no P-value

The pooled number of patients with some grade of diarrhoea is 97/199 (48.7%) in the GLN-supplemented group vs. 105/198 (53.0%) in controls, which is in agreement with the results from individual studies and the results from studies not suitable for meta-analysis.

Using a random effects analysis model, the aggregated result of 6 studies revealed an RR estimate of 0.97 (95% CI, 0.83 to 1.13, P=0.0.69, Figure 3.20). The result obtained is imprecise. There is not enough evidence to either support or refute that GLN supplementation has an effect on the number of patients presenting with ≥ grade 1 diarrhoea.

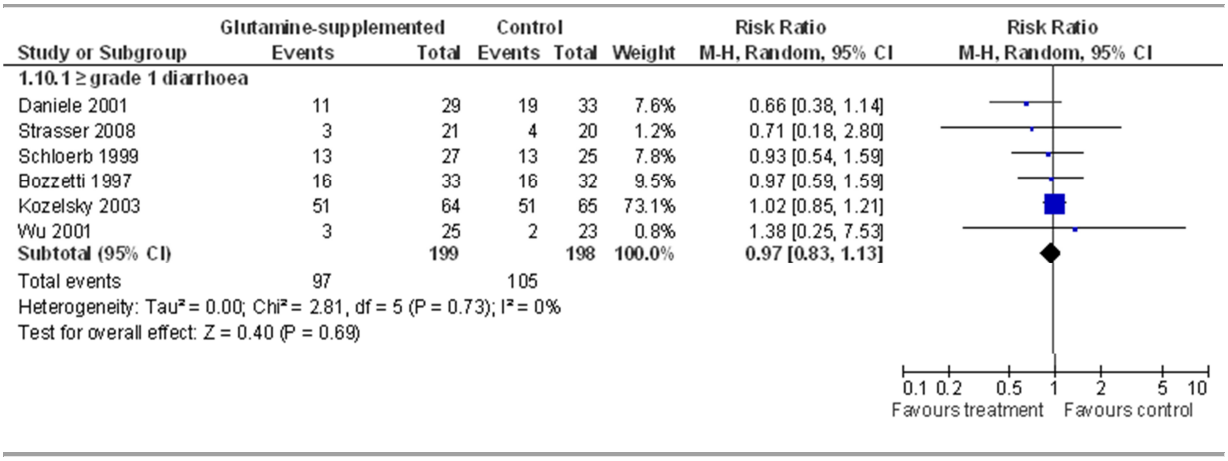


Figure 3.20: Forest plot of effect of GLN supplementation *versus* controls on number of patients with ≥ grade 1 diarrhoea

Sensitivity analysis confirms the original result obtained. A fixed model (0.93 RR, 95%CI, 0.78 to 1.11, P=0.42) and OR with random analysis model (0.83 OR, 95%CI, 0.53 to 1.31, P=0.42) did not challenge the overall conclusions made.

The 95% CI of the individual studies have good overlap, as illustrated in the forest plot (Figure 3.20). In addition there is no evidence of statistical heterogeneity as shown by the RevMan5 Chi-square test (Chi^2=2.81, df=5, P=0.73, I^2=0%, Figure 3.20).

Subgroup analysis could not identify any difference in estimated effect of GLN supplementation among predefined subgroups (APPENDIX 6.10) on number of patients presenting with diarrhoea of any grade. However, it is noted that five of the six included studies supplemented GLN via the oral route and one via the enteral route.[29] None of the included studies investigated the effect of parenteral GLN supplementation on the incidence of diarrhoea. Van Zaanen[106] supplemented GLN via the parenteral route, but did not report his data in a suitable format. However, he also reported similar results for both groups.

3.3.1.11 Diarrhoea ≥ Grade 2

RevMan5 Outcome 1.11. Diarrhoea: Number of patients with ≥ grade 2

Grade 2 diarrhoea is defined as an increase of 4 to 6 stools per day or nocturnal stools by the common toxicity criteria of the NCI[102,113,119] and CTCAE v3.[167]

A meta-analysis could only be done for 4 studies (N=248)[102,113,119,122] and 1 reported insufficient data.[48]

Two studies were judged to introduce a low risk of bias (Table 3.12).[102,122] Three studies were unclear about either allocation concealment[48,113,119] and/or blinding,[48,113] or sequence generation,[113] introducing a moderate risk of bias.

The cancer diagnoses ranged from colorectal cancer[48,102] to haematological malignancy[113] and two studies including mixed types as previously defined.[119,122]

None of the individual studies concluded a significant reduction in the number of patients presenting with ≥ grade 2 diarrhoea in the group receiving GLN supplementation compared to controls (Table 3.23).

Table 3.23: Summary of results of GLN-supplemented groups *versus* their controls regarding the number of patients with ≥ grade 2 diarrhoea

STUDY ID (Reference number)	DETAIL	GLN GROUP	CONTROL GROUP	COMMENTS
Daniele 2001 (102)	Common toxicity criteria of the NCI	7/29 (24%)	9/33 (27%)	-
Kozelsky 2003 (119)	Common toxicity criteria of the NCI Number of patients with ≥ grade 2 diarrhoea	35/64 (54%)	33/65 (51%)	-
Sornsuvit 2008 (113)	Common toxicity criteria of the NCI Number of patients with ≥ grade 2 diarrhoea	1/8 (12.5%)	1/8 (12.5%)	-
Strasser 2008 (122)	CTCAE v3.0 Diarrhoea ≥ grade 2	3/21 (14.2%)	4/20 (20%)	-
Decker-Baumann 1999 (48)	WHO classification (Grade 0-4)	No data	No data	NS

The pooled result estimates the number of patients presenting with at least grade 2 diarrhoea as 46/122 (37.7%) in the GLN-supplemented group vs. 47/126 (37.3%) in controls.

A random effects analysis model reached the same overall result with an RR estimate of 1.03 (95% CI, 0.77 to 1.39, P=0.84, Figure 3.21). The available evidence is weak and imprecise.

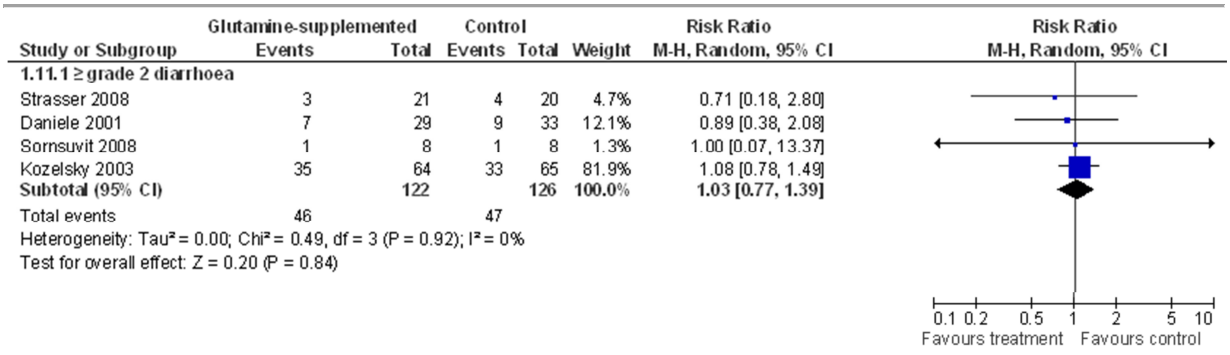


Figure 3.21: Forest plot of the effect of GLN supplementation *versus* controls on the number of patients with ≥ grade 2 diarrhoea

Sensitivity analysis was done. Changing the analysis model from random to fixed (1.01 RR, 95%CI, 0.74 to 1.37, P=0.96), and changing the effect measure from RR to OR (1.02 OR, 95%CI, 0.59 to 1.76, P=0.95) did not affect the overall result and conclusion.

Again there seems to be no statistical heterogeneity. This is illustrated by the complete overlap of the 95% CIs of individual studies in forest plot (Figure 3.21) and the I^2 statistic (I^2 =0%) together with a small Chi-square test result relative to its degree of freedom (Chi^2 =0.49, df=3, P=0.92).

There were too few studies per predefined subgroup to provide additional information on the effect of GLN supplementation on incidence of at least grade 2 diarrhoea for different routes (oral [102,119,122] vs. parenteral[113]), GLN dosages, intervention duration, cancer diagnoses/treatment and risk of bias (data not shown).

3.3.1.12 Diarrhoea ≥ Grade 3

RevMan5 Outcome 1.13: Diarrhoea: Number of patients with maximum grade (3/4) diarrhoea

According to the common toxicity criteria of the NCI, grade 3 diarrhoea is characterized by an increase of 7-9 stools per day or incontinence and grade 4 diarrhoea is an increase of ≥ 10 stools per day or grossly bloody diarrhoea or need for parenteral support for dehydration.[102,119] CTCAE v3 criteria are very similar to this.[167]

Three studies (*N*=256)[95,102,119] reported the number of patients per group presenting with ≥ grade 3 diarrhoea and one study[48] reported only median values.

Two studies were judged to be of low risk of bias across all methodological quality domains (Table 3.12).[95,102] Aspects about allocation concealment[48,119] and/or blinding[48] were unclear in two studies, introducing a moderate risk of bias.

Two of the studies included only colorectal cancer patients [48,102] and one study only breast cancer.[95] The cancer diagnoses were mixed in the remaining study, as previously defined.[119]

None of the 4 studies reporting data regarding the incidence of grade 3/4 diarrhoea reported a significant difference between the two groups (Table 3.24). Pooled incidence of maximum grade diarrhoea is 4/126 (3.1%) in GLN group vs. 8/130 (6.1%) in controls.

Table 3.24: Summary of results of four studies for GLN-supplemented groups *versus* their controls regarding the number of patients with maximum grade (3/4) diarrhoea

STUDY ID	DETAILS	EXPERIMENTAL GROUP	CONTROL GROUP	PUBLISHED P-VALUE
Bozzetti 1997	NCI criteria Number of patients with grade 3-4	2/33 (6%)	**5**/32 (16%)	NS
Kozelsky 2003	NCI criteria Number of patients with grade 4	2/64 (3%)	2/65 (3%)	P=0.76
Daniele 2001	NCI criteria Number of patients with Grade 4	0/29 (0%)	**1**/33 (3%)	-
Decker-Baumann 1999	WHO classification Number of patients with Grade 3	0 patients over 3 chemotherapy cycles	**2** patients over 3 chemotherapy cycles	-

Meta-analysis of three studies with the random effects model obtained an RR value of 0.54 (95% CI, 0.17 to 1.68, P=0.29, Figure 3.22). There is no strong evidence to either support or refute that GLN intervention has an effect on the incidence of maximum grade diarrhoea in oncology patients.

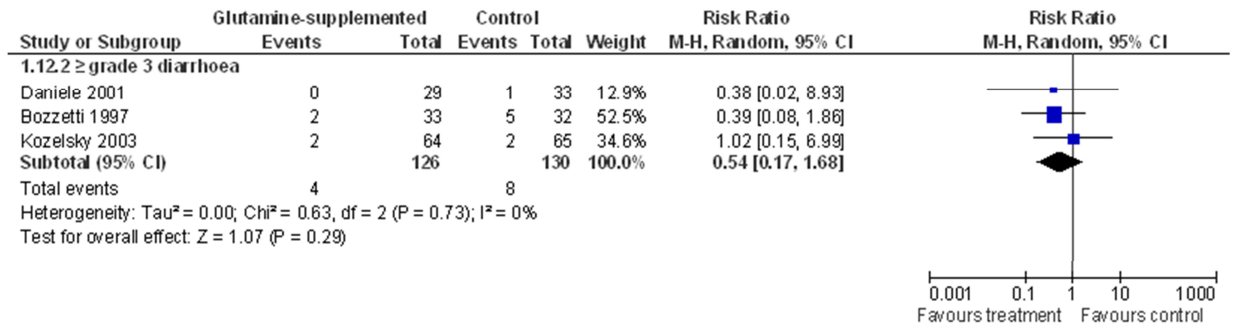


Figure 3.22: Forest plot of the effect of GLN supplementation *versus* controls on the number of patients with maximum grade (3/4) diarrhoea

Sensitivity analysis was carried out by changing the analysis model to fixed (0.53 RR, 95%CI, 0.18 to 1.61, P=0.27) and the effect measure from RR to OR (0.52 OR, 95% CI, 0.16 to 1.74, P=0.29). The overall result and conclusion were not affected by changes in analysis approach, indicating that the obtained result can be regarded with a higher degree of certainty, based on the limited data currently available.

Heterogeneity is indistinct and not statistically significant (Chi^2=0.63, df=2, P=0.73, I^2=0%, Figure 3.22).

There are too few studies per predefined subgroups to investigate differences in effect (data not shown). All three studies supplemented GLN via the oral route. No current data is available regarding the effect of GLN supplementation via the parenteral route on the number of patients presenting with maximum grade diarrhoea.

3.3.2 Primary Outcomes (Comparison 1 Animals: GLN-supplemented vs. Control)

3.3.2.1 Tumour Weight

RevMan5 Outcome 1.1: Tumour weight (g)

Twelve animal studies investigated tumour weight, 10 (*N*=269) indicated mean (SEM) in the published report;[5,74-76,78-82,86] and 2 did not publish sufficient data for meta-analysis,[3-4] but both of these authors provided sufficient unpublished data via e-mail correspondence.

All of the animal studies introduced at least a moderate risk of bias since none of the authors mentioned whether and how allocation concealment was achieved (Table 3.13). In addition none of the studies mentioned whether the assessor of tumour weight was blinded to which group the animals were randomized. In five studies a lack of blinding of caregivers resulted in a high risk of bias.[3-5,78-79] All of the studies indicated that the animals were randomized to groups; however, the method of blinding remains unclear. Two authors did not report how data of animals lost to follow-up (mainly due to mortality) were addressed in analysis.[74,78] One study[80] was judged to have a high risk of bias since the group sizes were indicated as ≥ 7. The exact group size is needed to calculate the standard deviation from the standard error, and this could lead to inaccuracy in the imputed values. In summary 7/12 (58.3%) studies was judged to introduce high risk of bias in the meta-analysis of tumour weight.

All animal models included only rats.

Six studies reported that GLN supplementation resulted in a significantly reduced ($P<0.05$) tumour weight compared to the controls.

- Day 21 after tumour implantation
  Randomized to diets 2 days before tumour implantation
  Mean (SEM) g
  (9.23 (0.73) GLN vs. 13.03 (2.27) Control, $P<0.05$)[86]
- Unpublished data obtained from author (E-mail correspondence with Suzanne Klimberg)
  Induction of tumour and randomization to diets at same time
  Mean (SEM) g
  Pre-fed (-1 to +16 weeks)
  (0.01 (0.00) GLN vs. 0.85 (0.54) FreAmine (FA), $P<0.05$)[3] *Dataset included in meta-analysis*
  Short fed (-1 to +1 weeks)
  (0.26 (0.09) GLN vs. 0.37 (0.11) FA, $P>0.05$)[3]

Post fed (+1 to +16 weeks)

(0.00 (0.00) GLN vs. 0.31 (0.11) FA,P<0.05)[3]

- Unpublished data obtained from author (E-mail correspondence with Yihong Kaufmann)

  Day 15 after tumour implantation

  Randomized to groups on day 0

  Mean (SEM) g

  (67.5 (15.8) GLN vs. 126.5 (23.1) FA, P<0.05)[4]

- 11 weeks after tumour induction

  Randomized to diets one week before tumour induction

  Mean (SEM) g

  (0.16 (0.11) GLN vs. 1.32 (0.54) FA, P<0.05)[5]

- Day 40 after tumour implantation

  Randomized to supplemental GLN day 25, Methotrexate (MTX) chemotherapy day 26 and 33

  Mean (SEM)

  (40.5 (2.9) GLN+ Methotrexate (MTX) vs. 51.8 (5.6) Glycine (GLY)+MTX, P<0.05)[78] *Dataset included in meta-analysis*

  (62.8 (5.2) GLN +Control vs. 60.8 (7.4) GLY + Control, P>0.05)[78]

- Day 14 after tumour implantation

  Randomized to diets one week before tumour implantation and exercise

  Mean (SEM)

  Sedentary rats: (6.7 (0.3) GLN vs. 9.4 (1.1) Control, P=0.0001)[81] Dataset included in meta-analysis

  Exercised rats: (swimming 3 hrs/day, 6 days/week): (4.9 (0.5) GLN vs. 8.8 (0.5) Control, NS)[81]

The remaining 6 studies reported no significant difference between groups (Table 3.25).

Table 3.25: Summary of data of six studies reporting no significant difference between GLN-supplemented groups *versus* their controls on tumour weight (Mean (SEM) g unless otherwise indicated)

STUDY ID	DETAIL	EXPERIMENTAL GROUP	CONTROL GROUP	COMMENTS
Austgen 1992 (74)	**Small tumour study:** Day 15 after tumour implantation *Randomization to TPN on day 11*	7.1 (0.8) Data set included in meta-analysis	6.9 (0.6)	-
	Large tumour study: Day 23 after tumour implantation *Randomized to diets on day 18*	23.7 (2.9)	25.2 (2.5)	
Bartlett 1995 (75)	Day 25 after tumour implantation *Randomized to diets on day 10*	40.10 (2.60)[a]	41.50 (2.60)[a]	-
Kaibara 1994 (76)	Day 15 after tumour implantation *Randomization to TPN on day 10*	TPN+GLN: 4.3 (0.5)	TPN: 4.5 (0.4)	-
		TPN+GLN+Mitomycin C: 3.6(0.5) Data set included in meta-analysis	TPN+Mitomycin C: 3.8(0.5)	
Klimberg 1996a (79)	7 weeks after tumour implantation *Randomized to diets/gavage on day of tumour implantation*	4.4 (0.8)	6.6 (1.7)	P=0.06
Robinson 1999 (80)	Day 14 after tumour implantation *Randomized to diets on day 0*	1.8 (0.5) ($N \geq 7$)	1.4 (0.2) ($N \geq 7$)	-
Yoshida 1995 (82)	Day 15 after tumour implantation *Randomized to TPN on day 10*	4.3 (0.5)	4.5 (0.4)	-

[a]Mean±SD

Pooling the results with a random effects meta-analysis model resulted in an MD value of -0.77 (95% CI, -1.47 to -0.07, P=0.03, Figure 3.23). The result obtained is imprecise as indicated by the 95% CI. It is, however, statistically significant (P<0.05), providing evidence that GLN-supplemented rats had a smaller tumour weight than controls.

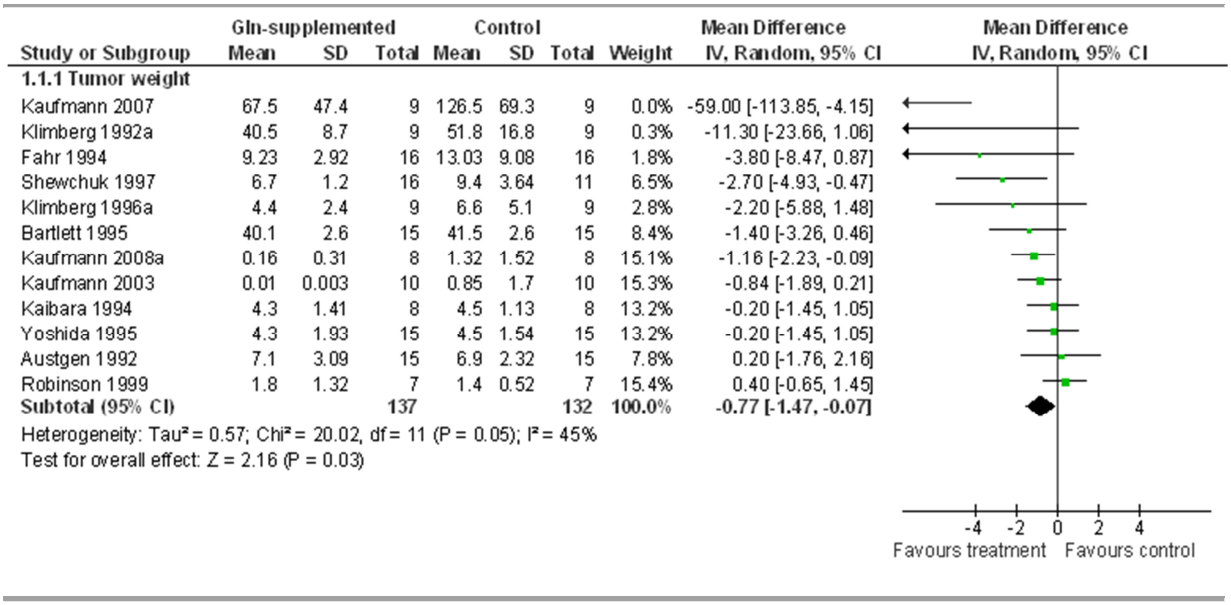


Figure 3.23: Forest plot of the effect of GLN supplementation *versus* controls on tumour weight

A sensitivity analysis of the random effects analysis model, changing to a fixed model, resulted in an MD value of -0.62 (95%CI, -1.07 to -0.16, P=0.008). In addition, changing the effect measure from MD to SMD revealed a point estimate of -0.46 (95% CI, -0.71 to -0.22, P=0.0002). According to rule of thumb[1] this can be expressed as a moderate effect (0.40 to 0.70). The overall result and conclusions were not affected by sensitivity analysis, indicating that obtained results can be regarded with a high degree of certainty.

A moderate, but significant degree of heterogeneity is present (Chi^2=20.02, df=11, P=0.05, I^2=45%, Figure 3.23). The heterogeneity within results of individual studies is clearly demonstrated by the poor overlap of 95% CI (Figure 3.23). Visual inspection of the funnel plot (Figure 3.24) indicates an asymmetrical cluster of studies at the top of the plot, with no studies at the bottom of the plot. It is probable that publication bias may be the underlying cause of this asymmetry, but even more so may the small size and poor methodological design of these animal studies introduce bias.

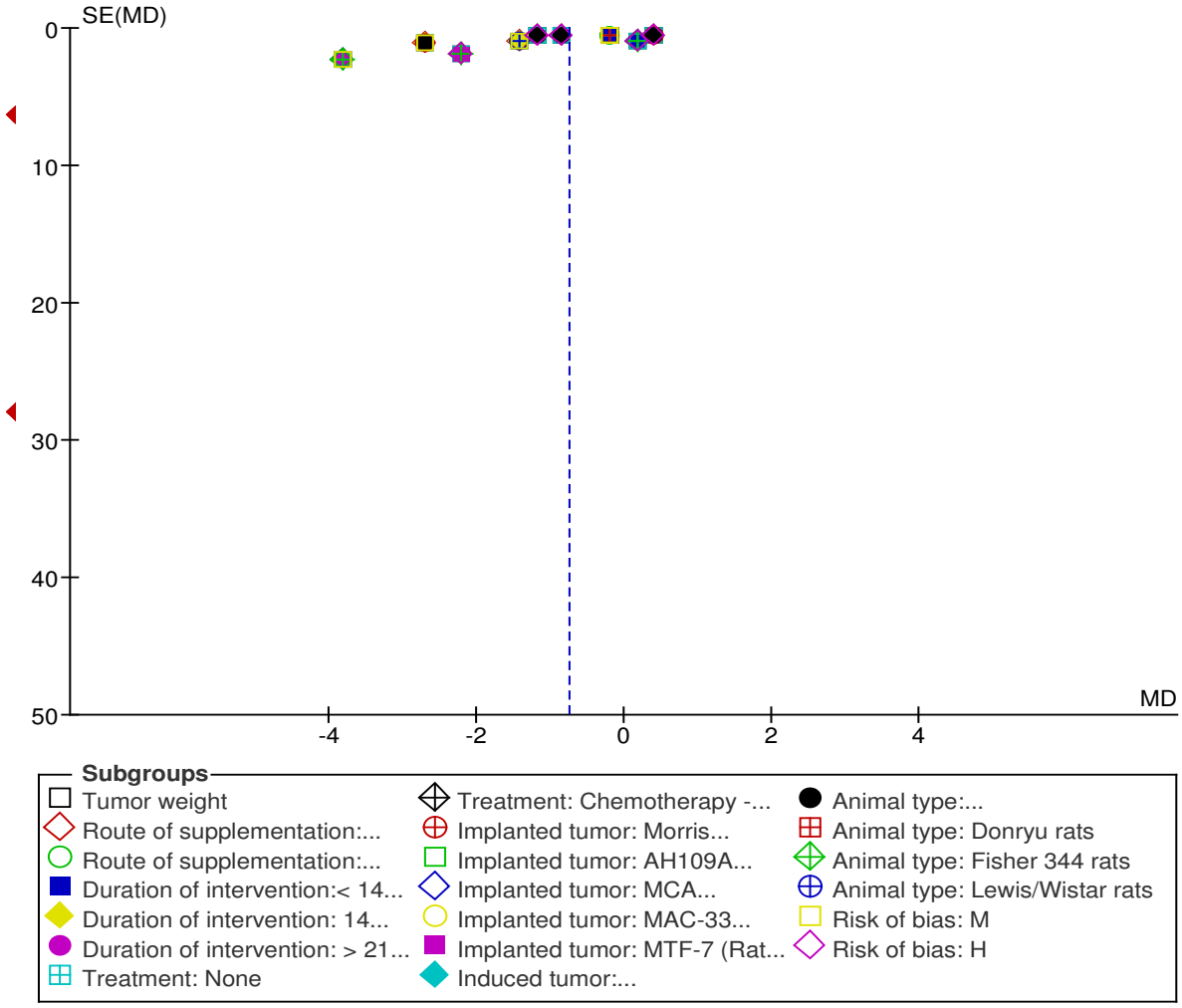


Figure 3.24: Funnel plot for animal studies using GLN supplementation (Tumour weight)

Heterogeneity was further explored through a predefined subgroup analysis (APPENDIX 6.10) to investigate whether GLN supplementation has a different effect in certain subgroups.

The subgroup for oral route (diet/gavage) of GLN supplementation retained statistical significance (-1.24 MD, 95%CI, -2.27 to -0.21, P=0.02, APPENDIX 6.10),[3-5,75,77-78,80-81,86] as opposed to the parenteral route (-0.13MD, 95% CI, -0.94 to 0.67, P=0.75, APPENDIX 6.10).[74,76,82] However, the heterogeneity remained significantly high in the oral route subgroup (Chi^2=17.84, df=8, P=0.02, I^2=55%). The subgroup for studies with the longest duration of intervention (>21 days) (-1.11 95% CI, -1.84 to -0.38, P=0.003, I^2=0%, APPENDIX 6.10)[3,5,79,86] remained significant as compared to a shorter intervention duration of 14-21 days (-2.18 MD, 95% CI, -5.04 to 0.69, P=0.14, APPENDIX 6.10)[4,75,81] and <14 days (0.03 MD, 95% CI, -0.60 to 0.67, P=0.92, APPENDIX 6.10).[74,76,78,80,82] The 95% CI of summary estimates of subgroups still

overlaps to a certain degree, indicating that the observed difference in effect between subgroups may possibly not be statistically significant. In addition the 95% CI of subgroups reaching statistical significance remains imprecise, indicating some degree of uncertainty with regard to the exact effect of GLN supplementation on tumour weight, and further investigation is warranted.

In only one study[78] the animals received chemotherapy as opposed to no treatment in the other 7 studies. Subgroups regarding risk of bias, the tumour type and animal type could not provide additional information (APPENDIX 6.10).

3.3.2.2 Tumour Volume

RevMan5 Outcome 1.2: Tumour volume

Nine (*N*=178 rats) of 10 animal studies reporting on tumour volume were included in meta-analysis.[3,5,71,77-79,86,87,90] One study did not provide sufficient data and is discussed only.[73] The data from two authors[3,71] were not published and received via e-mail correspondence.

In summary, five studies were judged to introduce a moderate risk of bias due to unclear aspects of methodological design (Table 3.13).[71,73,86-87,90] The remaining 5 studies introduced a high risk of bias due to lack of blinding.[3,5,77-79]

All studies included in the meta-analysis investigated the effect of GLN supplementation via the oral (diet/gavage) route in rats (Fischer 344 and Sprague Dawley Buffalo rats).

Six of the included studies reported a significantly lower tumour volume in the group receiving GLN supplementation as opposed to controls:

- <u>Day 21 after tumour implantation</u>
  Randomized to diets 2 days before tumour implantation
  Mean (SEM) (mL)
  (12.2 (1.3) GLN vs. 18.1 (2.3) Control, $P<0.05$)[86]
- <u>Unpublished data received from author</u> (E-mail correspondence with Suzanne Klimberg)
  Induction of tumour and randomization to diets at time 0
  Mean (SEM) (cc)
  <u>Pre-fed (-1 to +16 weeks)</u>
  (0.02 (0.00) GLN vs. 0.72 (0.43) FA, $P<0.05$)[3] Dataset included in meta-analysis

Short fed (-1 to +1 weeks)

(0.25 (0.22) GLN vs. 0.35 (0.17) FA, no P-value)[3]

Post fed (+1 to +16 weeks)

(0.00 (0.00) GLN vs. 0.75 (0.44) FA, $P<0.05$)[3]

- 11 weeks after tumour induction

  Randomized to diets one week before tumour induction

  Mean (SEM) (mL)

  (0.23 (0.15) GLN, 2.30 (0.61) FA, $P<0.05$)[5]

- Day 25 after tumour implantation

  Randomized to diets day 0

  Mean (SEM) (mL)

  (35.8 (4.7) GLN+MTX vs. 36.6 (3.0) Glycine (GLY)+MTX)[78]

  (32.4 (5.9) GLN+control vs. 32.8 (7.1) GLY+control, no P-value)[78]

  Day 40 after tumour implantation

  Randomized to supplemental GLN day 25, MTX chemotherapy day 26 and 33

  (54.2 (4.9) GLN+MTX vs. 68.5 (7.3) GLY+control, $P<0.05$)[78] Dataset included in meta-analysis

  (96.9 (11.9) GLN+control vs.95.6 (11.0) GLY+control, no P-value)[78]

- 7 weeks after tumour implantation

  Randomized to diets/gavage on day of tumour implantation

  Mean (SEM) (cc)

  (5.2 (1.0) GLN, 8.6 (2.3) FA, $P<0.05$)[79]

- Day 16 after tumour implantation

  Randomization to diets 2 weeks prior to tumour implantation. Initiation of chemotherapy injections on day 0 (CPT-11: day 0 & 7, 5-FU: day 1& 8)

  Mean (SEM) (cm^3)

  (-18.4 (5.8) % GLN vs. controls, $P<0.05$)[73]

The remaining 4 studies reported no significant difference ($P>0.05$) between the tumour volume of GLN-supplemented and control animals.

Table 3.26: Summary of results of four studies reporting no significant difference between GLN-supplemented groups *versus* their controls regarding tumour volume

STUDY ID	DETAILS	EXPERIMENTAL GROUP	CONTROL GROUP	PUBLISHED P-VALUE
Klimberg 1992 (77)	Day 26 after tumour implantation *24 Hours after MTX chemotherapy, randomized to diets day 23* Mean (SEM) (cm^3)	12.2 (2.9)	11.7 (2.3)	no P-value
	Day 27 after tumour implantation *48 Hours after MTC chemotherapy, randomized to diets day 23* Mean (SEM) (cm^3)	13.5 (2.3) dataset included in meta-analysis	12.7 (2.5)	no P-value
Rouse 1995 (87)	Day 24 after tumour implantation *Randomized to diets on day 21, randomized to MTX chemotherapy or control on day 23* Mean (SEM) (cc)	GLN+MTX 51.3 (6.3) dataset included in meta-analysis	GLY+MTX 42.3 (6.3)	no P-value
		GLN+control 51.9 (5.9)	GLY+control 42.7 (5.8)	no P-value
Rubio 1998 (90)	Day 25 after tumour implantation *Before MTX chemotherapy, randomized to diets on day 23* Mean (SEM) (cm^3)	12.2 (2.9)	11.7 (2.3)	P=NS
Xue 2007 (71) Unpublished data (E-mail correspondence with Vickie Baracos)	Day 0 *Initiation of CPT-11 chemotherapy and randomization to GLN supplementation* Mean (SEM) (cm^3)	2.39 (0.32)	2.56 (0.49)	-
	Day 5 *3 days after completion of chemotherapy* Mean (SEM) (cm^3)	1.32 (0.19)	1.42 (0.29)	-
	Day 9 *At end of study* Mean (SEM) (cm^3)	1.92 (0.26) Dataset included in meta-analysis	2.16 (0.35)	no P-value

When more than one different datasets were presented, the set representing the longest GLN supplementation duration was selected for inclusion in the meta-analysis, as indicated above. In some cases this would include GLN supplementation prior to tumour implantation/induction and after initiation of chemotherapy.

Combination of the results with a random effects analysis model and MD effect measure calculated a point estimate of -1.16 (95%CI, -2.19 to -0.12, P=0.03, Figure 3.25). These results corroborate a significantly reduced tumour volume in the GLN-supplemented animals as opposed to controls, which support the results obtained for tumour weight previously. However, caution is warranted with interpretation of these results, since uncertainty remains with regard to the precise affect of GLN on tumour volume as indicated by the wide range of values contained within the 95% CI.

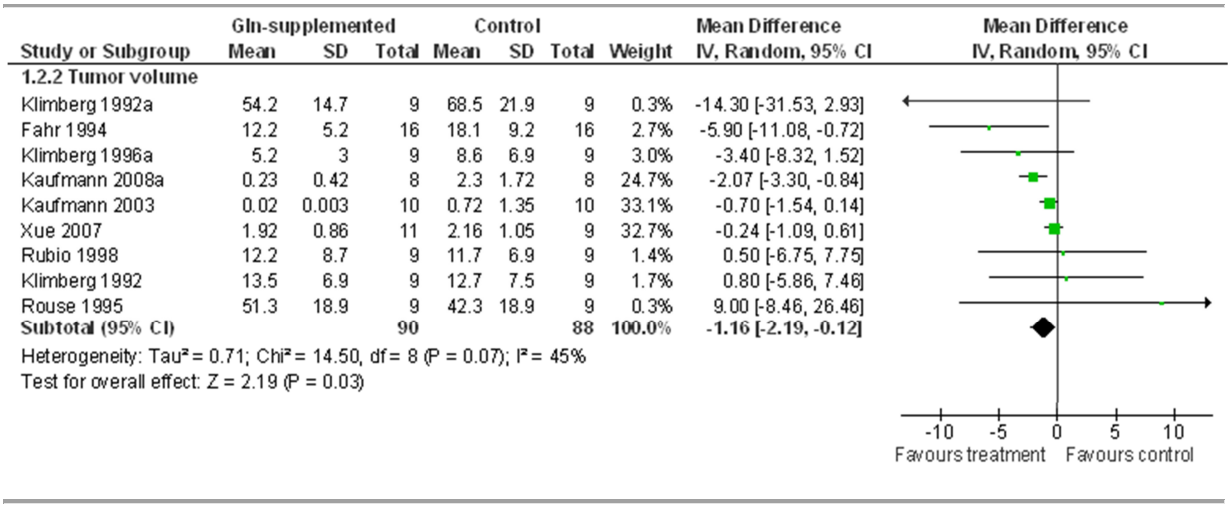


Figure: 3.25: Forest plot of the effect of GLN supplementation *versus* controls on tumour volume

Sensitivity analysis did not alter the overall results or conclusions for this outcome. A fixed effect analysis model yielded an MD of -0.85 (95% CI, -1.38 to -0.32, P=0.002). Changing the effect measure to SMD resulted in a point estimate of -0.42 (95% CI, -0.78 to -0.06, P=0.02). As a rule of thumb the point estimate indicates a moderate effect (0.40 to 0.70),[1] confirming the overall result and conclusion to be regarded with a high degree of certainty.

Moderate (I^2 = 45%), but significant (Chi^2=14.50, df=8, P=0.07, Figure 3.25) heterogeneity is evident. To explore this heterogeneity a predefined subgroup analysis was done (APPENDIX 6.10). Subgroup analysis of the seven studies which supplemented GLN at a higher dose of 1 g/kg/day, retained a significantly reduced tumour volume in the GLN-supplemented group (-1.51MD, 95%CI, -2.77 to -0.24, P=0.02, APPENDIX 6.10),[3,5,77,79,86-87,90] as opposed to the two studies exploring a lower GLN dose of 0.5 to 0.75 g/kg/day (-4.53 MD, 95%CI, -17.21 to 8.16, P=0.48, APPENDIX 6.10).[71,78] A longer GLN intervention duration (≥ 14 days ; Range: 2-11 weeks) significantly favoured the GLN-supplemented group (-2.00 MD, 95%CI, -3.61 to -0.39, P=0.01, APPENDIX 6.10),[3,5,78-79,86] as opposed to a shorter intervention duration (< 14 days) (-0.19 MD, 95% CI, -1.03 to 0.65, P=0.65, APPENDIX 6.10).[71,77,87,90] The difference between the duration of intervention subgroups may possibly be statistically significant, given that there is a poor overlap between the confidence intervals of the summary estimates on visual inspection. Tumour-bearing animals receiving no treatment gained a significant benefit from GLN supplementation (-1.76 MD, 95% CI, -3.27 to -0.25, P=0.02, APPENDIX 6.10)[3,5,79,86-87] in comparison with tumour-bearing animals receiving chemotherapy during GLN intervention (-0.25

MD, 95% CI, -1.09 to 0.59, P=0.56, APPENDIX 6.10).[71,77-78,90] The difference between summary effect in treatment subgroups was statistically significant [Qint = Q_{tot} (11.32) – (Q_1 (8.53) +Q_j (2.68)) = 0.11 vs. df (J-1=1).[1] When analyzing data according to the type of implanted/induced tumour and rat species, only DMBA-induced mammary gland tumours in Sprague-Dawley Buffalo rats reached borderline significance in reducing tumour volume in favour of GLN-supplemented animals (-1.31 MD, 95% CI, -2.64 to 0.03, P=0.05, APPENDIX 6.10),[3,5] but including the null value in the 95% CI. The same two studies were involved in both cases. Subgroups according to the risk of bias revealed that the five studies with a high risk of bias[3,5,77-79] yielded a significantly smaller tumour volume in the supplemented group (-1.44 MD, 95% CI, -2.72 to -0.15, P=0.03, APPENDIX 6.10), as opposed to studies with a moderate risk of bias,[71,86-87,90] which had no significant effect (-1.20 MD, 95% CI, -4.76 to 2.35, P=0.51, APPENDIX 6.10), also including the null value.

3.3.2.3 Tumour Volume/Weight Change

RevMan5 Outcome 1.3: Tumour volume/weight change

Five animal studies (*N*=104) reported sufficient continuous data regarding either tumour volume loss[71,77,87,90] after chemotherapy or tumour weight loss.[74] Another two studies published only percentages and comments.[72,73]

Five studies were judged to introduce a moderate risk of bias.[71-73,87,90] Two studies introduced a high risk of bias, since one author failed to explain loss to follow-up,[74] and in one study proper blinding was not carried out.[77]

Four of the seven studies reported a significant tumour volume loss in the rats receiving GLN supplementation when compared to controls after initiation of chemotherapy:

- Tumour volume loss

  Randomized to diets day 23

  Mean (SEM) (cm^3)

  Day 26 after tumour implantation, 24 hours after MTX chemotherapy

  (-2.4 (1.3) GLN vs. -0.1 (0.9) Control, P<0.05)[77]

  Day 27 after tumour implantation, 48 hours after MTX chemotherapy

  (-4.5 (1.6) GLN vs. -1.2 (0.6) Control, P<0.05)[77] Dataset included in meta-analysis

- Tumour volume loss (cc)

  Randomized to diets on day 21, randomized to MTX chemotherapy or control on day 23 Mean (SEM)

  Day 24 after tumour implantation, 24 hours after MTX chemotherapy

  (-0.8 (1.0) GLN+MTX vs. 9.5 (2.0) GLY+MTX, P<0.05)[87] Dataset included in meta-analysis

  (+5.7 (1.6) GLN+control vs. +8.4 (1.8) GLY+control, no P-value)[87]

- Tumour volume loss(cm^3)

  Randomized to diets on day 23

  Day 26 after tumour implantation, 24 hours after MTX chemotherapy

  Mean (SEM)

  (-2.4 (1.3) GLN, -0.1 (0.9) GLY, P<0.05)[90]

- Day 16 after tumour implantation

  Randomization to diets 2 weeks prior to tumour implantation

  Initiation of chemotherapy injections on day 0 (CPT-11: day 0 &7, 5-FU: day1& 8)

  "Both glutamine and *n*-3 PUFA diets significantly enhanced anti-tumour activity of CPT-11/5-FU chemotherapy as compared to control diet (P<0.05…)", data not shown.[73]

The remaining 2 studies reported similar results for both groups after initiation of chemotherapy (Table 3.27).

Table 3.27: Summary of results of two studies reporting similar results for GLN-supplemented groups *versus* their controls regarding tumour volume/weight change

STUDY ID	DETAILS	EXPERIMENTAL GROUP	CONTROL GROUP	PUBLISHED P-VALUE
Xue 2007 (73) Unpublished data from e-mail correspondence with *Vickie Baracos*	Tumour volume loss (cm^3) *Initiation of CPT-11 chemotherapy and randomization to GLN supplementation on day 0* Day 5, 3 days after completion of chemotherapy Mean (SEM)	1.07 (0.14)	1.14 (0.25)	-
	Day 9, at end of study	0.47 (0.08) Dataset included in meta-analysis	0.41 (0.21)	no P-value
Xue 2008 (72)	Reduction of tumour volume (%) *Initiation of chemotherapy injections and randomization to GLN supplementation on day 0* Mean (SEM) Day 5	-45.5 (1.5%)	-44.5 (4.4%)	
	Day 9	91.5 (8.1%) Dataset included in meta-analysis	83.2 (3.8%)	no P-value

The animals in one study reporting data on tumour weight change did not receive any treatment besides the GLN-supplemented TPN or standard TPN. The results of this study also did not reach significance:

- Tumour weight change (g) during TPN

  Mean (SEM)

  Small tumour study: Day 15 after tumour implantation

  Randomization to TPN on day 11

  (7.1 (0.8) GLN vs. 6.9 (0.6) Control, no P-value)[74] Dataset included in meta-analysis

  Large tumour study: Day 23 after tumour implantation

  Randomized to diets on day 18

  (16.7 (2.9) GLN, 18.2 (2.5) Control, no P-value)[74]

The results were combined with a random effects meta-analysis model with an SMD (Different scales of measurement) effect measure (-0.59 SMD, 95% CI, -1.30 to 0.11, P=0.10, Figure 3.26). The result was not statistically significant.

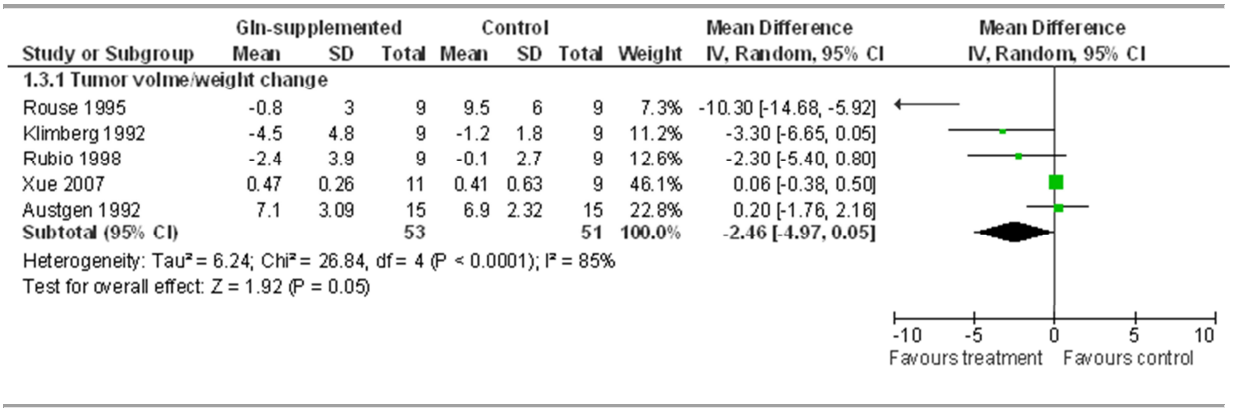


Figure 3.26: Forest plot of the effect of GLN supplementation *versus* controls on tumour volume loss/tumour weight change

Sensitivity analysis challenged the original results obtained. The fixed analysis model resulted in significant SMD value of -0.45 (95% CI, -0.86 to -0.05, P=0.03). Changing the effect measure to MD attained borderline significance with a point estimate of -2.46 (95%CI, -4.97 to 0.05, P=0.05). Further research is warranted to explore the effect of GLN supplementation on enhanced tumour volume loss during chemotherapy treatment.

There is evidence of considerable and significant heterogeneity (Chi^2=11.53, df=4, P=0.02, I^2=65%, Figure 3.26). There are not enough studies to explore this heterogeneity with a funnel plot. There were too few studies per subgroup to provide additional information (data not shown).

3.3.3 Secondary Outcomes

3.3.3.1 GLN Status – Effect of GLN Supplementation on Plasma GLN Levels in Human Subjects

Ten (23.8%, Table 3.28) of the human studies (*N*=42) reported on the GLN status of subjects after supplementation. The median (range) dose administered for these 10 studies is 24 (16-40) g GLN/day. In most cases GLN supplementation was initiated on day 1 or day -1 of either admission or underlying treatment for a duration ranging from 5 to 43 days. GLN status was reported in a wide range of units and therefore comments can only be made regarding the overall trends and significance reported in respective studies. According to 4 studies (day 14,[153] day 5,[48] day 7[104] and day 18[106]) there was no difference at baseline between the GLN and control groups, and neither did it change significantly during GLN intervention. In 6 studies (day 1, 3 & 5,[163] day 5,[59,120] day 8[29] and day 7[37,60]) the GLN level was significantly higher in the GLN group than in controls after treatment (Figure 3.27 and Figure 3.28). In one study[37] recovery of levels took place in controls as compared to GLN group on day 14 and remained that way up until day 28. Four of the studies reporting a significant change in GLN status, administered GLN via the parenteral route,[59,60,120,163] and one each via the enteral[29] and oral route.[37] One study reported no significant results, but a trend towards reduced GLN depletion in the GLN-supplemented group.[106]

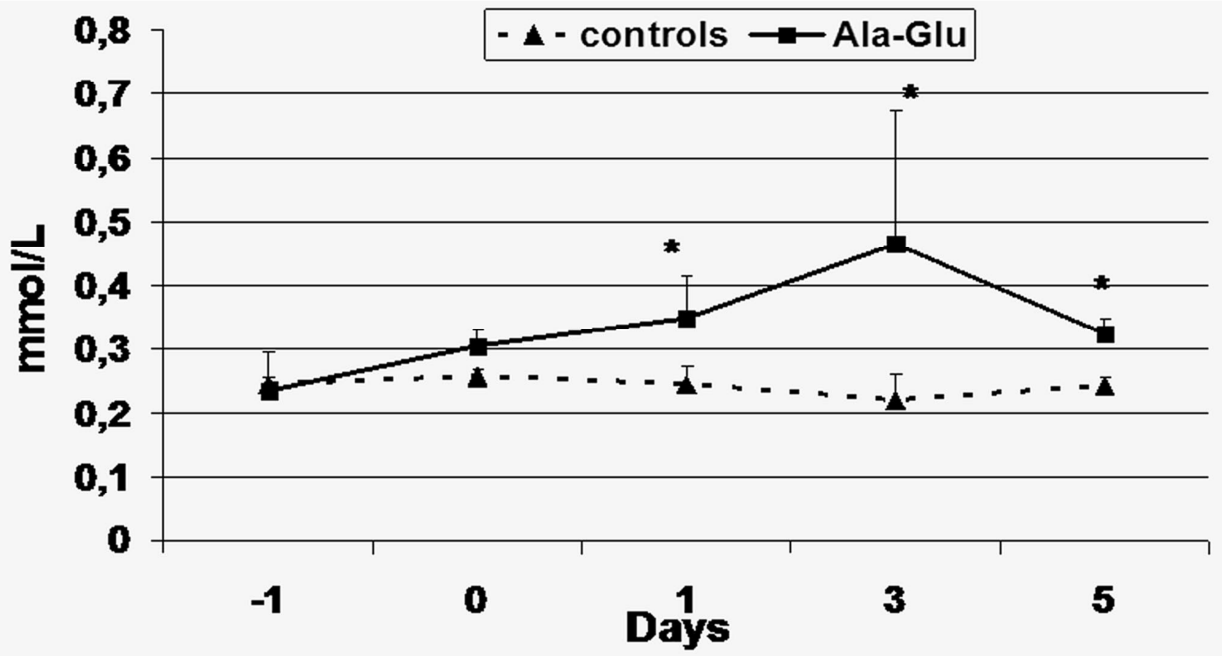


Figure 3.27: Glutamine plasma levels during the perioperative period. -1 (baseline), 0 (day of surgery), 1, 3, 5 (days after surgery); *minimum P=0.01 vs. controls.
Source:[163] Gianotti L, 2009:50.

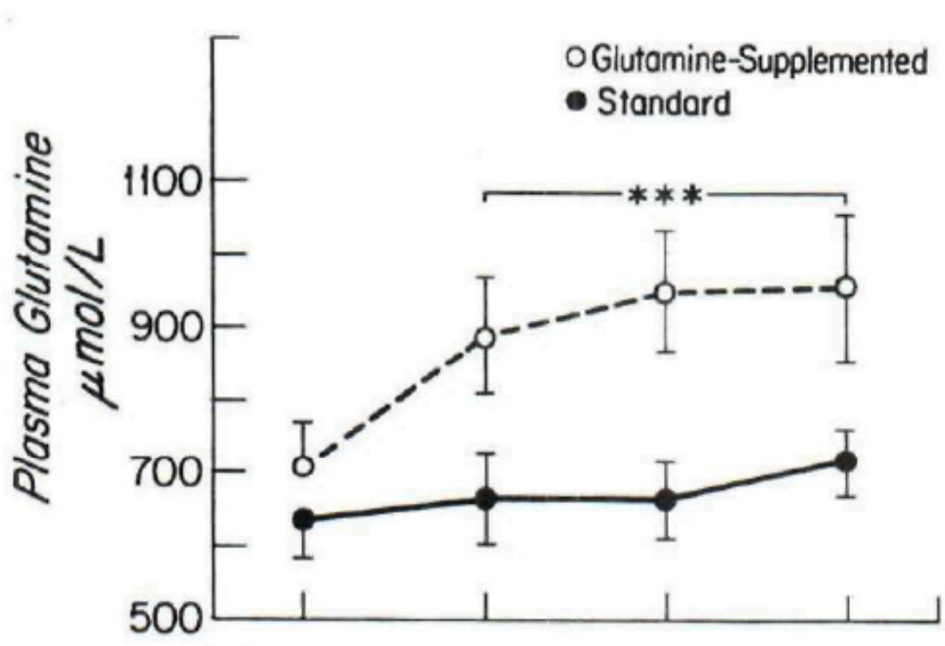


Figure 3.28: Plasma GLN concentrations over time in the initial 24 patients. GLN levels rose with GLN-supplemented nutrition (P<0.001).
Source:[60] Ziegler TR, 1992:116.

Table 3.28: GLN status of human subjects after GLN supplementation compared to control (Continued on next page)

STUDY ID (Reference number)	GLN SUPPLEMENTATION TIMING	ROUTE DOSE	MAIN RESULTS, FINDINGS OR CONCLUSIONS *Mean (SD), unless otherwise indicated*	MAIN SIMPLIFIED OUTCOME
Canovas 2000 (153)	Day of admission till day 14	**ORAL** 20 g GLN	No data presented	"GLN levels did not differ among groups, nor did they change during treatment."
Jebb 1994 (104) Serum GLN concentrations (µmol)	24 hrs prior to chemotherapy inception till 48 hrs after final infusion = 8 days	**ORAL** 16 g GLN/day	Study group: (day 5, 1st dose) Baseline: 0.53 (0.06) After dosing: NS changes (*N*=4), 1.33 (*N*=1) Control group: (day 5, 1st dose) Baseline: 0.68 (0.20) After dosing: NS changes (*N*=5) No P-value	NS changes in study or control group. "Only one patient showed 2-fold rise after 1st dose peaking at 15 min, declined with half-life of 17 min, achieving basal levels at 60 min."
Yoshida 1998 (37) Plasma levels (µmol/mL)	Day 1 of radio-chemotherapy (Rt days 1-5, 8-12, 15-20, Chemo days 1-5, 8-12) = 28 days	**ORAL** 30g GLN/day	Study group: Baseline:496.5 (22.9) Day 7: 494.2 (30.6) Day 14: 494.2 (30.6) Day 28: 466.4 (49.5) Control group: Baseline: 440.6 (42.4) Day 7: 399.5 (19.4) Day 14: 483 (30.4) Day 28: 470.3 (48.1) Day 7: P<0.05	"GLN levels in the plasma were decreased in the control patients 7 days after the initiation of radio chemotherapy, but GLN supplements prevented this reduction." "The GLN levels in the controls recovered to the levels in the GLN group on day 14 and remained the same in both groups on days 21 and 28."
Decker-Baumann 1999 (48) Plasma GLN concentrations (µmol/l)	1 day before and during chemotherapy =6 days every 4 weeks (3 cycles) = 18 days total	**TPN** 14-22 g GLN/day	Study group: Baseline: 609.3 (124.0) After 3rd cycle CT: 548.7 (65.6) Control group: Baseline: 597.5 (144.4) After 3rd cycle CT: 556.7 (71.8) No P-value	Values unchanged. NS difference.
Gianotti 2006 (163)	Preoperative day -1 to Postoperative day +5 (7 days)	**TPN** 0.40 g L-alanine-L-GLN/kg/day continuous infusion (20 hrs)	No data, only figure (Figure 3.27)	"In both groups the plasma level of GLN was below normal values (0.42 mmol/L). In treated patients, GLN level started to rise at day of surgery (day 0) compared to control group, although this difference was not significant. In the postoperative course, the plasma level of GLN increased over time in the Ala-GLN group compared to controls (minimum P=0.01). In control group, plasma GLN level did not have a significant variation during the study period."

STUDY ID (Reference number)	GLN SUPPLEMENTATION TIMING	ROUTE DOSE	MAIN RESULTS, FINDINGS OR CONCLUSIONS *Mean (SD), unless otherwise indicated*	MAIN SIMPLIFIED OUTCOME
Li 2009 (120) Plasma GLN concentration (mmol/l)	Day 1 of chemotherapy for 5 days.	**TPN** 20 g GLN/day	Study group: "GLN level significantly higher than in control group." No data, only figure presented. Control group: "Plasma GLN level decreased after chemo vs. pre-chemo, NS." P>0.05 No data, P< 0.001	"The GLN level was significantly higher in the GLN group than in control post chemo."
Stehle 1989 (59) Plasma & muscle concentration (mmol/L ICW)	Postoperative day 1 for 5 days	**TPN** ~19.6 g[a] GLN/day	Study group: Plasma Pre-op: 550.2 (16.1) Plasma Post-op: 517.2 (19.6) Muscle Pre-op: 18.9 (1.2) mmol/L ICW Muscle Post-op: 17.5 (1) mmol/L ICW Control group: Plasma Pre-op: 583.1 (26.3) Plasma Post-op: 532.1 (20.5) Muscle Pre-op: 19.7 (0.9) mmol/L ICW Muscle Post-op: 12.0 (0.6) mmol/L ICW P<0.001	"In the study group the intracellular GLN pool remained at almost the preoperative level (94.6(2.7%)), whereas in the control group it fell substantially (60.7(1.7%)). On the assumption that GLN is uniformly distributed in the muscle mass, which comprises 40% of body weight, and that muscle is 50% water, GLN depletion over 3 days would be 16.1(1.07) g in the control group and a trivial 2.12(1.05) g in the study group."
Van Zaanen 1994 (106) GLN + Glu Plasma levels (µmol/L)	Concomitantly with the chemotherapy or 1 day after BMT for 18(13-25) days	**TPN** 26 g GLN/day	Study group: 718 (507-1082)[b] Control group: 534 (395-924)[b] No P-value	NS
Ziegler 1992 (60) Plasma concentrations	Day +1 after BMT for 26(2 days	**TPN** ~40[a] g GLN/day	Study group: Rose 40% during first week of PN.	Rose significantly.
Wu 2001 (29) Plasma levels (nm/ml)	Within 48 hrs after operation for at least 7 days	**ENTERAL** ~31.2 g GLN/day (2400 ml Feed)	Study group: Baseline: 558.7 (62.5) D1: 386.0 (74.6) D8: 722.4 (116.5) Control group: Baseline: 584.1 (51.5) D1: 412.4 (65.3) D8: 555.0 (72.2) Baseline: NS D1: NS D8: P<0.05	"At baseline there was no significant difference between groups, but decreased 1day after operation in both groups. After 7 days of feeding, the study group had significantly higher plasma GLN levels."

[a] *Based on body weight of 70 kg.*

3.3.3.2 Timing, Dose, Route and Other Practice Issues Regarding GLN Administration

Route of administration

Twenty-two (52.3%, Figure 3.29, Table 3.29) of the included human studies administered GLN via the parenteral route as part of an all-in-one TPN solution (*N*=11),[59,61,106,110-111,123,134,136,142-143,145] or on its own with a vehicle (*N*=10)[48,60,98,101,109,113,120,141,163-164] supplemental towards the oral/enteral diet. In one study[140] the details regarding the make-up of parenteral solution was unclear.

The GLN-supplemented TPN was continuously infused in some studies over a period of either 4 hours,[101] 6 hours,[164] 8 hours,[48,98] 20 hours[163] or 24 hours.[111,145] The other studies did not publish details regarding infusion practices. Some authors reported using either a central[59,106,145] or peripheral[113,141] venous catheter for intravenous administration, and one reported using either of both.[163]

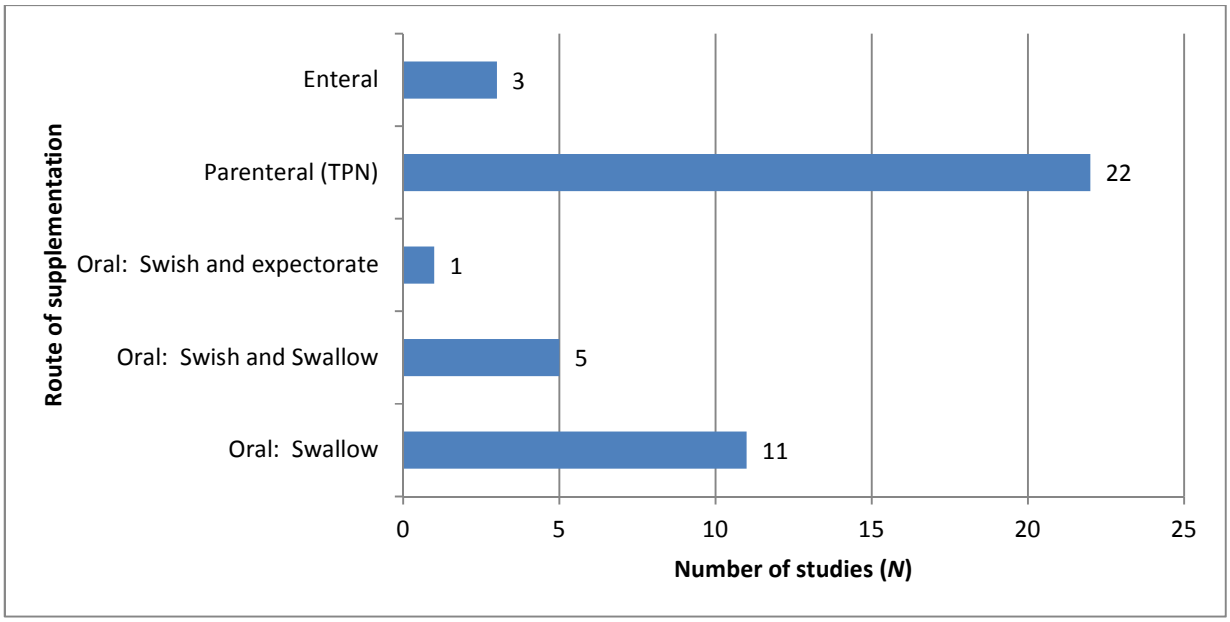


Figure 3.29: Number of studies per routes of administration.

Three studies administered a GLN-rich enteral formula, Alitraq[144] or Stresson Multi-fibre,[29,133] via a feeding tube. Nutritional goals were reached within 48[133] to 72 hours[29] following initiation of feed with a continuous flow of 90 – 100 ml per hour. In one study[144] enteral nutrition covered only 35% of daily energy requirements in addition to the hospital diet.

Oral supplementation was administered in 17 (40.4%, Figure 3.29) studies of which 16 used L-GLN powder and one Saforis.[94] Only 5 studies reported giving clear instructions to patients to swish the solution before swallowing either twice per day in the morning and evening with no relation to meals,[100,105] times 3 per day with no relation to meal times indicated[94] or times 4 per day after meals and before bedtime.[96,104] In two studies patients were instructed to remain null per os for 15 minutes[105] and 30 minutes[94] after taking the supplement.

One study instructed patients to swish and then expectorate the suspension 4 times per day after meals and before bedtime.[103]

The remaining 11 studies only reported that the supplement must be taken either twice per day,[119,130,162] times 3 per day after meals[95] or times 3 per day with no reference to meal times.[93,97,102,112] Three studies did not report how the daily dose must be divided.[37,122,153.]

The vehicle of administration varied between studies, including 5 ml Saforis,[94] or 160 ml Orasweet/Oraplus/Water (1:1:2) suspension,[100,119] or 240 ml normal saline[103] or any non-alcoholic fluids (water, juice, soup, milk, carbonated beverage, yogurt, suspension with orange flavour) of the patients' choice with a volume of 50 ml,[95] 100 ml,[112,153] 150 ml cold[96,104] or 240 ml,[130] and the vehicle being unclear in 5 studies.[37,102,105,122,162] Two studies also allowed soft/moist food for administration.[93,97]

Doses administered

For the purpose of this discussion the three enteral and one oral & expectorate studies will be grouped together with oral and oral, swish and swallow routes (*N*=20) vs. parenteral route (*N*=22).

Twelve studies (28.5%, Figure 3.30) supplemented GLN at a dose below 20 g per day (<0.28 g/kg/day), of which 9 studies were oral L-GLN, 1 oral Saforis and 2 studies parenteral L-glycyl-L-GLN dipeptide (Figure 3.31). More moderate doses of 20 – 30 g/day (0.28 – 0.42/kg/day)(Figure 3.30) were administered by 9 orally (all L-GLN) and 10 parenterally (8 L-alanyl-L-GLN, and 2 L-glycyl-L-GLN dipeptide, Figure 3.31), 45.2% in total. The highest dose L-GLN (31.2 g/day)[29] was administered enterally, so the maximum dose administered orally would be 30 g/day. The three studies,[60,111,143] supplementing L-GLN via the parenteral route, used

doses within the 30-40 g/day (0.44 – 0.57 g/kg/day) range. The other 4 studies within this range supplemented L-alanyl-L-GLN dipeptide via the parenteral route (Figure 3.31).

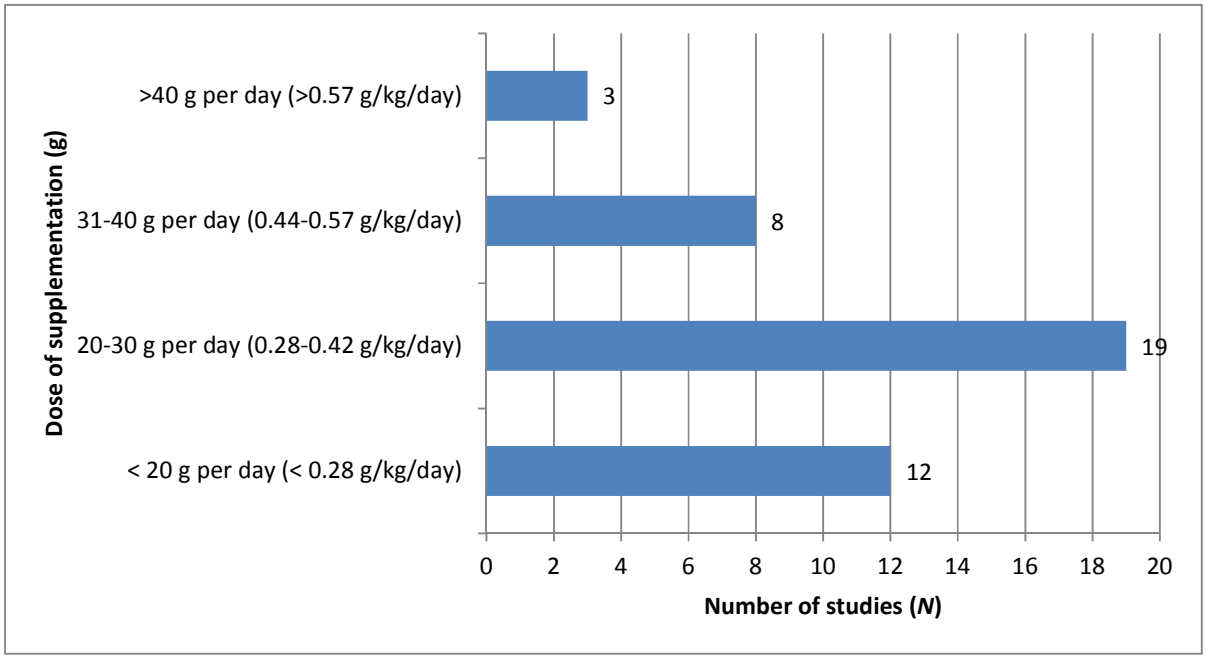


Figure 3.30: Number of studies per GLN dose administered.

The three studies in the highest GLN dose range of > 40 g/day (>0.57 g/kg/day, Figure 3.30) were all supplemented via the parenteral route as 2.0 ml dipeptiven/kg/day (L-alanyl-L-GLN),[61] 1 g L-alanine-L-GLN dipeptide/kg/day[141] and 50 g L-glycyl-L-GLN/day[140] (Figure 3.31).

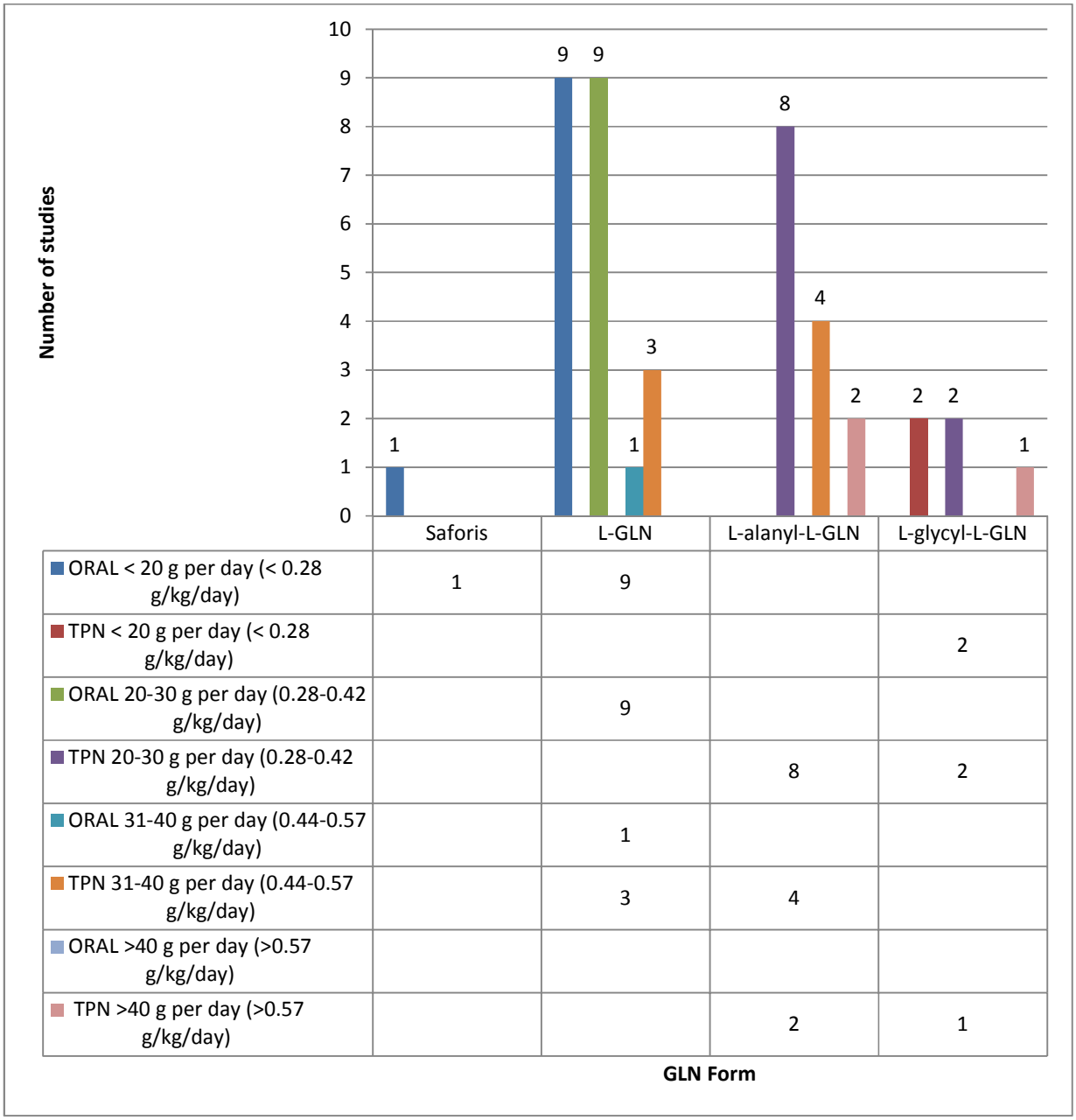


Figure 3.31: Number of studies per dose range, stratified by route and GLN form.

Timing of intervention

All 42 human studies reported the precise timing of intervention, including criteria for start and end of intervention (Table 3.29). Due to the large extent of heterogeneity in timing of interventions the total duration of interventions was stratified at 1, 2, 3 and more than 3 weeks (Table 3.5, Figure 3.32).

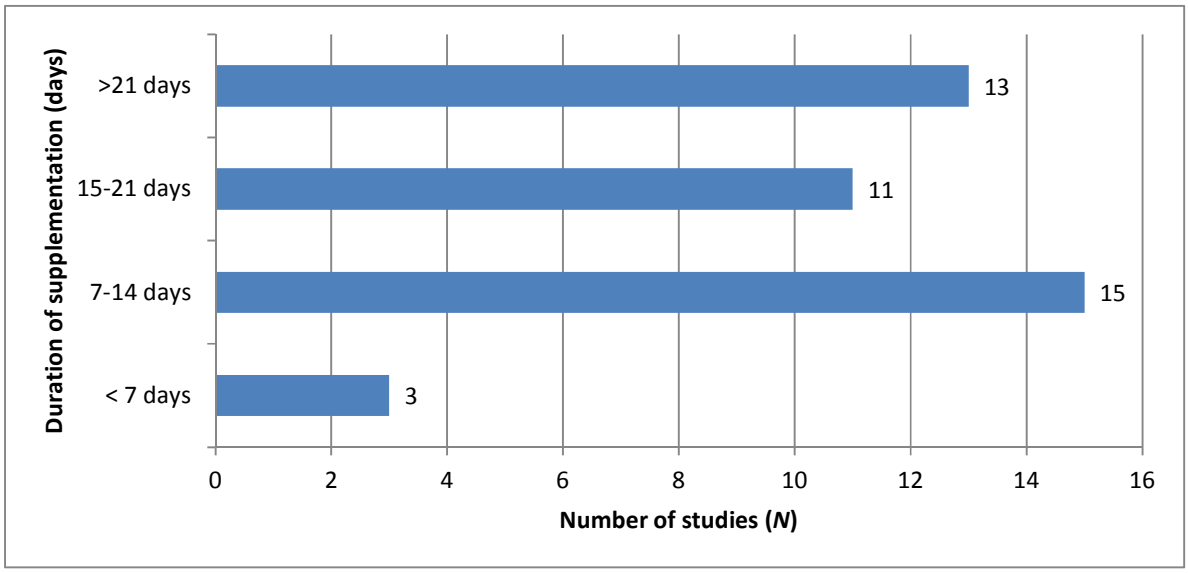


Figure 3.32: Number of studies, stratified by duration of GLN intervention.

In addition the duration was further stratified by route (oral vs. parenteral) and GLN form (Saforis, L-GLN, L-alanyl-L-GLN, L-glycyl-L-GLN) (Figure 3.33) to aid in the discussion of timing practices.

Studies (*N*=20) supplementing GLN via the oral route (oral, swish and swallow, swish and expectorate, enteral) exclusively used L-GLN (often referred to as crystalline GLN powder). Only one study used Saforis.[94] None of these oral studies supplemented GLN for less than 7 days. Six, seven and seven of the studies supplemented L-GLN via the oral route for 7-14 days, 15-21 days and > 21 days (ranging from 25 days to 24 weeks) respectively (Figure 3.33).

The remaining 22 studies supplemented either L-GLN (*N*=3), L-alanyl-L-GLN dipeptide (*N*=14) or L-glycyl-L-GLN dipeptide (*N*=5) via the parenteral route. The duration ranged from 5 days to 35 days. The studies using L-GLN all used longer duration for intervention of > 21 days (Figure 3.33). The studies using GLN dipeptides ranged from less than one week to more than 21 days, but most supplemented GLN for 7 to 14 days (Figure 3.33).

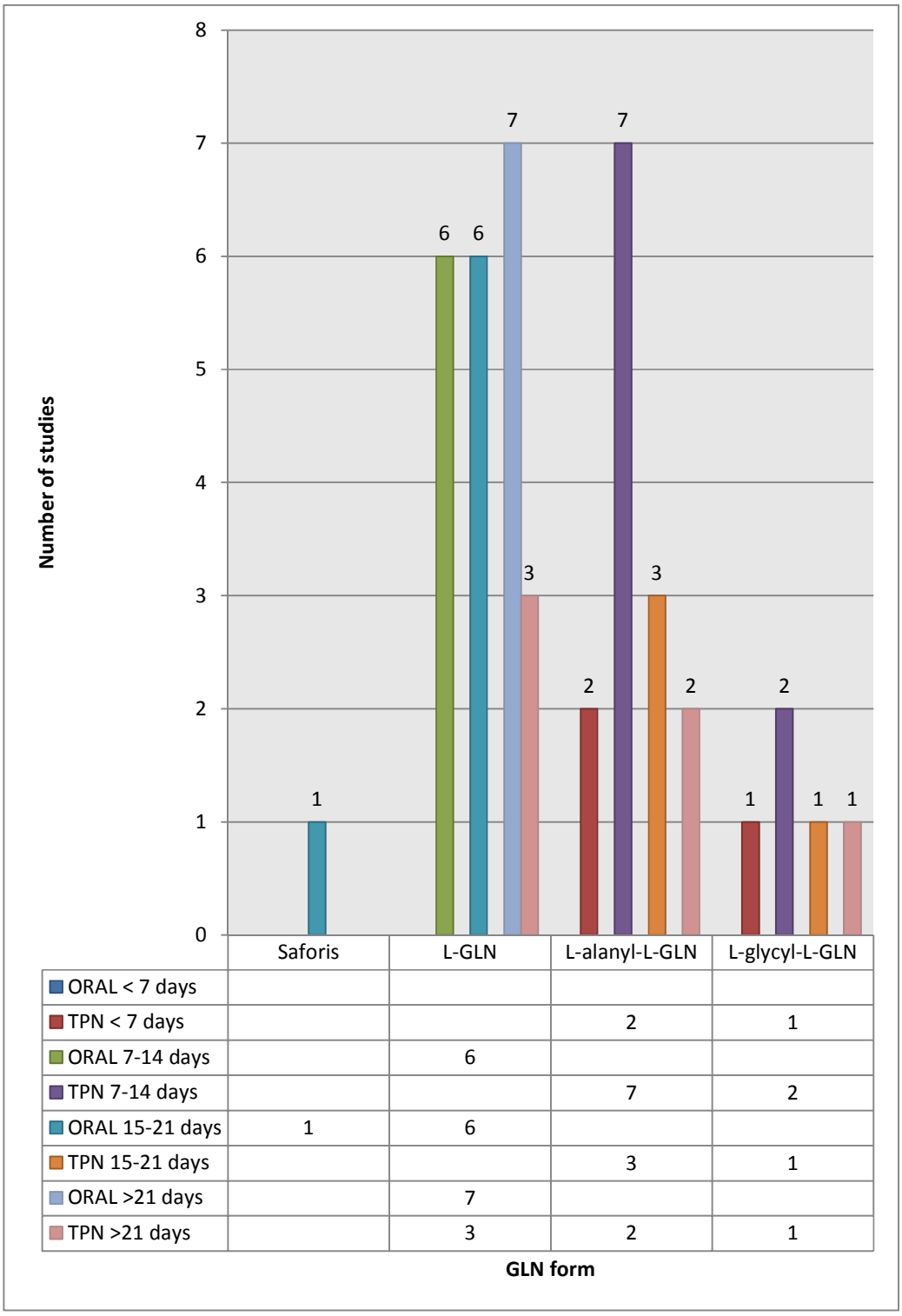


Figure 3.33: Number of studies per duration of GLN intervention, stratified by route and GNL form.

Criteria for starting and ending GLN supplementation were similar according to the underlying condition or treatment of the patients in the respective studies. For patients receiving no treatment,

GLN supplementation was started from admission to hospital or immediately after randomization in 2 oral studies.[130,162] The end criteria were stipulated as a specific number of days ranging from 15 days to 24 weeks.[130,162] In those receiving chemotherapy, oral GLN supplementation was started either one,[104] three[93] or five[102] days before commencement of treatment, or on the first day of chemotherapy.[94100,105,122] In all cases GLN intervention continued for at least the duration of chemotherapy [94,104,122] and in some for a period thereafter, ranging from 2 to 15 days.[93,100,102,104-105] In only one study was GLN supplemented in the chemotherapy-free period between cycles.[95]

GLN supplementation was initiated on the first day of RT in three oral studies,[37,103,119] lasting for the duration of RT[103] and some time thereafter.[37,119]

In BMT patients' oral supplementation was started on admission together with the preparative regime in three studies.[97,112,153] In another supplementation was started one day after BMT[96] and continued until discharge.[96,112] GLN supplementation was administered for up to 28 days in BMT patients[97] or until neutrophil recovery in one study.[153]

Enteral nutrition was only used in surgical patients, starting from either 7 days[144] in the preoperative period or the second[29] or third[133] postoperative day. Enteral nutrition was administered for a total duration of at least ten days[133] or up to seven[29] or ten[144] postoperative days.

GLN-supplemented TPN was started 1 day before[48] the inception of chemotherapy or on day one of chemotherapy initiation,[101,113120,134] continuing for at least the duration[48,113,120] of chemotherapy and some time thereafter.[101,134]

In surgical patients parenteral GLN supplementation was either started in the preoperative period for up to 5 days[141-142,163-164] or on the first postoperative day.[59,61,136] Supplementation was given for a specified number of days in the postoperative period ranging from 5 to 7 days.[59,61,136,141-142,163-164]

Patients undergoing BMT received parenteral GLN supplementation from day -7[109,140,145] in conjunction with the conditioning/preparative regime or in most cases from 1 day post BMT.[60,98,106,110-111,143] Only one study started supplementation on the day of BMT.[123] Parenteral

GLN supplementation was continued until neutrophil[109] or oral intake recovery[60,106,111,143,145] or a specified period of time ranging from 7 to 18 days[98,123,140] or discharge.[110]

Other practice issues

In two studies[103,119] patients were instructed to keep the oral suspension refrigerated and to shake it before use. The reason for this in one case was because the patients received a 2 week supply (112 g L-GLN)[119] to be mixed with vehicle (40 ml Orasweet, 40 ml Oraplus, 80 ml water) after randomization of which 8 ml (4 g GLN) was to be taken twice per day. The other[103] received a two day supply (16g L-GLN) mixed with vehicle (240 ml normal saline) to be swished and expectorated in 30 ml (2 g GLN) doses four times per day.

Sachets or foil packets were used for distribution of oral L-GLN powder supplements in some cases. The sachets usually contained L-GLN doses ranging from 3 g (single dose = 2 sachets),[102] 4 g,[96,104] 7 g[130] and 14 g.[162] One study used plastic cups for distribution of single 10 g L-GLN doses.[112] Many authors did not describe how doses were distributed for administration, but the single doses were specified as either 2 g,[100] 4 g[105] or 10 g.[93,95,97,122] Single daily doses of 20 g[153] and 30 g[37] were administered in only two studies.

Table 3.29 Timing, dose and other practice issues of included human RCTs (Continued)

STUDY ID (Reference number)	ROUTE & DOSE	MAX DOSE/DAY	GLN FORM	START	END	DURATION	OTHER PRACTICE ISSUES REPORTED
Anderson 1998 (100)	**ORAL, SWISH AND SWALLOW** 2g GLN/m^2 (4 ml/m^2) swish and swallow suspension x 2 per day (morning and evening).	4 g GLN/m^2/day	Crystalline amino acid powder (Ajinomoto USA, Teaneck, NJ)	Day 1 of chemotherapy	At least 14 days after chemotherapy	≥ 14 days	Vehicle sweetener (Ora sweet), Suspending agent (Ora Plus) GLN (500 mg/ml) mixed with Ora sweet, Ora Plus and water in a 2:1:1 ratio. Suspensions quite sweet with gritty texture similar to that experienced after brushing teeth with toothpaste.
Jebb 1994 (104)	**ORAL, SWISH AND SWALLOW** 4 g GLN x 4/day	16 g GLN/day	Powder	24 hrs prior to chemotherapy inception	48 hrs after final infusion	8 days total	Individual sachets dissolved into 150 ml water/cold fluids prior to consumption after meals and before bedtime. Patients instructed to use as mouthwash prior to swallowing.
Jebb 1995 (96)	**ORAL, SWISH & SWALLOW** 4 g GLN x 4/day	16 g GLN/day	Powder (BDH)	Day +1 post BMT	Continued until mucositis resolved or until discharged	LOS: GLN: 25.6 (2.2) Control: 28.3 (5.5)	Individual sachets dissolved into 150 ml water/cold fluids prior to consumption after meals and before bedtime. Patients instructed to use as mouthwash prior to swallowing.
Okuno 1999 (105)	**ORAL, SWISH AND SWALLOW** 4 g GLN x 2/day	8 g GLN/day	Powder (g)	Day 1 of 1st chemotherapy cycle	14 days	14 days	Advised to swish (10 seconds) and swallow. NPO 15 minutes afterwards.
Peterson 2007 (94)	**ORAL, SWISH AND SWALLOW** 2.5 g/5ml x 3 per day	7.5 g/day	Saforis (MGI Pharma, Inc. Bloomington, MN)	Day 1 of chemotherapy	Patients with no oral mucositis (OM): 14 days, Patients with OM: 5 days after resolution, or till end of treatment cycle: 21 days	~21 days	Study drug was orally swished for 30 seconds and then swallowed. Patients were instructed to refrain from eating or drinking for 30 min after dosing.

Table 3.29 Timing, dose and other practice issues of included human RCTs (Continued)

STUDY ID (Reference number)	ROUTE & DOSE	MAX DOSE/DAY	GLN FORM	START	END	DURATION	OTHER PRACTICE ISSUES REPORTED
Berk 2008 (162)	**ORAL** 14 g GLN x 2 per day. Co-interventions: 3 g HMB, 14g Arginine x 2 per day	28 g per day	HMB/Arg/GLN (Juven, MTI Biotech, Inc.)	After initial visit to institution.	For 8 weeks	8 weeks	Both placebo and HMB/ARG/GLN had an orange-drink taste. Patients took either twice a day for 8 weeks. Patients received 8 week supply of supplement in foil-sealed packets at initial visit.
Bozzetti 1997 (95)	**ORAL** 3 x 10 g GLN/day	30 g GLN/day	GLN powder	8 consecutive days during chemotherapy free period (days 5-12) between chemotherapy cycles (Day 1-4)	End of chemotherapy cycles Number of cycles median (range): GLN: 10 (3-15) Control: 10 (1-15)	~80 days total	Divided into 3 x 10 g daily doses dissolved in 50 ml cold water or other non-alcoholic fluids after the main meals.
Canovas 2000 (153)	**ORAL** 20 g GLN/day	20 g GLN/day	GLN powder (Adamin Glu, SHS, Barcelona, Spain)	Day of admission	When neutrophil count was > 500 cells/mm^3 or when TPN was required due to GI toxicity	14 days (Outcomes reported for day 1-14)	20 g GLN per day dissolved in 100 ml milk, fruit juice or water.
Choi 2007 (93)	**ORAL** 30 g GLN/day in 3 doses	30 g GLN/day	L-GLN crystalline powder (Daesang Wellife, Seoul, Korea)	3 days before inception of chemotherapy	Continued for 15 days	15 days	GLN powder is virtually tasteless, and can be mixed into any beverage or soft/moist food or water 3 x per day.
Coghlin Dickson 2000 (97)	**ORAL** 3 x 10 g GLN/day	30 g GLN/day	Pharmaceutical grade GLN powder (Ajinomoto USA, Inc, Teaneck, NJ)	First day of preparative regime (number of days unclear)	Discharge or not later than day 28 after transplant	LOS median (range): GLN: 21 (4-41) Control: 19 (3-53)	Powder mixed with liquid or soft solid food chosen by patient x 3 per day.
Daniele 2001 (102)	**ORAL** 6 sachets (18 g) per day. Two sachets (6 g) x 3/day	18 g GLN/day	Crystalline GLN powder (Bracco Pharmaceutical Company)	5 days before the first day of chemotherapy. Limited to first cycle.	15 consecutive days	15 days	Crystalline GLN powder in sachets (3 g GLN). 2 sachets x 3/day dissolved in water. No specific relation with meals was suggested.

Table 3.29 Timing, dose and other practice issues of included human RCTs (Continued)

STUDY ID (Reference number)	ROUTE & DOSE	MAX DOSE/DAY	GLN FORM	START	END	DURATION	OTHER PRACTICE ISSUES REPORTED
Kozelsky 2003 (119)	**ORAL** 4 g (8 ml) GLN x 2/day	8 g (16 ml) GLN/day	L-GLN	Day 1 or 2 of RT, for duration of RT (7 days)	2 weeks after RT or evidence of ≥ grade 3 diarrhoea	~21 days	Made up with 40 ml Ora-Sweet, 40 ml Ora-Plus and 80 ml water. To be taken morning and evening. 112 g was mixed with vehicle after randomization (2 week supply), to be kept refrigerated (Highly unstable in solution at room temperature).
May 2002 (130)	**ORAL** 7 g GLN/day x 2	14 g GLN/day Co-intervention: 3 g HMB, 14 g L-arginine.	L-GLN (Juven, Metabolic Technologies, Inc., Ames, Iowa)	Unclear	Unclear	4 - 24 weeks total	Supplement had tangy orange flavour when mixed with 240 ml water twice a day. Plain white foil packets, given in two equal doses that were mixed with 240 ml water.
Strasser 2008 (122)	**ORAL** 3 x 10 g GLN/day	30 g GLN/day	Pure GLN Powder (Baxter AG, Voletswil, Switzerland)	First day of chemotherapy	Duration of chemotherapy (at least 2 months)	74 days/11 wks (Median). Range: GLN: 1-33 Control: 2-28	30 g GLN per day in 2-3 doses in fluids (water, juice, soup, and yogurt). Patients received 4 week supply on day of each chemo cycle.
Yoshida 1998 (37)	**ORAL** 30g GLN/day	30 g GLN/day	Glutamine (Kyowa, Hakkoh, Tokyo, Japan)	Day 1 of radio-chemotherapy (RT days 1-5, 8-12, 15-20, Chemo days 1-5, 8-12)	Day 28	28 days	-
Huang 2000 (103)	**SWISH & EXPECTORATE** 2 g GLN (30 ml suspension) x 4/day	8 g GLN/day	L-GLN as crystalline amino acid powder (GIBCO-BRL, Grand Island, NY, USA)	Morning on Day 1 of first RT fraction	Bedtime of the 25th fraction of RT	Treatment time: GLN: 32 days Control: 33 days	GLN suspension (16 g L-GLN in 240 ml normal saline) Swished 30 ml (2 g GLN) for 3 minutes and expectorated before meals and at bedtime daily (4 times). Stored in refrigerator, shaking bottle before administration.

Table 3.29 Timing, dose and other practice issues of included human RCTs (Continued)

STUDY ID (Reference number)	ROUTE & DOSE	MAX DOSE/DAY	GLN FORM	START	END	DURATION	OTHER PRACTICE ISSUES REPORTED
Schloerb 1999 (112)	**ORAL** 3 x 10 g GLN/day **TPN:** 0.57g GLN/kg BW/day (2830 mg L-GLN/100 ml)	Oral: 30g GLN/day TPN: ~40[a] g GLN/day	L-GLN Oral: (Ajinonoto USA, Inc, Teaneck, NJ) TPN: Renamin (Baxter Health Care Corp, Deerfield, IL) TPN 2: TrophAmine (McGaw Laboratories, Irvine, CA)	Unclear. From randomization at admission (When TPN became necessary, patients who received GLN orally were given TPN with GLN.) (Low bacterial count diet)	Unclear. Discharge.TPN discontinued when patient consumed 50% of estimated nutrient requirements.	LOS mean (SD) days: GLN: 20.5 (15.4)	10 g GLN in small plastic cups, to be mixed with 100 ml liquid (water, carbonated beverage, fruit juice) x 3/day. TPN all in one at 100ml/h, 2.4 L/day.
Erdem 2002 (144)	**ENTERAL** 14.2 g GLN/L Co-interventions: 4.5 g arginine/L	14.2 g GLN/L	GLN-enriched elemental formula (Alitraq, 10g GLN/178g , Ross Laboratories, Columbus, Ohio)	7 Days pre-operative (for gastrointestinal malignancy)	10 days post-operative	17 days	In addition to hospital diet, 30 to 35% of daily E covered by enteral formula.
Hallay 2002 (133)	**ENTERAL** 1.3 g GLN/100 ml feed	~26 g GLN/day (2000 ml Feed)	GLN-rich Stresson Multi-fibre nutriment (Nutricia, Zoetermer)	Day 3 postoperative	Jejunal nutrition for at least 10 days. Jejunal nutrition was stopped only when per os calorie intake fulfilled patient's need.	≥10 days	A feeding tube was inserted into the second jejunum loop. Initial dose: (day 3 postop) 20 mL/h, max dose 80 - 100 mL/h. Day 5 postop: 1500 - 2000 ml , 30 ml/kg volume reached, flow of 90-100 ml/h.
Wu 2001 (29)	**ENTERAL** 1.3 g GLN/100 ml feed	~31.2 g GLN/day (2400 ml Feed)	Stresson (Nutricia) supplemented with GLN (1.30 g/100 ml). Also per 100 ml: Arg (0.89 g and omega-3 fatty acids (0.079 EPA, 0.030 DHA)	Within 48 hrs after operation.	At least 7 days	7 days total	Within 48 hrs after operation via needle catheter jejunostomy/nasogastric tube by continuous pump infusion. Diets were started at half strength 50 ml/hr. All patients reached their nutritional goals by 72 hrs after initiation.

Table 3.29 Timing, dose and other practice issues of included human RCTs (Continued)

STUDY ID (Reference number)	ROUTE & DOSE	MAX DOSE/DAY	GLN FORM	START	END	DURATION	OTHER PRACTICE ISSUES REPORTED
Blijlevens 2005 (109)	**TPN** 0.57g GLN/kg BW per day	~40[a] g GLN/day	GLN-dipeptide, L-alanyl-L-GLN (Dipeptiven, Fresenius-Kabi)	-6 days before SCT	Until bone marrow recovery (granulocyte count > 0.5 x 10^9/l), removal of CVC for any reason or because of intolerance	19 days (median duration of PN) GLN: 18.8 days Control: 17.9 days	Continuous infusion. Aminomix (Fresenius Kabi) of which a portion of amino acids has been replaced by 200 ml GLN-dipeptide.
Brown 1998 (140)	**TPN** 50 g GLN/day	50 g GLN/day	Glycl-L-GLN	Day -7 at start of conditioning	Discharge (outcomes reported up till day +18)	~25 days total	Daily infusion.
Cerchietti 2006 (101)	**TPN** 0.4 g (2 mL) GLN/kg BW/day	~28[a] g GLN/day	L-alanyl-L-GLN	During chemotherapy days (Days 1 to 5, 21 to 25, 35, 42, 49, 56)	End of antineoplastic treatment	10 days	Diluted in normal saline (1:5v/v) administered by intravenous infusion of 4 h on same days as the chemotherapy.
Da Gama Torres 2008 (123)	**TPN** 0.3-0.4 g GLN **dipeptide**/kg /day corresponding to 0.2-0.27 g **GLN**/kg/day	21-28[a] g GLN **dipeptide**/day 14-19[a] g GLN/day	L-alanyl-L-GLN dipeptide (Dipeptiven, Fresenius Kabi, Campinas, Brazil)	Day of stem cell infusion	6th day after infusion	7 days	500 ml of 10% crystalline amino acid solution with 500 ml of 50% dextrose and water, yielding a 25% concentration of glucose and a non-protein calorie/nitrogen relation of approximately 110 cal/g. GLN was added to PN.
Decker-Baumann 1999 (48)	**TPN** 0.4 g GLN peptide/kg body weight/day	14-22 g GLN/day	Glycyl-L-GLN	1 day before beginning and during chemotherapy (5 days)	Three courses of cytostatic therapy, repeated every 4 weeks	6 days every 4 wks (3 cycles) 18 days total	GLN administered as glycol-L-GLN i.v. over 8 h in a 10% solution.
Gianotti 2009 (163)	**TPN** 0.40 g/kg/day, 0.25 g of free GLN	~28[a] g/day	L-alanine-L-GLN dipeptide	Preoperative day -1	At least postoperative day +5	Mean (SD) duration: Ala-GLN: 7.1 (1.8) days	Intravenous infusion of L-alanine-L-GLN dipeptide in 500 ml 5% glucose vehicle. GLN was infused continuously through a peripheral or CVC over a period of 20 hrs

Table 3.29 Timing, dose and other practice issues of included human RCTs (Continued)

STUDY ID (Reference number)	ROUTE & DOSE	MAX DOSE/DAY	GLN FORM	START	END	DURATION	OTHER PRACTICE ISSUES REPORTED
Jo 2006 (142)	**TPN** 0.2 g GLN/kg/day	~14[a] g GLN/day	Glamin (15% amino acid solution), contains 2g/100 ml Glycyl -L-GLN dipeptide (Fresenius Kabi AG, Bad Homburg, Germany)	Day -2 Preoperative	Day + 5 postoperative (Excluding day of operation)	7 days total	10 ml Glamin (15% amino acid solution)/kg/day administered parenterally. TPN formula (30 kcal/kg/day with 1.3 g/kg per day amino acid).
Klek 2005 (61)	**TPN** 2.0 ml GLN/kg/day	~140[a] ml GLN/day	GLN-dipeptide (Dipeptiven, Fresenius-Kabi) L-Alanyl-L-GLN	24 hrs post-operative	At least 7 days or until enteral diet covered > 60% of protein & energy requirements	Time of PN mean (range) days: GLN: 8.5 (7-10) Control: 8.7 (7-11)	Standard PN supplemented with IV GLN. PN prepared as all-in-one admixtures.
Li 2009 (120)	**TPN** ~ 0.3 g/kg BW/day	20 g GLN/day	Alanyl-GLN dipeptide (Dipeptiven; Fresenius Kabi, Bad Homburg, Germany)	Day 1 of chemotherapy for 5 days.	Continued for 5 days	5 days	Prophylactic intravenous alanyl GLN dipeptide.
Marton 2010 (164)	**TPN** 0.5 g/kg/day	~35[a] g/day	Alanyl-GLN dipeptide (Dipeptiven; Fresenius Kabi)	Preoperative day -3 (Enteral nutrition (No GLN) initiated on day+1 for both groups)	Post-operative day +7 or until discharge from ICU	10 days	Continuous intravenous infusion for 6 hours.
Oguz 2007 (141)	**TPN** 1 g/kg/day (Hospital diet, enteral nutrition)	~70[a] g GLN/day	L-alanine-L-GLN dipeptide (Dipeptiven, Fresenius Kabi, Germany)	At least 5 days preoperative	At least 5 days postoperative	≥ 10 days GLN pre-op: 6 (2) days GLN post-op: 5 (1) days	Parenteral (via peripherally) GLN throughout duration of enteral nutrition.
O'Riordian 1994 (136)	**TPN** 0.18 g/kg/day	~12.6[a] g GLN/day	Dipeptide glycyl-GLN in amino acid solution (Glamin, Kabi Pharmacia, Erlangen, Germany)	Day +1 postoperative, 11 A.M. the morning after surgery.	Postoperative day +6	5 days	GLN supplemented (17% of nitrogen) amino acid solution with lipid emulsion and glucose with 0.2 g nitrogen/kg/day and 122 kJ/kg/day.

Table 3.29 Timing, dose and other practice issues of included human RCTs (Continued)

STUDY ID (Reference number)	ROUTE & DOSE	MAX DOSE/DAY	GLN FORM	START	END	DURATION	OTHER PRACTICE ISSUES REPORTED
Piccirillo 2003 (110)	**TPN** Study 1: 20 g L-GLN/day Study 2: 13.46 g L-GLN/day	Study 1: 20 g GLN/day Study 2: 13.46 g GLN/day	Study 1: Parenteral amino acid solution, Glamin (Fresenius Kabi) containing 30.27 g Glycyl-L-GLN Study 2: Dipeptiven (Fresenius Kabi) containing 20 g alynyl-GLN.	Day +1 after aPBSCT	Discharge	LOS median (range): GLN: 28 (23-70) Control: 27 (23-39)	Amino acid solution (containing GLN) added to standard TPN–bag.
Pytlik 2002a (98)	**TPN** 30 g dipeptide (20 g L-GLN)/day	30 g GLN/day	Alanyl-GLN dipeptide (Dipeptiven: Fresenius Kabi, Bad Homburg, Germany)	Day +1 post PBPC transplant	At least day +14 or discharge	LOS mean (SD) days: GLN: 13.8 (3.1) Control: 11.8 (2.2)	GLN dipeptide and non-glutamine amino acid solutions were dissolved in 900 ml normal saline. Administered for 8 hours daily.
Scheid 2004 (134)	**TPN** 30.27 g dipeptide (20 g L-GLN)/day	20 g GLN/day	Glycyl-GLN-dipeptide (Glamin, Baxter, Erlangen, Germany)	Criteria: Oral intake, % weight loss, central venous line in place/not	When oral intake ≥ estimated REE	Duration of TPN Median (Range) days: GLN: 13 (5-34) Control: 14 (3-34)	TPN solutions compounded in sterile bags.
Scheltinga 1991 (143)	**TPN** 0.57g GLN/kg BW/day (Low bacterial diet)	~40[a] g GLN/day	Crystalline L-GLN (Ajinomoto USA, Raleigh, NC)	BMT day +1	Oral intake > 50 % of E requirements	27(1 days	Commercially available solution + crystalline L-GLN.
Schloerb 1993 (111)	**TPN** 2830 mg GLN/100mL, 0.57 g GLN/kg BW/day (Low bacterial count diet)	~40[a] g GLN/day	Free L-GLN (Ajinomoto USA Inc, Teaneck, NJ)	Day +1 post BMT	When oral intake > 50% of nutritional requirements	Duration of PN mean (SEM), days: GLN: 30 (5) Control: 31 (3)	Combination of Renamin (Rich in essential L-amino acids) and free L-GLN as all-in-one at 100 ml/h (2.4L/day) The solution was sterilized by ultrafiltration because of degradation of GLN by heat and was stored at 5°C for up to 6 weeks without the loss of more than 5% of the GLN.

Table 3.29 Timing, dose and other practice issues of included human RCTs (Continued)

STUDY ID (Reference number)	ROUTE & DOSE	MAX DOSE/DAY	GLN FORM	START	END	DURATION	OTHER PRACTICE ISSUES REPORTED
Sornsuvit 2008 (113)	**TPN** 30 g GLN/day	30 g GLN/day	L-alanyl-L-GLN dipeptide (Dipeptiven, Fresenius Kabi Thailand, Bangkok, Thailand).	Day 1 of chemotherapy initiation	Consecutively for 5 days in each chemotherapy cycle (6 cycles)	Duration of TPN mean (SD) days: GLN: 16.5 (13.5) Control: 10.0 (9.0)	Placement of peripheral venous catheter performed on day of chemotherapy initiation. IV supplementation with 30 g/day GLN.
Stehle 1989 (59)	**TPN** 280 mg GLN/kg/day	~19.6 g[a] GLN/day	Dipeptide Ala-GLN	Postoperative day 1	Postoperative day 5	5 days	CVC. TPN supplemented with dipeptide Ala-GLN.
Sykorova 2005 (145)	**TPN** 0.5 GLN/kg BW/day	~35[a] g GLN/day	L-alanyl-L-GLN dipeptide (Dipeptiven, Fresenius Kabi)	Ad hoc: Next day after oral intake became inadequate. Prophylactic: Starting with the cytoreductive regime.	PN stopped after the leucocyte count grew over 1 x 10^9/l and oral intake became adequate again.	Unclear: LOS mean: 22 days	STD PN + commercially available GLN dipeptide in all-in-one bag. Infused continuously (24hrs/day) into CVC.
Van Zaanen 1994 (106)	**TPN** 40 g GLN/day (26 g free GLN)	40 g GLN/day	L-alanyl-L-GLN dipeptide	Concomitantly with the chemotherapy or 1 day after BMT.	When neutrophil count reached 0.5 x 10^9/l	TPN duration median (range) days: GLN: 18 (13-25) Control: 20 (11-25)	Administered through triple lumen CVC. TPN with Ala-GLN as all-in-one.
Ziegler 1992 (60)	**TPN** 0.57 g GLN/kg BW/day	~40[a] g GLN/day	Amino acid solution containing free L-GLN	Day +1 after BMT	Enteral consumption of > 50% of requirements for 3 consecutive days	TPN duration mean (SEM) days: GLN: 26 (2) Control: 28 (1)	*At libitum* food intake from a standardized diet with low bacterial content foods allowed.

[a]*Based on 70 kg adult; BW body weight.*

CHAPTER 4: DISCUSSION

4.1 GENERAL

The vast number of published randomized controlled trails and other study types (the latter excluded from this review) indicates the uncertainty, but moreover possible clinical importance regarding the use of supplemental GLN in the oncology setting. The 42 human trials included in this review have recruited a total of 2687 participants (mostly adults) and the 18 experimental studies included 441 animals (exclusively rats). This is the first systematic review to provide a data synthesis (APPENDIX 6.11) regarding the effect of GLN supplementation on clinical outcomes such as mortality, long term survival, length of hospital stay, body weight change and clinical infection and the duration and severity of diarrhoea in human subjects with cancer as well as *in vivo* tumour growth parameters (tumour weight, volume and volume/weight change) including unpublished data received from several authors via e-mail correspondence. In addition an updated data synthesis, including new evidence on the effect of GLN supplementation on the prevalence, severity and duration of mucositis in cancer patients, is presented and compared with results of previous reviews.

The majority of the human studies included in this review was of parallel design, recruiting participants from mainly single centres. The few multi-centre trials recruited the largest numbers of patients. For all 60 included studies, the country of conduct, financial support and particular design of studies have varied. There is a lack of trials investigating the effects of GLN supplementation (both oral/enteral and parenteral) in patients with a particular type of cancer (haematological, head and neck, breast, gastrointestinal and other) also receiving comparable treatment regimes on clinically important outcomes. Although the patient population was heterogenic, there is evidence of plasma GLN depletion in this population and certain side effects are common across treatments. In addition the dose of GLN supplementation as well as the GLN form (free L-GLN or dipeptide), timing and duration of intervention have varied across studies. This has resulted in high levels of inconsistency and significant heterogeneity in the results of the review, which limit the strength of the evidence and the ability to generalize to defined patient populations. Animal studies used a variety of rat species and tumour models, introducing high levels of inconsistency in results between studies, rendering evidence of low quality.

Unfortunately many of the studies complying with all inclusion criteria for this review did not present data in a usable form for inclusion in meta-analysis. Unpublished data were, however, requested and received from 12 authors. With regard to publication bias, studies with results in both directions have been published and included in this review. However, there were too few

(ranging from 3 to 14) studies in each meta-analysis to reasonably detect any existing publication bias for all of the outcomes concerned. Publication bias was explored, using a test for funnel plot assymmetry only when there were at least 10 studies included in the meta-analysis. When there are fewer studies, the power of the test is too low to distinguish chance from real assymmetry.[1] It is accepted that a certain degree of publication bias will exist, since unpublished trials and studies published in foreign languages have been excluded from this review and, furthermore, most studies had small sample sizes introducing bias caused by small study effects.

Furthermore, the assessment of mucositis incidence and severity is a subjective modality, even if the assessor used objective criteria. The appearance of mucositis and oral candidiasis is similar,[166] and may easily be mistaken by assessors who are not trained or experienced in the diagnoses of these lesions, affecting the validity of measurements. No referral was made to validating measurement techniques or criteria other than using a single person to do all assessments, or using a "trained" nurse. In some cases, the outcome assessment was based on the patient's own report of pain, presence of sores and ability to eat. Scores of mucositis were not always defined, but there was consistency in the number of categories used to describe the severity of mucositis, with the lowest score indicating no mucositis. The different scales/criteria used to dichotomize the severity of mucositis and diarrhoea are explored and summarized for clarity (APPENDIX 6.8).

The findings of this review should be considered in context with general medical and nutritional management of patients with cancer. This review has been split into two parts, one evaluating clinical outcomes and safety in patients with cancer, and the other investigating the growth of the tumour in tumour-bearing animals.

4.2 PRIMARY OUTCOMES

Mortality

This review indicates that GLN supplementation in patients with cancer had no effect on mortality during intervention, based on evidence provided by 16 studies including 1 523 participants. It is important to note that the upper 95% CI around the effect estimate on mortality approximates 1.37, suggesting the possibility of a worse outcome in the GLN group. Six studies[104,111-112,122,142,163] reported more deaths (1 to 2) in the GLN group than in control, but this was not statistically significant in the individual studies and it was rather associated with patients with advanced metastatic disease and multiple health problems in the GLN group or other reasons not associated with GLN supplementation. The diagnoses of the study population varied, but included

mainly gastrointestinal and haematological malignancy. Cancer treatment regimes varied from BMT, surgery and chemotherapy to some receiving no anti-cancer treatment at all. The dose and duration of intervention included wide ranges, using oral, enteral and parenteral routes. The sensitivity and subgroup analysis did not alter the results and conclusions. This is the first summary data regarding GLN and mortality in oncology patients.

In comparison with a meta-analysis of surgical and critically ill patients receiving GLN supplementation, these findings do not agree with the apparent association with reduced mortality (RR of 0.78 (95% CI, 0.58 to1.04) in the systematic review by Novak.[39] However, when Novak separated the result for critically ill patients from surgical patients, the association diminished ("no effect in surgical patients" (0.99 RR, 95% CI, 0.27 to 3.58), "associated with a trend toward reduced mortality in critically ill patients" (0.99 RR, 95% CI, 0.27 to 3.58)).[39]

The quality of the body of evidence is downgraded to being low, considering that several aspects of methodological designs was unclear in some of the studies and three studies included co-interventions. Two of the studies had a change in protocol due to unforeseen reasons. In addition to the risk of bias introduced by these methodological issues, the wide confidence interval indicates imprecision of the results obtained and uncertainty about the true effect of GLN on mortality. The funnel plot asymmetry further suggests the possibility of publication and other bias being present.

Survival at follow-up

Six studies reporting on survival beyond day 100 in 260 patients with mainly haematological malignancies receiving BMT, did not provide enough evidence to either support or refute that GLN is more or less effective than controls in reducing risk of death beyond day 100. Even though most of the studies had a low risk of methodological bias, the quality of evidence is seriously questioned due to considerable and significant levels of heterogeneity and inconsistency in the results of the individual studies unexplained by subgroup and sensitivity analysis. In addition the 95% CI was wide, indicating that we have little knowledge about the precise effect of GLN on long-term survival. The upper 95% CI for survival beyond 100 days approximates 1.19, indicating a possible worse outcome for the GLN-supplemented group. Findings of a Cochrane review investigating nutrition support for bone marrow patients reported that the results for oral GLN vs. placebo (1.51 OR; 95% CI, 0.88 to 2.6, P=0.13) and parenteral GLN vs. placebo (0.69

OR, 95% CI, 0.16 to 2.97, P=0.62) for survival up to day 100 post BMT were not significant.[148] This is in agreement with the results of the current review.

Length of hospital stay (LOS)

The length of hospital stay was not affected by GLN supplementation. The mean LOS in the GLN-supplemented group was on average 1.68 days (P=0.10) lower than in the controls, but the upper limit of the rather imprecise 95% CI suggested an apparent worse outcome for those receiving GLN supplementation. A significantly reduced LOS was obtained with subgroup analysis for higher doses >30 g GLN per day (-4.51 MD, P =0.0002) and a longer duration of intervention >21 days (-5.09 MD, P<0.0001). However, the quality of evidence has been downgraded due to imprecision of results (95% CI is wide, including 0% difference in effect) and the difference in summary effect between subgroups appear not to be statistically significant. Funnel plot asymmetry suggests that publication and other bias could, to some extent, account for some of the apparent benefit observed with respect to a reduced length of stay. Furthermore, considerable heterogeneity and inconsistency in results exist, with one study[98] reporting a significant increase in length of stay for those receiving GLN.

These results correlate with systematic results obtained for surgical and critically ill patients, which indicated an association with a reduced hospital stay of 2.6 days (95% CI, -4.5 to -0.7) in those receiving GLN, but also indicating significant levels of heterogeneity.[39] After subgroup analysis, GLN supplementation in surgical patients was associated with significantly reduced LOS (-3.54 days, 95% CI, -5.3 to -1.76), but not for the critically ill. A meta-analysis of the impact of GLN dipeptide on outcomes in elective surgical patients also found a significant shortening of LOS to be associated with GLN dipeptide therapy (-3.25 days; 95% CI, -4.87 to 1.62, P<0.0009).[168] Findings of a Cochrane review[148] investigating nutrition support for bone marrow patients reported that the results for oral GLN vs. placebo (-2.39 MD; 95% CI, -6.11 to 1.34, P=0.21) and parenteral GLN vs. placebo (0.22 MD; 95% CI, -1.29 to1.72, P=0.78) for hospital duration were not significant, and also indicated the possibility of a worse outcome for GLN-supplemented groups.[148]

Body weight change

The mean change in body weight was on average +0.63 kg more in the GLN group compared to controls, although this effect was not significant. The 95% CI was imprecise, also including the possibility of a worse outcome with GLN supplementation. In addition considerable and significant

heterogeneity exists within these results. Subgroup analysis could in part explain some of the heterogeneity observed, but overall the difference in effect between subgroups was not significant and very much conflicting. A statistically significant reduction in weight loss was observed in the GLN-supplemented group for the 4 studies supplementing a higher dose of GLN (21 – 30 g GLN/day, P=0.0003, APPENDIX 6.11) and also a shorter duration of supplementation ($\leq$ 21 days, 4 studies, P=0.02, APPENDIX 6.11). In contrast significantly more weight loss was observed in the subgroup including 2 studies supplementing the highest GLN dose (31 – 40 g GLN/day, P=0.03). Those receiving chemotherapy and/or RT in three studies as opposed to no treatment, surgery or BMT also concluded a significantly reduced weight loss in favour of the GLN group (2.37 MD, P=0.0006, APPENDIX 6.11). The quality of this evidence has been downgraded to low, based on the unexplained inconsistency and heterogeneity in results of individual studies and the imprecision of results obtained in addition to certain vague aspects of methodological design that may introduce a moderate to high risk of bias. In conclusion there is insufficient evidence to either support or refute that GLN supplementation is more or less effective than controls in preventing weight loss in cancer patients.

These results are in agreement with the findings of a Cochrane review[148] investigating nutrition support for bone marrow patients, reporting that the results for oral GLN vs. placebo (5.73 MD; 95% CI, -7.09 to 18.55, P=0.38) and parenteral GLN vs. placebo (-0.34; 95% CI, -1.40 to 0.72, P=0.53) for mean body weight change (%) from start to end of study were not significant and inconclusive.

Clinical infection

Currently the evidence from 12 studies, including 906 participants, is not sufficient to either support or refute that GLN supplementation is more or less effective than controls in reducing the incidence of clinical infection, but at least there is a trend at the 10% level (P=0.13) and further research on the number of cancer patients developing clinical infectious complications is warranted. The current evidence is of low grade, since some aspects of methodological quality remain unclear and in addition the apparent inconsistency in individual study results remain unexplained by subgroup analysis. Heterogeneity is, however, not significant and the 95% CI is fairly precise. The indirectness of poorly defined clinical infection as an outcome measure in some studies further necessitates the downgrading of the current evidence. The study population included mainly haematological, gastrointestinal (mixed, gastric, colon) and other mixed cancer diagnoses receiving mainly BMT or surgery and chemotherapy in one study. Most (11out of 12)

supplemented GLN via the parenteral route and used a wide range of GLN dose and the duration of GLN intervention also varied considerably. Subgroup analysis did not provide any additional information regarding the effect of GLN supplementation on clinical infection outcome.

There is no published summary finding with respect to GLN and its effect on clinical infection in oncology patients. In contrast with the results presented here, a systematic review[39] of surgical patients (0.36 RR; 95% CI, 0.14 to 0.92) and critically ill patients (0.86 RR; 95% CI, 0.68 to 1.08) reported a significant reduction in infectious complication rates when these results were aggregated (0.80 RR, 95% CI, 0.64 to 1.00, P=0.03).[39] The test for heterogeneity was also not significant for this meta-analysis.[39] A significant reduction in infectious complications in elective surgical patients receiving parenteral supplemented dipeptide was reported by Jiang[168] (0.42RR, 95% CI, 0.24 – 0.72, P<0.002), again with no significant heterogeneity in results. This result shows that patients with cancer are a unique entity, since the same results could not be obtained as for surgical and critically ill patients.

Mucositis

Oral and gastrointestinal mucositis is a common complication of drug and RT for cancer and may impact on the effectiveness of treatment because severe mucositis possibly will lead to dose reductions, increases in health care costs and may impair the quality of life of the cancer patient.[166] Several previous systematic reviews[57,117,148,169] have published results of meta-analyses regarding mucositis. All of these reviews have reported that there was insufficient evidence to either support or refute that GLN was more or less effective than controls for prevention of mucositis formation at any level of severity.

The current review provides new evidence, including additional RCTs[93-94,102,113] not cited in previous reviews. A significantly reduced formation of $\geq$ Grade 2 mucositis is indicated by the summary effect estimate for 9 studies, including 727 participants with a relative risk reduction of 24%, challenging the conclusions of previous reviews. The studies included in the meta-analysis incorporated a wide range of cancer diagnoses, but mainly chemotherapy and/or RT and only 1 study included patients with BMT. A wide range of GLN dosage of up to 30 g/day and a wide range of duration of GLN intervention ranging from at least 7 to >21 days were described in the individual studies. Only two (out of six) studies supplemented GLN via the parenteral route. After subgroup analysis the results remained significant for studies supplementing GLN at doses below 21 g/day (P=0.01, 5 studies, APPENDIX 6.11) and studies with a moderate risk of bias as

opposed to low risk of bias (P=0.04, 7 studies). The difference in the summary effects between subgroups was, however, not significant. Subgroup analysis for cancer diagnoses, treatment and duration of intervention could not provide additional information.

Six studies reported dichotomous data on the incidence of a maximum grade mucositis defined as either at least grade 3 or grade 4 mucositis. The aggregated result was not significant, but there was a trend (P=0.10) associating GLN supplementation with a reduced formation of maximum grade mucositis. Subgroup analysis of this data pool pointed toward a significant reduction of maximum grade mucositis with the parenteral route (2 studies, P=0.05, APPENDIX 6.11) and a longer (> 14 days) duration of intervention (4 studies, P=0.003, APPENDIX 6.11). Severe mucositis only develops later on in treatment; and that might explain why a significant reduction was only evident after 2 weeks of supplementation.

GLN supplementation was not associated with a shorter duration of mucositis based on data from 3 RCTs. In fact the summary effect indicated a longer duration of 1.05 days in the GLN-supplemented group. The result of the meta-analysis is inconclusive, but in addition to these 3 studies another 7 studies reported some data on duration of mucositis, of which only 2 studies[100,103] reported a significantly reduced duration in the GLN-supplemented group and the rest showed similar results for both groups.

The quality of evidence is downgraded due to the imprecision of the results as indicated by the wide 95% confidence intervals, the unexplained inconsistency and significant heterogeneity in the results of individual studies and the elusive methodological aspects of some of the studies. There is not sufficient evidence to either refute or support that GLN supplementation is more or less effective than controls in the prevention of maximum grade mucositis or reducing the duration of mucositis of any severity. These results are in agreement with results from previous reviews. A possible explanation for these results was proposed to be the insufficient delivery of GLN to the damaged tissues of the oral mucosa, as GLN has moderate solubility and undergoes nonenzymatic degradation under physiological conditions.[94] This may well be the case, but this matter has not been explored in any of the other studies included in this review. The significant results obtained with parenteral GLN subgroups may perhaps support such reasoning. There is, however, weak, but significant evidence that GLN supplementation may reduce the formation of clinically significant ($\geq$ grade 2) mucositis in oncology patients.

Diarrhoea

GLN supplementation is associated with a significantly reduced duration of diarrhoea (≥ grade 1) after aggregation of data of 4 studies, including 130 participants. The duration of diarrhoea is shortened by 1.26 days compared to controls. In contrast to these findings the data synthesis on the number of patients presenting with ≥ grade 1, ≥ grade 2 diarrhoea and ≥ grade 3 diarrhoea did not provide sufficient evidence to either support or refute that GLN supplementation is more or less effective than controls in preventing diarrhoea at any level of severity. The quality of evidence has been downgraded due to imprecision of the 95% CI and unexplained heterogeneity in results of individual studies in addition to certain unclear aspects of methodological design. These results are the first of its kind and suggest a possible role for GLN supplementation in reducing the duration of diarrhoea in cancer patients receiving treatment.

Tumour growth (Tumour weight, tumour volume and tumour weight/volume change)

Several narrative reviews [16,49-51] summarized preclinical data from experimental tumour-bearing animal models investigating the effect of GLN supplementation on tumour growth and metabolism. Although none of them embarked on meta-analysis, all of them concluded that there is no *in vivo* evidence to support that supplemental GLN causes a more aggressive tumour behaviour, like it is the case *in vitro*.[52] In fact, various animal models suggest the opposite, that supplemented GLN are actually associated with suppressed tumour genesis and tumour growth and possibly may enhance the tumouricidal effect of cancer therapy. The aggregation of data from experimental rat models included in this review associated GLN supplementation with a significantly slower tumour growth (reduced tumour weight and volume) in rats. The mean tumour weight was on average 0.77 g lower in the GLN-supplemented group as compared to controls. This result was statistically significant (9 studies, 178 rats, P=0.03). Subgroup analysis (APPENDIX 6.11), although the difference in effect between subgroups were not statistically significant, indicated a pronounced effect with the oral/diet/gavage route (7 studies, P=0.02) and longer duration (≥ 21 days, 9 studies, P=0.003). The mean tumour volume in the GLN group was on average 1.16 less compared to controls. This result was also statistically significant (9 studies, 178 rats, P=0.03). This effect was more pronounced, and statistically significant between subgroups, with a higher GLN dose (1 g GLN/kg/day, 7 studies, P=0.02), a longer duration (≥ 2 weeks, 5 studies, P=0.01) and for rats receiving no treatment (5 studies, P=0.02) as compared to those receiving chemotherapy. Another subgroup showing a larger, but still significant effect of GLN supplementation on tumour volume was the amount of risk introduced by methodological design. Studies introducing a high

risk of bias as opposed to a moderate risk of bias had a larger effect (1.44 cm^3 less) in the GLN-supplemented group as compared to controls. The mean tumour volume/weight change in the GLN group was on average 2.46 more (5 studies, 104 rats, APPENDIX 6.11) than in the controls, reaching borderline significance (P=0.05).

The evidence is estimated to be of low quality based on the imprecision of 95% confidence intervals and unexplained heterogeneity in results of some individual studies. In addition the methodological quality of studies is questionable, mainly due to poor reporting of methodology regarding allocation and blinding.

With the discrepancy between tumour weight/host weight ratio and cell kinetics in experimental versus human tumours, it is difficult to extrapolate laboratory findings to the clinical setting.[95] In addition preclinical studies showing various beneficial/protective effects of GLN supplementation in experimental rat models over the past two decades have been reported. These same effects have, however, not been shown successfully in clinical outcomes in human trials. A possible explanation for this may be the low quality of experimental studies overestimating the treatment effects. Few authors describe the methods and precautions taken to prevent the introduction of bias in their findings. The methodological design of experimental studies with regard to at least allocation concealment, adequate sequence generation, blinding, addressing incomplete outcome data and selective reporting remains elusive for most. It is possible that the data from these studies may be substantially distorted by experimental bias.[161] It has been reported previously that at least half of the 44% improvement in outcome of a stroke drug could be attributed to experimental bias, especially the failure to randomize to the allocation, failure to conceal treatment allocation to the surgeon or a failure to blind the assessment of outcome. Similar observations have been made for other experimental models where nonrandomized studies and studies without blinded assessment of outcome appear to give a relative overstatement of efficacy of 27% and 19% respectively.[161,170] Furthermore, it is expected that publication bias may play an even bigger role, with experimental studies showing little or no effect not being published. These experimental data should therefore be interpreted with caution, especially since the studies included in the current review almost 100% lack allocation concealment and blinding of caregivers (persons administering the drug) and outcome assessors. In addition it is unclear whether an adequate method of randomization has been used in those reporting to have “randomized” the rats to the study groups.

4.3 SECONDARY OUTCOMES

GLN status

There is evidence of reduced GLN plasma levels in cancer patients at baseline before initiation of treatment.[37,163] GLN supplementation significantly increased plasma GLN levels as compared to controls in six studies, of which four were supplemented via the parenteral route. The greatest effect is reported in the first week of supplementation. One study indicated that recovery of plasma levels in the control group took place by week two as compared to the GLN group, and remained that way up until day 28. Another three studies reported no significant difference at day 14 and 18. These results suggest that there might be significantly reduced GLN depletion within the first week of GLN supplementation during cancer treatment, but thereafter there does not seem to be any evidence of a significant difference in the plasma GLN levels of those receiving GLN supplementation and controls. This might explain why subgroups with a shorter duration of supplementation show significant benefits in some outcomes tested in this review. A pronounced effect was demonstrated after subgroup analysis of glutamine supplementation via the parenteral route, corresponding with the significant results obtained in enhanced plasma GLN levels via this route.

Route of administration

The route of administration is often determined by the setting, treatment regime and condition of the patient and needs to be adapted according to medical nutrition therapy protocol. A large proportion (52.3%) of human studies used the parenteral route for GLN administration, either as part of TPN or supplemental to oral or enteral diet. This review provided no significant evidence that parenteral route was superior to oral or enteral route, as suggested by several systematic reviews[21-22,39,41] in the critically ill. It is, however, noted that significant results were obtained for the parenteral route only after a subgroup analysis for route (oral vs. parenteral) investigating the effect of GLN supplementation on the number of patients presenting with $\geq$ 3 grade oral mucositis (P=0.05). In animal studies the reduced tumour weight was more pronounced in the oral route subgroup (P=0.02). However, in both cases the difference in summary effect between subgroups was not significant. There were too few studies per route reporting on the same outcome to determine whether swishing and swallowing is more beneficial than just swallowing the oral suspensions.

Dose administered

Forty-five percent of the studies in this review administered a moderate GLN dose of 20 to 30 g GLN/day (0.28–0.42 g/kg/day). Nineteen of the 22 parenteral studies administered a GLN dipeptide, and usually at a higher dose than the free GLN used for oral supplementation. The highest dose supplemented via the oral route was 30 g per day.[37,93,95,97,122] The highest dose supplemented via the enteral route was 31.3 g per day[29]. The highest dose administered via the parenteral route, was also the highest dose for this review (1 g alanyl-L-GLN dipeptide/kg/day, ~70 g/day).[141]

Subgroup analysis per dose showed a significant result for LOS only in the studies which supplemented GLN at a dose above 30 g/day (> 0.42 g/kg/day). Body weight loss was significantly less in the studies administering GLN at a dose of 21 to 30 g (0.3 -0.42 g/kg/day), but the weight loss was significantly more in the high dose subgroup (31-40 g/day; 0.44 – 0.57 g/kg/day). After subgroup analysis only the studies administering doses below 21 g/day (<0.30 g/kg/day) remained to have a significantly reduced incidence of $\geq$ grade 2 oral mucositis. However, the difference between summary estimates of subgroups was not statistically significant. These results are rather conflicting, as one would expect a dose response, but clearly this is not the case presented here, at least not the same for all outcomes.

The current recommendation for GLN supplementation in critical illness is 0.2-0.4 g/kg/day of L-GLN (0.3-0.6 g/kg/day alanyl-GLN dipeptide)[44] and for burns doses of up to 0.57 g/kg/day is recommended.[41] Significant reduction in at least grade 2 mucositis and the duration of diarrhoea was obtained with doses ranging up to 30 g/day (0.42/kg/day); therefore the effective dose will possibly be within the same range. No adverse effects was reported by any of the included trials, except for the trial by Pytlik,[98] which warns against a significantly worse outcome in some cases.

Timing of GLN supplementation

The timing of GLN intervention was determined by the underlying cancer treatment regime. None of the oral studies supplemented L-GLN for a duration less than 7 days. The number of days of oral supplementation ranged from 7 days to 24 weeks with an equal distribution between 1-2 weeks, 2-3 weeks and >3 weeks. TPN was supplemented for periods ranging from 5 to 35 days, but most supplemented GLN for a period of 7 to 14 days. It appeared to be general practice to start supplementation on the day of admission to the hospital for a period of up to 7 days before

the start of treatment in the case of BMT and surgery. For patients receiving chemotherapy and/or RT, the intervention was started on the first day of treatment and continued for at least the duration of treatment and in some cases until up to 15 days thereafter. In surgery patients supplementation is given up to 7 days in the preoperative period and/or from the second postoperative day for at least 7 to 10 days. The study characteristics are too heterogenic to comment on which approach to the timing of intervention would provide the most benefit. The difference in effect between the respective subgroups for the duration of intervention was not significant. The LOS was only significantly reduced in the subgroup administering GLN for more than 21 days. In contrast, body weight loss was only significantly reduced with interventions of less than 21 days. The incidence of maximum grade mucositis was only significantly reduced when GLN was supplemented for more than 14 days. In animal studies, reduced tumour weight and volume was pronounced in subgroups with longer duration of supplementation ($\geq$ 21 days and $\geq$ 14 days respectively). In critical illness it is recommended that GLN is supplemented for at least 5 days[40] and in burns for at least 2 to 3 weeks after injury.[41] There is little evidence for GLN supplementation of less than 7 days. The results of this review definitely lean towards a greater benefit after at least 14 days of supplementation, during which the plasma GLN levels seem to be most effected as well.

4.4 OVERALL COMPLETENESS AND QUALITY OF EVIDENCE

This systematic review, including 42 human studies of 2687 participants, confirms that GLN supplementation in doses of up to 0.57 g/kg/day in oncology patients receiving no or some treatment may be regarded as safe with no significant adverse effects. There is a lack of evidence focusing on GLN supplementation in specific cancers receiving comparable treatment regimes. The overall quality of evidence is low, since the review includes heterogenic data (regarding the patient diagnoses and treatment, the route, the dose and duration of GLN administration) and several aspects of methodological design of individual studies are uncertain. The lack of intention-to-treat analysis has introduced a high or at least moderate risk of bias in some studies. The results must further be downgraded due to the apparent imprecision of results (wide confidence intervals) obtained. The inconclusive and conflicting results of the individual studies and the unexplained heterogeneity in some further call for caution with the interpretation of results obtained in this review. Subgroup analysis suggests a larger effect for dose and duration of intervention in some cases, but the difference between subgroups appears not to be significant. A meta-regression might provide more information regarding subgroup analysis.

At best this review identified weak and unreliable evidence that GLN may be effective in reducing the duration of diarrhoea, but in contrast there was insufficient evidence to either support or refute that GLN is more or less effective than controls in reducing prevalence and severity of diarrhoea. Low-grade evidence also showed a reduced prevalence of severe mucositis ($\geq$ grade 2) in the GLN-supplemented group, but could not show a significantly reduced duration of oral mucositis or reduced formation of maximum grade mucositis. In addition weak and unreliable evidence indicated a trend towards a reduced length of hospital stay (especially at doses above 0.42 g/kg/day and for a duration of at least 3 weeks), less clinical infections (inconclusive subgroup analysis), and reduced incidence of maximum grade mucositis only when GLN was supplemented for more than 2 weeks. However, there is no strong evidence to either refute or support that GLN is more or less effective than controls in reducing deaths during intervention or until 3 year follow-up, neither in decreasing weight loss, or in reducing the duration of mucositis, or in preventing the formation of $\geq$ grade 3 mucositis. These results were compared with recent systematic reviews looking at interventions for treating oral mucositis in patients with cancer,[117,169] nutrition support for BMT patients [57,148] and GLN supplementation in serious illness.[39]

The systematic review of 18 animal studies, including 441 rodents, provides significant evidence that GLN supplementation of up to 2 g/kg/day decreases the tumour weight and tumour volume in tumour-bearing rats receiving chemotherapy or no treatment at all. Subgroups with longer duration of intervention (>21 days for tumour weight and > 14 days for tumour volume) had a more pronounced and precise effect, although the difference between subgroups were not significant. In addition this review provides evidence (based on results of 3 studies only) that GLN supplementation significantly increases tumour volume/weight loss after chemotherapy. However, this evidence is of low quality at best due to unexplained heterogeneity and inconsistency in results of individual studies and the imprecision of observed results. In addition the methodological quality of these experimental trials is questionable and may to some extent account for some of the apparent benefit observed. These results must be viewed as hypothesis generating, warranting further research regarding the mechanism of action of GLN in reducing tumour growth. These results are in agreement with current narrative reviews,[16,49,70] suggesting that GLN is primary fuel for natural killer cells and has a dichotomous effect on tumour growth via immunological and other pathways.

4.5 STRENGTHS AND LIMITATIONS

A major strength of this review is the incorporation of unpublished data from 12 studies. A major limitation of this review is the introduction of publication bias by excluding foreign language reports in selection criteria. The exclusion of results from these studies may affect the precision of the results obtained in a systematic review.[1] In addition only electronic databases were searched and no hand-searching was done of journals not listed on these databases as suggested by the Cochrane collaboration for systematic reviews.[1] The majority of studies had a small sample size, recruiting participants from a single site, introducing bias related to small study effects. However, within the context of this Master's thesis, within the limitations of available time, manpower and funding, this was thought to be acceptable. These limitations should be considered when interpreting the results of this review.

CHAPTER 5: CONCLUSIONS

5.1 CONCLUSIONS

The present data indicate that we know little about the precise effect of GLN supplementation in oncology. Currently there is only weak evidence suggesting a significant benefit in terms of a reduced incidence of clinically significant or severe mucositis and a reduced duration of diarrhoea in those at risk. There is a trend towards a reduced length of hospital stay, especially with higher doses and longer intervention periods. Another trend towards reduced infectious complications is also suggested, but not significant. There appears to be no significant indication of harm, since GLN supplementation was not associated with adverse effects, except for 3 studies[98,111,145] reporting an apparent worse outcome, cautioning against the routine use of GLN supplementation in oncology patients. It is, however, important to note that the upper 95% CI of aggregated data suggested a conspicuous worse outcome for GLN-supplemented patients with regard to mortality, long-term survival, LOS and clinical infection which are worrying. Literature indicates speculation regarding the effect of GLN on the growth of the tumour as opposed to benefit for the host, since it is clear that GLN is one of the primary and preferred fuels of tumour cells. Any nutrition provided to the patient will probably support the metabolism of the host and tumour. Whether exogenous supplementation of GLN stimulates more aggressive tumor growth in humans remains unclear. The animal data included in this review presents evidence that GLN supplementation results in a significantly reduced tumour weight and volume and increases the tumour volume/weight loss after chemotherapy. The reasons for this need to be further explored and were not part of the scope of this review. However, evidence of bias overestimating these effects in experimental studies caution against extrapolating these results towards the clinical setting and further well designed experimental trials with proper allocation concealment, sequence generation, blinding and powered group sizes are needed to clarify these effects.

The results presented here have further implications and considerations, especially in developing countries where funds are in short supply. Given the apparent lack of clinical benefit in terms of LOS and clinical infection rate in GLN-supplemented patients with cancer, it is likely that money will be wasted on expensive GLN-supplemented feeds in patients with cancer, especially in certain study populations where GLN supplementation was associated with an apparent worse outcome and even higher costs attributed to a longer stay, more antibiotics and the cost of the GLN dipeptide itself,[98] with the possibility that we are feeding the tumour as well.[33] However, Macburney[146] presented evidence of a cost saving in BMT patients (using data from Ziegler 1992[60]) due to the clinical benefits observed in these patients, while Sornsuvit[113] reported no significant difference in costs between groups. The cost saving mainly realizes from reduced

charges for room and board.[11] A reduced LOS will therefore translate into reduced costs, but that remains to be seen (and associated with GLN supplementation) in patients with cancer in general.

Another important consideration in oncology is the quality of life of the patient. Alleviating the painful and devastating effects of mucositis and diarrhoea might impact on the quality of life and mood[171] of patients. The significant benefits of GLN on these outcomes require consideration by practitioners.

5.2 IMPLICATIONS FOR PRACTICE

- A word of caution: The results of this review are based on RCTs including mainly adult patients. This review does not provide evidence for the safety of GLN supplementation in the long-term beyond 24 weeks via the oral route and 35 days via the parenteral route. In addition this review does not warrant the safety of GLN supplementation via any route without medical supervision or inclusion in a clinical trial. The upper limit of the 95% CI for mortality, long-term survival, LOS and clinical infection suggest a possible worse outcome. This trend is worrying and does not warrant routine GLN supplementation in this population.
- GLN supplementation via the parenteral route at doses of up to 1 g alanyl-GLN dipeptide/kg/day (70 g/day) for 10 days or 0.57 g L-GLN/kg/day for periods of up to 30 days is regarded as safe in oncology patients receiving treatment.
- GLN supplementation via the oral/enteral route at doses of up to 0.42 g L-GLN/kg/day (30 g/day) for periods of up to 10 weeks are regarded as safe in oncology patients receiving treatment
- There is weak evidence that GLN (L-GLN or dipeptide) supplementation at doses of 0.22-0.42 g/kg/day (16-30 g/day) for 14-21 days via oral or parenteral route in oncology patients with haematological malignancies, solid tumours or colorectal cancer may reduce the duration of diarrhoea in those receiving chemotherapy or BMT with/without chemotherapy and/or RT.
- There is weak evidence that GLN (L-GLN or dipeptide) supplementation at doses of 7.5-30 g/day (0.1-0.42 g/kg/day) for 8-21 days via oral or parenteral route in oncology patients with head and neck cancer, gastrointestinal cancer, breast cancer, haematological malignancy and solid tumours of different sites, may prevent the formation of severe oral mucositis ($\leq$ grade 2) in those receiving chemotherapy or BMT with/without chemotherapy and/or RT.
- GLN supplementation in tumour-bearing animals at doses of up to 2 g/kg/day for periods of up to 3 weeks may reduce the tumour weight and tumour volume and increase tumour volume/weight loss after chemotherapy.

- There is no strong evidence to either support or refute that GLN supplementation is either more or less effective than placebo/controls in: reducing deaths during intervention and up till 3 years follow-up; reducing the length of hospital stay; reducing body weight loss; reducing number of clinical infections; reducing the duration of mucositis; preventing formation of $\geq$ grade 3 mucositis or reducing the number of patients presenting with $\geq$ grade 1, 2 or 3 diarrhoea.

5.3 IMPLICATIONS FOR RESEARCH

- Authors must report principle aspects of methodological design which impact on the quality assessment of trials, especially allocation concealment, sequence generation, blinding of participants, caregivers and outcome assessors, percentages and reasons of dropouts and withdrawals and how these were addressed in analysis of results (ITT).
- Guidelines for good laboratory practice to be applied in experimental trials, and reported as such to aid in systematic review and meta-analysis of pre-clinical results. The optimal dose and timing for reducing tumour weight and volume in tumour-bearing animals should be further investigated as well as the underlying mechanisms for this phenomena.
- Future research should aim at translating the six foreign language studies identified as possibly eligible for inclusion (APPENDIX 6.4). The data of these RCTs may improve the strength and precision of results obtained in this review.
- Hence the observed safety of GLN supplementation, further research is warranted regarding the efficacy of GLN supplementation in oncology patients. Future research should aim at designing multi-centre, parallel design studies, including more specific patient populations in sufficient numbers to detect differences between intervention groups. The exact timing of GLN intervention providing the most clinical benefit relative to the treatment being received should be investigated. Well definded clinical outcomes should be the primary outcome of such trials.
- Clinical infections must be well defined in future trials, reporting number of patients with clinical infections as opposed to total number of infections per group.

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APPENDIX 6.1: Ethics Approval

UNIVERSITEIT·STELLENBOSCH·UNIVERSITY
jou kennisvennoot • your knowledge partner

10 March 2008

Ms E Van Zyl
Dept of Human Nutrition

Dear Ms Van Zyl

RESEARCH PROJECT: "GLUTAMINE SUPPLEMENTATION IN ONCOLOGY: A SYSTEMATIC REVIEW"

PROJECT NUMBER : N08/01/012

My letter dated 4 February 2008 refers.

At a meeting that was held on 5 March 2008, the Committee for Human Research ratified the approval of the above project by the Chairman.

Yours faithfully

FRANKLIN WEBER
RESEARCH DEVELOPMENT AND SUPPORT (TYGERBERG)
Tel: +27 21 938 9657 / E-mail: fweb@sun.ac.za

Copy to Supervisor/ Head of Department: Prof DL Labadarious

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Fakulteit Gesondheidswetenskappe • Faculty of Health Sciences

APPENDIX 6.2: Phase II - III Study Selection Outcome of 300 Full Text Reports (Continued)

INCLUDED IN SYSTEMATIC REVIEW	EXCLUDED PHASE III	EXCLUDED PHASE II

HUMANS (Y=YES, N=NO)	STUDY **INCLUSION** CRITERIA:											STUDY **EXCLUSION** CRITERIA						**INCLUDED or EXCLUDED**
	STUDY POPULATION			**OUTCOMES**														
	A **YES** needed in **EVERY** column			A **YES** needed in **AT LEAST ONE (or more)** row for inclusion								A **YES** in **ANY ROW** indicates exclusion						
STUDY ID ↓	Humans with cancer	GLN supplement	English language	GLN status	Mortality	Survival	Body Weight	Length of stay	Clinical infection	Mucositis	Diarrhoea	Outcomes not considered	Foreign language	Review article	Abstracts, letters, editorials, comments	No full text in SA, Not RCT	Not RCT (Phase III)	
Alexander 1990														Y				EXCLUDED
Alivizatos 2005	Y	Y	Y		Y			Y	Y							Y	Y	EXCLUDED
Algara 2007	Y	Y	Y														Y	EXCLUDED
Alpers 2006														Y				EXCLUDED
Alschuler 2008															Y	Y		EXCLUDED
Alvarez 2003														Y				EXCLUDED
Anderson 1998	Y	Y	Y							Y								INCLUDED
Anderson 1998a	N	Y	Y		Y	Y			Y	Y								EXCLUDED
Aosasa 1999	Y	Y	Y	N	N	N	N	N	N	N	N	Y						EXCLUDED
Aquino 2004	Y	Y	Y												Y			EXCLUDED
Aquino 2005	N	Y	Y		Y			Y	Y	Y								EXCLUDED
Arends 2006														Y				EXCLUDED
Arfons 2005														Y				EXCLUDED
Barndregt 2005														Y				EXCLUDED
Benes 2002	Y	Y	N				Y						Y					EXCLUDED
Benes 2002a	Y	Y	N								Y		Y					EXCLUDED
Berk 2008	Y	Y	Y		Y		Y											INCLUDED
Bertz 2008													Y	Y				EXCLUDED
Biganzoli 1996	Y	Y	Y								Y				Y			EXCLUDED
Bistrian 2004														Y				EXCLUDED
Blijlevens 2005	Y	Y	Y		Y			Y	Y	Y								INCLUDED
Boelens 2001														Y				EXCLUDED
Bongers 2007														Y				EXCLUDED
Bozzetti 1997	Y	Y	Y							Y	Y							INCLUDED
Bozzetti 1998															Y			EXCLUDED
Bozzetti 1999														Y				EXCLUDED
Bozzetti 2005														Y				EXCLUDED
Brown 1998	Y	Y	Y		Y		Y											INCLUDED

INCLUDED IN SYSTEMATIC REVIEW	EXCLUDED PHASE III	EXCLUDED PHASE II

HUMANS (Y=YES, N=NO)	STUDY **INCLUSION** CRITERIA:											STUDY **EXCLUSION** CRITERIA						INCLUDED or EXCLUDED
	STUDY POPULATION			**OUTCOMES**														
	A **YES** needed in **EVERY** column			A **YES** needed in **AT LEAST ONE (or more)** row for inclusion								A **YES** in **ANY ROW** indicates exclusion						
STUDY ID ↓	Humans with cancer	GLN supplement	English language	GLN status	Mortality	Survival	Body Weight	Length of stay	Clinical infection	Mucositis	Diarrhoea	Outcomes not considered	Foreign language	Review article	Abstracts, letters, editorials, comments	No full text in SA, Not RCT	Not RCT (Phase III)	
Buchman 1999														Y				EXCLUDED
Buchman 2001														Y				EXCLUDED
Campos 1996a													Y	Y				EXCLUDED
Candela 2006	Y	Y	Y				Y			Y			Y					EXCLUDED
Canovas 2000	Y	Y	Y	Y						Y	Y							INCLUDED
Cerchietti 2006	Y	Y	Y				Y			Y								INCLUDED
Choi 2007	Y	Y	Y							Y								INCLUDED
Choudry 2006														Y				EXCLUDED
Chuntrasakul 1998	N	Y	Y													Y	Y	EXCLUDED
Cockerham 2000	Y	Y	Y							Y							Y	EXCLUDED
Coëffier 2005														Y				EXCLUDED
Coghlin Dickson 2000	Y	Y	Y			Y		Y		Y	Y							INCLUDED
Colquhoun 1997	N	Y	Y									Y						EXCLUDED
Conklin 2000														Y				EXCLUDED
Culkin 2008	N	Y	Y															EXCLUDED
Da Gama Torres 2008	Y	Y	Y			Y		Y	Y									INCLUDED
Daniele 2001	Y	Y	Y							Y	Y							INCLUDED
Das 2007	Y	Y	Y									Y					Y	EXCLUDED
de Blaauw 1999			N										Y	Y				EXCLUDED
De Bustos 2003													Y				Y	EXCLUDED
Decker 2002														Y				EXCLUDED
Decker Baumann 1999	Y	Y	Y	Y			Y			Y	Y							INCLUDED
Duncan 2003														Y				EXCLUDED
Elia 1997														Y				EXCLUDED
Erdem 2002	Y	Y	Y		Y		Y											INCLUDED
Field 2000														Y				EXCLUDED

INCLUDED IN SYSTEMATIC REVIEW	EXCLUDED PHASE III	EXCLUDED PHASE II

HUMANS (Y=YES, N=NO)	STUDY **INCLUSION** CRITERIA:											STUDY **EXCLUSION** CRITERIA						**INCLUDED or EXCLUDED**
	STUDY POPULATION			**OUTCOMES**														
	A **YES** needed in **EVERY** column			A **YES** needed in **AT LEAST ONE (or more)** row for inclusion								A **YES** in **ANY ROW** indicates exclusion						
STUDY ID ↓	Humans with cancer	GLN supplement	English language	GLN status	Mortality	Survival	Body Weight	Length of stay	Clinical infection	Mucositis	Diarrhoea	Outcomes not considered	Foreign language	Review article	Abstracts, letters, editorials, comments	No full text in SA, Not RCT	Not RCT (Phase III)	
Filicko 2003														**Y**				**EXCLUDED**
Fish 1997	**Y**	**Y**	**Y**									**Y**						**EXCLUDED**
Ford 1997	**Y**	**Y**	**Y**					**Y**	**Y**							**Y**	**Y**	**EXCLUDED**
Fujita 2007	**Y**	**N**	**Y**									**Y**						**EXCLUDED**
Fürst 1989														**Y**				**EXCLUDED**
Fürst 2001														**Y**				**EXCLUDED**
Gallego 2006			**N**										**Y**	**Y**				**EXCLUDED**
Gianotti 2009	**Y**	**Y**	**Y**	**Y**	**Y**		**Y**	**Y**	**Y**									**INCLUDED**
Goeters 2002	**N**	**Y**	**Y**															**EXCLUDED**
Gottschlich 2006														**Y**		**Y**		**EXCLUDED**
Griffiths 1999														**Y**				**EXCLUDED**
Grover 2007														**Y**				**EXCLUDED**
Hallay 2002	**Y**	**Y**	**Y**		**Y**			**Y**										**INCLUDED**
Heslin 2000														**Y**				**EXCLUDED**
Heyland 1994														**Y**				**EXCLUDED**
Heyland 2001														**Y**				**EXCLUDED**
Heyland 2006	**N**	**Y**	**Y**															**EXCLUDED**
Heys 1996														**Y**				**EXCLUDED**
Heys 1999														**Y**				**EXCLUDED**
Heys 1999a														**Y**				**EXCLUDED**
Hornsby Lewis 1994	**N**	**Y**	**Y**	**Y**													**Y**	**EXCLUDED**
Huang 2000	**Y**	**Y**	**Y**				**Y**			**Y**								**INCLUDED**
Iestra 2002	**Y**	**N**	**Y**															**EXCLUDED**
Imoberdorf 1997														**Y**				**EXCLUDED**
Ishibashi 1995												**Y**			**Y**			**EXCLUDED**
Januszkiewicz 1996	**Y**	**Y**	**Y**	**Y**													**Y**	**EXCLUDED**
Jebb 1994	**Y**	**Y**	**Y**	**Y**						**Y**	**Y**							**INCLUDED**
Jebb 1995	**Y**	**Y**	**Y**					**Y**		**Y**	**Y**							**INCLUDED**

INCLUDED IN SYSTEMATIC REVIEW	EXCLUDED PHASE III	EXCLUDED PHASE II

HUMANS (Y=YES, N=NO)	STUDY **INCLUSION** CRITERIA:											STUDY **EXCLUSION** CRITERIA						INCLUDED or EXCLUDED
	STUDY POPULATION			**OUTCOMES**														
	A **YES** needed in **EVERY** column			A **YES** needed in **AT LEAST ONE (or more)** row for inclusion								A **YES** in **ANY ROW** indicates exclusion						
STUDY ID ↓	Humans with cancer	GLN supplement	English language	GLN status	Mortality	Survival	Body Weight	Length of stay	Clinical infection	Mucositis	Diarrhoea	Outcomes not considered	Foreign language	Review article	Abstracts, letters, editorials, comments	No full text in SA, Not RCT	Not RCT (Phase III)	
Jemaa 2004													Y	Y				EXCLUDED
Jiang 1999	Y	Y	Y	Y				Y	Y								Y	EXCLUDED
Jing-Xiang 2004	Y	Y	Y	N	N	N	N	N	N	N	N	Y						EXCLUDED
Jo 2006	Y	Y	Y		Y			Y										INCLUDED
Jones 1999	N	Y	Y															EXCLUDED
Jones 2008														Y				EXCLUDED
Katsuramaki 1998													Y	Y				EXCLUDED
Kelly 2008														Y				EXCLUDED
Kirk 2003														Y				EXCLUDED
Klek 2005	Y	Y	Y					Y	Y									INCLUDED
Klimberg 1996														Y				EXCLUDED
Klimberg 2005														Y				EXCLUDED
Köhler 2001	Y	N	Y															EXCLUDED
Koretz 2003														Y				EXCLUDED
Koretz 2008														Y				EXCLUDED
Kowanko 2008														Y				EXCLUDED
Kozelsky 2003	Y	Y	Y								Y							INCLUDED
Kulacoglu 2000															Y			EXCLUDED
Kuskonmaz 2008	Y	Y	Y							Y							Y	EXCLUDED
L GLN 2001														Y				EXCLUDED
Lauvin 1994													Y	Y				EXCLUDED
Lauvin 1995													Y				Y	EXCLUDED
Lecleire 2004													Y	Y				EXCLUDED
Lemos 2008	Y	Y	Y							Y					Y			EXCLUDED
Lenssen 2001														Y				EXCLUDED
Levy 2002														Y				EXCLUDED
Li 2006	Y	Y	N	Y						Y	Y		Y					EXCLUDED
Li 2009	Y	Y	Y	Y							Y							INCLUDED
MacBurney 1994	Y	Y	Y									Y						EXCLUDED

INCLUDED IN SYSTEMATIC REVIEW	EXCLUDED PHASE III	EXCLUDED PHASE II

HUMANS (Y=YES, N=NO)	STUDY **INCLUSION** CRITERIA:											STUDY **EXCLUSION** CRITERIA						INCLUDED or EXCLUDED
	STUDY POPULATION			**OUTCOMES**														
	A **YES** needed in **EVERY** column			A **YES** needed in **AT LEAST ONE (or more)** row for inclusion								A **YES** in **ANY ROW** indicates exclusion						
STUDY ID ↓	Humans with cancer	GLN supplement	English language	GLN status	Mortality	Survival	Body Weight	Length of stay	Clinical infection	Mucositis	Diarrhoea	Outcomes not considered	Foreign language	Review article	Abstracts, letters, editorials, comments	No full text in SA, Not RCT	Not RCT (Phase III)	
MacFie 2007															Y			EXCLUDED
Machtay 2005	Y	Y	Y									Y					Y	EXCLUDED
Marik 2007														Y		Y		EXCLUDED
Marton 2010	Y	Y	Y		Y				Y									INCLUDED
Maughan 1995	Y	Y	Y	Y						Y					Y			EXCLUDED
May 2002	Y	Y	Y		Y		Y											INCLUDED
McCarthy 2006	Y	Y	Y					Y								Y	Y	EXCLUDED
McClure 2002														Y				EXCLUDED
Medina 2001														Y				EXCLUDED
Melis 2004														Y				EXCLUDED
Mercadante 1998														Y				EXCLUDED
Miller 1999														Y		Y		EXCLUDED
Mizote 1992													Y				Y	EXCLUDED
Mobrahan 1992														Y				EXCLUDED
Morais 1995	Y	Y	Y				Y	Y	Y				Y					EXCLUDED
Morello 2002	Y	Y	Y								Y				Y		Y	EXCLUDED
Morlion 1998	N	Y	Y															EXCLUDED
Moskovitz 2004														Y				EXCLUDED
Murray 2002														Y				EXCLUDED
Muscaritoli 2002														Y				EXCLUDED
Muscartoli 1997	Y	Y	Y		Y						Y						Y	EXCLUDED
Napoli 1998															Y			EXCLUDED
Nelson 2001														Y		Y		EXCLUDED
Neu 1996														Y				EXCLUDED
Neu 2002														Y				EXCLUDED
Nitenberg 2000														Y				EXCLUDED
No author 2007	Y	Y	Y									Y				Y	Y	EXCLUDED
No author 2007a	Y	Y	Y									Y				Y	Y	EXCLUDED
Noble 2006															Y			EXCLUDED

INCLUDED IN SYSTEMATIC REVIEW	EXCLUDED PHASE III	EXCLUDED PHASE II

HUMANS (Y=YES, N=NO)	STUDY **INCLUSION** CRITERIA:											STUDY **EXCLUSION** CRITERIA						**INCLUDED or EXCLUDED**
	STUDY POPULATION			**OUTCOMES**														
	A **YES** needed in **EVERY** column			A **YES** needed in **AT LEAST ONE (or more)** row for inclusion								A **YES** in **ANY ROW** indicates exclusion						
STUDY ID ↓	Humans with cancer	GLN supplement	English language	GLN status	Mortality	Survival	Body Weight	Length of stay	Clinical infection	Mucositis	Diarrhoea	Outcomes not considered	Foreign language	Review article	Abstracts, letters, editorials, comments	No full text in SA, Not RCT	Not RCT (Phase III)	
Novak 2002															**Y**			**EXCLUDED**
O'Dwyer 2007															**Y**			**EXCLUDED**
Oguz 2007	**Y**	**Y**	**Y**		**Y**			**Y**	**Y**									**INCLUDED**
Okada 1988	**N**	**N**	**Y**									**Y**						**EXCLUDED**
Okuno 1999	**Y**	**Y**	**Y**							**Y**								**INCLUDED**
Okur 2006	**Y**	**Y**	**Y**				**Y**			**Y**							**Y**	**EXCLUDED**
O'Riordian 1994	**Y**	**Y**	**Y**						**Y**									**INCLUDED**
Pacifico 2005													**Y**	**Y**				**EXCLUDED**
Peng 2006	**Y**	**Y**	**N**							**Y**	**Y**		**Y**					**EXCLUDED**
Peterson 1995	**N**	**Y**	**Y**															**EXCLUDED**
Peterson 2007	**Y**	**Y**	**Y**							**Y**								**INCLUDED**
Phillips 1993														**Y**				**EXCLUDED**
Piccirillo 2003	**Y**	**Y**	**Y**					**Y**		**Y**								**INCLUDED**
Piccirillo 2004															**Y**			**EXCLUDED**
Pietsch 1999	**Y**	**Y**	**Y**									**Y**					**Y**	**EXCLUDED**
Posani 2000														**Y**		**Y**		**EXCLUDED**
Powell-Tuck 1997															**Y**			**EXCLUDED**
Powell-Tuck 1999	**N**	**Y**	**Y**															**EXCLUDED**
Poynton 1995															**Y**	**Y**		**EXCLUDED**
Pytlik 2002a	**Y**	**Y**	**Y**			**Y**	**Y**	**Y**	**Y**	**Y**	**Y**							**INCLUDED**
Pytlik 2002															**Y**	**Y**		**EXCLUDED**
Rathmacher 2004	**Y**	**Y**	**Y**									**Y**						**EXCLUDED**
Raynard 2002													**Y**	**Y**				**EXCLUDED**
Raynard 2005													**Y**	**Y**				**EXCLUDED**
Reeds 2001														**Y**				**EXCLUDED**
Richardson 2007	**Y**	**N**	**Y**									**Y**						**EXCLUDED**
Rombeau 1990														**Y**				**EXCLUDED**
Rubin 1987												**Y**						**EXCLUDED**
Savarese 2003														**Y**				**EXCLUDED**
Savy 2000														**Y**		**Y**		**EXCLUDED**

INCLUDED IN SYSTEMATIC REVIEW	EXCLUDED PHASE III	EXCLUDED PHASE II

HUMANS (Y=YES, N=NO)	STUDY **INCLUSION** CRITERIA:											STUDY **EXCLUSION** CRITERIA						INCLUDED or EXCLUDED
	STUDY POPULATION			**OUTCOMES**														
	A **YES** needed in **EVERY** column			A **YES** needed in **AT LEAST ONE (or more)** row for inclusion								A **YES** in **ANY ROW** indicates exclusion						
STUDY ID ↓	Humans with cancer	GLN supplement	English language	GLN status	Mortality	Survival	Body Weight	Length of stay	Clinical infection	Mucositis	Diarrhoea	Outcomes not considered	Foreign language	Review article	Abstracts, letters, editorials, comments	No full text in SA, Not RCT	Not RCT (Phase III)	
Savy 2002														Y		Y		EXCLUDED
Sax 1992															Y			EXCLUDED
Sax 2005														Y				EXCLUDED
Scheid 2001	Y	Y	Y									Y			Y			EXCLUDED
Scheid 2004	Y	Y	Y					Y										INCLUDED
Scheltinga 1991	Y	Y	Y				Y		Y									INCLUDED
Schloerb 1993	Y	Y	Y		Y		Y	Y	Y	Y								INCLUDED
Schloerb 1999	Y	Y	Y		Y	Y	Y	Y	Y	Y	Y							INCLUDED
Schloerb 2001														Y				EXCLUDED
Senkal 2004	Y	Y	Y	Y							Y						Y	EXCLUDED
Shirouzu 1996	Y	Y	Y	Y											Y			EXCLUDED
Siddiqui 2006														Y				EXCLUDED
Sigalet 2004														Y				EXCLUDED
Simpson 2005														Y				EXCLUDED
Singh 2002														Y		Y		EXCLUDED
Skubitz 1996	Y	Y	Y							Y							Y	EXCLUDED
Small 2005														Y		Y		EXCLUDED
Sornsuvit 2008	Y	Y	Y				Y	Y	Y	Y	Y							INCLUDED
Souba 1989														Y		Y		EXCLUDED
Souba 1990														Y				EXCLUDED
Souba 1993														Y				EXCLUDED
Souba 1993a														Y				EXCLUDED
Stehle 1989	Y	Y	Y	Y														INCLUDED
Steinbrunn 2006												Y			Y			EXCLUDED
Storey 2007														Y				EXCLUDED
Strasser 2008	Y	Y	Y		Y		Y				Y							INCLUDED
Sykorova 2005	Y	Y	Y			Y												INCLUDED
Tessier 2000													Y	Y				EXCLUDED
The third Oxford															Y			EXCLUDED
Topkan 2009	Y	Y	Y				Y			Y							Y	EXCLUDED

INCLUDED IN SYSTEMATIC REVIEW	EXCLUDED PHASE III	EXCLUDED PHASE II

HUMANS (Y=YES, N=NO)	STUDY **INCLUSION** CRITERIA:											STUDY **EXCLUSION** CRITERIA						INCLUDED or EXCLUDED
	STUDY POPULATION			**OUTCOMES**														
	A **YES** needed in **EVERY** column			A **YES** needed in **AT LEAST ONE (or more)** row for inclusion								A **YES** in **ANY ROW** indicates exclusion						
STUDY ID ↓	Humans with cancer	GLN supplement	English language	GLN status	Mortality	Survival	Body Weight	Length of stay	Clinical infection	Mucositis	Diarrhoea	Outcomes not considered	Foreign language	Review article	Abstracts, letters, editorials, comments	No full text in SA, Not RCT	Not RCT (Phase III)	
Vahdat 2001	**Y**	**Y**	**Y**									**Y**				**Y**		**EXCLUDED**
Van Acker 1999													**Y**	**Y**				**EXCLUDED**
Van Acker 2000	**N**	**Y**	**Y**	**Y**														**EXCLUDED**
Van der Hulst 1993	**N**	**Y**	**Y**									**Y**						**EXCLUDED**
Van Zaanen 1994	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**							**INCLUDED**
Von Meyenfeldt 1999														**Y**				**EXCLUDED**
Ward 2003	**Y**	**Y**	**Y**	**Y**													**Y**	**EXCLUDED**
Wilmore 1997														**Y**				**EXCLUDED**
Wilmore 1999														**Y**				**EXCLUDED**
Windle 2006														**Y**				**EXCLUDED**
Wischmeyer 2003														**Y**				**EXCLUDED**
Wischmeyer 2008														**Y**				**EXCLUDED**
Worthington 2008														**Y**				**EXCLUDED**
Wu 2001	**Y**	**Y**	**Y**	**Y**							**Y**							**INCLUDED**
Wu 2007	**Y**	**Y**	**Y**								**Y**				**Y**			**EXCLUDED**
Yao 2005	**N**	**Y**	**Y**					**Y**										**EXCLUDED**
Yoshida 1998	**Y**	**Y**	**Y**	**Y**														**INCLUDED**
Yoshida 2001														**Y**				**EXCLUDED**
Young 1993	**Y**	**Y**	**Y**									**Y**						**EXCLUDED**
Zheng 2007														**Y**				**EXCLUDED**
Ziegler 1990	**N**	**Y**	**Y**		**Y**													**EXCLUDED**
Ziegler 1992	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**		**Y**	**Y**	**Y**								**INCLUDED**
Ziegler 1998	**Y**	**Y**	**Y**											**Y**				**EXCLUDED**
Ziegler 1993												**Y**						**EXCLUDED**

INCLUDED IN SYSTEMATIC REVIEW	EXCLUDED PHASE III	EXCLUDED PHASE II

HUMANS (Y=YES, N=NO)	STUDY **INCLUSION** CRITERIA:											STUDY **EXCLUSION** CRITERIA						**INCLUDED or EXCLUDED**
	STUDY POPULATION			**OUTCOMES**														
	A **YES** needed in **EVERY** column			A **YES** needed in **AT LEAST ONE (or more)** row for inclusion								A **YES** in **ANY ROW** indicates exclusion						
STUDY ID ↓	Humans with cancer	GLN supplement	English language	GLN status	Mortality	Survival	Body Weight	Length of stay	Clinical infection	Mucositis	Diarrhoea	Outcomes not considered	Foreign language	Review article	Abstracts, letters, editorials, comments	No full text in SA, Not RCT	Not RCT (Phase III)	
Ziegler 2001														**Y**				**EXCLUDED**
Ziegler 2002														**Y**				**EXCLUDED**

INCLUDED IN SYSTEMATIC REVIEW	EXCLUDED PHASE III	EXCLUDED PHASE II

ANIMALS (Y=YES, N=NO)	STUDY **INCLUSION** CRITERIA:						STUDY **EXCLUSION** CRITERIA						**INCLUDED or EXCLUDED**
	Study **Population**			**Outcomes**									
	A **YES** needed in **EVERY** column			A **YES** needed in **AT LEAST ONE (or more)** row for inclusion			A **YES** in **ANY ROW** indicates exclusion						
STUDY ID ↓	Animals with cancer	GLN supplement	English language	Tumour volume	Tumour weight	Tumour volume loss	Outcomes not considered	Foreign language	Review article	Abstracts, letters, editorials, comments	No full text in SA, Not RCT	Not RCT (Phase III)	
Austgen 1992	**Y**	**Y**	**Y**		**Y**								**INCLUDED**
Bai 1996	**N**	**Y**	**Y**										**EXCLUDED**
Bartlett 1995	**Y**	**Y**	**Y**		**Y**								**INCLUDED**
Basivireddy 2004	**N**	**Y**	**Y**										**EXCLUDED**
Boyle 1996	**Y**	**N**	**Y**				**Y**						**EXCLUDED**
Boyle 1999	**Y**	**N**	**Y**				**Y**						**EXCLUDED**
Bunpo 2008	**N**	**Y**	**Y**										**EXCLUDED**
Campos 1994	**N**	**Y**	**N**					**Y**					**EXCLUDED**
Campos 1996	**N**	**Y**	**N**					**Y**					**EXCLUDED**
Cao 1999	**N**	**Y**	**Y**										**EXCLUDED**
Chance 1987	**Y**	**N**					**Y**						**EXCLUDED**
Chance 1987a		**N**											**EXCLUDED**
Chance 1988	**Y**	**N**					**Y**						**EXCLUDED**

INCLUDED IN SYSTEMATIC REVIEW	EXCLUDED PHASE III	EXCLUDED PHASE II

ANIMALS (Y=YES, N=NO)	STUDY **INCLUSION** CRITERIA:						STUDY **EXCLUSION** CRITERIA						**INCLUDED or EXCLUDED**
	Study **Population**			**Outcomes**									
	A **YES** needed in **EVERY** column			A **YES** needed in **AT LEAST ONE (or more)** row for inclusion			A **YES** in **ANY ROW** indicates exclusion						
STUDY ID ↓	Animals with cancer	GLN supplement	English language	Tumour volume	Tumour weight	Tumour volume loss	Outcomes not considered	Foreign language	Review article	Abstracts, letters, editorials, comments	No full text in SA, Not RCT	Not RCT (Phase III)	
Chance 1990	**Y**	**N**					**Y**						**EXCLUDED**
Chance 1991	**Y**	**N**					**Y**						**EXCLUDED**
Charland 1995	**N**	**Y**	**Y**								**Y**		**EXCLUDED**
Choudry 2006a	**Y**	**N**	**Y**				**Y**						**EXCLUDED**
Diestel 2005	**N**	**Y**	**N**					**Y**					**EXCLUDED**
Diestel 2007	**N**	**Y**	**Y**										**EXCLUDED**
Diestel 2007a	**N**	**Y**	**Y**										**EXCLUDED**
Erbil 2005	**N**	**Y**	**Y**										**EXCLUDED**
Ersin 2000	**N**	**Y**	**Y**										**EXCLUDED**
Erickson 1999	**Y**	**Y**	**Y**	**N**	**N**	**N**	**Y**						**EXCLUDED**
Fahr 1994	**Y**	**Y**	**Y**	**Y**	**Y**								**INCLUDED**
Fox 1988	**N**	**Y**	**Y**										**EXCLUDED**
Harari 1996	**N**	**Y**	**Y**										**EXCLUDED**
Harsha 2006	**N**	**Y**	**Y**										**EXCLUDED**
Holecek 2002	**N**	**Y**	**Y**										**EXCLUDED**
Hwang 2003	**N**	**Y**	**Y**										**EXCLUDED**
Inoue 1995	**Y**	**Y**	**Y**				**Y**						**EXCLUDED**
Jensen 1994	**N**										**Y**		**EXCLUDED**
Johnson 2003	**N**	**Y**	**Y**										**EXCLUDED**
Johnson 2003a	**N**	**Y**	**Y**										**EXCLUDED**
Kaibara 1994	**Y**	**Y**	**Y**		**Y**								**INCLUDED**
Kanauchi 1998	**N**										**Y**		**EXCLUDED**
Kaufmann 2003	**Y**	**Y**	**Y**	**Y**	**Y**								**INCLUDED**
Kaufmann 2007	**Y**	**Y**	**Y**		**Y**								**INCLUDED**
Kaufmann 2008	**Y**	**Y**	**Y**				**Y**				**Y**		**EXCLUDED**
Kaufmann 2008a	**Y**	**Y**	**Y**	**Y**	**Y**								**INCLUDED**
Klimberg 1992	**Y**	**Y**	**Y**	**Y**		**Y**							**INCLUDED**
Klimberg 1992a	**Y**	**Y**	**Y**	**Y**	**Y**								**INCLUDED**
Klimberg 1996a	**Y**	**Y**	**Y**	**Y**	**Y**								**INCLUDED**
Lana 2003	**Y**	**Y**	**Y**				**Y**						**EXCLUDED**

INCLUDED IN SYSTEMATIC REVIEW	EXCLUDED PHASE III	EXCLUDED PHASE II

ANIMALS (Y=YES, N=NO)	STUDY **INCLUSION** CRITERIA:						STUDY **EXCLUSION** CRITERIA						**INCLUDED or EXCLUDED**
	Study **Population**			**Outcomes**									
	A **YES** needed in **EVERY** column			A **YES** needed in **AT LEAST ONE (or more)** row for inclusion			A **YES** in **ANY ROW** indicates exclusion						
STUDY ID ↓	Animals with cancer	GLN supplement	English language	Tumour volume	Tumour weight	Tumour volume loss	Outcomes not considered	Foreign language	Review article	Abstracts, letters, editorials, comments	No full text in SA, Not RCT	Not RCT (Phase III)	
Le Bricon 1994	**Y**	**N**	**Y**				**Y**						**EXCLUDED**
Le Bricon 1995	**Y**	**N**	**Y**				**Y**						**EXCLUDED**
Leitao 2008	**N**	**Y**	**Y**										**EXCLUDED**
Lim 2009	**Y**	**N**	**Y**										**EXCLUDED**
Lin 1999	**N**	**Y**	**Y**										**EXCLUDED**
Luo 2004	**N**	**Y**								**Y**			**EXCLUDED**
Marks 1999	**N**	**Y**	**Y**										**EXCLUDED**
Mora 2002	**N**	**Y**	**Y**										**EXCLUDED**
Robinson 1999	**Y**	**Y**	**Y**		**Y**								**INCLUDED**
Rouse 1995	**Y**	**Y**	**Y**	**Y**		**Y**							**INCLUDED**
Rubio 1998	**Y**	**Y**	**Y**	**Y**		**Y**							**INCLUDED**
Satoh 2003	**N**	**Y**	**Y**										**EXCLUDED**
Scott 1992	**N**	**Y**	**Y**										**EXCLUDED**
Shewchuk 1997	**Y**	**Y**	**Y**		**Y**								**INCLUDED**
Shou 1991	**N**	**Y**	**Y**										**EXCLUDED**
Souba 1990a	**N**	**Y**	**Y**										**EXCLUDED**
Sukhotnik 2007	**N**	**Y**	**Y**										**EXCLUDED**
Sukhotnik 2009	**N**	**Y**	**Y**										**EXCLUDED**
Tanaka 2005	**N**	**Y**	**Y**							**Y**			**EXCLUDED**
Todorova 2003a	**Y**	**Y**	**Y**							**Y**			**EXCLUDED**
Todorova 2003	**Y**	**Y**	**Y**				**Y**						**EXCLUDED**
Todorova 2004	**Y**	**Y**	**Y**				**Y**						**EXCLUDED**
Todorova 2006	**Y**	**Y**	**Y**				**Y**						**EXCLUDED**
Winters 1994							**Y**						**EXCLUDED**
Xue 2007	**Y**	**Y**	**Y**	**Y**		**Y**							**INCLUDED**
Xue 2008	**Y**	**Y**	**Y**			**Y**							**INCLUDED**
Xue 2009	**Y**	**Y**	**Y**	**Y**		**Y**							**INCLUDED**
Yoshida 1995	**Y**	**Y**	**Y**		**Y**								**INCLUDED**
Yoshida 1997	**Y**	**Y**	**Y**				**Y**						**EXCLUDED**
Yu 1996	**N**	**Y**	**Y**										**EXCLUDED**

APPENDIX 6.3: Characteristics of Excluded Studies: Bibliographic Information and Reasons for Exclusion (Continued)

STUDY ID	STUDY REFERENCE	REASON FOR EXCLUSION
Alexander 1990	Alexander JW, Peck MD. Future prospects for adjunctive therapy: pharmacological and nutritional approaches to immune system modulation. Crit Care Med 1990;18(Suppl 2):S159-S164.	Review article
Algara 2007	Algara M, Rodríguez N, Viñals P, Lacruz M, Foro P, Reig A, et al. Prevention of radiochemotherapy-induced esophagitis with glutamine: results of a pilot study. Int J Radiat Oncol Biol Phys 2007;69(2):342-349.	Not RCT, cohort. 75 lung cancer patients receiving radio/chemotherapy and GLN powder orally at 30 g/day for 15 days. Outcome: acute esophageal toxicity.
Alivizatos 2005	Alivizatos V, Athanasopoulos P, Makris N, Karageorgos N. Early postoperative glutamine-supplemented parenteral nutrition versus enteral immunonutrition in cancer patients undergoing major gastrointestinal surgery. J BUON 2005;10(1):119-122.	Full text not available in SA library, not RCT.
Alpers 2006	Alpers DH. Glutamine: do the data support the cause for glutamine supplementation in humans? Gastroenterology 2006;130(2 Suppl 1):S106-S116.	Review article
Alschuler 2008	Alchuler L. More from Dr Alchuler ...: curb chemo side effects. Bottom Line Health 2008;22(5):14-14.	Full text not available in SA library, not RCT.
Alvarez 2003	Alvarez W, Mobarhan S. Finding a place for immunonutrition. Nutr Rev 2003;61(6 Part 1):214-218.	Review article
Anderson 1998a	Anderson PM, Ramsay NKC, Shu XO, Rydholm N, Rogosheske J, Nicklow R, et al. Effect of low-dose oral glutamine on painful stomatitis during bone marrow transplantation. Bone Marrow Transplant 1998;22(4):339-344.	RCT, but not all subjects have cancer. 195 consecutive patients undergoing BMT. 1.0 g GLN/m^2/dose swish and swallow suspension x 4 per day for 38 days. Outcomes: Mortality at day 28 and survival at day 100.
Aosasa 1999	Aosasa S, Mochizuki H, Yamamoto T, Ono S, Ichikura T. A clinical study of the effectiveness of oral glutamine supplementation during total parenteral nutrition: influence on mesenteric mononuclear cells. JPEN 1999;23(5 Suppl):S41-S44.	RCT, but outcomes not considered in objectives (Phase III). 15 colorectal cancer patients randomly divided into: either TPN with conventional GLN-free amino acid solution; or the same TPN with oral L-GLN at 30g/day for 5 preoperative days; or normal food control group. Outcomes: Comparison of plasma cytokine levels in peripheral blood and mesenteric blood: TNF- alpha and IL-10 (pg/mL). Comparison of Cytokine production of LPS stimulated M-MNC (pg/mL).
Aquino 2004	Aquino VM, Harvey A, Garvin J, Godder K, Nieder M, Adams R, et al. The use of supplemental glutamine to decrease morbidity in children undergoing stem cell transplantation: A pediatric blood and marrow transplant consortium study. Pediatr Res 2004;55(4):286A-286A.	Abstract only, no full text published.

STUDY ID	STUDY REFERENCE	REASON FOR EXCLUSION
Aquino 2005	Aquino VM, Harvey AR, Garvin JH, Godder KT, Nieder ML, Adams RH, et al. A double-blind randomized placebo-controlled study of oral glutamine in the prevention of mucositis in children undergoing hematopoietic stem cell transplantation: a pediatric blood and marrow transplant consortium study. Bone Marrow Transplant 2005;36(7):611-616.	RCT, but not all subjects had cancer. 120 children and adolescents (< 21 years) receiving BMT. 2 g GLN/m^2/dose (max dose 4 g) x 2 per day in 500 mg/ml oral solution until 28 days post transplant or until day of discharge. Outcomes included: Patients with episodes of bacteraemia, hospital days, mortality, mucositis grade (Modified Walsh score).
Arends 2006	Arends J, Bodoky G, Bozzetti F, Fearon K, Muscaritoli M, Selga G, et al. ESPEN guidelines on enteral nutrition: Non-surgical oncology. Clin Nutr 2006;25(2):245-259.	Review article
Arfons 2005	Arfons LM, Lazarus HM. Total parenteral nutrition and hematopoietic cell transplantation: an expensive placebo? Bone Marrow Transplant 2005;36(4):281-288.	Review article
Bai 1996	Bai MX, Jiang ZM, Lui YW, Wang WT, Li DM, Wilmore DW. Effects of alanyl-glutamine on gut barrier function. Nutrition 1996;12(11-12):793-796.	Animals without cancer.
Barndregt 2005	Barndregt K, Soeters P. Nutritional support. In: Gibney M, Elia M, Ljungqvist O, Dowsett J, editor(s). Clin Nutr. 1 edition. Oxford: Blackwell Publishing, 2005:115-131.	Review article (section of book)
Basivireddy 2004	Basivireddy J, Jacob M, Balasubramanian KA. Oral glutamine attenuates indomethacin-induced small intestinal damage. Clin Sci (Lond.) 2004;107(3):281-289.	Animals without cancer.
Benes 2002	Benes P, Pytlik R, Chocenská E, Pat'orková M, Klepetár J, Procházka B, et al. Article in Czech [Parenteral glutamine does not improve the nutritional status in patients during high-dose chemotherapy and autologous peripheral stem cell transplantation]. Vnitr Lek 2002;48(11):1039-1048.	Foreign language (article in Czech), would have been included.
Benes 2002a	Benes P, Pytlik R, Klepetar J, Pat'orkova M, Chocenska E, Prochazka B, et al. Poskozeni strevni resorpce cytostatickou lecbou - vliv parenteralniho glutaminu a pripravneho rezimu [Impaired intestinal resorbtion caused by cytostatic treatment - Effect of parenteral glutamine and the preparatory regime]. Ceska a Slovenska Gastroenterologie a Hepatologie 2002;56(5):190-195.	Foreign language (article in Czech), would have been included.
Bertz 2008	Bertz H. Ernährungsmedizinische aspekte der hämatopoetischen zelltransplantation [Nutrition in hematopoietic cell transplantation]. Onkologe 2008;14(1):38-44.	Foreign language (article in Dutch), review article.
Biganzoli 1996	Biganzoli L, Gavazzi C, Bozzetti F, Carnaghi C, Cappuzzo F, Baietta E. Glutamine (GLN) supplementation in cancer patients receiving chemotherapy: a double-blind randomized study (Abstract). Clin Nutr 1996;15(Suppl 1):8.	Abstract only, no full text published. Would have been included.

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Bistrian 2004	Bistrian BR. Practical recommendations for immune-enhancing diets 1,2. J Nutr: Arginine Metabolism: Enzymology, Nutrition, and Clinical Significance 2004;134(10S):2868S-2872S.	Review article
Boelens 2001	Boelens PG, Nijveldt RJ, Houdijk APJ, Meijer S, van Leeuwen PAM. Glutamine alimentation in catabolic state. J Nutr: Glutamine Metabolism: Nutritional and Clinical Significance 2001;131(9S):2569S-2577S.	Review article
Bongers 2007	Bongers T, Griffiths RD, McArdle A. Exogenous glutamine: the clinical evidence. Crit Care Med 2007;35(9 Suppl):S545-S552.	Review article
Boyle 1996	Boyle FM, Wheeler HR, Shenfield GM. Glutamate ameliorates experimental vincristine neuropathy. J Pharmacol Exp Ther 1996;279(1):410-415.	No GLN supplementation, only glutamate.
Boyle 1999	Boyle FM, Wheeler HR, Shenfield GM. Amelioration of experimental cisplatin and paclitaxel neuropathy with glutamate. J Neurooncol 1999;41(2):107-116.	No GLN supplementation, only glutamate.
Bozzetti 1998	Bozzetti F. Comments on: Why do patients with weight loss have a worse outcome when undergoing chemotherapy for gastrointestinal malignancies, Andreyev et al, Eur J Cancer 1998, 34, pp. 503-509. Eur J Cancer 1998;34(13):2132-2132.	Correspondence (Letter)
Bozzetti 1999	Bozzetti F. Lessons learned from studies on immune-nutrition in postoperative patients. Clin Nutr 1999;18(4):193-196.	Review article
Bozzetti 2005	Bozzetti F. Nutritional support in patients with cancer. In: Gibney MJ, Elia M, Ljungqvist O, Dowsett J, editor(s). Clinical Nutrition. 1 edition. Oxford: Blackwell Publishing, 2005:345-377.	Review article (section of book)
Buchman 1999	Buchman AL. Glutamine for the gut: mystical properties or an ordinary amino acid? Curr Gastroenterol Rep 1999;1(5):417-423.	Full text not available in SA library, review article.
Buchman 2001	Buchman AL. Glutamine: commercially essential or conditionally essential? A critical appraisal of the human data. Am J Clin Nutr 2001;74(1):25-32.	Review article
Bunpo 2008	Bunpo P, Murray B, Cundiff J, Brizius E, Aldrich CJ, Anthony TG. Alanyl- glutamine consumption modifies the suppressive effect of L-asparaginase on lymphocyte populations in mice. J Nutr 2008;138(2):338-343.	Animals without cancer.
Campos 1994	Campos FG, Mucerino DR, Waitzberg DL, Logulo AF, el Ibrahim R, Nadalin W, et al. Article in Portuguese [Protective effects of glutamine and elemental diet in acute actinic enterocolotis: histological evaluation]. Revista da Associaçâo Medica 1994;40(3):143-149.	Foreign language (article in Portuguese), animals without cancer.

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Campos 1996	Campos FG, Waitzberg DL, Mucerino DR, Gonçalves EL, Logulo AF, Habr-Gama A, et al. Protective effects of glutamine enriched diets on acute actinic enteritis. Nutrición Hospitalaria 1996;11(3):167-177.	Foreign language, animals without cancer.
Campos 1996a	Campos FG, Waitzberg DL, Logulo AF, Mucerino DR, Habr-Gama A. Article in Portuguese [The role of glutamine in nutrition in clinical practice]. Arquives de Gastroenterologia 1996;33(2):86-92.	Foreign language (article in Portuguese), review article.
Candela 2006	Candela CG, Castillo R, de Cos AI, Iglesias C, Martin MC, Aguado MJ, et al. Article in Spanish [Effects of parenteral glutamine in patients submitted to bone marrow transplantation]. Nutricion Hospitalaria 2006;21(1):13-21.	Foreign language (article in Spanish), would have been included.
Cao 1999	Cao Y, Kennedy R, Klimberg VS. Glutamine protects against doxorubicin-induced cardiotoxicity. J Surg Res 1999;85(1):178-182.	Animals without cancer.
Chance 1987	Chance WT, Cao L, Kim MW, Nelson JL, Fischer JE. Reduction of tumour growth following treatment with a glutamine antimetabolite. Life Sciences 1988;42:87-94.	No GLN supplementation (Acivicin (GLN metabolite)).
Chance 1987a	Chance WT, Cao L, Nelson JL, Foley-Nelson T, Fischer JE. Acivicin reduces tumour growth during total parenteral nutrition (TPN). Surgery 1987;102(2):386-394.	No GLN supplementation (Acivicin (GLN metabolite)).
Chance 1988	Chance WT, Cao L, Fischer JE. Insulin and Acivicin improve host nutrition and prevent tumour growth during total parenteral nutrition. Ann Surg 1988;208(4):524-531.	No GLN supplementation (Acivicin (GLN metabolite)).
Chance 1990	Chance WT, Cao L, Fischer JE. Response of tumour and host to hyperalimentation and anti-glutamine treatments. JPEN 1990;14(2):122-128.	No GLN supplementation (Acivicin (GLN metabolite)).
Chance 1991	Chance WT, Cao L, Zhang FS, Fischer JE. Clenbuterol plus acivicin decrease tumour growth and increase muscle mass in rats maintained on total parenteral nutrition. Am J Surg 1991;161(1):51-56.	No GLN supplementation (Acivicin (GLN metabolite)).
Charland 1995	Charland SL, Bartlett DL, Torosian MH. A significant methotrexate-Glutamine pharmacokinetic interaction. Nutrition 1995;11(2):154-158.	Full text not available in SA library, animals without cancer.
Choudry 2006	Choudry HA, Souba WW, Lin C, Meng Q, Karinch AM, Huang J, et al. Stimulation of Expression of the intestinal glutamine transporter ATB0 in tumour-bearing rats. Ann Surg Oncol 2006;13(12):1747-1753.	Animal subjects not receiving GLN supplementation.
Choudry 2006a	Choudry HA, Pan M, Karinch AM, Souba WW. Branched-chain amino acid-enriched nutritional support in surgical and cancer patients. J Nutr 2006;136(1 Suppl):314S-318S.	Review article

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Chuntrasakul 1998	Chuntrasakul C, Siltharm S, Sarasombath S, Sittapairochana C, Leowattana W, Chockvivatanavanit S, et al. Metabolic and inmmune effects of dietary arginine, glutamine and omega-3 fatty acids supplementation in immunocompromised patients. J Med Assoc Thai 1998;81(5):334-343.	Full text not available in SA library, not RCT.
Cockerham 2000	Cockerham MB, Weinberger BB, Lerchie SB. Oral glutamine for the prevention of oral mucositis associated with high-dose paclitaxel and melphalan for autologous bone marrow transplantion. Ann Pharmacother 2000;34:300-303.	Not RCT - Retrospective analysis of 21 consecutive patients receiving high-dose paclitaxel and melphalan preparative regimen for autologous peripheral blood SCT for metastatic breast cancer. First 9 patients received SCT without GLN oral rinse. Subsequent 12 patients were given 4 g GLN swish and swallow mouthwash every 4 hours around the clock (24 g GLN/day) starting on day -7. Outcomes (positive): Days mucositis, maximum grade mucositis, days on parenteral morphine pain relief and days on narcotic pain relief.
Colquhoun 1997	Colquhoun A, Newsholme EA. Aspects of glutamine metabolism in human tomour cells. Biochem Mol Biol Int 1997;41(3):583-596.	No humans or animals with cancer (*in vitro* study).
Conklin 2000	Conklin KA. Dietary antioxidants during cancer chemotherapy: Impact on chemotherapeutic effectiveness and development of side effects. Nutr Cancer 2000;37(1):1-18.	Review article
Coëffier 2005	Coëffier M, Déchelotte P. The role of glutamine in intensive care unit patients: Mechanisms of action and clinical outcome. Nutr Rev 2005;63(2):65-69.	Review article
Culkin 2008	Culkin A, Gabe SM, Bjarnason I, Grimble G, Madden AM, Forbes A. A double-blind, randomized, controlled crossover trial of glutamine supplementation in home parenteral nutrition. Eur J Clin Nutr 2008;62(5):575-583.	Humans without cancer.
Das 2007	Das S, Kar Mahapatra S, Gautam N, Das A, Roy S. Oxidative stress in lymphocytes, neutrophils, and serum of oral cavity cancer patients: modulatory array of L-glutamine. Support Care Cancer 2007;15(12):1399-1405.	Not RCT, case control study. 48 oral cancer patients and 48 healthy volunteers. 4 Groups: Control (normal healthy subjects, age and sex matched), cancer patients (1 day after operation), cancer patients (14 days after operation) and cancer patients (20 g oral L-GLN supplementation/day postoperative for 14 days) Outcomes: Oxidative stress (lipid peroxidation and antioxidant enzymes).
Decker 2002	Decker DM. GLUTAMINE: indicated in cancer care? Clin J Oncol Nurs 2002;6(2):112-115.	Review article
De Blaauw 1999	De Blaauw I, Deutz NE, von Meyenfeldt MF. Article in Dutch [Cachexia in cancer: disturbances in the protein and amino acid metabolism]. Ned Tijdschr Geneeskd 1999;143(27):1408-1413.	Foreign language (article in Dutch), review article.
De Bustos 2003	De Bustos FA, Mercé PAM. Tratamiento dietético-nutricional en la enteritis rádica crónica. A propósito de un caso clínico complejo [Dietetic-nutritional treatment in chronic radiation enteritis. A complex clinical case]. Nutrición Hospitalaria 2003;18(4):226-231.	Foreign language (article in Spanish), case report.

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Diestel 2005	Diestel CF. Efeito da suplementacao oral de l-glutamina na parede colonica de ratos submetidos a irradiacao abdominal [Effect of oral supplement of L- glutamine in colonic wall of rats subjected to abdominal irradiation]. Acta Cirurgica Brasileira 2005;20(Suppl 1):94-100.	Foreign language, animals without cancer.
Diestel 2007	Diestel CF, Marques RG, Lopes-Paulo F, Paiva D, Horst NL, Caetano CE, et al. Role of L- glutamine and glycine supplementation on irradiated colonic wall. Int J Colerectal Disease 2007;22(12):1523-1529.	Animals without cancer.
Diestel 2007a	Diestel CF, Marques Rg, Lopes-Paulo F, Paiva Dd, Horst Nl, Caetano Cer, et al. L-glutamine supplementation optimizes the repair of the colonic mucosa in rats subjected to abdominal irradiation. Nutr Res 2007;27(10):647-652.	Animals without cancer.
Duncan 2003	Duncan M, Grant G. Oral and intestinal mucositis - causes and possible treatments. Aliment Pharmacol Ther 2003;18(9):853-874.	Review article
Elia 1997	Elia M, Lunn PG. The use of glutamine in the treatment of gastrointestinal disorders in man. Nutrition 1997;13(7-8):743-747.	Review article
Erbil 2005	Erbil Y, Öztezcan S, Giris M, Barbaros U, Olgaç V, Bilge H, et al. The effect of glutamine on radiation-induced organ damage. Life Sciences 2005;78:376-382.	Animals without cancer.
Erickson 1999	Erickson R, Ross D, Medina J. Effects Of Glutamine On Head And Neck Squamous Cell Carcinoma. Otolaryngol Head Neck Surg 1999;121(4):348-354.	RCT, but outcomes not considered in objectives (phase III). 36 nu/nu mice with cell line UMSCC-38 and UMSCC-14A. The mice were paired as the tumour masses reached a geometric mean diameter of 4.5 mm to ensure equal tumour burden at beginning of intervention. Diets were modified from the standard Baker amino acid diet to be well pelleted, isonitrogenous and isocaloric. Study group: GLN rich diet(4.00 % GLN). Control group: GLN poor diet (0.5 % GLN). Duration: After pairing and randomization (masses GMD of4.5 mm) till GMD of 18mm or after 60 days on special diet. Outcomes: Tumour growth rate (GMD/day), weight gain (g/day) and daily intake (g/day).
Ersin 2000	Ersin S, Tuncyurek P, Esassolak M, Alkanat M, Buke C, Yilmaz M, et al. The prophylactic and therapeutic effects of GLUTAMINE- and arginine-enriched diets on radiation-induced enteritis in rats. J Surg Res 2000;89(2):121-125.	Animals without cancer.
Field 2000	Field CJ, Johnson I, Pratt VC. Glutamine and arginine: immunonutrients for improved health. Med Sci Sports Exerc 2000;32(7 Suppl):S377-S388.	Review article

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Filicko 2003	Filicko J, Lazarus HM, Flomenberg N. Mucosal injury in patients undergoing hematopoietic prognitor cell transplantation: new approaches to prohylaxis and treatment. Bone Marrow Transplant 2003;31(1):1-10.	Review article
Fish 1997	Fish J, Sporay G, Beyer K, Jones J, Kihara T, Kennedy A, et al. A prospective randomized study of glutamine-enriched parenteral compared with enteral feeding in postoperative patients. Am J Clin Nutr 1997;65:977-983.	Outcomes not considered in objectives.
Ford 1997	Ford C, Whitlock JA, Pietsch JB. Glutamine-supplemented tube feedings versus total parenteral nutrition in children receiving intensive chemotherapy. J Pediatr Oncol Nurs 1997;14(2):68-72.	Full text not available in SA library, not RCT.
Fox 1988	Fox AD, Kripke SA, De Paula J, Berman JM, Settle RG, Rombeau JL. Effect of a glutamine-supplemented enteral diet on methotrexate-induced enterocolitis. JPEN 1988;12(4):325-331.	Animals without cancer.
Fujita 2007	Fujita T, Yanaga, K. Association between glutamine extraction and release of citrulline and glycine by the small human intestine. Life Sciences 2007;80(20):1846-1850.	Subjects not receiving GLN supplementation.
Fürst 1989	Fürst P, Alpers S, Stehle P. Evidence for a nutritional need for glutamine in catabolic patients. Kidney Int Suppl 1989;27(Nov):S287-S292.	Review article
Fürst 2001	Fürst P. New developments in glutamine delivery. J Nutr 2001;131(9 Suppl):2562S-2568S.	Review article
Gallego 2006	Gallego LF, Lara MAJS, Bailon RS, Hernandez AG. Nitrogenous compounds of interest in clinical nutrition. Nutricion Hospitalaria 2006;Suppl 2:2114-2127.	Foreign language, review article.
Goeters 2002	Goeters C, Wenn A, Mertes N, Wempe C, Van Aken H, Stehle P, et al. Parenteral L-alanyl-L-glutamine improves 6-month outcome in critically ill patients. Crit Care Med 2002;30(9):2032-2037.	Humans without cancer.
Gottschlich 2006	Gottschlich MM. Adult enteral nutrition: formulas and supplements. In: Clinical nutrition in gastroentestinal disease. 1 edition. Thorofare: Slack Incorporated, 2006:503-518.	Full text not available in SA library, not RCT.
Griffiths 1999	Griffiths RD. Glutamine: establishing clinical indications. Curr Opin Clin Nutr Metab Care 1999;2(2):177-182.	Review article
Grover 2007	Grover Z, Tubman R, McGuire W. Glutamine supplementation for young infants with severe gastrointestinal disease. Cochrane Database Syst Rev 2007, Issue 1.	Review article
Harari 1996	Harari Y, Grossie VB, Castro GA. Nutritional support for adaptation to radiation-induced suppression of mucosal immunity in the intestine of rat. Radiat Res 1996;145(6):754-761.	Animals without cancer.

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Harsha 2006	Harsha WTF, Kalandarova E, McNutt P, Irwin R, Noel J. Nutritional supplementation with transforming growth factor-beta, glutamine, and short chain fatty acids minimizes methotrexate-induced injury. J Pediatr Gastroenterol Nutr 2006;42(1):53-58.	Animals without cancer.
Heslin 2000	Heslin MJ, Brennan MF. Advances in perioperative nutrition: cancer. World J Surg 2000;24(12):1477-1485.	Review article
Heyland 1994	Heyland DK, Cook DJ, Guyatt GH. Does the formulation of enteral feeding products influence infectious morbidity and mortality rates in the criticalli ill patients? A critical review of the evidence. Crit Care Med 1994;22(7):1192-1202.	Review article
Heyland 2001	Heyland DK, Novak F. Immunonutrition in the critically ill patient: More harm than good? JPEN 2001;25(2 Suppl):S51-S55.	Review article
Heyland 2006	Heyland DK, Dhaliwal R, Day AG, Muscedere J, Drover J, Suchner U, et al, Canadian Critical Care Trials Group. Reducing deaths due to oxidative stress (The REDOXS study): rationale and study design for a randomized trial of glutamine anioxidant supplementation in critically ill patients. Proc Nutr Soc 2006;65(3):250-263.	Not all patients have cancer. Mechanically ventilated, critically ill adults admitted to ICU. Parenteral 0.35 g GLN/kg body weight/day (provided as 0.50 g alanyl-GLN dipeptide/kg body weight/day or respective placebo solution. Outcomes: Mortality (28 days), duration of ICU stay, infectious complications, length of hospital stay, cost of care and antibiotic use.
Heys 1996	Heys SD, Gough DB, Khan L, Eremin O. Nutritional pharmacology and malignant disease: A therapeutic modality in patients with cancer. Br J Surg 1996;83(5):608-619.	Review article
Heys 1999	Heys SD, Ashkanani F. Glutamine. Br J Surg 1999;86(3):289-290.	Review article
Heys 1999a	Heys SD, Walker LG, Smith I, Eremin O. Enteral nutritional supplementation with key nutrients in patients with critical illness and cancer: a meta-analysis of randomized controlled clinical trials. Ann Surg 1999;229(4):467-477.	Review article
Holecek 2002	Holecek M, Skopec F, Sprongl L, Mráz J, Skalská H, Pecka M. Effect of alanyl-glutamine on leucine and protein metabolism in irradiated rats. Amino Acids 2002;22(1):95-108.	Animals without cancer.
Hornsby Lewis 1994	Hornsby-Lewis L, Shike M, Brown P, Klang M, Pearlstone D, Brennan MF. L-glutamine supplementation in home total parenteral nutrition patients: stability, safety, and effects on intestinal absorbtion. JPEN 1994;18(3):268.	Not RCT and only 3 of 7 subjects have cancer. 7 stable home TPN patients, no control. GLN (0.285g/kg) was added to TPN solutions for 4 weeks. Outcomes: Safety, plasma GLN levels and small bowel absorptive capacity.
Hwang 2003	Hwang J, Chan D, Chang T, Tsao T, Tsou S, Lu R, et al. Effects of oral arginine and glutamine on radiation-induced injury in the rat. J Surg Res 2003;109(2):149-154.	Animals without cancer.
Iestra 2002	Iestra JA, Fibbe WE, Zwinderman AH, van Staveren WA, Kromhout D. Body weight recovery, eating difficulties and compliance with dietary advice in the first year after stem cell transplantation: a prospective study. Bone Marrow Transplant 2002;29(5):417-424.	Subjects not receiving GLN supplementation.

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Imoberdorf 1997	Imoberdorf R. Immuno-nutrition: designer diets in cancer. Support Care Cancer 1997;5(5):381-386.	Review article
Inoue 1995	Inoue Y, Bode BP, Souba WW. Dietary regulation of the hepatic system glutamine transporter in tumour bearing rats. Am J Surg 1995;169(1):173-178.	Outcomes not considered in objectives.
Ishibashi 1995	Ishibashi N, Yoshida S, Kaibara A, Noake T, Kakegawa T. Effect of glutamine supplementation on tissue glutathione levels during chemotherapy. FASEB Journal 1995;9(3):A482-A482.	Abstract only, no full text published and outcomes not considered in objectives.
Januszkiewicz 1996	Januszkiewicz A, Essén P, McNurlan MA, Calder GA, Andersson K, Wernerman J, et al. Effect of a short-term infusion of glutamine on muscle protein metabolism postoperatively. Clin Nutr 1996;15(5):267-273.	Not RCT, no placebo or control group. 6 patients undergoing elective surgery for colon cancer. GLN-glucose infusion given for 5.5hrs, 2-3 days postoperatively (0.285 g/kg body weight). Outcomes: GLN concentration in plasma and skeletal muscle.
Jemaa 2004	Jemaa Y, Lecleire S, Petit A, Déchelotte P. Prise en charge nutritionnelle périopératoire en chirurgie de l'adulte [Perioperative nutritional support in adult patient]. Nutrition Clinique et Metabolisme 2004;18(3):137-146.	Foreign language (article in French), review article.
Jensen 1994	Jensen JC, Schaefer R, Nwokedi E, Bevans DW 3rd, Baker ML, Pappas AA, et al. Prevention of chronic radiation enteropathy by dietary glutamine. Ann Surg Oncol 1994;1(2):157-163.	Full text not available in SA library, animals without cancer.
Jiang 1999	Jiang ZM, Cao JD, Zhu XG, Zhao WX, Yu JC, Ma EL, et al. The impact of alanyl-glutamine on clinical safety, nitrogen balance, intestinal permeability, and clinical outcome in postoperative patients: A randomized, double-blind, controlled study of 120 patients. JPEN 1999;23(5):S62-S66.	RCT, but all subjects do not have cancer. 120 major gastrointestinal surgery patients who needed PN for 6 days. Parenteral Ala-GLN 0.50 g/kg/day or parenteral isonitrogenous, isocaloric solution for 6 days post surgery. Outcomes: Clinical safety, hospital stay, nitrogen balance, IP and plasma amino acid profile.
Jing-Xiang 2004	Jing-Xiang S, Xiao-Huang T, Lie W, Chen-Jing L. Glutamine dipeptide-supplemented parenteral nutrition in patients with colorectal cancer. Clin Nutr Suppl 2004;1(Suppl 1):49-53.	RCT, but outcomes not considered in objectives (phase III). 40 consecutive adult patients with colorectal cancer. Control group for immune parameters: Matched group with benign GIT diseases preop. Parenteral Ala-GLN (0.3-0.4 g/kg per day) or standard parenteral nutrition without Ala-GLN from second postoperative day until 7 days postop. Outcomes: Changes of cellular immune function parameters in patients with cancer (CD3, CD4, CD8, NK, IL2R (%)), changes of humeral immune function in patients with colorectal cancer (IgG, IgM, IgA (g/L) and nitrogen balance.
Johnson 2003	Johnson AT, Kaufmann YC, Luo S, Todorova V, Klimberg VS. Effect of glutamine on glutathione, IGF-I, and TGF-B1. J Surg Res 2003;111(2):222-228.	Animals without cancer.
Johnson 2003a	Johnson AT, Kaufmann Y, Luo S, Babb K, Hawk R, Klimberg VS. Gut glutathione metabolism and changes with 7,12-DMBA and glutamine. J Surg Res 2003;115(2):242-246.	Animals without cancer.

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Jones 1999	Jones C, Palmer TE, Griffiths RD. Randomized clinical outcome study of critically ill patients given glutamine-supplemented enteral nutrition. Nutrition 1999;15(2):108-115.	Humans without cancer.
Jones 2008	Jones NE, Heyland DK. Pharmaconutrition: a new emerging paradigm. Curr Opin Gastroenterol 2008;24(2):215-222.	Review article
Kanauchi 1998	Kanauchi O, Mitsuyama K, Saiki T, Agata K, Nakamura T, Iwanga T. Preventative effects of germinated barley foodstuff on methotrexate-induced enteritis in rats. Int J Mol Med 1998;1(6):961-966.	Full text not available in SA library, animals without cancer.
Katsuramaki 1998	Katsuramaki T, Hirata K, Isobe M. Article in Japanese [Nutrition in cancer patients]. Nippon Geka Gakkai Zasshi 1998;99(3):187-192.	Foreign language (article in Japanese), review article.
Kaufmann 2008	Kaufmann Y, Todorova VK, Luo S, Klimberg VS. Glutamine affects gluthathione recycling enzymes in a DMBA-induced breast cancer model. Nutr Cancer 2008;60(4):518-525.	Full text not available in SA library (one year embargo on e-journal full text), not RCT.
Kelly 2008	Kelly KM. Bringing evidence to complementary and alternative medicine in children with cancer: Focus on nutrition-related therapies. Pediatr Blood Cancer 2008;50(2 Suppl):490-493.	Review article
Kirk 2003	Kirk HJ, Heys SD. Immunonutrition. Br J Surg 2003;90(12):1459-1460.	Review article
Klimberg 1996	Klimberg VS, McClellan JL. glutamine, cancer, and its therapy. Am J Surg 1996;172(5):418-424.	Review article
Klimberg 2005	Klimberg VS. Is glutamine effective in enhancing host immune response to tumours? J Nutr 2005;135(12):2920S.	Review article
Koretz 2003	Koretz RL. Immunonutrition: can you be what you eat? Curr Opin Gastroenterol 2003;19(2):134-139.	Review article
Koretz 2008	Koretz RL. Parenteral nutrition and urban legands. Curr Opin Gastroenterol 2008;24(2):210-214.	Review article
Kowanko 2008	Kowanko I, Long L, Hodgkinson B, Evans D. The effectiveness of strategies for preventing and treating chemotherapy- and radiation-induced oral mucositis in patients with cancer (Structured abstract). Database of Abstracts of Reviews of Effects 2008, Issue 3.	Review article
Kulacoglu 2000	Kulacoglu H. Are the elemental diets beneficial for colonic anastomoses? Eur Surg Res 2000;32(5):322-322.	Correspondence (letter to the editor).
Kuskonmaz 2008	Kuskonmaz B, Yalcin S, Kucukbayrak O, Cetin N, Cetin M, Tezcan I, et al. The effect of glutamine supplementation on hematopoietic stem cell transplant outcome in children: a case control study. Pediatr Transplant 2008;12(1):47-51.	Not RCT, case control. 21 children receiving allogeneic HSCT and cytotoxic-conditioning regimen. Parenteral GLN (Dipeptiven) supplementation (0.4 g/kg per day from day -9 till day +21). Outcomes: Myeloid Engraftment, drug-related adverse effects, GVHD, VOD, SOS and infection.

STUDY ID	STUDY REFERENCE	REASON FOR EXCLUSION
Köhler 2001	Köhler h, Klowik M, Brand O, Göbel U, Schroten H. Influence of glutamine and glycyl-glutamine on in vitro lymphocyte proliferation in children with solid tumours. Support Care Cancer 2001;9(4):261-266.	Subjects not receiving GLN supplementation (*in vitro* study).
Lana 2003	Lana SE. The effects of oral glutamine supplementation on plasma glutamine concentrations and PGE2 concentrations in dogs experiencing radiation-induced mucositis. Am J Vet Res 2003;1(4):259-265.	Outcomes not considered in objectives.
Lauvin 1994	Lauvin R, Picot D, Hellegouarc'h R. Glutamine and cancer. Medecine et Nutrition 1994;30(4):199-204.	Foreign language, review article.
Lauvin 1995	Lauvin R, Lefeuvre C, Picot D, Hellegouarch R. Chronic radiation-induced enteritis - a retrospective study of nutrition and management in 26 patients. Semaine des Hopitaux 1995;71(7-8):197-204.	Foreign language retrospective study.
Lecleire 2004	Lecleire S. Nutrition du patient cancereux: la pratique clinique [Nutrition in patients with cancer]. Cahiers de Nutrition et de Dietetique 2004;39(4):247-252.	Foreign language, review article.
Leitao 2008	Leitáo RFC, Ribeiro RA, Lira AMS, Silva LR, Bellaguarda EAL, Macedo FDB, et al. Glutamine and alanyl-glutamine accelerate the recovery from 5-fluorouracil-induced experimental oral mucositis in hamster. Cancer Chemother Pharmacol 2008;61(2):215-222.	Animals without cancer.
Lemos 2008	Lemos Junior HP, Atallah AN, Soares BGDO, de Lemos ALA. Glutamine supplementation in enteral or parenteral nutrition for the incidence of mucositis in colorectal cancer (Protocol). Cochrane Database Syst Rev 2004, Issue 1.	Review article (protocol only).
Lenssen 2001	Lenssen P, Bruemmer B, Aker SN, McDonald GB. Nutrient support in hematopoietic cell transplantation. JPEN 2001;25(4):219-228.	Review article
Levy 2002	Levy J, Turkish A. Protective nutrients. Curr Opin Gastroenterol 2002;18(6):717-722.	Review article
Le Bricon 1994	Lebricon T, Cynober L, Baracos VE. Ornithine alpha-ketoglutarate limits muscle protein breakdown without stimulating tumour-growth in rats bearing yoshida ascites hepatoma. Metabolism 1994;43(7):899-905.	No GLN supplementation (ornithine alpha-ketoglutarate).
Le Bricon 1995	Le Bricon T, Cynober L, Field CJ, Baracos VE. Supplemental nutrition with ornithine alpha-ketoglutarate in rats with cancer-associated cachexia: Surgical treatment of the tumour improves efficacy of nutritional support. J Nutr 1995;125(12):2999-3010.	No GLN supplementation (ornithine alpha-ketoglutarate).
Lim 2009	Lim V, Korourian S, Todorova VK, Kaufmann Y, Klimberg VS. Glutamine prevents DMBA-induced squamous cell cancer. Oral Oncol 2009;45:148-155.	Outcomes not considered in objectives, animals without cancer.

STUDY ID	STUDY REFERENCE	REASON FOR EXCLUSION
Lin 1999	Lin CM, Abcouwer SF, Souba WW. Effect of dietary glutamate on chemotherapy-induced immunosuppression. Nutrition 1999;15(9):687-696.	Animals without cancer.
Li 2006	Li Y, Yu Z, Liu F, Tan L, Wu B, Li J. Oral glutamine ameliorates chemotherapy-induced changes of intestinal permeability and does not interfere with the antitumour effect of chemotherap in patients with breast cancer: a prospective randomized trial. Tumouri 2006;92(5):396-401.	Foreign language, would have been included.
Luo 2004	Luo S, Kaufmann Y, Todorova VK, Luo OK, Babb KB, Hawk RM, et al. Glutamine supplementation overcomes gut glutathione transport depression by breast cancer carcinogen. Breast Cancer Res Treat 2004;88(Suppl 1):S161-S161.	Abstract only, no full text published. Animals without cancer.
L GLN 2001	L-glutamine. Altern Med Rev 2001;6(4):406-410.	Review article
MacBurney 1994	MacBurney M, Young LS, Ziegler TR, Wilmore DW. A cost-evaluation of glutamine-supplemented parenteral nutrition in adult bone marrow transplant patients. J Am Diet Assoc 1994;94(11):1263-1266.	RCT, but outcomes not considered in objectives (phase III). Same BMT patient population as Ziegler 1992. TPN + 0.57 g GLN/kg per day GLN vs. standard TPN starting day +1. Outcomes: Total hospital charges (Total $).
MacFie 2007	MacFie J, Gatt M. L-alanine-L-glutamine supplementation improves the outcome after colorectal surgery for cancer. Colerectal Dis 2007;9(9):853.	Correspondence
Machtay 2005	Machtay M, Washam C, Devine P. Pilot study of accelerated radiotherapy with concurrent chemotherapy for stage III non-small cell lung cancer. Semin Oncol 2005;32(2 Suppl 3):S9-S12.	Outcomes not considered in objectives, not RCT. 5 lung cancer patients receiving accelerated radio/chemotherapy with concurrent chemotherapy + GLN (10 mg x 3 per day) to decrease esophagitis. No control group. Primary outcome: To determine the safety and feasibility of accelerated fractionation RT with concurrent carboplatin/paclitaxel chemotherapy.
Marik 2007	Marik PE. Maximizing efficacy from parenteral nutrition in critical care: appropriate patient populations, supplemental parenteral nutrition, glucose control, parenteral glutamine, and alternative fat sources. Curr Gastroenterol Rep 2007;9(4):345-353.	Full text not available in SA library, review article.
Marks 1999	Marks SL, Cook AK, Reader R, Kass PH, Theon AP, Greve C, et al. Effects of glutamine supplementation of an amino acid-based purified diet on intestinal mucosal integrity in cats with methotrexate-induced enteritis. Am J Vet Res 1999;60(6):755-763.	Animals without cancer.
Maughan 1995	Maughan TS. Glycyl-glutamine supplementation and high dose therapy. Eur J Cancer 1995;31(Suppl 6):S234-S234.	Abstract only, no full text published. RCT of patient population receiving high dose therapy and 50 g/day Glycyl-GLN.
McCarthy 2006	McCarthy MAS. Perioperative immunonutrition in head and neck cancer: a feasibility study. University of Washington Ph.D 2006:160 pages.	Full text not available in SA library, pilot study.

STUDY ID	STUDY REFERENCE	REASON FOR EXCLUSION
McClure 2002	McClure MW. An overview of holistic medicine and complementary and alternative medicine for the prevention and treatment of BPH, prostatitis, and prostate cancer. World J Urol 2002;20(5):273-284.	Review article
Medina 2001	Medina MA. Glutamine and cancer. J Nutr 2001;131(9 Suppl):2539S-2542S.	Review article
Melis 2004	Melis GC, ter Wengel N, Boelens PG, van Leeuwen PA. Glutamine: recent developments in research on the clinical significance of glutamine. Curr Opin Clin Nutr Metab Care 2004;7(1):59-70.	Review article
Mercadante 1998	Mercadante S. Parenteral versus enteral nutrition in cancer patients: indications and practice. Support Care Cancer 1998;6(2):85-93.	Review article
Miller 1999	Miller AL. Therapeutic considerations of L-glutamine: a review of the literature. Altern Med Rev 1999;4(4):239-248.	Full text not available in SA library, review article.
Mizote 1992	Mizote H, Hikita S, Yoshida S, Katsuki N, Fujita H, Kakegawa T. Article in Japanese [The strategy against post-operative infection in the patients with esophageal cancer]. Gan To Kagaku Ryoho 1992;19(2):167-172.	Foreign language (article in Japanese), not RCT.
Mobrahan 1992	Mobrahan S. Glutamine: a conditionally essential nutrient or another nutritional puzzle. Nutr Rev 1992;50(11):331-333.	Review article
Morais 1995	Morais AA, Santos JE, Faintuch J. Article in Portuguese [Comparitive study of arginine and glutamine supplements in malnourished surgical patients]. Rev Hosp Clin Fac Med Sao Paulo 1995;50(5):276-279.	Foreign language (article in Portuguese). Would have been included.
Mora 2002	Mora Lde O, Antunes LM, Francescato HD, Bianchi ML. The effects of oral glutamine on cisplatin-induced genotoxicity in Wistar rat bone marrow cells. Mutat Res 2002;518(1):65-70.	Animals without cancer.
Morello 2002	Morello E, Fabris P, Billio A, Amato B, Coser P. Parenteral glutamine supplementation in haematological patients treated with high dose chemotherapy (HD-CT). Blood 2001;100(11 Part 1):414A-414A.	Abstract only, no full text published. 107 patients treated with high dose chemotherapy for haematologicalal malignancies. GLN supplementation (parenteral L-alanyl-L-GLN at 20g/day for 10 days) or no GLN supplementation. Outcome: Chemotherapy-induced diarrhoea.
Morlion 1998	Morlion BJ, Stehle P, Wachtler P, Siedhoff HP, Köller M, König W, et al. Total parenteral nutrition with glutamine dipeptide after major abdominal surgery. Ann Surg 1998;227(3):302-308.	Not all patients have cancer, outcomes are considered in objectives.
Moskovitz 2004	Moskovitz DN, Kim YI. Does perioperative immunonutrition reduce postoperative complications in patients with gastrointestinal cancer undergoing operations? Nutr Rev 2004;62(11):443-447.	Review article
Murray 2008	Murray SM, Pindoria S. Nutrition support for bone marrow transplant patients. Cochrane Database Syst Rev 2008, Issue 2.	Review article
Muscaritoli 2002	Muscaritoli M, Grieco G, Capria S, Iori AP, Fanelli FR. Nutritional and metabolic support in patients undergoing bone marrow transplantation. Am J Clin Nutr 2002;75(2):183-190.	Review article

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Muscartoli 1997	Muscartoli M, Micozzi A, Conversano L, Martino P, Petti MC, Cartoni C, et al. Oral glutamine in the prevention of chemotherapy-induced gastrointestinal toxicity. Eur J Cancer 1997;33:319-320.	Not RCT, case control. 14 adult patients with acute myelogenous leukaemia admitted for intensive remission induction chemotherapy. Treatment: EORTC-GIMEMA AML10 chemotherapy protocol + 18 g oral GLN (3 days prior to chemotherapy until neutrophil recovery or initiation of TPN). Case controls with same underlying disease and matched for sex and chemotherapy. Outcomes: Diarrhoea (severity & duration) and length of stay.
Napoli 1998	Napoli M. Chemo effect alleviated. Health Facts 1998;23(10):6.	Newsletter.
Nelson 2001	Nelson MA, Frishman WH, Seiter K, Keefe D, Dutcher J. Cardiovascular considerations with anthracycline use in patients with cancer. Heart Disease 2001;3(3):157-168.	Full text not available in SA library, review article.
Neu 1996	Neu J, Shenoy V, Chakrabarti R. Glutamine nutrition and metabolism: Where do we go from here? FASEB Journal 1996;10(8):829-837.	Review article
Neu 2002	Neu J, DeMarco V, Li N. Glutamine: clinical applications and mechanisms of action. Curr Opin Clin Nutr Metab Care 2002;5(1):69-75.	Review article
Nitenberg 2000	Nitenberg G, Raynard B. Nutritional support of the cancer patient: issues and dilemmas. Crit Rev Oncol Hematol 2000;34(3):137-168.	Review article
No authors 2007	None listed. A pilot study investigating the effects of glutamine and vincristine-induced neuropathy in pediatric patients with cancer. J Soc Integr Oncol 2007;5(4):182-182.	Full text not available in SA library, not RCT.
No author 2007	None listed. Coenzyme Q During anthracycline-based chemotherapy: A case series with evaluation of cardotoxicity and clinical response. J Soc Integr Oncol 2007;5(4):188-188.	Full text not available in SA library, not RCT.
Noble 2006	Noble DW, Avenell A. Glutamine-containing parenteral nutrition: Another piece in the jigsaw? Crit Care Med 2006;34(3):893-894.	Letter
Novak 2002	Novak F, Heyland DK, Avenell A, Drover JW, Su X. Glutamine supplementatain in serious illness: a systematic review of the evidence. Crit Care Med 2002;30(9):2022-2029.	Review article
O'Dwyer 2007	O'Dwyer ST. Glutamine supplementation in colorectal cancer. Colerectal Dis 2007;9(9):852.	Correspondence
Okada 1988	Okada H, Yamamoto K, Tsutano S, Nakamura S. A new group of antibiotics, hydroxamic acid antimycotic antibiotics. 1. Precursor-initiated changes in productivity and biosynthesis of neoenactins NL1 and NL2. J Antibiot 1988;41(7):869-874.	Outcomes not considered in objectives.

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Okur 2006	Okur A, Ezgü FS, Tümer L, Cinasal G, Oguz A, Hasanoglu A, et al. Effects of oral glutamine supplementation on children with solid tumours receiving chemotherapy. Pediatr Hematol Oncol 2006;23(4):277-285.	Not RCT, case-control study. 21 children with various solid tumours, aged 1 - 17 years, receiving chemotherapy. Oral GLN (4 g/m2/day) for all 5 days of chemotherapy course. Patients accepted as own control during another course of the same chemotherapy without GLN supplementation. Outcomes: Anthropometric, haematological, immunological, and nutritional parameters; occurrence of stomatitis; fever and need for antibiotic therapy.
Pacifico 2005	Pacifico S, Leite HP, Carvalho WB de. Glutamine supplementation: is it beneficial to critically ill children? Revista de Nutricao 2005;18(1):95-104.	Foreign language, review article.
Peng 2006	Peng YL, Gong QF, Wand ZQ. Article in Chinese [The prospective study on application of parenteral nutrition with alanyl-glutamine dipeptide in chemotherapy of gastrointestinal neoplasms patients.] Ai Zheng 2006;25(8):1044-1047.	Foreign language (article in Chinese), RCT. 72 gastrointestinal neoplasm patients receiving chemotherapy. Parenteral nutrition with alanyl-GLN dipeptide. Control unclear. Outcomes: Side effects of chemotherapy, serum albumin, serum pre-albumin, IgG, IgA, IgM, C3, X4 levels and nitrogen balance. Would have been included.
Petersson 1995	Peterson B, Hultman E, Andersson K, Wernerman J. Human skeletal muscle protein: effect of malnutrition, elective surgery and total parenteral nutrition. Clin Sci (Lond.) 1995;88(4):479-484.	Humans without cancer.
Phillips 1993	Phillips MC, Olson LR. The immunologic role of the gastroentestinal tract. Crit Care Nurs Clin North Am 1993;5(1):107-120.	Review article
Piccirillo 2004	Piccirillo N, De Matteis S, Sorà F, Laurenti L, Chiusolo P, Leone G, et al. Glutamine parenteral supplementation in stem cell transplant. Bone Marrow Transplant 2004;33(4):455.	Correspondence
Pietsch 1999	Pietsch JB, Ford C, Whitlock JA. Nasogastric tube feedings in children with high-risk cancer: a pilot study. J Pediatr Hematol Oncol 1999;21(2):111-114.	Not RCT. 17 children receiving intensive CTX or BMT and enteral tube feedings containing free GLN or TPN. Outcomes: Complications and hospital charges comparing nasogastric feeds with TPN.
Posani 2000	Posani T. Review: supplemented enteral nutrition reduces infectious complications and length of hospital stay in patients with critical illness. Evid Based Nurs 2000;3(1):23.	Full text not available in SA library, review article.
Powell-Tuck 1997	Powell-Tuck J. The third Oxford Glutamine workshop, University of Oxford, Oxford, UK, 20 March 1996. Nutrition 1997;13(7/8):725.	Editorial comment
Powell-Tuck 1999	Powell-Tuck J, Jamieson CP, Bettany GE, Obeid O, Fawcett HV, Archer C, et al. A double-blind, randomised, controlled trial of glutamine supplementation in parenteral nutrition. Gut 1999;45(1):82-88.	Humans without cancer.
Poynton 1995	Poynton CH, Maughan TS, et al. Glutamine supplementation in bone marrow and peripheral blood stem cell transplantation. Bone Marrow Transplant 1995;15:204.	Abstract only, no full text published.

STUDY ID	STUDY REFERENCE	REASON FOR EXCLUSION
Pytlik 2002	Pytlik R, Benes P, Gregora E, Pajorková M, Chocenská E, Procházka B, et al. No role for parenteral glutamine supplementation in autologous stem cell transplant patients: results of a triple-blinded study. Bone Marrow Transplant 2002;29(Suppl 2):S20-S20.	Abstract only, no full text published.
Rathmacher 2004	Rathmacher JA, Nissen S, Panton L, Clark RH, Eubanks MP, Barber AE, et al. Supplementation with a combination of beta-hydroxy-beta-metylbutyrate (HMB), arginine, and glutamine is safe and could improve haematologicalal parameters. JPEN 2004;28(2):65-75.	RCT, but outcomes not considered in objectives (phase III). 49 volunteers with advanced solid tumours (stage IV) with weight loss > 5% and 3 month survival prognosis. Chemotherapy and RT was acceptable during the study. 3 g HMB, 14 g arginine, 14 g GLN daily (given in 2 equal oral doses) or placebo mixture containing nonessential amino acids, alanine (11g), glutamic acid (1.75 g), glycine (6.10 g) and serine (4.22 g) for 4 – 24 weeks. Outcomes: Blood chemistry, haematology, liver function tests, adverse events and circumplex emotional profile.
Raynard 2002	Raynard B, Nitenberg G, Gory-Delabaere G, Bourhis JH, Bachmann P, Bensadoun RJ, et al. Article in French [Standards, options and recommendations for nutritional support in bone marrow transplant patients]. Bull Cancer 2002;89(4):381-198.	Foreign language (article in French), review article.
Raynard 2005	Raynard B. Les compléments nutritionnels oraux en cancérologie (en dehors de la période périopératoire) [Oral nutritional supplements in oncology and hematology]. Nutrition Clinique et Metabolisme 2005;19(2):102-105.	Foreign language (article in French), review article.
Reeds 2001	Reeds PJ, Jahoor F. The amino acid requirements of disease. Clin Nutr 2001;20(Suppl 1):15-22.	Review article
Richardson 2007	Richardson M, Martel L, Martensson L. Outpatient transfusion practice and factors leading to inpatient transfusion in a pediatric hematology/oncology program. J Pediatr Oncol Nurs 2007;24(1):46-51.	Outcomes not considered in objectives.
Rombeau 1990	Rombeau JL. A review of the effects of glutamine-enriched diets on experimentally induced enterocolitis. JPEN 1990;14(4Suppl):100S-105S.	Review article
Rubin 1987	Rubin H, Nomura T. Use of lymph in cell culture to model hormonal and nutritional constraints on tumour growth in vivo. Cancer Res 1987;47(18):4924-4931.	Outcomes not considered in objectives (*in vitro* study).
Satoh 2003	Satoh J, Tsujikawa T, Fujiyama Y, Bamba T. Nutritional benefits of enteral alanyl-glutamine supplementation on rat intestinal damage induced by cyclophosphamide. J Gastroenterol Hepatol 2003;18(6):719-725.	Animals without cancer.
Savarese 2003	Savarese DM, Savy G, Vahdat L, Wischmeyer PE, Corey B. Prevention of chemotherapy and radiation toxicity with glutamine. Cancer Treat Rev 2003;29(6):501-513.	Review article

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Savy 2000	Savy GK. Applications for glutamine supplementation in oncology. On-Line 2000;8(4):9-11.	Full text not available in SA library, review article.
Savy 2002	Savy GK. **Glutamine** supplementation. Heal the gut, help the patient. J Infus Nurs 2002;25(1):65-69.	Full text not available in SA library, review article.
Sax 1992	Sax HC. Clinical and metabolic efficacy of glutamine-supplemented parenteral nutrition after bone-marrow transplatation. A randomized, double-blind, controlled study. JPEN 1992;16(6):589-590.	Correspondence
Sax 2005	Sax HC. Immunonutrition and upper gastrointestinal surgery: what really matters. Nutr Clin Practice 2005;20(5):540-543.	Review article
Scheid 2001	Scheid C, Kremer G, Hermann K, Heck G, Holsing A, Fuchs M, et al. Glutamine-enriched parenteral nutrition accelerates neutrophil recovery after chemotherapy for acute myeloid leukemia. Blood 2001;98(11):221B-221B.	Abstract only, no full text published. RCT including patients with AML undergoing intensive induction and consolidation chemotherapy. Comparison of standard GLN free TPN with GLN-supplemented TPN. Outcomes: Neutrophil recovery, neutropenia and neutropenic fever.
Schloerb 2001	Schloerb PR. Immune-enhancing diets: Products, components, and their rationales. JPEN 2001;25(2):S3-S7.	Review article
Scott 1992	Scott TE, Moellman JR. Intravenous glutamine fails to improve gut morphology after radiation injury. JPEN 1992;16(5):440-444.	Animals without cancer.
Senkal 2004	Senkal M, Haaker R, Deska T, Hummel T, Steinfort C, Zumtobel V, et al. Early enteral gut feeding with conditionally indispensable pharmaconutrients is metabolically safe and is well tolerated in post-operative cancer patients - a pilot study. Clin Nutr 2004;23(5):1193-1198.	Not RCT, prospective clinical trial. 20 patients with gastrointestinal cancers, planned to undergo elective gastrointestinal surgery. All patients received the test supplement containing GLN dipeptide (30 g/500 ml), antioxidant vitamins (C, E, and B-carotene), maltodextrine, tributyrine, sodium, zinc, and selenium within 2-3 hrs after surgery continuously via jejunostomy tube for 3 days (500 ml/day). Outcomes: Metabolic effects (substrate monitoring, haematology, liver/kidney parameters) and tolerance (nausea, vomiting, flatulence, constipation, diarrhoea).
Shirouzu 1996	Shirouzu Y, Yoshida S, Matsui M, Ishibashi N, Noake T, Yoshizumi T, et al. Effect of glutamine supplement on immune function in advanced esophageal cancer patients with radio-chemotherapy [abstract]. Clin Nutr 1996;15(Suppl 1):27-28.	Abstract only, no full text published. 12 patients receiving radio/chemotherapy for advanced esophageal cancer. Control and GLN group (30 g/day orally for 28 days). Outcomes: Plasma GLN levels and lymphocyte count.
Shou 1991	Shou J, Lieberman MD, Hofmann K, Leon P, Redmond HP, Davies H, et al. Dietary manipulation of methotrexate-induced enterocolitis. JPEN 1991;15(3):307-312.	Animals without cancer.
Siddiqui 2006	Siddiqui R, Pandya D, Harvey K, Zaloga GP. Nutrition modulation of cachexia/proteolysis. Nutr Clin Practice 2006;21(2):155-167.	Review article
Sigalet 2004	Sigalet DL, Mackenzie SL, Hameed SM. Enteral nutrition and mucosal immunity: implications for feeding strategies in surgery and trauma. Can J Surg 2004;47(2):109-116.	Review article

STUDY ID	STUDY REFERENCE	REASON FOR EXCLUSION
Simpson 2005	Simpson F, Doig GS. Parenteral vs. enteral nutrition in the critically ill patient: a meta-analysis of trials using the intention to treat principle. Intensive Care Med 2005;31(1):12-23.	Review article with meta-analysis.
Singh 2002	Singh R, Gopalan S, Sibal A. Immunonutrition. Indian J Pediatr 2002;69(5):417-419.	Full text not available in SA library, review article.
Skubitz 1996	Skubitz KM, Anderson PM. Oral glutamine to prevent chemotherapy induced stomatitis: a pilot study. J Lab Clin Med 1996;127(2):223-228.	Not RCT, case control. Patients experiencing stomatitis after a course of chemotherapy were offered the opportunity to enter the study if no other clinical parameters precluded receiving the same chemotherapy doses during the next course of treatment. 14 patients received a suspension of L-GLN 4 gm swish and swallow orally x2 per day from day 1 of second chemotherapy course for 28 days or for 4 days past the resolution of any post-chemotherapy stomatitis. Outcomes: Total number of days of mucositis, severity of mucositis (numbers of days at each grade), patient's subjective impression of whether the mucositis was more severe, the same or less severe with GLN supplementation and neutrophil count.
Small 2005	Small S. Use of oral glutamine in cancer symptom management. Oncol Nutr Connect 2005;13(4):18-23.	Full text not available in SA library, review article.
Souba 1989	Souba WW, Copeland EM 3rd. Hyperalimentation in cancer. CA Cancer J Clin 1989;39(2):105-114.	Full text not available in SA library, review article.
Souba 1990	Souba WW, Klimberg VS, Copeland EM 3d. Glutamine nutrition in the management of radiation enteritis. JPEN 1990;14(4 Suppl):106S-108S.	Animals without cancer.
Souba 1990a	Souba WW, Herskowitz K, Austgen TR, Chen MK, Salloum RM. Glutamine nutrition: theoretical considerations and therapeutic impact. JPEN 1990;14(5 Suppl):237S-243S.	Review article
Souba 1993	Souba WW. Intestinal glutamine-metabolism and nutrition. J Nutr Biochem 1993;4(1):2-9.	Review article
Souba 1993a	Souba WW. Glutamine and cancer. Ann Surg 1993;218(6):715-728.	Review articl
Steinbrunn 2006	Steinbrunn T, Kudlich T, Schauber J, Melcher R, Luehrs H, Gostner A, et al. Glutamine, butyric acid, and antioxidants in combination inhibit proliferation and induce differentiation in colorectal tumour cell lines independent of apoptosis. Gastroenterology 2006;130(4):A447-A447.	Abstract only, no full text published. Outcomes not considered in objectives (*in vitro* study).
Storey 2007	Storey B. The role of oral glutamine in pediatric bone marrow transplant. J Pediatr Oncol Nurs 2007;24(1):41-45.	Review article
Sukhotnik 2007	Sukhotnik I, Agam M, Shamir R, Shehadeh N, Lurie M, Coran AG, et al. Oral glutamine prevents gut mucosal injury and improves mucosal recovery following lipopolysaccharide endotoxemia in a rat. J Surg Res 2007;143(2):379-384.	Animals without cancer.

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Sukhotnik 2009	Sukhotnik I, Mogilner JG, Karry R, Shamian B, Lurie M, Kokhanovsky N, et al. Effect of oral glutamine on enterocyte turnover during methotrexate-induced mucositis in rats. Digestion 2009;79(1):5-13.	Animals without cancer.
Tanaka 2005	Tanaka A, Takahira Y, Endo T, Takeuchi K. Prophylactic effect of L-glutamine against intestinal derangement induced by 5-fluorouracil or indomethacin in rats. Gastroenterology 2005;128(4):A348-A348.	Abstract only, no full text published. Animals without cancer.
Tessier 2000	Tessier C, Corda B, Marty J. Article in French [Wasting and postoperative infection in cancer patients]. Pathologie Biologie 2000;48(8):725-732.	Foreign language (article in French), review article.
The third Oxford	The third Oxford glutamine workshop, University of Oxford, Oxford, UK, 20 March 1996. Nutrition 1997;13(7/8):725-725.	Index page
Todorova 2003	Todorova VK, Harms SA, Luo S, Kaufmann Y, Babb KB, Klimberg VS. Oral glutamine (AES-14) supplementation inhibits PI-3k/Akt signaling in experimental breast cancer. JPEN 2003;27(6):404-410.	Outcomes not considered in objectives.
Todorova 2003a	Todorova VK, Kaufmann Y, Luo S, Harms SA, Babb KB, Klimberg VS. Glutamine supplementation reduces glutathione levels and stimulates apoptosis in DMBA-induced mammary tumours. J Surg Res 2003;114(2):276.	Abstract only, no full text published. Outcomes not considered in objectives.
Todorova 2004	Todorova VK, Harms SA, Kaufmann Y, Luo S, Luo KQ, Babb K, et al. Effect of dietary glutamine on tumour glutahione levels and apoptosis-related proteins in DMBA-induced breast cancer of rats. Breast Cancer Res Treat 2004;88(3):247-256.	Outcomes not considered in objectives.
Todorova 2006	Todorova VK, Kaufmann Y, Luo S, Klimberg VS. Modulation of P53 and c-myc in DMBA-induced mammary tumours by oral glutamine. Nutr Cancer 2006;54(2):263-273.	Outcomes not considered in objectives.
Topkan 2009	Topkan E, Yavuz MN, Onal C, Yavuz AA. Prevention of acute radiation-induced esophagitis with glutamine in non-small cell lung cancer patients treated with radiotherapy: Evaluation of clinical and dosimetric parameters. Lung Cancer 2009;63:393-399.	Not RCT, cohort. Data from 41 patients with stage III lung carcinoma treated with thoracic irradiation were retrospectively analyzed. 22 Patients received prophylactic powdered GLN in doses of 10g/8h. Outcomes: Acute radiation-induced esophagitis incidence and its correlation with clinical/dosimetric factors relative to treatment with GLN.
Vahdat 2001	Vahdat L, Papadopoulus K, Lange D, Leuin S, Kaufmann E, Donovan D, et al. Reduction of paclitaxel-induced peripheral neuropathy with glutamine. Clin Cancer Res 2001;7(5):1192-1197.	Not RCT, cohort. 45 patients with stage iv breast cancer receiving high dose paclitaxel at 825mg/m-2 over 24 hrs. The first cohort of patients did not receive GLN. The second cohort received 10 g oral GLN x 3 per day for 4 days starting 24hrs after completion of chemotherapy. Outcomes: Neuropathy (baseline, 2 weeks after chemo).
Van Acker 1999	Van Acker BA, von Meyenfeldt MF, Soeters PB. Article in Dutch [Glutamine as a key ingredient in protein metabolism]. Ned Tijdschr Geneeskd 1999;143(38):1904-1908.	Foreign language (article in Dutch), review article.

STUDY ID	STUDY REFERENCE	REASON FOR EXCLUSION
Van Acker 2000	Van Acker BAC, Hulsewé KWE, Wagenmakers AJM, von Meyenfeldt MF, Soeters PB. Response of glutamine metabolism to glutamine-supplemented parenteral nutrition. Am J Clin Nutr 2000;72:790-795.	RCT, but not all patients have cancer. 23 preoperative patients received either 60 umol GLN/kg body weight or isonitrogenous, isocaloric standard TPN. Outcomes: Appearance rate of GLN (total and endogenous) and plasma GLN concentration.
Van der Hulst 1993	Van der Hulst RRWJ, van Kreel BK, von Meyenfeldt MF, Brummer RM, Arends J, Deutz NEP, et al. Glutamine and the preservation of gut intergrity. Lancet 1993;341(8857):1363-1365.	RCT, but not all subjects had cancer. 20 patients needing TPN randomly allocated to TPN+GLN-dipeptide (0.23 g GLN/kg body weight) or standard TPN. Outcomes: IP.
Von Meyenfeldt 1999	von Meyenfeldt MF. Nutritional support during treatment of biliopancreatic malignancy. Ann Oncol 1999;10(Suppl 4):273-277.	Review article
Ward 2003	Ward E, Picton S, Reid U, Thomas D, Gardener C, Smith M, et al. Oral glutamine in paediatric oncology patients: a dose finding study. Eur J Clin Nutr 2003;57(1):31-36.	Not RCT. 13 patients undergoing treatment for paediatric malignancy. Dose finding study determined by patient acceptability, plasma GLN and ammonia levels. Doses of 0.35, 0.5 and 0.65 g/kg were well tolerated.
Wilmore 1997	Wilmore DW. Metabolic support of the gastrointestinal tract - potential gut protection during intensive cytotoxic therapy. Cancer 1997;79(9):1794-1803.	Review article
Wilmore 1999	Wilmore DW, Schloerb PR, Ziegler TR. Glutamine in the support of patients following bone marrow transplantation. Curr Opin Clin Nutr Metab Care 1999;2(4):323-327.	Review article
Windle 2006	Windle EM. Glutamine supplementation in critical illness: evidence, recommendations, and implications for clinical practice in burn care. J Burn Care Res 2006;27(6):764-772.	Review article
Winters 1994	Winters R, Matthews R, Ercal N, Krishnan K. Glutamine protects chinese-hamster ovary cells from radiation killing. Life Sciences 1994;55(9):713-720.	Outcomes not considered in objectives (*in vitro* study).
Wischmeyer 2003	Wischmeyer PE. Clinical applications of L-glutamine: past, present, future. Nutr Clin Pract 2003;18(5):377-385.	Review article
Wischmeyer 2008	Wischmeyer PE. Glutamine: role in critical illness and ongoing trials. Curr Opin Gastroenterol 2008;24(2):190-197.	Review article
Worthington 2008	Worthington HV, Clarkson JE, Eden OB. Interventions for preventing oral mucositis for patients with cancer receiving treatment. Cochrane Database Syst Rev 2008, Issue 4.	Review article with meta-analysis.
Wu 2007	Wu M, Chen Y, Chen T, Chen J, Yang Y, Wang K. The effect of glutamine in preventing concurrent chemotherapy and radiotherapy induced acute diarrhea for cervical cancer: A randomized trial. Int J Radiat Oncol Biol Phys 2007;69(3 Suppl 1):S390-S390.	Abstract only. 49 patients with cervical cancer undergoing concurrent chemotherapy and RT received either 10 g in 100 ml normal saline, orally, x 3/day, or GLN-free suspension. Outcomes: Grading of acute toxicities and medications (diarrhoea).

STUDY ID	STUDY REFERENCE	REASON FOR EXCLUSION
Yao 2005	Yao GX, Xue XB, Jiang ZM, Yang NF, Wilmore DW. Effects of perioperative parenteral glutamine-dipeptide supplementation on plasma endotoxin level, plasma endotoxin inactivation capacity and clinical outcome. Clin Nutr 2005;24(4):510-515.	RCT, but not all patients had cancer diagnoses. 40 patients undergoing gastrointestinal operations received either standard TPN or isonitrogenous TPN + alanyl-GLN dipeptide (0.5 g GLN/kg/day, 21 g GLN/day) from day -1 to day + 3 (5 days). Outcomes: Plasma endotoxin level, plasma CD14 level and EIC, length of hospital stay and sepsis.
Yoshida 1997	Yoshida S, Ishibashi N, Noake T, Shirouzu Y, Oka T, Shirouzu K. Glutamine and arginine metabolism in tumour-bearing rats receiving total parenteral nutrition. Metabolism 1997;46(4):370-373.	Outcomes not considered in objectives.
Yoshida 2001	Yoshida S, Kaibara A, Ishibashi N, Shirouzu K. Glutamine supplementation in cancer patients. Nutrition 2001;17(9):766-768.	Review article
Young 1993	Young LS, Bye R, Scheltinga M, Ziegler TR, Jacobs DO, Wilmore DW. Patients receiving glutamine-supplemented intravenous feedings report an improvement in mood. JPEN 1993;17(5):422-427.	RCT, but outcomes not considered in objectives (phase III). Subset of patients from trial by Ziegler 1992. Outcomes: Change in total mood score from baseline to predischarge (POMS), handgrip-strength, POMS scores.
Yu 1996	Yu JC, Jiang ZM, Li DM, Yang NF, M-X B. Alanyl- glutamine preserves hepatic glutathione stores after 5-FU treatment. Clin Nutr 1996;15(5):261-265.	Animals without cancer.
Zheng 2007	Zheng Y, Li F, Qi B, Luo B, Sun H, Liu S, et al. Application of perioperative immunonutrition for gastrointestinal surgery: a meta-analysis of randomized controlled trials. Asia Pac J Clin Nutr 2007;16(Suppl 1):253-257.	Review article with meta-analysis.
Ziegler 1990	Ziegler TR, Benfell K, Smith RJ, Young LS, Brown E, Ferrari-Baliviera E, et al. Safety and metabolic effects of l-glutamine administration in humans. JPEN 1990;14(4):137S-146S.	Not RCT. 8 consecutive cancer patients treated according to an identical allogeneic BMT protocol. Patients 1 and 2 received TPN with zero GLN/kg/day, patients 3-6 received TPN with 0.285 g GLN/kg/day, and patients 7-8 received TPN with 0.570 g GLN/kg/day. Outcomes: Safety and metabolic effects of GLN (vital signs, mental status, medications, antibiotics, infections, organ dysfunction, transfusion requirements, fluid intake and output, nutrient intake and nitrogen balance.
Ziegler 1993	Ziegler TR, Young LS. An amino acid helps bone-marrow transplant patients. RN 1993;56(1):72-101.	Full text not available in SA library, review article.
Ziegler 1998	Ziegler TR, Bye RL, Persinger RL, Young LS, Antin JH, Wilmore DW. Effects of glutamine supplementation on circulating lymphocytes after bone marrow transplantation: a pilot study. Am J Med Sci 1998;315(1):4-10.	RCT, but outcomes not considered in objectives (phase III). Data obtained from subset from Ziegler 1992. 20 adults receiving allogeneic BMT for haematological malignancies. Only the subjects with flow cytometry studies performed within 14 days after hospital discharge were eligible for analysis. Outcomes: Weekly leucocyte and lymphocyte counts (cells/uL) during hospitalization, flow cytometry data , T (cells/uL) (CD3+, CD4+, CD8+, CD16+, CD19+cells) and total leucocyte count (cells/uL).

STUDY ID	STUDY REFERENCE	REASON FOR EXCLUSION
Ziegler 2001	Ziegler TR. Glutamine supplementation in cancer patients receiving bone marrow transplantation and high dose chemotherapy. J Nutr 2001;131(9 Suppl):2578S-2584S.	Review article
Ziegler 2002	Ziegler TR. Glutamine supplementation in bone marrow transplantation. Br J Nutr 2002;87(Suppl 1):S9-S15.	Review article

TLC: Total lymphocyte count, IL: Interleukin.

APPENDIX 6.4: Characteristics of Excluded Studies Reported in Foreign Languages: Possibly Eligible Studies Recommended for Future Review (Continued)

STUDY ID (*N*=25)	BIBLIOGRAPHIC IN FORMATION	COMMENTS
Benes 2002	Benes P, Pytlik R, Chocenská E, Pat'orková M, Klepetár J, Procházka B, et al. Article in Czech [Parenteral glutamine does not improve the nutritional status in patients during high-dose chemotherapy and autologous peripheral stem cell transplantation]. Vnitr Lek 2002;48(11):1039-1048.	Possibly eligible
Benes 2002a	Benes P, Pytlik R, Klepetar J, Pat'orkova M, Chocenska E, Prochazka B, et al. Poskozeni strevni resorpce cytostatickou lecbou - vliv parenteralniho glutaminu a pripravneho rezimu [Impaired intestinal resorbtion caused by cytostatic treatment - Effect of parenteral glutamine and the preparatory regime]. Ceska a Slovenska Gastroenterologie a Hepatologie 2002;56(5):190-195.	Possibly eligible
Bertz 2008	Bertz H. Ernährungsmedizinische aspekte der hämatopoetischen zelltransplantation [Nutrition in hematopoietic cell transplantation]. Onkologe 2008;14(1):38-44.	Review article
Campos 1994	Campos FG, Mucerino DR, Waitzberg DL, Logulo AF, el Ibrahim R, Nadalin W, et al. Article in Portuguese [Protective effects of glutamine and elemental diet in acute actinic enterocolotis: histological evaluation]. Revista da Associaçâo Medica 1994;40(3):143-149.	Animals without cancer
Campos 1996	Campos FG, Waitzberg DL, Mucerino DR, Gonçalves EL, Logulo AF, Habr-Gama A, et al. Protective effects of glutamine enriched diets on acute actinic enteritis. Nutr Hosp 1996;11(3):167-177.	Review article
Campos 1996a	Campos FG, Waitzberg DL, Logulo AF, Mucerino DR, Habr - Gama A. Article in Portuguese [The role of glutamine in nutrition in clinical practice]. Arquives de Gastroenterologia 1996;33(2):86-92.	Review article
Candela 2006	Candela CG, Castillo R, de Cos AI, Iglesias C, Martin MC, Aguado MJ, et al. Article in Spanish [Effects of parenteral glutamine in patients submitted to bone marrow transplantation]. Nutr Hosp 2006;21(1):13-21.	Possibly eligible
de Blaauw 1999	De Blaauw I, Deutz NE, von Meyenfeldt MF. Article in Dutch [Cachexia in cancer: disturbances in the protein and amino acid metabolism]. Ned Tijdschr Geneeskd 1999;143(27):1408-1413.	Review article
De Bustos 2003	De Bustos FA, Mercé PAM. Tratamiento dietético-nutricional en la enteritis rádica crónica. A propósito de un caso clínico complejo [Dietetic-nutritional treatment in chronic radiation enteritis. A complex clinical case]. Nutr Hosp 2003;18(4):226-231.	Case report
Diestel 2005	Diestel CF. Efeito da suplementacao oral de l-glutamina na parede colonica de ratos submetidos a irradiacao abdominal [Effect of oral supplement of L- glutamine in colonic wall of rats subjected to abdominal irradiation]. Acta Cirurgica Brasileira 2005;20(Suppl 1):94-100.	Animals without cancer
Gallego 2006	Gallego LF, Lara MAJS, Bailon RS, Hernandez AG. Nitrogenous compounds of interest in clinical nutrition. Nutr Hosp 2006;Suppl 2:2114-2127.	Review article
Jemaa 2004	Jemaa Y, Lecleire S, Petit A, Déchelotte P. Prise en charge nutritionnelle périopératoire en chirurgie de l'adulte [Perioperative nutritional support in adult patient]. Nutrition Clinique et Metabolisme 2004;18(3):137-146.	Review article
Katsuramaki 1998	Katsuramaki T, Hirata K, Isobe M. Article in Japanese [Nutrition in cancer patients]. Nippon Geka Gakkai Zasshi 1998;99(3):187-192.	Review article
Lauvin 1994	Lauvin R, Picot D, Hellegouarc'h R. Glutamine and cancer. Medecine et Nutrition 1994;30(4):199-204.	Review article
Lauvin 1995	Lauvin R, Lefeuvre C, Picot D, Hellegouarch R. Chronic radiation-induced enteritis - a retrospective study of nutrition and management in 26 patients. Semaine des Hopitaux 1995;71(7-8):197-204.	Retrospective study

STUDY ID (*N*=25)	BIBLIOGRAPHIC IN FORMATION	COMMENTS
Lecleire 2004	Lecleire S. Nutrition du patient cancereux: la pratique clinique [Nutrition in patients with cancer]. Cahiers de Nutrition et de Dietetique 2004;39(4):247-252.	Review article
Li 2006	Li Y, Yu Z, Liu F, Tan L, Wu B, Li J. Oral GLN ameliorates chemotherapy-induced changes of intestinal permeability and does not interfere with the antitumour effect of chemotherapy in patients with breast cancer: a prospective randomized trial. Tumouri 2006;92(5):396-401.	Possibly eligible
Mizote 1992	Mizote H, Hikita S, Yoshida S, Katsuki N, Fujita H, Kakegawa T. Article in Japanese [The strategy against post-operative infection in the patients with esophageal cancer]. Gan To Kagaku Ryoho 1992;19(2):167-172.	Not RCT
Morais 1995	Morais AA, Santos JE, Faintuch J. Article in Portuguese [Comparitive study of arginine and glutamine supplements in malnourished surgical patients]. Rev Hosp Clin Fac Med Sao Paulo 1995;50(5):276-279.	Possibly eligible
Pacifico 2005	Pacifico S, Leite HP, Carvalho WB de. Glutamine supplementation: is it beneficial to critically ill children? Revista de Nutricao 2005;18(1):95-104.	Review article
Peng 2006	Peng YL, Gong QF, Wand ZQ. Article in Chinese [The prospective study on application of parenteral nutrition with alanyl-glutamine dipeptide in chemotherapy of gastrointestinal neoplasms patients.] Ai Zheng 2006;25(8):1044-1047.	Possibly eligible
Raynard 2002	Raynard B, Nitenberg G, Gory-Delabaere G, Bourhis JH, Bachmann P, Bensadoun RJ, et al. Article in French [Standards, options and recommendations for nutritional support in bone marrow transplant patients]. Bull Cancer 2002;89(4):381-198.	Review article
Raynard 2005	Raynard B. Les compléments nutritionnels oraux en cancérologie (en dehors de la période périopératoire) [Oral nutritional supplements in oncology and hematology]. Nutrition Clinique et Metabolisme 2005;19(2):102-105.	Review article
Tessier 2000	Tessier C, Corda B, Marty J. Article in French [Wasting and postoperative infection in cancer patients]. Pathologie Biologie 2000;48(8):725-732.	Review article
Van Acker 1999	Van Acker BA, von Meyenfeldt MF, Soeters PB. Article in Dutch [Glutamine as a key ingredient in protein metabolism]. Ned Tijdschr Geneeskd 1999;143(38):1904-1908.	Review article

APPENDIX 6.5: Study ID, Bibliographic Information and Source of Included Studies (Continued)

STUDY ID (REFERENCE NUMBER)	REFERENCE	DATABASE CITATIONS	SOURCE OF FULL TEXT
Anderson 1998 (100)	Anderson PM, Schroeder G, Skubitz KM. Oral glutamine reduces the duration and severity of stomatitis after cytotoxic cancer chemotherapy. Cancer 1998; 83(7):1433-1439.	Medline; EBSCO Host; SCI; Cochrane.	Wiley InterScience *(Published data only)*
Austgen 1992 (74)	Austgen TR, Dudrick PS, Sitren H, Bland KI, Copeland E, Souba WW. The effects of glutamine-enriched total parenteral nutrition on tumour growth and host tissues. Ann Surg 1992;215(2):107-113.	Reference list	Journals@Ovid *(Published data only)*
Bartlett 1995 (75)	Bartlett DL, Charland S, Torosian MH. Effect of glutamine on tumour and host growth. Ann Surg Oncol 1995;2(1):71-76.	Medline; EBCSO; SCI.	International Library *(Published data only)*
Berk 2008 (162)	Berk L, James J, Schwartz A, Hug E, Mahadevan A, Samuels M, et al. A randomized, double-blind, placebo-controlled trial of a β-hydroxyl β-methyl butyrate, glutamine, and arginine mixture for the treatment of cancer cachexia (RTOG 0122). Support Care Cancer 2008;16:1179-1188.	Personal files	SpringerLINK *(Published data only)*
Blijlevens 2005 (109)	Blijlevens NMA. A randomized, double-blind, placebo-controlled, pilot study of parenteral glutamine for allogeneic stem cell transplant patients. Support Care Cancer 2005;13(10):790-796.	EBCSO; SCI.	SpringerLINK *(Published and unpublished data: e-mail correspondence with NMA Blijlevens N.Blijlevens@hemat.umcn.nl, 14 Dec 2009)*
Bozzetti 1997 (95)	Bozzetti F, Biganzoli L, Gavazzi C, Cappuzzo F, Carnaghi C, Buzzoni R, et al. Glutamine supplementation in cancer patients receiving chemotherapy: a double-blind randomized study. Nutrition 1997;13(7-8):748-751.	Medline; EBCSO; SCI; Cochrane.	ScienceDirect *(Published data only)*
Brown 1998 (140)	Brown SA, Goringe A, Fegan C, Davies SV, Giddings J, Whittaker JA, et al. Parenteral glutamine protects hepatic function during bone marrow transplantation. Bone Marrow Transplant 1998;22(3):281-284.	Medline; EBCSO; SCI; Cochrane.	Academic Search Premier *(Published data only)*
Canovas 2000 (153)	Canovas G, León-Sanz M, Gómez P, Valero MA, Gomis P, La Huerta JJ. Oral glutamine supplements in autologous hematopoietic transplant: impact on gastrointestinal toxicity and plasma protein levels. Haematologica 2000;85(11):1229-1230.	Medline; EBSCO; SCI; Cochrane.	ScienceDirect *(Published data only*
Cerchietti 2006 (101)	Cerchietti LCA, Navigante AH, Lutteral MA, Castro MA, Kirchuk R, Bonomi M, et al. Double-blind, placebo-controlled trial on intravenous L-alanyl-L-glutamine in the incidence of oral mucositis following chemoradiotherapy in patients with head-and-neck cancer. Int J Radiat Oncol Biol Phys 2006;65(5):1330-1337.	SCI	ScienceDirect *(Published and unpublished data: e-mail correspondence with Leandro Cerchietti lec2010@med.cornell.edu, 15 June 2009)*
Choi 2007 (93)	Choi K, Lee SS, Oh SJ, Lim SY, Jeon WK, Oh TY, et al. The effect of oral glutamine on 5-fluoroucil/leucovorin-induced mucositis/stomatitis assessed by intestinal permeability test. Clin Nutr 2007;26(1):57-62.	Medline; EBCSO; SCI; Cochrane.	ScienceDirect *(Published data only)*

STUDY ID (REFERENCE NUMBER)	REFERENCE	DATABASE CITATIONS	SOURCE OF FULL TEXT
Coghlin Dickson 2000 (97)	Coghlin Dickson TM, Wong RM, Offrin RS, Shizuru JA, Johnston LJ, Hu WW, et al. Effect of oral glutamine supplementation during bone marrow transplantation. JPEN 2000;24(2):61-66.	Medline; ProQuest; EBCSO; SCI; Cochrane.	Proquest Medical Library *(Published data only)*
Da Gama Torres 2008 (123)	Da Gama Torres HO, Vilela EG, da Cunha AS, Goulart EM, Souza MH, Aquirre AC, et al. Efficacy of glutamine-supplemented parenteral nutrition on short term survival following allo-SCT: a randomized study. Bone Marrow Transplant 2008;41(12):1021-1027.	Medline	International library *(Published and unpublished data: e-mail correspondence with torresh@medicina.ufmg.br, 11 Oct 2009)*
Daniele 2001 (102)	Daniele B, Perrone F, Gallo C, Pignata S, De Martino S, De Vivo R, et al. Oral glutamine in the prevention of fluoroucil induced intestinal toxicity: a double-blind, placebo-controlled, randomised trial. Gut 2001;48(1):28-33.	SCI	Proquest Medical Library *(Published data only)*
Decker Baumann 1999 (48)	Decker-Baumann C, Buhl K, Frohmüller S, von Herbay A, Dueck M, Schlag PM. Reduction of chemotherapy-induced side-effects by parenteral glutamine supplementation in patients with metastatic colorectal cancer. Eur J Cancer 1999;35(2):202-207.	Medline; EBCSO; SCI; Cochrane.	ScienceDirect *(Published data only)*
Erdem 2002 (144)	Erdem NZ, Yasti AC, Alti M, Gozolan AU, Dolapci M, Kama NA, et al. The effects of perioperative oral enteral support with glutamine-added elemental formulas in patients with gastrointestinal cancers. A prospective, randomized, clinical study. Nutr Res 2002;22(9):977, 12p.	EBSCO; SCI.	ScienceDirect *(Published data only)*
Fahr 1994 (86)	Fahr MJ, Kornbluth J, Blossom S, Schaeffer R, Klimberg VS. Harry M. Vars Research Award. Glutamine enhances immunoregulation of tumour growth. JPEN 1994;18(6):471-476.	Medline	US Health Sciences Library Open shelf *(Published data only)*
Gianotti 2009 (163)	Gianotti L, Braga M, Biffi R, Bozzetti F, Mariani L. Perioperative intravenous glutamine supplementation in major abdominal surgery for cancer. A randomized multicenter trial. Ann Surg 2009;250(5):684-690.	Pubmed	Journals@Ovid *(Published data only)*
Hallay 2002 (133)	Hallay J, Kovacs G, Sz SK, Farkas M, Lakos G, Sipka S, et al. Changes in the nutritional state and immune-serological parameters of esophagectomized patients fed jejunely with glutamine-poor and glutamine-rich nutrients. Hepatogastroenterology 2002;49(48):1555-1559.	SCI	US Health Sciences Library Open shelf *(Published and unpublished data: e-mail correspondence with Judit Hallay jhallay@dote.hu,15 June 2009)*
Huang 2000 (103)	Huang EY, Leung SW, Wang CJ, Chen HC, Sun LM, Fang FM, et al. Oral glutamine to alleviate radiation-induced oral mucositis: a pilot randomized trial. Int J Radiat Oncol Biol Phys 2000;46(3):535-539.	EBSCO; Medline.	ScienceDirect *(Published data only)*
Jebb 1994 (104)	Jebb SA, Osborne RJ, Maughan TS, Mohideen N, Mack P, Mort D, et al. 5-fluorouracil and folinic acid-induced mucositis: no effect of oral glutamine supplementation. Br J Cancer 1994;70(4):732-735.	Medline; EBCSO; SCI; Cochrane.	US Health Sciences Library Open shelf *(Published data only)*
Jebb 1995 (96)	Jebb SA, Marcus R, Elia M. A pilot study of oral glutamine supplementation in patients receiving bone marrow transplants. Clin Nutr 1995;14(3):162-165.	Medline; EBCSO; SCI; Cochrane.	ScienceDirect *(Published data only)*

STUDY ID (REFERENCE NUMBER)	REFERENCE	DATABASE CITATIONS	SOURCE OF FULL TEXT
Jo 2006 (142)	Jo S, Choi S, Heo J, Kim E, Min M, Choi D, et al. Missing effect of glutamine supplementation on the surgical outcome after pancreaticduodenectomy for periampullary tumours: a prospective, randomized, double-blind, controlled clinical trial. World J Surg 2006;30(11):1974-1982.	Medline; Cochrane.	Proquest Medical Library *(Published data only)*
Kaibara 1994 (76)	Kaibara A, Yoshida S, Yamasaki K, Ishibashi N, Kakegawa T. Effect of glutamine and chemotherapy on protein metabolism in tumour-bearing rats. J Surg Res 1994;57(1):143-149.	Medline; EBSCO; SCI.	US Health Sciences Library Open shelf *(Published data only)*
Kaufmann 2003 (3)	Kaufmann Y, Kornbluth J, Feng Z, Fahr M, Schaefer RF, Klimberg VS. Effect of glutamine on the initiation and promotion phases of DMBA-induced mammary tumour development. JPEN 2003;27(6):411-418.	Medline; ProQuest; EBSCO; SCI.	Proquest Medical Library *(Published and unpublished data: e-mail correspondence with Suzanne Klimberg Klimbergsuzanne@uams.edu, 11 May 2009)*
Kaufmann 2007 (4)	Kaufmann Y, Klimberg VS. Effect of glutamine on gut glutathione fractional release in the implanted tumour model. Nutr Cancer 2007;59(2):199-206.	Medline; EBSCO; SCI.	Academic Search Premier *(Published and unpublished data: e-mail correspondence with Yihong Kaufmann KaufmannYihong@uams.edu, 16 Oct 2009)*
Kaufmann 2008a (5)	Kaufmann Y, Spring P, Klimberg VS. Oral glutamine prevents DMBA-induced mammary carcinogenesis via upregulation of gluthathione production. Nutrition 2008;24(5):462-469.	Medline; EBSCO; SCI.	ScienceDirect *(Published and unpublished data: e-mail correspondence with Yihong Kaufmann KaufmannYihong@uams.edu, 8 Aug 2009)*
Klek 2005 (61)	Klek S, Kulig J, Szczepanik AM, Jedrys J, Kolodziejczyk P. The clinical value of parenteral immunonutrition in surgical patients. Acta Chirurgica Belgica 2005;105(2):175-179.	Medline; Cochrane.	International Library *(Published data only)*
Klimberg 1992 (77)	Klimberg VS, Pappas AA, Nwokedi E, Jensen JC, Broadwater JR, Lang NP, et al. Effect of supplemental dietary glutamine on methotrexate concentrations in tumours. Arch Surg 1992;127(11):1317-1320.	Medline; EBSCO; SCI.	US Health Sciences Library Open shelf *(Published data only)*
Klimberg 1992a (78)	Klimberg VS, Nwokedi E, Hutchins LF, Pappas AA, Lang NP, Broadwater JR, et al. Glutamine facilitates chemotherapy while reducing toxicity. JPEN 1992;16(6 Suppl):83S-87S.	Medline; EBSCO; SCI.	US Health Sciences Library Open shelf *(Published data only)*
Klimberg 1996a (79)	Klimberg VS, Kornbluth J, Cao Y, Dang A, Blossom S, Schaeffer RF. Glutamine suppresses PGE2 synthesis and breast cancer growth. J Surg Res 1996;63(1):293-297.	Medline; EBSCO; SCI.	ScienceDirect *(Published data only)*
Kozelsky 2003 (119)	Kozelsky TF, Meyers GE, Sloan JA, Shanahan JA, Dick SJ, Moore RL, et al. Phase III double-blind study of glutamine versus placebo for the prevention of acute diarrhoea in patients receiving pelvic radiation therapy. J Clin Oncol 2003;21(9):1669-1674.	SCI	Jco.ascopubs.org by JS Gericke Library *(Published data only)*
Li 2009 (120)	Li Y, Ping X, Yu B, Liu F, Ni X, Li J. Clinical trial: prophylactic intravenous alanyl-glutamine reduces the severity of gastrointestinal toxicity induced by chemotherapy - a randomized crossover study. Aliment Pharmacol Ther 2009;30:452-458.	Reference list	International library *(Published data only)*

STUDY ID (REFERENCE NUMBER)	REFERENCE	DATABASE CITATIONS	SOURCE OF FULL TEXT
Marton 2010 (164)	Marton S, Gosh S, Papp A, Bogar L, Koszegi T, Juhasz V, et al. Effect of glutamine in patients with esophagus resection. Dis Esophagus 2010;23:106-111.	Pubmed	Wiley Interscience *(Published data only)*
May 2002 (130)	May PE, Barber A, D'Olimpio JT, Hourihane A, Abumrad NN. Reversal of cancer-related wasting using oral supplementation with a combination of beta-hydroxy-beta-methylbutyrate, arginine, and glutamine. Am J Surg 2002;183(4):471-479.	Medline; EBSCO; SCI; Cochrane.	ScienceDirect *(Published data only)*
O'Riordian 1994 (136)	O'Riordian MG, Fearon KC, Ross JA, Rogers P, Falconer JS, Bartolo DC, et al. Glutamine-supplemented total parenteral nutrition enhances T-lymphocyte response in surgical patients undergoing colorectal resection. Ann Surg 1994;220(2):212-221.	Medline	Journal@Ovid *(Published data only)*
Oguz 2007 (141)	Oguz M, Kerem M, Bedirli A, Mentes BB, Sakrak O, Salman B, et al. L-alanin-L-glutamine supplementation improves the outcome after colorectal surgery for cancer. Colerectal Dis 2007;9(6):515-520.	Medline; EBSCO; ProQuest; Cochrane.	Academic Search Premier *(Published data only)*
Okuno 1999 (105)	Okuno SH, Woodhouse CO, Loprinzi CL, Sloan JA, LaVasseur BI, Clemens-Schutjer D, et al. Phase III controlled evaluation of glutamine for decreasing stomatitis in patients receiving fluorouracil (5-FU)-based chemotherapy. Am J Clin Oncol 1999;22(3):258-261.	Reference list	Journal@Ovid *(Published data only)*
Peterson 2007 (94)	Peterson DE, Jones JB, Petit II RG. Randomized, placebo-controlled trial of safaris for prevention and treatment of oral mucositis in breast cancer patients receiving antracycline-based chemotherapy. Cancer 2007; 109:322-31.	Reference list	Wiley Interscience *(Published data only)*
Piccirillo 2003 (110)	Piccirillo N, De Matteis S, Laurenti L, Chiusolo P, Sora F, Pittiruti M, et al. Glutamine-enriched parenteral nutrition after autologous peripheral blood stem cell transplantation: effects on immune reconstitution and mucositis. Haematologica 2003;88(2):192-200.	SCI	National Library *(Published and unpublished data: e-mail correspondence with Nicola Piccirillo n.piccirillo@rm.unicatt.it, 16 June 2009)*
Pytlik 2002a (98)	Pytlik R, Benes P, Patorková M, Chocenská E, Gregora E, Procházka B, et al. Standardized parenteral alanyl-glutamine dipeptide supplementation is not beneficial in autologous transplant patients: a randomized, double-blind, placebo controlled study. Bone Marrow Transplant 2002;30(12):953-961.	Medline; Proquest; SCI; Cochrane.	Academic Search Premier *(Published data only)*
Robinson 1999 (80)	Robinson LE, Bussière FI, Le Boucher J, Farges MC, Cynober LA, Field CJ, et al. Amino acid nutrition and immune function in tumour-bearing rats: a comparison of glutamine-, arginine- and ornithine 2-oxoglutarate- supplemented diets. Clin Sci (Lond) 1999;97(6):657-669.	Medline; EBSCO; SCI.	National Library *(Published data only)*
Rouse 1995 (87)	Rouse K, Nwokedi E, Woodliff JE, Epstein J, Klimberg VS. Glutamine enhances selectivity of chemotherapy through changes in glutathione metabolism. Ann Surg 1995;221(4):420-426.	Medline; EBSCO; SCI.	Journals@Ovid *(Published data only)*
Rubio 1998 (90)	Rubio IT, Cao Y, Hutchins LF, Westbrook KC, Klimberg VS. Effect of glutamine on methotrexate efficacy and toxicity. Ann Surg 1998;227(5):772-778.	Medline; EBSCO; SCI.	Journals@Ovid *(Published data only)*

STUDY ID (REFERENCE NUMBER)	REFERENCE	DATABASE CITATIONS	SOURCE OF FULL TEXT
Scheid 2004 (134)	Scheid C, Hermann K, Kremer G, Holsing A, Fuchs M, Waldschmidt D, et al. Randomized, double-blind, controlled study of glycyl-glutamine-dipeptide in the parenteral nutrition of patients with acute leukemia undergoing intensive chemotherapy. Nutrition 2004;20(3):249-254.	Medline; EBSCO; SCI; Cochrane.	ScienceDirect *(Published data only)*
Scheltinga 1991 (143)	Scheltinga MR, Young LS, Benfell K, Bye RL, Ziegler TR, Santos AA, et al. Glutamine-enriched intravenous feedings attenuate extracellular fluid expansion after standard stress. Ann Surg 1991;214(4):385-393.	Medline; EBSCO; SCI; Cochrane.	Journals@Ovid *(Published data only)*
Schloerb 1993 (111)	Schloerb PR, Amare M. Total parenteral nutrition with glutamine in bone marrow transplantation and other clinical applications (a randomized, double-blind study). JPEN 1993;17(5):407-413.	Medline; EBSCO; SCI; Cochrane.	US Health Sciences Library *(Published data only)*
Schloerb 1999 (112)	Schloerb PR, Skikne BS. Oral and parenteral glutamine in bone marrow transplantation: a randomized, double-blind study. JPEN 1999;23(3):117-122.	Medline; Proquest; EBSCO; SCI; Cochrane.	ProQuest Medical Library *(Published data only)*
Shewchuk 1997 (81)	Shewchuk LD, Baracos VE, Field CJ. Dietary L-glutamine supplementation reduces the growth of the Moris Hepatoma *7777* in excercise-trained and sedentary rats. J Nutr 1997;127(1):158-166.	Medline; SCI.	ProQuest Medical Library *(Published data only)*
Sornsuvit 2008 (113)	Sornsuvit C, Komindr S, Chuncharunee S, Wanikiat P, Archararit N, Santanirand P. Pilot Study: effects of parenteral glutamine dipeptide supplementation on neutrophil functions and prevention of chemotherapy-induced side-effects in acute myeloid leukaemia patients. J Int Med Res 2008;36(6):1383-1391.	Medline	National Library *(Published data only)*
Stehle 1989 (59)	Stehle P, Zander J, Mertes N, Albers S, Puchstein C, Lawin P, et al. Effect of parenteral glutamine peptide supplements on muscle glutamine loss and nitrogen balance after major surgery. Lancet 1989;1(8632):231-233.	Medline; SCI.	ProQuest Medical Library *(Published data only)*
Strasser 2008 (122)	Strasser F, Demmer R, Bohme C, Schmitz SFH, Thuerlimann B, Cerny T, et al. Prevention of docetaxel- or paclitaxel-associated taste alterations in cancer patients with oral glutamine: A randomized, placebo-controlled, double-blind study. Oncologist 2008;13(3):337-346.	SCI	www.TheOncologist.com *(Published data only)*
Sykorova 2005 (145)	Sykorova A, Horacek J, Zak P, Kmonicek M, Bukac J, Maly J. A randomized, double blind comparative study of prophylactic parenteral nutritional support with or without glutamine in autologous stem cell transplantation for haematologicalal malignancies - three years' follow-up. Neoplasma 2005;52(6):476-482.	Medline; SCI.	International Library *(Published and unpublished data: e-mail correspondence with Prof Jiri Horacek horacek@fnhk.cz, 15 June 2009)*
Van Zaanen 1994 (106)	Van Zaanen HC, van der Lelie H, Timmer JG, Fürst P, Sauerwein HP. Parenteral glutamine dipeptide supplementation does not ameliorate chemotherapy-induced toxicity. Cancer 1994;74(10):2879-2884.	Medline; EBSCO; SCI; Cochrane.	Wiley Interscience *(Published data only)*
Wu 2001 (29)	Wu GH, Zhang YW, Wu ZH. Modulation of postoperative immune and inflammatory response by immune-enhancing enteral diet in gastrointestinal cancer patients. World J Gastroenterol 2001;7(3):357-362.	Medline; EBSCO; SCI; Cochrane.	Pubmed *(Published data only)*

STUDY ID (REFERENCE NUMBER)	REFERENCE	DATABASE CITATIONS	SOURCE OF FULL TEXT
Xue 2007 (71)	Xue HY, Sawyer MB, Field CJ, Dieleman LA, Baracos VE. Nutritional modulation of antitumour efficacy and diarrhoea toxicty related to irinotecan chemotherapy in rats bearing the ward colon tumour. Clin Cancer Res 2007;13:7146-7154.	SCI	Paper copy from other SA Library *(Published and unpublished data: e-mail correspondence with Vickie Baracos vickieb@cancerboard.ab.ca, 14 June 2009)*
Xue 2008 (72)	Xue H, Sawyer BM, Field CJ, Dieleman LA, Murray D, Baracos VE. Bolus oral glutamine protects rats against CPT-11-induced diarrhoea and differentially activates cytoprotective mechanisms in host intestine but not tumour. J Nutr 2008;138(4):740-746.	Reference list	ProQuest Medical Library *(Published data only)*
Xue 2009 (73)	Xue H, Le Roy S, Sawyer MB, Field CJ, Dieleman LA, Baracos VE. Single and combined supplementation of glutamine and n-3 polyunsaturated fatty acids on host tolerance and tumour response to 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin (CPT-11)/5-fluorouracil chemotherapy in rats bearing Ward colon tumour. Br J Nutr 2009;102:434-442.	Reference list	ProQuest Medical Library *(Published data only)*
Yoshida 1995 (82)	Yoshida S, Kaibara A, Yamasaki K, Ishibashi N, Noaka T, Kakegawa T. Effect of glutamine supplementation on protein metabolism and glutathione in tumour-bearing rats. JPEN 1995;19(6):492-497.	Medline	ProQuest Medical Library *(Published data only)*
Yoshida 1998 (37)	Yoshida S, Matsui M, Shirouzu Y, Fujita H, Yamana H, Shirouzu K. Effects of glutamine supplements and radiochemotherapy on systemic immune and gut barrier function in patients with advanced esophageal cancer. Ann Surg 1998;227(4):485-491.	Medline; EBSCO; SCI; Cochrane.	Journals@Ovid *(Published data only)*
Ziegler 1992 (60)	Ziegler TR, Young LS, Benfell K, Scheltinga M, Hortos K, Bye R, et.al. Clinical and metabolic efficacy of glutamine-supplemented parenteral nutrition after bone marrow transplantation. A randomized, double-blind, controlled study. Ann Intern Med 1992;116(10):821-828.	Medline; EBSCO; SCI; Cochrane.	Academic Search Premier *(Published and unpublished data: e-mail correspondence with Thomas R Ziegler TZIEG01@emory.edu, 14 June 2009)*

APPENDIX 6.6: Characteristics of Included Studies

HUMAN STUDIES

STUDY ID	Anderson 1998 (100)	
SETTING	Randomized, double-blind, crossover clinical trial in Minnesota, 1 May 1993 – 26 April 1996. Randomly assigned to two courses of GLN and two courses of glycine, patients served as their own controls over four courses of chemotherapy.	
POPULATION	24 cancer patients receiving chemotherapy who previously had experienced moderate to severe oral mucositis associated with at least 1 prior course of cancer chemotherapy. Inclusion: All ages and diseases; need to have at least 2 or more courses of chemotherapy scheduled for future. 24 patients eligible and enrolled, 13 completed and included in analysis. Diagnoses (Chemotherapy): Sarcoma (CAD) 3; Ewing's Sarcoma (VAdrC) 3; Osteosarcoma (IA, CDDPAdr, MTX) 5; Rhabdomyosarcoma (VAdrC) 1; Neuroblastoma (VAdrC) 1. Age range: 4–43 yrs (16 children, 8 adults)	
INTERVENTION	Route: Oral, swish and swallow. Study group: GLN (*N*=13): 2g GLN/m^2 (4 ml/m^2) swish and swallow suspension x 2/day (morning and evening). Control group: Placebo (*N*=13): Glycine suspension with same administration schedule. Start criteria: On the day of chemotherapy. End criteria: At least 14 days after chemotherapy. Duration: At least 14 days (2 weeks). (If mouth sores made swallowing difficult, swishing and spitting the suspension out was permitted (1 patient in end)).	
OUTCOMES	Outcomes included: • None Narrative: Patient reported; MECOG grading system (grade 0-4): • Duration of mouth pain/stomatitis (days) • Severity of mouth pain (number of days of stomatitis ≥ grade 2 requiring that the patient's diet be modified to soft food) No data reported as mean (SD).	Other: • None
OTHER	Funding: Grants from the Viking's Children's Fund, Hedberg Foundation, Children's Cancer Research fund, and Mayo Clinic Cancer Center. Analysis: At time of final analysis, much of the data regarding all four courses for each patient was missing. Paired data analysis was used, using the first complete outcome data pair using patients as their own control.	

CAD: Cyclophosphamide, doxorubicin, and dacarbazine; CDDPAdr: Cisplatin and doxorubicin; IA: Ifosfamide and doxorubicin; MECOG: Modified Eastern Cooperative Oncology Group MTX: High dose methotrexate; SD: Standard deviation; VAdrC: Vincristine, doxorubicin, and cyclophosphamide

STUDY ID	Berk 2008 (162)
SETTING	Randomized, double-blind, placebo-controlled multicenter trial in USA, December 2002 to October 2004.

STUDY ID	Berk 2008 (162)	
POPULATION	Patients receiving treatment at any RTOG (*N*=23 institutions) full member, affiliate member, or community clinical oncology programme (CCOP, *N*=15 institutions) member institution were candidates for inclusion. Eligible patients had a stage III or IV solid cancer or currently metastatic cancer of any initial stage. They must have had at least 2% to 10% maximum weight loss over the previous 3 months. They must have Zubrod performance status of 0-2, a life expectancy of at least 3 months and not be on any concurrent appetite-enhancing drugs. 472 patients randomized, 197 analyzed. Primary disease site (HMB/Arg/GLN + Placebo): Lung (67+75), others (153+151) Concurrent chemotherapy (HMB/Arg/GLN + Placebo): Yes (114+126), no (98+96) Evidence of metastases (HMB/Arg/GLN + Placebo): Yes (114+126), no (98+96) Degree of weight loss (3 months prior) (HMB/Arg/GLN + Placebo): 2-5% (98+97), 6-10% (122+129) Age mean (range) yrs: HMB/Arg/GLN 67 (23-91), Placebo 65 (35-90) Gender (HMB/Arg/GLN + Placebo): Male (145+143), female (75+83) Stratified 3 months prior to study entry according to degree of weight loss, primary disease site, concurrent chemotherapy and evidence of metastases.	
INTERVENTION	Route: Oral Study group: HMB/Arg/GLN (*N*=106): Supplement consisted of 3 g HMB, 14 g Arg and 14 g GLN. Both placebo and HMB/Arg/GLN had an orange-drink taste. Patients took either twice a day for 8 weeks. Patients received 8 week supply of supplement at initial visit. Control group: Placebo (*N*=91): Isonitrogenous, isocaloric mixture containing 7.72 g L-alanine, 4.28 g glycine, 2.96 g serine, 1.23 g L-glutamic acid, and 30.52 g gelatin. Start criteria: Initial visit to institution. End criteria: Randomized to either supplement for 8 weeks.	
OUTCOMES	Outcomes included: • Mortality during intervention (*N*) Narrative: • Change in body weight (%)	Other: • Change in lean body mass and circumference measurements
OTHER	Funding: MTI Biotech, Inc. supplied and distributed both the placebo and active supplements.	

Arg: Arginine; CCOP: Community Clinical Oncology Program; HMB: Beta-hydroxy-beta-methylbutyrate; RTOG: Radiation Therapy Oncology Group

STUDY ID	Blijlevens 2005 (109)
SETTING	Randomized, double-blind, placebo-controlled, parallel pilot study in UK, July 1999 - July 2002.
POPULATION	32 allogeneic SCT candidates prepared with Ida, CY and TBI. Ages 18-65 yrs. 35 recruited, 32 enrolled. Type of donor: HLA-matched T-cell-depleted sibling graft. Diagnoses: AML 9; ALL 7; NHL 7; MDS 3; CML 2; MPD 2; CLL 1; chronic myelofibrosis 1 Age (yrs) mean (range): GLN-dipeptide 49 (25-64); Placebo 48 (28-57) Gender (M:F): GLN: 11:9; Placebo: 9:7 Exclusion criteria: Inborn error of amino acid metabolism, insulin-dependent diabetes mellitus and inflammatory bowel disease
INTERVENTION	Route: TPN Study group: GLN dipeptide (*N* = 16): Aminomix of which portion of amino acids has been replaced by a 200 ml GLN-dipeptide (L-alanyl-L-GLN, Dipeptiven, Fresenius-Kabi) isonitrogenous and isocaloric solution equivalent to 0.57g/kg/day GLN dipeptide. Control group: Placebo (*N*=16): Standard Aminomix (Fresenius Kabi, Nederland BV) given parenterally. Start criteria: From SCT day - 6. End criteria: Until bone marrow recovery (granulocyte count > 0.5 x 10^9/l) or removal of CVC for any reason or because of intolerance. Duration: 19 days (median) GLN: 18.8 days Control: 17.9 days

STUDY ID	Blijlevens 2005 (109)	
OUTCOMES	Outcomes included: • Mortality(*N*) • LOS (Mean) + unpublished SD • Unpublished number of patients with clinical infections Narrative: • Clinical events (number of events) • DMS (day +21)	Other: • CRP • Morphine use(*N*) • GVHD (*N*) • MBI (L/R ratio, day +24). • Days of PN • MBI, DMS, DGS assessments at day +7 • Albumin levels • Duration of neutropenia
OTHER	Funding: Educational grant of Fresenius-Kabi Nederland BV. Other: Reasons for stopping PN (GLN + Placebo): CVC removal 8+9; ANC > 500/mm^3 6+4; intolerance 1+2 or doctor's decision 1+1.	

ALL: Acute lymphoblastic leukaemia; AML: Acute myeloid leukaemia; ANC: Absolute neutrophil count; CLL: Chronic lymphocytic leukaemia, CML: Chronic myelogenous leukaemia; CRP: C-reactive protein; *CVC: Central venous catheter; CY: Cyclophosphamide, DGS: Daily gut score; Ida: Idarubicin; NHL: Non-Hodgkin's lymphoma; MBI: Mucosal barrier injury; MDP: Myeloproliferative disorder; MDS: Myelodysplastic syndrome; SD: Standard deviation; SCT: Stem cell transplant; TBI: Total body irradiation*

STUDY ID	Bozzetti 1997 (95)	
SETTING	Randomized, double-blind, placebo-controlled, parallel study in Italy, April 1993 - October 1995.	
POPULATION	67 patients, aged ≥ 70 years with advanced breast cancer treated with oral doxifluridine plus LV. Sites of metastatic disease (GLN + placebo): Soft tissues 31+29; skin 7+7; lymph nodes 14+14; breast 10+8; viscera 18+17; liver 8+8; Lung 6+4; pleural effusion 2+3; other 2+2 and bone 11+9. No of sites (GLN + Placebo): **1** (11+12); **2** (17+15) and **≥3** (4+4) No of treatment for metastatic disease (GLN + Placebo): **0** (18+23); **1** (8+5); **2** (4+3) and **>2** (3+1) Chemotherapy: Oral doxifluridine (600 mg/m2) plus LV (25 mg dose), both x2 per day for 4 consecutive days (day 1 -4) every 12 days. Age mean (range) yrs: GLN: 73.5 (70-88); Placebo: 73.5 (70-86) Exclusion criteria: Pretreated with pelvic irradiation and history of diarrheic habitus.	
INTERVENTION	Route: Oral Study group: GLN (*N* =33): 30 g GLN/day (divided into 3 x 10 g daily doses dissolved in 50 ml water) for 8 consecutive days during chemotherapy-free period (day 5 - 12). Control group: Placebo (*N*=32): Maltodextrin placebo given on the same schedule as GLN. Mean duration of treatment: GLN: 3.5 mo (range 1 - 5 mo); Placebo: 3 mo (range 1 - 6) Median 10 cycles: GLN range 3-15; Control range 1 -15	
OUTCOMES	Outcomes included: *Physician assessment, NCI criteria* • Diarrhoea, number of patients with > grade 1 diarrhoea (N) • Number of patients with maximum grade (3-4) diarrhoea (N) Narrative: • Mean duration of diarrhoea • Grade 1-2 mucositis (no data)	Other: • Tumour response (evaluated by the WHO criteria) complete response, partial response, stable disease and progressive disease • Nausea and vomiting • Gastric pain
OTHER	Funding: No mention.	

LV: Leucovorin; NCI: National Cancer Institute, Bethesda, Maryland; WHO: World Health Organization

STUDY ID	Brown 1998 (140)
SETTING	Randomized, double-blind, placebo-controlled, parallel prospective study in UK.

STUDY ID	**Brown 1998 (140)**	
POPULATION	34 patients undergoing BMT. Disease (GLN+control): NHL 6+7; Hodgkin's 5+3; AML 2+3; CML 2+2; Myeloma 2+1; ALL 1+0. Conditioning regime (GLN+Control): BEAM 10+9; CYTBI 4+5; BUCY 4+2. Type of transplant (GLN+Control): Autologous blood stem cells 11+8; autologous marrow 4+4; allogeneic marrow 3+4. Age mean (range) yrs: GLN: 41 (19-62); Control: 32 (16-55) Gender (M:F): GLN: 11:7; Control: 9:7	
INTERVENTION	Route: TPN Study group: GLN (n =18): Daily infusion of 50 g glycl-L-GLN. Control group: Control (n =16): Daily infusion of an isonitrogenous mixture of non-essential amino acids (no GLN). Start criteria: From the start of conditioning (day -7 before BMT) End criteria: Until discharge from the BMT unit (Outcomes reported up to day +18); ~25 days	
OUTCOMES	Outcomes included: • Mortality (*N*) Narrative: • Weight loss (%) (Median (Range))	Other: • Protein C levels • Albumin levels • Markers of thrombin and plasmin generation (Thrombin-antithrombin, prothrombin fragment F1+2 and plasmin-antiplasmin levels) • Nutritional status (daily caloric and protein intake)
OTHER	Funding: No mention. Other: Extra treatment received (GLN +control): Warfarin 9+2; Vitamin K 10+11	

BEAM: (BCNU 300 mg/m², etoposide 200 mg/m², cytarabine 200 mg/m², melphalan 140 mg/m²; BUCY: Busulphan 14 or 16 mg/kg, CY 120 mg/kg; CYTBI: TBI 14.4 Gy over 8 fractions, CY 120 mg/kg

STUDY ID	**Canovas 2000 (153)**	
SETTING	Randomized, controlled, double-blind, prospective, parallel study in Madrid, Spain.	
POPULATION	Patients undergoing high dose chemotherapy and autologous hematopoietic transplantation. "There were no differences between groups in demographic characteristics, primary haematological disease, initial nutritional assessment, chemotherapy (including melphalan) or infectious prophylaxis."	
INTERVENTION	Route: Oral Study group: GLN (*N*=?): 20 g GLN (Adamin Glu, SHS, Barcelona, Spain) per day dissolved in 100 ml milk, fruit juice or water. Control group: DXM (*N*=?): 20 g dextrinomaltose/day. Other: WP (*N*=?): 20 g whole protein/day. Start criteria: Day of admission. End criteria: When neutrophil count was > 500 cells/mm³ or when TPN was required due to gastrointestinal toxicity. Duration: Unclear, but outcomes reported for days 1, 7 and 14.	
OUTCOMES	Outcomes included: • None Narrative: Physician assessment; NCOG criteria • Diarrhoea (Duration, incidence, severity) • Stomatitis (Duration, incidence, severity) (median (range)) • Serum GLN	Other: • Vomiting • Plasma proteins (Day 1, 7, 14) • Number of patients requiring TPN
OTHER	Funding: No mention	

DXM: Dextrinomaltose; NCOG: Northern California Oncology Group;WP: Whole protein

STUDY ID	**Cerchietti 2006 (101)**
SETTING	Randomized, double-blind, placebo controlled, parallel study in Argentina.

STUDY ID	Cerchietti 2006 (101)	
POPULATION	32 patients (≥18 years age) with histological documented diagnoses of squamous head and neck cancer, clinically unresectable tumour, committed to a treatment of induction chemotherapy plus CRT, and performance status ≤ 2. 29 enrolled. Primary tumour (GLN + placebo): Oral cavity/sinus 5+4; nasopharynx 2+2; oropharynx 6+8 and other 1+1. Staging (GLN +Placebo): **III** (4+5); **IV** (10+10). Antineoplastic treatment: Induction chemotherapy consisting of two inpatient cycles of cisplatin 100 mg/m2 (3 hour infusions on days 1 and 21) + continuous infusion of 5-FU 1000 mg/m2 (days 1 -5 and days 21 - 25) followed by concurrent outpatient regimen for CRT. RT (70 Gy over 5 weeks) started on day 28. Concomitant chemotherapy consisted of a weekly administration (days 35, 42, 49 and 56) of a 1 -h infusion of cisplatin 30 mg/m2 followed by a 1 -h infusion of 5-FU 300 mb/m2. Age mean(range) yrs: GLN: 56 (42-75); Placebo: 55 (40-74) Exclusion criteria: Patients whose primary disease precluded an adequate evaluation of the oral mucosa; patients with severe renal or hepatic insufficiency.	
INTERVENTION	Route: TPN Study group: GLN (*N*=14): L-alanyl-L-GLN 0.4 g/kg weight/day (2 mL/kg weight/day) diluted in normal saline (1:5v/v) administered by intravenous infusion of 4 hrs on same days as the chemotherapy. Control group: Placebo (*N*=15): Normal saline administered with same dose, dilution and infusion rate. All patients received oral liquid-food supplementation equivalent to 20% of the basal metabolic rate at rest measured at the start of the treatment by bio-electrical impedance. Patients with mucositis WHO grade 4 or weight loss ≥ 5% respective to the weight at day 1 of treatment were given feeding tubes. Criteria for administration of intervention: Days 1-5, 21-25, 35, 42, 49, 56) = 14 days total	
OUTCOMES	Outcomes included: Physician assessment, OMS in addition to WHO scale • Patients with severe objective mucositis (OMS >1.49) (*N*) • Patients with mucositis WHO grade 4 (*N*) • Body weight change (kg) Narrative: • OMS (mean of three highest scores) • Incidence of infections (no data)	Other: • Incidence of mucositis-related hospitalizations (*N*) • Need for feeding tube (*N*) • Time free of severe functional mucositis (WHO score grade 3 - inability to swallow solid food or 4 - no form of oral alimentation possible) • Incidence of pain (mean of three highest Numeric Rating Scale values) • Use of opioid analgesic medication • Performance status change • Tumour response • Intensity of CRT delivered • Laboratory results.
OTHER	Funding: Supplements were gifts from Fresenius Argentina S.A., saline solutions and intravenous sets were gifts of Roux-Ocefa S.A. and research funds from the Institute Angel Roffo and the Angel Roffo Foundation. Pilot study to determine incidence of local and systemic adverse events, compliance, comparison between scales to quantify mucositis, nutritional status and staff training. Patients were randomly assigned (1:1 ration) to receive either 300 or 400 mg/kg body weight of intravenous L-alanyl-L-GLN.	

5-FU: 5-Flourouracil; CRT: Chemo-radiation therapy; OMS: Objective mucositis score; RT: Radiation therapy; WHO: World Health Organization

STUDY ID	Choi 2007 (93)
SETTING	Randomized, controlled, parallel study in Seoul, Korea, September 2003 - August 2005. Open label trial.

STUDY ID	Choi 2007 (93)	
POPULATION	51 patients with histological confirmed advanced/metastatic cancer received FU/LV chemotherapy. Patients had not received the chemotherapy previously or prior RT or concurrent RT. Inclusion criteria: Age > 18 yrs; ECOG performance status ≤ 1; ANC ≥ 1500/mm^3; platelet count ≥ 100,000/mm^3, serum creatinine concentration ≤ 1.25 times the upper normal limit, or creatinine clearance > 60 mL/min, normal liver function tests, free of signs of systemic infection, not taken antibiotics/alcohol 1 week prior testing, not taken non-steroidal anti-inflammatory drugs 2 weeks prior and during intestinal permeability test. Histological type (GLN + BSC): Adenocarcinoma 12+16; squamous cell carcinoma 7+9 and others 3+4. Primary tumour site (GLN +BSC): Stomach 3+6; esophagus 5+6; colorectum 2+6; periampulla 2+3; biliary 4+4; head and neck 3+2 and others 3+2. Chemotherapy: Daily administration of 100 mg/m^2 LV over 30 min followed by 500 mg/m^2 FU continuous infusion for 5 days. Oral cryotherapy: 30 min 4 x per day during chemotherapy. Age Mean (Range)yrs: GLN: 54 (26-73), BSC: 54 (26-79) Gender (M:F): GLN: 14:18, BSC: 19:10	
INTERVENTION	Route: Oral Study group: Oral GLN group (*N* = 22): 30 g GLN (L-GLN, Daesang Wellife, Seoul, Korea) per day. Administered by mixing virtually tasteless powder with any food/drink/water 3 x per day. Control group: Best Supportive Care group (*N* = 29): Unclear Start criteria: 3 days before the inception of chemotherapy. End criteria: Continued for 15 days total.	
OUTCOMES	Outcomes included: Physician assessment, CTCAE v3.0 grades • Mucositis/stomatitis, some degree (N) • Mucositis/stomatitis, ≥ grade 2 (N) • Mucositis/stomatitis, grade 4 (N)	Other: • Intestinal permeability (Measuring of the Cr-EDTA urinary excretion after oral challenge) on day 7 after discontinuation of chemotherapy.
OTHER	Funding: No mention. 2nd Control Group (*N*=18) of healthy volunteers to establish normal IP values.	

ANC: Absolute neutrophil count; BSC: Best supportive care; CTCAE: Common terminology criteria for adverse events; ECOG: Eastern Cooperative Oncologic Group; FU/LV: Flourouracil/Leucovorin; RT: Radiation therapy

STUDY ID	Coghlin Dickson 2000 (97)
SETTING	Randomized, double-blind, placebo-controlled, prospective, parallel study in Stanford, California, June 1995 - August 1997.
POPULATION	58 autologous and allogeneic BMT or PBPC transplant patients. Disease (GLN + placebo): ALL 1+1; ANLL 2+2; CML 5+3; MDS 0+2; MM 10+12 and NHL 12+9. Regimen type (GLN + placebo): Non-FTBI 1+1; FTBI 28+28. Graft type (GLN+placebo): Allogeneic 11+13; Autologous 18+16. Age median (range) yrs: GLN: 46 (17-58); Placebo: 48 (21-59) Gender (M:F): GLN: 14:15; Placebo: 18:11
INTERVENTION	Route: Oral Study group: GLN (*N*=29): 3 x 10 g doses per day of pharmaceutical grade GLN powder (Ajinomoto USA, Inc, Teaneck, NJ). Powder mixed with liquid or soft solid food. Control group: Placebo (*N*=29): 3 x 10 g doses per day of powdered sugar in similar manner. Not isonitrogenous. Start criteria: First day of preparative regimen. End criteria: Until discharge or no later than day 28 after transplant. LOS median (range): GLN: 21 (4-41); Placebo: 19 (3-53) Follow –up(median (range)) months: GLN: 21 (1 - 35); Placebo: 13 (1-35); Minimum of 11 months follow-up for surviving patients.

STUDY ID	Coghlin Dickson 2000 (97)	
OUTCOMES	Outcomes included: Physician assessment; Stanford University Hospital BMT toxicity scale • Patients with mucositis grades 2-4 (*N*) • Two year survival rate(*N*) Narrative: • Length of hospitalization (days) • Days of mucositis (days) • Mucositis grades (median grade) • Diarrhoea (days stool output > 500 mL/day) (Median(range) only)	Other: • Number of TPN days. • Number days until > 1000 ml fluid orally per day. • Time to WBC engraftment (WBC > 1000/mL3) • Lowest serum albumin. • Highest creatinine, total billurubin. • Tolerance and consumption of GLN.
OTHER	Funding: Small Grants Program of Stanford University Hospital's Nursing Management Department. Other: TPN was initiated when oral intake < 50% of caloric needs. If a patient was unable to take anything in oral form, the provision of GLN or placebo was stopped until the patient was able to resume oral intake. Generally, if the patient stopped taking the GLN or placebo, it was not restarted.	

ALL: Acute lymphoblastic leukaemia; ANLL: Acute non-lymphocytic leukaemia; BMT: Bone marrow transplant, CML: Chronic lymphocytic leukaemia; FTBI: Full total body irradiation; MDS: Myelodysplastic syndrome; MM: Multiple myeloma; NHL: Non-Hodgkin's lymphoma; PBPC: Peripheral blood prognitor cell; WBC: White blood cell

STUDY ID	Da Gama Torres 2008 (123)	
SETTING	Randomized, controlled, double-blind, parallel study in Brazil. October 2001 - September 2004.	
POPULATION	53 consecutive leukaemia patients aged 18-64 who underwent HLA-identical sibling allogeneic SCT after myeloblative conditioning regimen. Disease status (GLN + Control): CML 17+16; AML 5+6; ALL 2+3 and Myelodisplasia 3+1. Graft source (GLN +placebo): Peripheral blood 19+19; bone marrow 8+7 Age mean (SD) yrs: GLN: 37.33 (11.06); Control: 35.96 (8.71) Gender (M: F): GLN: 13:14; Control: 12:14.	
INTERVENTION	Route: TPN All patients were allowed to eat at libitum, but oral intake was not recorded. Study group: GIPN (*N*=27): 500 ml of 10% crystalline amino acid solution with 500 ml of 50% dextrose and water, yielding a 25% concentration of glucose and a non-protein calorie/nitrogen relation of approximately 110 cal/g. A dose of 0.3 - 0.4 g/kg/day of L-alanyl-L-GLN dipeptide (Dipeptiven, Fresenius Kabi, Campinas, Brazil) corresponding to 0.2 -0.27 g/kg/day of GLN was added to PN. Control group: PN (*N*=26): The corresponding amount of standard amino acids was removed from the solution to prepare isonitrogenous solutions. Start criteria: Day of stem cell infusion (day 0) End criteria: Sixth day after infusion (day +6) Patients who needed to continue on PN after day+6 were kept on standard solutions.	
OUTCOMES	Outcomes included: • Clinical infection rate (*N*) (addition or change in antimicrobials). • Survival on day+100 (*N*) • Length of stay (Mean) (Unpublished *SD provided by author)*	Other: • Duration of neutropenia (< 0.5 x 10^9/l.)(days) • IP (lactulose and mannitol urinary excretion fraction) on admission, day +6, day +14. • Survival on day +180 • Incidence of acute GVHD (*N*)
OTHER	Funding: GLN was supplied without charge by Fresenius Kabi, Campinas, Brazil.	

ALL: Acute lymphoblastic leukaemia; AML: Acute myeloid leukaemia; CML: Chronic lymphocytic leukaemia; GVHD: Graft-versus-host-disease; SD: Standard deviation

STUDY ID	Daniele 2001 (102)
SETTING	Randomized, double-blind, placebo-controlled, parallel study in Italy, June 1996 - April 1998.

STUDY ID	**Daniele 2001 (102)**	
POPULATION	70 chemotherapy naive patients with colorectal cancer scheduled to receive chemotherapy with FU/FAcid as treatment for advanced metastatic colon cancer or as adjuvant therapy after surgical resection of colon cancer. ECOG scale <2 for performance status, normal creatinine clearance. Treatment setting (GLN + placebo): Adjuvant 8+10; advanced 21+23 Age median (range) yrs: GLN: 63 (44-76); Placebo: 61 (35-75) Gender (M:F): GLN: 17:12; Placebo: 19:14 Chemotherapy: Daily administration of 100mg/m^2 FAcid followed by 450 mg/m^2 FU for 5 days. FAcid given by IV infusion over 30 min followed by IV bolus dose of FU between 9 - 11 am.	
INTERVENTION	Route: Oral Study group: GLN (*N* = 35): Crystalline GLN powder (Bracco Pharmaceutical Company) in 3 g sachets (2 sachets x 3 per day = 18 g GLN per day). Control group: Placebo (*N*=35): Crystalline maltodestrin powder (Bracco Pharmaceutical Company) in 3 g sachets (2 sachets x 3 per day = 18 g placebo). Start criteria: 5 days before the first day of chemotherapy. End criteria: After 15 consecutive days. Study was limited to the first chemotherapy cycle.	
OUTCOMES	Outcomes included: Patient diary (number, consistency, presence of faecal blood of stools); Common toxicity criteria of NCI • Incidence of different grades of diarrhoea (*N*) • Duration of diarrhoea (days) • Number of patients with > grade 2 stomatitis (*N*) Narrative only: • Mean duration of stomatitis (No SD)	Other: • Intestinal absorption (D-xylose absorption test) • Intestinal permeability (cellobiose-mannitol permeability test) • Nausea, vomiting and haematologicalal toxicity. • Loperamide use (number of tablets) • Toxicity (patient diary): Nausea, vomiting, abdominal pain, number of loperamide tablets.
OTHER	Funding: Partly by Ministero della Sanita and Regione Campania.	

ECOG: Eastern Cooperative Oncologic Group; FU/FAcid: Flourouracil/ Folic acid; IV: Intravenous; NCI: National Cancer Institute, Maryland, Bethesda; SD: Standard deviation

STUDY ID	**Decker Baumann 1999 (48)**
SETTING	Randomized, controlled, parallel study in Berlin, Germany.
POPULATION	24 patients with metastatic colorectal carcinoma. Chemotherapy: three courses of cytostatic therapy. 5-FU (550 mg/m^2/day) by continuous 5-day infusion and CFO (170 mg/m^2/day) as an IV bolus injection on each of these days. Chemotherapy was repeated every 4 weeks. Response to chemotherapy (GLN + Control): Partial response 3+4; no change 5+5 and progressive disease 4+3. Age mean (SD) yrs: GLN: 56.1 (9.6); Control: 58.4 (7.2) Gender (M:F): GLN: 8:4; Control: 7:5
INTERVENTION	Route: TPN Study Group: GLN group (*N*=12): GLN administered as glycl-L-GLN IV over 8 h in a 10% solution at 0.4 g/kg body weight per day (14-22 g GLN/day). Control group: Controls (*N*=12): Without GLN supplementation. Start criteria: One day before the beginning and during chemotherapy. End criteria: Duration of three courses chemotherapy (5 days chemo per cycle). Duration: 6 days every 4 weeks (3 cycles)

STUDY ID	**Decker Baumann 1999 (48)**	
OUTCOMES	Outcomes included: • Body weight change from baseline (kg) Narrative: • Plasma GLN concentrations (umol/L) *Physician assessment; WHO criteria:* • Mucositis incidence, severity • Diarrhoea incidence, severity (Data presented in figure, no SD	Other: • Plasma pre-albumin and RBP change from baseline (mg/100ml). • Ratio of villous height to crypt depth. • Chemotherapy-induced side effects graded according to WHO criteria: Gastrointestinal and duodenal mucosa ulcerations. • GLN concentration in duodenal mucosa. • Energy and protein ingestion.
OTHER	Funding: Supported by Pharmacia & Upjohn GmbH, Erlangen, Germany.	

CFO: Calcium-folinate, IV: Intravenous; RBP: Retinol-binding protein; SD: Standard deviation; WHO: World Health Organization

STUDY ID	**Erdem 2002 (144)**	
SETTING	Randomized, controlled, parallel study at the 4th Surgical Department of Ankara Numune Education and Research Hospital, Turkey, May 1995 - March 1997.	
POPULATION	32 adult patients who underwent operations for gastrointestinal system (esophagus, stomach, colon, rectum) malignancies with no distant metastases. Patients had no previous operation for the matter and required both pre- and postoperative nutritional support. Tumour site (GLN + Control): Esophagus 1+0; stomach 8+7; colon 1+2 and rectum 6+7. Surgical procedure (GLN+Control): Total gastrectomy 3+3; subtotal gastrectomy 5+2; colectomy 1+2; anterior resection of the rectum 6+3 and unresectable tumour 1+6. Age mean (SD): GLN: 52.94 (13.09); Control: 56.50 (11.22) Gender (M:F): GLN: 7:9; Control: 8:8	
INTERVENTION	Route: Enteral Enteral formulas covered 30 - 35% of requirements and hospital diet 65 - 75% in all patients. Study group (SG): Alitraq (*N*=16): Patients in SG received GLN enriched elemental formula (Alitraq, 10g GLN/178g , Ross Laboratories, Columbus, Ohio) + hospital diet (14.2 g GLN/L, 4.5 g arginine/L). Control group (CG): Ensure (*N*=16): Patients in CG received GLN-free polymeric enteral formula (Ensure, Ross Laboratories, Columbus, Ohio) + hospital diet. Start criteria: 7 days preoperative. End criteria: 10 days postoperative. After exclusion: GLN (*N*=15); Control (*N*=10).	
OUTCOMES	Outcomes included: • Mortality during intervention (*N*) • Body weight change from baseline (kg)	Other: • Initiation of oral intake postoperatively (days). • Blood sampling (total protein, albumin, prealbumin and transferrin) change from baseline. • Baseline, pre-operative day 7, postoperative day 1 and 10: Anthropometric measurements (MUAC, TST, BMI). • PNI (1 & 2), NRI
OTHER	Funding: No mention.	

MUAC: Mid-upper-arm circumference; NRI: Nutritional risk index; PNI: Prognostic nutritional index; TST: Triceps skinfold thickness

STUDY ID	**Gianotti 2009 (163)**
SETTING	Prospective, randomized, multicentre (11 hospitals) trial in Italy, July 2005 to December 2007.

STUDY ID	Gianotti 2009 (163)	
POPULATION	Eligible patients were adult, well nourished (preoperative weight loss < 10% of usual body weight), with documented cancer of gastrointestinal tract, and a candidate for major surgery. Exclusion criteria: Denied written informed consent, Child-Pugh class C, New York heart Association class (NYHA) > 3, renal insufficiency (hemodyalisis, plasma creatinine >3 mg/dL, or both), respiratory insuffiency (arterial blood PaO_2 <70 mm Hg), Karnofsky performance status <80, American Society of Anesthesiology score (ASA) > 3, ongoing infection, immunosuppressive diseases (including steroid use), emergency operation, or pregnancy. 555 patients were eligible for the study, 428 patients were randomized and analyzed on an intention-to-treat basis. Age mean (SD) years: Ala-GLN 65.0 (9.6), Control 64.4 (10.0) Gender (M:F): Ala-GLN 129:83; Control 131:85 BMI: Ala-GLN 25.7 (4.0); Control 26.2 (3.2) % weight loss: Ala-GLN 1.4 (2.4), Control 1.4 (2.7) Co-morbidities (Ala-GLN+Control): **1** (73+67); **2** (28+31) and **≥ 3** (6+8) Surgical procedures (Ala-GLN+Control): Left colectomy (60+50); rectal resection (39+48); right colectomy (28+32); subtotal gastrectomy (26+22); total gastrectomy (14+19); pancreatoduodenectomy (20+16); liver resection (10+13); abdomino-perineal amputation (2+9); ileal resection (5+4); distal pancreatectomy (4+3); subtotal colectomy (2+2); multiple procedures (14+19). Neoadjuvant therapy: Ala-GLN 18; Control 20	
INTERVENTION	Route: TPN Both groups received preoperative fluids and electrolytes as required. In both groups no artificial nutritional support was allowed unless the patient could not start a progressive oral feeding within 7 days after surgery. Patients were allowed to resume oral natural food. Study group: Ala-GLN group (*N=212*): Intravenous infusion of L-alanine-L-GLN dipeptide (0.40g/kg/day, 0.25 g of free GLN) in 500 ml 5% glucose vehicle. GLN was infused continuously through a peripheral or CVC over a period of 20 hrs. Control group: (*N*=216): Received only the vehicle. Start criteria: Morning before operation, day -1, after randomization. End criteria: GLN infusion continued for at least 5 days in the postoperative course. Mean (SD) duration: 7.1 (1.8) days	
OUTCOMES	Outcomes included: • Length of hospital stay • Infectious complication rate Narrative: • Plasma levels of GLN (day -1 (baseline), day 0 (day of surgery), and on days +1, +3, and +5 after operation).	Other: • Postoperative complication rates. • Need for postoperative artificial nutrition support.
OTHER	Funding: Supported by the Italian Society of Parenteral and Enteral Nutrition (SINPE)	

CVC: Central venous catheter

STUDY ID	Hallay 2002 (133)
SETTING	Randomized, placebo-controlled, parallel study in Second Department of Surgery, Medical and Health Science Centre, School of Medicine, University of Debrecen, Hungary, 1998-2001.
POPULATION	36 patients suffering from esophageal cancer. All patients received cobalt irradiation (15 Gy) plus ceftriaxon and metronidazol preoperatively. Age range (mean) yrs: GLN: 43-70 (55.1); Control: 34-71 (52.5)
INTERVENTION	Route: Enteral A feeding tube was inserted into the second jejunum loop. Study group: Group 1 – Stresson Multi Fibre (*N*=23): GLN rich (1.3 g/100 mL) Stresson Multi Fibre nutriment (Nutricia, Zoetermer). Control group: Group II, Nutrison Multi Fibre (*N*=13): GLN poor Nutrison Multi Fibre diet (Nutricia, Zoeterner) Start criteria: Day 3 postoperative. (Initial dose 20 mL/h, max dose 80 - 100 mL/h); Day 5 postoperative. (1500 - 2000 ml , 30 ml/kg volume reached, flow of 90-100 ml/h) End criteria: Jejenul nutrition for at least 10 days.

STUDY ID	Hallay 2002 (133)	
OUTCOMES	Outcomes included: • Mortality (*N*) Narrative: • Hospital stay (No SD) • Clinical infection (No data)	Other: • CRP (mg/L) • Protein (g/L) • Albumin g/L) • Prealbumin (mg/L) • RBP (mg/L) • IgA (g/L), IgM (g/L), IgE (g/L), IgG (g/L) • Phagocyte activity (cpm) • C3, C4 (g/L), Cd3, CD4, CD8, CD19, CD56 (%) • BMI kg/m'2. • ICU stay • Fibrinogen (g/L)
OTHER	Funding: No mention Other: Continued per os intake of fluids (enriched with different nutriments) after day 7 postoperative X-ray examination. Jejenul nutrition was stopped only when per os calorie intake fulfilled patient's need.	

CRP: C-reactive protein; ICU: Intensive care unit

STUDY ID	Huang 2000 (103)	
SETTING	Randomized, placebo-controlled, parallel pilot study in Department of Radiation Oncology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung Hsien, Taiwan, July 1997 to June 1998.	
POPULATION	17 patients with head and neck cancer receiving RT. At least one half of the oral cavity mucosa needed to be included in the fields of irradiation. Patients had to tolerate solid food at study entry. Exclusion criteria: Mouth sores, diabetes, trismus, underwent chemotherapy, used other prophylactic drugs or mouthwashes and Karnofsky's performance status < 70. Diagnoses (GLN + Placebo): Nasopharyngeal cancer 3+2; oropharyngeal cancer 0+3 and oral cavity cancer 5+4. Radiation schedule: 1.8 Gy/fraction, 5 fractions per week and 25 fractions for initial fields. Gender (M:F): GLN: 6:2; Placebo: 7:2 Age mean (SD) yrs: GLN: 47 (8); Placebo: 54 (12)	
INTERVENTION	Route: Swish and expectorate. Study group: GLN (*N* = 8): GLN suspension (16 g L-GLN in 240 ml normal saline) of which 30 ml (2 g GLN) to be swished for 3 minutes and expectorated before meals and at bedtime daily (4 times). 8 g GLN per day. Control group: Placebo (*N*=9): 30 ml normal saline swished x 4 daily in same way as GLN group. Start criteria: Morning of the first fraction of RT. End criteria: Bedtime of the 25th fraction of RT.	
OUTCOMES	Outcomes included: Physician assessment; RTOG/EORTC criteria • Objective severity of mucositis (grade 0-4) Narrative: • Subjective severity (grade 0-4, patient complaint). • Body weight change (kg) • Mucositis duration (No SD)	Other: • Treatment time to 45 Gy (days)(No SD) • WHO steps (3) for pain of Mucositis (N)
OTHER	Funding: No mention.	

RT: Radiation therapy; RTOG/EORTC: Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer; SD: Standard deviation; WHO: World Health Organization

STUDY ID	Jebb 1994 (104)
SETTING	Randomized, double-blind, cross-over study in UK.

STUDY ID	Jebb 1994 (104)	
POPULATION	28 patients with advanced, metastatic, gastrointestinal cancer. Primary sites (Male: Female): Stomach 3: 0; colon 11:6; rectum 0:5, pancreas 0:1; gall bladder 0:1 and unknown 1:0. All patients received folinic acid (20 mg.m-2) IV bolus followed by 5-FU (400 or 425 mg.m-2) IV bolus daily for 5 days x 4 weeks. After dropout: Patients (*N*=17) Consumption of dose, mean (SD): 93 (11%)	
INTERVENTION	Route: Oral swish and swallow Study group: GLN (*N*=28): 16 g GLN (BDH) daily divided into 4 equal doses taken after meals and before bed. Control group: Placebo (*N*=28): Polycal (Cow and Gate), a glucose polymer, given to the same schedule. Presentation: Individual sachets dissolved into 150 ml water/cold fluids immediately prior to consumption. Patients instructed to use as mouthwash prior to swallowing. Start criteria: 24 hrs prior to treatment. End criteria: 48 hrs after final infusion of chemotherapy - 8 days total.	
OUTCOMES	Outcomes included: Physician assessment, WHO classification • Observer mucositis some degree (*N*) • Observer mucositis score ≥2 (*N*) • Observer mucositis grade ≥ 3 (*N*) • Mortality (*N*) Narrative only: • Mean score for patient-reported mouth comfort (mucositis) • Serum GLN levels (no data)	Other: • WBC (x 10^9/l), haemoglobin (g/l) and platelets (x10^9/l). • Mean score for patient-reported ease of eating and stool consistency, no of stools per day.
OTHER	Funding: No mention.	

FU: Flourouracil; IV: Intravenous; WBC: White blood cell; WHO: World health Organization

STUDY ID	Jebb 1995 (96)	
SETTING	Randomized, placebo-controlled, parallel pilot study in UK.	
POPULATION	24 patients (12 pairs matched for current treatment) receiving autologous BMTs, following 6 days of conditioning treatment with BCNU, etoposide and melphalan. Diagnoses included: AML, CML, HD, myeloma and NHL. (No data on characteristics presented). Groups after dropout: GLN (*N*=8); Control (*N*=8) Consumption, mean (SD): GLN: 69 (15%), Placebo: 76 (15%)	
INTERVENTION	Route: ORAL, swish & swallow Study group: GLN (*N*=12): 16 g GLN (BDH) per day divided into 4 equal doses taken after meals and before bedtime. Control group: Placebo (*N*=12): Polycal (Cow and Gate), a glucose polymer, given to the same schedule. Presentation: Individual sachets dissolved into 150 ml water/cold fluids immediately prior to consumption. Patients instructed to use as mouthwash prior to swallowing. Start criteria: Day 1 post-transplant End criteria: Continued until mucositis resolved or until discharged. Length of hospital stay, mean (SD): GLN: 25.6 (2.2); Control: 28.3 (5.5)	
OUTCOMES	Outcomes included: Nurses assessment; "Observer Mucositis assessment" tool (grade 0-4) • Duration of mucositis score ≥3 (days) • Hospital days (days) • Mean duration of diarrhoea (> 4 loose stools) (days) Narrative only: • Total mucositis score (Observer- & patient-reported, mean (SD)) • Patient-reported mucositis score ≥ 3 duration (days)	Other: • Neutropenia, < 0.5 x 10^9/l (days) • Platelets< 50 x 10^9/l (days) • TPN use (days) • IV diamorphine use (days) • 6 Month follow-up: complete response, partial response, relapse (*N*)

STUDY ID	Jebb 1995 (96)
OTHER	Funding: No mention. Other: Total enteral intake was not formally assessed. Patients commenced PN when < 1000 kcal consumed orally. At the peak of mucositis about 25% of patients were unable to swallow comfortably and were only able to use either solution as a mouthwash. This persisted for 2-5 days.

AML: Acute myeloid leukaemia; CML: Chronic myelogenous leukaemia; HD: Hodgkin's disease; Non-Hodgkin's lymphoma; SD: Standard deviation; TPN: Total parenteral nutrition

STUDY ID	Jo 2006 (142)	
SETTING	Prospective, randomized, double-blind, controlled, parallel study at the Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, February 2004 - March 2005.	
POPULATION	143 consecutive patients admitted for operations for alleged/suspected periampullary tumours; 99 (initial examination) became interim candidates (suitable preoperatively), 66 (interim examination) enrolled postoperatively; 60 (final examination) after pathology reports. Exclusion criteria: Age > 70, < 30; presence of debilitating disease other than DM at and/or within 2 months before diagnoses of periampullary tumours; history of drug/alcohol abuse; restricted oral intake or TPN > 2 weeks preoperative; pregnancy or + serum beta-hCG; patient refusal; concurrent tumours; combined resection of other intra-abdominal organs; operations other than PD and benign pathologic findings. Age mean (SD) yrs: GLN: 56.8 (9.4); Control: 56.9 (10.3) Gender (M:F): GLN: 1.5:1; Control: 0.6:1.0	
INTERVENTION	Route: TPN Study group: GLN group (*N*= 2): 10 ml Glamin (15% amino acid solution)/kg/day (contains 0.2 g GLN/kg/day in form of glycyl -L-GLN dipeptide) (Fresenius Kabi AG, Bad Homburg, Germany). Control group: Control group (*N*=28): Commercially available isonitrogenous (1.3 g/kg/day amino acid) and isocaloric (30 kcal/kg/day) TPN formula. Start criteria: From second preoperative day after randomization. End criteria: 5th postoperative day, excluding day of operation. End of follow-up: Discharge	
OUTCOMES	Outcomes included: • Hospital mortality (*N*) Narrative: • Hospital stay (Median (Range))	Other: • Time to soft diet (days) • Postoperative complications (*N*) • Anthropometric measures (MAC, TSF, MAMC) • Chemical profiles (TLC, total protein, CRP, haemoglobin and cholesterol)
OTHER	Funding: No mention. Other: Patients were discharged when they could tolerate a soft diet and with no unresolved complications.	

CRP: C-reactive protein; DM: Diabetes mellitus, MAC: Mid-arm circumference, MAMC: Mid-arm muscle circumference; TLC: Total lymphocyte count; TPN: Total parenteral nutrition

STUDY ID	Klek 2005 (61)
SETTING	Prospective, randomized, placebo-controlled, parallel clinical study at 1st Department of General and GI Surgery of Jagiellonian University in Cracow, January 2001- August 2003.

STUDY ID	Klek 2005 (61)	
POPULATION	105 patients operated on for gastric carcinoma randomized, 15 dropped out (*N*=90). All patients underwent gastric resection with lymphadenectomy and qualified for postoperative PN. Inclusion criteria: Age 18-90 years, total/subtotal gastrectomy for gastric cancer, Karnoffsky grade > 60, contra-indications for enteral nutrition after surgery, no indications for nutritional treatment preoperatively and indications for PN postoperative. Exclusion criteria: Severe grade malnutrition, recent history of severe heart, lung, kidney or liver failure, history of recent immunosuppressive therapy, confirmed metastases to CNS, contra-indications for PN and unexplained vomiting/diarrhoea. Types of surgical procedures (Group A+B+C): Subtotal gastric resection 7+8+6 and total resection 22+24+23. Type of lymphadenectomy (Group A+B+C): **D-2** (14+16+13); **D-3** (15+16+16) Gender (M:F) = 51:39 Mean age (range) yrs: 61.9 (38-77)	
INTERVENTION	Route: TPN Protein: 0.15 -0.5g N/kg BW. Energy: Q=130 – 170 kcal/g N. Control Group: Group A (*N*=35): Standard PN. Study Group: Group B (*N*=34): Standard PN supplemented with IV GLN (Dipeptiven, Fresenius-Kabi) at dose of 2.0 ml/kg per day. Other: Group C (*N*=36): Standard PN supplemented with IV omega-3-unsaturated fatty acids (Omegaven, Fresenius-Kabi). Start criteria: 24 hours after surgery for at least 7 days. End criteria: Until enteral diet covered > 60% of protein and energy requirements. After dropout/withdrawal: GLN: *N*=30; Control: *N*=30; Omega: *N*=30	
OUTCOMES	Outcomes included: • Number of patients with clinical infection (Wound infection)(*N*) Narrative: • Length of post-operative hospital stay (Mean(Range))	Other: • Time of TPN (days)(No SD) • Laboratory analysis of liver and kidney function (serum SGOT, SGTP, urea and creatinine) • Cost of hospital stay (PLN, Euro) • Change in serum albumin (g/L), prealbumin (mg/dl) and TLC (cells/mm'3) from day postoperative day 3 to 7/12. • Patients with postoperative complications in general (*N*) • Nutritional assessment (BMI)
OTHER	Funding: No mention	

CNS: Central nervous system; PN: Parenteral nutrition; SD: Standard deviation; TLC: Total Lymphocyte count; TPN: Total parenteral nutrition

STUDY ID	Kozelsky 2003 (119)
SETTING	Randomized, placebo-controlled, double-blind, parallel study from 14 institutions in USA, February 1998 - October 1999.
POPULATION	129 patients, > 18 yrs, with histological confirmed adenocarcinoma or squamous cell carcinoma. Inclusion criteria: Must receive RT to entire pelvis at an NCCTG-approved radiation oncology facility. Planned dose 45 - 53.5 Gy (daily dose 1.7-2.1 Gy). Exclusion criteria: Pregnancy; GLN allergy; history of pelvic RT; history of abdominal-perineal resection; planned concurrent chemotherapy other than FU. Primary tumour site % (GLN+Placebo): Rectal cancer 6+6; prostate cancer 66+65; gynecologic cancer 27+28 and other 2+2. Use of FU % (GLN+Control): Not used 94+94; bolus 3+2 and continuous infusion 3+5. Gender % (M:F): GLN: 69:31; Control: 68:32 Age median (mean) yrs: GLN: 69.5 (67.5); Control: 69.0 (65.4)
INTERVENTION	Route: Oral Study group: GLN (*N*=64): 4 g L-GLN (8 ml) x 2 per day (morning and evening) 7 days per week during RT and for 2 weeks thereafter. Control group: Placebo (*N*=65): Identical appearing placebo (glycine) which was administered according to the same schedule. Both medications made up with 40 ml Ora-sweet and 80 ml water. Start criteria: First or second day of RT, for duration of RT. End criteria: 2 weeks after RT or evidence of grade 3 or worse diarrhoea, rectal bleeding, or abdominal cramping.

STUDY ID	Kozelsky 2003 (119)	
OUTCOMES	Outcomes included: Physician assessment; NCI criteria • Number of patients with > grade 1 diarrhoea (*N*) • Number of patients with maximum grade diarrhoea (*N*) • Number of patients with at least grade 2 diarrhoea (*N*)	Other: • Number of patients with no abdominal cramping (*N*) • Number of patients with at least grade 2 abdominal cramping (*N*) • Antidiarrhoeal agent used (Dipenoxylate, Loperamide) • Stools per day, maximum stools per day • Quality of life data (UNISCALE) • Follow-up data
OTHER	Funding: Supported in part by Public Health Service grant	

FU: Flourouracil; NCCTG: North Central Cancer Treatment Group; NCI: National Cancer Institute, Maryland, Bethesda; RT: Radiation therapy

STUDY ID	Li 2009 (120)	
SETTING	Randomized, double-blind, cross-over clinical trial in China.	
POPULATION	44 patients with gastric or colorectal cancer. Eligibility criteria: Diagnoses with gastric or colorectal cancer; WHO-developed side effect grade 2 or higher in previous screening chemotherapy cycle and aged between 40-69 yrs. Exclusion criteria: GLN allergy, abdominal-pelvic RT in their medical history; renal and/or liver function insufficiency; administration of antibiotic therapy for specific indication of fever and use of analgesics or/and anti-diarrheic. Diagnosis (GLN+Control): Gastric cancer 16+14 and colorectal cancer 6+8. Patients received same chemotherapy in cycle 1 and 2. Gastric cancer patients (*N*=26) received FAM regimen. Colorectal cancer patients (*N*=18) received FOLFOX-4 regimen. A new course of chemotherapy could begin if the ANC was > 1500/mm^2; the platelet count was > 100 000/mm^3. If after a 1-week delay, toxicities were < grade 1, treatment resumed. If the toxicity did not resolve in 1 week, a second one-week delay was allowed. No patient had treatment interrupted lasting more than 3 weeks. Gastrointestinal toxicity at baseline (screening cycle): Nausea/vomiting GLN: 2.61 (0.15); Control: 2.59 (0.13) Diarrhoea GLN: 2.69 (0.20); Control: 2.76 (0.19) Age, mean (SD)(Range) yrs: GLN: 56.1 (5.9)(48-68); Control: 56.5 (6.2)(46-68) Gender(M:F): GLN: 13:9; Control: 11:11	
INTERVENTION	Route: TPN Study group: With GLN (*N*=22): Prophylactic intravenous 20 g (~ 0.3 g/kg BW/day) of alanyl GLN dipeptide (Dipeptiven; Fresenius Kabi, Bad Homburg, Germany) Control group: Without GLN (*N*=22): No GLN, only chemotherapy. Unclear whether placebo was given. Start criteria: Day one of chemotherapy. End criteria: Continued for 5 days.	
OUTCOMES	Outcomes included: • None Narrative: *Patient record; WHO side effect grading system* • Diarrhoea (Mean (SD)) • Plasma GLN concentration (No data, only figure)	Other: • Leucopenia, neutropenia and thrombocytopenia. • Plasma endotoxin concentration. • Recombinant human granulocyte colony-stimulating factor dose.
OTHER	Funding: Grant from Jiangsu Province's Outstanding Medical Academic Leader.	

BW: Body weight; FAM regimen (5-FU 600 mg/m^2, IV, day 1-5; doxorubicin 30 mg/m^2, IV, day 1; mitomycin 10 mg/m^2, IV, day 1), FOLFOX-4 regimen (oxaliplatin 85mg/m^2, day 1; folinic acid 200 mg/m^2, day 1, 2; 5-FU 400 mg/m^2 bolus + 600 mg/m^2 infusion over 22 hrs, day 1, 2); SD: Standard deviation; WHO: World Health Organization

STUDY ID	**Marton 2010 (164)**	
SETTING	Prospective, randomized, double-blind, and controlled trial in Hungary, 2006 to 2008.	
POPULATION	All patients with esophagus resection because of cancer were included. Exclusion criteria: Patients with benign disease Diagnoses : All patients had advanced stage esophageal cancer. Age (yrs) median (interquartile range): GLN 56 (48-64), Control 58 (44-75) Gender (M/F): GLN 20/10, Control 14/11	
INTERVENTION	Route: TPN The two groups were on the same nutritional regime after surgery. Through a jejuna stoma implanted during surgery, patients first received rehydrating saline solution at 10 mL/h following surgery; then 15 kcal/kg on day 1, 20 kcal/kg on day 2, 25 kcal/kg on day 3, and finally 30 kcal/kg from day 4 onwards in the form of enteral nutrients (Fresubin, Fresenius) until oral nutrition. Study group: GLN group (group C) (*N*=30): Received 0.5g/kg GLN (Dipeptiven, Fresenius) in the form of continuous intravenous infusion for 6 hours. Control group: Control group (*N*=25): Given placebo. Start criteria: 3 days prior to surgery End criteria: 7 days following surgery Duration: 10 days Study end point: discharge from ICU or day 7 following surgery. Patients were not followed after the 7th day.	
OUTCOMES	Outcomes included: • ICU mortality (*N*) Narrative: • Infectious complication (no data)	Other: • Biochemical parameters (serum total protein, albumin, pre-albumin, RBP, transferrien and transferrien saturation) • Inflammatory response (TNF-α, IL-6, IL-8 and CRP)
OTHER	Funding: No mention	

CRP: C-reactive protein; ICU: Intensive care unit; RBP: Retinol binding protein, TNF: Tumour necrosis factor

STUDY ID	**May 2002 (130)**
SETTING	Randomized, double-blind, parallel, nitrogen-controlled study at three study sites: North Shore University Hospital, Manhasset, New York; the Veteran Affairs Medical Center, Reno, Nevada; and the University of Nevada School of Medicine, Las Vegas, Nevada, January 1999 - May 2000.
POPULATION	49 patients with advanced solid tumours (stage IV), weight loss > 5%, ≥ 3 month survival prognosis. Chemotherapy and RT were acceptable during the study. Diagnoses (Control+HMB/Arg/GLN): Breast 1 + 1, carcinoid 1 +0, head and neck 3 + 0, colon 5 +8, lung 4+2, ovarian 2+0, pancreatic 4+4, prostate 2+5, stomach 2+1, liver 1+1, uterine 0+1 and gall bladder 0+1. Age mean (SD) yrs: HMB/Arg/GLN: 65.9 (2.0); Control: 66.1 (2.1) Gender (M:F): HMB/Arg/GLN: 19:5; Control: 16:9 Exclusion criteria: Taking appetite stimulants, corticosteroid analogues, omega 3 fatty acids or their cogeners or TPN in previous 3 months.
INTERVENTION	Route: Oral Study group: HMB/Arg/GLN (*N*=24): 3 g HMB, 14 g L-arginine, 14 g, L-GLN daily. Control group: Control (*N*=25): Isonitrogenous mixture with nonessential amino acids, L-alanine (11g), L-glutamic acid (1.75g), L-glycine (6.10 g) and L-serine (4.22 g) Duration: 24 weeks 4 weeks: HMB/Arg/GLN (*N*=18); Control (*N*=14) 24 weeks: HMB/Arg/GLN (*N*=7); Control (*N*=2)

STUDY ID	**May 2002 (130)**	
OUTCOMES	Outcomes included: • Body weight (kg) change at 4 weeks follow-up. • Mortality (*N*) at 24 weeks follow-up. Narrative: • Body weight change (kg) at 24 weeks	Other: • Safety (missing data) • Health status (Short form-30 health survey; Functional assessment health survey) • Serum chemistries • FFM through air displacement plethysmography in subset of subjects • Oral caloric and protein intake (ckal/day) • FFM change at 24 weeks
OTHER	Funding: Grant from National Institutes of Health and Metabolic Technologies.	

Arg: Arginine; FFM: Fat-free mass; HMB: Beta-hydroxy-beta-methylbutyrate

STUDY ID	**O'Riordian 1994 (136)**	
SETTING	Randomized, placebo-controlled, parallel study in UK.	
POPULATION	22 patients (aged 18-80) with presumptive preoperative diagnoses of carcinoma, undergoing colonic resection were included, 20 included postop. Exclusion criteria: renal failure (creatinine > 180 umol/L), hepatic failure (bilirubin > 40 umol/L, alanine aminotransferase > 100 units/L and gamma glutamyl transferase > 100 > 100 units/L), resection for irritable bowel disease, patients receiving systemic steroids, woman of childbearing potential. All patients received prophylaxis against infection 24 hrs preoperatively and in addition a low-dose subcutaneous heparin from 4 hrs preoperatively continuing for duration of study. Age median(range)yrs: GLN TPN: 65 (59-78); Control TPN: 69 (58-74) Gender (M:F): GLN TPN: 5:5; Control TPN: 7:3	
INTERVENTION	Route: TPN Oral intake of patients restricted to clear fluids during TPN. Study group: GLN TPN, Group II (*N*=10): Isocaloric, isonitrogenous solution with GLN-supplemented amino acid solution (Glamin, Kabi Pharmacia, Erlangen, Germany) providing 0.18 g/kg per day dipeptide glycyl-GLN (17% of nitrogen) Control group: Control TPN, Group I (*N*=10): Conventional TPN based on an amino acid solution (Vamin 18EF, Kabi Pharmacia, Milton Keynes, England) with lipid emulsion (Intralipid, Kabi Pharmacia) and glucose (Baxter, Newbury, England) with 0.2 g nitrogen/kg per day and 122 kJ/kg per day. Start criteria: 11 am the morning after surgery, postoperative day 1. End criteria: For 5 days, postoperative day 6.	
OUTCOMES	Outcomes included: • Incidence of infection requiring initiation of antibiotic therapy (*N*)(*N*=22)	Other: • PHA stimulated lymphocyte DNA synthesis • Nitrogen balance
OTHER	Funding: Wilkie Scholarship (University of Edinburgh), the Leverhulme Trust, and by the generous assistance of Kabi Pharmacia.	

TPN: Total parenteral nutrition

STUDY ID	**Oguz 2007 (141)**
SETTING	Prospective, randomized, controlled, parallel study at Coloproctology Unit, Department of General Surgery, Gazi University, Ankara, Turkey, January 2001 - January 2005.
POPULATION	109 patients with colorectal cancer, undergoing surgery and given enteral nutrition. Exclusion criteria: Metabolic disorders (hyperthyroidism, DM), emergency surgery, abdominoperineal resection. Associated disorders (GLN+Control): COLD 8+6; heart failure 7+4; liver disorder 3+2. Age mean (SD)yrs: GLN: 52 (12); Control: 57 (17) Gender (M:F): GLN: 40:17; Control: 31:21

STUDY ID	Oguz 2007 (141)	
INTERVENTION	Route: TPN All patients were allowed to eat normal hospital food. Requirements calculated as follow: 30kcal/kg/day energy and 1g/kg/day protein. Study group: GLN group (*N=57*): Parenteral L-alanine-L-GLN (1 g/kg per day, Dipeptiven, Fresenius Kabi, Germany) together with oral polymeric enteral nutrition (Ensure, Abbott, Zwolle, The Netherlands). Control group: Control group (*N*=52): Received only enteral nutrition (Ensure, Abbott) orally - a standard isonitrogenous and isocaloric formula. Duration: Supplemental enteral nutrition - at least 5 days pre- and postoperatively in both groups according to nutritional status of patients. In GLN group, parenteral L-ala-L-GLN was supplemented throughout the duration of enteral nutrition in all. Pre- and postoperative nutritional support in GLN group was 6 (2) and 5 (1) days respectively; while it was 7 (1) and 6 (1) days in the control group. Enteral nutrition schedule: 5 days pre-op: 1000ml/day; 2 days post-op: 500 ml/day and 3-5 day post-op: 1000 ml/day	
OUTCOMES	Outcomes included: • Postoperative complications (infections)(*N*) • Length of hospital stay (days) • Mortality (*N*)	Other: • Duration of preoperative and postoperative enteral nutrition support (days) • SGA, BMI • Serum albumin, protein • Associated disorders • Localization of pathology • Techniques of anastomosis • Wound dehiscence, pulmonary embolism and anostomotic leak (major/minor)
OTHER	Funding: No mention	

BMI: Body mass index; COLD: Chronic obstructive liver disease; DM: Diabetes Mellitus; SGA: Subjective global assessment

STUDY ID	Okuno 1999 (105)
SETTING	Randomized, placebo-controlled, double-blind, parallel study in USA, November 1995 - March 1997.
POPULATION	134 adult patients scheduled to receive their first 5 day course of 5-FU-based chemotherapy on an NCCTG treatment clinical trial. Chemotherapy regimen (GLN+placebo): 5-FU 370+LCF 100 = 0+1; 5-FU 370+ CF 500 = 0+1; 5-FU 370 + CF20 +LEV = 60 +60; 5-FU 425 + CF 20 + LEV = 2 +3; 5-FU 450 +LEV = 4 +3. All patients: Oral cryotherapy (ice chips) 5 min before chemotherapy for 30 minutes. Age, mean(median) yrs: GLN: 67.0 (67.0); Placebo: 60.8 (60.5) Gender (M:F): GLN: 36:30; Placebo: 36:32 Patients were stratified according to denture use, smoking history and chemotherapy regimen.
INTERVENTION	Route: Oral, swish and swallow Study group: GLN (*N*=66): 4 g GLN x 2 per day. Advised to swish (10 seconds) and swallow. NPO 15 minutes afterwards. Control group: Placebo (*N*=68): Identical appearing placebo with same instructions. Start criteria: First day of the first chemotherapy cycle for 14 days. Follow-up (by physician): 4-5 weeks after beginning of each chemotherapy cycle by "historical means." Follow-up (self-reporting by patient): During 21 days after the first dose of chemotherapy, "daily diary questionnaire based on NCCTG criteria."

STUDY ID	Okuno 1999 (105)	
OUTCOMES	Outcomes included: *Physician assessment, "Mucositis grading criteria"* • Number of patients with some degree mucositis (*N*) • Number of patients with maximum grade mucositis (*N*) • Number of patients with at least grade 2 mucositis (*N*) Narrative: Patient-reported, NCCTG criteria • Mucositis grade and duration. • Physician assessment mucositis mean (no SD)	Other: • Patient-reported: Use of medication to alleviate mucositis.
OTHER	Funding: no mention.	

FU: Flourouracil; LCF: Levo citrovorum factor; CF: Citrovorum factor; LEV: Levamisole; NCCTG: North Central Cancer Treatment Group

STUDY ID	Peterson 2007 (94)	
SETTING	Multicentre, randomized, double-blind, placebo controlled, cross-over phase III trial in Russia.	
POPULATION	326 adult patients developing WHO grade ≥ 2 oral mucositis during chemotherapy screening cycle with an ECOG performance status ≤ 2 and histopathologically confirmed breast cancer suitable for treatment with anthracycline-bases chemotherapy were eligible to participate. Patients must be scheduled to receive at least 2 additional cycles of the same chemotherapy with no dose reduction. Eligible patients must present with normal oral mucosa (WHO grade 0) at baseline, have completed any previous RT involving the oral or esophageal mucosa at least six weeks before study entry, and have recovered from all previous RT toxicities. All patients received aclovir (oral 200 mg x 2/day) prophylaxis during the study. Exclusion criteria: Receiving or scheduled to receive any other topical or systemic treatments specifically targeting mucositis, including growth factors, cytokines, cryotherapy, sucralfate, or prostaglandins. Patients with uncontrolled diabetes mellitus, current evidence of drug/alcohol abuse, active mouth or gingival sores. Patients who are pregnant, lactating or at risk of pregnancy or participation in a clinical trial for treatment or prevention of mucositis within 4 weeks of study entry. Chemotherapy regimen, 21 day cycle (GLN+Placebo): CAF (101+97), FAC (37+42), AC (25+24). Initial diagnoses (GLN+placebo): Adenocarcinoma (162+163), estrogen receptor-positive (1+0). Age median (range) yrs: GLN: 50 (27-74), Placebo: 50 (24-73) Gender: Only Caucasian females. Patients were stratified according to: chemotherapy regimen before randomization in 1:1 ratio	
INTERVENTION	Route: Oral, swish and swallow Study group: GLN (*N*=136): Saforis (MGI Pharma, Inc. Bloomington, MN) administered at a dose of 2.5 g/5 ml x 3 per day (Total dose = 7.5 g). Saforis is composed of GLN in a novel, proprietary drug delivery system (UpTec). Compared with other available forms of GLN, Saforis has been shown to facilitate the uptake of > 100 times more GLN by epithelial oral mucosal cells. Study drug was orally swished for 30 seconds and then swallowed. Patients were instructed to refrain from eating or drinking for 30 min after dosing. Control group: Placebo formulation (*N*=163): Matched texture and characteristics of the active drug. Administered at 5 ml x 3 per day (Total dose = 15 ml). Start criteria: First day of chemotherapy End criteria: Continued for 14 days in patients who did not develop oral mucositis, or until 5 days after the resolution of oral mucositis for patients who experienced oral mucositis, or to the end of the treatment cycle (21 days).	
OUTCOMES	Outcomes included: Investigator assessment, WHO oral mucositis scale: • Number of patients with ≥ grade 1 mucositis (*N*) • Number of patients with maximum grade mucositis (*N*) • Number of patients with at least grade 2 mucositis (*N*) Narrative: • Oral mucositis score mean (SD)	Other: • Worst ulceration score • Ulceration score > 0 • Adverse events (*N*) • Patient self-reported ability to eat solid foods

STUDY ID	Peterson 2007 (94)
OTHER	Funding: Clinical component funded by Aesgen, Inc. After completion of phase II clinical study, Aesgen, Inc. was acquired by MGI Phrama, Inc. in September 2004. MGI Pharma, Inc. then provided funding for completion of data analyses and related study activities.

AC: doxorubicin and cyclophosphamide; CAF: CY, doxorubicin, and 5-flourouracil; ECOG: Eastern Cooperative Oncologic Group; FAC: 5-Flourouracil, doxorubicin, CY; SD: Standard deviation; WHO: World Health Organization

STUDY ID	Piccirillo 2003 (110)	
SETTING	Randomized, blinded, controlled, parallel study at the Hematology Institute, Università Cattolica del Sacro Cuore, Rome, Italy. STUDY 1: October 1998 - August 1999; STUDY 2: September 1999	
POPULATION	Patients received relatively heterogeneous conditioning regimes for aPBSCT. **STUDY 1:** 27 consecutive patients submitted to selected/unselected aPBSCT. Disease (GLN+Placebo): NHL 5+5; AML 4+1; MM 1+6; Osteasarcoma 1+0; CLL 0+1; HD 1+2. Status at transplant (GLN+Control): CR 5+6; PR 6+6; PD 1+3. Gender (M:F): GLN: 7:5; Placebo: 10:5 Age (yrs): GLN: 37.5 (17-66); Placebo: 47 (18-56) **STUDY 2:** 21 consecutive patients. Disease (GLN+Placebo): NHL 3+4; MM 4+4; Osteosarcoma 1+0; AML 0+2; HD 2+1. Status at transplant (GLN+Placebo): CR 1+3; PR 7+5; PD 2+3. Gender (M:F): GLN: 5:5; Placebo: 8:3 Age (yrs): GLN: 31.5 (22-61); Placebo: 49 (27-61)	
INTERVENTION	Route: TPN **STUDY 1:** All patients received PN composed of Intralipid 10% (500 ml); 33% glucose solution (1000ml); hydrosoluble and liposoluble vitamins. Study group: GLN group (*N*=12): Glamin (Fresenius Kabi) 1000mL/die - a parenteral amino acid solution containing 20 g free GLN. Control group: Placebo group (*N*=15): Received placebo - Freamine 8.5% (Fresenius Kabi, Uppsala, Sweden) Start criteria: From day +1 after aPBSCT. **STUDY 2:** (New commercial premixed nutrition bag and glutamine solution lead to design of second study) All patients received premixed balanced commercial PN bag - Kabimix 1830 (Fresenius Kabi, Uppsala, Sweden); hydrosoluble and liposoluble vitamins. Study group: GLN group (*N*=10): Dipeptiven (Fresenius Kabi) 100 mL/die - a parenteral solution containing 13.46 g alanyl-GLN added to PN - bag. Control group: Placebo group (*N*=11): Received only PN bag. (Not isonitrogenic) Start criteria: From day +1 after aPBSCT. End criteria: Discharge	
OUTCOMES	Outcomes included: • None Narrative: • Severity and duration of mucositis (DMS) • Duration of hospitalization. (Mean (Range))	Other: • Time to neutrophil, lymphocyte, platelet recovery. • Days of non-prophylactic antibiotics. • Days with body tempr. > 38 °C. • Reticulocyte recovery (> 1%). • Number of RBC and platelets infused. • Reconstitution of the lymphocyte subsets. • Costs of GLN- enriched PN
OTHER	Funding: Associazione Italiana per le Ricerca sul Cancro, Milan, Italy.	

aPBSCT: Autologous peripheral blood stem cell transplantation; AML: Acute myeloid leukaemia; CLL: Chronic lymphocytic leukaemia; CR: Complete remission; DMS: Daily mucositis score; HD: Hodgkin's disease; MM: Multiple myeloma; NHL: Non-Hodgkin's lymphoma; PN: Parenteral nutrition; PD: Progressive diease; PR: partial remission; RBC: Red blood cell

STUDY ID	Pytlik 2002a (98)
SETTING	Randomized, double-blind, placebo-controlled, parallel study in the Czech Republic.

STUDY ID	Pytlik 2002a (98)	
POPULATION	40 consecutive autologous transplant patients. Inclusion criteria: Peripheral blood prognitor cells (PBPC) collection of at least 1 x 10^6/kg CD34+ cells (with or without supplemental bone marrow); adequate organ function; any conditioning regimen other than paclitaxel with carboplatin used for ovarian cancer in OVCT trial. Diagnoses (GLN+Placebo): Lymphoma 6+10; Myeloma 6+5; CLL 2+1; AML 2+0; Multiple sclerosis 3+1; Solid tumour 2+2. Age mean (SD): GLN: 49 (12); Control: 42 (14) Gender (M:F): GLN: 14:7; Control: 11:8	
INTERVENTION	Route: TPN Study group: GLN (*N*=21): parenteral 30 g of dipeptide alanyl-GLN per day (containing 20 g GLN). Control group: Placebo (*N*=19): parenteral isonitrogenous amino acid solution. Start criteria: Day + 1 End criteria: Day +14 or to discharge	
OUTCOMES	Outcomes included: • Days of post-transplant hospitalization • Survival at 24 months (*N*). • Number of patients with infections (*N*) *Trained Nurse assessment, (Nebraska Oral Assessment Score)* • Mucositis (days) • Diarrhoea (days). Narrative: • Body weight change (kg)	Other: • Febrile days • Days on antibiotics, opioids, vancomycin, amphotericin-B. • Days of TPN, • Immunological parameters (x 10^9/L) • Economic calculations • Number of patients with no positive blood cultures, relapse at 24 months follow-up • Oral Xylose absorption test • Platelets > 20x 10^9/l (days) • Red cell, platelet concentrates • Mean oral energy intake in days +1 to +10
OTHER	Funding: Czech Ministry of Public Health Grant	

AML: Acute myeloid leukaemia; CLL: Chronic lymphocytic leukaemia; TPN: Total parenteral nutrition

STUDY ID	Scheid 2004 (134)
SETTING	Randomized, double-blind, controlled parallel study in London, UK.
POPULATION	54 adult patients with AML scheduled to receive myelosuppressive chemotherapy, or had begun chemotherapy not more than 3 d before inclusion into the study. Exclusion criteria: Sepsis; HIV; pregnancy; severe liver or renal failure (bilirubin, glutamic-pyruvic transminase, or alkaline phosphatase > 4 x the upper normal limit or creatine/urea > 3 x upper normal limit); known inherited disturbance of amino acid metabolism. Chemotherapy (GLN+Control): TAD 7+8; HAM 11+10; IdaFLAG 2+2, G-SCF use 7+8. Age median (range) yrs: GLN: 47.5 (18-75); Control: 54 (21-72) Gender (M:F): GLN: 9:5; Control: 10:6
INTERVENTION	Route: TPN Total daily dose of amino acids was limited to 2 g/kg BW max. Lipids (Intralipid 20%, Baxter) and 50% glucose were added to obtain caloric dose of 0.75-fold the estimated REE for each compound, for a total of 1.5-fold REE of non-protein calories. Study group: GLN-TPN (*N*=20): Glycyl-GLN-dipeptide at dose of 30.27 g (20 g free GLN) per day using 1000 ml of a standard amino acid solution (Glamin, Baxter, Erlangen, Germany). Control group: S-TPN (*N*=20): 1000 ml of a GLN-free standard solution (Vamin, Fresenius-Kabi Stans, Switzerland). Solutions had similar amino acid content (other than glycyl-GLN-dipeptide). Start criteria: 1. When oral food intake became impossible due to hyperemesis and/or mucositis. 2. Weight loss of 1 - 2% body weight per week with central venous line in place. 3. Weight loss > 2 % body weight per week (CVC initiated). End criteria: TPN stopped when patient's oral intake met or exceeded estimated REE.

STUDY ID	Scheid 2004 (134)	
OUTCOMES	Outcomes included: • None. Narrative: • Length of hospital stay (days) Median (range).	Other: • Duration of neutropenia • Incidence and duration of neutropenic fever • Immunocompetent cell recovery (CD4+, CD8+ lymphocytes) and monocyte activation
OTHER	Funding: No mention.	

CVC: Central venous catheter; HIV: Human immunodeficiency virus; TAD: Thioguanine (2x100 mg/m², days 3-9), cytarabine (100 mg/m², days 1&2, 2x100 mg/m², days 3-8) and daunorubicin (60 mg/m², days 3-5), HAM: high-dose cytarabine (2x3000 mg/m², days 1-3) and mitoxantrone (10 mg/m², days 3-5), IdaFLAG: Ida 98 mg/m², days 1,3&5), fludarabine (25 mg/m², days 2-5), cytarabine (2x100 mg/m², days 1-5), and granulocyte colony-stimulating factor (G-CSF; 400 ug/m² from day 0); REE: Resting energy expenditure

STUDY ID	Scheltinga 1991 (143)	
SETTING	Randomized, double-blind, controlled parallel study in Kansas City, Kansas.	
POPULATION	20 subjects with haematological malignancies in remission enrolled in allogeneic BMT protocols in the Brigham and Woman's Hospital. Diagnoses (GLN+Control): CML 5+5; AML 4+4; misc 1+1. Age mean (SD) yrs: GLN: 36 (3); Control: 33 (3) Gender (M: F): GLN: 5:5; Control: 4:6. Conditioning regimen (7 day period): Cytosine arabinoside (3 g/m², x2 per day, for 3 days), CY (1800 mg/m², x1 per day, for 2 days), TBI (175 cGy x 2 per day for 4 days, total dose of 1400 cGy (*N*=18)). 2 subjects received only CY (3.6 g/m²) and busulfan (16 mg/kg). GVHD prophylaxis: ST-1 immunotoxin or methotrexate and cyclosporine combo. All patients underwent programme for reduction of endogenous flora of skin and gut.	
INTERVENTION	Route: TPN Patients were allowed *ad libitum* low-bacterial diet throughout hospitalization. Caloric requirements based on basal energy requirements from standard tables x 1.5 (BMT patients). Protein intake maintained at 1.5 g/kg/day. NPE - 70% glucose, 30% lipid emulsion (Intralipid, Kabi Vitrum, Stockholm, Sweden). Control group: STD (*N*=10): Standard parenteral nutrition solution containing commercially available amino acid mixture (Novamine, Kabi Vitrum, Stockholm, Sweden). Study group: GLN (*N*=10): Isonitrogenous, isocaloric GLN-supplemented intravenous feedings. Commercial available solution (Renamine, Baxter Health Care Corp., McGaw Park, IL) + crystalline L-GLN (0.57g/kg/day, Ajinomoto USA, Raleigh, NC). 3rd group: Healthy controls - no intervention. Start criteria: After BMT Day 0. End Criteria: Oral intake > 50% of E requirements. Duration: 27 (1) days	
OUTCOMES	Outcomes included: • Body weight change (kg) • Clinical infection (fever > 38.5 °C)	Other: • Oral calories and nitrogen intake per day (cal/g). • TPN use (days). • Body composition. • Total body water. • Extracellular water. • Total quantity of blood products (platelets, red blood cells), steroids and diuretics. • Number of nonprophylactic antibiotic agents per day + number of days.
OTHER	Funding: Marc R Scheltinga was supported by grant from the Department of Surgery of the Vrije Universiteit in Amsterdam, The Netherlands. The study was supported by the National Institute of Health Trauma Grant.	

AML: Acute myeloid leukaemia; BMT: Bone marrow transplant; CML: Chronic myelogenous leukaemia; CY: Cyclophosphamide; GVHD: Graf-versus-host-disease; TBI: Total body irradiation

STUDY ID	Schloerb 1993 (111)	
SETTING	Randomized, double-blind, controlled parallel study in Kansas City, 9 September 1991 - 19 July 1992.	
POPULATION	29 BMT patients enrolled at the University of Kansas Medical Center. Diagnoses (GLN+control): AML 6+5; CML 4+1; HL 2+2; NHL 1+4; MM 0; carcinoma breast 1+1; seminoma 1+0. BMT (GLN+control): Allogeneic 7+6; autologous 9+7. Age mean (range) yrs: GLN: 37.6 (19-55); Control: 35.6 (19-55) Gender (M:F): GLN: 9:7; Control: 8:5 Conditioning regimen: All autologous BMT patients + autologous BMT patients with leukaemia prepared with either CY and TBI (12Gy, 2 Gy x2 per day for 3 days) or CY and busulfan. All lymphoma patients with autologous BMT received CY, carmustine, and etoposide. Breast CA and seminoma patients with autologous BMT received CY and thiotepa. GVHD prophylaxis: Cyclosporin A, methylprednisone and IV methotrexate. Viral prophylaxis: acyclovir if positive serumtitus for herpes or cytomegalovirus.	
INTERVENTION	Route: TPN All patients received low bacterial count diet. Calories provided as 1.5 times REE. Protein requirement was 3.2 g/kg body weight, dextrose 14g/kg body weight and remaining calories as fat. TPN solutions consisted of 4.7% amino acids, 20% dextrose, electrolytes, trace elements, and multivitamins, administered with 20% fat emulsion (Intralipid, Kabi Pharmacia, Stockholm, Sweden), all in one. Study group: GLN-supplemented parenteral nutrition (*N*=16): Combination of Renamin (Baxter Health Care)(Rich in essential L-amino acids) and free L-GLN (Ajinomoto USA Inc, Teaneck, NJ)(2830 mg/100mL)(0.57 g/kg/day). Control group: Standard parenteral nutrition (*N*=13): Isocaloric, isonitrogenous standard TPN solution Travasol (Baxter Health Care Corporation, McGaw Park IL). Start criteria: After BMT. End criteria: TPN discontinued when patient consumed ½ of estimated nutrient requirements on any given day. Follow-up until discharge.	
OUTCOMES	Outcomes included: • Mortality (*N*) • Patients with clinical infections (*N*) • Change in weight (kg) • Length of stay after transplant (days) Narrative: Physician assessment; Criteria: Unclear – "The oral mucosa was examined periodically for the presence and severity of mucositis and was graded as a function of the degree of inflammation." • Cumulative mucositis score	Other: • Total days on antibiotics • Patients receiving corticosteroids, amphoteriein B (*N*) • Blood, platelet transfusions (*N*) • Days until average neutrophil count > 0.5 x 10^9/L • Patients with no positive cultures (*N*) • Patients with positive stool, throat cultures (*N*) • Change in total body water (L) (Deuterium dilution and Bioimpedance • Change in extracellular water (L) • Average daily maximal temperature (°C)
OTHER	Funding: Curaflex Infusion Services, Rancho Cucamonga, CA; Ajinomoto USA Inc, Teaneck, NJ, RJL Systems, Mt, Clemens, MI, and in part by Research Grant from National Institutes of Health.	

AML: Acute myeloid leukaemia; BMT: Bone marrow transplant; CML: Chronic myelogenous leukaemia; CY: Cyclophosphamide; HL: Hodgkin's lymphoma; NHL: Non-Hodgkin's lymphoma; MM: Multiple myeloma; REE: Resting energy expenditure; TBI: Total body irradiation; TPN: Total parenteral nutrition

STUDY ID	Schloerb 1999 (112)
SETTING	Randomized, double-blind, controlled parallel study in Kansas City, October 1993 - August 1995 and August 1996 - May 1997.

STUDY ID	Schloerb 1999 (112)	
POPULATION	66 patients who received BMT or HSC at University of Kansas Medical Center. Haematological malignancies = 43 (42% received allogeneic and 58% received autologous HSC transplants), solid tumours = 23 (21 breast carcinomas) (100% received autologous grafts). Age (yrs): Haematological, allogeneic: GLN: 37.6 (11.1); Control: 37.8 (5.7). Haematological, autologous: GLN: 44.5 (11.2); Control: 42.2 (11). Solid Tumour, autologous: GLN 46.8 (10.3); Control: 45.8 (9.9) Gender (M:F): GLN: 10:18; Control: 9: 29 Conditioning regimen: Leukaemia = CY and TBI (12 Gy- 2 Gy x 2/day for 3 days) or CY and busulfan. Lymphoma + autologous HSC = CY, carmustine and etoposide. Breast CA, Ewing's sarcoma, ovarian CA + autologous HSC = CY and thiotepa.	
INTERVENTION	Route: Oral Low bacterial count diet, oral antibiotics. Calories were provided at 1.3 times the REE (Harris Benedict). Protein 3.4 g/kg body weight, dextrose 12 g/kg body weight and remaining calories provided as fat emulsion (Intralipid, Pharmacia & Upjohn, Clayton, NC) Study group: GLN (*N*=35): L-GLN (Ajinonoto USA, Inc, Teaneck, NJ) 3 x 10 g per day + 100 ml liquid. Isonitrogenous, isocaloric TPN (Schloerb 1993) (0.57g/kg GLN) Change in experimental protocol was necessary for last 5 GLN patients. TPN-base solution was changed to TrophAmine (McGaw Laboratories, Irvine, CA) due to interruption in funding. Control group: Control (*N*=31): Glycine 3 x 10 g per day in 100 ml liquid. Standard TPN. Start criteria: Unclear (From randomization at admission) TPN was started when necessary. End criteria: Unclear (Until discharge). Patients were followed daily until hospital discharge. TPN discontinued when patient consumed ½ of estimated nutrient requirements on any given day.	
OUTCOMES	Outcomes included: • Mortality (*N*) • Weight change (kg) • Length of hospital stay (days) • Sepsis (*N*) Physician assessment; "Incidence and severity of mucositis based on review of progress notes in each individual chart" • Mucositis of any degree (*N*) • Diarrhoea of any degree (*N*)	Other: • Days oral amino acids • Oral GLN g/kg/day • Days TPN • TPN GLN g • Days Average neutrophil count > 500 • GVHD (N) • Positive blood culture (*N*)
OTHER	Funding: Amino acids provided by Ajinomoto Inc, Teaneck, NJ; Trophamine base solution for TPN by McGaw Laboratories, Inc; Financial support by American Home Therapies.	

CY: Cyclophosphamide; GVHD: Graft-versus-host-disease; HSC: Human stem cell; TBI: Total body irradiation; TPN: Total parenteral nutrition

STUDY ID	Sornsuvit 2008 (113)
SETTING	Randomized, controlled, parallel study in Bangkok, Thailand.
POPULATION	16 patients with AML admitted to Ramathibodi Hospital, Mahidol University, Chiangmai, Thailand, to receive chemotherapy, were enrolled in the study. Exclusion criteria: Renal failure (serum creatinine > 2.5 mg/dl), hepatic failure (serum glutamic oxalacetic transaminase > 100 IU/l), glutamate supplements within 1 month from start of study. Chemotherapy regimen (GLN+Control): Ara-C + Ida 5+5 and other 3+3. Use of G-CSF (no of cycles): GLN: 3; Control: 3 Age (yrs): GLN: 49.5 (17.6); Control: 35.5 (13.4) Gender (M:F): GLN: 5:3; Control: 6:2
INTERVENTION	Route: TPN Study group: GLN (*N*=8): IV supplementation with 30 g/day GLN (Dipeptiven, Fresenius Kabi Thailand, Bangkok, Thailand). Control group: Control (*N*=8): Equivalent quantity (25 g/day) of standard amino acid mixture (Aminosol, Thai Otsuka, Bangkok, Thailand). Start criteria: On the day of chemotherapy initiation. End criteria: Consecutively for 5 days (days 1-5) in each chemotherapy cycle.

STUDY ID	**Sornsuvit 2008 (113)**	
OUTCOMES	Outcomes included: Physician assessment; Anderson 1998, Jebb 1994, subjectively • Mucositis grades and duration (WHO and NCI criteria) • Diarrhoeal ≥ grade 2 duration (days) • Body weight change (kg) • Infection (*N*) • Length of hospital stay (days) Narrative: • Mucositis mean	Other: • Serum albumin, total protein change. • Days neutropenia (ANC < 500/ul), lymphopenia and leucopenia. • Neutrophil phagocytosis activity and neutrophil SAG generation. • Neutropenic fever (> 38°C during neutropenia). • Number and duration of TPN cycles. • Total cost of hospital stay.
OTHER	Funding: Supported by funds from Faculty of Graduate Studies of Mahidol University and Cerebos Awards.	

Ara-C: Cytarabine; ANC: Absolute neutrophil count; Ida: Idarubicin; NCI: National Cancer Institute, Maryland, Bethesda; TPN: Total parenteral nutrition; WHO: World Health Organization

STUDY ID	**Stehle 1989 (59)**	
SETTING	Randomized, controlled, parallel study in Federal Republic of Germany.	
POPULATION	12 patients admitted for elective resection of carcinoma of colon or rectum. Exclusion criteria: Clinical or laboratory evidence of malnutrition or systemic disease. Gender (M:F): GLN: 3:3; Control: 4:2 Age mean (SD) yrs: GLN: 52 (6); Control: 52 (6).	
INTERVENTION	Route: TPN TPN (all): Amino acid solution (10% Intrafusion, Pfrimmer) 0.23 g nitrogen/kg and 166 KJ/kg daily. NPE (140 KJ/kg per day) came equally from glucose and a fat emulsion (20% Intralipid, Kabi-Vitrum) Study group: Ala-GLN group (*N*=6): TPN supplemented with dipeptide Ala-GLN (280 mg/kg/day; 54 mg nitrogen/kg per day) and glycyl-L-tyrosine (50 mg/kg/day; 5.9 mg nitrogen/kg/day). Control group: Control group (*N*=6): Received corresponding amounts of free alanine and glycine nitrogen. Start criteria: Postoperative day 1. End Criteria: Postoperative day 6 (5 days total)	
OUTCOMES	Outcomes included: • None Narrative: • Maintenance of intracellular GLN (%) • Change in GLN plasma and intracellular muscle concentration.	Other: • Mean nitrogen balance (g/day) • Cumulative nitrogen balance, day 5 (g)
OTHER	Funding: Grants from BMWi and Pfrimmer and Co Other: Co-intervention: Glycyl-L-tyrosine	

STUDY ID	**Strasser 2008 (122)**
SETTING	Randomized, double-blind, placebo-controlled, parallel study in Switzerland, March 2004 - March 2006.
POPULATION	52 adult patients with cancer who were receiving first time taxane-based chemotherapy. Inclusion criteria: Written consent, self-feeding, ECOG performance status score ≤ 2, no previous surgery or RT of the oral/nasal region, no oral candidiases, no zinc deficiency, creatinine clearance of ≥ 30 ml/min. Cancer type (GLN+placebo): Prostate 9+2; lung 3+6; breast 3+6 and other 6+6. TaxCh (GLN+Placebo): Docetaxel 11+7; paclitaxel 10+13; weekly schedule 18+14; adjuvant 1+5 and palliative 20+15. Pre-existing neuropathy at baseline: GLN 4 (grade2), 2 (grade 2); Control: 4 (grade1) Age median (range) yrs: GLN: 67 (49-83); Placebo: 63 (40-73) Gender (M:F): GLN: 16:5; Placebo: 12:8

STUDY ID	**Strasser 2008 (122)**	
INTERVENTION	Route: Oral Study group: GLN (*N*=21): 30 g GLN per day (Pure Powder; Baxter AG, Voletswil, Switzerland) in 2- 3 doses in fluids. Control group: Placebo (*N*=20): 30gr maltodextrin per day in 2- 3 doses in fluids. Start criteria: Patients received 4 week supply on first day of chemotherapy (Day 1) End criteria: Duration of TaxCh, at least 2 months. Median study duration: 74 days/11 weeks Range: GLN: 1-33 weeks; Control: 2- 28 weeks Total dose median (range): GLN: 1 430 g (70-6110); Control: 2 175 g (50-5760)	
OUTCOMES	Outcomes included: • Mortality(*N*) *Physician assessment; CTCAE v 3.0* • Number of patients with ≥ grade 1 and at least grade 2 diarrhoea Narrative: • Change in body weight	Other: • Difference in maximal Dysgeusia from baseline (100 mm patient-reported visual analogue scale - very good - bad) • Number of patients with at least grade 2 adverse events: • Performance status, sensory neuropathy, arthralgia, myalgia, anorexia, nausea, vomiting (CTCAE v 3.0) • Objective and subjective taste recognition tests (sweet, salty, bitter, sweet)
OTHER	Funding: Swiss Institute of Applied Cancer Research, Eastern Switzerland Foundation of Clinical Cancer Research, Baxter Switzerland, Bristol-Myers Squibb Switzerland.	

CTCAE: Common Terminology Criteria for Adverse Events, version 3.0; ECOG: Eastern Cooperative Oncologic Group; RT: Radiation therapy

STUDY ID	**Sykorova 2005 (145)**	
SETTING	Randomized, double-blind, controlled parallel study in the Czech Republic, 2000 - 2003.	
POPULATION	44 patients who received autologous SCT for haematological malignancies. All patients received standard conditioning regimens and standard supportive care. Diagnoses (GLN+Control): NHL 8+7; MM 6+8; HL 4+4 and AML 6+1. Conditioning regimen (GLN+Control): BEAM 12+11; MEL 100(200) 6+8; BUCY 2 4+1; TBI +CP 2+0. Age median(range) yrs: GLN: 49 (18-69); Control: 51 (19-69) Gender (M:F): GLN: 13:11; Control: 8:12	
INTERVENTION	Route: TPN First randomization: 2 Groups (No GLN supplementation) P Group, Prophylactic PN (*N*=21) starting with cytoreductive regimen; C-Group, PN given ad hoc (*N*=23) initiated the next day after oral intake < 50 % of REE x 1.3 (Harris Benedict) Next randomization: Control group: B group (*N*=20) Standard PN Study group: A Group (*N*=24): Isocaloric, isonitrogenous prophylactic PN containing 0.5 g GLN/kg BW per day (L-alanyl-L-GLN dipeptide - Dipeptiven, Fresenius Kabi) Start criteria: Day 1 of cytoreductive regimens. End criteria: PN stopped when leucocyte count > 1 x 10^9/l or oral intake > 50% of E requirements Follow-up: 3 years (median: 38 months)	
OUTCOMES	Outcomes included: • Three years follow-up: Overall survival (*N*)	Other: • Event-free survival • Disease-free survival • Outcomes for groups C & P (no GLN supplementation)
OTHER	Funding: Internal Grant Agency of Czech Ministry of Public Health.	

AML: Acute myeloid leukaemia; BEAM: (BCNU 300 mg/m² iv on day -6; Etoposide 200 mg/m² iv for 4 consecutive days (day -5 to day -2); Cytarabine 200 mg/m² x 2 per day iv for 4 consecutive days (day -5 to day-2); Melphalan 140 mg/m² iv on day -1); MEL 100: Melphalan 100 mg/m² iv on day -1; MEL 200: Melphalan 200 mg/m² iv on day -1; BUCY 2: Busulphan 4 mg/kg for 4 consecutive days (day -3 to day -2); TBI+CP: Total body irradiation 1.5 Gy x 2 per day for 4 consecutive days (day -7 to day -4) and Cyclophsophamide 60mg/kg iv for 2 consecutive days (day -3 to day -2); HL: Hodgkin's lymphoma; MM: Multiple myeloma; ; PN: Parenteral nutrition; REE: Resting energy expenditure

STUDY ID	**Van Zaanen 1994 (106)**	
SETTING	Randomized, double-blind, placebo-controlled, parallel study in Stuttgart, Germany.	
POPULATION	15 patients with haematological malignancy receiving intensive chemotherapy (20 cycles) with an expected neutropenic period of approximately 3 weeks. "Patients were randomized at each treatment cycle and not per individual. In each group, 10 treatment cycles were studied. Some patients randomized more than once." Diagnoses (GLN+Control): AML new onset 5+2; AML relapse 4+3; ALL new onset 0+2; ALL relapse 0+1; Burkitt lymphoma 0+1 and Chronic myeloid leukaemic blast crisis 1+1. Chemotherapy: Combinations of cytarabine, amsacrine, CY, vincristine and anthracyclines. Age median (range)yrs: GLN: 49.2 (20-77); Control: 49.2 (32-72) Gender (M:F): GLN: 2:8; Control: 4:6	
INTERVENTION	Route: TPN Control group: Standard TPN (*N*=10): Isonitrogenous (0.272 g nitrogen/kg body weight) and isoenergetic (2200 kcal NPE/day) Amino acid solution (Vamin 18 without electrolytes, Kabi Pharmacia, The Netherlands). Study group: TPN+Glutamine (*N*=10): TPN with 40 g L-alanyl-L-GLN dipeptide/day (26 g free GLN). Start criteria: Concomitantly with the chemotherapy or day +1 after BMT. End criteria: When neutrophil count = 0.5 x 10^9/l. TPN duration median (range) days: GLN: 18 (13-25); Control: 20 (11-25)	
OUTCOMES	Outcomes included: • Mortality during intervention (*N*) • Survival at 1 year (*N*) Narrative: • GLN and glutamate plasma concentrations (umol/L) • Weight gain per cycle (kg) • Length of stay in hospital (days) Physician assessment; WHO classification: • Mucositis mean • Diarrhoea, some degree • Microbiologically documented infections (Blood, CVC, bronchial lavage)(*N* of events) Data presented as median (range)	Other: • Daily food intake • Duration of TPN, neutropenia (days) • No. of platelet transfusions • Days with fever (> 38.5 °C) • Systemic antibiotics other than prophylactic • Colonization (nose/throat, stool) • Toxicity of GLN-dipeptide.
OTHER	Funding: Fresenius AG, Germany provided alanine-GLN dipeptide.	

ALL: Acute lymphoblastic leukaemia; AML: Acute myeloid leukaemia; BMT: Bone marrow transplant; CVC: Central venous catheter; CY: Cyclophosphamide, TPN: Total parenteral nutrition; WHO: World Helath Organization

STUDY ID	**Wu 2001 (29)**
SETTING	Randomized, controlled, double-blind, parallel study in Shanghai, China.
POPULATION	48 adult patients with gastrointestinal malignancies, scheduled for major abdominal surgery due to their cancer. Exclusion criteria: Clinically relevant alterations of the pulmonary, cardiovascular, renal, intestinal or hepatic function; history of recent immunosuppressive therapy (including preoperative RT); immunological disease; ongoing infection; intestinal obstruction or emergency surgery. Site of cancer (GLN+Control): Stomach 14+13; colorectum 8+7 and pancreas 3+3. Type of operation (GLN+Control): Gastrectomy 14+13; Miles or Dixon procedure 8+7 and pancreaduodenectomy 3+3. Age mean (SD) yrs: GLN: 55.2 (12.1); Control: 52.6 (9.8) Gender (M:F): GLN: 16:9; Control: 15:8

STUDY ID	Wu 2001 (29)	
INTERVENTION	Route: Enteral Goals for enteral nutrition delivery: 146 kJ/kg/day and 2.2 g protein/kg/day. All patients received IV fluid. Study group: Supplemented (*N*=25): Immune-enhancing enteral diet, Stresson (Nutricia) supplemented with GLN (1.30 g/100 ml), arg (0.89 g/100 ml) and omega-3 fatty acids (0.079 EPA/100 ml; 0.030 DHA/100ml). Control group: Control (*N*=23): Standard diet, Nutrison (Nutricia), Isonitrogenous (added protein powder) and isocaloric. Contains 0.40 g GLN/100 ml and 0.16 g arg/100 ml) Start criteria: Within 48 hrs after operation via needle catheter jejunostomy/nasogastric tube by continuous pump infusion. Diets were started at half strength 50 ml/hr. All patients reached their nutritional goals by 72 hrs after initiation. End criteria: Minimum of 7 days (until day +8).	
OUTCOMES	Outcomes included: Criteria: Unclear • Diarrhoea, some degree (*N*) Narrative: • Plasma levels GLN (nm/mL)	Other: • Nausea, vomiting, abdominal cramping/distension. • Polymorphyonuclear cell function, nitric oxide. • Plasma levels: alpha 1 antitrypsin, fibrinogen, arg, CRP (g/L), Prostaglandin E-2 (pg/mL). • Lymphocyte subsets: CD3, CD4, CD8 (%). • Natural killer cells (%). • Circulating cytokine level IL-1, IL-2, IL-6 and TNF - alpha (pg/mL).
OTHER	Funding: No mention.	

CRP: C-reactive protein; IL: Interleukin

STUDY ID	Yoshida 1998 (37)	
SETTING	Randomized, controlled, parallel study in the first Department of Surgery, Kurume University, School of Medicine, and Department of Radiology, St. Mary's Hospital and Japan, April 1994 - September 1996.	
POPULATION	11 of 128 patients with esophageal cancer, stage IV, caused by tumour invasion into the adjacent organs (T4). Two patients without any adjacent organ invasion (T3) were also included because they selected neoadjuvant CRT rather than surgical resection. Age (yrs): GLN: 61.6 (5.3); Control: 60.7 (2.6) Gender (M:F): GLN: 6:1; Control: 5:1 CRT: hyper-fractioned irradiation (1.2 Gy x 2 per day) from days 1 -5, days 8-12 and days 15-20. Total dose: GLN: 36.2 (0.9), Control: 36.0 (0) Chemotherapy: IV infusion on days 1-5, days 8-12. Total dose: 5FU: GLN: 5214 (145) mg; Control: 5750 (519) mg. Cisplatin: GLN: 150 (10) mg, Control: 154 (7) mg	
INTERVENTION	Route Oral GLN vs. IV Placebo When anorexia resulted from CRT, IV infusions of glucose and amino acids were started. Study group: Oral GLN (*N*=7): Oral GLN (30g/day, Glumine, Kyowa, Hakkoh, Tokyo, Japan) Control group: Control (*N*=6): Standard amino acid solution (30 g/day Amiparen, Otsuka Pharmaceutical, Tokushima, Japan) administered intravenously = isonitrogenous. Start criteria: Day 1 of CRT. End criteria: Day 28.	
OUTCOMES	Outcomes included: • None Narrative: • GLN plasma levels (umol/mL)	Other: • Immunoglobulin A levels in plasma (ug/mL) • WBC, Total Lymphocytes, T-cell, B-cell (/mm'3) • Gut barrier function (PSP %) • Tumour response • Mean calorie and nitrogen intake • Glutamate plasma levels • Immunoglobulin A levels in saliva; • Mitogenic activity of Lymphocytes
OTHER	Funding: Research fund from Otsuka Pharmaceutical Co. (Tokoshima, Japan)	

CRT: Chemo-radiation therapy; IV: Intravenous; WBC: White blood cell

STUDY ID	Ziegler 1992 (60)	
SETTING	Randomized, double-blind, controlled parallel study in Brigham and Woman's Hospital, Boston, Massachusetts USA.	
POPULATION	52 adults receiving allogeneic BMT for haematological malignancies, 45 included in analysis. Inclusion criteria: No evidence of non-neoplastic systemic disease deemed to require IV nutritional support after BMT. Patients were hospitalized 1 week before BMT for conditioning regimen. Diagnoses (GLNPN+PN): AML 11+9; CML 11+10; ALL 1+1; Myelodysplasia 1+0 and HL 0+1. Age median (range) yrs: GLNPN: 35.5 (20-49); PN: 32.1 (20-48) Gender (M:F): GLNPN: 8:16; PN: 8:13 All patients underwent daily intestinal decontamination with oral vancomycin, gentamicin and nystatin. Daily mouth and skin care regimen of 0.12% chlorohexidine gluconate to inhibit microbial colonization. IV acyclovir for herpes prophylaxis. GVHD prophylaxis: ex-vivo T-lymphocyte depletion of marrow with ST-1 immunotoxin or IV methotrexate and cyclosporin. Pre-BMT conditioning regimen: High-dose chemotherapy and TBI (1300 cGy) or chemotherapy alone.	
INTERVENTION	Route: TPN *Ad libitum* oral intake of low-bacterial diet allowed. PN estimated maintenance energy based on BER x 1.5. NPE - 70% dextrose, 30% lipid emulsion (Intralipid, Kabi Vitrum, Stockholm, Sweden) PN protein = crystalline amino acid acids at 1.5 g protein/kg body weight per day. Isonitrogenous and isocaloric. Study group: GLN-supplemented parenteral nutrition (*N*=24): Amino acid solution containing free L-GLN at 0.57 g GLN/kg body weight per day. Control group: Standard parenteral nutrition (*N*=21): Commercially available amino acid solution (GLN - free). Start criteria: BMT on day 0. Started on Day +1 after BMT. End criteria: Enteral consumption of > 50% of requirements for 3 consecutive days.	
OUTCOMES	Outcomes included: • Patients with clinical infections (*N*) • Length of stay after BMT (days) • Mortality (*N*) • Survival at 100 days (*N*) Narrative: • Plasma GLN levels Physician assessment; "Oral mucosa was examined for presence and severity of mucositis three times weekly and graded as a function of the degree of inflammation", grade 0-5 • Cumulative mucositis score	Other: • Nitrogen balance (days 4-11 in initial 23 patients) (g/day). • Total days on antibiotics. • GVHD, grade 1-3 • Patients receiving corticosteroids, amphtericin B • Blood, platelet transfusions (*N*) • Days until average neutrophil count of $\geq$ 0.5 x 10^9/L • Days until recontamination. • Patients with no positive cultures (N) • Patients with positive stool, throat cultures • Duration of PN (days) • Oral calories (kcal/day), nitrogen (g/day), 7 days, entire course • Days until leukocyte count $\geq$ 0.5 x 10^9/L or 1.0 x 10^9/L. • Average daily maximal temperature (°C).
OTHER	Funding: National Institutes of Health Trauma Grant, Grant CA 39542, M. Larry Lawrence Foundation. Robert Wood Johnson Foundation grant (Dr. Jacobs)	

ALL: Acute lymphoblastic leukaemia; AML: Acute myeloid leukaemia; BMT: Bone marrow transplant; CML: Chronic myelogenous leukaemia; GVHD: Graft-versus-host-disease; HL: Hodgkin's lymphoma; PN: Parenteral nutrition; TBI: Total body irradiation

ANIMAL STUDIES

STUDY ID	**Austgen 1992 (74)**	
SETTING	Randomized, placebo-controlled, parallel experimental study in USA.	
POPULATION	36 male Fischer 344 rats (250 g) (small tumour study < 5% of weight); 24 (large tumour study 10% of weight). All rats had bilateral flank implantations of a viable methylcholanthrene- induced fibrosarcoma. Exclusion: If palpable differences of tumour size were noted.	
INTERVENTION	Route: TPN Animals were allowed free access to standard chow and water up until day of starting TPN, when all chow was removed. Diets were isonitrogenous, isocaloric and isovolumic (Calories: 80% dextrose, 20% lipid)(Nitrogen: 80% commercially available crystalline AA solution) Study group: TPN+GLN (*N*=15): GLN enriched TPN. Remaining 20% of nitrogen was provided as GLN (222 ml of 3% GLN per liter) Control group: TPN–GLN (*N*=15): Standard GLN-free TPN. Remaining 20% of nitrogen was provided as an equimolar mixture of three other nonessential amino acids (serine, proline, glycine). Over 6 hour period the delivery rate of TPN was increased to its final flow rate of 2.5 ml/hr in both groups in both studies. During TPN animals were allowed free access to water. Start criteria: Small tumour study (*N*=36) day 11 after tumour implantation. Large tumour study (*N*=24) day 18 after Tumour End Criteria: Small tumour study; day 15. Duration: 5 days Large tumour study; day 23. Duration: 6 days	
OUTCOMES	Outcomes included: • Tumour weight (g)	Other: • All outcomes for large tumour study (most studies used smaller tumours) • Tumour glutaminase activity (µmol/mg prot/hour) • Tumour DNA flow cytometrics • Tumour weight change (g) • Tumour DNA content (mg/g) • Tumour glutathione content (µmol/g)
OTHER	Funding: NIH Grants and an American Cancer Society Career Development Award	

STUDY ID	**Bartlett 1995 (75)**
SETTING	Randomized, placebo-controlled, parallel experimental study in USA.
POPULATION	30 female Lewis/Wistar rats (weighing 175-200 g) were subcutaneously inoculated on the left flank with mammary adenocarcinoma cells (MAC-33).
INTERVENTION	Route: Diet All animals received standard rat chow (22.0% protein/4.20 kcal/g) *ad libitum* by mouth for 10 days after tumour implantation. Study diets isonitrogenous and isocaloric. Study group: GLN (*N*=15): 3.0% GLN-enriched diet (3.0% GLN/25.0% protein/4.2% kcal/g); 30 g GLN/kg diet. Control group: Glycine (*N*=15): 3.0% glycine-enriched diet (3.0% glycine/25.0% protein/4.20 kcal/g) Start criteria: On day 10 after tumour implantation. End criteria: Maintained on diet for 25 days.

STUDY ID	**Bartlett 1995 (75)**	
OUTCOMES	Outcomes included: • Tumour weight (g)	Other: • Tumour DNA, RNA (ug/100 mg tissue) • Tumour/carcass ratio • Lung metastasis (*N*) • Liver (g) • Hamstring muscle (g) • Distal ileum (g) • Liver, muscle and Ileum prot, RNA and DNA content • Flow cytometry data.
OTHER	Funding: American Cancer Society Career Development Award, Merit Review Grant from the Veterans Administration, Research grant from Clintec, Inc.	

STUDY ID	**Fahr 1994 (86)**	
SETTING	Randomized, placebo-controlled, parallel study in USA.	
POPULATION	32 male Fischer-344 rats, aged 25 - 30 weeks. After 1 week acclimatization to the animal care facility and 2 days after randomization for pregavage of defined diets, all animals underwent unilateral flank implantation with fibrosarcoma cells.	
INTERVENTION	Route: Gavage All rats were pair-fed rat chow and allowed water *ad libitum*. Study group: TUM+GLN (*N*=16): GLN (1g/kg/d) by gavage 3 x per day. Control group: TUM+Gly (*N*=16): Isonitrogenous amount of glycine by gavage. Start criteria: 2 days before tumour implantation. End criteria: Day 23 (21 days after tumour implantation)	
OUTCOMES	Outcomes included: • Tumour weight (g) • Tumour volume (mL)	Other: • Tumour glutaminase (umol/mg/h) • *In vitro* study results • Body weight (kg) • Tumour GSH • Chow, Gavage, nitrogen intake (g) • Arterial GLN and GSH metabolism. • Ketamine study • Mitoses/high powered field
OTHER	Funding: Research Advisory Group grants from the Veterans Administration Merit Award Review Board and the American Cancer Society.	

STUDY ID	**Kaibara 1994 (76)**
SETTING	Randomized, placebo-controlled, parallel study in Japan.
POPULATION	32 male Donryu rats. AH109A rat ascites hepatoma cells were subcutaneously implanted in the back of the rats on Day 0. On day 10 all rats were catheterized for TPN. Two groups (*N*=8) received mitomycin C (0.5mg/kg) chemotherapy for 5 days.
INTERVENTION	Route: TPN Rats were fed rat chow and water *ad libitum* from day 0-10. All TPN was isocaloric (250 kcal/kg/day) and isonitrogenous (1.5 g N/kg/day). Control group: STPN (*N*=8): Standard TPN + saline injection. Study group: GTPN (*N*=8): GLN-supplemented (Alanyl-GLN = 1.5 g/100 ml diet) TPN + saline. Control group: STPN+mitomycin C (*N*=8): 0.5 mg/kg/day for 5 days. Study group: GTPN+mitomycin C (*N*=8): 0.5 mg/kg/day for 5 days Start criteria: After cannulation into the jugular vein on day 10. End criteria: After 5 days TPN (Day 14)

STUDY ID	Kaibara 1994 (76)	
OUTCOMES	Outcomes included: • Tumour weight (Day 15) (g) (STPN vs. GTPN) Narrative: • Estimated tumour weight (Day 10)(g) • Tumour weight (g) (STPN+MMC vs. GTPN+MMC)	Other: • Tumour growth rate from tumour weight (%) • Tumour fractional synthesis rate (%/day) • Body weight (Day 0, 10, 15) Body weight change (g) • Carcass weight change (g) • Tumour growth rate from tumour volume (%) • Endogenous Leucine production (umole LEU/kg/hr)
OTHER	Funding: No mention.	

STUDY ID	Kaufmann 2003 (3)	
SETTING	Randomized, placebo-controlled study in USA.	
POPULATION	81 female Sprague-Dawley rats (35-40 days old) At time 0, age 55 days, rats were gavaged with a one-time dose of 80 mg/kg DMBA in corn oil (</= 2 ml per rat) to induce tumour growth.	
INTERVENTION	Route Gavage All rats were pair-fed standard rat (3% of protein as GLN) chow and given water *ad libitum*. Study and Control groups: GLN + DMBA, FA + DMBA, H2O + DMBA. Rats randomly received either GLN (Sigma Chemical Co., St Louis, MO) at 1 g/kg per day or an isonitrogenous amount of Freamine (FA, McGaw, St.Louis, MO) or water by gavage 3 x per day with diets delivered at maximum rate of 20 ml/100 g for 24 hours or 4 ml/gavage. Start and end criteria: Rats were further randomized into 3 groups: PreFed (-1 to +16 weeks) Shortfed (-1 to +1 weeks) PostFed (+1 to +16 weeks)	
OUTCOMES	Outcomes included: • Tumour weight and volume (Significant, but no data) (Mean (SEM) provided by author)	Other: • Tumour development (*N*) • Body weight (g) • Total nitrogen intake (g/kg/day) • Arterial GLN and GSH metabolism • Gut GLN and GSH metabolism • NK cell cytotoxicity.
OTHER	Funding: No mention. (Primary reviewer wanted to exclude due to absence of tumour at time of GLN supplementation start. 2 Secondary reviewers disagreed.)	

DMBA: 7,12-dimethylbenz[a] anthracene

STUDY ID	Kaufmann 2007 (4)
SETTING	Randomized, placebo-controlled study in USA.
POPULATION	34 female Fisher-344 rats. MTF-7 mammary tumour was implanted subcutaneously. Control groups received SHAM operation.
INTERVENTION	Route: Gavage All rats were pair-fed a predefined diet of chow and given water *ad libitum*. Diets were isocaloric and isonitrogenous. Study group: GLN+TUMOUR (*N*=9): 1 g GLN/kg per day by gavage (3 x per day). Control group: FA+TUMOUR (*N*=9): Isonitrogenous amount of Freamine to same schedule. Other: GLN+SHAM (*N*=8): Same GLN supplementation, no tumour. Other: FA+SHAM (*N*=8): Same Freamine, no tumour. Start criteria: Day 0. End criteria: after 2 weeks animals were sacrificed on day 15 for analysis.

STUDY ID	**Kaufmann 2007 (4)**	
OUTCOMES	Outcomes included: • None Narrative: • Tumour weight (mg) (Significant, but no data presented, only figure)	Other: • Mean body weight • Calorie and nitrogen intakes from chow and gavage • Arterial GLN, Glu, GSH concentration • Gut GLN, Glu, GSH extraction, Gut mucosa GSH concentration.
OTHER	Funding: VA Merit Review Award to VS Klimberg	

STUDY ID	**Kaufmann 2008a (5)**	
SETTING	Randomized, placebo-controlled study in USA.	
POPULATION	198 female Sprague-Dawley rats (35-40 days old). Chemically-induced rat mammary gland carcinogenesis model using 7,12-dimethylbenz[a] anthracene (DMBA). At age 50 days rats were gavaged with one-time dose of 20 mg of DMBA in 1 ml of sesame oil or 1 ml sesame oil alone.	
INTERVENTION	Route: Gavage All rats were pair-fed the purified research diet TD96163 (GLN = 1.84% of protein) and water *ad libitum*. Initial randomization into 6 groups (*N*=8): Study Group: DMBA+GLN Control group: DMBA+FA Rats received either GLN (Sigma Chemical Co., St Louis, MO, USA) at 1 g/kg/day or an isonitrogenous amount of Freamine (FA, McGaw, St. Louis, MO, USA) or H2O by gavage 3 x per day. Other: DMBA + H_2O, OIL + GLN, OIL + FA OR OIL + H_2O Second randomization into 1-, 2-, 4- or 11 wk (after DMBA administration) groups (*N*=unclear (8)): Start criteria: 1 wk before DMBA administration. End criteria: Rats were sacrificed at 1, 2, 4 and 11 wk after DMBA administration.	
OUTCOMES	Outcomes included: • Tumour volume (mL) (11 wks) • Tumour weight (g) (11 wks)	Other: • Tumourigenesis: number of rats with tumour development (*N*). • Mean body weight (g), chow intake, nitrogen intake • Arterial GLN, GSH metabolism • Gut GLN and GSH metabolism, Gut mucosa GSH concentration, Breast tissue GSH concentration • NK cell cytotoxicity.
OTHER	Funding: Supported by VA merit award to Dr VS Klimberg. (Primary reviewer wanted to exclude due to absence of tumour at time of GLN supplementation. 2 Secondary reviewers disagreed)	

DMBA: 7,12-dimethylbenz[a] anthracene; FA: Freamine

STUDY ID	**Klimberg 1992 (77)**
SETTING	Randomized, placebo-controlled, parallel trial in Arkansas, USA.
POPULATION	36 male Fischer 344 rats (300g). All animals received flank implantation of 3-methylcholanthrene-induced fibrosarcoma cells. On day 25 after implantation all animals received a single intraperitoneal dose of 20 mg/kg of methotrexate.
INTERVENTION	Route: Gavage All animals were allowed *ad libitum* intake of chow and water and were pair-fed. Study group: +GLN (*N*=18): 1 g/kg per day of GLN by gavage. Control group: -GLN(*N*=18): Isonitrogenous amount of Glycine by gavage. Start criteria: Day 23 after implantation. End criteria: 24 hours (day 26) and 48 hours (day 27) after initiation of methotrexate

STUDY ID	Klimberg 1992 (77)	
OUTCOMES	Outcomes included: • Initial tumour volume (cm^3) • Tumour volume loss (cm^3)	Other: • Metaphase mitoses per high-power field. • Tumour glutaminase activity (µm/mg/hour) Methotrexate concentrations in tumour, serum. • Body weight and food intake • Arterial GLN concentration.
OTHER	Funding: No mention.	

STUDY ID	Klimberg 1992a (78)	
SETTING	Randomized, placebo controlled parallel study in Arkansas, USA.	
POPULATION	30 male Fischer 344 rats (300 g). On day 0 (after one week acclimatization) rats were randomized to flank implantation of methyl-cholanthrene-induced fibrosarcoma cells. All rats were allowed *ad libitum* intake of standard rat food and water during 1 week acclimatization before tumour implantation. On day 26 and 33 after implantation rats received 5 mg/kg methotrexate by intraperitoneal injection (total dose = 10mg/kg).	
INTERVENTION	Route: Diet Rats were pair-fed nutritionally complete elemental, isonitrogenous (0.00417 g N/mL) - 30% from added GLN (*N*=15) or Glycine(*N*=15), 70% from Aminosyn) and isocaloric (1.25 nonprotein kcal/ml, 1 kcal/mL from dextrose and 0.25 kcal/mL from fat) from day 25 after tumour implantation. Diets were administered twice daily (30 mL) *ad libitum* (max 60 mL/day) Study group: GLN+MTX (*N*=9): Diet containing 0.5 g/kg per day GLN (0.66%) + methotrexate chemo. Control group: GLY+MTX (*N*=9): Diet containing isonitrogenous amount of Glycine (GLN free, 0%) + same Methotrexate schedule. Other: GLN+CTRL (*N*=6): GLN-supplemented diet + isotonic intraperitoneal injection of saline to same schedule as methotrexate. Other: GLY+CTRL (*N*=6): Glycine supplemented diet + same saline injection schedule. Start criteria: Day 25 after tumour implantation. Rats were pair-fed for 2 weeks. End criteria: Day 40	
OUTCOMES	Outcomes included: • Tumour volume (mL) • Tumour weight (g)	Other: • Tumour glutaminase activity (umol/min/mg)Mitoses per 20 HPF (g) • Mortality (*N*) • Body weights and food intake • Arterial GLN concentration • Gut metabolism (Glutaminase, S-phase DNA) • Haematology (HCT, WBC, PLT) • Morbidity (Positive blood cultures)
OTHER	Funding: Grant from Merit Review Board of Veterans Administration	

MTX: High dose methotrexate

STUDY ID	Klimberg 1996a (79)
SETTING	Randomized, placebo-controlled, parallel study in Arkansas, USA.
POPULATION	18 female Fisher 344 rats (150-200 g) underwent unilateral flank implantation with MTF-7 rat mammary tumour cells (Clone of the 13762NF rat mammary adenocarcinoma). All rats received predefined diet of chow and water *ad libitum* during 1 week acclimatization before tumour implantation.
INTERVENTION	Route: Gavage During study all animals were pair-fed chow, which contains 3% of its protein as GLN, thus excluding GLN-deprived rats. Study group: TUM+GLN (*N*=9): GLN (Sigma Chemical Co., St Louis, MO) at 1 g/kg per day by gavage 3 times a day. Control group: TUM+FA (*N*=9): Isonitrogenous amount of Freamine (McGaw, St Louis, MO) by gavage 3 times a day (contains no GLN) Start criteria: Day of tumour implantation. End criteria: After 7 weeks.

STUDY ID	**Klimberg 1996a (79)**	
OUTCOMES	Outcomes included: • Tumour volume (cc) • Tumour weight (g)	Other: • Axillary metastasis (*N*) • Mitoses per HPF • Nutrition (mean body weight, chow and gavage intake, nitrogen intake) • Arterial GLN and Glutathione metabolism. • PGE2 concentrations and NK cell cytotoxicity.
OTHER	Funding: Grant from American Cancer Society	

STUDY ID	**Robinson 1999 (80)**	
SETTING	Randomized, controlled, parallel study in France.	
POPULATION	80 female rats of buffalo strain. 16 rats served as healthy controls, the rest (*N*=64) were implanted with the Morris hepatoma *7777*. *7* day adaptation period before tumour implantation.	
INTERVENTION	Route: Diet *5* Groups (*N* ≥ *7*) Diets were isoenergetic (gross energy 15.48 MJ/kg of diet) and isonitrogenous (26.1% crude protein, inclusive amino acid supplements) All rats were given free access to water and experimental powder. Study group: GLN (40 g/kg of diet): From day 0 - 14 rats consumed *2.7* g (0.1 g/kg per day GLN. Control group: Tumour control (control mixture of amino acids). Other: Arg; Healthy control; OKG Start criteria: Tumour implantation (day 0) End criteria: 14 days after tumour implantation (day 14)	
OUTCOMES	Outcomes included: • Tumour weight (g)	Other: • Arg, OKG, Healthy control groups • Plasma and skeletal muscle amino acids • Immune variables • Peritoneal macrophage isolation in culture • Splenocyte isolation and activation • Indirect immunofluorescence (phenotype) assay • Macrophage cytostatic activity • NK cell cytotoxicity assay • Mechanisms of immune cell cytotoxicity and cytostasis.
OTHER	Funding: Natural Sciences and Engineering Research Council of Canada.	

STUDY ID	**Rouse 1995 (87)**
SETTING	Randomized, placebo-controlled, parallel study in Arkansas.
POPULATION	36 male Fischer 344 rats. 1 week acclimation to animal care facility. First randomization: On day 0 of study, rats were randomized to implantation of fibrosarcoma cells.
INTERVENTION	Route: Gavage Second randomization: On day 21 after tumour implantation, rats were randomized to receive pair-fed chow diets with supplemental GLN (GLN) or Glycine (GLY) by gavage: GLN (*N*=18) 1 g/kg/day ORAL elemental GLN by gavage GLY (*N*=18) 1 g/kg/day glycine by gavage Third randomization: On day 23 (after 2 days pre-feeding) rats were randomized to 4 groups receiving an intraperitoneal injection of Methotrexate (MTX) (20mg/kg) or saline (CON): Study group: GLN + MTX (*N*=9) Control Group: GLY + MTX (*N*=9) Other: GLN+CON (*N*=9); GLY+CON (*N*=9) Start criteria: Day 21 after tumour implantation. End criteria: Day 24.

STUDY ID	**Rouse 1995 (87)**	
OUTCOMES	Outcomes included: • Tumour glutaminase activity (um/mg/min) • Tumour volume loss (cc).	Other: • Body weights and food intake • Arterial GLN concentration • Tissue glutathione levels
OTHER	Funding: Grant from Merit Review Board of the Veterans Administration	

MTX: High dose methotrexate

STUDY ID	**Rubio 1998 (90)**	
SETTING	Randomized, placebo-controlled, parallel study in Arkansas.	
POPULATION	18 male Fischer 344 rats. 1 week acclimatization to animal care facility. On day 0 of study, rats were implanted with fibrosarcoma cells. On day 25 (after 2 days pre-feeding) all rats received a single intraperitoneal dose of methotrexate (MTX) (20 mg/kg)	
INTERVENTION	Route: Gavage On day 23 after tumour implantation, rats were randomized to receive pair-fed chow diets: Study group: GLN (*N*=9): 1 g/kg/day elemental GLN by gavage. Control group: GLY (*N*=9): 1 g/kg/day glycine by gavage. Start criteria: Day 23 (48 hrs pre-feeding) End criteria: Day 26 (24 hours after initiation of MTX)	
OUTCOMES	Outcomes included: • Tumour volume (before MTX initiation, after 48 hrs prefeeding with GLN) • Tumour volume loss (cm^3) Narrative: • Tumour glutaminase activity (µm/mg/hour) (No data)	Other: • Human study - Not RCT. • MTX6 in gut (nmol/g) • Metaphase mitoses per high-power field
OTHER	Funding: No mention.	

MTX: High dose methotrexate

STUDY ID	**Shewchuk 1997 (81)**	
SETTING	Randomized, controlled, parallel study in Edmonton, Canada.	
POPULATION	59 female Sprague-Dawley rats (197±2g) of the Buffalo strain. After 1 week of feeding, all rats were implanted with the MH *7777* subcutaneously. Rats in each diet group were randomly assigned to one of two exercise groups and trained progressively to swim 3 hrd/day(6 days/wk) or remained sedentary for 14 days.	
INTERVENTION	Route: Diet All rats had free access to water and received nutritionally complete purified isonitrogenous, isocaloric diets. Study group: +GLN (*N*=32): 20 L-GLN g/kg diet + cornstarch (20 g/kg diet) Control group: -GLN (*N*=27): 40 g Glycine per kg diet. Start criteria: 7 days before tumour implantation. End criteria: 14 days after tumour implantation (and randomization to exercise groups)	
OUTCOMES	Outcomes included: • Tumour weight (g) (Sedentary rats only)	Other: • Plasma amino acid concentration • Soleus muscle amino acid concentration • Glucose and amino acid metabolism by MH*7777* cells • Splenic phenotypes and natural killer cytotoxic activity • Mitogen responses • All outcomes for exercise trained rats (data presented separately)
OTHER	Funding: Grants from Natural Science and Engineering Council of Canada and the Central Research Fund at the University of Alberta.	

STUDY ID	**Xue 2007 (71)**
SETTING	Randomized, controlled, parallel study in Alberta, Canada.

STUDY ID	Xue 2007 (71)	
POPULATION	22 female Fisher 344 rats (weight 150-180 g), aged 11 -12 weeks Ward colorectal carcinoma implanted in all rats. CPT-11 chemotherapy injections at 75 (*N*=5), 100 (*N*=9), 125 (*N*=21) and 150 (*N*=7) mg/kg/day to determine the maximum tolerated dose in control rats only. When tumours reached ~ 2.0 cm^3, CPT -11 injections (125 mg/kg x 3 per day) were initiated (day 0).	
INTERVENTION	Route: Diet Constant portion of diet: Modified American Institute of Nutrition-76 basal diet mix (40% of calories as fat; 1.1% of total fatty acids as omega 3 (3.2% EPA, 0.8% DHA)) Acclimatization period: Rodent chow: control diet (50:50 w/w). Randomization to test diets after 1 week. 4 Groups (of interest). Study group: GLN (*N*=11): GLN containing diet (2% w/w) = 0.75 g GLN/kg body weight per day. Study group: Control diet+Oral GLN bolus (*N*=11): Oral GLN bolus (3% solution w/v) = 0.75 g/kg; given by oral gavage (30 min before each CPT-11 injection) Study group: Control diet+IV GLN (*N*=11): IV GLN infusion (3% solution w/v) = 0.75 g GLN/kg per day. Control group: CON (*N*=11): Control amino acid mixture contained an equimolar mixture of alanine, serine, glycine and histidine and was isonitrogenous with GLN-enriched diet. Other supplementation groups (*N*=11): Fish oil and prebiotics. Start criteria: Diet: After one week of acclimatization/7 days prior to the first dose of CPT-11. Oral Bolus/IV infusion: before each daily CPT-11 injection. End criteria: Day 9	
OUTCOMES	Outcomes included: • None Narrative: • Tumour volume/growth • Tumour response to CPT-11 (Data not shown)	Other: • Diarrhoea • Body weight • GLN concentrations in colonic mucosa, plasma, tumour • Mortality
OTHER	Funding: Canadian Institutes of Health Research, The Natural Sciences and Engineering Research Council of Canada, Crohn's and Colitis Foundation of Canada.	

STUDY ID	Xue 2008 (72)	
SETTING	Randomized, placebo-controlled, parallel study in Alberta, Canada.	
POPULATION	48 female Fisher 344 rats (body weight, 150-180 g), 11 - 12 weeks old. Pieces of the Ward colorectal carcinoma were transplanted subcutaneously on the flank. When tumour volume reached ~2 cm^3, 3 consecutive daily CPT-11 IV injections (125 mg/kg/day) were initiated.	
INTERVENTION	Route: Gavage Initially rats were fed the nonpurified Rodent Laboratory Chow (HarianTeklad). Constant portion of study diets: Semipurified Modified American Institute of Nutrition-76 basal diet mix (40% of calories as fat, PUFA: SFA ratio, 0.35). During adaptation period the Lab Chow and study diet was mixed 50:50 for 1 wk, followed by transition to 100% semipurified diet for 2 wks before tumour implantation. Study group: GLN (*N*=6, 12): GLN was administered by oral gavage (0.75 g/kg/day) 30 min before each daily CPT-11 injection. GLN was made as 3% (wt:v) solution immediately before use. Control group: Sham (*N*=6, 12): The sham treatment group was gavaged with isovolemic sterile water. Start criteria: Day of the first CPT-11 injection (day 0) End criteria: Day 0 (*N*=6,6); day 3 (*N*=6,6); day 9 (*N*= 12,12)	
OUTCOMES	Outcomes included: • None. Narrative: • Tumour volume (%)	Other: • Mortality • Diarrhoea • Cytoprotective mechanisms
OTHER	Funding: Canadian Institutes of Health Research, national Sciences and Engineering Council	

CPT-11: 7-ethyl-10-[4-(1-piperidinol)-1-piperidno] carbonyloxy-camptothecin

STUDY ID	Xue 2009 (73)	
SETTING	Randomized, controlled, parallel study in University of Alberta, Canada.	
POPULATION	42 female Fisher 344 rats (150-180 g body weight and 11-12 weeks old). Ward colorectal carcinoma were transplanted subcutaneously on the flank of the rats. When tumours reached 1.2 % of bw (2.3 cm^3) chemotherapy was initiated (CPT-11/5-FU combination) intravenously once a week for two weeks. Day 0 and 7 (50 mg/kg CPT-11), day 1 and 8 (50 mg/kg 5-FU). Atropine (1 mg/kg, subcutaneously) before each CPT-11 injection.	
INTERVENTION	Route: Diet All diets contained 262 g protein and 15-48 x 10^3 kJ energy/kg (40% of energy from fat, PUFA:SFA = 0.35). The constant portion consisted of the pre-mixed modified AIN -76 basal ingredients; the variable portion was formulated to allow addition of selected fat/fiber/amino acid elements. Water and food was available for *ad libitum* consumption. During acclimatization period the Rodent Laboratory Chow was mixed 50:50, w/w) with control diet for 1 week, followed by transition to experimental diets. Control group: Control diet (CON, *N*=12). The control amino acid mixture contained an equimolar mixture of alanine, serine, glycine and histidine and was isonitrogenous with GLN-enriched diet. Study group: GLN diet (GLN, *N*=10): 2 g GLN/100 g diet (2%, w/w total diet) Other: Ω-3 PUFA (fish oil) diet (*N*=10) (2.3 g Fish oil/100 g diet (0.8%, w/w total diet)); GLN+Ω-3 PUFA diet (*N*=10) and reference group (*N=7*) had no tumour implantation or chemotherapy. Start criteria: 2 weeks prior to tumour implantation + 16 days, post-tumour, pre-chemo period. End criteria: 13 days after completion of chemotherapy.	
OUTCOMES	Outcomes included: • None Narrative: • Effects of dietary treatments during the pre-chemotherapy period (2 weeks pre-feeding, tumour implantation, 16 days pre-chemo = 30 days) on tumour growth inhibition (%) • Tumour response to chemotherapy. (Data presented in figure only)	Other: • Survival/mortality during intervention (*N*) • Effects of dietary treatments on GSH stores in tumour tissue following CPT-11/5-FU therapy (Total GSH, umol/g) • All data for reference group, FO group and GLN+FO group. • Effects of dietary treatments during pre-chemotherapy period on body weight, food intake and leucocyte, neutrophil and lymphocyte concentrations. • Effects during the post-chemotherapy period on blood cell counts, body weight, food intake, muscle weight, GSH stores in host tissue following CPT-11/5-FU therapy (total GSH, GSSG, reduced GSH, rGSH/GSSG ratio)
OTHER	Funding: Canadian Institutes of Health Research, The Natural Sciences and Engineering Research Council of Canada and the Crohn's and Colitis Foundation of Canada	

PUFA: Polyunsaturated fatty acid; SFA: Saturated fatty acid; CPT-11: 7-ethyl-10-[4-(1-piperidinol)-1-piperidno] carbonyloxy-camptothecin; 5-FU: 5-Flourouracil

STUDY ID	Yoshida 1995 (82)
SETTING	Randomized, controlled, parallel, experimental study in Kukuoka, Japan.
POPULATION	46 male Donryu rats (150-170 grams) randomly divided into two groups: Tumour-bearing (TB) (*N*=30) and non-tumour-bearing (NTB) (*N*=16). On day 0 AH109A rat ascites hepatoma cells were inoculated into the TB rat subcutaneously. On day 10 all rats were catheterized. Rats from both groups randomized to STPN or GTP. 4 Groups: NTB+STPN (*N*=8) NTB+GTPN (*N*=8) TB+STPN (*N*=15) TB+GTPN (*N*=15)

STUDY ID	**Yoshida 1995 (82)**	
INTERVENTION	Route: TPN All rats received rat food for 1 week. After tumour implantation (*N*=30) rats had access to a stock diet and water *ad libitum* for 10 days. 2 groups of interest: Study group: TB+GTPN (*N*=15): 1.5 g/100 ml diet of standard amino acid solution replaced by 1.1 g of alanyl-GLN (Otsuka Pharmaceutical Co)/100 ml = 25% of total nitrogen as GLN. Control group: TB+STPN (*N*=15): Isonitrogenous and 90% of calories given as glucose, and 10% as fat. Start criteria: Day 10 after tumour implantation. End criteria: Day 15 (6 days)	
OUTCOMES	Outcomes included: • Tumour weight (g)	Other: • Tumour Fractional synthesis rate (%/day) • Tumour BrdU (% gated) • Body weight, body weight change • GLN levels in plasma, muscle, jejunum, tumour • Fractional synthesis rate (muscle, liver, jejunum, colon) • Whole body protein breakdown rate • Glutathione levels
OTHER	Funding: Research fund in 1st Department of Surgery, Kurume University. Otsuka Pharmaceutical Co (Tokushima, Japan) - glucose, lipid solution, multiple vitamins and alanyl GLN gifts	

APPENDIX 6.7: Risk of Bias Tables for Included Studies

HUMAN STUDIES

RISK OF BIAS TABLE	Anderson 1998 (100)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"The study was designed as a randomized, placebo, double-blind, cross-over study"; "Patients were assigned randomly to two courses of GLN and two courses of glycine." Comment: Method unclear, probably done based on the fact that the study is described as such.
Allocation concealment?	Unclear	No mention Comment: Unclear
Blinding? (Mucositis/Diarrhoea)	Yes	"Double-blind". "Participants were provided with "mouth sore study suspension". "In addition to the patients, the nurses and oncologists involved in the care of these patients also were blinded." Comment: Probably done
Incomplete outcome data addressed?	Yes	2 weeks: 11/24 (45%) did not complete at least two courses of identical chemotherapy, take prescribed doses and complete mouth pain calendar (6 progressive disease; 1 pharmacy error, 4 incomplete questionnaires/withdrawal). Paired data analysis using patients as their own controls. Comment: 11 pairs excluded from data analysis.
Free of selective reporting?	Yes	Feedback on primary outcomes
Free of other bias?	Yes	"If mouth sores made swallowing difficult, swishing and spitting the suspension out was permitted (1 patient in end)." Comment: Could introduce bias, only 1 patient acceptable.

RISK OF BIAS TABLE	Berk 2008 (162)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"This was a randomized … trial." "Randomization was performed using the Zelen treatment allocation scheme to balance patients factors other than institution." Comment: Probably adequate
Allocation concealment?	Yes	"MTI Biotech, Inc. … and distributed both placebo and HMB/Arg/GLN supplements to institutions in a foil packet identified by patient case number only." Comment: Probably adequate
Blinding? (Mucositis/Diarrhoea)	Yes	"This was a … double-blinded trial." "All study personnel and patients were blinded to treatment assignment for the duration of the study." Comment: Adequate
Incomplete outcome data addressed?	Yes	Withdrawal/dropout from randomization to analyses: Total 275/472 (58.2%); HMB/Arg/GLN 146/237 (61.6%); Placebo 129/235 (54.8%). Reasons are described in detail. "Patients not completing the 8-week assessment were treated as missing data." Intention-to-treat analysis done. Comment: Adequate
Free of selective reporting?	Yes	None apparent
Free of other bias?	No	"MTI Biotech, Inc. supplied and distributed both the placebo and HMB/Arg/Gln supplements.." Comment: Funding by pharmaceutical industry cold introduce bias due to conflict of interest. Co-interventions introduce bias towards making assumptions about the effect of GLN alone on outcomes.

RISK OF BIAS TABLE	Blijlevens 2005 (109)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Randomization performed by hospital pharmacy." " Patients were randomly allocated to receive either…" Comment: Method unclear, Centralized in pharmacy, probably adequate.

RISK OF BIAS TABLE	Blijlevens 2005 (109)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Allocation concealment?	Yes	No mention. Comment: Centralized, probably adequately concealed
Blinding? (Mucositis/Diarrhoea)	Yes	"Double-blinded"
Incomplete outcome data addressed?	Yes	Mortality 8/32 (25 %) (4 in each group) "If these items (DMS, DGS) were not performed on a specific day, the scores obtained on the day before was used." Comment: Adequate
Free of selective reporting?	Yes	All outcomes reported.
Free of other bias?	Yes	Twenty (63%) of the 32 patients were men. Educational grant of Fresenius-Kabi Nederland BV. Comment: To be considered in interpretation of results

RISK OF BIAS TABLE	Bozzetti 1997 (95)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"All the patients, after oral informed consent, were randomized to receive GLN of placebo." "Randomization was handled in double-blind manner by an individual who had no further involvement in the study." Comment: Method unclear, probably adequate based on centralized randomization.
Allocation concealment?	Yes	"Randomization was handled in double-blind manner by an individual who had no further involvement in the study." Comment: Method unclear, probably done.
Blinding? (Mucositis/Diarrhoea)	Yes	"Double-blind"
Incomplete outcome data addressed?	Yes	Day 1: 2/35 (2.9%)(GLN) refused to take GLN from first cycle due to intolerance, were not considered in analysis. Comment: Study groups still comparable at baseline.
Free of selective reporting?	Yes	Primary outcomes reported.
Free of other bias?	Yes	"GLN was taken regularly by 29 patients. Two patients received only one dose each day, partially due to nausea, and one refused to continue taking the AA during the next cycles of chemotherapy. One patient took a reduced dose by mistake and another patient stopped the treatment even though no toxicity had occurred." "Placebo was taken irregularly by 7 patients for a total of 10 cycles: 3 by mistake, 7 because of nausea or diarrhoea. Two patients refused to continue treatment, reporting intolerance to the compound." Comment: Could introduce bias, but in end studies are compared to others with lower doses…

RISK OF BIAS TABLE	Brown 1998 (140)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	"Patients were randomized to receive either…" Comment: Method unclear.
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	Yes	"Double blind"
Incomplete outcome data addressed?	Yes	1/34 (0.02%) mortality (control). 8/34 (23%), 4 from each group, 26% withdrawal/dropout in total. Reasons: 7 on patients request due to non-specific upper gastrointestinal symptoms with the sensation of abdominal fullness, 1 due to problems with supply of amino acid solution. Data analyzed on ITT basis. Comment: Adequately addressed.
Free of selective reporting?	Yes	None apparent

RISK OF BIAS TABLE	Brown 1998 (140)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Canovas 2000 (153)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	"Randomized study"," Patients divided into 3 groups" Comment: Method unclear.
Allocation concealment?	Unclear	No mention.
Blinding? (Mucositis/Diarrhoea)	Yes	"Double-blinded"
Incomplete outcome data addressed?	Yes	No withdrawals apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Cerchietti 2006 (101) (Unpublished correspondence from author in brackets in italics)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear (Yes)	"Randomly assigned to groups" *("Randomization done according to computer-generated randomization scheme (in a 1:1 ratio in blocks of 10). The statisticians were in charge of generating this scheme and they informed the research pharmacist what treatment she must prepare for a particular patient.")* Comment: Method unclear (*Adequate*)
Allocation concealment?	Unclear (Yes)	*("Only the statistician and the research pharmacist knew what treatment the patients were receiving during the whole duration of the trial.")* Comment: No mention (*Adequate*)
Blinding? (Mucositis/Diarrhoea)	Yes	"Double-blind methodology used". "Solutions prepared by pharmacy, physical aspects of solutions similar." (*Patients, caregivers and outcome assessors were blinded to treatment allocation for the whole duration of the study and 3 months after.)*
Incomplete outcome data addressed?	Yes	No withdrawals apparent.
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	"Supplements were gifts from Fresenius Argentina S.A." Comments: To be considered in interpretation of results

RISK OF BIAS TABLE	Choi 2007 (93)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Randomization by clinical trials office using computer-driven procedure, using stage of therapy as stratifying variable." Comment: Adequate
Allocation concealment?	Yes	"Packages containing anonymous treatment supplies for each patient were provided by the clinical trials office." Comment: Centralized, probably adequate
Blinding? (Mucositis/Diarrhoea)	Unclear	"Open label trial" Comment: Unclear
Incomplete outcome data addressed?	Yes	No withdrawals apparent. "All statistical analyses were performed according to the intention-to-treat principle." Comment: Adequate
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	BSC group not defined. Comments: Probably not an issue

RISK OF BIAS TABLE	Coghlin Dickson 2000 (97)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Randomized study"; "The BMT or PBPCT patients were registered with the data managers and randomly assigned to receive GLN or placebo." Comment: Method unclear, centralized, probably adequate.
Allocation concealment?	Yes	"Patients were registered with the data managers." Comments: Appears to be centralized
Blinding? (Mucositis/Diarrhoea)	Yes	"Double-blind"; "Patients who were blinded to the study drug"; "The patients were not given the opportunity to compare." Comment: Adequate
Incomplete outcome data addressed?	Yes	No withdrawals apparent. Follow-up at two years: 10/29 (30%) GLN; 13/29 (55%) Placebo due to Mortality. Comment: No loss to follow-up apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Unclear	"Except for the patient-study drug consumption, which was obtained daily during hospitalization, all data collection was obtained from the medical chart after the patients were discharged." Supplementation not isonitrogenous. Comment: Could introduce bias (but not for mortality/survival) with regard to standardized and consistent assessment of outcomes, extend unclear.

RISK OF BIAS TABLE	Da Gama Torres 2008 (123)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Patients were randomly assigned either to receive GIPN or PN"; Random numbers were generated by the statistical program EPIIFO 6.04 and were drawn to assign patients to one of the two groups. Comment: Adequate
Allocation concealment?	Yes	"Randomization was performed by pharmacist in charge of preparing PN solutions." Comment: Adequate
Blinding? (Mucositis/Diarrhoea)	Yes	"Patients and clinical transplantation team blinded to assigning arm"; "PN solutions were not identified for GLN supplementation."
Incomplete outcome data addressed?	Yes	No withdrawals apparent. Follow-up at day 180: 7/27 (26%)GIPN, 14/26 (54%) PN (mortality). No withdrawals for reasons other than mortality apparent. Comment: Adequate
Free of selective reporting?	Yes	Non apparent
Free of other bias?	Yes	"GLN was supplied without charge by Fresenius Kabi, Campinas, Brazil." Comment: To be considered in interpretation of results

RISK OF BIAS TABLE	Daniele 2001 (102)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Randomized trial"; "Patients randomized by phoning the clinical trials office where clinicians used computer-driven procedure with stage of therapy (adjuvant/advanced) as stratifying variable." Comment: Adequate
Allocation concealment?	Yes	"Patients randomized by phoning the clinical trials office…" Comment: Adequate
Blinding? (Mucositis/Diarrhoea)	Yes	"Double-blind"; "Patients, clinicians, and statistician were blinded to assigned treatment"; "Packages containing the anonymous supply of treatment (GLN or placebo) for patients provided by the clinical trials office"; "The organoleptic features of GLN and placebo as well as their appearance were identical." Comment: Adequate

RISK OF BIAS TABLE	Daniele 2001 (102)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Incomplete outcome data addressed?	Yes	After treatment: 8/70 (11%)(6 GLN, 2 Placebo) "did not perform the post-treatment functional assessment". "Of these, administration of the study treatment was not completed because of severe heartburn (one case), myocardial infarction (one case), severe stomatitis (two cases), intense nausea (one case), and emergency surgery (one case); one patient refused treatment soon after randomization and one patient was excluded because he erroneously received chemotherapy at a dose lower than planned." Comment: Reasons given for withdrawals, no differences in baseline characteristics between two arms.
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Decker-Baumann 1999 (48)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	"Patients, who were randomly allocated to groups…" Comment: Method unclear
Allocation concealment?	Unclear	No mention.
Blinding? (Mucositis/Diarrhoea)	Unclear	"Endoscopist did not know to which group the patients belonged." Comment: Blinding of patients unclear.
Incomplete outcome data addressed?	Yes	3/24 (12.5%) (1 GLN, 2 Control) because of severe side effects, the chemotherapy dose had to be reduced. Comment: Reasons given, data excluded from analysis, evenly distributed between groups, adequate.
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	"Supported by Pharmacia & Upjohn GmbH, Erlangen, Germany" Comment: To be considered in interpretation of results

RISK OF BIAS TABLE	Erdem 2002 (144)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	"Randomized, clinical study"; "Patients were randomized to …" Comment: Method unclear
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	Unclear	No mention
Incomplete outcome data addressed?	Yes	"One patient in the SG and six patients in the CG were unresectable at the operation; and one in each group died postoperatively. All these seven patients were excluded from the study." Comment: Baseline characteristics provided for the 32 included patients – still comparable.
Free of selective reporting?	Yes	None apparent
Free of other bias?	No	Co-intervention: 4.5 g/L arginine in SG. Comment: Changes in outcome measures cannot be attributed to GLN alone

RISK OF BIAS TABLE	Gianotti 2009 (163)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Randomization was done by a computer-generated permuted-block sequence and balanced by participating centers in a block of 10." Comment: Adequate

RISK OF BIAS TABLE	Gianotti 2009 (163)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Allocation concealment?	Yes	"Each center had a specific code with an independent randomization list. From these lists, sealed envelopes were produced. Each envelope contained an individual random code with the assigned treatment. The envelopes were opened the morning before surgery day -1." Comment: Adequate
Blinding? (Mucositis/Diarrhoea)	Unclear	"Independent observers performed data collection using standard case report forms and also registered the occurrence and type of complication according to definition… This was done to avoid possible bias due to lack of study blindness. Comment: No blinding after opening of randomization envelopes, but measures in place to reduce bias.
Incomplete outcome data addressed?	Yes	555 Eligible patients, 428 randomized. Not randomized (23%) due to predefined exclusion criteria. Reasons given. "All analysis were carried out on an intention-to-treat basis." Comment: Adequate
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Hallay 2002 (133) (Unpublished correspondence from author in brackets in italics)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear (Yes)	"Randomized trial". *"Randomization was performed with help of an envelope randomized system, carried out personally by anesthesiologist."* Comment: Unclear (*Adequate*)
Allocation concealment?	Unclear (Yes)	No mention. *"There was adequate allocation concealment."* Comment: Probably done
Blinding? (Mucositis/Diarrhoea)	Unclear (Yes)	No mention. "*Subjects, caregivers, outcome assessors, nurses responsible for nutrition of patients, assistants of laboratory, who carried out laboratory measurement were all blinded to treatment allocation.*"
Incomplete outcome data addressed?	Unclear	3/36 (8%) mortality (2 GLN, 1 control). Comment: No mention of how it was addressed in analysis.
Free of selective reporting?	Yes	None apparent
Free of other bias?	No	Co-intervention: Arginine (0.8 g/L), Large difference in sizes of groups 23 vs.13. Comment: Changes in outcome measures cannot be attributed to GLN alone.

RISK OF BIAS TABLE	Huang 2000 (103)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Randomized trial". "Patients were sequentially randomized to two treatment arms." Comment: Probably adequate
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	Unclear	No mention
Incomplete outcome data addressed?	Yes	No dropout apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Jebb 1994 (104)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	"Patients were randomized to receive either GLN or placebo with the first cycle of treatment and the alternative supplement with 2nd cycle." Comment: Method unclear

RISK OF BIAS TABLE	Jebb 1994 (104)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	Yes	"Double-blind trial …" "Patients and investigator were unaware of the randomization order for each subject."
Incomplete outcome data addressed?	Unclear	After second treatment cycle: 11/28 (39.2 %). 6 patients died before the second cycle was due (4 GLN, 2 Placebo); of which 4 deaths was attributed to treatment toxicity (2 GLN, 2 Placebo). At the end of the first cycle of treatment 5 patients had evidence of progressive disease and treatment was either changed or withdrawn (1 GLN, 4 Placebo). Comment: Reasons for dropouts given, but no mention of how this was addressed in analysis.
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	"The mean ((SD) consumption of the dose was 93(11% of that described." Comment: Comparable with other studies

RISK OF BIAS TABLE	Jebb 1995 (96)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"One patient in each pair was randomly allocated to receive GLN and the other a placebo…" Comment: Method unclear, centralized, probably adequate
Allocation concealment?	Yes	"The pairing of patients and their randomization was handled by two individuals who had no further involved in the study." Comment: Adequate concealment
Blinding? (Mucositis/Diarrhoea)	Yes	"Randomly allocated… in a double-blind manner."
Incomplete outcome data addressed?	Yes	8/24 (33.3 %), 4 patient pairs (4 from each group) were excluded from analysis as one or both patients of the pair consumed less than 50% of the prescribed dose. Comment: Reasons given, pairs excluded from analysis.
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	"Mean consumption was 69 (15) % for GLN and 76 (15) % for the placebo, which were not statistically significant." "At the peak of mucositis about 25% of patients were unable to swallow comfortably and were only able to use either solution as a mouthwash. This persisted for 2-5 days." Comment: Should be considered in interpretation of results, comparable with other studies.

RISK OF BIAS TABLE	Jo 2006 (142)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Candidates were then randomly allocated to either the GLN or the control group by permuted block randomization." Comment: Adequate
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	Yes	"All these preoperative evaluations were carried out in double-blind (patient and staff) manner." "The preoperative nutritional status was blindly assessed by one dietitian and the postoperative TPN formula was uniformly prepared for all patients, to minimize bias."
Incomplete outcome data addressed?	Yes	Exclusions: Pre-operative 44/143 (53%), operative 33/143 (39.1%) and pathologic 6/143 (7.2%), reasons are given (*N*=60, 32 GLN, 28 Control). 1/60(1.7%) mortality (GLN group), none other apparent. Comment: Acceptable.
Free of selective reporting?	Yes	None apparent

RISK OF BIAS TABLE	Jo 2006 (142)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Free of other bias?	Unclear	"At the halfway point in this study, when the two groups numbered approximately 30 patients each, GLN solution was no longer available from our domestic supplier. As a result, we had no choice but to stop the study…" Comment: No information on how this was addressed.

RISK OF BIAS TABLE	Klek 2005 (61)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	"Patients were randomly allocated to one of three groups." Comment: Method unclear
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	Unclear	No mention
Incomplete outcome data addressed?	Yes	6/69 (8%) (5 Controls, 1 GLN). Group A: One because of central venous site infection, in the other four PN, the amount of proteins and energy was smaller than assumed. Group B: One withdrew consent. Comment: Reasons given, excluded from analyses.
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	Only groups of interest included in systematic review. Comment: No apparent bias.

RISK OF BIAS TABLE	Kozelsky 2003 (119)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Randomized clinical trial" "Before random assignment to treatment arms, patients were stratified by the following…" Comment: Method unclear, study described as such and double-blind methodology used, probably adequate.
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	Yes	"Double-blind study" "Patients were randomly assigned to groups in double-blind manner."
Incomplete outcome data addressed?	Yes	No dropout apparent during intervention
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Li 2009 (120)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Randomized, double-blind, crossover design used" "All patients receiving chemotherapy scored grade 2 or higher during a screening cycle were randomized to either control or GLN group during the next chemotherapy cycle (chemotherapy cycle 1). Patients were crossed over to the alternate treatment during chemotherapy cycle 2." Comment: Method unclear, described as such, probably adequate
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	Yes	"Double blind design"
Incomplete outcome data addressed?	Yes	No dropout apparent
Free of selective reporting	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Marton 2010 (164)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Following sealed envelope block randomization, the patients were divided into two groups." "A randomized… trial…" Comment: Adequate
Allocation concealment?	Yes	"Following sealed envelope…" Comments: Probably adequate
Blinding? (Mucositis/Diarrhoea)	Yes	"A… double-blind…trial." Comment: Adequate
Incomplete outcome data addressed?	Yes	No apparent withdrawal/dropout except for ICU mortality. ICU mortality: GLN 5/30 (16.7%), Control 6/25 (24%) Comment: Adequate
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	May 2002 (130)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Patients were randomly assigned" "Nutritional supplements were randomly assigned to each patient, within each study site, using computer-generated random numbers prior to the start of the study…" Comment: Adequate
Allocation concealment?	Yes	"…using computer-generated random numbers prior to the start of the study…" Comment: Adequate
Blinding? (Mucositis/Diarrhoea)	Yes	"... in a double- blind fashion." "Each supplement had an indistinguishable tangy orange flavor..." "Each patient received the supplements in plain white foil packets..." "Each dose was supplied in a spate packet that was allocated by subject number."
Incomplete outcome data addressed?	Yes	Week 4: 17/49(34%) 11 control; 6 HMB/GLN/Arg Week 12: 16/49 (32%) 7 Control; 9 HMB/GLN/Arg) Week 24: 7/49 (14 %) 5 Control; 2 HMB/GLN/Arg Total = 81%. Mortality in two cases. Rest's reasons not specified - only states various reasons/not due to adverse events according to questionnaires. "Patients had to complete 4-week follow-up visit to be included in the analyses. The last value carried forward procedure was used to analyze the IT analysis. Comment: Dropouts addressed, reasons discussed, not specified.
Free of selective reporting?	Yes	None apparent
Free of other bias?	No	Co-interventions: Beta-hydroxy-beta methylbutyrate, arginine. Comment: Outcomes cannot be attributed to GLN alone.

RISK OF BIAS TABLE	O'Riordian 1994 (136)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	"Patients were randomized to receive either conventional TPN or GLN-supplemented TPN." Comment: Method unclear
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	Yes	"TPN solutions prepared by pharmacy staff" "The clinicians responsible for patient management were blinded to which TPN regimen was used."

RISK OF BIAS TABLE	O'Riordian 1994 (136)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Incomplete outcome data addressed?	Yes	2/22 (9 %) (1 GLN, 1 Control) Two patients became incapable of evaluation with respect to the immunological and metabolic studies. The first patient (group II), who was a habitual smoker with a previous history of multiple myocardial infarctions, developed fluid overload with left ventricular failure and a probable respiratory infection on the 4th postoperative day. A second patient (group I) became confused on the 2nd postoperative day and removed his central line. Both of these patients were included when assessing the infection rate in the study. Comment: No dropouts for included outcome.
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	Funding "… and by the generous assistance of Kabi Pharmacia" Comment: To be considered in interpretation of results

RISK OF BIAS TABLE	Oguz 2007 (141)	
DOMAIN	**JUDGMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	"Patients were randomized and analyzed in two groups…" Comment: Method unclear
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	No	No mention. Comment: Probably not since only study group received PN.
Incomplete outcome data addressed?	Yes	5/114 (4.5%), all control group. "Five patients in the control group could not tolerate enteral nutrition and thus number of subjects was not equal between the two groups." Comment: Excluded from analyses
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Okuno 1999 (105)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	"At study entry, patients were stratified by…" "The patients were subsequently randomized to receive … GLN or…" "Patients were randomized…" Comment: Method unclear
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	Yes	"…in double-blind manner" "Identical appearing placebo"
Incomplete outcome data addressed?	Yes	"No loss to follow-up …" No dropout apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Peterson 2007 (94)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	"…randomized…Phase III trial"; Eligible patients were randomized to receive either Saforis or placebo…" Comment: Method unclear
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	Yes	"The placebo formulation matched the texture and characteristics of the active drug…"; "…double-blind…" Comment: Probably done

RISK OF BIAS TABLE	Peterson 2007 (94)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Incomplete outcome data addressed?	Yes	Withdrawal/dropout during first treatment cycle was 8 (5%) in Saforis and 13 (8%) in placebo. Reasons not given. ITT analysis done. Comment: Adequate
Free of selective reporting?	-	None apparent
Free of other bias?	Yes	Funding provided by Aesgen Inc. and later by MGI Pharma (Suppliers of Saforis). Comment: To be considered in interpretation of results

RISK OF BIAS TABLE	Piccirillo 2003 (110) (Unpublished correspondence from author in brackets in italics)	
DOMAIN	**JUDGMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear (Yes)	"Randomized study" "Patients were randomized into two groups." *("Block randomization was obtained by N. Piccirillo, using a dedicated software and it was truly random.")* Comment: Unclear (*Adequate*)
Allocation concealment?	Unclear (Yes)	No mention. *("Allocation concealment was adequate.")*
Blinding? (Mucositis/Diarrhoea)	Yes	"The DMS was recorded daily from day 0 to discharge by one of us who was blind to the treatment with GLN." *("Subjects, care-givers and outcome assessors were blinded to treatment allocation.")*
Incomplete outcome data addressed?	Yes	No dropout apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Unclear	New commercial premixed nutrition bag and glutamine solution lead to design of second study. Comment: To be considered in interpretation of results

RISK OF BIAS TABLE	Pytlik 2002a (98)	
DOMAIN	**JUDGMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Randomized … study" "Forty consecutive, eligible patients were randomized." "Patients were randomized to receive parenterally either 30 g of dipeptide alanyl-GLN daily… or an isonitrogenous amonicide solution…" "Randomization was performed by random-number method in the hospital pharmacy and was stratified according to diagnoses." Comment: Adequate
Allocation concealment?	Yes	"Randomization was performed by random-number method in the hospital pharmacy and was stratified according to diagnoses." Except for the hospital pharmacist, all other personnel involved in the trial were blinded, as were the patients." Comment: Adequate
Blinding? (Mucositis/Diarrhoea)	Yes	"Double-blind… study" "Except for the hospital pharmacist, all other personnel involved in the trial were blinded, as were the patients." "GLN dipeptide and non-GLN amino acid solutions … were indistinguishable from each other."
Incomplete outcome data addressed?	Yes	No dropout apparent during intervention. "All analyses were done on an intention-to-treat basis and…" 24 months: 7/40 (1.8%) mortality (1placebo, 6GLN) "All analyses were done on an intention-to-treat basis and…"
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Scheid 2004 (134)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"A randomized… study" "Patients were randomized to receive a standard TPN or a glycyl-GLN-containing TPN in case a TPN would be indicated on clinical grounds at any time during treatment based on predefined criteria…" "Patients were randomized at baseline and maintained their allocation to S-TPN or GLN-TPN throughout subsequent cycles of chemotherapy." Comment: Method unclear, centralized, probably adequate
Allocation concealment?	Yes	No mention Comment: Centralized, double blinded, probably adequate
Blinding? (Mucositis/Diarrhoea)	Yes	"...Double-blind... study" "TPN preparations were compounded in sterile bags in the central TPN unit of the hospital pharmacy by a pharmacist not involved in patients care. Bags were labelled without indication of their content as GLN-TPN or S-TPN to ensure proper blinding of physicians and patients."
Incomplete outcome data addressed?	Yes	"A total of 127 chemotherapy cycles to 54 patients was given. During 45 cycles in 33 patients, a parenteral nutrition was applied. Forty of these cycles in 30 patients were included in analysis, and five were excluded because salvage chemotherapy regimens were used. No further patients had to be excluded from the study for other reasons." 24/54 (44%) "Analyses were planned as per treatment and not as ITT, because of the expected high proportion of randomized patients not receiving parenteral nutrition during their chemotherapy treatment." Comment: Predefined criteria used, reasons given.
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Scheltinga 1991 (143)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"A… randomized controlled trial was performed." "After completion of this regimen, they were randomized to receive either standard parenteral nutrition… or an isocaloric, isonitrogenous nutrient solution enriched with crystalline L-GLN…" "…patients were randomized by the research pharmacist." Comment: Centralized, probably truly random
Allocation concealment?	Yes	No mention. "…patients were randomized by the research pharmacist." Comment: Centralized, probably adequate concealment
Blinding? (Mucositis/Diarrhoea)	Yes	"A double-blind … trial was performed" "Patients, investigators, physicians, and nurses were blinded to randomization."
Incomplete outcome data addressed?	Yes	No dropout apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Schloerb 1993 (111)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"A randomized, ... study" "A research pharmacist, by using a random number table, randomized patients to receive either standard TPN or TPN with L-GLN." Comment: Adequate
Allocation concealment?	Yes	"A research pharmacist, by using a random number table…" Comment: Centralized, adequate
Blinding? (Mucositis/Diarrhoea)	Yes	"…double blind study." "The research nurse who recorded the data on these patients was unaware which TPN solution the patients received."

RISK OF BIAS TABLE	Schloerb 1993 (111)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Incomplete outcome data addressed?	Unclear	"Data were tested for statistical outliers, defined as those data falling outside 1.5 times the interquartile distance from median value for the two groups. Two such values for length of stay were excluded from analysis. (Both died and were from TPN with GLN group)." 4/29 (13.7%) mortality (3 GLN, 1 Control), reasons are given. Comment: Unclear how mortality was addressed in analyses of outcomes.
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	Funding from Ajinomoto USA Inc (Suppliers of L-GLN). Comment: To be considered in interpretation of results.

RISK OF BIAS TABLE	Schloerb 1999 (112)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"A randomized, … study" "…66 patients with… were randomized, double-blinded, to either oral GLN or glycine-control…" "Using a random number table, patients received either glycine or GLN orally." Comment: Adequate
Allocation concealment?	Yes	No mention Comment: Centralized, randomized, double-blinded, probably adequate
Blinding? (Mucositis/Diarrhoea)	Yes	"…Double-blind study". "Glycine or L-GLN were weighed out as 10 g portions in small plastic cups. Unused amino acids were returned to hospital pharmacy." "…preparation of TPN solutions in hospital pharmacy."
Incomplete outcome data addressed?	Yes	14/66 (21%) mortality (6 Control, 8 GLN) did not indicate significant correlation with either GLN or glycine administration for either haematological malignancies or patients with solid tumours. All but one of the patients who died required mechanical ventilation. "To reduce variability in determining patient outcomes, before breaking the randomization code, mortality data were excluded in assessing the data." Comment: Reasons given, excluded from analyses.
Free of selective reporting?	Yes	None apparent
Free of other bias?	Unclear	"Amino acids provided by Ajinomoto Inc, Teaneck, NJ; Trophamine base solution for TPN by McGaw Laboratories, Inc; Financial support by American Home Therapies." "Change in experimental protocol was necessary for last 5 GLN patients. TPN base solution was changed to TrophAmine (McGaw Laboratories, Irvine, CA) due to interruption in funding." Comment: Could introduce bias, should be considered in interpretation of results.

RISK OF BIAS TABLE	Sornsuvit 2008 (113)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	"Placement of a peripheral venous catheter was performed on the day of chemotherapy initiation, followed immediately by randomization to receive intravenous supplementation with either … GLN… or an equivalent quantity of a standard amino acid mixture…" Comment: Method unclear
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	Unclear	No mention
Incomplete outcome data addressed?	Yes	No dropout apparent. "Efficacy of the treatment periods was evaluated using the intention-to-treat approach." Comment: Addressed
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Stehle 1989 (59)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	"Patients admitted for elective resection of carcinoma of colon or rectum were randomly allocated to an experimental group and a control group." Comment: Method unclear
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	Unclear	No mention
Incomplete outcome data addressed?	Yes	No dropout apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	No	Grants from BMWi and Pfrimmer and Co. Comment: Should be considered in interpretation of results. Co-intervention: glycyl -L- tyrosine Comment: Changes in outcomes cannot be attributed to GLN alone.

RISK OF BIAS TABLE	Strasser 2008 (122)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"A randomized… study" "Patients were randomized before TaxCh: unique patient numbers assigned continuously and corresponded to the randomization number." "Identical boxes containing GLN or maltodextrin were labeled before the study start with randomization numbers." Comment: Adequate
Allocation concealment?	Yes	"The randomization list was prepared by an independent person and was kept inaccessible to the study team in a locked container. Sealed envelopes for each number were stored in a locked container accessible to clinicians for emergencies." Comment: Adequate
Blinding? (Mucositis/Diarrhoea)	Yes	"…Double-blind study".
Incomplete outcome data addressed?	Yes	11/52 (21%) In GLN group: 1 withdrew consent, 2 had tumour progression, death, 2 had tumour complications (painful bone metastasis, abdominal fullness), 2 had taxane toxicity, 2 patient wish to withdraw, 3 had taxanes stopped (patient wish, abdominal pain, diarrhoea). In Placebo group: 2 had taxanes withdrawn, 2 withdrew consent, 1 tumour progression, death, 1 co-morbidity, depression, 1 bloating, nausea, 1 vomiting, 2 patient wish. "Forty-one patients complied with the study procedures, completing the diary for the primary outcomes and the medication log, and were analyzed." Comment: Addressed
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	Funding by Baxter Switzerland, Bristol-Myers Squibb Switzerland. Comment: To be considered in interpretation of results.

RISK OF BIAS TABLE	**Sykorova 2005 (145)** (Unpublished correspondence from author in brackets in italics)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear (Yes)	"A randomized... study" "Patients were randomized to receive either prophylactic PN.. or PN initiated ad hoc..." "In each group, patients were further randomized to receive either standard PN, or isocaloric , isonitrogenous PN containing GLN..." "Randomization was performed and PN prepared in the nutritional centre of our hospital and distributed to the transplant unit as all-in-one bag." (*"Eligible patients were assigned using closed envelopes."*) Comment: Method unclear (Adequate)
Allocation concealment?	Yes	"Randomization was performed and PN prepared in the nutritional centre of our hospital and distributed to the transplant unit as all-in-one bag." Comment: Centralized

RISK OF BIAS TABLE	**Sykorova 2005 (145)** (Unpublished correspondence from author in brackets in italics)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Blinding? (Mucositis/Diarrhoea)	Yes	"Except for the responsible member of the nutrition centre, all other personnel involved in the study were blinded, as were the patients." "Double-blind".
Incomplete outcome data addressed?	Yes	At discharge: 1/44 mortality. One patient died during transplant hospital stay of early septic complication. Comment: Addressed 3 years: - 12/44 (27%) mortality (9 GLN, 3 Control) 5 patients died after discharge of disease progression and related complications, in 4 patients CR was followed by relapse resistant treatment and eventually fatal, and another 2 patients with CR died of sepsis without a relapse. Comment: Survival is only outcome included
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	**Van Zaanen 1994[106]**	
DOMAIN	**JUDGMENT**	**DESCRIPTION**
Adequate sequence generation?	No	"Randomized pilot study" "Patients were randomized at each treatment cycle and not per individual." Comment: Probably not truly random.
Allocation concealment?	Unclear	"Patients were randomized on the decision of the pharmacist by balanced assignment to receive either Standard TPN or TPN with ala-GLN." Comment: Centralized, method unclear
Blinding? (Mucositis/Diarrhoea)	Yes	"Double-blind"
Incomplete outcome data addressed?	Yes	During study: 2/20 (10%) mortality (Control group), both excluded from analyses. Comment: Reasons given, addressed One year follow-up: 8/20 (40%) mortality (4GLN, 4Control). In the GLN group 3 patients had refractory leukaemia and one patient died of *Cytomegalovirus pneumonia*. In the Standard TPN group, two patients died during the study period, one with herpes simplex virus pneumonia and a refractory leukaemia at day 12 of the neutropenic period and the other one at day 9 of the neutropenic period because of sepsis with *Streptococcus mitis*. Two patients died of refractory leukaemia within the year after the study. Comment: Reasons given, addressed
Free of selective reporting?	Yes	None apparent
Free of other bias?	No	"15 patients with 20 treatment cycles were studied." "Patients were randomized at each treatment cycle and not per individual." Some patients randomized more than once. "Two patients underwent three treatment cycles and were randomized x 3, one patient x 2 and rest only once." "Fresenius AG, Germany provided alanine-GLN dipeptide." Comment: Could introduce bias, should be considered in interpretation of results.

RISK OF BIAS TABLE	**Wu 2001 (29)**	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	"A randomized… clinical trial" "…patients with gastrointestinal cancer were randomized into two groups…" Comment: Method unclear
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	Yes	"Double-blind clinical trial".
Incomplete outcome data addressed?	Yes	No dropout apparent

RISK OF BIAS TABLE	Wu 2001 (29)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Free of selective reporting?	Yes	None apparent
Free of other bias?	No	Co-interventions: Arginine and omega 3 fatty acids. Comment: Could introduce bias, outcome cannot be attributed to GLN alone.

RISK OF BIAS TABLE	Yoshida 1998 (37)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	"Thirteen patients with esophageal cancer were randomly placed in either a control or a GLN group." Comment: method unclear
Allocation concealment?	Unclear	No mention
Blinding? (Mucositis/Diarrhoea)	No	"Oral GLN was administered in GLN group." "A standard amino acid solution was administered intravenously to the control patients to make control isonitrogenous with GLN group." Comment: No mention of blinding, probably not since study group received oral supplement and control IV placebo.
Incomplete outcome data addressed?	Yes	No loss to follow-up during study period apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	Supported by research fund from Otsuka Pharmaceutical Co. (Tokoshima, Japan) Comment: Should be considered in interpretation of results.

RISK OF BIAS TABLE	**Ziegler 1992 (60)** (Unpublished correspondence from author in brackets in italics)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear (Yes)	"Eligible study patients were randomly assigned to a control or experimental group…" *(Double-blind randomization table by research pharmacist)* Comment: Method unclear (*Adequate*)
Allocation concealment?	Unclear (Yes)	*("There was allocation concealment.")* Comment: No mention (*Adequate*)
Blinding? (Mucositis/Diarrhoea)	Yes	"All persons other than the research pharmacist were blinded to randomization."
Incomplete outcome data addressed?	Yes	4/45 (9%) (2 GLN, 2 Control) "All data were tested for statistical outliers, defines as those data falling outside of 1.5 times the interquartile distance from the median value for the two groups. Four such values were excluded from analysis. Nitrogen balance data for one control patients… and three patients were hospitalized for a prolonged periods and were clinically dissimilar from the rest of the study group. Two GLN-treated patients were kept in hospital after engraftment because of hepatitis and uncontrolled hypertension; only data on their length of hospital stay (73 and 60 days, respectively) were excluded from analysis. A control patient who rejected the first BMT and received a second transplant (LOS, 95 days) were excluded from all data analysis." Comment: Reasons given, excluded from analysis. Day 100: No loss to follow-up apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

ANIMAL STUDIES

RISK OF BIAS TABLE	Austgen 1992 (74)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Randomized in 2 groups to receive STD or GLN TPN." "Randomization of animals was done such that animal weight and tumour size were equivalent between groups at the time of central line insertion, day 7 after tumour implantation." Comment: Randomization method unclear, probably adequate for animal study.
Allocation concealment?	Unclear	No mention.
Blinding?	Unclear	No mention.
Incomplete outcome data addressed?	Unclear	Day 5: 36 rats reported to be used for small tumour study - data presented for only 15 rats in each group, no reasons given = 16% dropout, 8% in each group. Comment: Unclear
Free of selective reporting?	Yes	Primary outcomes reported.
Free of other bias?	Yes	None apparent.

RISK OF BIAS TABLE	Bartlett 1995 (75)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Animals were randomized to groups." Comment: Randomization method unclear, probably adequate for animal study.
Allocation concealment?	Unclear	No mention.
Blinding?	Unclear	No mention.
Incomplete outcome data addressed?	Yes	No dropouts apparent.
Free of selective reporting?	Yes	Primary outcomes reported.
Free of other bias?	Yes	None apparent.

RISK OF BIAS TABLE	Fahr 1994 (86)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Rats were randomized to receive GLN or…" Comment: Randomization method unclear, probably adequate for animal study.
Allocation concealment?	Unclear	No mention
Blinding?	Unclear	"Pair-fed." Comment: Unclear
Incomplete outcome data addressed?	Yes	No dropouts apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Kaibara 1994 (76)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"The animals were randomized into 4 groups according to the diet and treatment." Comment: Randomization method unclear, probably adequate for animal study.
Allocation concealment?	Unclear	No mention
Blinding?	Unclear	No mention

RISK OF BIAS TABLE	Kaibara 1994 (76)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Incomplete outcome data addressed?	Yes	No dropouts apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	**Kaufmann 2003 (3)** (Unpublished correspondence from Suzanne Klimberg (Klimbergsuzanne@ uams.edu, 11 May 2009) in brackets in italics)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Rats randomly received GLN… or Freamine…" "Rats were further randomized into 3 groups: PreFed, ShortFed and PostFed." *(The rats were randomized.)* Comment: Method of randomization unclear, probably adequate for animal study. Author only reaffirmed randomization, but not method.
Allocation concealment?	Unclear	No mention
Blinding?	Unclear (No)	"Pair-fed." No mention *(The rats were blinded to what they were getting, but how could the researchers be blinded to what they were gavaging the rats with?)* Comment: Outcomes assessors not blinded
Incomplete outcome data addressed?	Yes	No dropouts apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Kaufmann 2007 (4)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Rats were randomized into 1 of 4 groups." Comment: Randomization method unclear, probably adequate for animal study.
Allocation concealment?	Unclear	No mention
Blinding?	No	"Pair-fed" No mention Comment: Probably not, based on methods used in other studies by same author.
Incomplete outcome data addressed?	Yes	No dropout apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	**Kaufmann 2008a (5)** (Unpublished correspondence from Yihong Kaufmann (KaufmannYihong@ uams.edu, 8 Aug 2009) in brackets in italics)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Rats randomly received GLN or ..." "Rats were randomized to one of six groups…" *("In this study rats were randomly divided to the different tested groups before the study started.")* Comment: Randomization method unclear, probably adequate for animal study.
Allocation concealment?	Unclear	No mention
Blinding?	(Unclear) No	No mention. *("After randomization, all the groups were identified.")* Comment: No
Incomplete outcome data addressed?	Yes	No dropout apparent

RISK OF BIAS TABLE	**Kaufmann 2008a (5)** (Unpublished correspondence from Yihong Kaufmann (KaufmannYihong@ uams.edu, 8 Aug 2009) in brackets in italics)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	**Klimberg 1992 (77)**	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Rats were randomized to receive…GLN or…" Comment: Randomization method unclear, probably adequate for animal study.
Allocation concealment?	Unclear	No mention
Blinding?	No	"Pair-fed" No mention Comment: Probably not, based on other studies by same authors.
Incomplete outcome data addressed?	Yes	No dropout apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	**Klimberg 1992a (78)**	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Rats were randomized to flank implantation of…" "Animals were then randomized to a nutritionally complete elemental diet that was GLN enriched or…" Comment: Randomization method unclear, probably adequate for animal study.
Allocation concealment?	Unclear	No mention
Blinding?	No	"Rats were then pair-fed for 2 weeks." Comment: Unclear. Probably not, based on other studies by same authors.
Incomplete outcome data addressed?	Unclear	Day 40: 10/18(56%) mortality (4GLN+MTX, 6GLY+MTX) Comment: Unclear whether and how this was addressed in analysis.
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	**Klimberg 1996a (79)**	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"The rats were randomized on the day of implantation to receive GLN…or…" Comment: Randomization method unclear, probably adequate for animal study.
Allocation concealment?	Unclear	No mention
Blinding?	No	"Pair-fed" No mention Comment: Probably not, based on other studies by same author.
Incomplete outcome data addressed?	Yes	No dropout apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Robinson 1999 (80)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"After 7 day adaptation period, rats were randomly allocated to serve as healthy controls… or were implanted with the Morris hepatoma 777…" "Rats were randomly allocated to one of five treatments…" Comment: Randomization method unclear, probably adequate for animal study.
Allocation concealment?	Unclear	No mention
Blinding?	Unclear	No mention
Incomplete outcome data addressed?	Yes	No dropout apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	No	Values reported as mean (SEM). Group sizes indicated as ≥ 7 per group. N=7 used for calculation of SD from SEM. E-mail correspondence unsuccessful in obtaining more specific data regarding group sizes. Comment: Could introduce bias, since the exact number of rats per group is not used in calculation of SD from SEM.

RISK OF BIAS TABLE	Rouse 1995 (87)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"On day 0 of the study, 36 rats were randomized to flank implantation of… fibrosarcoma cells." "On day 21 after tumour cell implantation, rats were randomized to receive pair-fed chow diets with supplemental GLN or GLY by gavage." "On day 23, after 2 days prefeeding, rats were randomized to one of the following four groups receiving an… of MTX… or saline…" Comment: Randomization method unclear, probably adequate for animal study.
Allocation concealment?	Unclear	No mention
Blinding?	Unclear	"Rats were randomized to receive pair-fed chow diets…" Comment: Unclear
Incomplete outcome data addressed?	Yes	No dropout apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Rubio 1998 (90)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"…rats were randomized to 48 hours of prefeeding with GLN… or an isonitrogenous amount of glycine." Comment: Randomization method unclear, probably adequate for animal study.
Allocation concealment?	Unclear	No mention
Blinding?	Unclear	"Pair-fed" No mention
Incomplete outcome data addressed?	Yes	No dropout apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Shewchuk 1997 (81)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Rats… were randomly assigned to one of two groups." "Rats in each diet group were randomly assigned to one of two exercise groups…" Comment: Randomization method unclear, probably adequate for animal study.
Allocation concealment?	Unclear	No mention
Blinding?	Unclear	No mention
Incomplete outcome data addressed?	Yes	No dropout apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	**Xue 2007 (71)** (Unpublished correspondence from Vickie Baracos (vickieb@ cancerboard.ab.ca) in brackets in italics)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	No mention
Allocation concealment?	Unclear	No mention
Blinding?	Unclear *(Unclear)*	No mention *("The diets, once prepared, are indistinguishable from one another and these were prepared independently by a person not involved in the study in any other way. The persons conducting the feeding of the animals every day had only diet number A,B,C,D.")* Comment: Blinding of assessment of tumour volume/weight still unknown.
Incomplete outcome data addressed?	Yes	No dropout apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Xue 2008 (72)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	No mention
Allocation concealment?	Unclear	No mention
Blinding?	Unclear (Yes)	"Diarrhoea assessments were conducted by a researcher unaware of the study treatments." *(Caregivers probably blinded, from studies by same author. Xue 2007)* Comment: Blinding of assessor of tumour weight unknown.
Incomplete outcome data addressed?	Unclear	Mortality 2 Sham Comment: No mention of how this was addressed in analysis of other outcomes.
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Xue 2009 (73)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"Two weeks prior to tumour implantation, rats were randomly assigned to one of four diets…" Comment: Randomization method unclear, probably adequate for animal study.
Allocation concealment?	Unclear	No mention

RISK OF BIAS TABLE	Xue 2009 (73)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Blinding?	Unclear	No mention (*Caregivers probably blinded, from studies of same author. Xue 2007)* Comment: Blinding of assessor of tumour weight unknown.
Incomplete outcome data addressed?	Unclear	Days 9-17 of study: 7/22 (31.8%) Mortality, (5/12 Control, 2/10 GLN). "As tumour burden was markedly reduced with CPT-11/5-FU treatment, the observed short-term mortality was considered to be attributable to the chemotherapy rather than cancer progression." Comment: Unclear how dropout due to mortality was addressed in analysis of other outcomes.
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	None apparent

RISK OF BIAS TABLE	Yoshida 1995 (82)	
DOMAIN	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	"…rats were randomly divided into 2 groups: tumour-bearing and non-tumour-bearing." "Each group was subdivided into two groups according to the TPN infusate,…" Comment: Randomization method unclear, probably adequate for animal study.
Allocation concealment?	Unclear	No mention
Blinding?	Unclear	No mention
Incomplete outcome data addressed?	Yes	No dropout apparent
Free of selective reporting?	Yes	None apparent
Free of other bias?	Yes	Otsuka Pharmaceutical Co (Tokushima, Japan) - glucose, lipid solution, multiple vitamins and alanyl GLN gifts. Comment: Could introduce bias and needs to be considered in the interpretation of results.

APPENDIX 6.8: Summary of Criteria Used to Assess Presence and Severity of Oral Mucositis/stomatitis

PATIENT REPORTED (SUBJECTIVE)	**0**	**1 (I)**	**2 (II)**	**3 (III)**	**4 (IV)**	**5 (V)**
Modified Eastern Cooperative Oncology Group (MECOG) grading criteria [100,103,113] *(Mouth sore? Yes/No; How soreness affected eating)*	No pain	Painful mucositis, no change in oral intake	Painful mucositis, soft foods only	Painful mucositis, liquids only	Painful mucositis, no oral intake (except meds)	-
Scoring system for patient-reported symptoms. (Scored from 1-5)[104,113] Mouth comfort/Ease of eating	No change from normal/Eating as normal	Slightly sore mouth/Eating is uncomfortable	Sore mouth/Pain on chewing	Painful mouth with ulcers/Soft food or liquids only	Severe pain with ulcers and inflammation/Unable to eat or drink	-
Patient mucositis assessment (How does your mouth feel today?)[96]	No change from normal	Slightly sore mouth/Little or no difficulty in eating	Sore mouth/Pain when chewing or swallowing	Considerable mouth pain with redness, ulcers and inflammation	Severe pain/Unable to eat or drink	-
The North Central Cancer Treatment Group *(N*CCTG) criteria for reporting mucositis.[105]	Criteria not published					

PHYSICIAN/NURSE ASSESSMENT (OBJECTIVE)	**0**	**1 (I)**	**2 (II)**	**3 (III)**	**4 (IV)**	**5 (V)**
Daily mucositis score (DMS)[109,110] *Lesions, erythema, oedema, pain, bleeding, dryness and production of viscous mucous* Score for each item of 0*(N*ormal) to 3(severe)	-	Score 1-7, mild mucositis	Score 8-14, moderate mucositis	Score $\geq$ 15, severe mucositis (Comparable with WHO grade III)	-	-
World Health Organization (WHO) scale [48,94,101,104,106] *Symptoms (pain), functions (ability to drink and eat), presence of lesions (ulcers, erythema)*	None	Soreness with/without erythema, but no ulceration	Erythema, ulcers. Patients can swallow solid diet	Ulcers, extensive erythema. Patients cannot swallow solid diet.	Oral mucositis to the extent that alimentation is not possible	-

PHYSICIAN/NURSE ASSESSMENT (OBJECTIVE)	0	1 (I)	2 (II)	3 (III)	4 (IV)	5 (V)
Objective mucositis score (OMS)[101] *(Grading of oral erythema, and ulceration size in nine oral sites)* OMS < 1.5 = not severe OMS ≥ 1.5 = severe	Oral erythema = none Ulceration size = 0	Oral erythema = mild Ulceration size = < 1 cm	Oral erythema = severe Ulceration size = 1-3 cm	Ulceration size = >3 cm		
Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0)[93]	Clinical: No mucositis	Function/symptom: Minimal symptoms, normal diet. Clinical: erythema of mucosa	Function/symptom: Symptomatic, but can eat and swallow modified diet. Clinical: patched ulceration or pseudomembranes	Function/symptom: Symptomatic and cannot eat. Clinical: confluent ulceration or pseudomembranes, bleeding with minor trauma	Function/symptom: Symptoms associated with life-threatening consequences. Clinical: tissue necrosis, significant spontaneous bleeding.	Death
Mucositis grading criteria[105]	No mucositis	Soreness/erythema	Erythema/ulcers/can eat solids	Ulcers/requires liquid diet only	Alimentation not possible	-
Toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC) (Objective)[103,166]	No mucositis. No change over baseline	Injected mucosa. Injection, may experience mild pain not requiring analgesic	Patchy mucositis that may produce inflammatory serosanguinitis discharge; may experience moderate pain requiring analgesia	Confluent, fibrinous mucositis, may include severe pain requiring narcotic	Ulceration, hemorrhage, or necrosis	-
Observer Mucositis Score[96]	Normal mucosa. No inflammation /ulceration	"Chewed tongue" syndrome. < 3 sites inflammation/ulceration	> 3 sites inflammation/ulceration	Overall inflammation/ulceration of the mouth and/or throat. Profuse saliva production	Overall inflammation/ulceration of the mouth and/or throat. Thick stringy saliva requiring manual removal from mouth and/or saline nebulizers	-
Common toxicity criteria of the National Cancer Institute, Bethesda, Maryland (NCI)[95,102,]	None	Painless ulcers, erythema, or mild soreness in the absence of ulcers	Painful erythema, edema, or ulcers but eating or swallowing possible	Painful erythema, edema, or ulcers requiring IV hydration	Severe ulceration or requiring parenteral or enteral nutritional support or prophylactic intubation	Death related to toxicity

PHYSICIAN/NURSE ASSESSMENT (OBJECTIVE)	**0**	**1 (I)**	**2 (II)**	**3 (III)**	**4 (IV)**	**5 (V)**
Nebraska Oral Assessment Score "Individual item scores (Grade 1 to 3) were summed to obtain an overall mucositis score. The following indexes were recorded: MUCPEAK – the peak oral mucositis score reached in each individual patient. MUC9, MUC10, MUC11, MUC12, MUC13, MUC14 – the number of days with mucositis of at least the particular score"[98]	*Voice Quality* *Swallowing* *Lips* *Tongue* *Saliva* *Mucous membranes* *Gingiva* *Teeth, dentures*	Normal Normal Smooth, pink, moist Pink, moist, palpillated Watery Pink and moist Pink, stippled, firm Clean, no debris	Deep or raspy Some pain on swallowing Dry or cracked Coated or without papillae and shiny with or without redness Thick or ropy Reddened or coated without ulcerations Edematous with or without redness Plaque or debris in localized areas	Difficulty talking or painful Unable to swallow Ulcerated or bleeding Blistered or cracked Absent Ulcerations with or without bleeding Spontaneous bleeding or bleeding with pressure Generalized plaque or debris	- - - - - - - -	- - - - - - - -
Gastrointestinal toxicity classified according to Northern California Oncology Group *(*NCOG)[153] Criteria not published.						
Stanford University Hospital BMT toxicity scale. *"The number of days of mucositis included days of grades 2-4 mucositis. Grades 0-1 were not considered significant mucositis."*[97] Criteria not published.						
"Incidence and severity of mucositis was based on review of each patient chart by one individual: Diagnoses, treatment and frequent mention of mucositis in progress notes were accepted as presence of mucositis."[112]						
"Oral mucosa was examined for the presence and severity of mucositis and graded as a function of the degree of inflammation."[60,111].						

APPENDIX 6.9: Summary of Criteria Used to Assess Presence and Severity of Diarrhoea

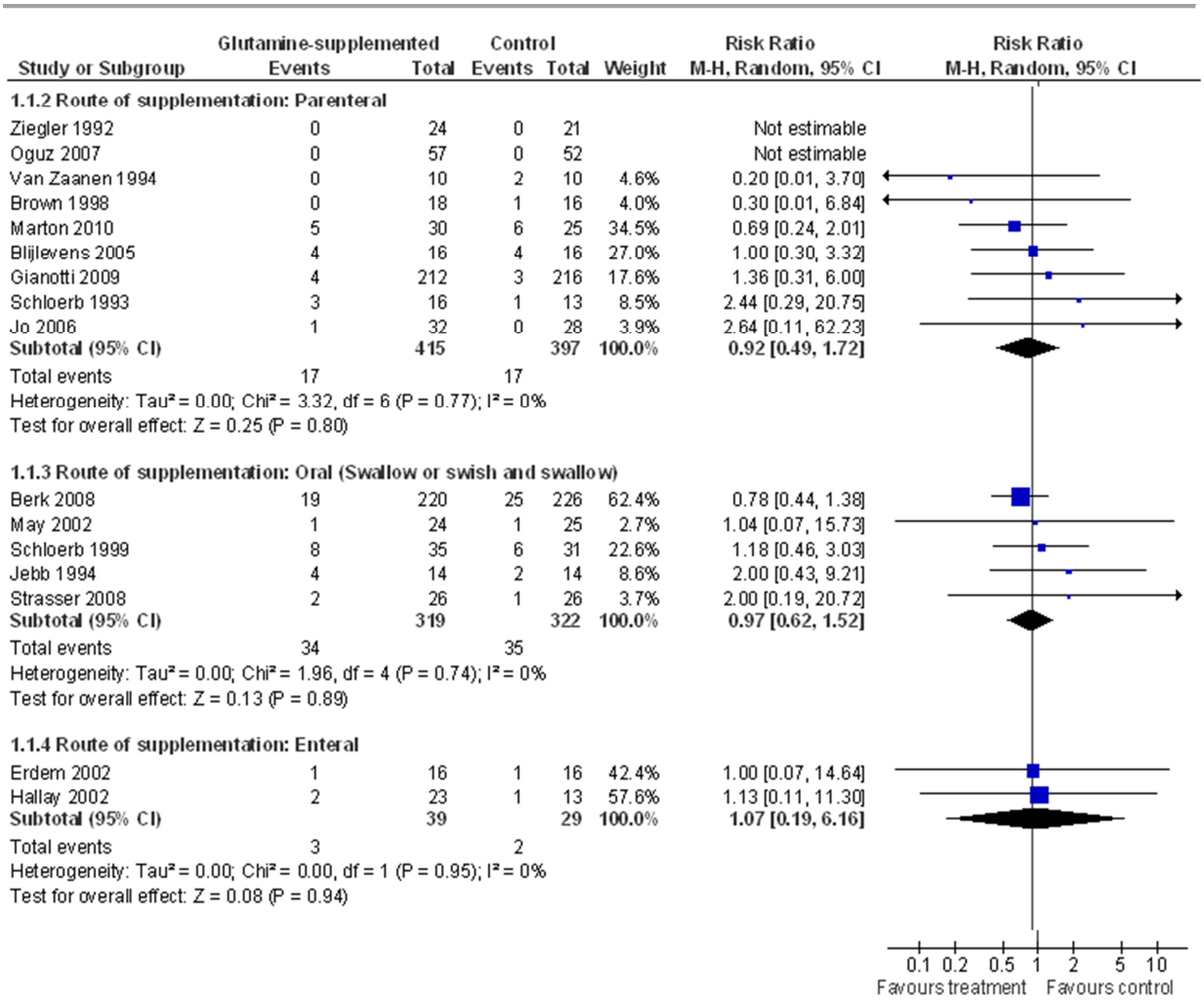
	0	1	2	3	4	5
Scoring system for patient-reported symptoms. "Stool consistency" (1-5)[102,104]	Normal stools[104]	Soft but formed stools[104]	Unformed stools[104]	Watery stools[104]	Watery and blood-stained stools[104]	-
	<u>Patient diary:</u>[102] Number of stools; consistency of stools (normal; soft; watery) Presence of faecal blood (yes/no) Abdominal pain (yes/no) Number of loperamide tablets (2 mg)					
Common toxicity criteria of the National Cancer Institute, Bethesda, Maryland (NCI)[95,102,113,119]	None[95] (No diarrhoea or increased stool frequency)[119]	Increase of 2-3 (or < 4) stools/d compared to pre-therapy[95] (over pretreatment)[119]	Increase of 4-6 stools/d or nocturnal stools[95,119]	Increase of 7-9 stools/d or incontinence[95,119] (or need for parenteral support for dehydration)[102]	Increase of ≥ 10 stools/d or grossly bloody diarrhoea, or need for parenteral support[95,119] for dehydration (Physiological consequences requiring intensive care of haemodynamic collapse)[102]	-(Diarrhoea resulting in the death of the patient)[119]
Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0)[122,167]	-	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline[167]	Increase of 4 – 6 stools per day over baseline; IV fluids indicated <24 hrs; moderate increase in ostomy output compared to baseline; not interfering with ADL[167]	Increase of ≥7 stools per day over baseline; incontinence; IV fluids ≥24 hrs; hospitalization; severe increase in ostomy output compared to baseline; interfering with ADL[167]	Life-threatening consequences (e.g. hemodynamic collapse)[167]	Death[167]
Diarrhoea[98]	-	> 3 loose stools daily	-	-	-	-
Diarrhoea[96]			> 4 loose stools per day			
World Health Organization (WHO) classification (Grade 0-4)[48,106,120]						
Gastrointestinal toxicity classified according to Northern California Oncology Group (NCOG)[153]						
Stool output of volume > 500 ml in 24-hour period was notable diarrhoea[97]						
Unclear[29,112]						

APPENDIX 6.10: Subgroup analysis

Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.1: Mortality during intervention – Number of deaths during intervention

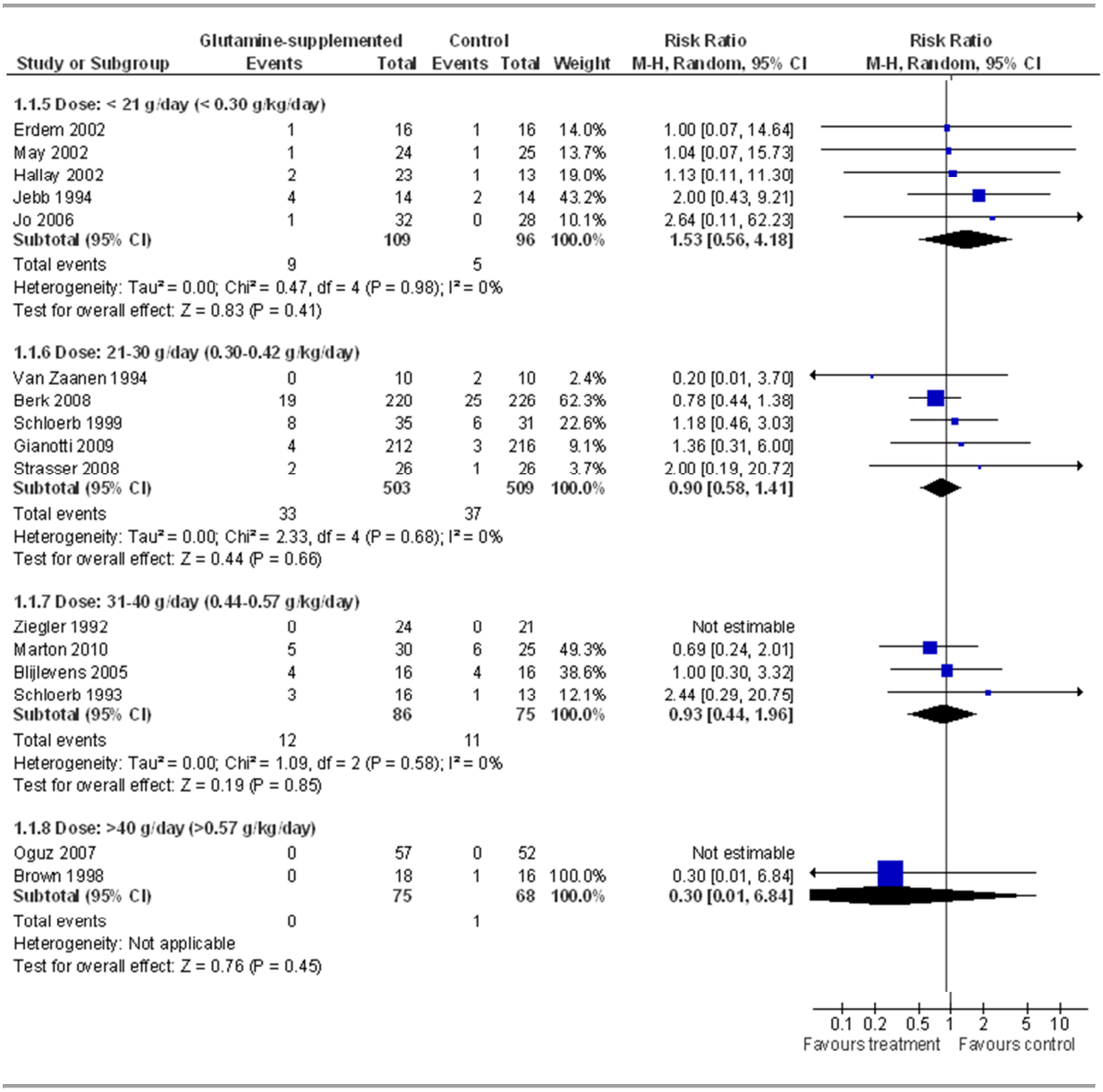
Subgroup analysis



Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.1: Mortality during intervention – Number of deaths during intervention

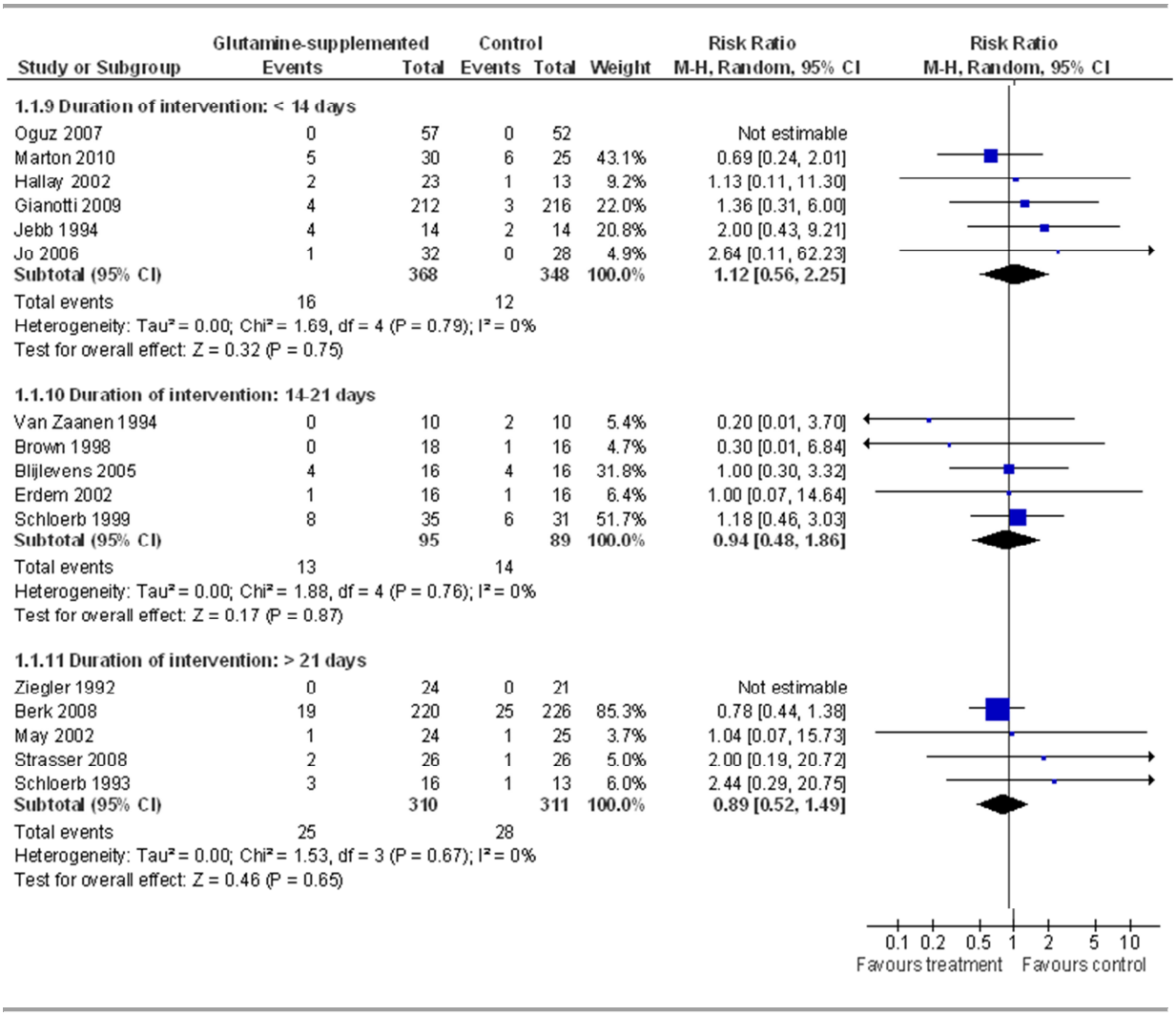
Subgroup analysis



Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.1: Mortality during intervention – Number of deaths during intervention

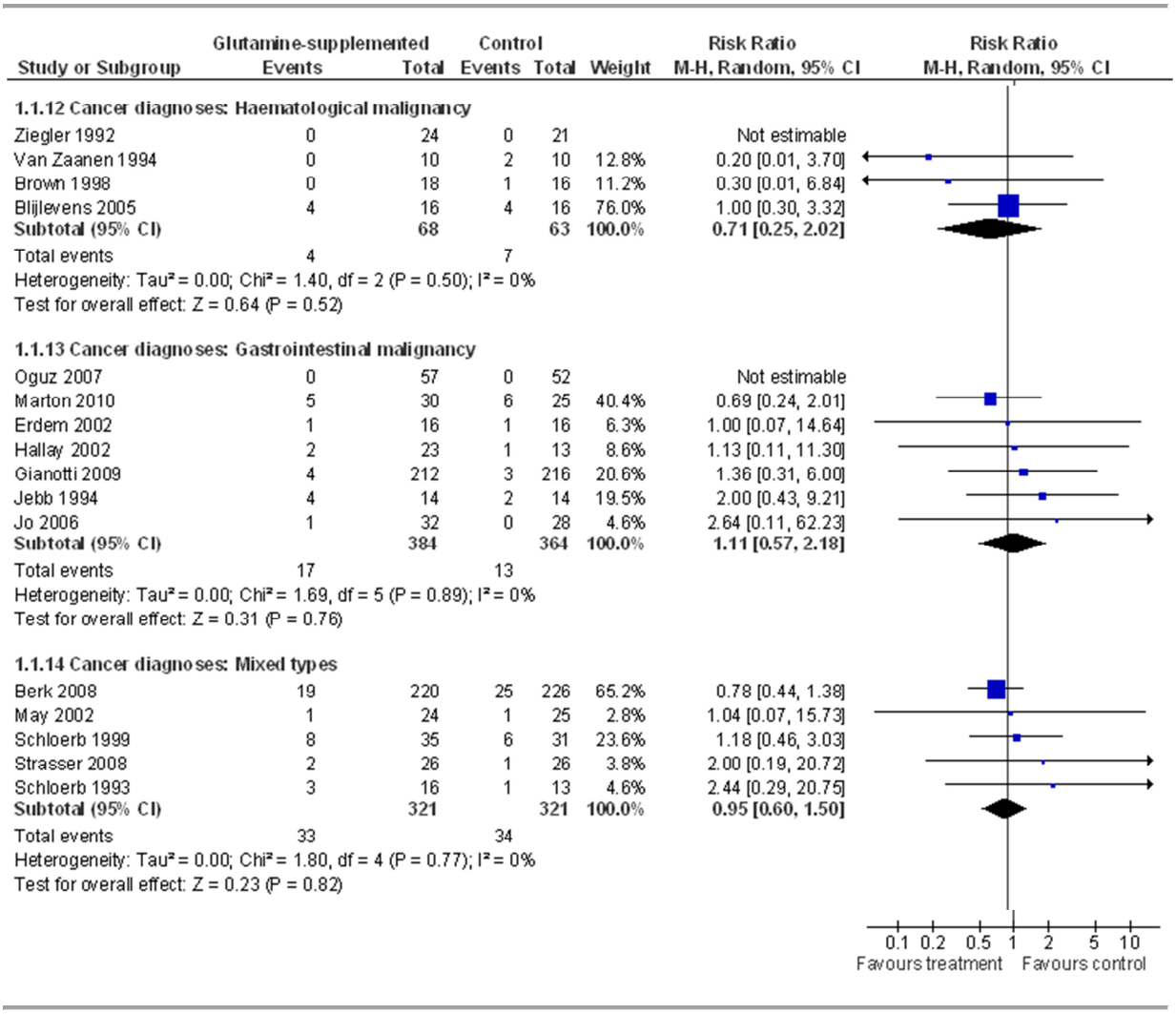
Subgroup analysis



Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.1: Mortality during intervention – Number of deaths during intervention

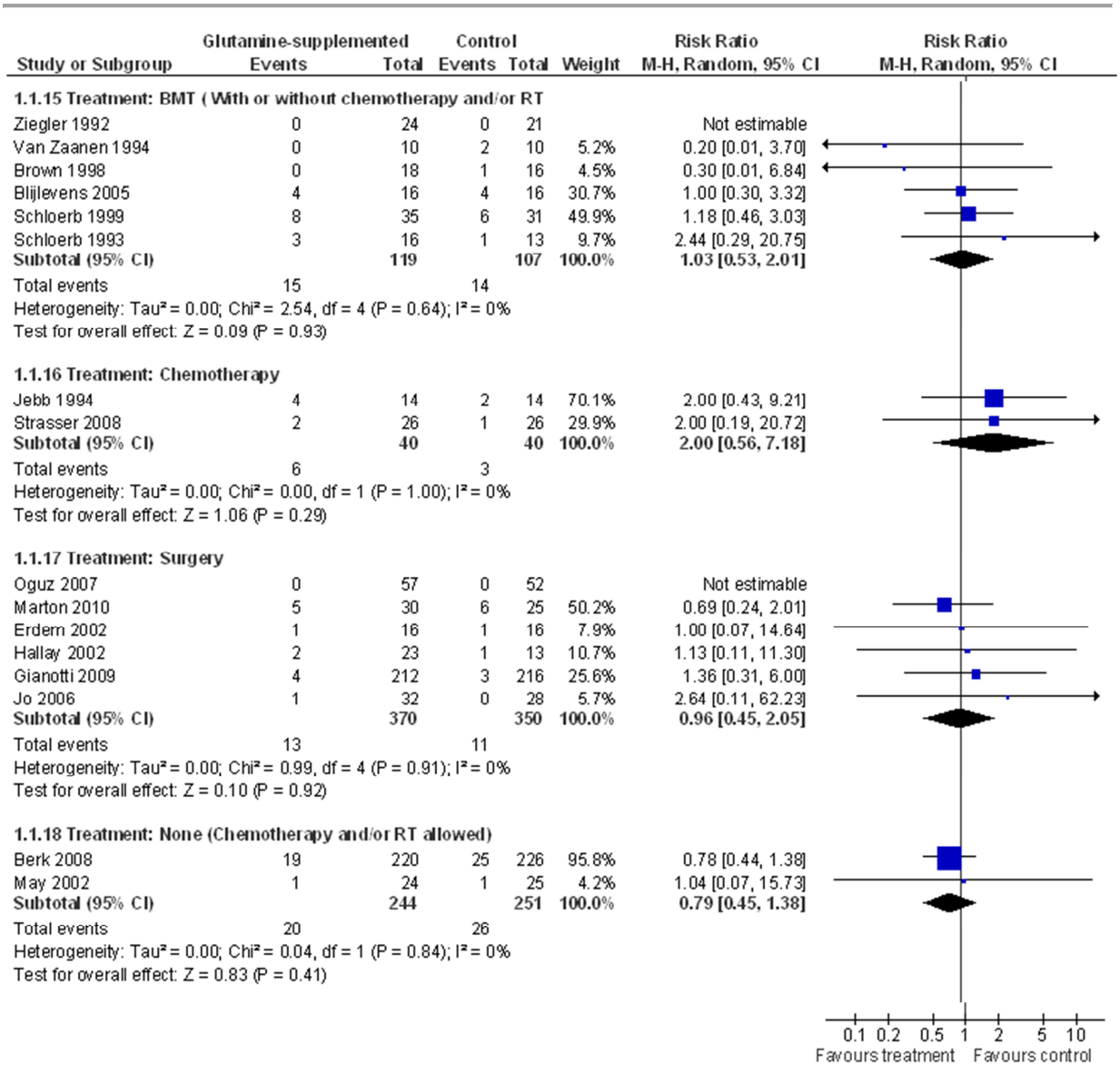
Subgroup analysis



Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.1: Mortality during intervention – Number of deaths during intervention

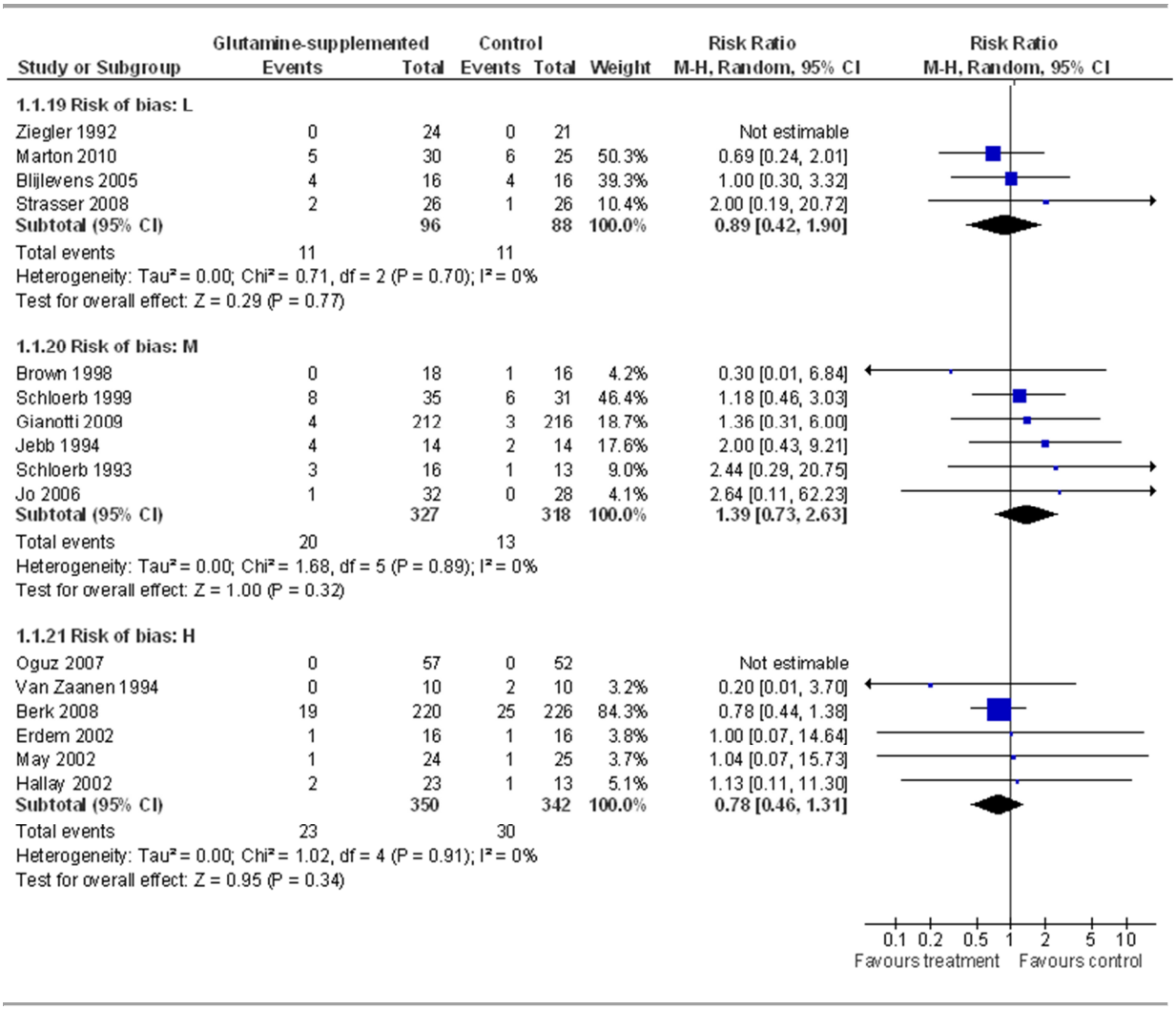
Subgroup analysis



Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.1: Mortality during intervention – Number of deaths during intervention

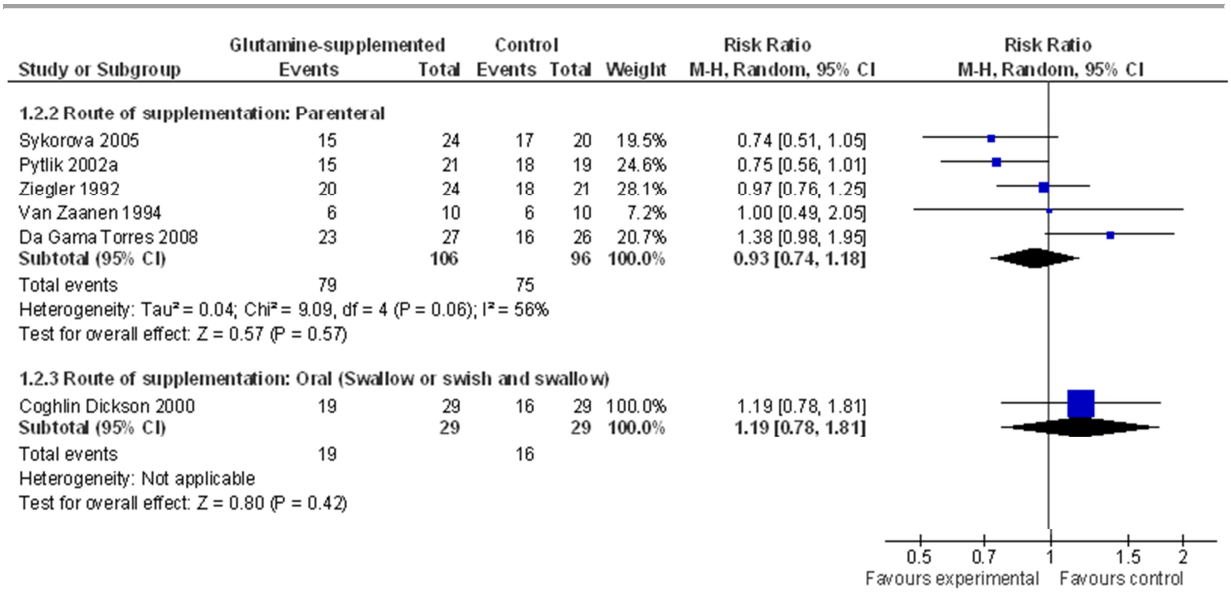
Subgroup analysis

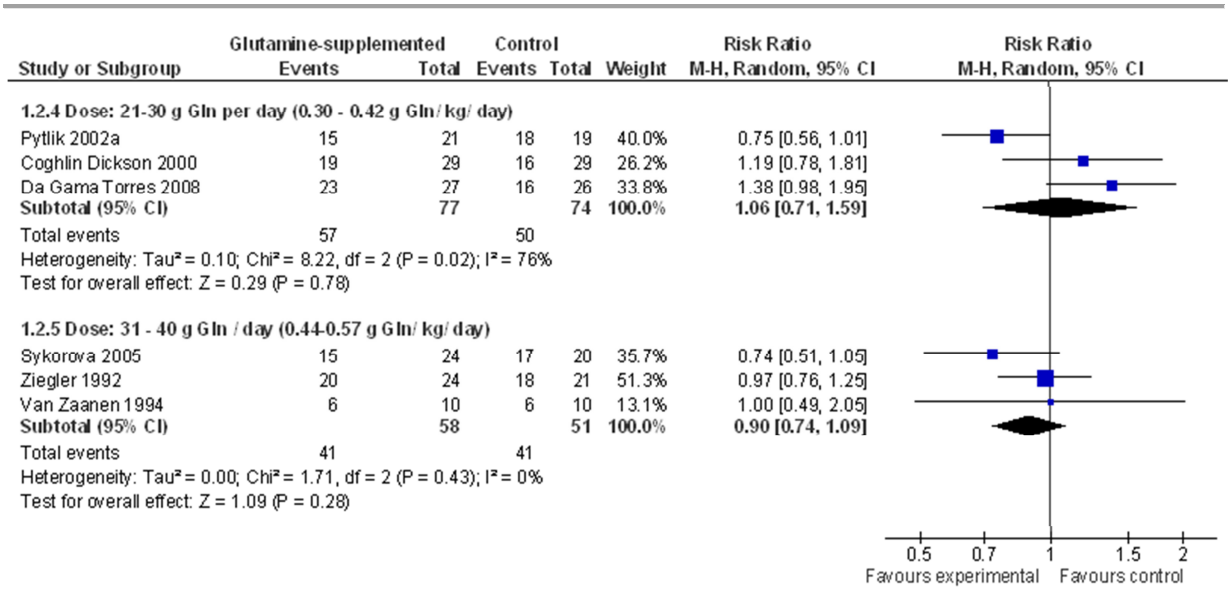


Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.2: Survival at follow-up

Subgroup analysis

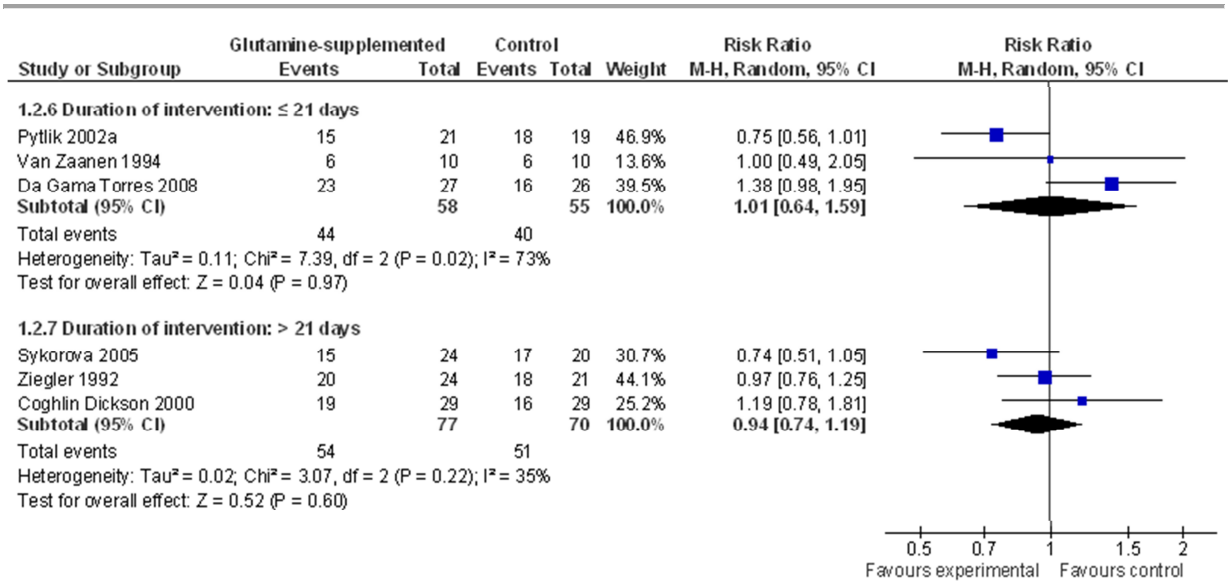


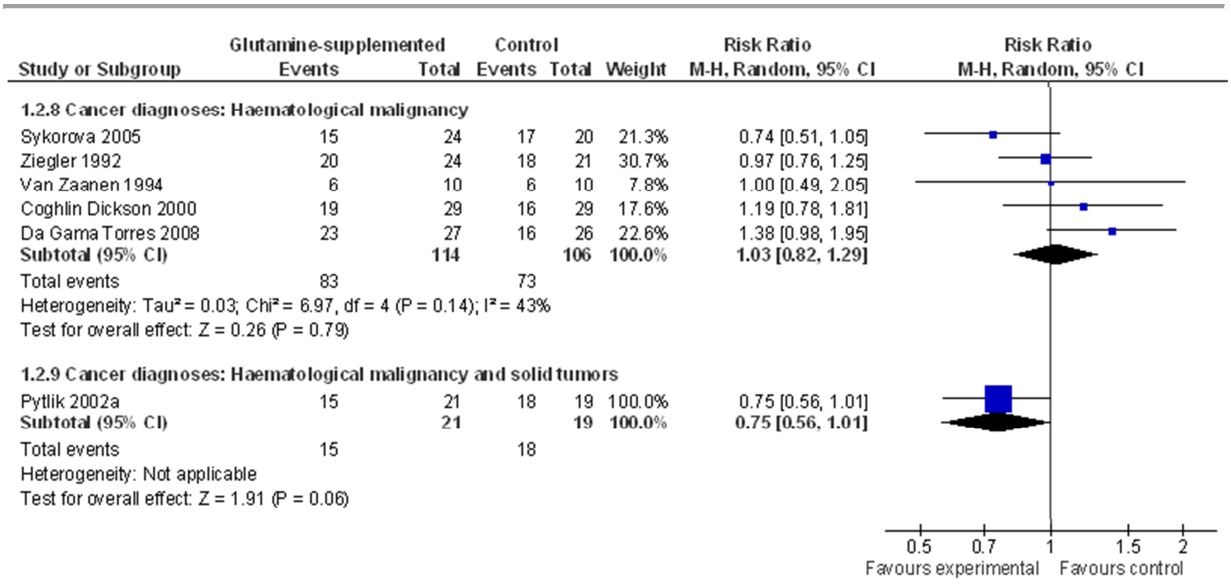


Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.2: Survival at follow-up

Subgroup analysis

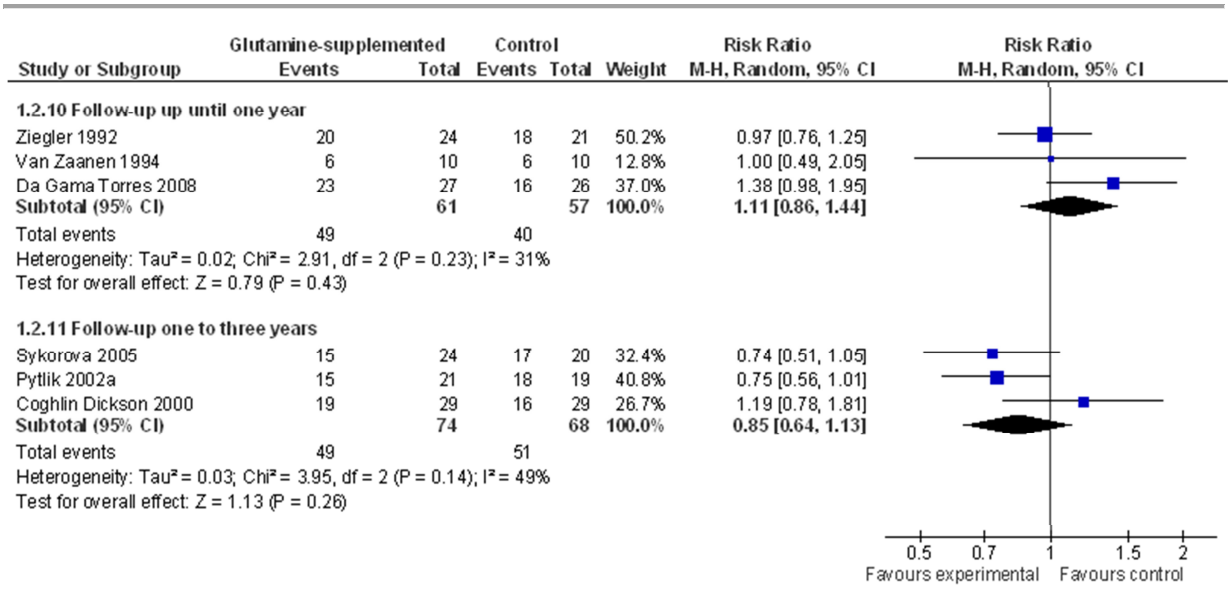


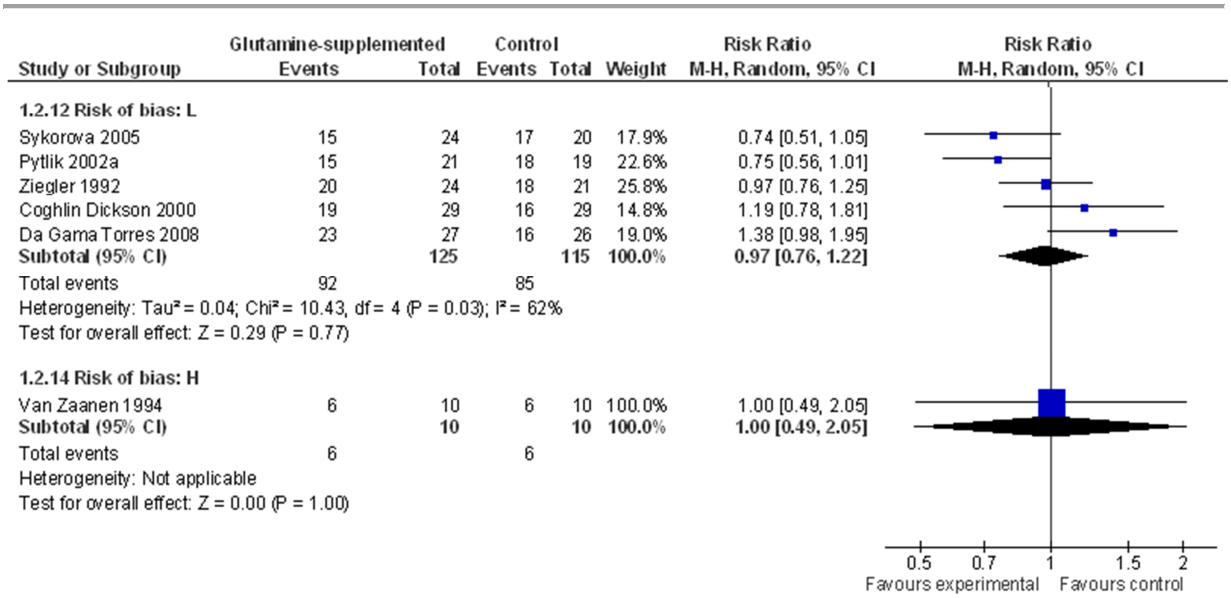


Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.2: Survival at follow-up

Subgroup analysis

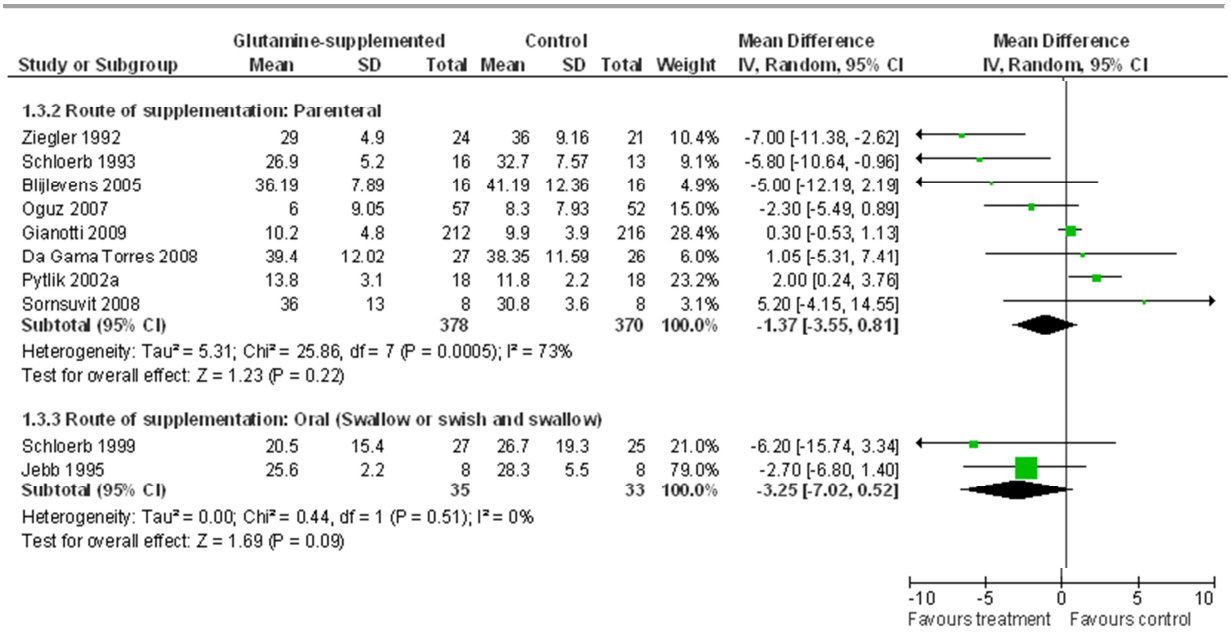


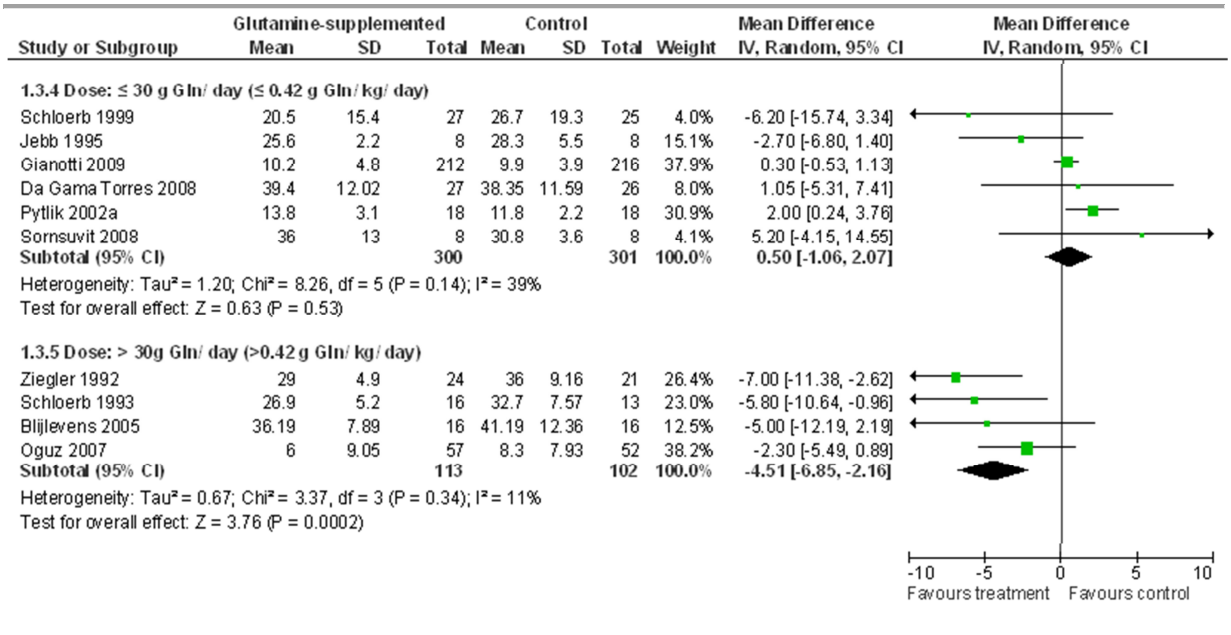


Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.3: Length of hospital stay - Mean duration of hospital stay (days) from admission/day 0 to discharge:

Subgroup analysis

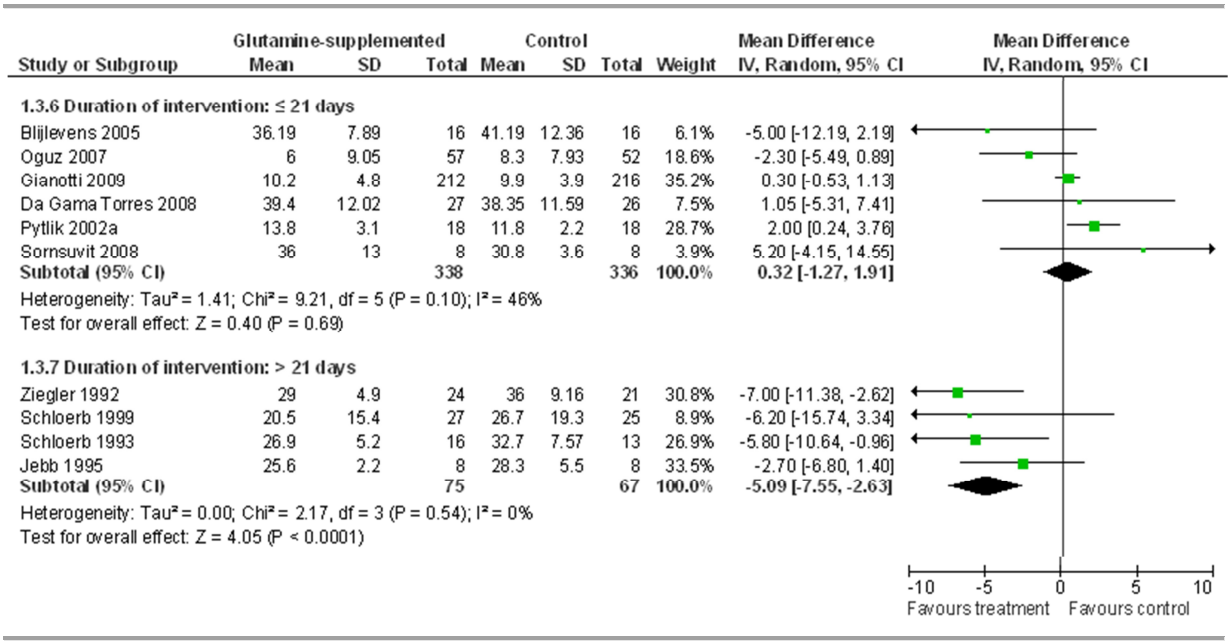


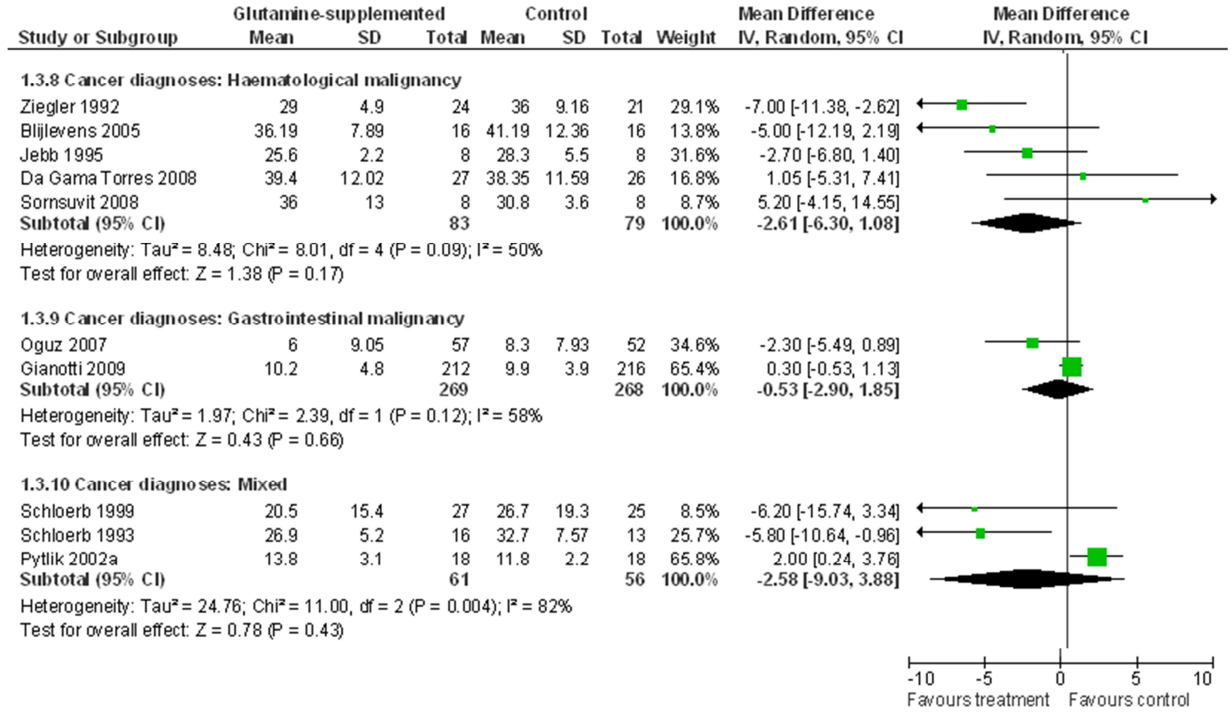


Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.3: Length of hospital stay - Mean duration of hospital stay (days) from admission/day 0 to discharge:

Subgroup analysis

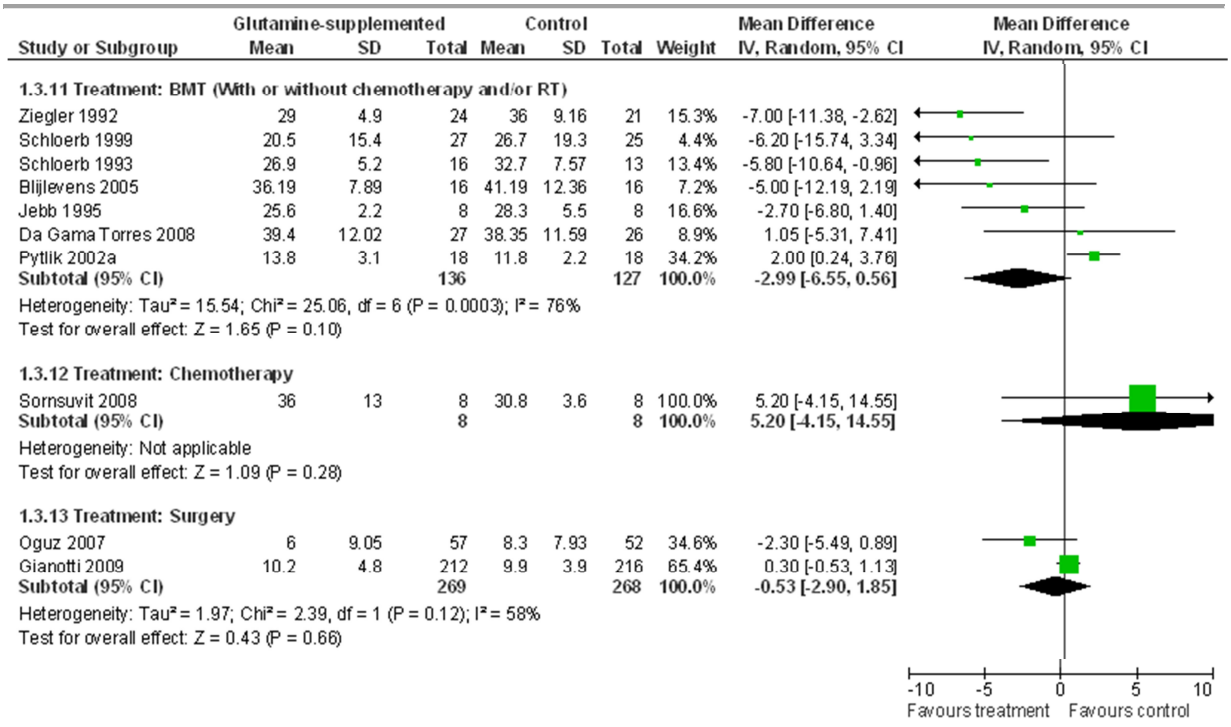


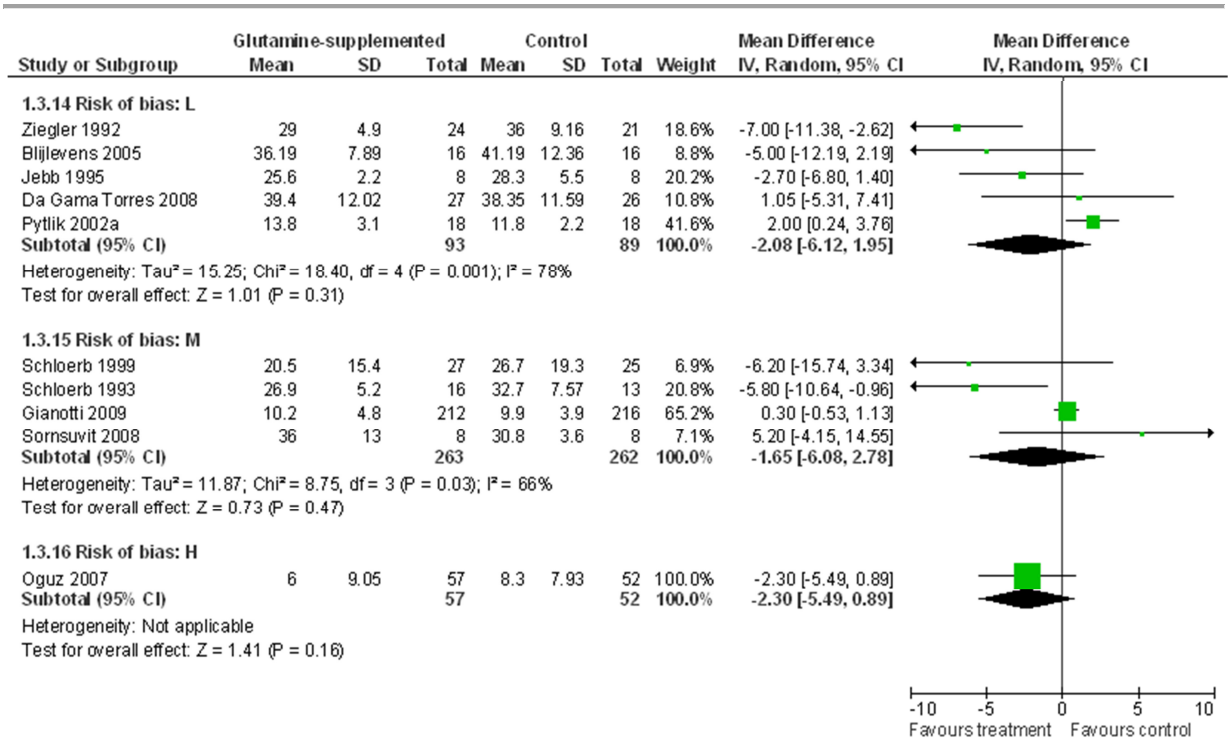


Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.3: Length of hospital stay - Mean duration of hospital stay (days) from admission/day 0 to discharge:

Subgroup analysis

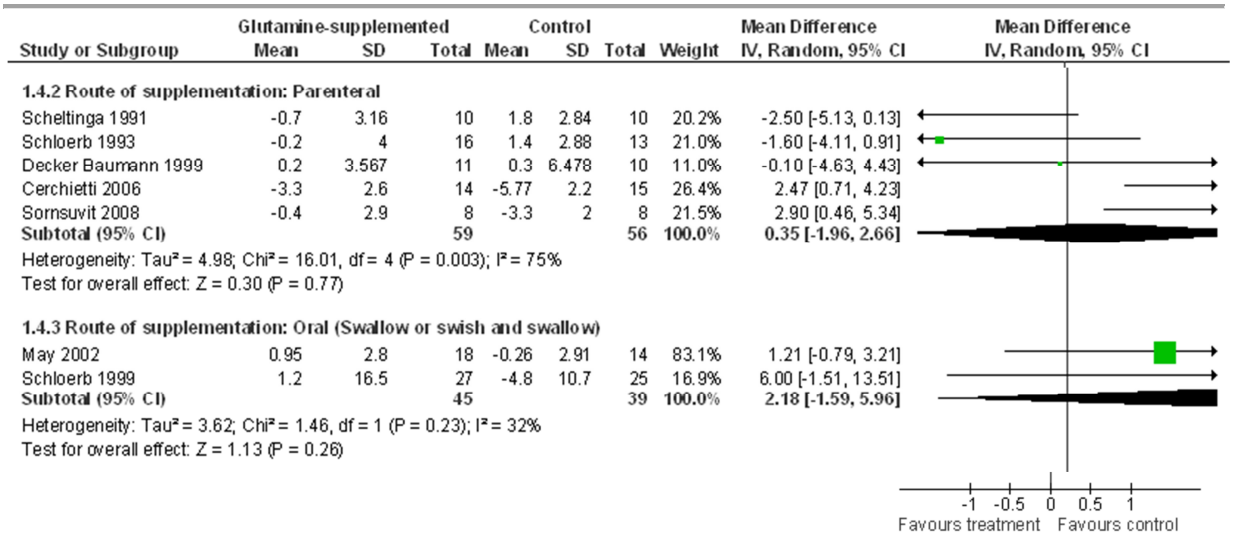


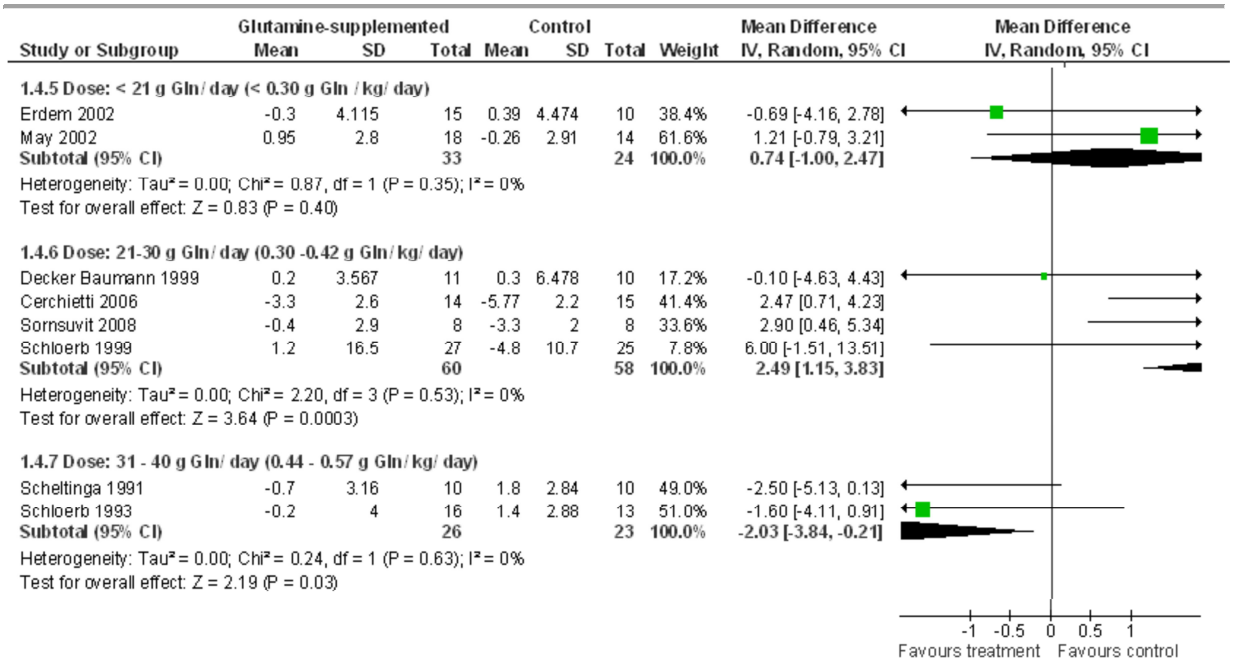


Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.4: Nutritional status: Body weight change – Difference in mean change in body weight (kg) from baseline to end of study between groups

Subgroup analysis

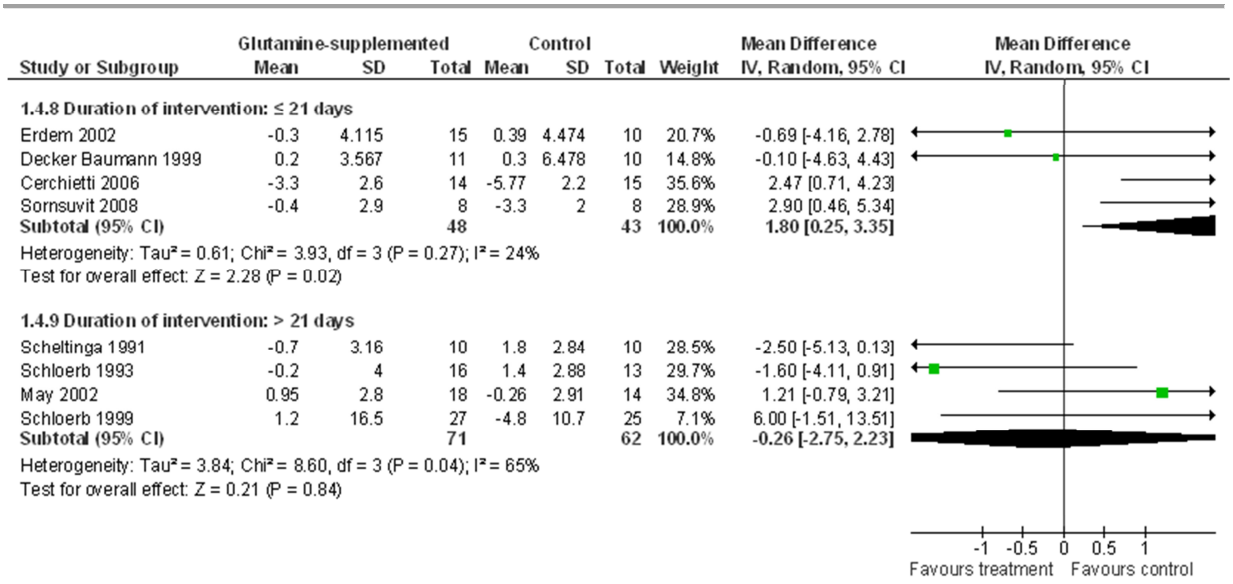


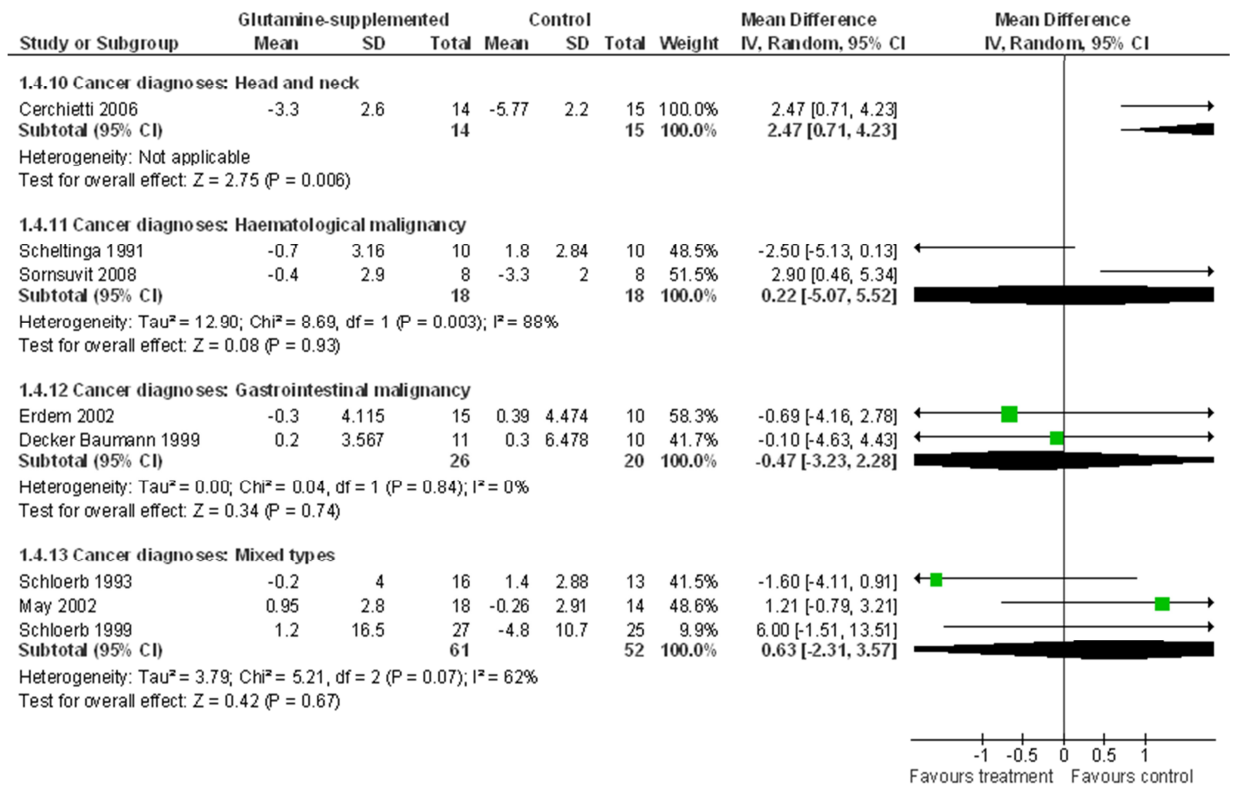


Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.4: Nutritional status: Body weight change – Difference in mean change in body weight (kg) from baseline to end of study between groups

Subgroup analysis

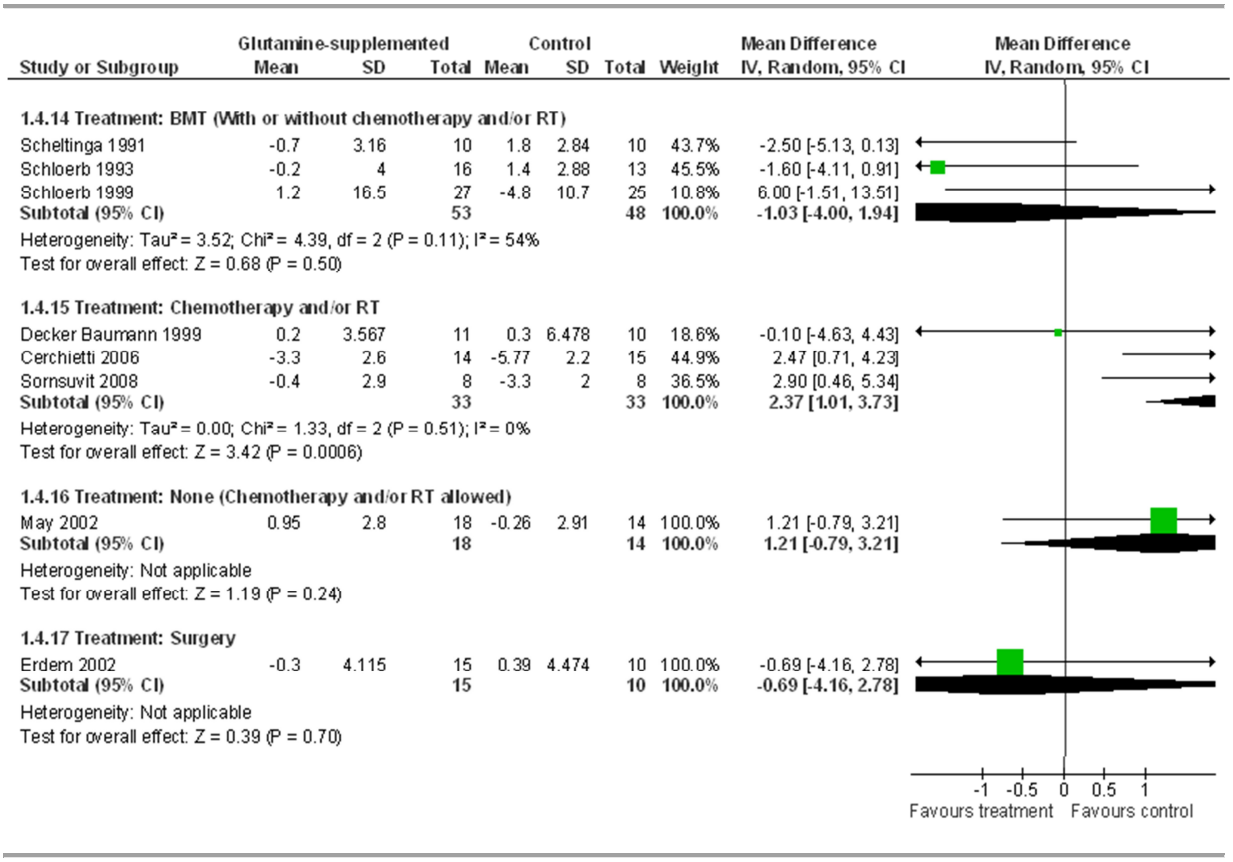




Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.4: Nutritional status: Body weight change – Difference in mean change in body weight (kg) from baseline to end of study between groups

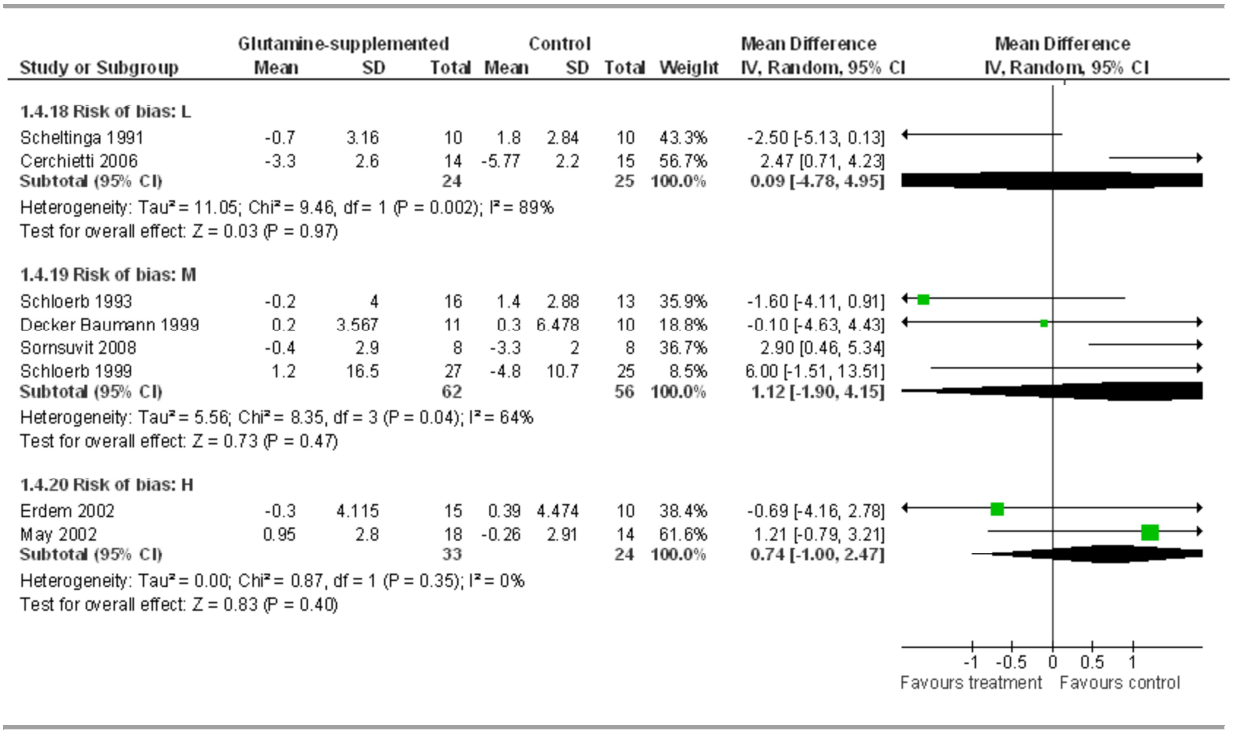
Subgroup analysis



Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.4: Nutritional status: Body weight change – Difference in mean change in body weight (kg) from baseline to end of study between groups

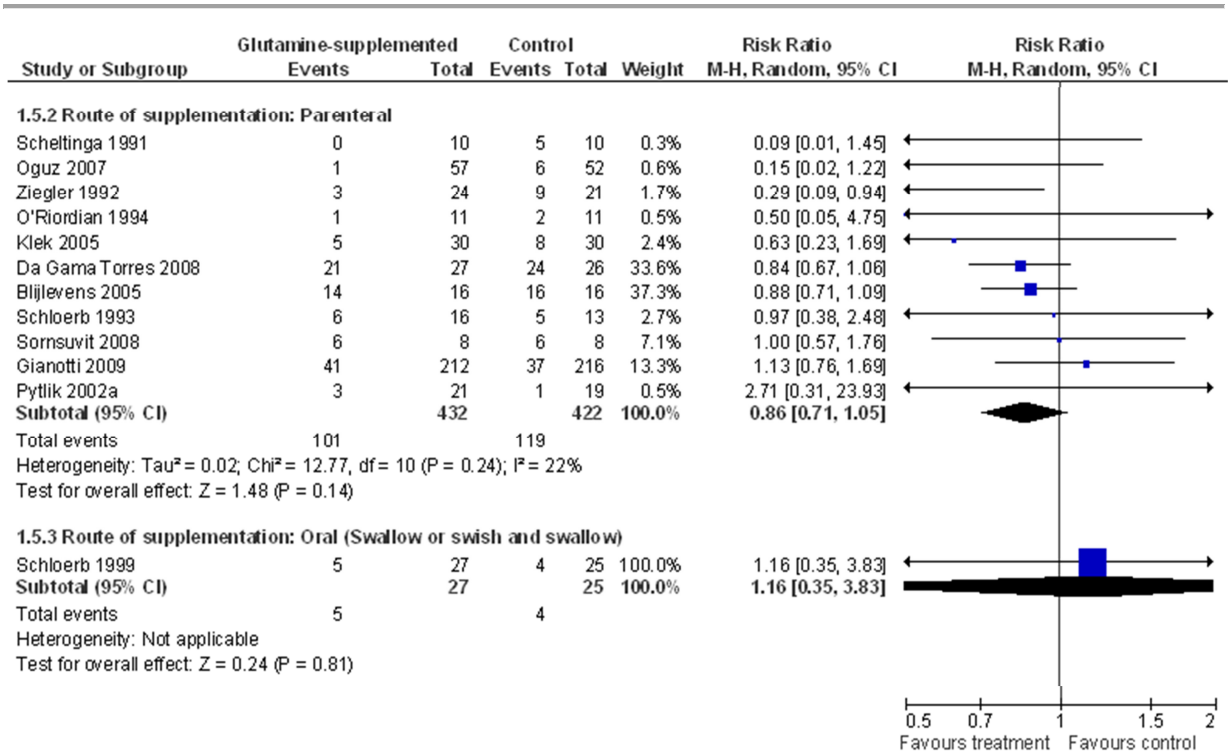
Subgroup analysis

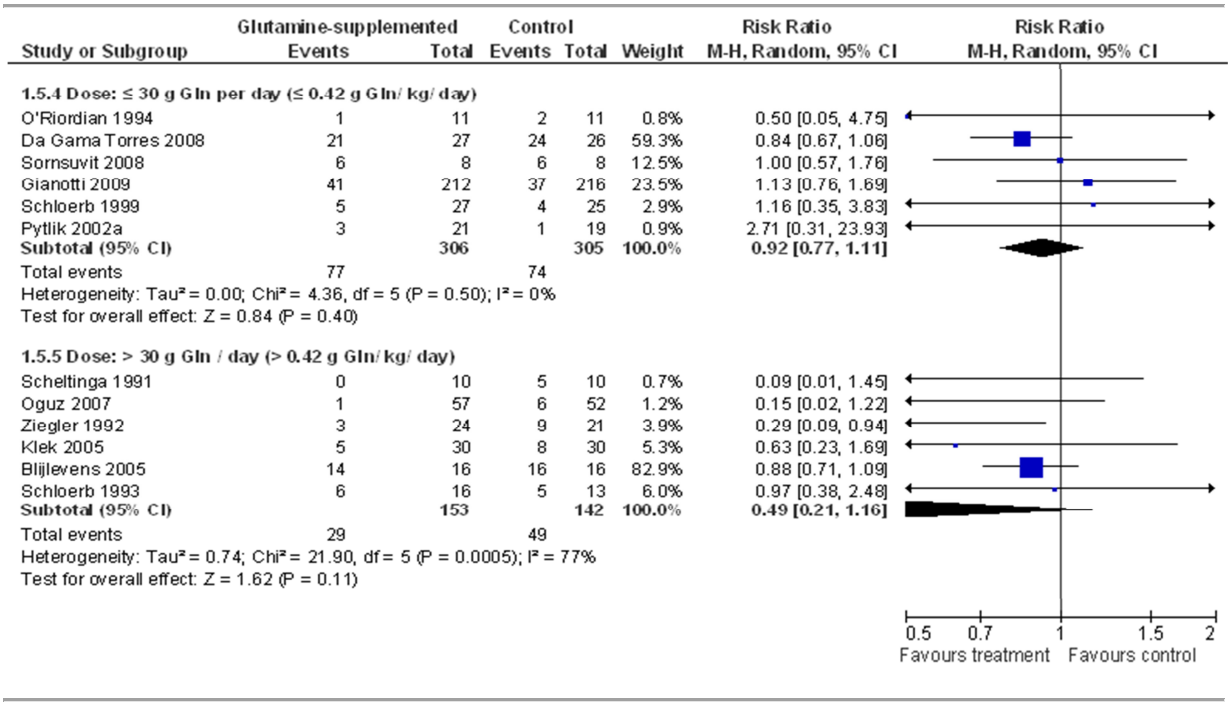


Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.5: Number of patients who developed clinical infection during intervention

Subgroup analysis

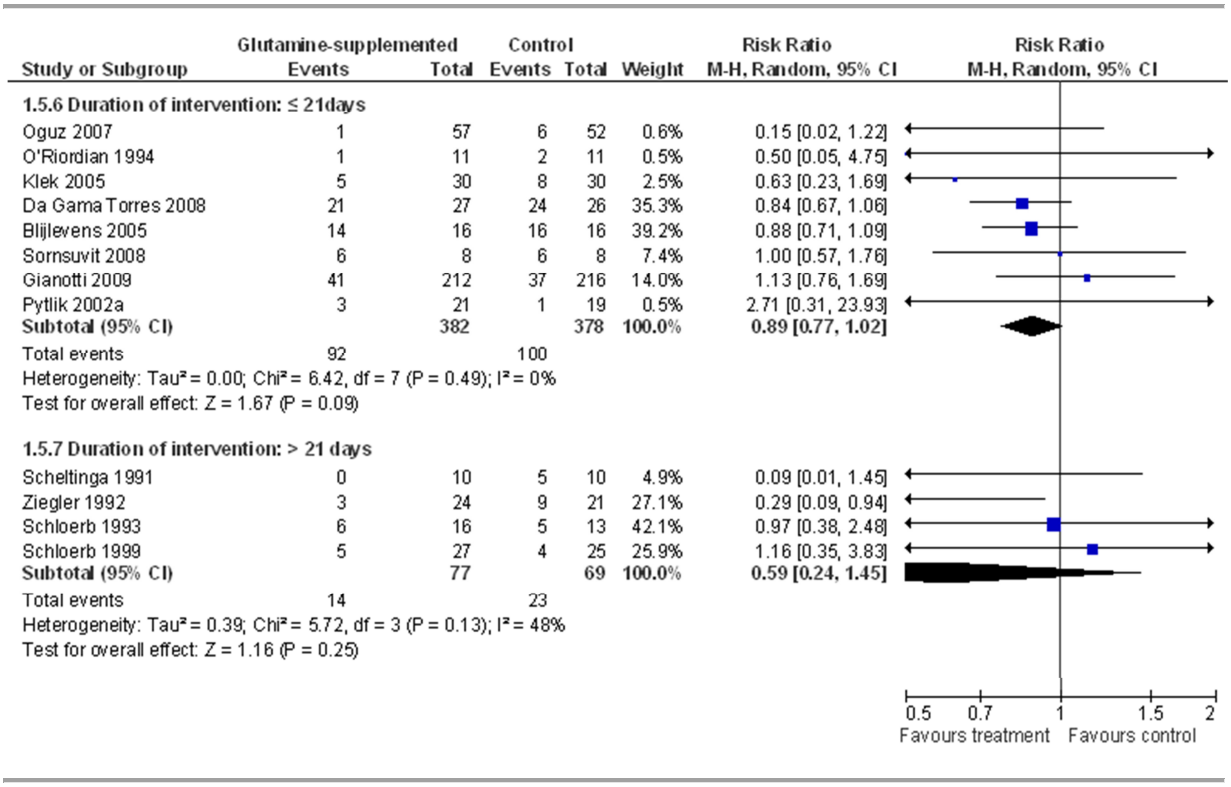




Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.5: Number of patients who developed clinical infection during intervention

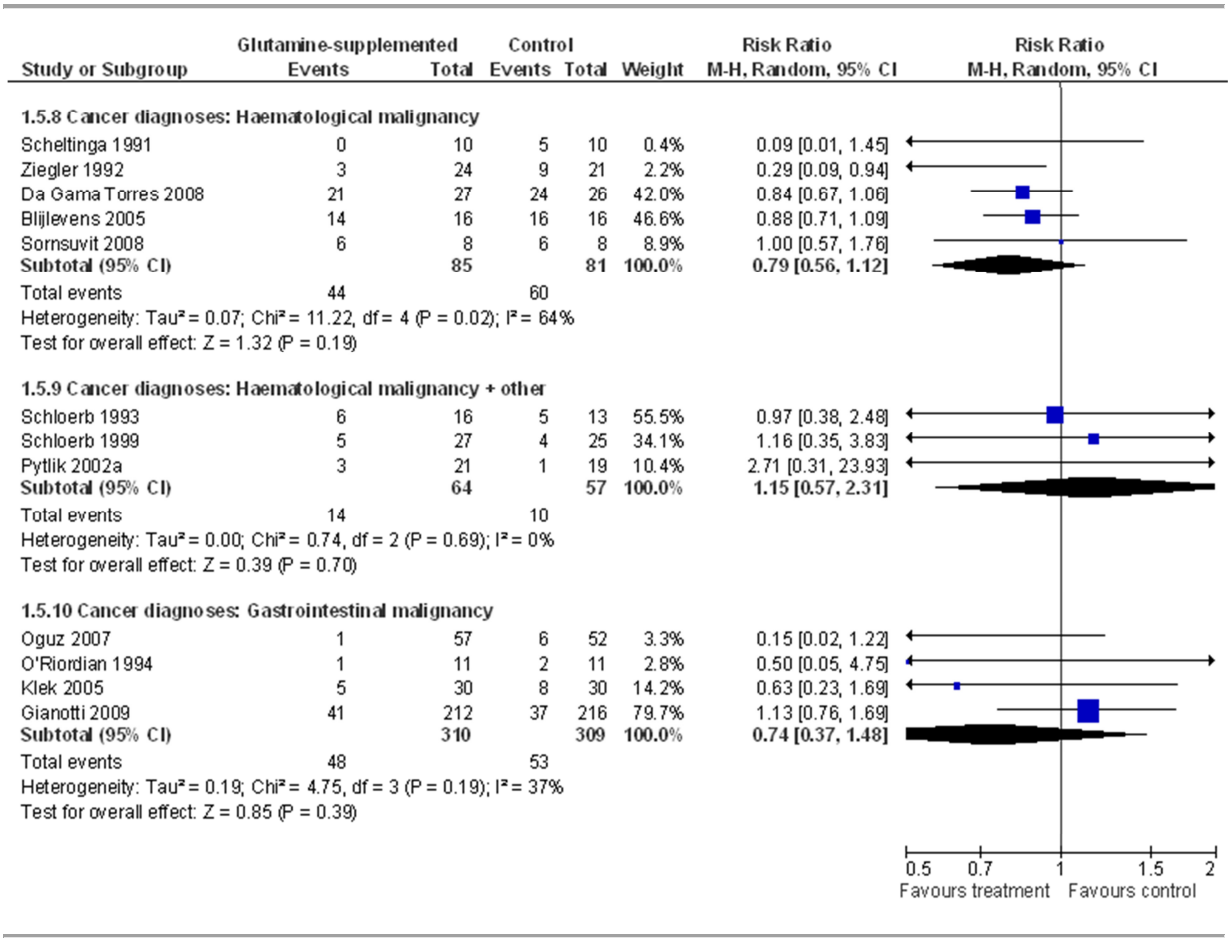
Subgroup analysis



Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.5: Number of patients who developed clinical infection during intervention

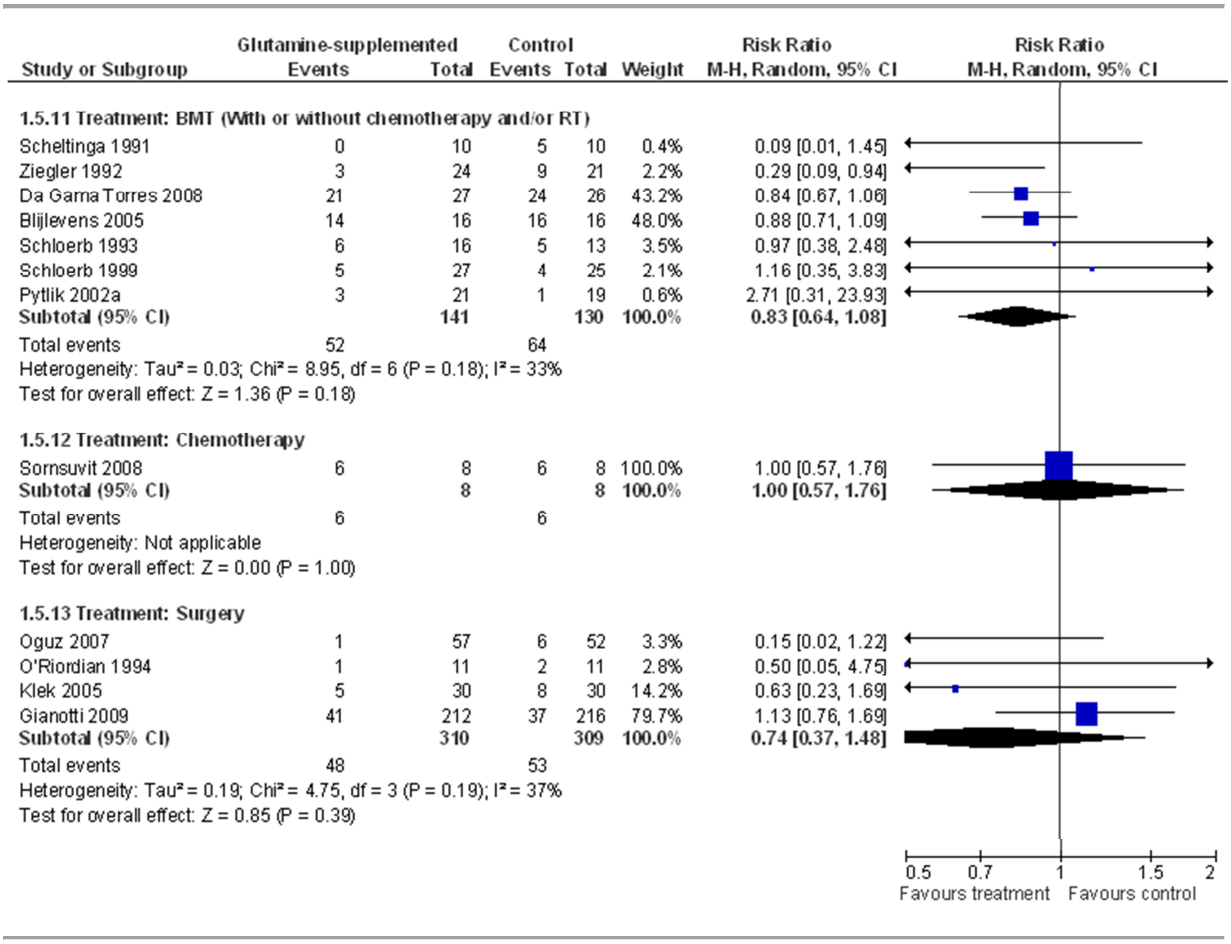
Subgroup analysis



Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.5: Number of patients who developed clinical infection during intervention

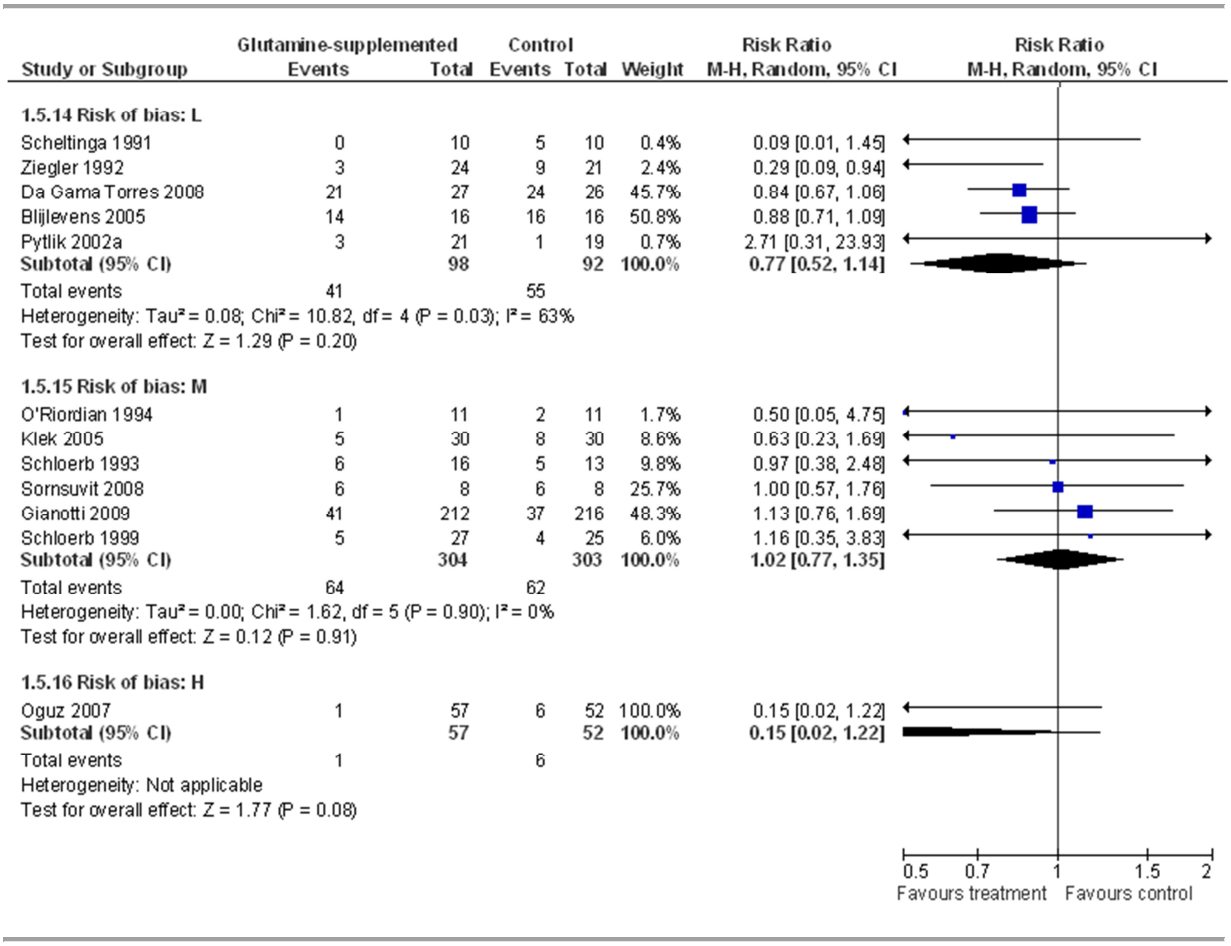
Subgroup analysis



Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.5: Number of patients who developed clinical infection during intervention

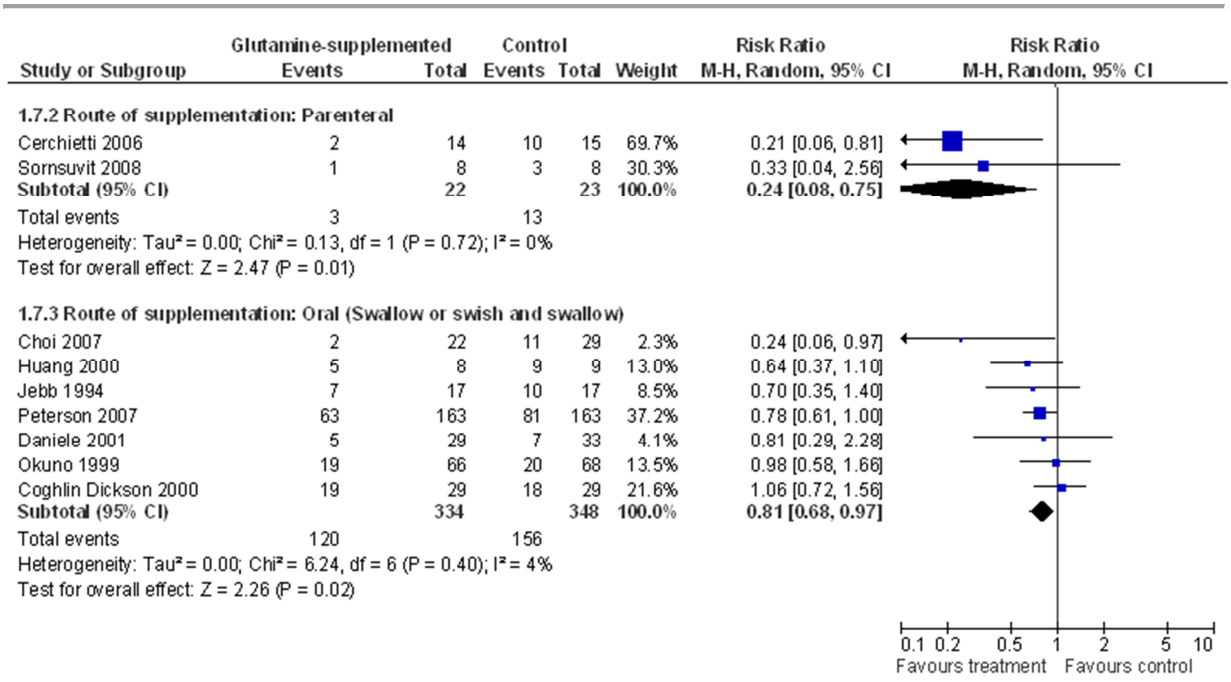
Subgroup analysis

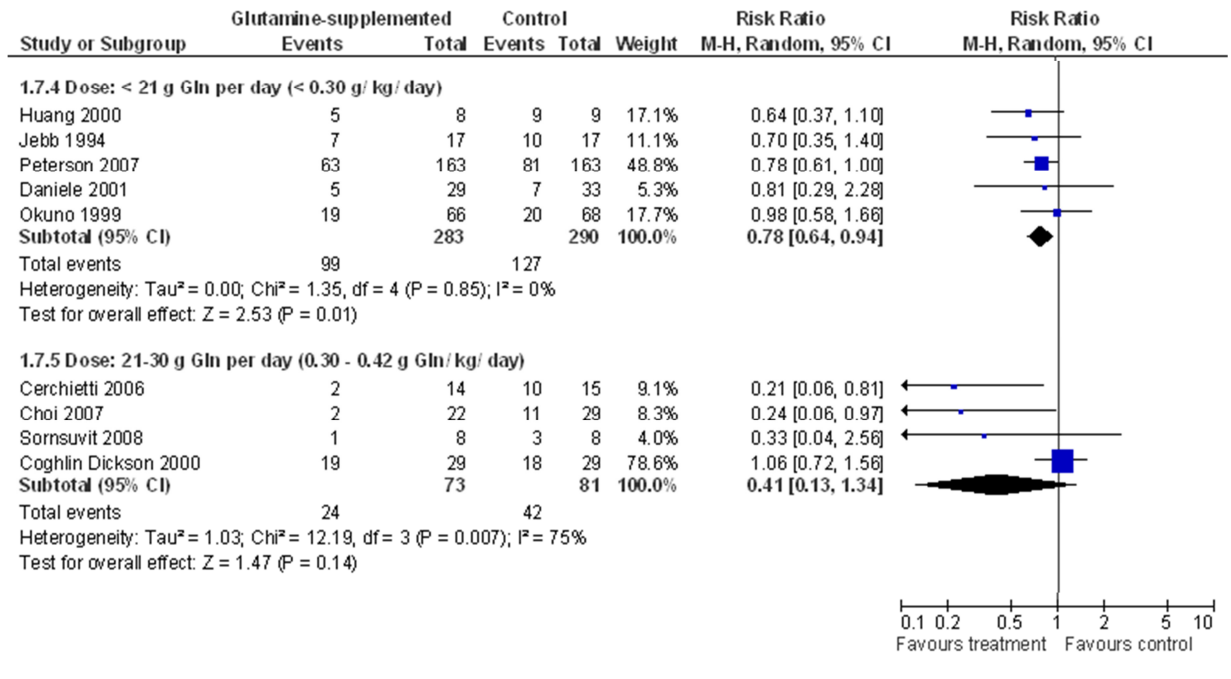


Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.7. Mucositis: Number of patients with ≥ grade 2

Subgroup analysis

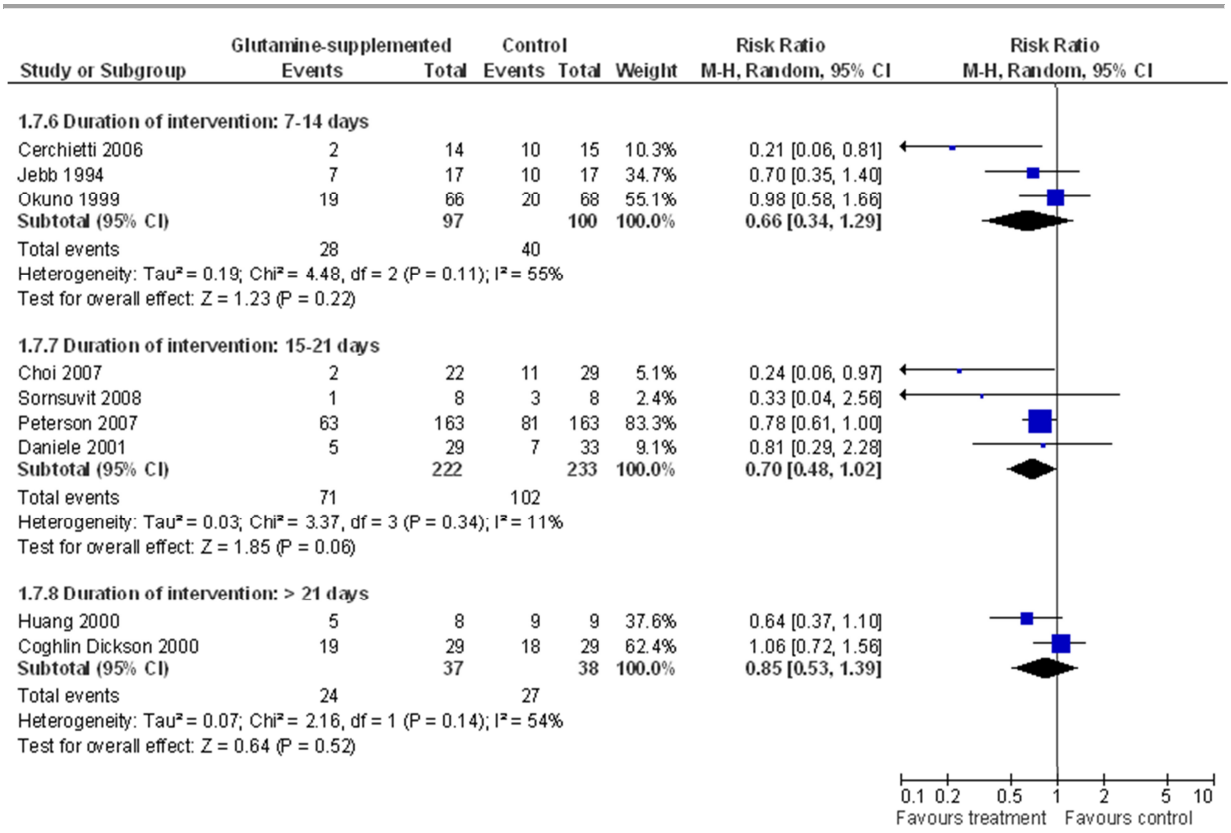




Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.7. Mucositis: Number of patients with ≥ grade 2

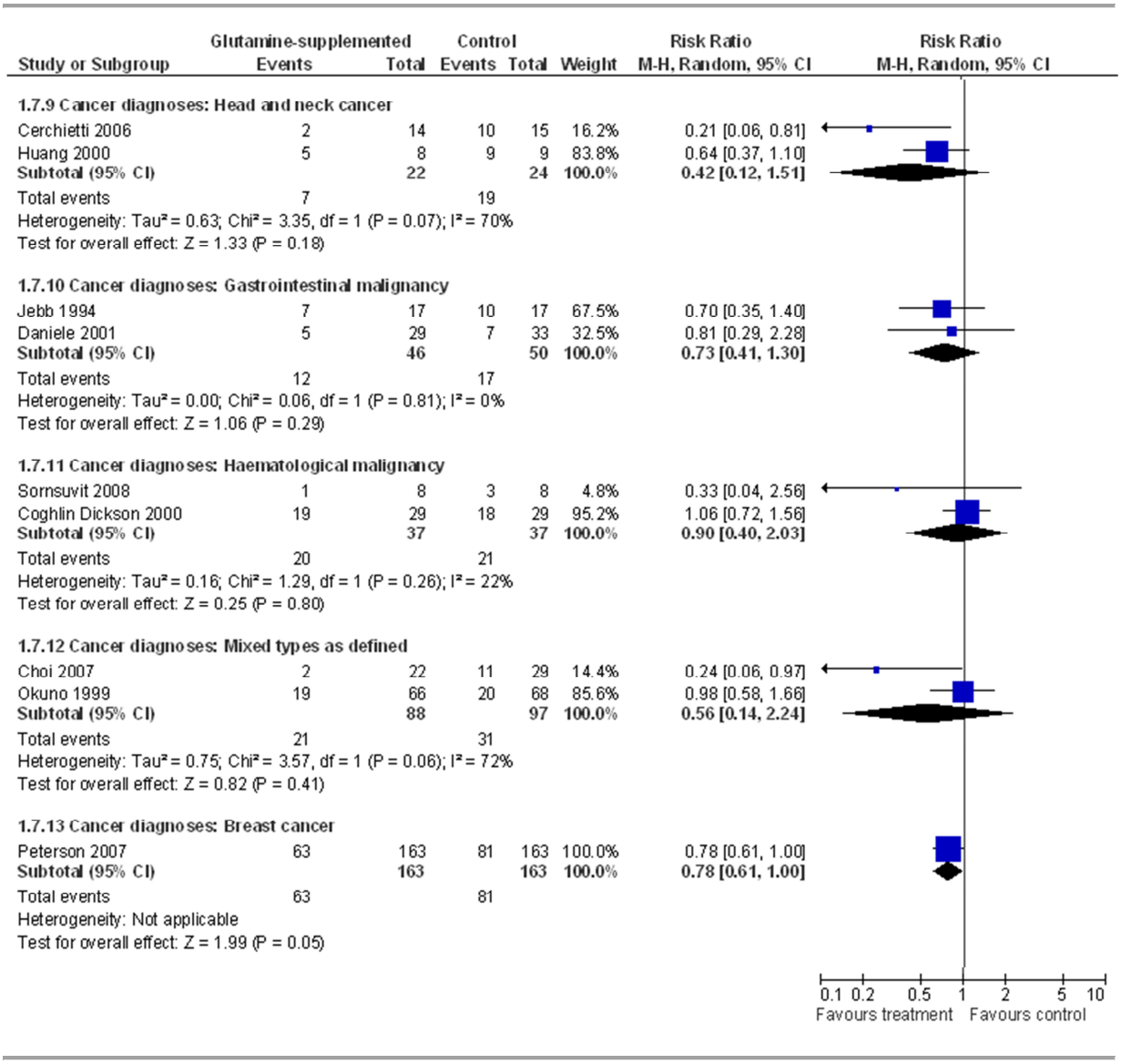
Subgroup analysis



Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.7. Mucositis: Number of patients with ≥ grade 2

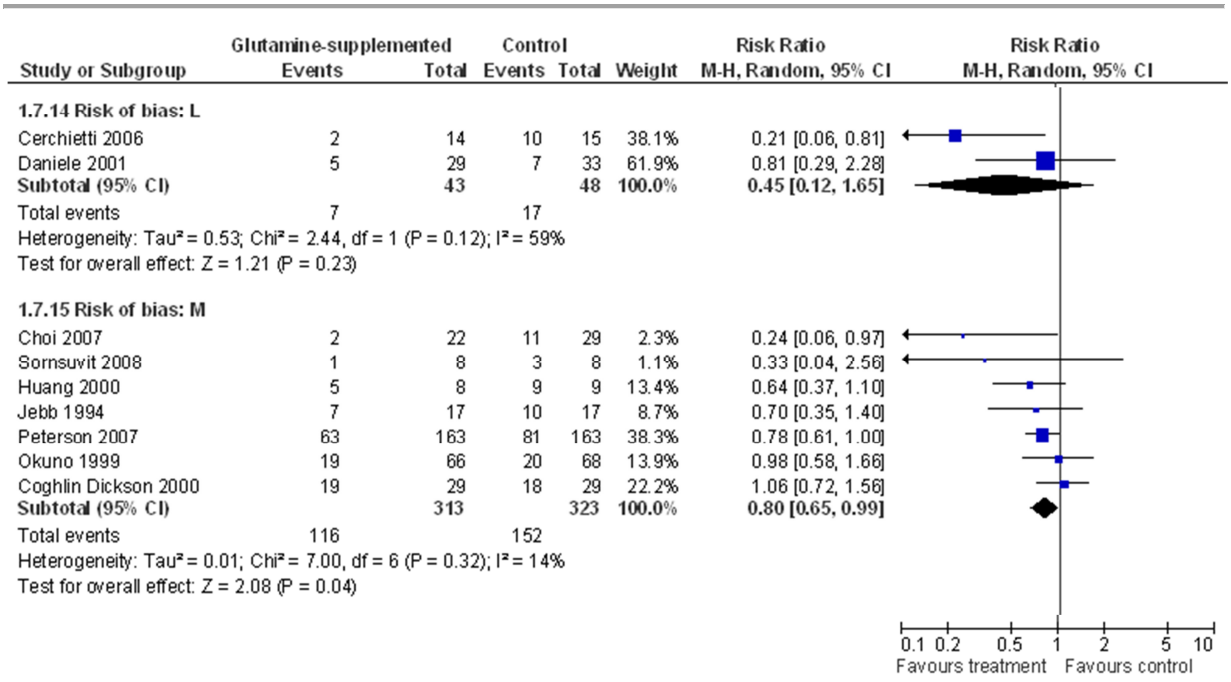
Subgroup analysis



Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.7. Mucositis: Number of patients with ≥ grade 2

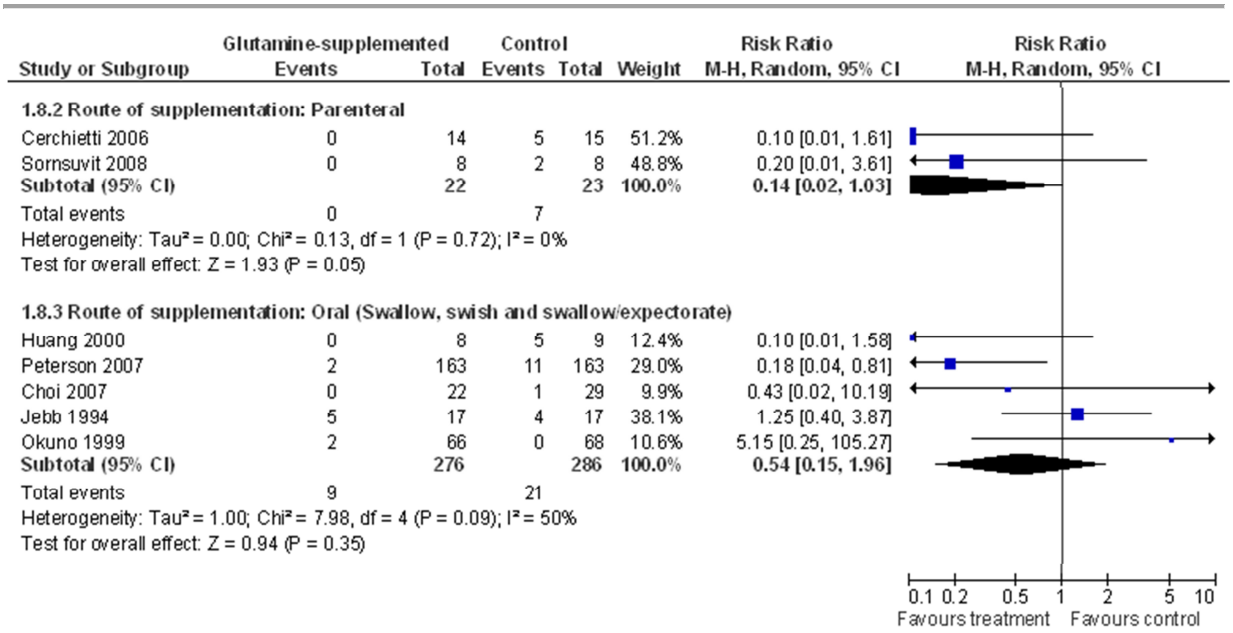
Subgroup analysis

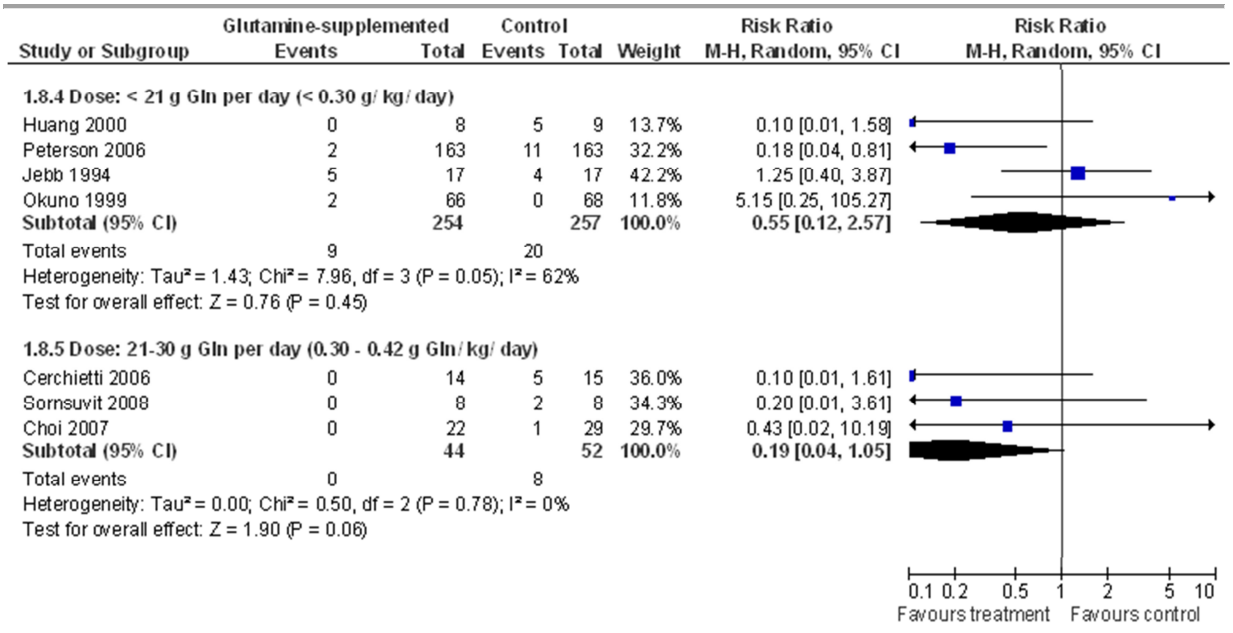


Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.8. Mucositis: Number of patients with maximum grade (3/4)

Subgroup analysis

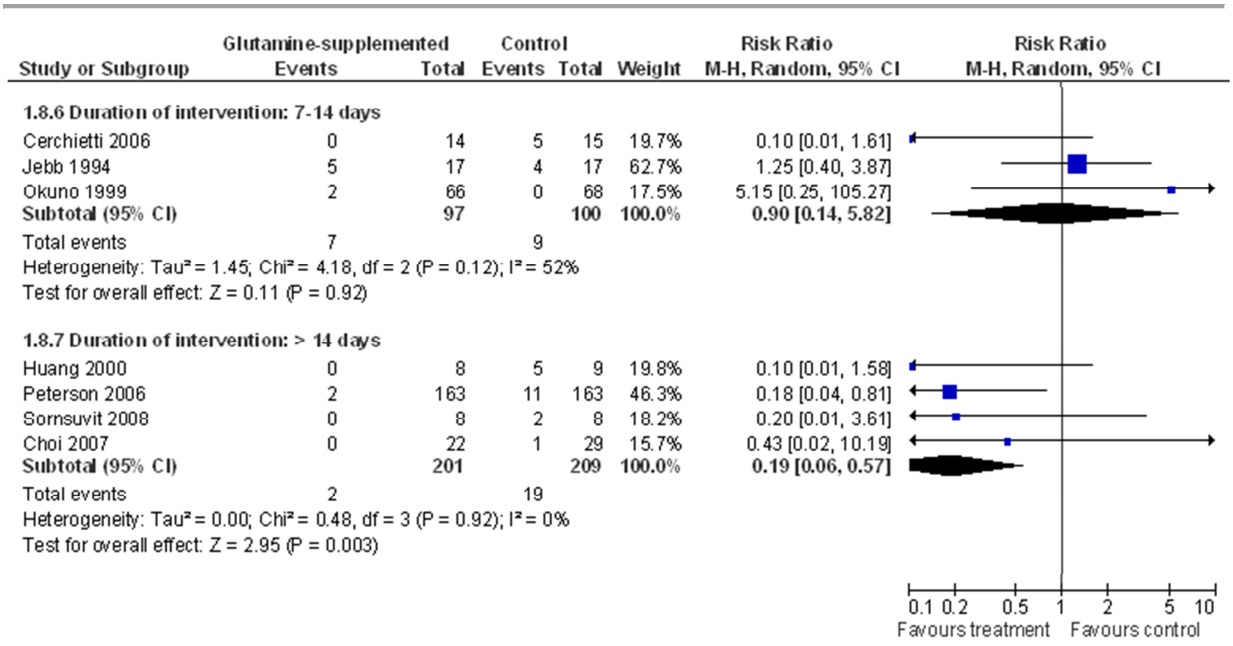


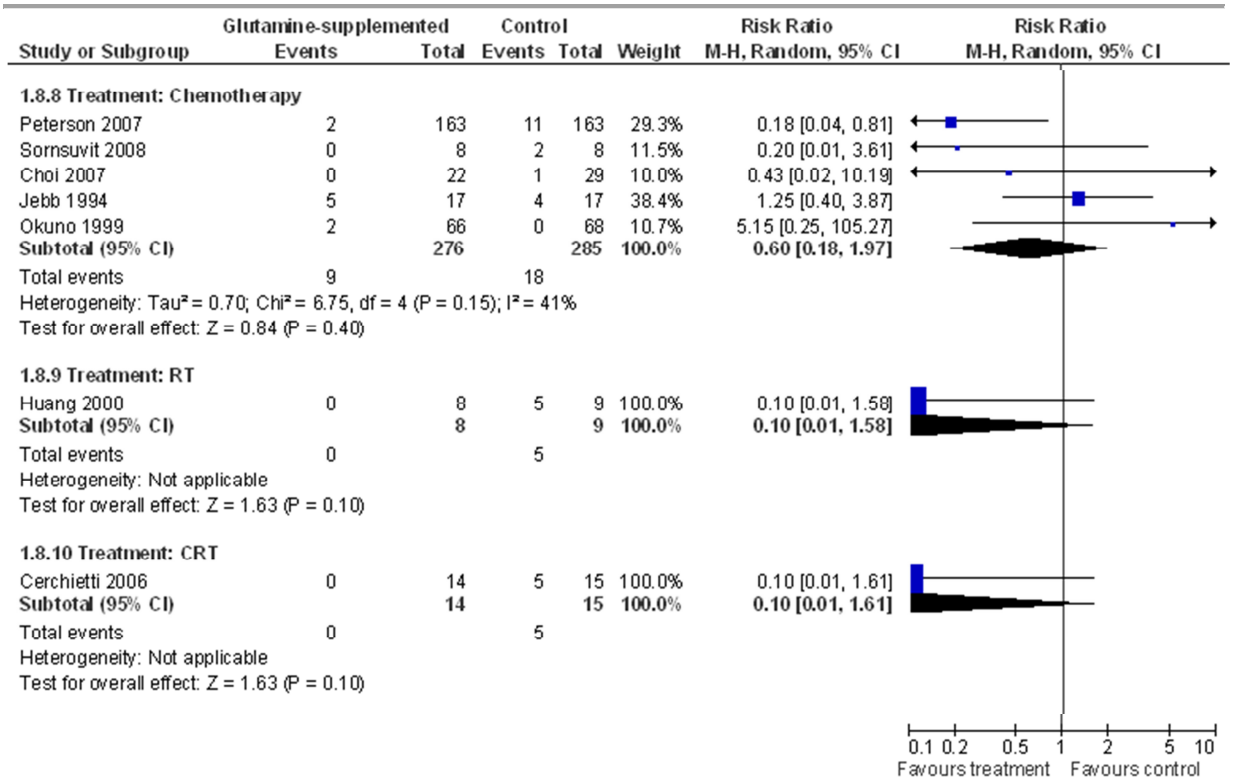


Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.8. Mucositis: Number of patients with maximum grade (3/4)

Subgroup analysis

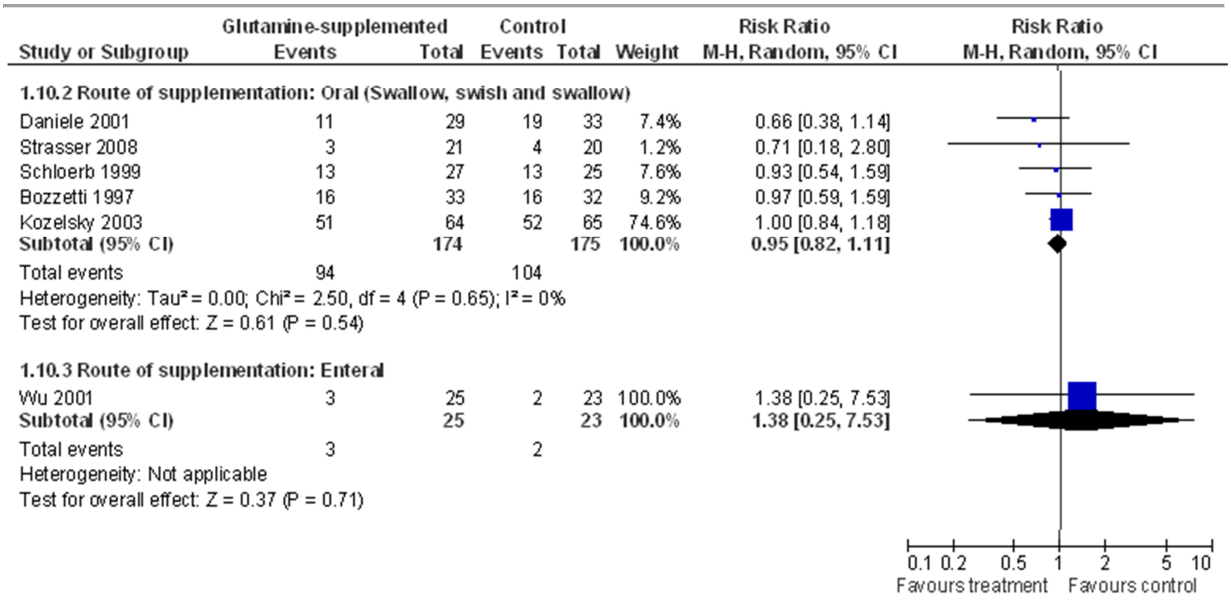


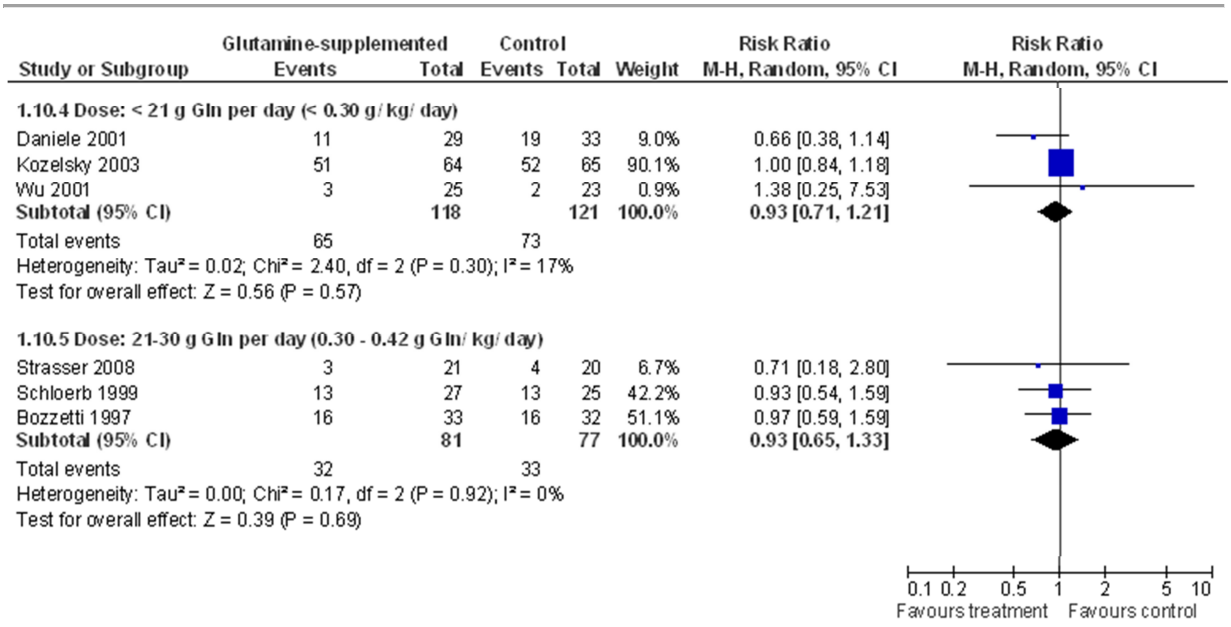


Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.10. Diarrhoea: Patients with ≥ grade 1

Subgroup analysis

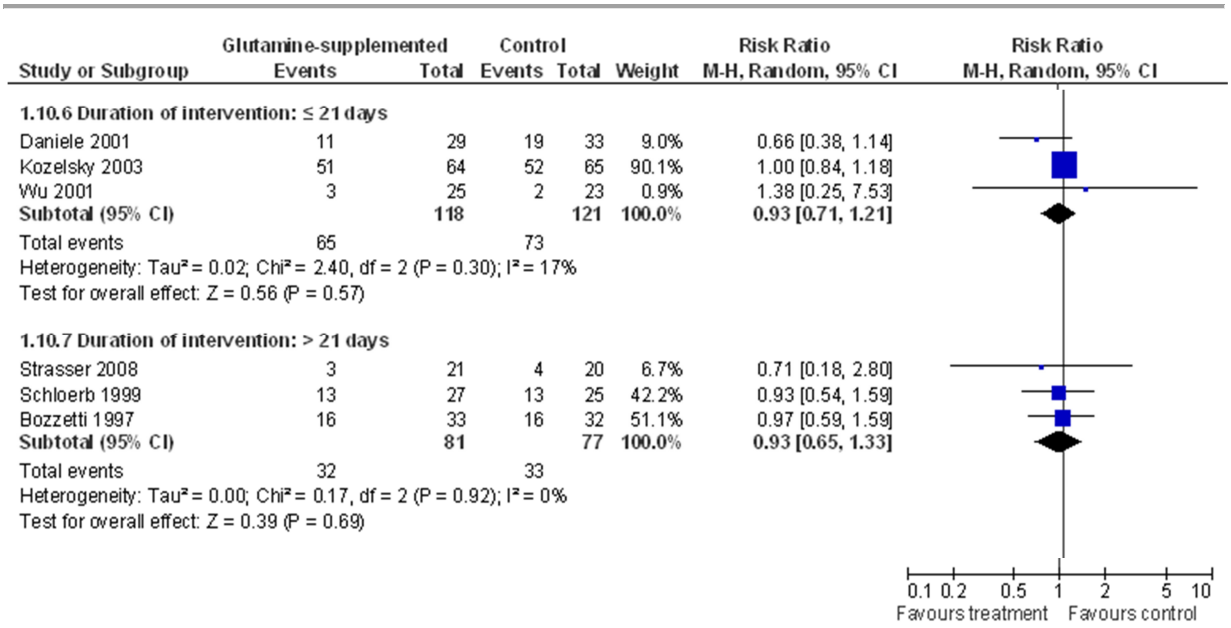


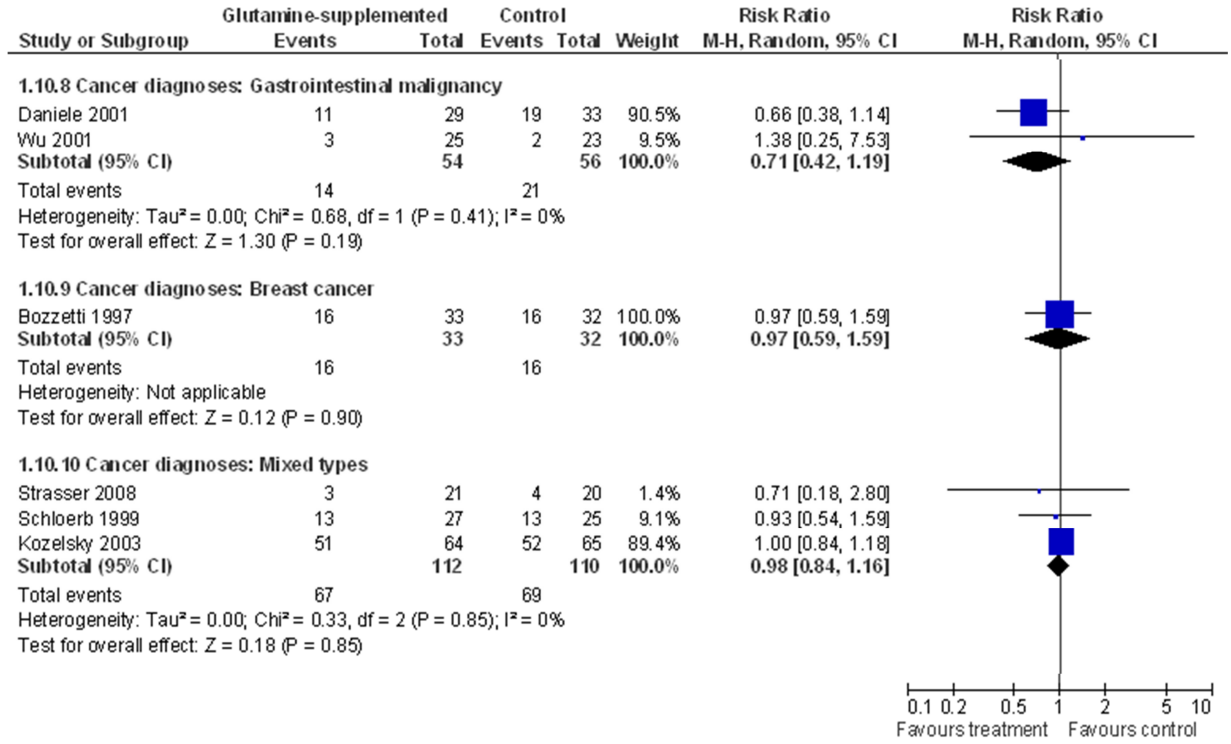


Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.10. Diarrhoea: Patients with ≥ grade 1

Subgroup analysis

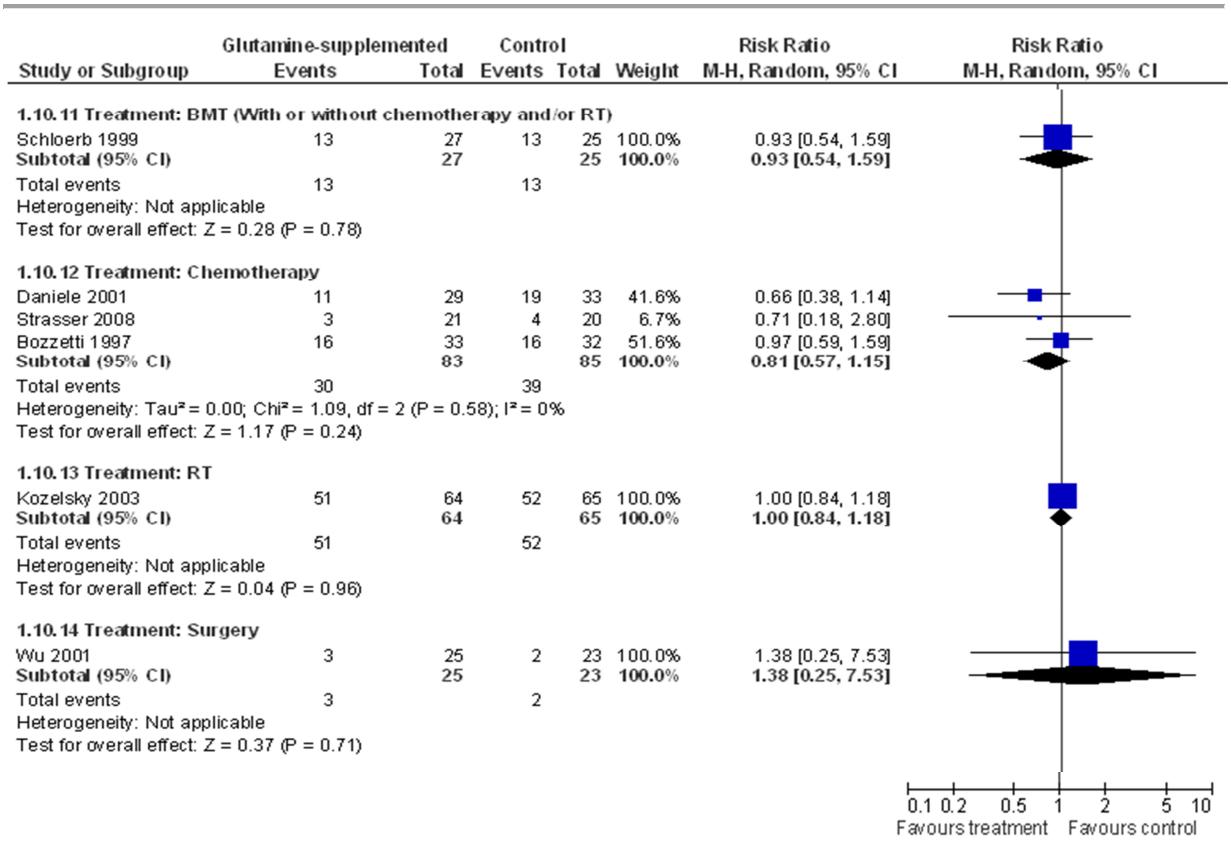


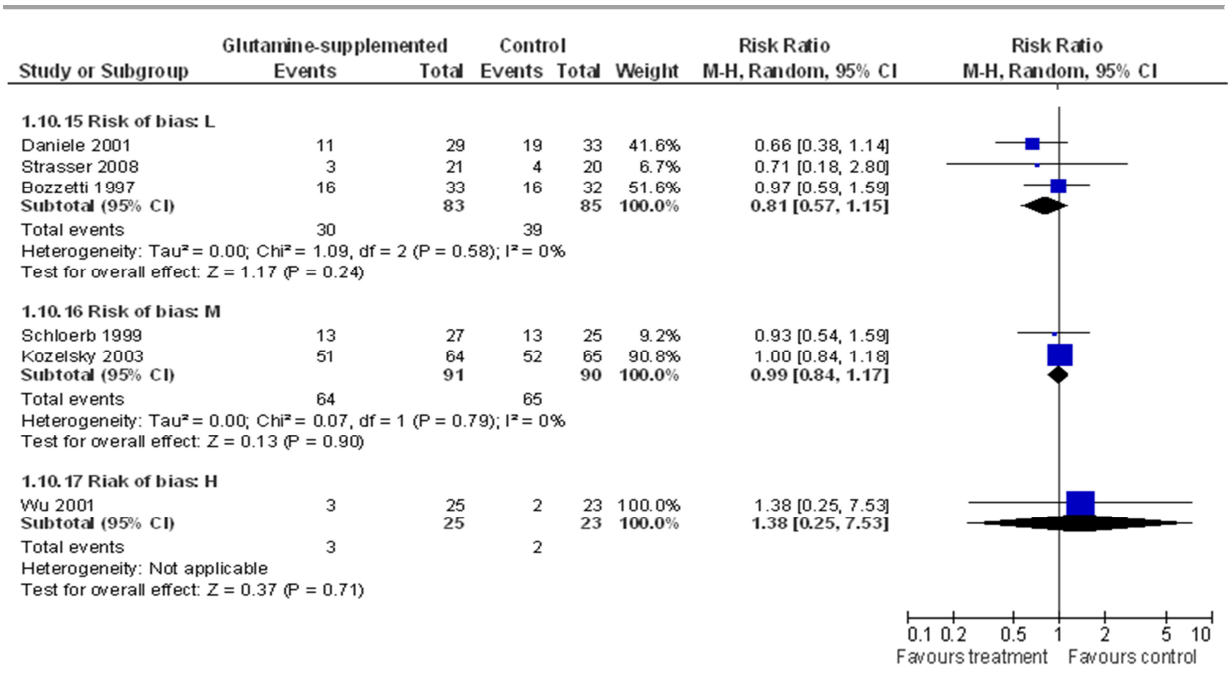


Comparison 1 Humans: GLN-supplemented vs. control

RevMan5 Outcome 1.10. Diarrhoea: Patients with ≥ grade 1

Subgroup analysis

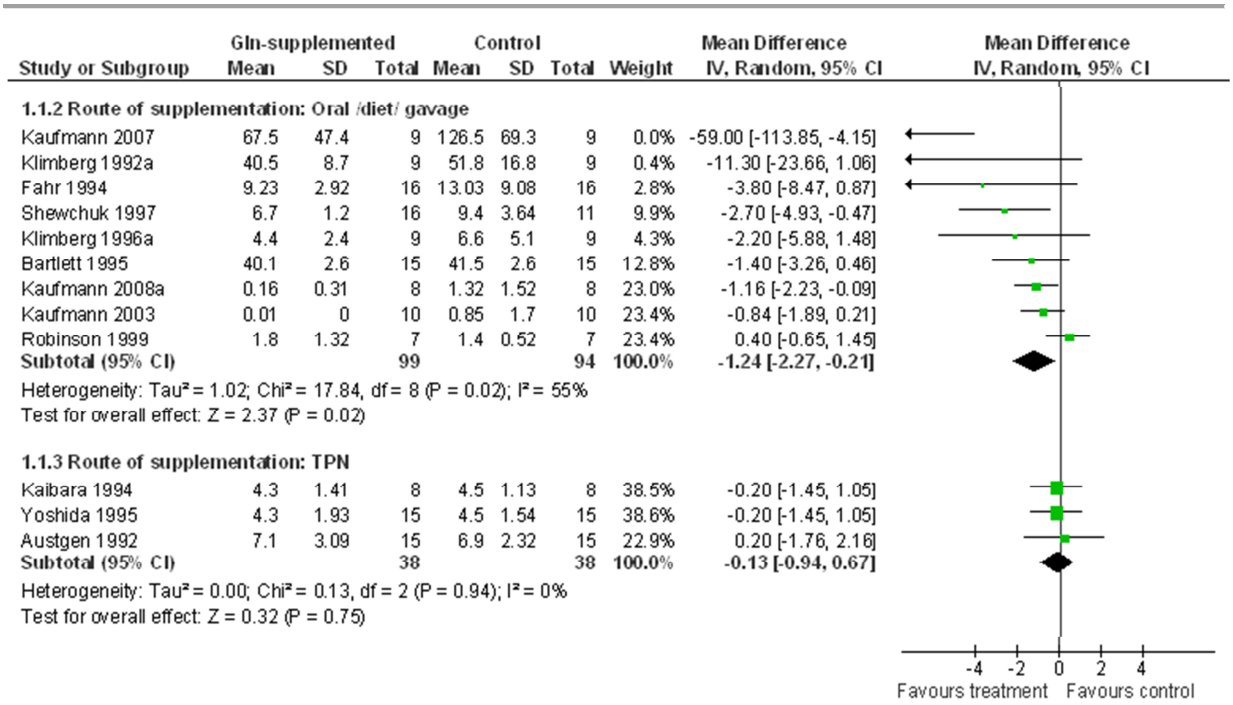


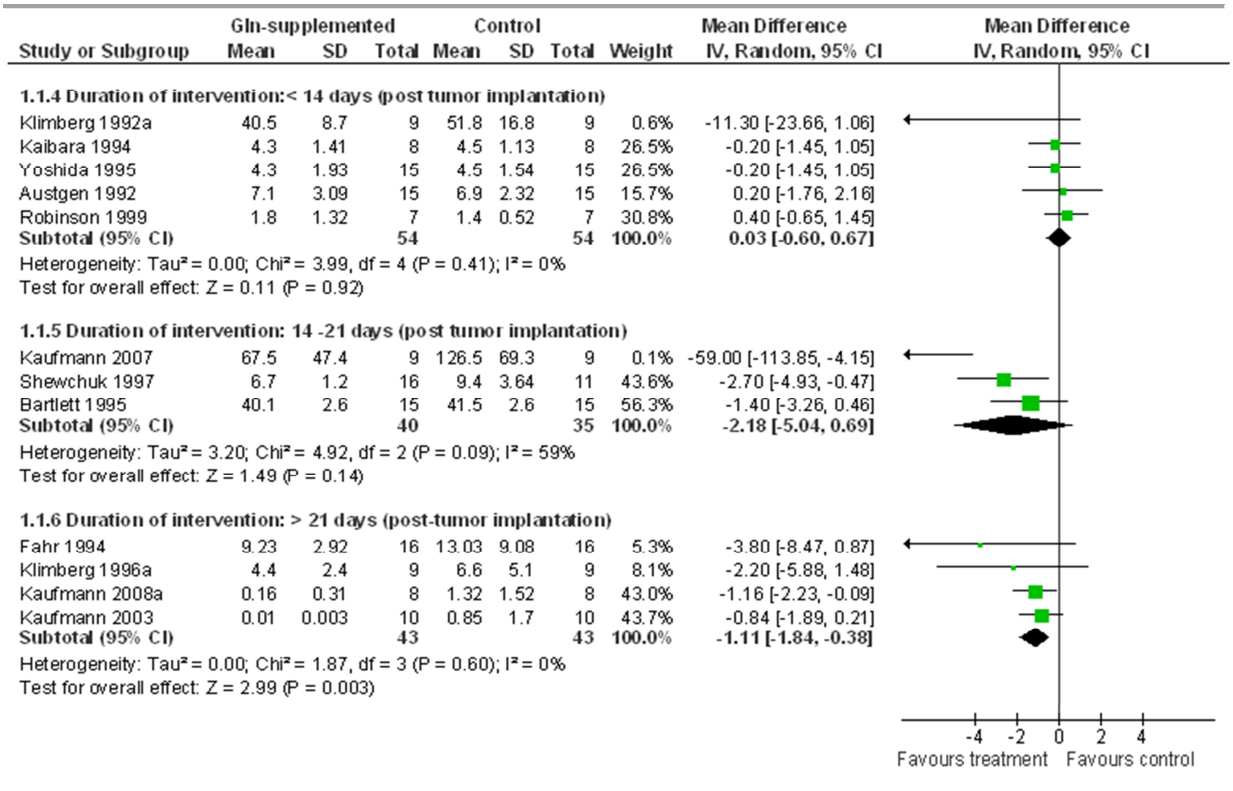


Comparison 1 Animals: GLN-supplemented vs. control

RevMan5 Outcome 1.1: Tumour weight (g)

Subgroup analysis

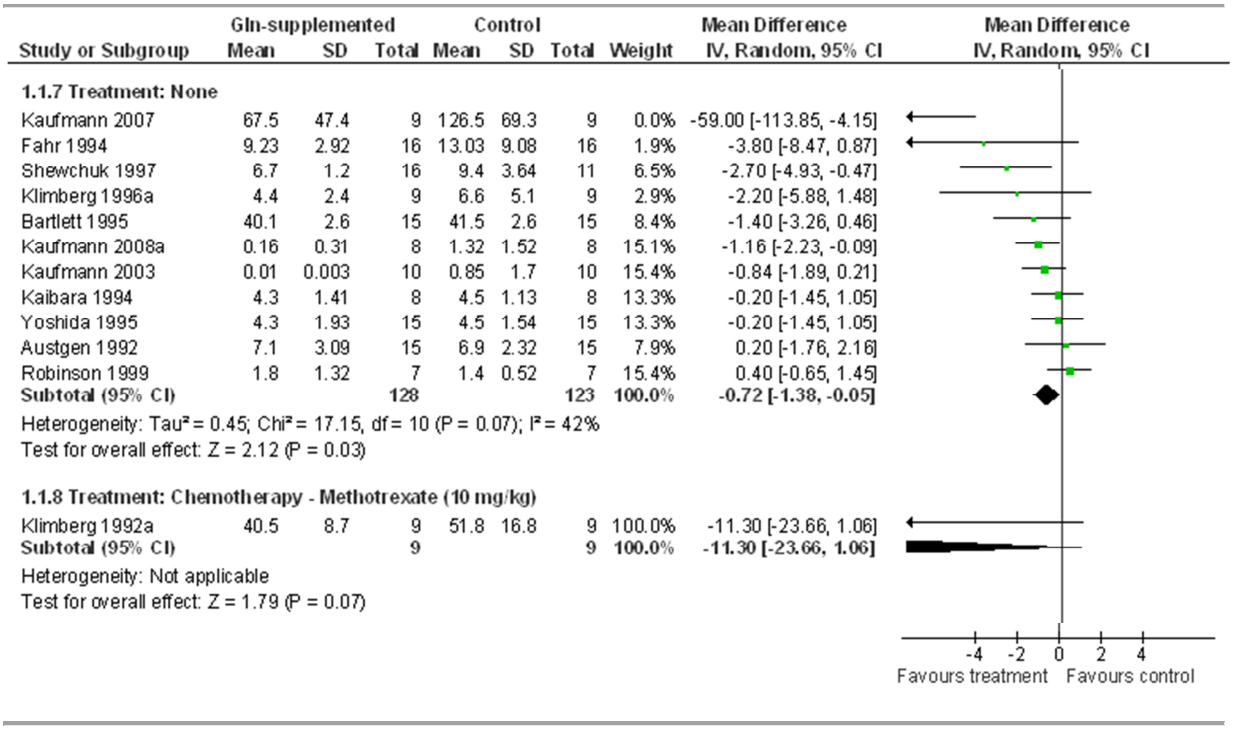




Comparison 1 Animals: GLN-supplemented vs. control

RevMan5 Outcome 1.1: Tumour weight (g)

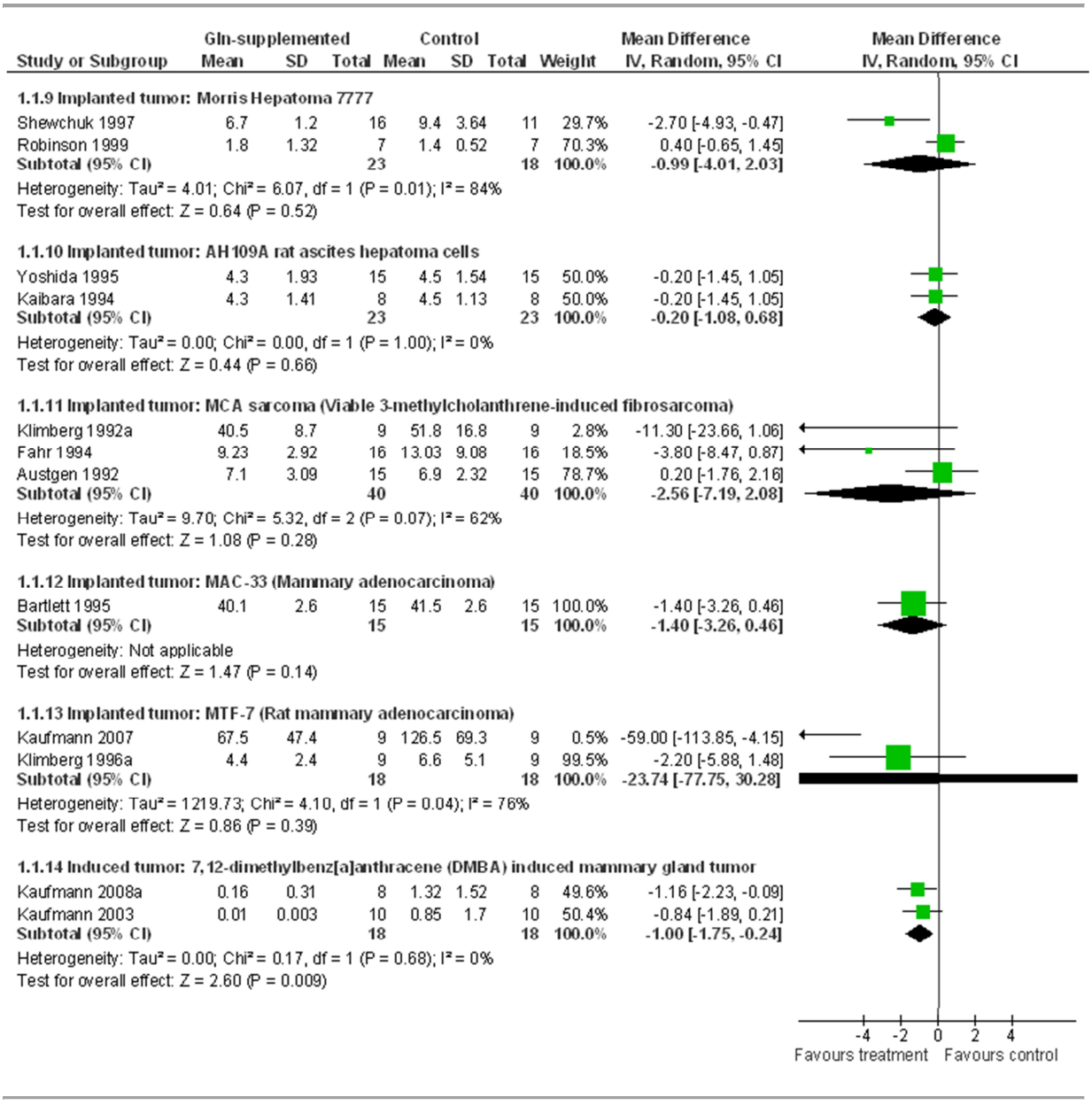
Subgroup analysis



Comparison 1 Animals: GLN-supplemented vs. control

RevMan5 Outcome 1.1: Tumour weight (g)

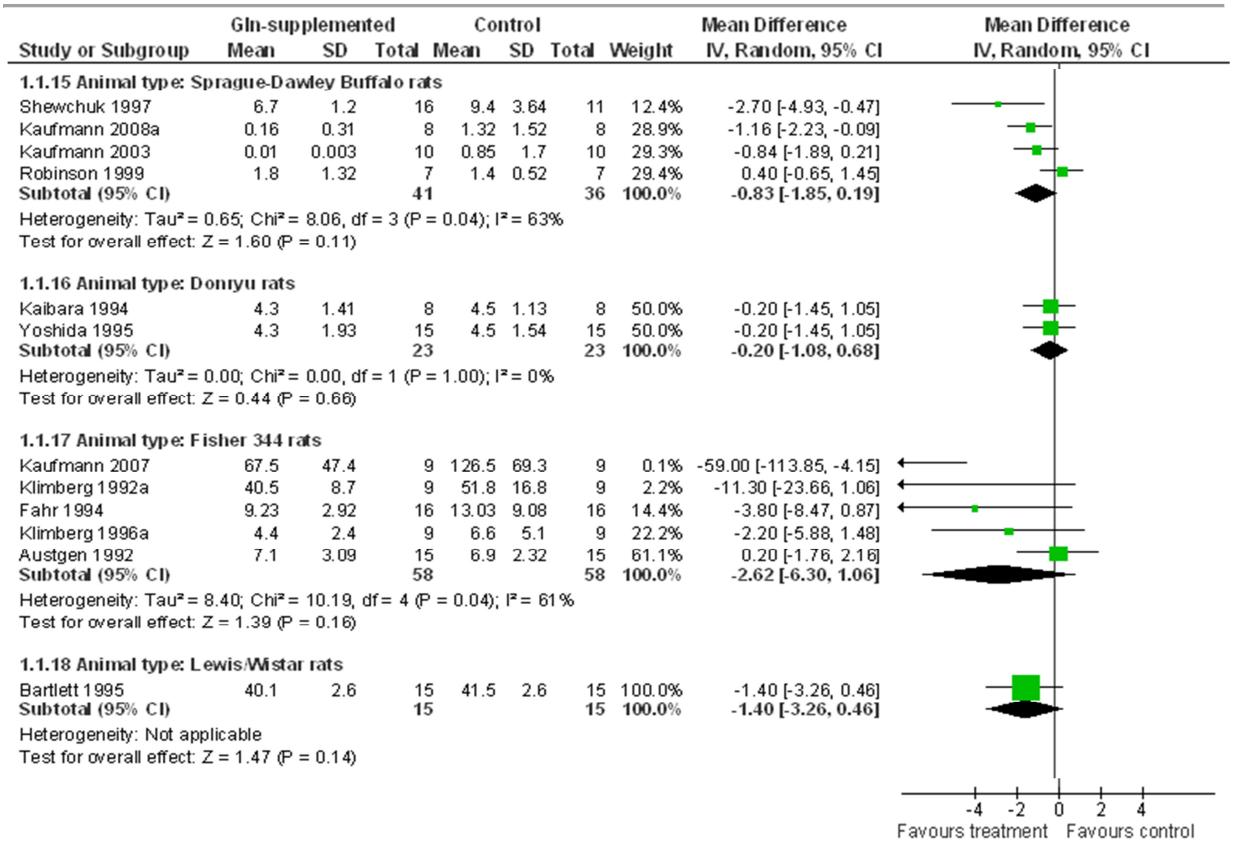
Subgroup analysis

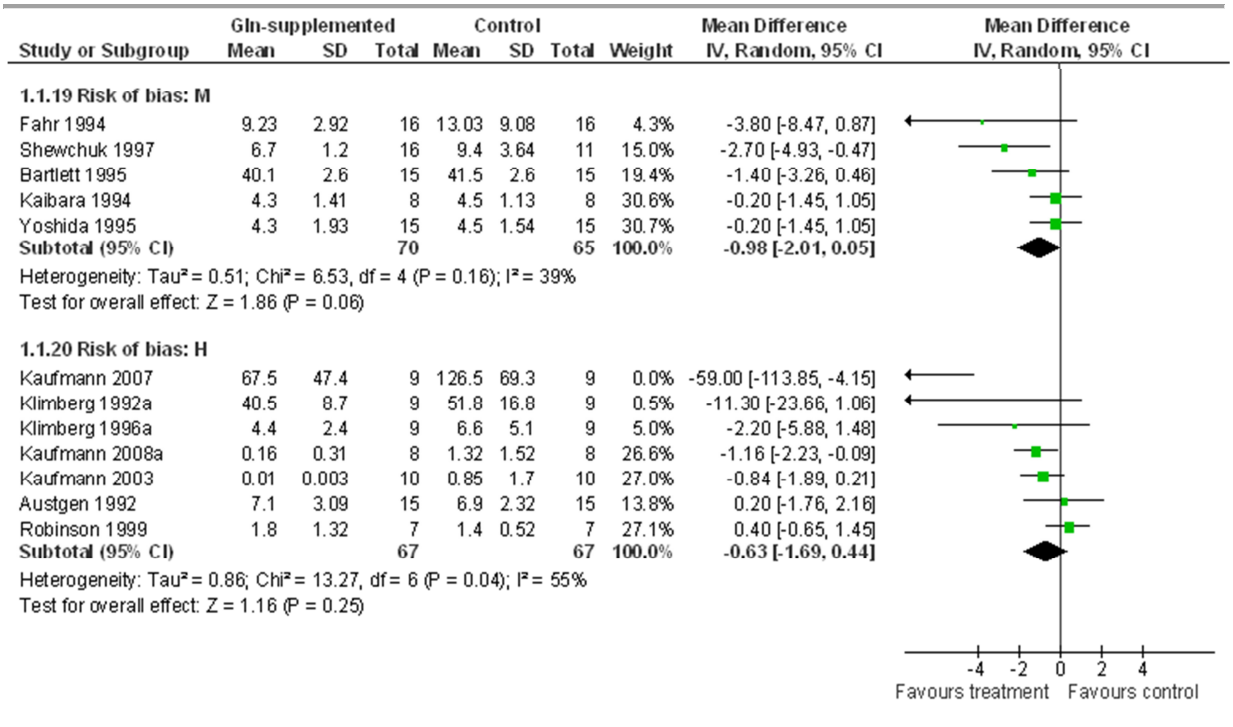


Comparison 1 Animals: GLN-supplemented vs. control

RevMan5 Outcome 1.1: Tumour weight (g)

Subgroup analysis

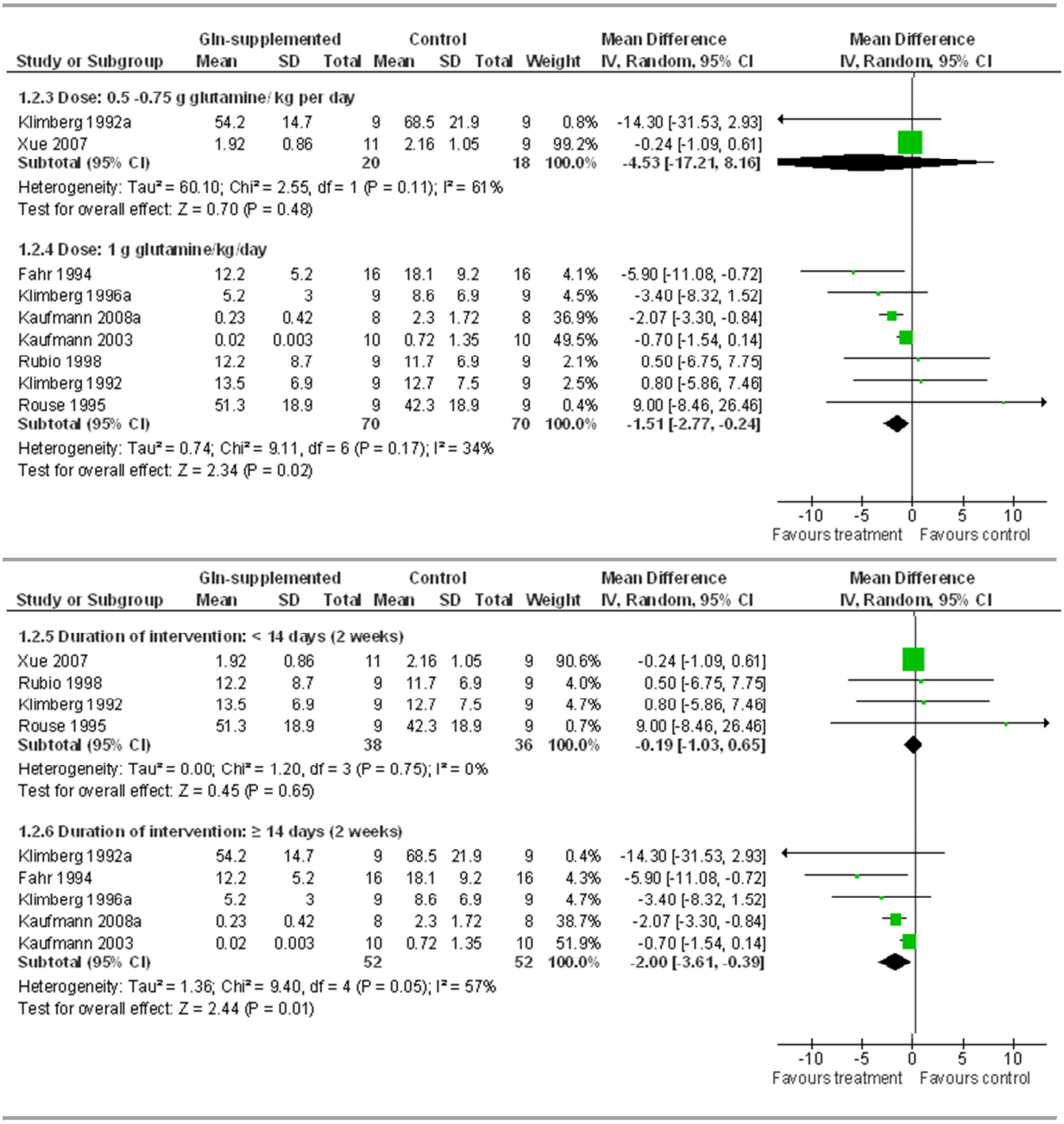




Comparison 1 Animals: GLN-supplemented vs. control

RevMan5 Outcome 1.2: Tumour volume (mL, cc)

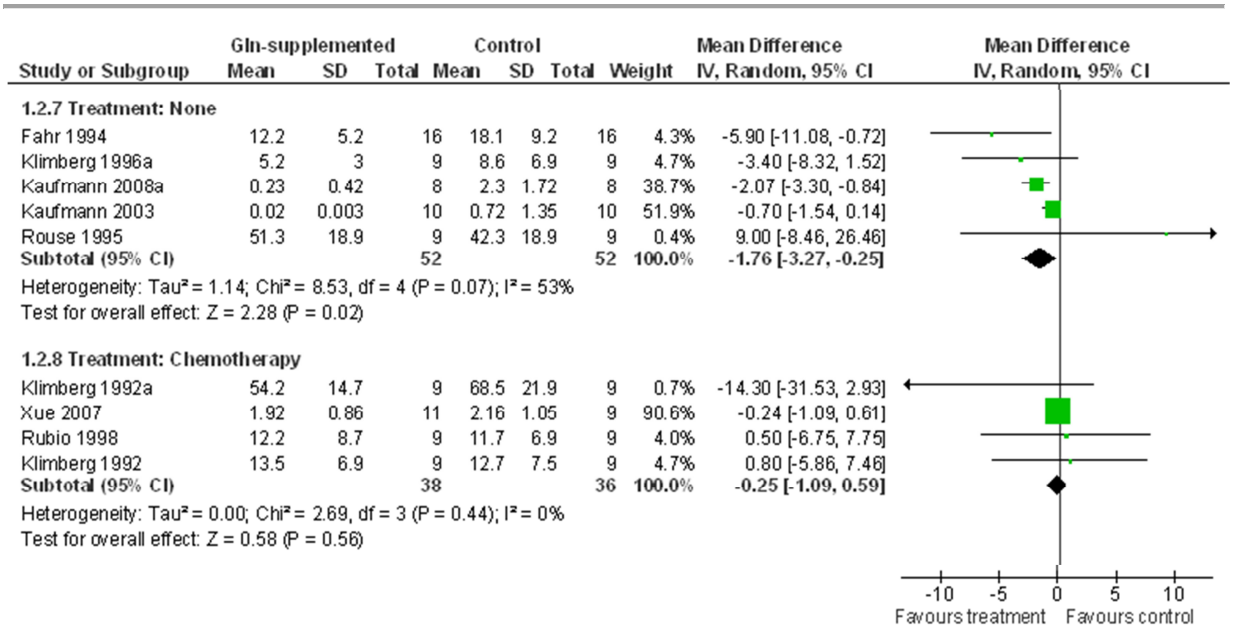
Subgroup analysis

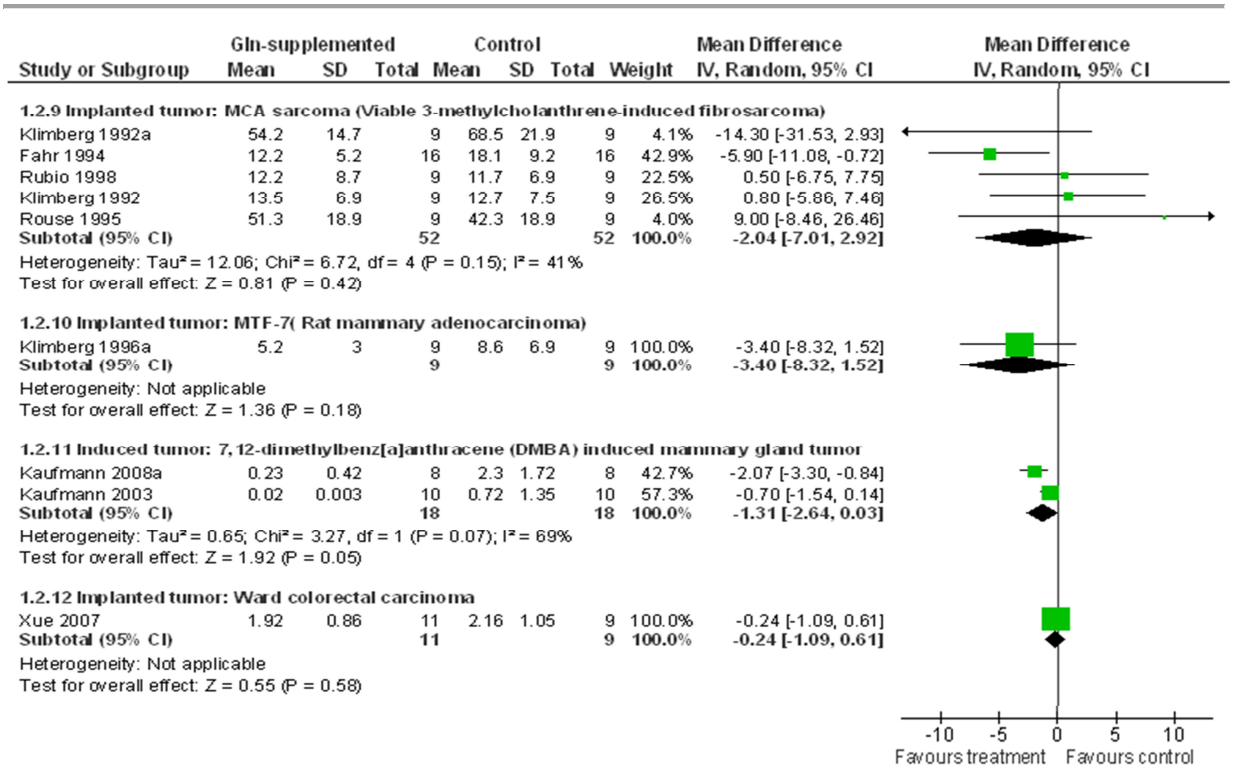


Comparison 1 Animals: GLN-supplemented vs. control

RevMan5 Outcome 1.2: Tumour volume

Subgroup analysis

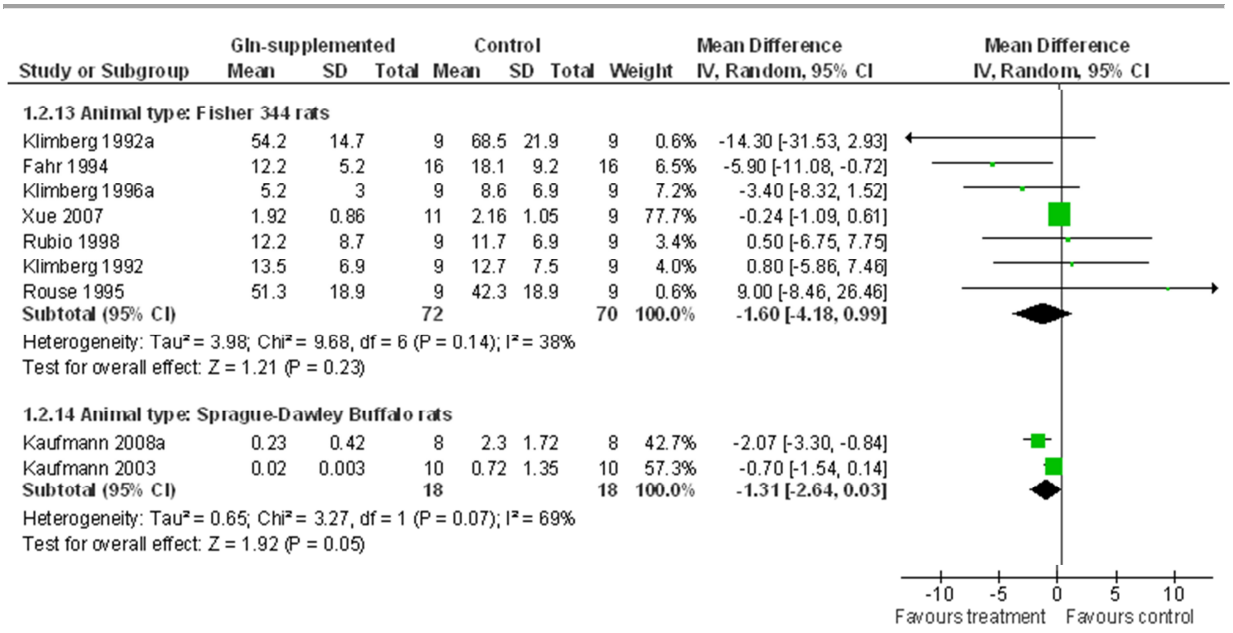


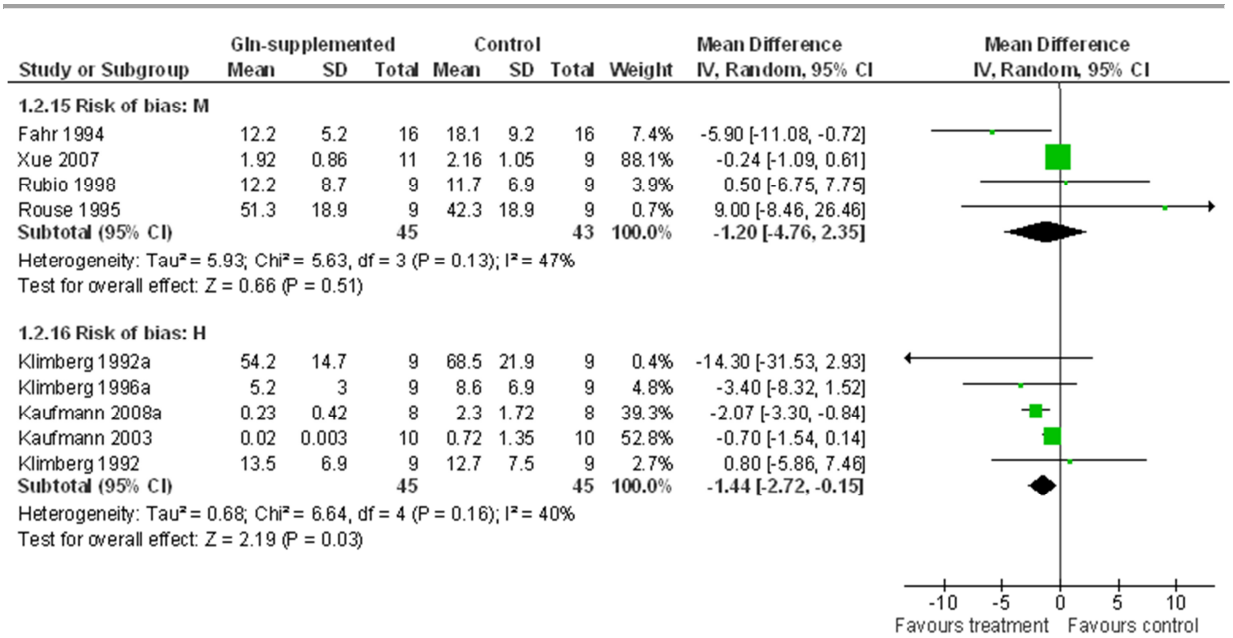


Comparison 1 Animals: GLN-supplemented vs. control

RevMan5 Outcome 1.2: Tumour volume

Subgroup analysis





APPENDIX 6.11: Summary of findings

GLN supplementation compared to no GLN supplementation in **humans** with cancer

Patients or population: Humans (Mainly adults) with cancer receiving either no treatment at all, chemotherapy and/or RT, surgery or BMT with/without chemotherapy and/or RT.

Settings: Various single and some multi-centres in several countries

Intervention: GLN supplementation (Oral, Parenteral, Enteral)

Comparison: Placebo/standard care

OUTCOMES	ILLUSTRATIVE COMPARATIVE RISKS (95% CI)		RELATIVE EFFECT (95% CI)	NUMBER OF PARTICIPANTS (STUDIES)	QUALITY OF THE EVIDENCE (GRADE)	COMMENTS
	ASSUMED CONTROL RISK (ACR)	CORRESPONDING INTERVENTION RISK = 100 x ACR x RR				
	No GLN	With GLN				
MORTALITY	7 per 100[1a]	7 per 100 (5 to 10)	**RR 0.96** (0.67 to1.37)	1521 (16 studies)	Low[1b]	P=0.81
SURVIVAL AT FOLLOW-UP	73 per 100[2a]	71 per 100 (57 to 87)	**RR 0.97** (0.78 to 1.19)	260 (6 studies)	Low[2b]	P=0.75
LENGTH OF STAY (LOS)	The mean LOS ranged across control groups[3a] from **8.3 to 41.19** days	The mean LOS in the GLN group was on average **1.68** days less (95% CI -3.67 to + 0.31)	**MD -1.68**	816 (10 studies)	Low[3b]	P=0.10
	Dose: >30 g GLN/day (>0.42g GLN/kg/day)		**MD -4.51**	215 (4 studies)	Low[4b]	Subgroup analysis (P=0.0002)
	The mean LOS ranged across control groups[4a] from **8.3 to 41.19** days	The mean LOS in the GLN group was on average **4.51** days lower (95% CI -6.58 to -2.16)				
	Duration of intervention: >21 days		**MD -5.09**	142 (4 studies)	Low[5b]	Subgroup analysis (P<0.0001)
	The mean LOS ranged across control groups[5a] from **26.7 to 36** days	The mean LOS in the GLN group was on average **5.09** days lower (95% CI -7.55 to -2.63)				

OUTCOMES	ILLUSTRATIVE COMPARATIVE RISKS (95% CI)		RELATIVE EFFECT (95% CI)	NUMBER OF PARTICIPANTS (STUDIES)	QUALITY OF THE EVIDENCE (GRADE)	COMMENTS
	ASSUMED CONTROL RISK (ACR)	CORRESPONDING INTERVENTION RISK = 100 x ACR x RR				
	No GLN	With GLN				
BODY WEIGHT CHANGE (BWC)	The mean BWC ranged across control groups[6a] from **+1.8 to -5.77** kg	The mean BWC in the GLN group was on average **0.63** kg more (95% CI -0.99 to 2.25)	**MD 0.63**	224 (8 studies)	Low[6b]	P=0.45
	Dose: 21 -30 g GLN/day (0.3 -0.42 g GLN/kg/day)		**MD 2.49**	114 (4 studies)	Low [7b]	Subgroup analysis (P=0.0003)
	The mean BWC ranged across control groups[7a] from **+0.3 to -5.77** kg	The mean BWC in the GLN group was on average **2.49** kg more (95% CI -1.15 to 3.83)				
	Dose: 31 – 40 g GLN/day (0.44 -0.57g GLN/kg/day)		**MD -2.03**	49 (2 studies)	Low[8b]	Subgroup analysis (P=0.03)
	The mean BWC ranged across control groups[8a] from **+1.4 to +1.8** kg	The mean BWC in the GLN group was on average **2.03** kg **less** (95% CI -3.84 to -0.21)				
	Duration of intervention: ≤21 days		**MD 1.80**	91 (4 studies)	Low[9b]	Subgroup analysis (P=0.02)
	The mean BWC ranged across control groups[9a] from **+0.3 to -5.77** kg	The mean BWC in the GLN group was on average **1.80** kg **more** (95% CI 0.25 to3.35)				
	Treatment: Chemotherapy and/or RT		**MD 2.37**	66 (3 studies)	Low[10b]	Subgroup analysis (P=0.0006)
	The mean BWC ranged across control groups[10a] from **+0.3 to -5.77** kg	The mean BWC in the GLN group was on average **2.37** kg **more** (95% CI 1.01 to 3.73)				
CLINICAL INFECTION	28 per 100[11a]	24 per 100 (20 to 29)	**RR 0.87** (0.73 to 1.04)	906 (12 studies)	Low[11b]	P=0.13
MUCOSITIS DURATION	The mean duration of mucositis ranged across control groups[12a] from **1.5 to 7 days**	The mean difference in duration of mucositis in the intervention group was **1.05 days more** (95% CI -2.58 to 4.68)	**MD 1.05 Std MD 0.17**	68 (3 studies)	Low[12b]	P=0.57
≥ GRADE 2 MUCOSITIS	46 per 100[13a]	35 per 100 (27 to 44)	**RR 0.76** (0.60 to 0.97)	727 (9 studies)	Low[13b]	P=0.03
	Dose : <21 g GLN/day (<0.30 g GLN/kg/day)		**RR 0.78** (0.64 to 0.94)	573 (5 studies)	Low[14b]	Subgroup analysis (P=0.01)
	44 per 100[14a]	34 per 100 (28 to 41)				
	Risk of bias: Moderate		**RR 0.80** (0.65-0.99)	636 (7 studies)	Low[15b]	Subgroup analysis (P=0.04)
	47 per 100[15a]	38 per 100 (31 to 47)				
MAXIMUM GRADE MUCOSITIS (3/4)	9 per 100[16a]	4 per 100 (1 to **11**)	**RR 0.41** (0.14 to 1.19)	607 (7 studies)	Low[16b]	P=0.10
	Route of supplementation: Parenteral		**RR 0.14** (0.02 to1.03)	45 (2 studies)	Low[17b]	Subgroup analysis (P=0.05)
	30 per 100[17a]	4 per 100 (1 to **31**)				
	Intervention >14 days		**RR 0.19**	410	Low [18b]	Subgroup

OUTCOMES	ILLUSTRATIVE COMPARATIVE RISKS (95% CI)		RELATIVE EFFECT (95% CI)	NUMBER OF PARTICIPANTS (STUDIES)	QUALITY OF THE EVIDENCE (GRADE)	COMMENTS
	ASSUMED CONTROL RISK (ACR)	CORRESPONDING INTERVENTION RISK = 100 x ACR x RR				
	No GLN	With GLN				
	9 per 100[18a]	2 per 100 (1 to 5)	(0.06 to 0.57)	(4 studies)		analysis (P=0.003)
DIARRHOEA DURATION	The mean duration of diarrhoea ranged across control groups[19a] from **2.8 to 4.3**	The mean difference in duration of diarrhoea in the intervention group was **1.26 days less** than in controls (95% CI, -2.28 to-0.24)	**-1.26 MD SMD -0.38**	130 (4 studies)	Moderate[19b]	P=0.02
>GRADE 1 DIARRHOEA	53 per 100[20a]	51 per 100 (44 to **59**)	**RR 0.97** (0.83 to 1.13)	397 (6 studies)	Low[20b]	P=0.69
≥ GRADE 2 DIARRHOEA	37 per 100[21a]	38 per 100 (29 to **52**)	**RR 1.03** (0.77 to 1.39)	248 (4 studies)	Low[21b]	P=0.84
MAXIMUM (≥3) GRADE DIARRHOEA	6 per 100[22a]	3 per 100 (1 to **10**)	**RR 0.54** (0.17 to1.68)	256 (3 studies)	Low[22b]	P=0.29

[1a] Including mainly gastrointestinal and haematological malignancy, but also a wide range of mixed types of cancer. Including BMT, surgery and chemotherapy treatment, but also no treatment in 2 studies. Wide range of dose and duration of GLN intervention. 9 studies TPN, 5 studies oral and 2 studies Enteral route.

[1b] Methodological design: Adequate sequence generation 11/16 (68.8%), allocation concealment 10/16 (62.5%), blinding 13/16 (81.3%), incomplete outcome data addressed 13/16 (81.3%), free of selective reporting 16/16 (100%), free of other bias 9/16 (56.3%), of which study discontinued x1, change in GLN product x1, co-intervention x4, problematic cross-over study design x1. Imprecision of results (wide confidence interval). Unexplained inconsistency in results. 0% heterogeneity. Asymmetry on funnel plot.

[2a] Haematological malignancy and solid tumours undergoing BMT with/without chemotherapy and/or RT. 5 studies TPN, 1 oral route. Wide range of dose between 21-40 g GLN /day. Wide range of duration of intervention.

[2b] Methodological design: Adequate sequence generation 5/6 (83.3%), allocation concealment 5/6 (83.3%), blinding 6/6 (100%), incomplete outcome data addressed 6/6 (100%), free of selective reporting 6/6 (100%), free of other bias 5/6 (83.3%), which had a problematic cross-over study design with some patients randomized more than 2 times. It was unclear how this was addressed in analyses. Imprecision of results (wide confidence interval). Unexplained heterogeneity and inconsistency in results.

[3a] Including haematological malignancies, gastrointestinal cancer, colorectal cancer and mixed types. Including mainly BMT, but also chemotherapy and surgery treatments. 8 studies TPN, 2 Oral route. Wide range of dose and duration of GLN intervention.

[3b] Methodological design: Adequate sequence generation 8/10 (80%), allocation concealment 8/10 (80%), blinding 7/10 (70%), incomplete outcome data addressed 9/10 (90%), free of selective reporting 10/10 (100%), free of other bias 9/10 (90%), of which change in GLN product x1. Imprecision of results (95% CI very wide, including 0% difference in effect). Unexplained heterogeneity and inconsistency in results. Asymmetry on funnel plot.

[4a] Including haematological malignancies, colorectal cancer and mixed types. Including mainly BMT, but also surgery. TPN only. Wide range of duration of intervention.

[4b] Methodological design: Adequate sequence generation 3/4 (75%), allocation concealment 3/4 (75%), blinding 3/4 (75%), incomplete outcome data addressed 3/4 (75%), free of selective reporting 4/4 (100%), free of other bias 4/4 (100%). Imprecision of results (wide confidence interval). Difference between subgroups not statistically significant (Overlap of confidence intervals). Good consistency in results.

[5a] Including haematological malignancies and mixed types. BMT only. 2 studies TPN, 2 studies oral route. Wide range of GLN dose.

[5b] Methodological design: Adequate sequence generation 4/4 (100%), allocation concealment 4/4 (100%), blinding 4/4 (100%), incomplete outcome data addressed 3/4 (75%), free of selective reporting 4/4 (100%), free of other bias 3/4 (75%), of which change in GLN product x1. Imprecision of results (wide confidence interval). Difference between subgroups not statistically significant (Overlap of confidence intervals). Good consistency in results.

[6a] Including a wide range of cancer diagnoses. Including BMT, chemotherapy and/or RT, surgery or no treatment at all. 5 studies TPN, 2 oral and 1 enteral route. Wide range of doses up to 40 g GLN/day. Wide range duration of intervention.
[6b] Methodological design: Adequate sequence generation 5/8 (62.5%), allocation concealment 5/8 (62.5%), blinding 5/8 (62.5%), incomplete outcome data addressed 7/8 (87.5%), free of selective reporting 8/8 (100%), free of other bias 5/8 (62.5%), of which change in GLN product x1, co-intervention x2. Imprecision of results (wide confidence interval including 0% difference in effect). Unexplained heterogeneity and inconsistency in results.
[7a] Including a wide range of cancer diagnoses. Including mainly chemotherapy and/or RT, but also one study with BMT. 3 studies TPN, 1 oral route. Wide range duration of intervention.
[7b] Methodological design: Adequate sequence generation 2/4 (50%), allocation concealment 2/4 (50%), blinding 2/4 (50%), incomplete outcome data addressed 4/4 (100%), free of selective reporting 4/4 (100%), free of other bias 3/4 (75%), of which change in GLN product x1. Imprecision of results (wide confidence interval). Difference between subgroups not significant (Overlap of confidence intervals). Unexplained inconsistency in results.
[8a] Including haematologicalal malignancy and mixed types. Only BMT. Only TPN. ≥21 days duration.
[8b] Methodological design: Adequate sequence generation 2/2 (100%), allocation concealment 2/2 (100%), blinding 2/2 (100%), incomplete outcome data addressed 1/2 (50%), free of selective reporting 2/2 (100%), free of other bias 2/2 (100%). Imprecision of results (wide confidence interval). Difference between subgroups not significant (Overlap of confidence intervals). Good consistency in results. Only two studies.
[9a] Including haematological, gastrointestinal and head and neck cancer diagnoses. Including chemotherapy and/or RT and surgery. 3 studies TPN, 1 study enteral. Includes wide range of GLN up to 30 g/day.
[9b] Methodological design: Adequate sequence generation 1/4 (25%), allocation concealment 1/4 (25%), blinding 1/4 (25%), incomplete outcome data addressed 4/4 (100%), free of selective reporting 4/4 (100%), free of other bias 3/4 (75%), of which co-intervention x1. Imprecision of results (wide confidence interval). Difference between subgroups not significant (Overlap between confidence intervals). Unexplained inconsistency and heterogeneity of results.
[10a] Including haematological, gastrointestinal and head and neck cancer diagnoses. TPN only. GLN dose of 21-30 g/day. Duration of <21 days.
[10b] Methodological design: Adequate sequence generation 1/3 (33.3%), allocation concealment 1/3 (33.3%), blinding 1/3 (33.3%), incomplete outcome data addressed 3/3 (100%), free of selective reporting 3/3 (100%), free of other bias 3/3 (100%). Imprecision of results (wide confidence interval). Difference between subgroups not significant (Overlap of confidence intervals). Inconsistency in results.
[11a] Including mainly haematological, gastrointestinal (mixed, gastric, colon) and other mixed cancer diagnoses. Including mainly BMT, surgery and 1 study with chemotherapy treatment. 11 studies TPN, 1 study oral. Wide range of dose and duration of GLN intervention.
[11b] Methodological design: Adequate sequence generation 8/12 (66.6%), allocation concealment 8/12 (66.6%), blinding 8/12 (66.6%), incomplete outcome data addressed 11/12 (91.7%), free of selective reporting 12/12 (100%), free of other bias 11/12 (91.7%), of which change in GLN product x1. Imprecision of results (wide confidence interval). Some inconsistency in results.
[12a] Including mainly haematological malignancy mixed with solid tumour diagnoses. Included BMT and chemotherapy. 2 studies TPN, 1 study oral. Wide range of GLN dose up to 30 g/day. Wide range of duration in GLN intervention.
[12b] Methodological design: Adequate sequence generation 2/3 (66.6%), allocation concealment 2/3 (66.6%), blinding 2/3 (66.6%), incomplete outcome data addressed 3/3 (100%), free of selective reporting 3/3 (100%), free of other bias 3/3 (66.6%). Imprecision of results (wide confidence interval). Unexplained heterogeneity and inconsistency in results. Only 3 studies.
[13a] Including a wide range of cancer diagnoses. Including mainly chemotherapy and/or RT and 1 study BMT. 2 studies TPN, 6 studies oral. Wide range of GLN dose up to 30 g/day. Wide range of duration of GLN intervention from 7 to >21 days.
[13b] Methodological design: Adequate sequence generation 5/9 (55.6%), allocation concealment 4/9 (44.4%), blinding 6/9 (66.7%), incomplete outcome data addressed 8/9 (88.9%), free of selective reporting 9/9 (100%), free of other bias 8/9 (88.9%), of which spurious data collection for medical charts x 1. Good consistency in results.
[14a] Including a wide range of cancer diagnoses. Including chemotherapy and RT. Oral route only. Wide range of duration of GLN intervention.
[14b] Methodological design: Adequate sequence generation 2/5 (40%), allocation concealment 1/5 (20%), blinding 4/5 (80%), incomplete outcome data addressed 4/5 (80%), free of selective reporting 5/5 (100%), free of other bias 5/5 (100%). Difference in effect between subgroups not significant (Overlap of confidence intervals).
[15a] Including wide range of cancer diagnoses. Including BMT chemotherapy and/or RT. 1 study TPN, 6 studies oral route. Wide range of GLN dose up to 30 g/day. Wide range of duration of GLN intervention from 7 to >21 days.

[15b] Methodological design: Adequate sequence generation 3/7 (42.8%), allocation concealment 2/7 (28.5%), blinding 4/7 (57.1%), incomplete outcome data addressed 6/7 (85.7%), free of selective reporting 7/7 (100%), free of other bias 6/7 (85.7%), of which spurious data collection for medical charts x 1. Difference in effect between subgroups not significant (Overlap of confidence intervals).

[16a] Including a wide range of cancer diagnoses. Including chemotherapy and/or RT. 2 studies TPN, 5 studies oral route. Wide range of GLN dose up until 30 g/day. Wide range of duration of GLN intervention from at least 7 days.

[16b] Methodological design: Adequate sequence generation 3/7 (42.8%), allocation concealment 2/7 (28.5%), blinding 4/7 (57.1%), incomplete outcome data addressed 6/7 (85.7%), free of selective reporting 7/7 (100%), free of other bias 7/7 (100%). Imprecision of results (wide confidence interval). Unexplained heterogeneity and inconsistency in results.

[17a] Including haematological malignancy and head and neck cancer. Including RT and/or chemotherapy. GLN dose of 21-30 g/day. Wide range of duration of GLN intervention from at least 7 days.

[17b] Methodological design: Adequate sequence generation 1/2 (50%), allocation concealment 1/2 (50%), blinding 1/2 (50%), incomplete outcome data addressed 2/2 (100%), free of selective reporting 2/2 (100%), free of other bias 2/2 (100%). Imprecision of results (wide confidence interval). Difference between subgroups not significant (Overlap of confidence intervals). Good consistency in results.

[18a] Including a wide range of cancer diagnoses. Including RT or chemotherapy. 1 study TPN, 3 studies Oral route. Wide range of dose up until 30 g/day.

[18b] Methodological design: Adequate sequence generation 2/4 (50%), allocation concealment 1/4 (25%), blinding 1/4 (25%), incomplete outcome data addressed 4/4 (100%), free of selective reporting 4/4 (100%), free of other bias 4/4 (100%). Difference between subgroups not significant (Overlap of confidence intervals). Good consistency in results.

[19a] Wide range of cancer diagnoses including mainly haematological malignancy, but also solid tumours, colon cancer and breast cancer. Including treatment with BMT or chemotherapy. 2 studies each TPN and oral route. Wide range of GLN dose up to 30 g/day. Wide range of duration of GLN intervention.

[19b] Methodological design: Adequate sequence generation 3/4 (75%), allocation concealment 3/4 (75%), blinding 3/4 (75%), incomplete outcome data addressed 4/4 (100%), free of selective reporting 4/4 (100%), free of other bias 4/4 (100%). Imprecision of results (wide confidence interval). Data from only 4 studies.

[20a] Including wide range of cancer diagnoses (gastrointestinal, colon, breast, mixed solid, haematological). Including BMT, chemotherapy, RT and surgery treatments. 5 studies oral, 1 study enteral. Wide range GLN dose up to 30 g/day. Wide range of duration of GLN intervention.

[20b] Methodological design: Adequate sequence generation 5/6 (83.3%), allocation concealment 4/6 (66.7%), blinding 6/6 (100%), incomplete outcome data addressed 6/6 (100%), free of selective reporting 6/6 (100%), free of other bias 4/6 (66.7%), change in GLN product x1, co-intervention x1. Imprecision of results (wide confidence interval). Unexplained inconsistency in results.

[21a] Including haematological malignancy, colorectal cancer, and other mixed types of diagnoses. Including mainly chemotherapy, but also RT. 1 study TPN, 3 studies oral. Wide range GLN dose up to 30 g/day. Wide range of duration of GLN intervention of at least 15 days.

[21b] Methodological design: Adequate sequence generation 3/4 (75%), allocation concealment 2/4 (50%), blinding 3/4 (75%), incomplete outcome data addressed 4/4 (100%), free of selective reporting 4/4 (100%), free of other bias 4/4 (100%). Imprecision of results (wide confidence interval). Data from only 4 small studies.

[22a] Including colon, breast and mixed types of cancer diagnoses. Including chemotherapy and RT. Only oral route. Wide range GLN dose up to 30 g/day. Wide range of duration of GLN intervention of at least 15 days.

[22b] Methodological design: Adequate sequence generation 3/3 (100%), allocation concealment 2/3 (66.7%), blinding 3/3 (100%), incomplete outcome data addressed 3/3 (100%), free of selective reporting 3/3 (100%), free of other bias 3/3 (100%). Imprecision of results (wide confidence interval). Data from only 3 small studies.

GLN supplementation compared to no GLN supplementation in **animals** with cancer

Patients or population: Any animals (exclusively rats) with cancer receiving no treatment or chemotherapy.

Settings: Experimental studies

Intervention: GLN supplementation (Oral, Parenteral)

Comparison: Placebo/standard care

OUTCOMES	ILLUSTRATIVE COMPARATIVE RISKS (95% CI)		RELATIVE EFFECT (95% CI)	NUMBER OF PARTICIPANTS (STUDIES)	QUALITY OF THE EVIDENCE (GRADE)	COMMENTS
	ASSUMED CONTROL RISK	CORRESPONDING INTERVENTION RISK				
	No GLN	With GLN				
TUMOUR WEIGHT (g)	The mean tumour weight ranged across control groups[1a] from **0.85 to 126.5.**	The mean tumour weight in the GLN group was on average **0.77 g less.** (95% CI, -1.47 to -0.07)	**MD -0.77** (-1.47 to -0.07)	269 (12 studies)	Low[1b]	P=0.03
	Route of supplementation: Oral/Diet/Gavage		**MD -1.24** (-2.27 to -0.21)	193 (9 studies)	Low[2b]	Subgroup analysis (P=0.02)
	The mean tumour weight ranged across control groups[2a] from **0.85 to 126.5.**	The mean tumour weight in the GLN group was on average **1.24 g less.** (95% CI, -2.27 to -0.21)				
	Duration of intervention: ≥ 21 days		**MD -1.11** (-1.84 to -0.38)	86 (4 studies)	Low[3b]	Subgroup analysis (P=0.003)
	The mean tumour weight ranged across control groups[3a] from **0.85 to 13.03**	The mean tumour weight in the GLN group was on average **1.11 g less.** (95% CI, -1.84 to -0.38)				

OUTCOMES	ILLUSTRATIVE COMPARATIVE RISKS (95% CI)		RELATIVE EFFECT (95% CI)	NUMBER OF PARTICIPANTS (STUDIES)	QUALITY OF THE EVIDENCE (GRADE)	COMMENTS
	ASSUMED CONTROL RISK	CORRESPONDING INTERVENTION RISK				
	No GLN	With GLN				
TUMOUR VOLUME (ml/cc)	The mean tumour volume ranged across control groups[4a] from **0.72 to 68.5.**	The mean tumour volume in the GLN group was on average **1.16 cm³ less.** (95% CI, -2.19 to-0.12)	**MD -1.16**	178 (9 studies)	Low[4b]	P=0.03
	Dose: 1 g GLN/kg/day (vs. 0.5–0.75 g/kg/day)		**MD -1.51**	140 (7 studies)	Low[5b]	Subgroup analysis (P=0.02)
	The mean tumour volume ranged across control groups[5a] from **0.72 to 42.3.**	The mean tumour volume in the GLN group was on average **1.51 cm³ less.** (95% CI, -2.77 to -0.24)				
	Duration: ≥ 14 days (vs. < 14 days)		**MD -2.00** (-3.61 to -0.39)	104 (5 studies)	Low[6b]	Subgroup analysis (P=0.01)
	The mean tumour volume ranged across control groups[6a] from **0.72 to 68.5.**	The mean tumour volume in the GLN group was on average **2.00 cm³ less.** (95% CI, -3.61 to -0.39)				
	Treatment: None (vs. Chemotherapy)		**MD -1.76** (-3.27 to -0.25)	104 (5 studies)	Low[7b]	Subgroup analysis (P=0.02)
	The mean tumour volume ranged across control groups[7a] from **0.72 to 42.3.**	The mean tumour volume in the GLN group was on average **1.76 cm³ less.** (95% CI, -3.27 to -0.25)				
	Risk of bias: High (vs. Moderate)		**MD -1.44** (-2.72 to -0.15)	90 (5 studies)	Low[8b]	Subgroup analysis (P=0.03)
	The mean tumour volume ranged across control groups from **0.72 to 68.5**	The mean tumour volume in the GLN group was on average **1.44 cm³ less.** (95% CI, -2.72 to -0.15)				
TUMOUR VOLUME LOSS	The mean tumour volume loss ranged across control[9a] groups from **-1.2 to +9.5.**	The mean tumour volume loss in the GLN group was on average **2.46 cm³ more.** (95% CI, -4.97 to 0.05)	**MD -2.46** (-4.97 to 0.05)	104 (5 studies)	Low[9b]	P=0.05

[1a] Wide range of rat species, with wide range of implanted/induced tumour models. Rats in only one study received chemotherapy treatment. 9 studies oral route, 3 studies TPN. Wide range of duration of intervention. Wide range of GLN dose used.

[1b] Methodological design: Adequate sequence generation 12/12 (100%), allocation concealment 0/12 (0%), blinding 0/12 (0%), incomplete outcome data addressed 10/12 (83%), free of selective reporting 12/12 (100%), free of other bias 11/12 (91.6%). Imprecision of results (wide confidence interval). Unexplained heterogeneity and inconsistency in results. Subgroup analysis for dose not possible. Asymmetry in funnel plot.

[2a] Wide range of rat species, with wide range of implanted/induced tumour models. Rats in only one study received chemotherapy treatment. Wide range of duration of intervention. Wide range of GLN dose used.
[2b] Methodological design: Adequate sequence generation 9/9 (100%), allocation concealment 0/9 (0%), blinding 0/9 (0%), incomplete outcome data addressed 8/9 (88.9%), free of selective reporting 9/9 (100%), free of other bias 8/9 (88.9%). Significant unexplained heterogeneity in results. Imprecision of results (wide confidence interval). Difference in summary effect between subgroups not statistically significant.
[3a] Wide range of rat species, with wide range of implanted/induced tumour models. Rats received no other treatment. Only oral route. Wide range of duration of intervention. Wide range of GLN dose used.
[3b] Methodological design: Adequate sequence generation 4/4 (100%), allocation concealment 0/4 (0%), blinding 0/4 (0%), incomplete outcome data addressed 4/4 (100%), free of selective reporting 4/4 (100%), free of other bias 4/4 (100%). Significant unexplained heterogeneity in results. Imprecision of results (wide confidence interval). Difference in summary effect between subgroups not statistically significant. Only 4 studies.
[4a] Wide range of rat species (Fischer 344, Sprague Dawley Buffalo), with wide range of implanted/induced tumour models. 5 studies no other treatment, 4 studies chemotherapy treatment. Only oral route. Wide range of duration of intervention. Wide range of GLN dose (0.5-1 g GLN/kg body weight/day).
[4b] Methodological design: Adequate sequence generation 8/9 (88.9%), allocation concealment 0/9 (0%), blinding 0/9 (0%), incomplete outcome data addressed 8/9 (88.9%), free of selective reporting 9/9 (100%), free of other bias 9/9 (100%). Difference in summary effect between subgroups not statistically significant. Unexplained heterogeneity and inconsistency in results.
[5a] Wide range of rat species (Fischer 344, Sprague Dawley Buffalo), with wide range of implanted/induced tumour models. 5 studies no other treatment, 2 studies chemotherapy treatment. Only oral route. Wide range of duration of intervention.
[5b]Methodological design: Adequate sequence generation 7/7 (100%), allocation concealment 0/7 (0%), blinding 0/7 (0%), incomplete outcome data addressed 7/7 (100%), free of selective reporting 7/7 (100%), free of other bias 7/7 (100%). Imprecision of results (wide confidence interval). Difference in summary effect between subgroups not statistically significant. Unexplained inconsistency in results.
[6a] Wide range of rat species (Fischer 344, Sprague Dawley Buffalo), with wide range of implanted/induced tumour models. 4 studies no other treatment, 1 studies chemotherapy treatment. Only oral route. Wide range of GLN dose (0.5-1 g GLN/kg body weight/day).
[6b] Methodological design: Adequate sequence generation 5/5 (100%), allocation concealment 0/5 (0%), blinding 0/5 (0%), incomplete outcome data addressed 4/5 (80%), free of selective reporting 5/5 (100%), free of other bias 5/5 (100%). Significant unexplained heterogeneity in results. Imprecision of results (wide confidence interval).
[7a] Wide range of rat species (Fischer 344, Sprague Dawley Buffalo), with wide range of implanted/induced tumour models. Only oral route. Wide range of duration of intervention. GLN dose of 1 g GLN/kg body weight/day.
[7b] Methodological design: Adequate sequence generation 5/5 (100%), allocation concealment 0/5 (0%), blinding 0/5 (0%), incomplete outcome data addressed 5/5 (100%), free of selective reporting 5/5 (100%), free of other bias 5/5 (100%). Significant unexplained heterogeneity in results. Imprecision of results (wide confidence interval).
[8a] Wide range of rat species (Fischer 344, Sprague Dawley Buffalo), with wide range of implanted/induced tumour models. 3 studies no other treatment, 2 studies chemotherapy treatment. Only oral route. Wide range of duration of intervention. Wide range of GLN dose (0.5-1 g GLN/kg body weight/day).
[8b] Methodological design: Adequate sequence generation 5/5 (100%), allocation concealment 0/5 (0%), blinding 0/5 (0%), incomplete outcome data addressed 4/5 (80%), free of selective reporting 5/5 (100%), free of other bias 5/5 (100%). Difference in summary effect between subgroups not statistically significant. Imprecision of results (wide confidence interval).
[9a] Fischer 344 rats only. Implanted MCA sarcoma and ward colorectal carcinoma. 1 study no other treatment, 4 studies chemotherapy. 1 study TPN, 4 studies oral route. Wide range of duration of intervention (4 studies < 7 days). Wide range of GLN dose.
[9b] Methodological design: Adequate sequence generation 4/5 (80%), allocation concealment 0/5 (0%), blinding 0/5 (0%), incomplete outcome data addressed 4/5 (80%), free of selective reporting 5/5 (100%), free of other bias 5/5 (100%). Imprecision of results (wide confidence interval).