A HISTORICAL PERSPECTIVE OF ALLOGENEIC AND AUTOLOGOUS IMMUNOHAEMATOPOIETIC STEM CELL TRANSPLANTATION IN SOUTH AFRICA AND A STUDY OF THE NON-HAEMATOLOGIC CONSEQUENCES

— BY —

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Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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AUTHENTICATION

It is hereby certified that this thesis is my own work and has not been presented for any degree at another University.

Date **Lucille Wood**

DEDICATION

To my mother Judith Ellen Posthumus and father Sydney Wood.

SUMMARY

HISTORICAL PERSPECTIVE

Stem cell therapy was commenced after using rabbits as research models. Once this process was successful, the first human transplant was done in 1974.

Certain prerequisites were necessary and these were achieved - a protected environment, an apheresis unit, protocols and accreditation with International Registries.

Initially, unmanipulated bone marrow and peripheral blood stem cells were used together with immunosuppressive drugs followed by the use of Cyclosporin A then the addition of *ex vivo* Campath®.

AUDIT OF ACUTE ASSOCIATIONS (468 subjects in initial cohort)

NEPHROLOGY

Creatinine was used as an indication of renal function. Of the 76 available for analysis, 47% had acute kidney injury. Dialysis had a poor outcome as reported in the literature. Renal complications occurred frequently mostly due to infection.

CARDIOLOGY

A total of 119 individuals were available for analysis. Echocardiograms and electrocardiograms were part of pre-transplant assessment. Left ventricular systolic dysfunction predicted for increasing post transplant problems. Cardiac complications occurred at a lower frequency than other post-transplants side-effects consistent with the published data.

DERMATOLOGY

Cases were evaluated on a daily basis and referred to a dermatologist when necessary.

To confirm Graft-Versus-Host Disease (GVHD), a skin biopsy was done to differentiate it from drug hypersensitivity or viral infections.

The exposure to *ex vivo* Campath® significantly improved outcome by reducing the incidence and severity of GVHD. Quality of life was enhanced with substantial cost saving.

GASTROENTEROLOGY

Foregut symptoms occurred in 90% of patients. Nutritional problems were encountered. Altered liver functions were relatively common attributable to drugs, sepsis and conditioning regimens. Liver biopsies were not performed in this series and endoscopy performed only when necessary.

A STUDY ON LATE COMPLICATIONS (55 subjects)

RESPIRATORY

Spirometry and diffusing capacity were done in this cohort. All the lung function studies were within the predicted normal range apart from some marginal reduction in diffusing capacity. In none of these patients did late consequences such as Bronchiolitis Obliterans Organising Pneumonia and Late Onset Non-Infectious Pulmonary complications occur. Cytomegalovirus reactivation was common but early intervention prevented serious complications.

IMMUNOLOGY
An *in vitro* functional study was done.

Both the innate and adaptive systems were evaluated. Taken into consideration were the type of transplant, age from transplant, diagnosis and conditioning.

The granulocyte Burst-test was done for the innate profile. Reduced activity was shown in all the subgroups. It appears as if the innate response of the granulocytic cells never recovered due to reduced granulocytic function *in vitro*.

The adaptive responses were evaluated *in vitro* and only the autografts showed better CD4+ and CD8+ cytokine production. No major differences were seen in other groups.

Normal cytokine production by CD4+ and CD8+ T cells were present when these were activated *in vitro* to produce regulatory cytokines, implying that their lymphoid component was intact post-transplant.

BONE DISEASE
Here both the Dual energy X-ray Absorptiometry (DXA) and Quantitative Computed Tomography (QCT) were used to evaluate bone mineral density. There was a discrepancy present between the two modalities. DXA showed no osteoporosis but QCT 22%. Biomarkers were normal in all. There was no history of fracture and no objective evidence of vertebral fractures using vertebral fracture assessment.

Although QCT was used for the study, DXA remains the gold standard in South Africa.

CONCLUSION
This doctoral provided information on the non-haematological consequences in South Africa with the use of Campath® *ex vivo*.

OPSOMMING

HISTORIESE PERSPEKTIEF

Stamsel terapie is voortgesit nadat konyne aanvanklik as navorsingsmodelle gebruik is. Na suksesvolle voltooiing van hierdie proses, is die eerste menslike oorplanting gedoen in 1974.

Sekere voorvereistes was nodig en hierdie was bereik – 'n beskermde omgewing, 'n aferese eenheid, protokolle en akkreditasie by Internasionale Registers.

Aanvanklik is ongemanipuleerde beenmurg- en perifere bloed stamselle gebruik, tesame met immuunonderdrukkende middels, gevolg deur die gebruik van Sikloporien A en daarna die toevoeging van *ex vivo* Campath®.

OUDIT VAN AKUTE ASSOSIASIES (468 GEVALLE IN DIE OORSPRONKLIKE GROEP)

NEFROLOGIE

Kreatinien is gebruik as 'n aanduiding van nierfunksie. Van die 76 gevalle beskikbaar vir ontleding, het 47% akute nierbeserings gehad. Dialise het 'n swak uitkoms gehad soos gerapporteer in publikasies. Nier komplikasies het gereeld voorgekom, meestal as gevolg van infeksie.

KARDIOLOGIE

'n Totaal van 119 gevalle was beskikbaar vir ontleding. Eggokardiogramme en elektrokardiogramme was deel van die pre-oorplanting assessering. Linker ventrikulêre disfunksie was voorspelbaar van verhoogde post-oorplanting probleme. Kardiale komplikasies het konstant volgens publikasies minder geredelik voorgekom as ander post-oorplantings newe-effekte.

DERMATOLOGIE

Gevalle is op 'n daaglikse basis geëvalueer en verwys na 'n dermatoloog wanneer nodig.

'n Velbiopsie is gedoen om "Graft-Versus-Host" siekte (GVHD) te bevestig en dit te onderskei van middel hipersensitiwiteit of virale infeksies.

Die blootstelling aan *ex vivo* Campath® het uitkomste aansienlik verbeter deur die voorkoms en erns van GVHD te verminder. Kwaliteit van lewe is verhoog met aansienlike koste besparing.

GASTROENTEROLOGIE

Boonste gastro-intestinale simptome het voorgekom in 90% van die pasiënte. Wanvoeding het voorgekom.. Abnormale lewerfunksies was relatief algemeen toeskryfbaar aan middels, sepsis en kondisionerings protokolle. Lewer biopsies is nie in hierdie reeks uitgevoer nie en endoskopie slegs wanneer dit noodsaaklik was.

DIE STUDIE VAN LAAT KOMPLIKASIES (55 GEVALLE)

RESPIRATORIES

Spirometrie en diffusie kapasiteit is gedoen in hierdie groep. Al die longfunksie ondersoeke was binne die voorspelde normale waardes behalwe 'n paar marginale afnames in die diffusie kapasitiet. In geen van hierdie pasiënte het laat nagevolge soos Bronchoilitis Obliterans Organiserende Pneumonie en Laat Aanvangs Nie-infektiewe Long komplikasies voorgekom nie. Sitomegaal virus heraktivering was algemeen maar vroeë intervensie het ernstige komplikasies voorkom.

IMMUNOLOGIE

'n *In vitro* funksionele studie is gedoen.

Beide die spesifieke en nie-spesifieke immuun stelsels is geëvalueer. Die tipe oorplanting, tyd vanaf oorplanting, diagnose en kondisionering is in ag geneem.

Die "Granulocyte Burst" toets is gedoen vir die nie-spesifieke profiel. Verminderde aktiwiteite is bewys in al die subgroepe. Dit wil voorkom asof die nie-spesifieke respons van die granulosiete nooit herstel nie as gevolg van die verlaagde *in vitro* granulosiet funksie.

Die spesifieke immuun respons is *in vitro* geëvalueer en slegs die outotransplantaat het beter CD4+ en CD8+ sitokiene produksie getoon. Geen groot verskille is gesien in ander groepe nie.

By CD4+ en CD8+ T selle was normale sitokiene produksie teenwoordig toe dit *in vitro* geaktiveer is om regulatoriese sitokiene produksie te produseer, wat beteken dat hul limfoïede komponent na oorplanting ongeskonde was.

BEEN SIEKTE

Beide die Dubbele energie x-straal absorpsiemetrie (DXA) en Kwantitatiewe rekenaar tomografie (QCT) is hier gebruik om been mineraal digtheid te evalueer. Daar was 'n teenstrydigheid teenwoordig tussen die twee modaliteite. DXA het geen osteoporose getoon nie maar QCT het 22% getoon. Biomerkers was normaal in albei. Daar was geen geskiedenis van frakture en geen objektiewe bewyse van vertebrale frakture met Vertebrale Fraktuur Assessering nie.

Alhoewel QCT gebruik is vir die studie bly DXA die goue standaard in Suid-Afrika.

GEVOLGTREKKING

Hierdie doktoraal verskaf inligting oor die nie-hematologiese gevolge in Suid-Afrika met die gebruik van Campath® *ex vivo*.

ACKNOWLEDGEMENTS

This research project would not have been possible but for the courage of our patients and their families. They continue to be an example of immense fortitude during difficult periods of treatment where outcomes could not be guaranteed. Giving informed consent was founded on trust and confidence in the dedication of the multidisciplinary "Haem–Team". This ethos reflected an appreciation of the commitment of striving for perfection as the goal of delivering a cure by means of individualised, yet inclusive and holistic treatment. It is a privilege to have been part of this unique experience.

A number of people have contributed to this work over the many years, the freedom with which they gave time and shared their knowledge was an invaluable example of all that is best in scientific, academic and intellectual endeavour.

While it would be impossible to thank all individuals who have contributed it is a pleasure to accord recognition to Dr Michael Hayhurst, Mr Wayne Banfield, Dr David Stickles, Dr Agnes Hurter, Professor Elvis Irusen and Mr David Maree for stimulating interaction in the elucidation of the complexities of pulmonary function. Professor Patrick Bouic and Mr Devon Allies helped to document innate and adaptive immune status thereby defining patterns of reconstitution. Dr Richard Tuft and his colleagues generously contributed radiological studies focused on serial changes in the skeleton, with active participation from Professor Stephen Hough and Mrs Riana Eager. Dr Esmé Hitchcock and Mr Gerben van den Hoogen carried out biochemical determinations to explore bone turnover which were complementary to the bone mineral density determinations defined by imaging. Professor Charles Swanepoel provided thought-provoking stimulation into the effects of these procedures on kidney function. Dr Philip Barlow Mills patiently reviewed changes in the cardiovascular system including the heart, Dr Ranks Lehloenya and team for their encyclopaedic knowledge of the skin in its many guises. Dr Melvyn Letier played a central role in the assessment of the many and varied disturbances in the gastrointestinal tract, including nutritional management. Infectious diseases were constantly monitored and debated with Professor Arderne Forder. Professor Peter Beighton provided guidance to the content and balance of the thesis. He is a special and most widely respected colleague of long standing.

Technologists in the dedicated transplantation laboratory skilfully gathered data on routine haematological parameters including bone marrow aspiration and trephine biopsy, flow cytometry, conventional karyotyping and molecular genetics. Helpful comments provided by Dr Munro Marx and his associates were greatly appreciated.

The tissue-typing, which was essential for this work, was carried out co-operatively with Dr MC Botha. Latterly, Professor Ernette du Toit collaborated with Professor Peter Jacobs in establishing the South African Bone Marrow Registry. This endeavour resulted in continued productive interaction with Mrs Terry Schlaphoff and Mrs Veronica Borrill. This working relationship extended to studies for chimerism using short terminal repeat assay and the results have been reported jointly.

From inception it was clear that, particularly in a developing country, accountability was mandatory for these new, costly and high-risk procedures. This viewpoint received strong endorsement from many pioneers in the field over long periods of time. Specifically, a steady stream of acknowledged investigators and transplant physicians established involvement initially with the International and subsequently with the then Autologous Bone Marrow Transplant Registries.

This process culminated in their fusion to form the Centre for International Blood and Marrow Transplant Research directed first by Professor Mortimer Bortin and subsequently by Professor Mary Horowitz. Important benefits were achieved by sharing results with scientists in the European Group for Blood and Transplant Research and the National Donor Program. Registry reporting meant that external and, therefore critical and impartial, audit regularly took place at the highest level. Successful participation in this review process has been maintained at all times, leading to more than three decades of unbroken reaccreditation. To these friends and co-investigators the author gratefully acknowledges their encouragement and the opportunity of working together over the decades.

Progress was facilitated by the ever present compassion of Mrs Yvonne Reynolds, Mr Mike Weber and Mr Richard Vergunst in association with liaison psychiatrists Dr Elizabeth Peter-Ross and Dr Jan Swanepoel. In areas of particular ethical difficulty the beneficial advice was received from Professor Solly Benatar and the staff at the University of Cape Town Bioethics Centre.

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Finally, the stimulus to start, persist with and carry the entire project to a clear and logical conclusion reflects the encouragement of my primary supervisor Professor Peter Jacobs. Over the last 40 years, since introducing these procedures into South Africa, he has ensured that the highest first world academic standards were never compromised in any area under his influence. Simultaneously, research and patient care, commensurate with the best international practices, have been steadfastly maintained by continued interaction with his own role model and Nobel Laureate Professor E Donnall Thomas.

These ideals are recognised and they acknowledge the ever present, but subtle, mentorship necessary for completion of this undertaking. To Professor Peter Jacobs personally as an example of an individual having non - negotiable principles and as a universally respected scientist and academician, I record my special and genuine gratitude. Grateful thanks to Professor Elvis Irusen for his willingness to take over the role as my supervisor at an advanced stage of this dissertation, due to the sad and untimely death of Professor Peter Jacobs.

ABBREVIATIONS

AA	Aplastic anaemia
ABO	Blood groups
A/C	Adults/Children
AKI	Acute kidney injury
ALL	Acute lymphocytic leukaemia
ALLO	Allogeneic transplant
ALT	Alanine aminotransferase
AML	Acute myeloid leukaemia
AST	Aspartate aminotransferase
ATRA	All-trans retinoic acid
ATG	Antithymocyte globulin
ATS	American Thoracic Society
AUTO	Autologous transplant
BAL	Bronchoalveolar lavage
BD	Twice daily
BEAM	Carmustine, Etoposide, Cytosine Arabinoside, Melphelan
BFM	Berlin-Frankfurt-Munster/Muenster
BM	Bone marrow
BMBx	Bone marrow aspirate and trephine biopsy
BMD	Bone mineral density
BOOP	Bronchiolitis Obliterans with organising pneumonia
BU	Busulfan
Bu Cy	Busulfan cyclophosphamide
Bu Cy2	Busulfan 2 days Cyclophosphamide
Bu Cy4	Busulfan 4 days Cyclophosphamide
Bx	Biopsy
CBV	Cyclophosphamide, Carmustine, Etoposide
CD4/CD8	Cluster of differentiation
CIBMTR	Center for International Blood and Marrow Research
CIC	Center Identification Code
CLL	Chronic lymphocytic leukaemia
CML	Chronic myeloid leukaemia
CMML	Chronic myelomonocytic leukaemia
CMV	Cytomegalovirus
COP	Cryptogenic organising pneumonia
CR	Complete remission
CRP	C-Reactive protein
CTR IV	Cytosine Arabinoside, Etoposide, Adriamycin®
CTR V	Cytosine Arabinoside, Idarubicin
CVC	Central venous catheter
CXR	Chest X-ray
CY	Cyclophosphamide
CyA	Cyclosporin A
DIC	Disseminated intravascular coagulation
DILI	Drug induced liver injury
DLBC	Diffuse large B-cell lymphoma
DLCO	Diffusing capacity for carbon monoxide
DLS	Date last seen
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribonucleic acid
DOB	Date of birth
DOT	Date of transplant

DPD/Creatinine	Deoxypiridinoline
DQB1	Alleles
DRB1	Gene
DXA	Dual energy x-ray absorptiometry
E Coli	*Escherichia coli*
EBMT	European Group for Blood and Bone Marrow Transplant
EBV	Epstein Barr virus
ECCS	European Community of Coal and Steel Workers programme
ECG	Electrocardiogram
ECHO	Echocardiogram
EMLA	Lidocaine prilocaine eutectic mixture
EORTC	European Organisation for Reseach and Treatment of Cancer
ERS	European Respiratory Society
ESR	Erythrocyte sedimentation rate
ET	Essential Thrombocytosis
ETI	Etoposide, 6-Thioguanine, Idarubicin
FA	Fanconi Anaemia
FAB	French-American-British
FBC	Full blood count
FDA	Food and Drug Administration
FEF25-75	Forced expiry flow rate at 25 – 75% of forced vital capacity
FEV1	Forced expiratory volume in the first second
FEV1/FVC	Forced expiratory ratio
FISH	Fluorescent in situ hybridisation
FLAG	Fludarabine, Cytosine Arabinoside, G-CSF
Fmlp	Formyl-Methionyl-Leucyl-Phenylalanine
FVC	Forced vital capacity
G	Gram
G-CSF	Granulocyte colony-stimulating factor
GEC	Gastroenterology consultation
Geog	Geographical
GGT	Gamma-glutamyl transpeptidase
GI	Gastrointestinal
GIT	Gastrointestinal Tract
GM-CFUc	Granulocyte macrophage colony-forming unit
GM-CSF	Granulocyte macrophage colony-stimulating factor
GVHD	Graft-versus-host disease
HCL	Hairy cell leukaemia
HES	Hydroxyethyl Starch
H Pylori	*Helicobacter Pylori*
"Haem Team"	Haematology Team
HIV	Human immunodeficiency virus
HL	Hodgkin's lymphoma
HLA	Human Leucocyte Antigen
HYDREA®	Hydroxyurea
IBM	International Business Machines
IBS	Irritable Bowel Syndrome
ICE	Ifosfamide, Carboplatin, Etoposide
IFN	Interferon
IL4	Interleukin 4
INF	Infections
IFN-y	Interferon gamma
INR	International Normalised Ratio
IQR	Interquantile range
ISCH	International Society for Clinical Densitometry

ISCT	International Society for Cell Therapy
IVI	Intravenous infusion
J Lines	Jacobson lines
JACIE	Joint Accredited Committee of ISCT
Kg	Kilogram
L	Litre
LBBB	Left bundle branch block
LD	Lymphocyte depleted
LDH	Lactic Dehydrogenase
LFT	Liver function test
LL	Lymphocytic lymphoma
LONIP	Late onset non-infectious pulmonary complications
LP	Lumbar puncture
LTFU	Lost to follow up
LVEF	Left ventricular ejection fraction
m^2	Meter squared
MDS	Myelodysplastic syndrome
MESNA	2-Mercaptoethanesulfonate
MFC	Mitomycin 5-Fluorouracil
Mg	Milligram
MINI-ICE	Idarubicin, Cytosine Arabinoside, Etoposide
Ml	Milliliter
MM	Multiple myeloma
MS	Mixed-cellularity
MUD	Matched unrelated donor
MYELOPROLIF	Myeloproliferative syndrome
N	Number
Ng	Nanogram
NHANES 111	Third National Health and Nutrition Examination Survey
NHL	Non-Hodgkin's lymphoma
NK cells	Natural killer cells
NMDP	National Marrow Donor Program
NS	Nodular sclerosing
N & V	Nausea and vomiting
OBS	Observations
PA	Posterior Anterior
PBSC	Peripheral blood stem cells
PCR	Polymerase chain reaction
PEFR	Peak Expiratory Flow Rate
PET	Positron emission tomography
PKC	Protein Kinase C-activator
PMA	Phorbol-myristate acetate
PPI	Proton pump inhibitor
PR	Partial remission
PRA	Pure Red Cell Anaemia
Pro-BNP	Brain natriuretic peptide
p-value	Probability value
P Vera	Polycythemia Vera
Q1	1^{st} Quantile
Q3	3^{rd} Quantile
QCT	Quantitative computed tomography
RA	Refractory Anaemia
RAI	Staging developed by Dr Kanti Rai
RAEB	Refractory Anaemia with excess blasts
RARS	Refractory Anaemia with ringed sideroblasts

RBBB	Right bundle branch block
REAL	Revised European American Lymphomas
RFC	Rituximab, Fludarabine, Cyclophosphamide
RV	Residual volume
SABMR	South African Bone Marrow Registry
SCID	Severe combined immunodeficiency
SCT	Stem cell transplant
SD	Standard deviation
SS	Sickle cell anaemia
T	Transfusion
TC	Transplant Centre
T cell	T-Lymphocyte
TH1	"T" helper cell
TH2	"T" helper cell
THAL	Thalassaemia
TLC	Total lung capacity
TLCO	Carbon monoxide transfer factor
TNC	Total nucleated cells
TPA	Tissue plasminogen activator
TPN	Total Parenteral Nutrition
TTP	Thrombotic Thrombocytopenic Purpura
USA	United States of America
VECD	Vincristine,Epirubicin, Cyclophosphamide,Dexamethasone
VFA	Vertebral fracture assessment
VOD	Veno-occlusive disease
Vol Excl	Voluntary exclusion
WHO	World Health Organisation
µg	Microgram

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SYNOPSIS

CONTENT

The inception and evolution of stem cell therapy in Cape Town formed the subject of this thesis. In chapter one, an overview is given of the study done. In chapter two, the historical development of the Transplant Team and their organisational facilities are outlined. International accreditation is a major factor in the transplantation process and chapter three deals with accountability to various registries. In chapter four, technical details of the procedure are provided and chapter five outlines the complications that may occur. In chapter six overall survival data is presented from the audit of the complete cohort of transplanted patients. Chapter seven outlines the results from the acute associations and late effects occurring in the non-haematopoietic tissue. This is followed by a concluding summary.

ETHICAL APPROVAL

Special attention was given at all times to ethical considerations. The patients who participated in the protocols did so with fully informed consent documented as part of the written case record. The experimental work in this thesis was systematically translated into clinical research programmes over more than twenty years. These were carried out collaboratively, presented and reported jointly with Professor Peter Jacobs. Specifically there was full participation from both investigators in data collection, analysis, critical review and literature citation leading to preparation of the manuscripts involving response to editorial review and publication. Inclusion of the segments, needed to keep perspective balanced, was approved by supervisors, the Health Ethics Research and PhD Evaluation Committees as in no way conflicting with scientific principles. Rather, such studies constituted an integral part of the long-running project and did not infringe the rules of information sharing. In particular they were fully compliant with the regulations pertaining to plagiarism.

ILLUSTRATION

All tables and figures have been listed. Permission was granted and acknowledged as appropriate. The remainder was obtained from the Haematology Department archives.

METHOD FOR REFERENCES

The Vancouver Referencing Style was used, cited at the end of each chapter and then alphabetically at the end of the thesis.

IMMUNOLOGY RESULTS

Because of immune system terminology, repetition of words was unavoidable.

CHAPTER 1

THE PROJECT IN OVERVIEW

FOCUS

Stem cell transplantation is an increasingly used life-saving procedure. Laboratory and clinical advances continue to broaden the indications and improve outcome whilst simultaneously exposing a greater number of recipients, and donors, to complications which may be relatively common early associations or only emerge as late effects. Awareness of haematopoietic, and non-haematopoietic, sequelae underlie the concept of survivorship which can be viewed as cure with care. These correlations, which are the core of this long running clinical and laboratory research project, were acknowledged by a systematic audit, to define the complete data set. More than three decades of paediatric and adult experience with allogeneic and autologous grafting carried out by a single internationally accredited team have been recorded. Initially, the donor source was bone marrow but subsequently it was extended to the use of peripheral and umbilical cord blood. These modifications facilitated clear delineation of the physiologic pattern by which immunohaematopoietic recovery took place. It was in this cohort that the two distinct groups of tissue injury were defined outside the process of blood formation. In the first three months after transplant almost uniform and typically reversible acute abnormalities of kidney and cardiovascular system function were analysed together with a range of short lived reactions in the skin. Gastroenterological problems, including the challenge of maintaining nutrition, were also seen. With longer survival, typically beyond one year, a quite different spectrum of late effects emerged in the lung, skeleton and in the process of immunologic reconstitution. Accordingly, in a group of surviving transplant volunteers a selected panel of tests was performed. This study was the main theme of this PhD thesis.

1.1 INTRODUCTION

By way of orientation and justification a brief note is needed for better appreciation of the reason behind the introduction of new haematologic high technology into South Africa. This process took place at a time when transplantation had recently emerged in first world academic centres. It had tentatively been accepted as a realistic therapy which had clinical applicability.

The reason and decision can be traced back to two events.

The entire field was given enormous impetus at the University of Cape Town Medical School by the success of the first human heart allograft carried out in the Groote Schuur Hospital by Professor Christiaan Neethling Barnard.

In this environment, pulsating with advances in immunology and tissue typing required to support expansion of cardiac and other solid organ exchanges, came the University Council decision to formally and fully integrate every aspect of clinical and laboratory haematology. This innovative restructuring was made possible by the establishment of an entirely new and autonomous department for the comprehensive study of blood and bone marrow. It was modelled on the facility at University of Washington in Seattle led by Professor E Donnall Thomas and his rapidly growing multidisciplinary team. The motivation was based in scientific, academic and intellectual linking of numerous already strong disciplines in the school. Equally relevant and important, was the potential of major benefit for all patients in South Africa, where this new technology was destined to find a specialised niche.

Looking back on the foregoing circumstances, those who shared the vision and supported the imperative will be gratified with the current position enjoyed by the South African immunohaematopoietic stem cell transplantation programmes in both local and international settings.

The author was involved in these endeavours and seized the opportunity to systematically and serially document her early experience.

The process represents a practical means of highlighting possibilities for further research into phenomena that have received less attention than initially thought to be appropriate. Patient survivals increased and the short and longer term adverse impacts on a number of systems outside the blood and bone marrow increasingly became evident. There was already a worldwide appreciation of how previously unsuspected injury, ranging from occult to severe, produced increasing morbidity and mortality. These variables constitute a poorly defined group but nevertheless are an important arena which presents ethical, scientific and clinical questions which need to be addressed.

Historically two consecutive phases can be described.

The entire intact database, from the time that the first human stem cell transplant was carried out in 1974, records a detailed description of all technical, nursing and medical aspects as they evolved over the last 37 years.

From this exercise was synthesised a consolidated model that could be applied, clinically and in a structured post-transplant follow-up protocol, to precisely document those pathological differences that were already taking place. At the time these were largely unappreciated, yet, had a potential impact on the conventional endpoints of outcome.

Building on this foundation, a carefully organised and systematic analysis was undertaken in a series of patients to delineate these underemphasised risk factors for both quality and duration of life. This endeavour occupied a period of 15 years during which time the observations were focused on the concept of survivorship**.** Stated differently, these phenomena which encapsulated wide ranging changes in the internal environment affecting a number of primarily non-haematopoietic organ systems, gathered sufficient momentum to justify a formal research application.

This imperative shifted focus from the traditional retrospective preoccupation with events that have already resulted in recognisable symptoms and signs defined as side-effects or complications, to an approach seeking to recognise potential risk factors. These influenced quality of life and, being amenable to intervention, dictated a more innovative restructuring in the years following stem cell grafting.

The rationale for these clinicopathological studies that were cross-disciplinary by intention and design, was to examine the impact of profound alterations in physical and mental status which are an integral part of the subtly altered lifestyle that follows these procedures. These have only recently started to find acceptance but appear to be potentially avoidable consequences with steadily improving benefits. This is being made possible by increasing sophistication and understanding in diagnosis followed by proactive management. The whole area is of immense and ever escalating importance where, for example, the source of immunohaematopoietic stem cell has moved from marrow to blood, now to umbilical cord and, likely in the future, to amniotic fluid.

Patient preparation continues to oscillate between myeloablative and reduced intensity or essentially only immunosuppressive regimens. The concept of autografting as a form of gene therapy and the challenge of embryonic stem cells opens up a whole new landscape, both conceptually and functionally, that offers movement from replacement to regenerative medicine. Central to this aspect of biology are the recipients. While fully supportive of the rapidly growing and wider frontiers, there is the obligation to give careful attention to the frequently, overlooked changes in persons who survive these costly and hazardous interventions. Into this equation must now be factored potential risk to donors over long periods of follow up.

For these reasons, the scope of this 37-year research imperative focused firstly on establishing the facility, then refining the protocols and confirming conventional outcomes in children and adults. Once achieved, early associations that were typically reversible were identified. Focus then shifted priority to quantitating these problems as more long survivors were added to the follow-up clinic. It was at this point that the stimulus arose to study selected extra haematopoietic organ systems following stem cell transplantation.

1.2 REFERENCE COHORT (1972-1994)

The 22-year observational data set, which comprised consecutive individuals registered from the start of the experimental programme, which was translated into clinical practice in 1974. Every person, from that date until 2010, was reported to the International and Autologous Bone Marrow Transplant Registries which merged to form the the Centre for International Blood and Marrow Transplant Research. This practice continues to the present date.

The same outcomes were concurrently recorded in the European Group for Blood and Bone Marrow Transplant registry. When donors were sourced from the National Marrow Donor Program, these transplants were additionally reported to the latter registry**.**

This audit of technical details was used to recognise changes within each of the selected organ systems which occurred with sufficient frequency as to pose a future risk. It was the motivation for the exploration of lesions outside the usual end points of transplantation. Although all patients were reported to the registries, none of the data was obtained from them.

There followed a 15 year segment (1995-2010) generated by all patients detailed in the comprehensive record (**Annexure 1)**. These were registered in the Department of Haematology and Bone Marrow Transplant Unit incorporating The Searll Research Laboratory for Cellular and Molecular Biology in the Constantiaberg Medi-Clinic. This became a designated satellite of the Division of Clinical Haematology - Department of Internal Medicine at Stellenbosch University and Tygerberg Academic Hospital.

1.3 AUDIT OF ACUTE ASSOCIATIONS

The audit contained data extracted from the patients' records. Four organ systems were chosen as they were affected most frequently and sufficient data was available to analyse these. This damage typically occurred in the neutropenic period after which, almost universally, resolution took place in the majority of cases.

1.3.1 NEPHROLOGY

Biochemical disturbances were a frequent occurrence which were not only exacerbated by fluid and electrolyte imbalance, but also by a wide range of infections where nephrotoxic antibiotics compounded abnormalities in biochemical tests. Whether any of these drugs as well as immunosuppressive regimens, particularly cyclosporin A, affected these special approaches additionally to routine treatment were evaluated. This calcineurin inhibitor was largely reserved for the matched unrelated allografts in the present study and not routinely employed.

1.3.2 CARDIOLOGY

The heart can precipitously malfunction and was specifically monitored during the first three months after transplantation. This was undertaken by constant clinical review supported by a selected series of tests including 12 lead electrocardiogram and echocardiogram. Thereafter, serial studies were continued in order to characterise any emerging adverse effects.

1.3.3 DERMATOLOGY

The skin has a large surface and disturbances arose in two distinct ways. Frequent rashes reflected a range of allergies and viral infections most of which were readily recognisable, transient and had little lasting importance. A notable problem was the engraftment syndrome typically in persons undergoing autologous transplantation and not initially recognised.

Particularly challenging manifestations were found after allogeneic grafting in the form of graft-versus-host disease (GVHD). Here a uniquely different regimen has been standardised following introduction and continuous use of *ex vivo* T cell depletion using the Campath® series of monoclonal antibodies. This approach was named the in-the-bag technique.[1] The innovation created a distinct model when compared to unmanipulated marrow used in many other centres. The latter required a range of immunosuppressive agents after the procedure, notably corticosteroids and cyclosporin A, among others.[2]

The only variation in the present programme was a subset created by introduction of matched unrelated volunteer donors with varying degrees of histoincompatibility. In this setting, immunologic phenomena, exemplified by graft-versus-host disease, were anticipated and a tapering dose of cyclosporin A was used to blunt the expression of an unusual variant or *forme frust* of the acute syndrome. This distinctive cutaneous lesion was different in duration and severity from the more traditional acute and chronic manifestations of the immunologically mediated change arising with infusion of unmanipulated grafts.[1] Contrastingly, cyclosporin A given to donors and recipients produced a superficially similar benefit but did not abrogate either acute or chronic variant[3] – these were a feature only of the Campath® in-the-bag technique.

1.3.4 GASTROENTEROLOGY

Specific focus was given to the incidence and management of mucositis with pain control and hygiene extending to the foregut and small bowel that overshadowed colonic dysfunction. Typically, both were self-limited and reversed promptly with engraftment. The aetiology was often viral ranging from herpes simplex to cytomegalovirus and concurrently disturbed liver enzymes, particularly cannalicular enzymes such as alanine aminotransferase (ALT) which corrected as the marrow recovered.

Liver biopsy was unnecessary in almost all cases as dysfunction and injury were meticulously surveyed and their normalization documented. A particular aspect was a long-term experience of enteral nutrition analysed in terms of practical endpoints that included weight stability and serum albumin estimation. Over this entire period, reversal to intravenous supplementation was seldom necessary. The point had practical relevance in safety and quality of life as well as cost containment. Specifically, the early placement of a fine-bore nasal feeding tube, into the jejunum rather than into stomach, was proven to be effective. This outcome is thought to represent a significant contribution from the study.[4] This approach had virtually no concomitant bacterial or fungal infectious complications. Similarly there were no problems with thromboembolism which can complicate parenteral nutrition. Serious efforts to examine supplements, particularly the impact of glutamine, were not possible due to lack of funding. In selected cases, laboratory studies were supplemented by endoscopy with biopsy but these were few and insufficient for any significant conclusion.

1.4 STUDY POPULATION REQUIREMENTS

The sustained reporting to registries which ensured international surveillance, transparency and accountability, has remained unchanged. Optimum communication culminated in fully informed consent for each of the management protocols.

All these activities were notified to the Institutional Review Board, known as the Committee for Clinical Governance which resided in the Mediclinic Corporation. The cross-sectional research project on a group of post transplant volunteers was approved by Stellenbosch University Committees for Health, Research, Ethics and PhD evaluation. It is important and relevant to note that no new drugs were tested nor were any randomised trials undertaken.

1.5 CHARACTERISATION OF LATE EFFECTS

The reason the following three organs were selected for the study was due to the availability of specialised assistance to do these tests with no cost to the patients. Additionally, we had very little data available on pulmonology, immunology and bone disease due to financial constraints. A group of surviving volunteers was recruited for this study **(Annexure 2)**.

1.5.1 PULMONOLOGY

The majority of the cohort was clinically asymptomatic in terms of exercise tolerance and none had a proven diagnosis of lung pathology. Accordingly the starting point for the evaluation of any silent injury was consideration of potential damage from exposure to a range of chemotherapy programmes that had been given to obtain disease control prior to transplantation.

The next stop was to reflect on the toxicity profile of agents, including radiotherapy, and high-dose multiple drug administration for immunosuppression during the preparatory phase. The airways and pulmonary parenchyma are targets for damage some of which may be subtle yet irreversible. Accordingly, a series of specific studies was selected for the ability to demonstrate impaired function with reference to bacterial as well as viral insult.

The patients were at risk of reactivation of the cytomegalovirus. This possibility received extra attention including consideration of the relative importance of laboratory studies ranging from serology to the polymerase chain reaction. Such information was the basis for pre-emptive intervention. The approach included therapy with valganciclovir in an attempt to prevent progression to the potentially lethal complication of pneumonitis or other, less frequent, inflammatory changes in the gut and liver.

A second more ominous lesion reflected acute and subsequently chronic graft-versus-host disease. These immunologic phenomena were shown to be of much less frequency and severity in patients conditioned with the monoclonal antibodies.[1] This observation provided a strong impetus for the evaluation of subclinical changes in the respiratory system and comparison of findings to published data, particularly non-infectious sequelae.[5]

Many of the carefully selected tests were limited for financial reasons in the early phases of transplantation.

For this reason, collaboration with the Division of Pulmonology at Stellenbosch University and Tygerberg Academic Hospital was undertaken to define lung function and correlate any changes with the type of procedure or, secondarily, the underlying disease where protocol management might have included potentially damaging interventions.

1.5.2 IMMUNOLOGY

Although opportunities were not initially available to critically characterise innate and adaptive competence, there was nevertheless appreciation that integrity in both aspects were generally known to be impaired. Risk factors included the type of procedure, conditioning and the use of post-transplant and extended immunosuppressive regimens.

Again, an opportunity was created to contrast selected function tests between cohorts of patients prepared for transplantation with *ex vivo* CD 52 antibody.

1.5.3 BONE DISEASE

The fundamental comparison started with simultaneous dual energy x-ray absorptiometry (DXA) and quantitative computed tomography (QCT) imaging and extended as relevant results accumulated. These were supplemented with biochemical measurements of bone turnover.

1.6 ORGANISATION AND TIMELINES

These were compliant with the university rules and regulations for doctoral studies. Specifically, each segment of work was interpreted statistically and the local results analysed. The entire thesis is supported by selective review of the literature. In 2011 new ethics approval was needed and took approximately six months to finalize. Appoval was obtained from Tygerberg Hospital where the studies were done. Patient recruitment occupied from April-July 2012. The various tests started in August 2012 and completed February 2013. Audit data was collected January 2012 to January 2013. Statistical analysis was done from March 2013 to July 2013. Literature review and writing the thesis followed next. The preliminary analysis was completed during the course of 2013 and the completed project submitted for examination in 2014.

1.7 SUMMARISING COMMENT

This project has occupied 37 years (1972-2010). In the initial two decades, the aim was to participate, as an international centre, in setting up the transplant programme in South Africa. During that period the clinical programmes were refined, ensuring that data was available on every case from inception. Once the necessary infrastructure was standardised, analysis was done on audited data to establish outcome on both children and adult patients. Concurrently, identification of non-haematologic consequences in selected major target organs was undertaken and these were then systematically defined. The comprehensive project led to a model for immunohaematopoietic stem cell transplantation that has specific relevance in South Africa.

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CHAPTER 2

TRANSPLANT PROCEDURES IN SOUTH AFRICA

A SHORT HISTORICAL PERSPECTIVE

FOCUS

Physiologically, irreversible bone marrow failure is incompatible with life despite availability of short-term therapy with red cells and platelets. Pathophysiologically, the aetiology may be congenital or acquired and benign versus malignant. Therapeutically, the challenge is to favour autologous reconstitution wherever possible. Alternatively, replacement of this entire organ system with one or other form of stem cell grafting may be undertaken. Experimental haematology has demonstrated, by means of animal models and in vitro culture systems, the existence of cells with repopulating capacity that home to niches in the bone marrow microenvironment. Translational research established that clinical applicability and ongoing scientific endeavour improve outcome, broaden indication, refine technique and now increasingly focus attention on patient consequences in the concept of survivorship. Three circumstances predicated the decision in 1972 to commit the future direction for the newly established and autonomous Department of Haematology in the Faculty of Health Sciences at the University of Cape Town to immunohaematopoietic stem cell transplantation under the Headship of Peter Jacobs as Foundation Professor. There was already, in other parts of the world, a clear demonstration that these procedures were potentially life-saving when properly used. Protocol studies for haematologic malignancy were introduced locally and some of these would require transplantation to achieve outcomes comparable to international standards of care. No such treatment options existed on the African continent. It was relevant that a research environment, including tissue-typing, existed at the Groote Schuur Hospital to sustain the impetus created following the first human heart transplant by Professor Christiaan Barnard.[1]

2.1 INTRODUCTION

Formal inclusion of this brief historical orientation in the thesis is based on three facts. The awareness of side effects only became possible with accumulating experience of bone marrow transplantation in South Africa. In the start-up period, many short term symptoms and signs were recognised as therapy-related and reversed with engraftment: these are interpreted as early associations. The focus of this study was damage outside the blood and bone marrow, which was sufficiently recognised to justify a research study designated as late effects. In recognition of this time frame, the Faculty Committees accepted the relevance of preceding development as integral to this whole PhD study.

2.2 THE PHYSICAL FACILITY – GROOTE SCHUUR HOSPITAL

Initially the Department of Haematology had no beds or clinic space. There was only a small laboratory for blood clotting tests in C8 and space on the F floor adjoining the Falconer Lecture Theatre for routine haematological service including morphology. Brief comment is given to this start-up allocation since the unusual *esprit de corps* which was engineered was sufficient to anchor all members of staff through the difficult period ahead as the "Haem Team". Space for research and development did not exist: an office was provided on the fifth floor in the somewhat remote Anatomy block on the medical school campus. Thereafter, with a research building being developed under the chairmanship of Professor Eugene Dowdle, it was possible to obtain a dedicated area for the first time and concurrently to integrate the administrative components. This challenging mandate was important in the forging of a functional discipline that would permanently and inseparably fuse clinical and laboratory medicine in line with the accepted world standard.

How did the bone marrow transplantation unit develop?

As the momentum gradually increased, it was possible to create an outpatient clinic, group in-patients on the D floor and to consolidate the staff, including dedicated nurses and pharmacists. Representatives from the Departments of Physiotherapy, Logopedics and Social Work were significant additions. These innovations established the future hallmark of the Department of Haematology – a morning report followed by walking rounds in which the entire group discussed the referrals that always accommodated each patient and their families.

It was now time to take the next step.

The legacy in which benign or malignant disorders of the haematopoietic system were treated by the admitting medical firm resulted in a lack of any statistical base and random management. With willingness by the haematologists to accept responsibility for all these cases within the Teaching Hospitals Group, it became possible to change this entrenched practice. The development provided an opportunity to work very closely with other departments including Radiotherapy and Nuclear Medicine, as well as Pharmacology and the major disciplines. This resulted in the introduction of combined clinics for lymphoma, myeloma and leukaemia where protocols had a final common pathway to one or other form of transplantation.

Shortly there followed the opportunity to document and report outcomes to European and American Transplant Registries. Such a model remains evident today and has become part of other transplant facilities which are developing in South Africa.

As results were published from major centres in Europe, Australasia and particularly the United States of America, the emerging role of bone marrow transplantation moved up the South African agenda. It was unlikely that these procedures could have been started without some special dispensation, and cardiac and renal groups came to the rescue by making space available in the newly built Heart Tower. At the same time a day ward was created as the link between the transplant unit and dedicated beds for chemotherapy.

This was the status of the new haematological programme at a time when money was allocated to re-build Groote Schuur Hospital. The government of the day decreed that it was to match and be based largely on the recently completed gigantic Tygerberg Academic counterpart.

Despite numerous petitions to use a completely different or radial design with central facilities and specialised service, this fell on deaf ears and the plans proceeded for the new monolith. Nevertheless haematology received a fair share of general bed allocation and input to planning design with the result that almost all of the previous limitations were overcome.[2]

2.3 HAEMATOLOGY CONSOLIDATED – NEW GROOTE SCHUUR HOSPITAL

Without exaggeration the many long hours, days, weeks and eventually years to commissioning the redeveloped facility launched the completely redesigned Department of Haematology finally into the modern era. This was achieved by integrating various activities by having all the staff, except the developmental positions, in a single geographical area. This new arrangement made possible the ability to deliver appropriate interaction between the reverse isolation unit, custom-built for administering high-intensity chemotherapy programmes, managing neutropenia and meeting international standards for bone marrow transplantation.

Throughout this exercise there was the sharing of engineering knowledge on such things as filters and all the associated aspects of laminar down-flow units which were magnanimously made available by our American colleagues in Seattle. The net result was a highly efficient and state-of-the-art protected environment for safe bone marrow grafting with emphasis on reverse isolation that played an important role during periods of profound low white cell counts.

2.4 CREATING AN INFRASTRUCTURE – APHERESIS TECHNOLOGY

Advances in immunohaematology, accelerated by better understanding of transplantation biology, gave impetus to the provision of a sound blood transfusion practice. At this time packed red cells were delivered in glass bottles and platelets supplied as single units without any quality control either by the user or prescribed by the service provider! The regular visits to the MD Anderson Hospital and Tumour Institute in Houston brought contact with Professor Jeane Porter Hester.

She had been developing the physics and engineering facilities required for continuous blood flow separation in collaboration with International Business Machines (IBM). It was clear that, if the serious intention was to set up this new and innovative programme then, somehow, comparable advances needed to be available in the department. Fortunately, donations were obtained and the model 2990 apheresis system, with a disposable polycarbonate bowl, was delivered for introduction of this technology in Africa.[3] Paradoxically, although these instruments are now commonplace in commercial blood banks the first use, and all the refinements, took place in Groote Schuur Hospital.[4] This close association continues to the current day and the prototype South African apheresis unit was named in respect and honour of this extraordinarily capable lady colleague and scientist.

To be fully compliant with prevailing transfusion regulations, both hospital and university administration obtained authorisation from the Department of Health for the establishment of a dedicated research programme in this area. There was a solitary proviso that the products, other than stem cells, would not be stored and it is a matter of record that volunteers flocked to donate essential granulocytes[5] and platelets.[6] It was now possible to add expertise that had been accumulated at the Royal Marsden Hospital in the United Kingdom via an exchange programme that brought nursing staff, with a particular interest in these procedures, to the unit.

This was an important acknowledgement by the licensing authority because, although with restricted approval of the Western Province Blood Transfusion Service in the form of their medical director, it created a precedent. The arrangement was vested within the Teaching Hospitals Group – the Professor and the Nursing Coordinator with the latter given overall charge of this special area. At no time, from the first trial run to the establishment of a flawlessly operated system that was constantly updated and refined, was any serious adverse event encountered. Indeed this became the acknowledged training centre for South Africa and extended to new groups embarking on similar approaches.

2.5 MULTIDISCIPLINARY MANAGEMENT TEAM

Based on the constantly changing complex symptoms and signs arising during the course of transplantation, typically preceded by complicated treatment programmes dictated by the underlying disease, it was early acknowledged that the model so well developed in Seattle would be employed as reference point.[7] A number of visitors from academic medical centres worldwide personally coordinated much of that development. The Haematology Staff ("Haem Team") defined a holistic or cross-disciplinary approach that recognised and constructively responded to the crucial role played by the paramedicals. Impressively, one of the concessions made by hospital management to this somewhat unusual continuum was of a trained and experienced scientist constantly moving between clinics, dedicated ward and transplantation unit with very particular focus on rapid access through the outpatients. This was achieved by creating a Chief Professional Nurse level post designated Haematology Coordinator. This position had already been widely acknowledged and implemented by transplant units elsewhere in the world and shown to function most efficiently.

Added to the growing team was the increasingly integrated contribution from Physiotherapy, Logopedics, Pharmacy, Dietetics and Social work.

Consultants from other specialities, notably from the Infectious Diseases service played an important role as did a psychiatrist. This step blurred the previous emphasis between physical and other emotional disturbances that required careful attention in maintaining quality of life of the patients.

2.6 RELOCATION TO A PRIVATELY BASED ACADEMIC CENTRE

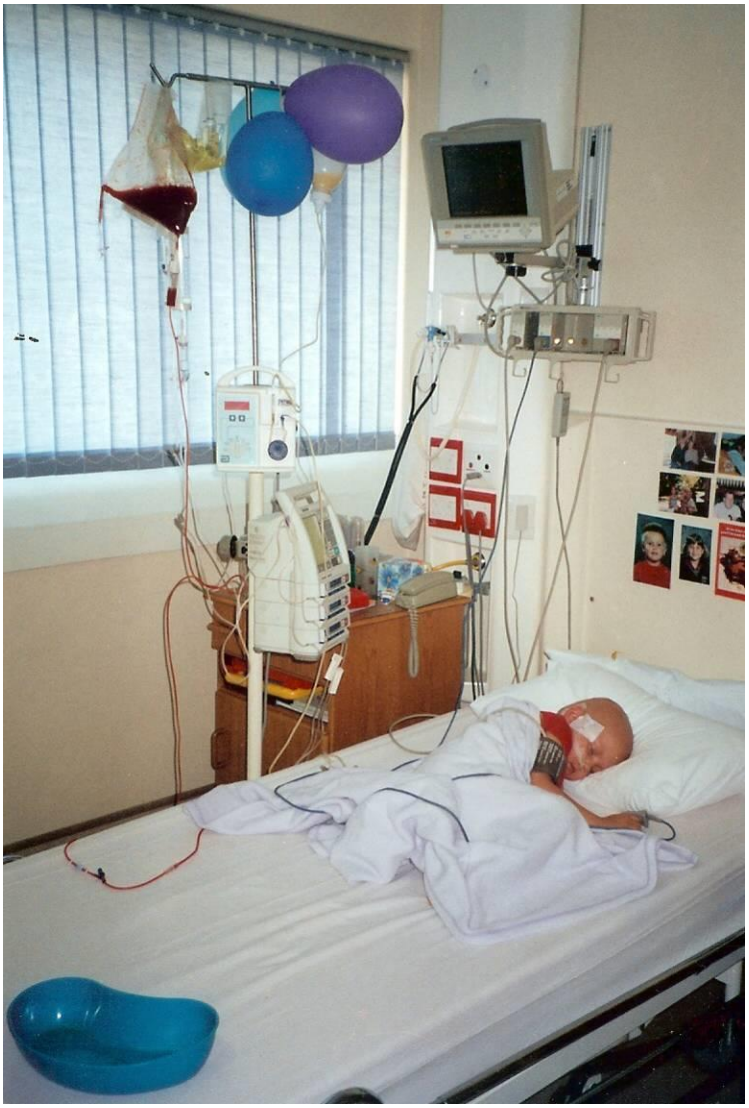
Changing circumstances in health care delivery provided an opportunity to test the new concept that the established level of first world haematology could be duplicated in the private sector. To this end a University style department was successfully created by building a corresponding facility incorporating all the matching technology in the Wynberg Hospital. With steady growth, relocation became necessary to accommodate the need for space which at that time was only available at the Mediclinic Constantiaberg.

The initial renovation of a paediatric ward to include an eight-bed reverse isolation unit founded on the experience of the prototype in Groote Schuur Hospital and refined in Wynberg proved efficient and functional. This self-sufficient unit had access control and comprised eight twin rooms – one of each pair for the patient with monitoring facilities including dialysis and linked to a central nursing station; the other for medication storage and preparation for procedures. **(Figure 1)** A complement of dedicated and trained professional nursing staff operated the unit as part of the holistic team: in-house special transplantation training was provided. This approach consolidated the holistic core of an integrated or cross-disciplinary team with strong management based in the daily report.

A self-sufficient transplantation laboratory with all the necessary equipment and expertise for cryopreservation of grafts was included as well as space for consulting rooms and a conference centre. With additional pressure to centralise the increasing number of haematology referrals, a completely new wing was built and named the Sunflower Haematology Ward. This achievement facilitated continued reporting of every consecutive case to registries and unbroken accreditation as an internationally designated transplant, harvest and donor centre.

The final step was the submission and approval of the recognition as a satellite within the recently established Division of Clinical Haematology in the Department of Internal Medicine of the Faculty of Health Sciences at Stellenbosch University.

FIGURE 1
CHILD RECEIVING STEM CELLS IN A PROTECTED ENVIRONMENT



Courtesy of child's parents

2.7 SUMMARISING COMMENT

This is the brief historical background to the introduction and development of immunohaematopoietic stem cell transplantation in South Africa. Careful attention to the clinical management, over time, shifted attention from primarily haematopoietic patterns of recovery to more subtle deviations outside blood and bone marrow. As such, evolution of the protected environment played a significant role in the comprehensive care of recipients. Control of external factors that impacted on injury to non-haematopoietic tissues – some common, rapidly reversible and others of late onset. It is unrealistic to try and interpret the organ changes without an awareness of how stepwise improvements in the physical facility were modified to keep pace with demands generated by actual experience. Stated differently, the direct involvement in setting up and testing the prototype was part of arriving at the final model used in this country. Superimposed on this structure was the systematic generation of protocols, based on worldwide standards that were consolidated with experience. At all stages survival was matched to corresponding outcome published from reputable units in Europe and America. This model was used to promote dissemination elsewhere in South African Universities and ultimately spread to the private sector.

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CHAPTER 3

ACCOUNTABILITY AND ACCREDITATION

FOCUS

Continued acceptance of case referrals by registries that generated the research audit, was of such importance that it has been assigned separate presentation. Three crucial confirmatory statements apply to the study and thesis, each in their entirety. All the experimental and first two decades of clinical work had prior approval and ongoing authorisation by the local University of Cape Town Ethics and Research Committees. Consecutive reporting of details for each and every patient documented to time of death or remaining on study, under constant review by these groups, guaranteed compliance with conduct in keeping activities aligned to international codes. The participation in each, as a designated investigator, ensured observation of rules governing patient care as standard of practice and clinical research. Concurrently, on the basis of completed demographic capture sheets that have been subject to critical analysis, there has been accreditation remaining unbroken over three decades. These collaborative experiences, that include both joint reporting and incorporation of data into international publications, span the entire research project of some 37 years and continue actively to the present. This status reflects independent review of all aspects that included inspection by committees from the Centre for International Blood and Marrow Transplant Research and from the National Marrow Donor Program.

3.1 INTRODUCTION

Although the importance of data verification was appreciated and implemented when transplantation was started, the major impact of this exercise on the evolving research project could not have been anticipated at the time when marrow grafting was first undertaken. Commitment to transparency and external review subsequently added authenticity to the entire listing of patients, in the reference cohort, and of post-transplant volunteers participating in the thesis protocol. For this reason, the approval from the different registries remained an important criterion. Accreditation was done on a yearly basis depending on compliance to certain requirements. Reporting now only involves the Center for International Blood and Marrow Transplant Research (CIBMTR), the European Group for Blood and Bone Marrow Transplant (EBMT) and the National Marrow Donor Progam (NMDP) if donors were recruited from the latter registry

3.2 INTERNATIONAL BONE MARROW TRANSPLANT REGISTRY

This registry was the only peer review group available when allografting was introduced in South Africa. Once application was approved, including review of curriculum vitae of the investigators, a probationary period was followed by membership with our data incorporated into the registry's reports.[1] This registry became part of the CIBMTR and was discontinued.

3.3 AUTOLOGOUS BONE MARROW TRANSPLANT REGISTRY

As technology became better established and dual reporting fully compliant, our designated team was assigned a centre number and each individual case was registered.[2] We initially reported to them but this registry was subsequently incorporated into CIBMTR.

3.4 CENTER FOR INTERNATIONAL BLOOD AND MARROW TRANSPLANT RESEARCH – (TRANSPLANT CENTRE – TC – 348) - CIBMTR

With the explosion of hospitals all over the world undertaking stem cell grafting, a profusion in the number of registries has resulted in this parent body being established. Reporting continues in full compliance with the updated rules and regulations.[3]

3.5 EUROPEAN GROUP FOR BLOOD AND BONE MARROW TRANSPLANT – EBMT

From the time that our team was established, with both investigators officially designated as members, it continued to operate as an audited and accredited transplant, donor and harvest centre. (Centre identification code – CIC-772).[4] Reporting was done simultaneously with CIBMTR.

3.6 JOINT ACCREDITATION COMMITTEE OF ISCT – EBMT – JACIE

Close participation in as many of the activities of the above organisation has continued. As we are not a member of the European Community, we are excluded due to being geographically based in South Africa. Nevertheless, we have maintained standards designed to fully comply with those set out in the guidelines for demonstrating excellence in stem cell transplantation. Although some aspects, notably enrolment in clinical trials, remain desirable it is still not possible in terms of prevailing European Commission Directives. Efforts to formally register with JACIE were precluded due to financial reasons but nevertheless standard operating procedures were followed according to publication guidelines.[5]

3.7 THE SOUTH AFRICAN BONE MARROW REGISTRY - SABMR

Continuous expansion of the program activity created a problem with finding suitable histocompatible donors. This led to the establishing of the Bone Marrow Transplant Registry.[6] The initiative was undertaken by Professor Peter Jacobs jointly with Professor Ernette du Toit, Director of Tissue Immunology and Dr Arthur Bird, Director of Blood Transfusion Services. This registry has grown steadily and it is currently housed in the Provincial Laboratory for Tissue Immunology where all tissue typing is performed. This laboratory has international endorsement for immunogenic competence and interacts widely particularly with the World Marrow Donor Association. All donors coming into our service did so via the local laboratory with which we held regular scheduled meetings.

3.8 THE NATIONAL MARROW DONOR PROGRAM – TC 475 - NMDP

Difficulties were encountered with access to this large group of volunteers in the United States. After application and processing of curriculum vitae and a lengthy evaluation, we were designated as the initial and probably still the only centre in South Africa enjoying transplantation privileges, particularly harvest and donor, accreditation.[7] Our representation on their Council has ensured continued close interaction and, as an outgrowth, our status and links with the South African Bone Marrow Registry have made it possible to increasingly include members of their staff in this international collaborative venture. We reported patients only if a donor was recruited from them.

3.9 AMERICAN SOCIETY FOR BLOOD AND MARROW TRANSPLANTATION

Active membership is maintained with this group where interaction and concurrent availability of data is being explored through a combined process that will notably be included in the Centre for International Blood and Bone Marrow Transplant Research.

3.10 ALTERNATIVE SOURCES OF STEM CELLS

It has been a privilege to enjoy access to Eurocord[8] and international Netcord foundation largely through searches conducted worldwide on our behalf by the South African Bone Marrow Registry.

Throughout this period, there has been an increasing awareness of the apparently unlimited potential of alternative donor sources – not only from blood and bone marrow but also umbilical cord and now amniotic fluid. Here again accountability will be a challenge for years to come and this can be based on the precedent set by this prototype unit.

3.11 SUMMARISING COMMENT

It is currently not clear how future development will extend to umbilical cord blood and amniotic fluid or how these donor sources will interface with the shift in use of immunohaematopoietic stem cells. This brings into sharp focus the ethical and moral issues that surround the use of these human biological reagents in adult as opposed to embryonic stem cells. Such differences need to continue being researched but with responsible regulation. The future landscape will accommodate naturally the place of restorative, in contrast to regenerative, medicine. Against this reality is confidence in the proactive way that all aspects of these technologies were documented from inception. This research design has anchored use of this precious human resource in South African clinical practice. It also provides a framework on which to develop the responsible use of these procedures, particularly as their deployment escalates, even in this under-resourced area of the globe. The overarching impact of this wide ranging registry participation is two-fold. In the first instance, that quality of programme continues to match highest possible international standards. Secondly, there is long term uniformity of outcome to the relevant extent that the cohort selected for more advanced testing of specific organ system, extrapolates back to both the reference sample and to other centres that participate in registry reporting.

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CHAPTER 4

TECHNICAL DETAILS OF THE STEM CELL TRANSPLANT PROCESS.

FOCUS

This section sets out the minutae that had been standardised via constant refinement to include ongoing review by international colleagues from the time the programme was introduced. It defined the methodology used throughout all aspects of the transplant process. To that specific end a consolidated perspective, or audit, is provided from personal observations that focused attention on the outcome of these various types of immunohaematopoietic stem and progenitor cell transplantation. Procedures have remained largely uniform from inception and were updated only as needed. Then, based on observations, changes in non-haematopoietic tissues and organ systems that had been identified as potentially at risk were additionally exploredd. There emerged two patterns. Early associations that were almost universal, transient and corrected with myeloid recovery proactively managed as integral to this routine protocol. Late effects were quite different, separate and needed additional methods to define functional and structural alterations previously not reported locally.

4.1 INTRODUCTION

This section of the thesis provides a description of the transplant process. Protocols were established for each step contributing to the successful outcome of this procedure.

4.2 PROTECTED ENVIRONMENT - THE PHYSICAL PLANT

Advice from the Fred Hutchinson Cancer Center in Seattle, resulted in building a dedicated prototype unit with a safe air exchange facility which incorporated the capacity to exhaust room volume and to do so safely.[1]

Each two-room suite was made up of a patient bed with necessary monitoring equipment interlinked to a constantly staffed medical and nursing workstation. Individual temperature and humidity stabilisation was instituted. Microbiological studies and maintenance of the filters were a regular routine. Initially 16 beds were commissioned in the new Groote Schuur Hospital but subsequently downsized to eight in the privately based Mediclinic Constantiaberg.

Access and exit were controlled by the Unit Manager. Linen changes were part of staff and visitor regulation in which appropriate gowning required change into surgical scrubs. The numbers of visitors were restricted and determined by the condition of the patients with particular concerns about risk of post-transplant infections.[2]

4.3 PSYCHOSOCIAL SUPPORT

In adults as well as children one of the earliest deficiencies was that of good, explicit and simple but reliable communication with patients and their families.[3] This step, when viewed superficially, might be regarded as non-medical and perhaps only remotely related to the underlying haematologic disorder. However, chemotherapy or radiotherapy and the frightening use of the words such as leukaemia and bone marrow transplantation were found to be a major cause for distress. This problem presents an ever-increasing focal point in the provision of comprehensive treatment.

This supportive process was started on the basis of experience in Europe and USA and wisdom was brought to bear by the first liaison psychiatrist with whom we were able to work regularly. He patiently cultivated very wide ranging interaction with the patients and all immediate relatives.

Emphasis was placed upon the involvement of paramedical professional members of the transplant team and included paediatricians. This holistic view was subsequently consolidated by encouraging participation of psychologists and, of special benefit, a bereavement counsellor.[4] Reciprocally, support was available in the reverse direction whereby access to these members of the team was not only ongoing but could be activated, directly by patients of all ages or their families at any time.

4.4 TRANSPLANT DECISIONS

Departmental staff conferences were routinely convened to ensure that all medical and haematologic criteria had been carefully checked and ratified prior to proceeding with this procedure. Simultaneously, information was provided for the patient and the family[5] and this procedure included discussion with interested and involved parties, specifically the psychosocial counsellor, psychologists and psychiatrists. All necessary consent forms were completed including living wills or advance directives as part of the clinical record.[6] Separate agreements were signed for material collection as required as part of international studies. Where appropriate, clinical trials were formally registered with attention to ethics and research approval.[7]

Participation within the European Group for Blood and Bone Marrow Transplant (EBMT) and subsequently by the Centre for International Blood and Marrow Transplant Research (CIBMTR) and links to the National Donor Program (NMDP) required the assignment of a unique haematology departmental accession and registry reporting number to ensure autonomy of all patients who were registered.

In addition to these administrative requirements, important transplant related data were checked, these included histocompatibility and blood groups. HLA typing in year 2000 used serology testing for class I and class II by molecular high resolution. In 2002 high resolution was introduced for class I and in both donor and recipient, five loci were tested (HLA–A,B,C, DRBI and DQBI). Planning for plasma exchange was necessary to remove isoagglutinins before a procedure where there was ABO incompatibility or remove antibody where platelet refractoriness was present. In the latter situation these complications could be reversed with apheresis and infusing gammaglobulin[8] followed by platelet infusion and laparoscopic splenectomy.[9]

These decisions were consolidated into a personalised patient calendar with the addition of consultation for cardiac assessment, chest radiology, simulation and consultation with radiation oncologists, placement of central venous lines[10] and provision for later use of an antibiotic lock. [11] Dates were finalised for autologous harvest with cryopreservation in patients undergoing matched-unrelated volunteer donor procedures and also for autologous transplants. The timetable also included the conditioning regimen to be used.

Fully informed consent documents were signed by both recipient and donor for the chemotherapy, harvesting and the transplant itself.

4.5 RECIPIENT PREPARATION

General requirements were physical examination including chest radiology, cardiac assessment (echocardiogram and electrocardiogram); height and weight were calculated to body surface area using the ideal body mass index.

The following laboratory studies needed to be performed to ensure that the patient was in a suitable condition for the transplant: full blood count with manual differential and reticulocyte production index, appropriate biomarkers, biochemical profile defined as creatinine and electrolytes together with glucose estimation.

Liver function and enzyme tests were performed and confirmation that viral screens for cytomegalovirus, hepatitis, HIV I and II, as well as Epstein Barr virus serology, remained negative. If not already in position, central venous lines were placed by the Hickman technique which, if inactive, the line was locked with saline containing 5000 units/L of heparin, 10,000 mg/L of vancomycin, 10,000 mg/L of ceftazidime or alternatively 20,000 mg/L of tazobactam or 5000 mg/L of gentamycin (Heparin – antibiotic lock), constituted in 100 ml, using a tenth of the dose. Our pharmacist used 50 ml and adjusted the dose accordingly and then provided 5 ml of this solution in a syringe to insert 2.5 ml into each lumen. If required, bone marrow aspiration and trephine biopsy and lumbar puncture were also undertaken. Psychosocial counselling included quality of life assessment and assurance that all information about the procedure had been provided. At this time, we also ensured that storage of sperm and ova had been carried out and if the patient was entered in study protocols, that these be continued. Where indicated, a visit to the dentist and referral to maxillofacial surgical colleagues were also included. Repeat family discussions or conferences were held as needed.

4.6 DONOR PREPARATION

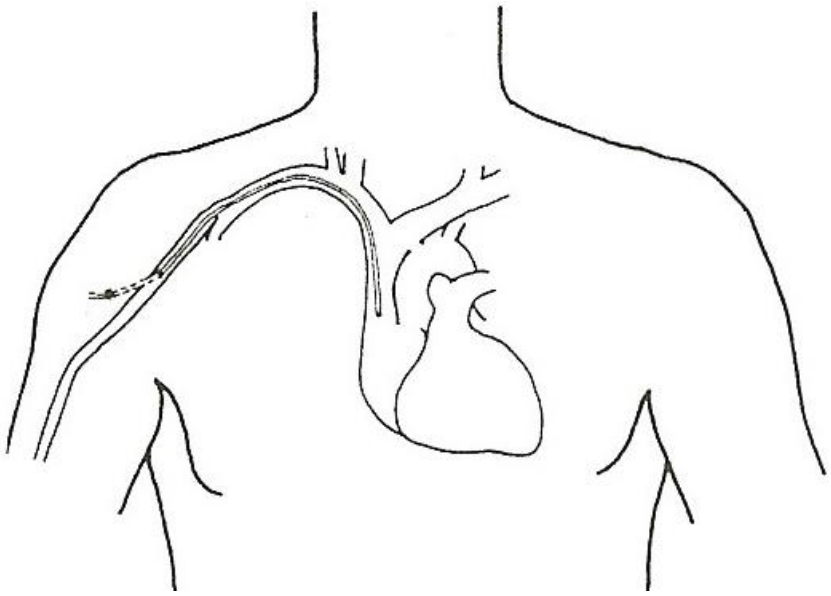
The following investigations and blood tests needed to be done to ensure that the donor qualified for the procedure. Physical examination with chest radiology and cardiology clearance and the collection of blood specimens for full blood count with manual differential, biochemical profile, viral screens for cytomegalovirus, hepatitis, Epstein Barr and immunodeficiency virus together with creatinine estimation.[12] Five days before apheresis collection, we commenced granulocyte colony-stimulating factor (G-CSF) 300 µg (Roche) subcutaneously daily advising the donor that some side effects were possible. Large volume apheresis collection required that a minimum of 2 x 10^6/kg CD34 positive stem cells and 6.5 x 10^8/kg mononuclears are recovered with these being characterised by flow cytometry and in vitro clonogenic assay using the GM-CFUc to get a minimum of 5 x 10^4/kg body weight.[13]

4.7 PLACING AND MANAGEMENT OF CENTRAL VENOUS OR J LINES

In a distressed and already anxious patient, one of the most unpleasant events is the spectre of regular intravenous therapy. Based on a pilot and then a feasibility study, long stay intravenous access was pioneered by placing, under local anaesthetic, a paediatric nasogastric feeding tube into the right subclavian or preferably superior vena cava.[14] **(Figure 2)**

FIGURE 2

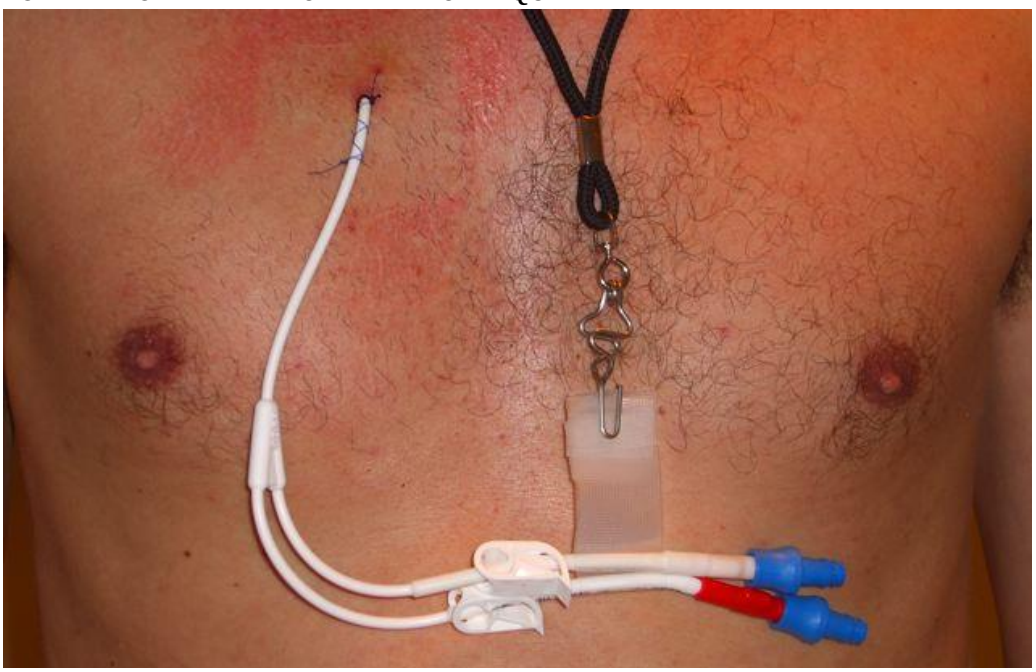
PROTOTYPE INTRAVENOUS ACCESS [14]



This approach was subsequently replaced by the Hickman procedure which is safe, reliable and has virtually no side-effects.[15] The line was tunnelled to leave the teflon cuff about 1 cm above the placement incision. It was important to loop and secure the line to the anterior chest wall to avoid accidental dislocation or loss. **(Figure 3)** Colonisation, although always a matter for concern, proved to be a relatively minor problem in that only a few catheters had ever been removed on this suspicion.[15]

FIGURE 3

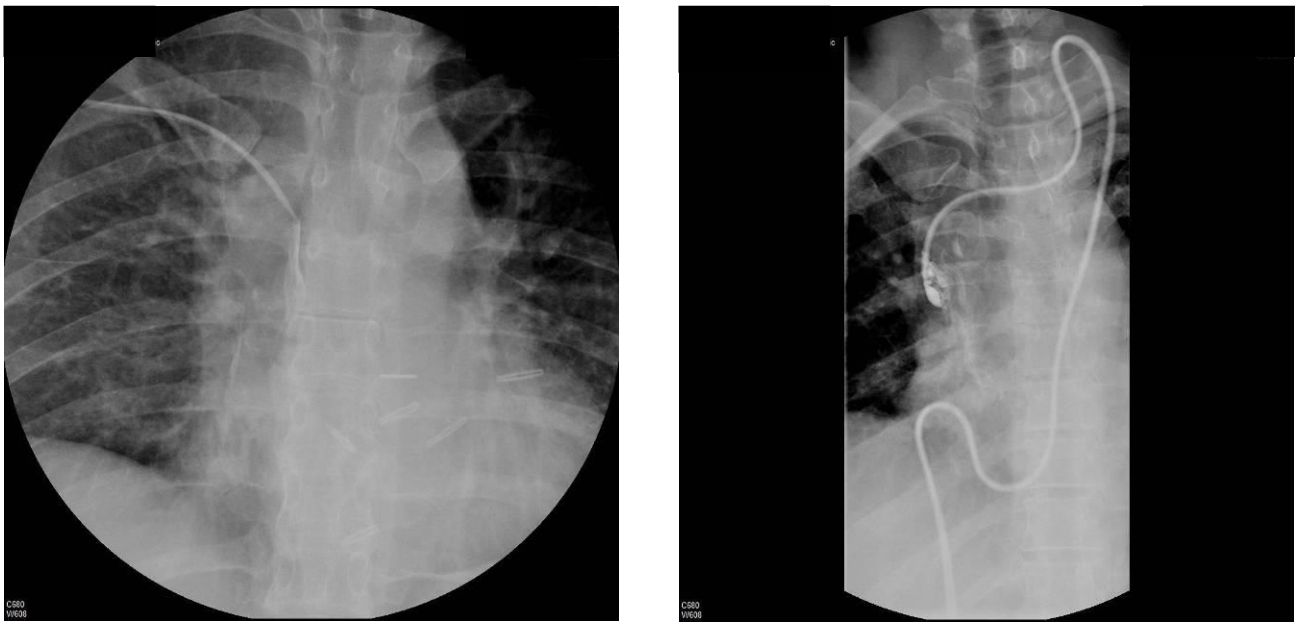
J LINE IN OPERATION – THE HICKMAN TECHNIQUE[15]



It was relevant, in each instance where cultures were negative, that removal did not result in resolution of the fever; an alternative site for the infection was subsequently identified. This observation in no way detracted from maintaining a very high index of sterility but, in contrast to many of the published recommendations that in this situation the lines should automatically be removed, the Haematology Team has recorded a substantially different, and less intrusive, outcome. Use of a heparin-antibiotic lock may explain this finding.[11] Low-level anticoagulation with 2.5 mg of warfarin on alternative days without prolongation of the INR contributed to reducing deposition of fibrin around the lines.

When deposition did occur, radiological studies typically demonstrated a fibrin sheath. **Figure 4a** shows a normal linogram while **Figure 4b** shows occlusion due to fibrin sheath around the tip of the catheter in the vena cava. This could be readily lysed by installation of recombinant human tissue plasminogen activator into both channels by leaving it *in situ* for 24 hours and then aspirating the intraluminal material.[16] In periods when not actively used, heparinised saline containing antibiotics was instilled into the lumens and may have contributed to an unusually low sepsis rate. Removal of the line was a simple outpatient procedure provided the teflon cuff had been correctly placed within a centimetre of the insertion site.

FIGURE 4
LINOGRAM TO IDENTIFY OBSTRUCTION – FIBRIN SHEATH



(a) (b)

Courtesy Dr Derek Solomon – Tuft and Partners

4.8 CONDITIONING

Although more trials are needed to recommend the routine use of CD52 antibody, it has been shown that it reduces GVHD without increasing graft rejection. There was however an increase in relapse rate, especially in AML but not affecting overall survival. There is ongoing debate about the use of Campath® in-the-bag and although it is not the norm, it is increasingly being used in other transplant centres.

In this transplant centre, graft-versus-host disease ceased to be a dominant problem when Campath® 1G, and subsequently 1H, was added to bone marrow in-the-bag.[17] This GVHD differed from the fully developed entity with only erythroderma being found in approximately 10 - 20% of peripheral blood stem cell grafts when exposed to Campath® 1H – but not 1G – *ex vivo*.

Conditioning programmes were another area that attracted early interest because, as with the prior drug therapy, these different approaches brought with them alternative forms of morbidity and mortality. Since the graft-versus-host disease may have been related to conditioning, a question remained as to whether our established regimen combining cyclophosphamide with total body and total nodal irradiation had an impact on this complication.

This method was different from the alternative standard approach using only busulphan and cyclophosphamide: the Bu-Cy2[18] or Bu-Cy4 regimens. Considerable literature exists but opinions are divergent and the two methods are variably reported to be beneficial – or disadvantageous – in one or other disease category. There is no consensus favouring either approach. Pragmatically, patients were allocated by disease category, after reading and signing an informed consent.

On rare occasions individuals were precluded from the drugs because of specific sensitivities directed at lung or bladder: here the standard became etoposide combined with melphalan. Other options included mitoxantrone,[19] BEAM, [20] CBV,[21] and ICE[22] or even more complex regimens[23] particularly when only haplo-identical family donors were used.[24] In an extensive analysis of published data, there were no significant differences in relapse rates and graft-versus-host disease or probabilities in survival for the period from 1995–2002.[25,26]

A comprehensive review from Sweden supported the observations but emphasized a higher risk of veno-occlusive disease and haemorrhagic cystitis with the busulphan regimens: there also has to be a minimum of 24 hours separating the busulphan and the cyclophosphamide to avoid drug interaction.[27] Furthermore, in a study from Johns Hopkins Hospital (Santos, personal communication) 16 mg/kg of busulphan was given over four days followed by a rest period of 24 hours and two days of cyclophosphamide at a dose of 60 mg/kg per day. Therefore this replaced the earlier regimen in which cyclophosphamide was given at 50 mg/kg of ideal body weight to a total of 200 mg/kg: (the Bu-Cy4 protocol). This approach applied to both adults and children irrespective of disease category.[18]

The interval between the two drugs and the slightly reduced dose of cyclophosphamide diminished most of the problems. Preferably intravenous busulphan was given as 3.2 mg/kg daily as a single infusion in place of the oral route. All individuals were and should be premedicated with standard anti-convulsant regimen to prevent

seizures; this was commenced one day before the busulphan. In unusual circumstances etoposide can be given at a dose of 60mg/kg continuously over 18 hours between the busulphan and the cyclophosphamide: this is not our current practice but noted for information.

A crucial point is that the patients received MESNA and if any haematuria persisted, the dose of uromitexan was extended in 24-hour blocks until no further bleeding was evident. Urine output must have exceeded 75 ml per hour and furosemide was given as needed. Platelets were maintained above 50 x 10^9/L.

In Fanconi Anaemia recent data favoured non-radiotherapy conditioning regimens in the form of fludarabine, antilymphocyte globulin and cyclophosphamide.[28,29]

In all age groups, benign disorders, notably haemoglobinopathies and thalassaemia as well as bone marrow failure, the same approach was used. In collaborative amyloid studies, preparation did not include radiotherapy. Only melphalan was used based on the data from Dr Phillip Hawkins and also the group in Boston: at a dose of 220mg/m^2 (personal communication). It was found that two divided doses, or over two days (100mg/m^2) had better patient acceptability and this became the subsequent standard. An alternative is to employ melphalan 140 mg/m^2 over 15 minutes and etoposide 2.5 g/m^2 as a one hour infusion. The etoposide was used because of better solubility and bioequivalence between the two formulations.[30]

Radiotherapy was retained as a myeloablative option for malignant conditions.

Where blood group differences exist between donor and recipient plasma exchange may be needed. In these circumstances survival is unaffected but haematopoietic regeneration is often delayed.[31] Thereafter only AB plasma and platelets were given with red cells limited to group O. Selective decontamination of the gut was standard practice and antibiotic regimens varied as a result of constant review. The use of ofloxacin or ciprofloxacin over co-trimoxazole was favoured and the current choice is levofloxacin at 500 mg orally daily.[32]

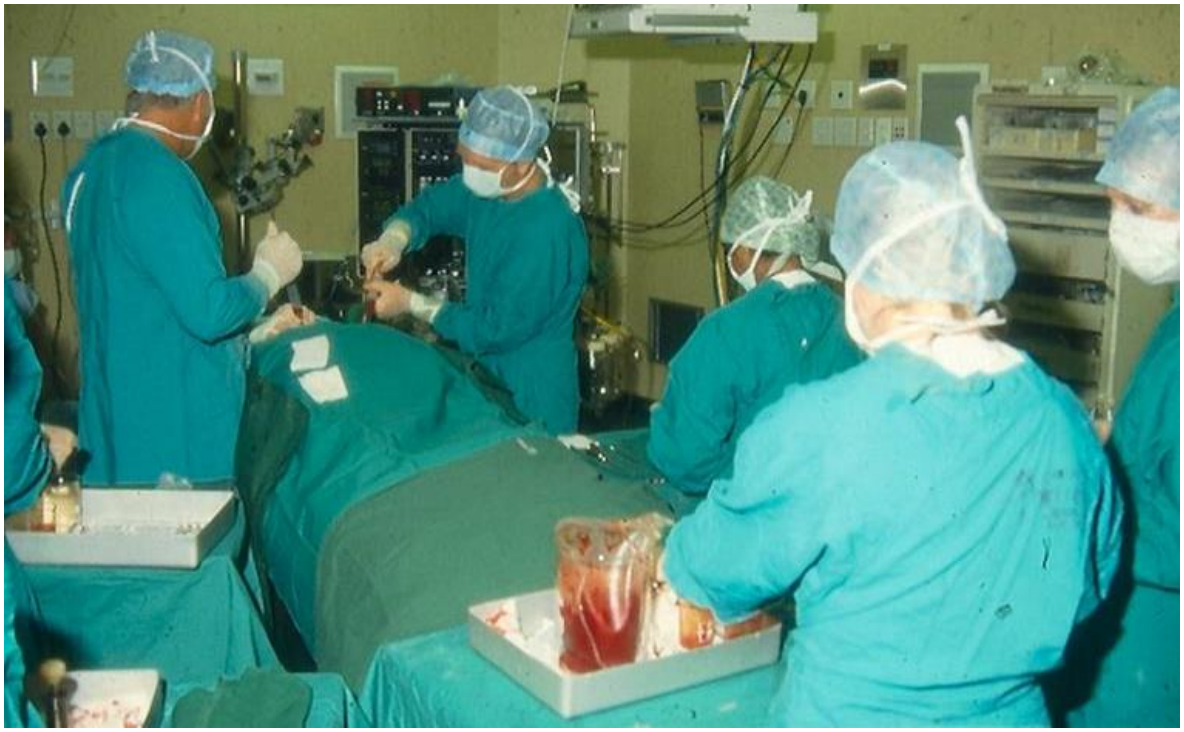
4.9 SEQUENTIAL EVOLUTION OF SEVEN DEVELOPMENTAL PHASES

These involved the main areas which had an influence on our program.

4.9.1 UNFRACTIONED BONE MARROW AS THE GRAFT SOURCE

This was, from inception and for many years thereafter, the worldwide standard. The stem cell harvesting was a dramatic process which required a general anaesthetic. Two teams working in parallel aspirated from sternum and both iliac crests, between 10 and 15 ml of marrow rich blood for every kilogram recipient lean body mass, in appropriate anticoagulant.[33] **(Figure 5)** Recipients were immunosuppressed, marrow infused intravenously and an engraftment period monitored in reverse isolation or a protected environment.

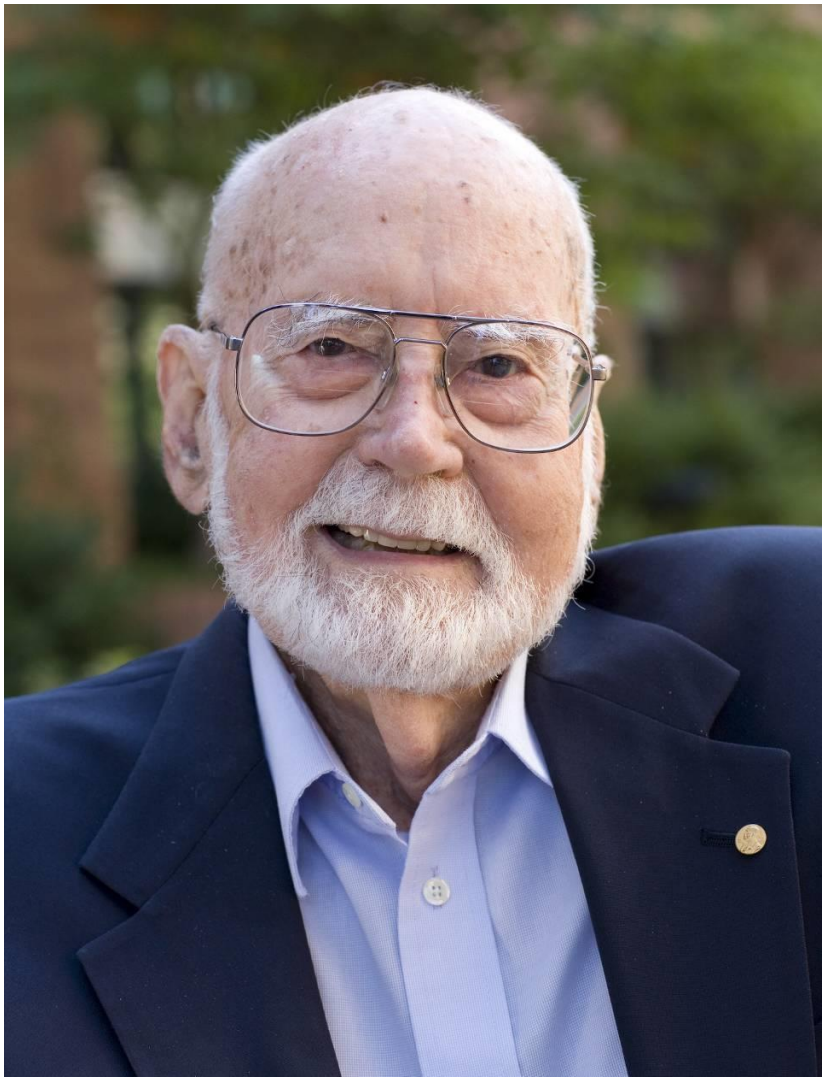
FIGURE 5
THE FIRST HUMAN ALLOGRAFT IN SOUTH AFRICA – HARVESTING OF STEM CELLS



These efforts had widespread support throughout the Faculty. In reflecting on this defining aspect of the study there is recalled the same absolute dedication to scientifically approaching this challenge with a quiet and understated confidence that was so reminiscent of the leadership of Professor E Donnall Thomas **(Figure 6)** on which was modelled this first South African venture of transplant immunology, biology and haematology. These attributes are still shared and are a constant reminder of his continued influence through many graduates and fellows from the Fred Hutchinson Cancer Institute, Seattle USA, which remains a reference point for an exemplary standard of care.

Much of our local achievement in Cape Town was anchored in the major and continuous guidance provided by this legendary father of bone marrow transplantation. To the present day his example and ready help are enjoyed by our group – the Seattle connection.

FIGURE 6
DR E DONNALL THOMAS – NOBEL LAUREATE



(Courtesy: Meg E. Bender, Project Coordinator, Clinical Transplantation Support, Clinical Research Division – Fred Hutchinson Cancer Research Center).

4.9.2 REJECTION AND NONENGRAFTMENT

These were significant impediments to survival but early recognition and repeat procedure with altered conditioning and enhanced post-transplant corticosteroid with methotrexate was standard of this era.

4.9.3 GRAFT-VERSUS-HOST DISEASE

An acute syndrome (GVHD) occurred typically within the first 90 days. A major immunologic phenomenon which caused enormous morbidity and significant mortality.[34]

4.9.4 THE INFLUENCE OF CYCLOSPORIN A[35]

This drug came when effective intervention was desperately needed. Access to the product was obtained through years of close association with pharmaceutical company Sandoz in Basel. This reflected collaboration with Swiss investigators and led to systematic studies with Dr Jean Borel which demonstrated a reduction in

the incidence and severity of acute graft-versus-host disease but not abrogation. This advance was based on a rabbit model and extended to clinical practice.[36]

Wide and unpredictable variability existed in pharmacokinetics and dynamics when cyclosporin A was used in matched unrelated transplants. These transplants were started on 5 mg/kg as 2-hour infusion BD on day two and switched to an oral dose of 100 mg 12 hourly once the individual was able to swallow.

The target was 1500 μg/ml at 120 minutes using the C2 assay[37] from giving the oral tablet or completing the intravenous administration. Individual differences in recipient drug handling were thought to explain unpredictable occurrences of skin rash in the allografts.[38]

4.9.5 IMPACT OF CAMPATH® MONOCLONAL ANTIBODIES

This was carried out in close collaborative studies with Professor Herman Waldman and Dr Geoff Hale first in Cambridge and then continuing subsequently in Oxford - actively ongoing to the present moment. These could, even in an understated way, be chronicled as momentous to the point of revolutionising interventional approaches to these lymphocyte-mediated inflammatory attacks on many body systems. [39]

4.9.5.1 HISTORY OF CAMPATH®

In 1973 Herman Waldman started research on how the immune system could choose between immunity and tolerance. He then became aware in 1974 of monoclonal antibodies and in 1978 realised that such an antibody could be useful in bone marrow transplants, GVHD being a major complication caused by the contamination of bone marrow by "T lymphocytes". T cells can be depleted by using anti-CD 52 antibodies directed against the CD52 antigen found on T and B lymphocytes, monocytes and dendritic cells. Herman Waldman was joined by Geoff Hale at Cambridge University and subsequently they continued in Oxford. They initially produced the monoclonal antibody Campath® I-M, a rat antibody that was capable of dissolving T cells by activating complement. It was effective in preventing GVHD but caused graft rejection. Then followed Campath® I-G developed for *in vivo* depletion of host cells to prevent rejection. In 1988, this was humanised by genetic engineering to create Campath® 1-H. Herman Waldman constituted a Campath® Users' Group of which Professor Peter Jacobs was a member.

The "Haem Team" had the privilege and unique position of being assigned a particular responsibility by the Campath® Users' Group of following up on their reported effect of adding the first of these, which was a mouse lytic immunoglobulin, to the graft in-the-bag – the *ex vivo* approach. This process has been refined by gradually improving the choice of reagents and currently the standard is the humanised opsonic protein that has the interesting capacity of binding to the CD52 receptor making these cells suitable for selective removal upon infusion by the reticuloendothelial system.[39]

Campath® 1H was first approved by the Food and Drug Administration (FDA) in 2001 and in 2007, it was also approved as first-line therapy for CLL. Commercialisation of the product then took place.

The *ex vivo* use of this CD52 antibody was very successful in preventing GVHD. This approach was subsequently followed in our department and use of unmanipulated marrow was discontinued.

4.9.6 MOBILISED PERIPHERAL BLOOD WITH APHERESIS HARVESTING

This procedure was a natural addition to the rapidly advancing achievements for improved technology.[40] Here again the Haematology Team was ideally placed to participate actively in this transition because of the early involvement with continuous flow cell separators and laboratory studies that documented the nomadic nature of the immunohaematopoietic stem cell.[41] A short period was dedicated to systematically testing and demonstrating that, for practical and clinical purposes, this source was interchangeable with marrow although some subtle differences exist. A composition of various graft sources are seen using a modified table from Gluckman in the hand book from the Bone Marrow Registry. **(Table 1)** Notably local concern was focused on immunologic effects in donors.[42] This point in the research programme included evaluation of differing cryobiology techniques and scrupulous attention to characterisation and behaviour of the recovered material both in terms of cell numbers and using clonogenic assay of long-term colony initiating population, which were required for re-establishment of immunohaematopoietic function.[43] In parallel, introduction of flow cytometry to document the numbers of CD34 expressing population.[44]

TABLE 1
COMPOSITION OF THE GRAFT SOURCE[45]

	VOLUME COLLECTED	MEDIAN CD34 CONTENT	MEDIAN CD3 CONTENT	TARGET CELL DOSE
BONE MARROW	10-20 mL/kg	2-3 x 10^6/kg*	25 x 10^6/kg	>2 x 10^8 TNC/kg
PERIPHERAL BLOOD	150-400 mL	8 x 10^6/kg	250 x 10^6/kg	5-10 x 10^6 $CD34^+$/kg
UMBILICAL CORD BLOOD	8—160 mL	0.2 x 10^6/kg	2.5 x 10^6/kg	>3 x 10^7 TNC/kg

**Per kg recipient body weight*

4.9.7 CORD BLOOD

This alternative source of stem cells became routinely available over the last five years for the use in this transplant facility. It is determined by patient size and availability and is largely interchangeable with conventional marrow and peripheral blood. In all these situations corresponding values are now internationally standardised.[45] It was mostly considered in paediatric patients as the total nucleated cell (TNC) dose is small. It was seldom used as first choice and usually a fully matched donor cannot be found. It is very expensive, there is delayed engraftment and there is no possibility that more product can be obtained if needed.

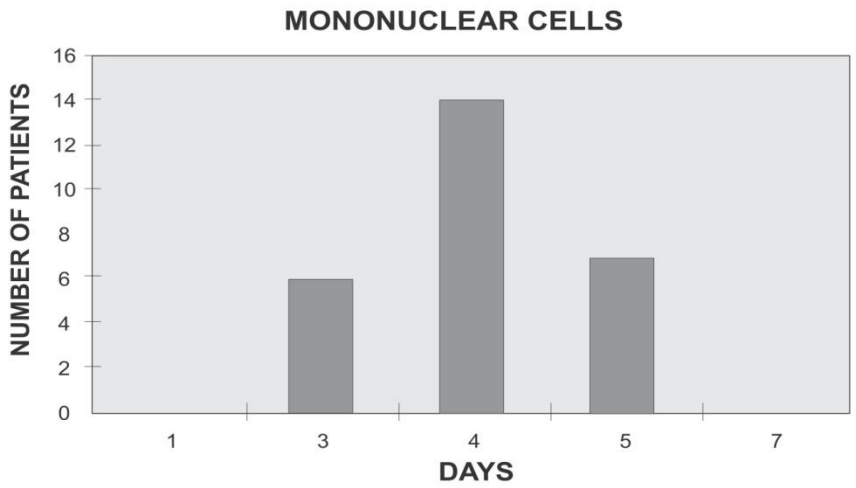
4.10 DETAILS OF THE GRAFT

A study was done in our department to characterise the response to the stimulatory peptide with most allogeneic donors achieving a peak mononuclear **(Figure 7a)** and CD34 cell population by day four. **(Figure 7b)** Recovery of circulating immunohaematopoietic stem and progenitor cells rests on standardised apheresis methodology[40] that takes into account appropriate use of stimulatory peptide to mobilise the required CD 34+ population.[46] Data analysis from the series of donors showed a correlation between total number of mononuclear cells and the CD34 positive numbers.[13]

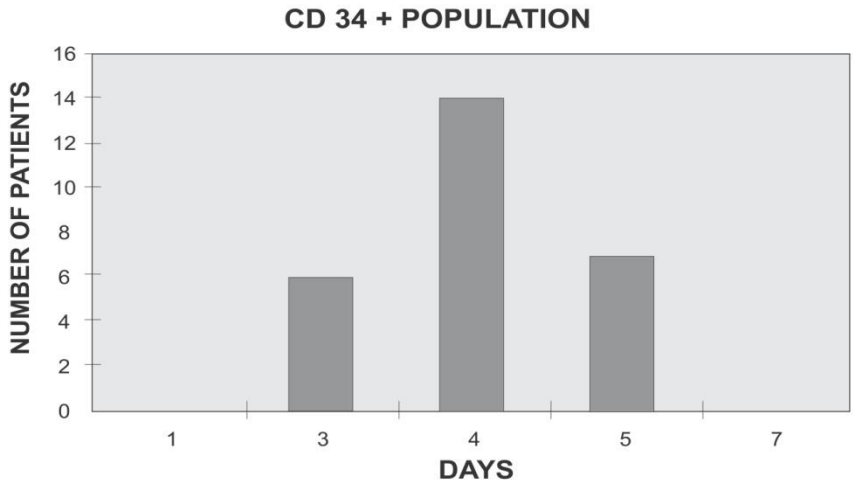
Following mobilisation with G-CSF, the appearance of peripheral blood mononuclear cells paralleled the all-important CD34 population considered to identify the earlier progenitor and stem cells. At this point the graft was harvested with greatest concentration of the desirable population for haematopoietic reconstitution.

FIGURE 7
DETAILS OF ALLOGENEIC DONATION

a)



b)



In the 10 donors harvested for allografts, the mononuclear cells were sufficient but the required CD34 levels were not always achieved. The GM-CFUc did not always establish viability (5 x 10^4/kg) although patients were still engrafted.[13] **(Table 2)**

TABLE 2

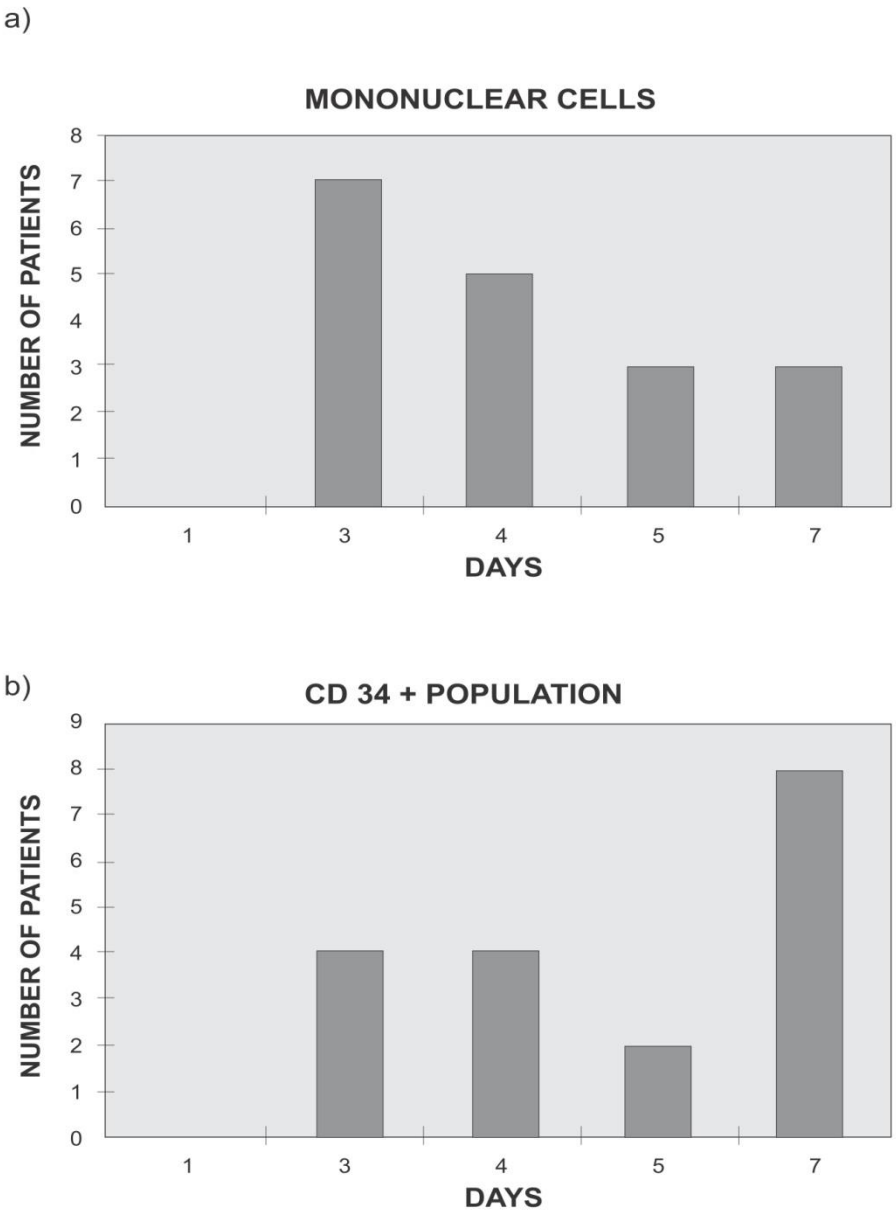
ALLOGRAFT COLLECTION AND INFUSION DATA

Number and	Mononuclear	CD3	CD 34+	GM-CFUc	Days to peripheral blood response				
subject	cells (x 10^8kg)	(x 10^8/kg)	(x 10^6/kg)	(x 10^4/kg)	Neutrophils		Platelet		
					(x 10^9/kg)		(x 10^9/kg)		
					0.5	1.0	>20	>50	>100
1. LC	6.8	4.9	2.2	0.9	-	-	-	-	-
2.AdB	7.6	4.4	4.4	5.5	15	15	27	27	34
3. CS	9.1	5.8	4.2	3.5	11	11	29	38	49
4. CB	12.5	7.5	9.9	4.7	8	8	8	8	13
5. HdP	7.0	5.5	3.0	1.9	13	13	3	13	23
6. AP	6.6	4.5	4.2	2.8	12	12	14	21	45
7. VS	7.0	4.3	15.1	7.2	10	10	19	30	44
8. PF	8.8	6.6	14.6	14.7	12	12	30	37	54
9. HdW	8.1	6.7	3.6	2.3	11	11	21	37	-
10. RA	6.7	4.4	10.2	1.9	11	11	13	13	-
11. JM	8.2	5.6	5.8	1.9	12	12	10	10	10
12. FK	7.7	4.7	3.6	3.6	13	13	13	13	18
13. PO	7.5	5.4	4.6	2.8	12	12	12	15	15
14. EG	6.6	4.8	3.9	1.0	12	14	21	21	-
15. IvZ	7.1	5.2	4.6	4.2	12	12	14	14	24
16. JF	7.3	5.6	0.8	5.1	12	12	14	14	35
17. MM	9.4	5.8	1.3	12.8	11	11	20	20	78
18. CvdW	9.8	7.2	4.1	9.6	12	12	14	14	14
19. ZV	9.1	6.9	2.8	6.5	11	12	56	77	100
20. SK	8.2	5.3	1.1	12.5	7	31	7	61	61
21. GF	7.6	5.7	2.6	7.4	12	12	12	14	100
22. CT	6.7	5.1	3.2	11.3	12	12	6	6	15
23. DH	7.4	5.6	2.8	2.3	13	13	7	14	37
24.KvD	8.9	6.9	3.1	8.6	12	12	14	14	21
25. MG	7.9	2.1	8.7	31.1	12	12	62	62	100
26. LL	8.3	6.3	6.1	13.2	9	22	54	54	100
27. KL	9.8	5.4	1.4	4.7	12	12	14	14	21
Median	6.6	476	3.9	1.0	12	12	17.5	17.5	62
Range	6.6 - 12.5	206 - 750	2.7 - 11	1.0 - 31.1	7 - 15	8 - 31	3 - 62	6 - 77	10 - 100+
Mean	8.0	54.8	5.3	6.8	11.5	13.0	20	25.7	46
Standard deviation	1.3	112.7	1.7	6.34	1.6	4.4	15.39	18.87	32.9

The same pattern of response in peripheral blood was seen as when graft was harvested from the patient for subsequent reinfusion. In these cases the CD4+ autologous transplant cells were less uniformly predicted by the mononuclear cells which were recovered. The explanation of this bimodal distribution remained unclear.[13] **(Figures 8a and b)** Differences in autografts were thought to reflect previous chemotherapy and a second peak was noted on day seven which was unexplained.

In autografts no further manipulation was necessary whereas in allogeneic transplants the Campath® monoclonal antibodies IG or IH were added under standard conditions.[17]

FIGURE 8
DETAILS OF AUTOLOGOUS DONORS



In the autografts, mononuclear cell numbers were achieved but with CD34 cells this was not always the case. Viability of cells was achieved on most of those who were tested.[13] **(Table 3)**

TABLE 3

AUTOGRAFT COLLECTION AND INFUSION DATA

Number and subject	**Total in bag**			**Days to peripheral blood response**				
				Neutrophils		**Platelet**		
				(x10^9/kg)		**(x 10^9/kg)**		
	Mononuclear	CD34+	GM:CFUc	0.5	1	>20	> 50	>100
	cells (x10^{8}kg)	(x 10^6/kg)	(x 10^4/kg)					
1. RL	7.7	1.08	5.8	12	12	34	41	72
2. SC	8.7	0.89	10.6	12	12	33	47	100
3. ST	9.6	2.58	1.5	13	13	19	19	39
4. DC	6.7	6.7	5.1	14	14	100	100	100
5. SF	6.0	22.6	a	a	a	a	a	a
6. GC		1.43	a	a	a	a	a	a
7. KB	7.0	3.0	a	a	a	a	a	a
8. MA	9.7	2.24	a	a	a	a	a	a
9. TS	6.3	1.4	a	a	a	a	a	a
10. FB [a]	8.6	1.2	9.9	12	12	15	19	21
11. HR [a]	8.4	1.2	16	22	22	100	100	100
12. CvM	6.0	1.18						
13. PB [a]	11.77	0.46	0	15	15	63	100	100
14. JS	12.13	2.08						
15. DE [a]	6.6	1.39	3.0	12	14	100	100	100
Mean	8.2	3.3	7.4	14	14	58	60.8	72
SD	2.0	5.5	5.0	3.4	3.3	37.5	38	34.7
Range	6.0 - 12.13	0.89 - 2.256	1.5 - 16	12-22	12 - 22	15 - 100+	19 - 100+	21 - 100+
Median	7.5	1.4	5.1	13	13	57.5	59.5	60.5

[a] Patients have to date not received their stored graft.

In those individuals where the source material was not to be immediately infused, the cells were cryopreserved. The details of cryopreservation had been extensively studied and standardised using a combination of DMSO, an intracellular cryoprotectant and hydroxyethyl starch (HES), an extracellular cryoprotectent. This included storage with appropriate inventory control.[47] The cells were then stored in a minus 80^{o}C freezer. The allogeneic cells were infused following addition of Campath® into the bag. Practical indications of graft recovery were increments in the monocytes followed in 24 to 36 hours by neutrophils then by reticulocytes. This process was expressed as the production index.[48]

4.11 INFUSION OF MARROW OR CIRCULATING STEM CELLS

The patients were premedicated for Campath® containing grafts with 100 mg hydrocortisone and 12.5 mg phenergan® intravenously and 500 mg paracetamol orally one hour before infusion. The dose was adjusted for children. This premedication was also used in autografting because of the side effects of dimethyl sulphoxide (DMSO).

Campath® 20 mg was added to each collection bag. No attempt was made to adjust the amount of antibody for contained cells since the rationale is that excess immunoglobulin may have an immunosuppressive effect on the recipient lymphoreticular system *in vivo*. Marrow or peripheral blood stem cells were given intravenously over 60 minutes but the rate needed to be slowed down if abdominal cramps, diarrhoea, rigors or vomiting occurred. In these circumstances, 10 to 20 mg of hyoscine hydrobromide was generally efficacious.

Blood pressure and pulse rate as well as oxygen saturation were monitored throughout the infusion. Cryopreserved cells were thawed in a water bath immediately prior to administration. Transient rises in blood pressure could be avoided by giving 10mg furosemide intravenously with every alternate bag and a careful watch on fluid balance to prevent under or over hydration was maintained. It was important to warn the patients about the unpleasant smell of demethyl sulphoxide (DMSO).

4.12 BLOOD TRANSFUSION AND RELATED PRODUCT PROTOCOLS

Following conditioning patients often developed side effects which led to diarrhoea and vomiting causing dehydration. The chemotherapy was myeloablative and red cells, white cells and platelets were reduced until regeneration occurred.

4.12.1 FLUID AND ELECTROLYTE BALANCE

This was crucial and was monitored on a continuous 24-hour basis. In practical terms requirements were calculated and charted during the daily morning report but measured more frequently if necessary. Correlation was with daily body weight together with specific gravity and osmolality of blood and urine as needed. Additions were made to 24 hour replacement using isotonic or physiologic saline with the flexibility of using potassium or magnesium riders to maintain physiologic blood levels. Particular attention was paid to static creatinine levels with appropriate revision when any potentially nephrotoxic antibiotics or other medication was given. Regular review with a nephrology consultant was undertaken in order to anticipate or correct changes in metabolic status which required correction by haemofiltration or haemodialysis.

4.12.2 ANAEMIA

Although anaemia could be caused by reasons other than myeloablative chemotherapy, it remained the most important. Complications such as vaginal and GIT bleeding, haemorrhagic cystitis, renal failure, severe infections, graft rejection and myelodysplasia could all contribute to anaemia.

When symptomatic this was preferentially treated by erythropoietin where possible.[49] In more acute situations, determined by patient symptoms or clinical signs rather than arbitrary laboratory values, leukodepleted units were used.[50] No post-transfusion levels were done. All products were irradiated to reduce the risk of transfusion related acute graft-versus-host disease. [51]

4.12.3 PLATELETS

Platelets were leucocyte reduced and irradiated single apheresis donations.[52] A minimum number of 5 x 10^{11}/L were infused over 15 minutes and 15 minutes later a post-infusion value was established. From the post infusion result an increment in the platelet numbers and daily blood counts were used to follow platelet survival. All at risk thrombocytopenic patients, predicated by fever, uraemic platelet dysfunction and counts under 10 x 10^9/L, received the anti-fibrinolytic agent tranexamic acid or cyclokapron® orally or parenterally as a continuous intravenous infusion between 25 and 50mg per kilogram per 24 hours.

4.12.4 NEUTROPENIA

This was defined as an absolute neutrophil count below 0.5 x 10^{11}/L. Treatment commenced with 300 µg of stimulatory peptide in the form of granulocyte (Roche) or granulocyte-monocyte colony-stimulating factor (Sanofi Aventis) intravenously to achieve confirmed white cell levels above 10 x 10^9/L and be apyrexial.

It was recognised that this approach was controversial and needed to be implemented in the context of clinical status and the presence or absence of pyrexia.[53] Furthermore, given the uncertainty about late effects occurring in donors, this intervention necessitated additional informed consent.[54]

4.13 INTEGRATED FUNCTION OF THE CROSS-DISCIPLINARY MANAGEMENT TEAM

Based on the combined ward rounds that characterise leading centres throughout the world, the cardinal commitment was concurrent information sharing at all levels of staff with inclusion of the patient and family members. In addition, to maintain teaching programmes, these activities were balanced with nursing in-service requirements. To sustain such interaction the multidisciplinary or "Haem Team" started each day by reviewing overnight status, responded positively to decisions required and planned management covering the next 24-hour period. The participatory presence of infectious disease consultants and paramedical professionals including nurses, occupational and physiotherapists and an academic dietician[55] was integral to these staff meetings.

In this forum, that by design included dedicated pharmacists, treatment protocols were scrutinised and potential drug interactions were identified. Similarly, nutritional status including management of mucositis and the placement of naso-jejunal, as opposed to naso-gastric, fine bore feeding tubes followed as part of standardised care. This approach virtually obviated recourse to total parenteral nutrition (TPN) in favour of the well tested and equally efficient enteral route. [56]

Cardinal to the whole exercise was the overarching organisational responsibility of a medical scientist to co-ordinate movement between various departments and data recording.

4.14 PROTOCOL FOR DISCHARGE AND FIRST THREE MONTHS

On transfer to the outpatient post-transplant follow-up clinic, patients attended weekly. They were also seen at any time that symptoms developed and received locally manufactured stabilised human serum[57] as a means of providing polyclonal immunoglobulins drawn from the community as an alternative to commercially available gammaglobulin. In this period, anti-viral therapy with valaciclovir[58] and pneumocystis prophylaxis with oral

co-trimoxazole[59] was given with awareness that side-effects, particularly vasculitis, can be encountered with the latter agent. Screening for reactivation of cytomegalovirus was routine with polymerase chain reaction to anticipate early treatment thereby preventing progression to organ involvement.[60]

The discharge period was a high-risk time. Experiences with recipients being monitored, even by experienced doctors and nurses outside the multidisciplinary transplant group, were regarded sometimes as suboptimal, as complications did not always receive urgent intervention. Specific problems were consistent and delayed presentation with typically neutropenic fever often necessitating urgent admission to high or even intensive care facilities with a concomitant and substantial increase in morbidity and mortality.[61]

As experience accumulated, our team became aware of two slightly different sets of complications which were separated rationally on a temporal basis. The realisation led to the careful construction of rigidly structured protocols for scheduled monitoring.[62]

The crucial post-graft follow-up very rapidly became a contentious area and was to some extent influenced by sophistication of the host transplantation referral. If this was back to care in a remote area, and irrespective of the perceived skills and competence of the attending medical and nursing consultants, a number of deaths occurred early on due to late diagnosis and fulminating sepsis.

This was not a function of poor quality support but simply the treacherous nature of infections in patients whose immunologic competence was still compromised often years after they had their procedure. Accordingly it became obligatory practice to keep the patients in the immediate geographical area of Cape Town with weekly visits for a minimum of three months. **(Table 4)** Based on the reduction of adverse incidents such surveillance became entrenched practice. Currently a strict protocol exists for intensive follow up in the first twelve weeks and gradually decreasing over time. The highest risk for sudden onset of complaints remained in the first 12 post-transplant weeks. Infection of central venous line, which was often a nidus leading to fulminating sepsis, reflected a slow return to viable immune status and dictated close supervision with meticulous data recording and review by the nurse co-ordinator. When indicated patients were promptly referred for special studies.

TABLE 4
TYPE AND FREQUENCY OF SURVEILLANCE IN THE FIRST THREE MONTHS POST TRANSPLANT

EXAMINATION	**FREQUENCY**
Full clinical examination	Weekly
Central venous line hygiene	Weekly
Routine haematology (FBC, INR)	Weekly
Renal biochemistry (BUN, creatinine) electrolytes.	Weekly
Liver function and enzymes (AST, ALT, bilirubin, LDH, albumin)	Weekly
CMV	Weekly
EBV; Hepatitis A, B and C	When indicated
Chimerism by short terminal repeat assay	Weekly
Infusion of stabilised human serum	Weekly

4.15 LOGISTICS FOR POST-TRANSPLANTATION SURVEILLANCE

The value of such organisational detail cannot be over emphasised and remains of paramount importance as extension of the above outlined shorter-term obligation for care of these individuals. In the start-up days of the programme, the late effects of these profoundly new monitoring interventions were not clearly formulated yet their appreciation came to contribute significantly to the more recently emerging awareness of survivorship.

This particular aspect of structured care has in the last four or five years attracted increasing investigation. With world interest in harmonising, for purposes of centre recognition a Joint Accreditation Committee of the originally Spanish-based group, an opportunity may now become available, although primarily focused on the European Economic Community, to seek registration for South Africa.[63]

If successful, such attention to detail, aimed at technological standardisation, may help explain reported variations of graft content. The challenges brought about by the rapidly escalating acquired immunodeficiency disease syndrome arose with ominous overtones of new unusual infections and not the least of which is multi-resistant tuberculosis.[64] Of particular sub-Saharan impact, this situation understandably had widespread social and economic implications as budgets required readjustment to deal with this epidemic. Consequently, many of these immunohaematopoietic interventions suddenly required greater justification and, unlike the initial phase, patients were being denied ready access to life-saving treatment in state facilities because of limited funds. The situation in the private sector was similar as managed health care providers were reluctant to spend these amounts of money on individual members.

4.16 PROTOCOL FOR EXTENDED FOLLOW-UP

Unpredictability of complications necessitated a careful surveillance structured system with comprehensive written case notes. It was from this matrix that some of the side effects were indirectly noted and could then subsequently be documented over time. **(Table 5)**

TABLE 5
LONG-TERM MONITORING RECOMMENDATIONS POST-TRANSPLANT

CATEGORY	EVALUATIONS
History and physical exam	Chronic GVHD-focused review of systems and examination Complete skin examination Range of motion testing, if indicated Weight, height, vital signs, medical photographs List of all medications and supplements
Laboratory testing	Complete blood count with differential and reticulocyte count Comprehensive chemistry panel Fasting lipogram Virology screen (hepatitis, cytomegalovirus) Immunisation titers Endocrine evaluations (thyroid, sex hormones) Iron profile Immunosuppressive drug levels ABO typing (if ABO-mismatched) Chimerism testing
Other studies	Bone marrow aspirate and biopsy Skin biopsy Pulmonary function tests Schimer's test – to determine moisture of eyes Dual energy X-ray absorptiometry – for bone density Chest X-ray (chest computed tomography scan if indicated)
Secondary cancer screening	Mammogram (if age >35 years) Pap smear in females (if age >21 years or sexually active) Prostate specific antigen in males (if age >45 years) Colonoscopy (if age >50 years) Stool for occult blood (if age >50 years)
Subspecialist exams	Ophthalmology Oral medicine (dentist) Gynaecology

The follow-up programme required immediate referral should there be any clinical change. Routine monthly assessment needed to be done taking into account quality of life[65] and psychosocial circumstances. This follow up protocol resembles that from groups elsewhere in the world notably the European Organization for Research and Treatment of Cancer (EORTC).[66]

It was during this period of audit reporting to international registries that changes were first but consistently noted in this reference cohort. Collectively these drew attention to a wide range of complaints. These often only minimally and not infrequently occurring as solitary events, interfered with lifestyle but nevertheless gave emphasis to the important study concept of survivorship.[67]

4.17 PERSPECTIVES

On this basis more than a quarter century of research and development necessary to import bone marrow transplantation to South Africa, a prototype reverse isolation unit and a thoroughly standardised protocol emerged. This set the standards for haematopoietic reconstitution following allograft or, more importantly, *ex vivo* T cell depletion using the Campath® series of monoclonal antibodies. [17,14]

It was this structured approach that provided the reference point for the management of patients in both academic and state services and subsequently in the private sector. Ongoing analysis was focused on the quality of life with psychiatric and psychosocial components and the subsequent evolution of the general support group extending to psychology specialists. Systematic analysis of survival data much of which, focused in the early days simply on haematopoietic recovery, once secure and reproducible, drew attention to concurrent injury to non-haematopoietic tissue. These phenomena separated broadly, on a timescale, into two categories.

There was an almost universal deterioration in renal function, potential chemotherapy related changes in cardiovascular status, an abundance of cutaneous manifestations and gastrointestinal tract symptoms including alterations in liver biochemistry. All of these complications were predictable, transient and, being anticipated, could be managed proactively. As a result of this experience, these problems were designated as the acute associations. This audited database was analysed and reported in this subsection on non-haematopoietic sequelae.

There were different findings in other organ systems that presented late, needed new laboratory methods and were therefore not researched in the initial audit but became the specific focus of the present investigation. In particular, this part of the overall project depended on specialised studies that were not at first available in respect of lung function, immunologic reconstitution and unusual findings in bone mineral density following transplantation.

Immunosuppression was delivered by exclusive exposure to anti CD52 Campath® series of monoclonal antibodies given, not *in vivo*, but added to the incoming graft *ex vivo* or in-the-bag. The exception was unrelated donors who received cyclosporin A briefly. This alternative preparatory regimen also focused attention on the possibility of immune recovery which followed a different pattern from conventional approaches and was therefore studied in both innate and adaptive pathways.

4.18 SUMMARISING COMMENT

These details of the long established management programme in the Department of Haematology continued to undergo constant updating but have been relatively stable for at least five years preceding commencement of data collection for this research project. The next step was to confirm that this entirely reproducible regimen generated results comparable to published literature.

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CHAPTER 5

COMPLICATIONS OF HAEMATOPOIETIC STEM CELL TRANSPLANTATION

FOCUS

In this segment of the long-term study, it became clear that there was a wide range of outcomes following transplantation. There was, if carefully observed by following each case by the author, a disquieting pattern. For want of a sufficiently large number of individuals the initial step was descriptive with systematic and meticulous documentation of the symptoms and signs that could subsequently be correlated with adverse events. This, as more data accumulated over a prolonged follow-up and serial analysis, started to crystallise into a format that could be used objectively to define endpoints more sharply. Upon these could be structured a formal study of selected non-haematopoietic complications which have been alternatively viewed as the frequent occurrence of acute associations and of less obvious late effects. Stated differently, survivorship or cure with care did not only concentrate on marrow recovery but also on complications in non-haematopoietic organs.

5.1 INTRODUCTION

This section of the thesis includes acute and late complications. Only a few will be discussed in detail. An audit involved mainly the acute associations while a cohort of surviving volunteers was studied for late effects. The magnitude of the problem was constantly given reality by reporting to International Registries. This participation in regular outcome analysis, gauged from the audit and accrediting peer review, established a reliable reference point from which to start accumulating a local experience. The perspective of the professional nurse, working as an integral component of the multidisciplinary "Haem Team", was of several dominant problems.

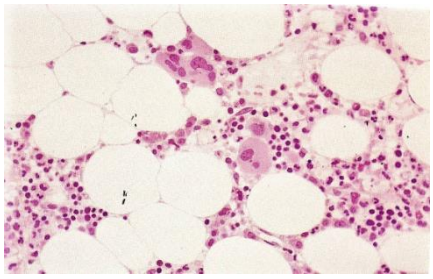
5.2 BACKGROUND TO TRANSPLANTATION COMPLICATIONS

Interest in these bone marrow transplantation related phenomena started slowly with the observation that some individuals experienced little by way of side effects while, apparently unpredictably in others, a range of newly encountered symptoms and signs occurred. This was at a time when these procedures were relatively novel worldwide and experience at the University of Cape Town in the newly established Department of Haematology was just awakening. While working in the wards directly with our patients, the initial excitement was simply the appearance of monocytes and neutrophils in the peripheral blood. In those days, bone marrow aspiration and particularly trephine biopsies were in vogue. The earliest morphologic evidence of engraftment could be demonstrated in bone marrow trephine biopsy.[1] Here, the general intertrabecular hypoplasia is given relief by groups of normoblasts along the endosteal lining in specialised areas or haematopoietic microenvironment termed the niches. Gradually, these aggregations became more heterogenous by the appearance of myeloid lineage and megakaryocytes simultaneously by repopulation of the previously vacant areas when the marrow returned to normal. **(Figure 9)**

It soon became apparent, again without any clear separation between individuals, that some patients would continue to regenerate and leave hospital to lead normal lives. In contrast, others may lose even this rudimentary evidence of recommencing haematopoiesis and need to be treated alternatively or even re-transplanted.

FIGURE 9

PHOTOMICROGRAPH: NUCLEATED RED CELLS OR NORMOBLASTS IN THEIR NICHES



Courtesy Professor Barbara Bain

Table 6 shows the acute complications that may occur post-transplant.

TABLE 6

ACUTE COMPLICATIONS OCCURRING DURING FIRST 100 DAYS POST-TRANSPLANT

Complication	**Tests to be performed**	**Time to complication**
CARDIAC		
Arrhythmias	ECG	Any time during first 100 days
Cardiomyopathy	ECG & ECHO	Any time during first 100 days
Failure	ECG & ECHO, chest x-ray	Any time during first 100 days
RENAL		
AKI	Renal function tests twice week	Any time during first 100 days
Haemorrhagic cystitis	Monitor haematuria	72 hours or more than 2 weeks
Obstructive uropathy	Cystoscopy	
Hydronephrosis		
Acute renal failure		
GIT		
Mucositis	Oral swabs	Pre-transplant to 3-4 weeks
Dysphagia		
Diarrhoea	Stool culture	
Pain	Keep pain ladder	During mucositis
Anorexia	Weight albumin	Pre-transplant to 3-4 weeks
Change in taste		Pre-transplant up to 100 days
Hepatic		
Hepatitis	Viral serology	2-3 weeks
Liver failure	Hepatic enzymes	
Endothelial		
VOD	Hepatic enzymes LDH Ultrasound	30-60 days
Capillary leak syndrome	Weight albumin	30-60days
Engraftment syndrome	Weight renal & liver function	At engraftment
Diffuse alveolar haemorage	Chest x-ray, CT Chest BAL	19 Days (Auto); 12Days (Allo)
Microangiopathy	FBC, LDH. Liver biochemistry	60 Days
	Viral screens Vital signs	
Idiopathic pneumonia syndrome	Chest x-ray, CT chest, BAL	26 Days
	Lung biopsy	
Various Infections		
Bacterial	Blood cultures, tissue swabs	Usually during neutropenia but may occur at any time.
Fungal		
Viral	Viral blood tests	1-4 weeks

5.2.1 REJECTION AND NON-ENGRAFTMENT

Rejection is an immune response with both human and cellular components involved. It can be diagnosed by few peripheral cells in the circulation (not regenerating adequately) or loss of these occurring later. It can be associated with mixed chimerism within lymphocyte populations. Bone marrow biopsy will have infiltrating T cells perhaps accompanied by infiltrating eosinophils, plasma cells and neutrophils, occurring usually in allogeneic and matched unrelated donor transplants.

These were significant impediments to survival but early recognition and repeat procedure with altered conditioning and enhanced post-transplant corticosteroids with methotrexate was the standard of this era.

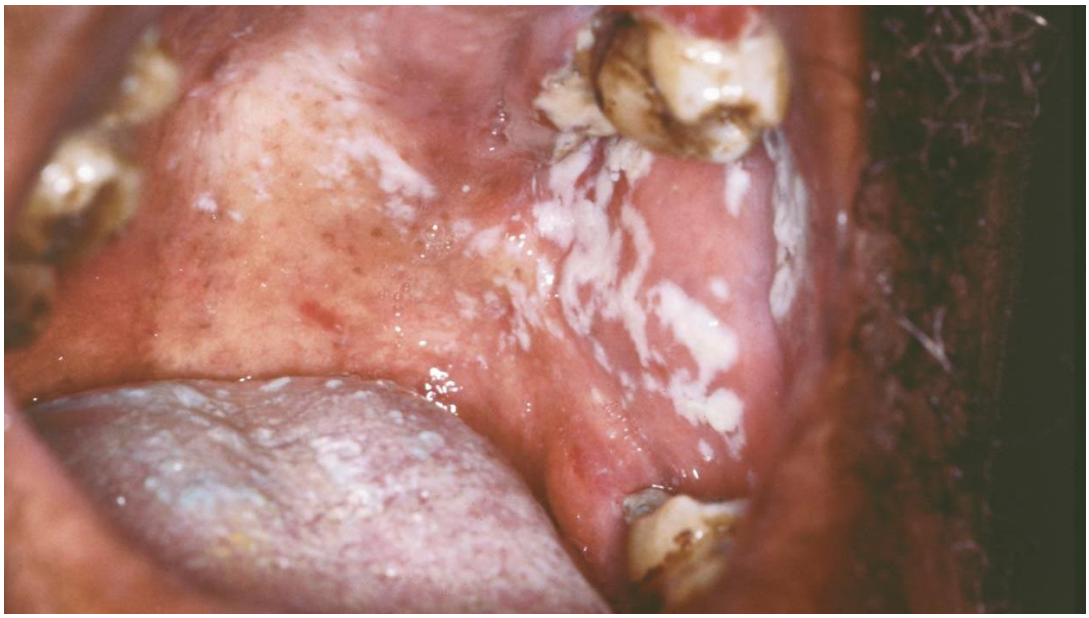
This was the first personal contact with the feared consequences previously described as either primary graft failure or secondary rejection, depending upon the time sequence. These have not been a problem since exposing haematopoietic stem and progenitor cells to the Campath® series of monoclonal antibodies in-the-bag.[2] Additional advantages might theoretically be derived from exposing haematopoietic stem and progenitor cells to the radiotherapy, both total nodal and total body, used as an anti-tumour agent in patients with haematologic malignancy.[3] The short terminal repeat assay was established in order to anticipate any risk of graft loss and our results are consistent with published data on chimerism.[4]

When rejection of the graft occurred the procedure could be repeated but conditioning changed to four days cyclophosphamide 50 mg/kg IVI over three hours and overlapped with ATG 30 mg/kg daily for three days – chemotherapy was given during the night and followed with protein during the day, as it could cause allergic reaction.

5.2.2 GASTROENTEROLOGY

These were relatively short lived normally returning to normal once regeneration occurred. Painful mouth due to mucositis with sloughing of epithelial layers predicated a need for a high level of pain control. This step was best managed with patient education and personal attention to scrupulous hygiene. Rotating mouth wash with alternating hydrogen peroxide and warm sodium bicarbonate facilitated removal of dead tissue which was a nidus for infection. The pathogens were most often viral with ulcers and fungal superinfection extending down the oesophagus and contaminating of trachea with aspiration to the pulmonary parenchyma. **(Figure 10)**

FIGURE 10
EXAMPLE OF OROPHARYNGEAL MUCOSITIS WITH SUPERIMPOSED FUNGAL INFECTION

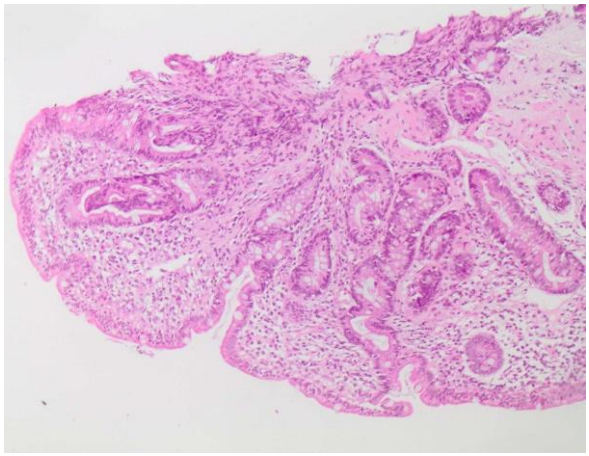


Dry mouth could be a problem and was often helped by 30 mg pilocarpine hydrochloride orally each day[5] with artificial saliva which is now routinely available. Patients were routinely commenced on maxalon 10mg daily, kytril® 1mg daily and phenobarbitone 10 mg three times daily one week before commencement of conditioning. Nausea and vomiting constituted a problem and adequate antiemetics needed to be provided. These were changed as necessary in order to give the individual adequate cover. A further ongoing challenge was that of diarrhoea and it was necessary to recognize that *Clostridium Difficile* infection could occur and that eradication regimens were uniform but not universally effective. Furthermore, there has been considerable interest in the use of anti-diarrhoea therapy in cancer patients where three principles apply: firstly, to reduce fluid loss by inhibiting intestinal secretion, then by promoting absorption and decreasing intestinal motility.

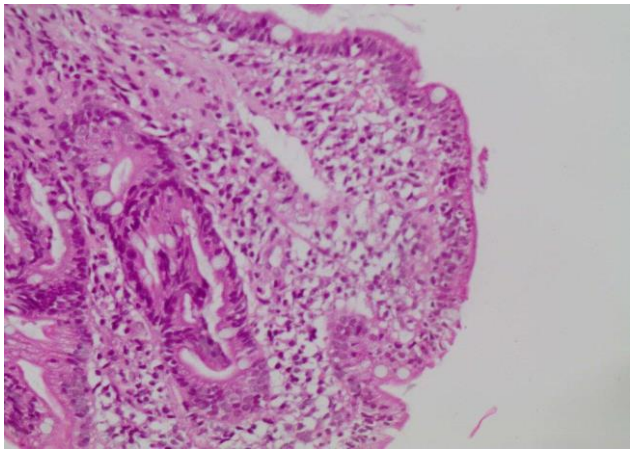
The available therapeutic options are classified as bowel transit inhibitors (loperamide and codein) intraluminal agents (cholestyramine) and post-absorptive or antisecretory drugs (clonidine). These drugs were selected, singly or in combination, after consultation with the gastroenterologist on an individual basis.[6,7]

As part of the bowel abnormalities, suboptimal nutrition emerged as a significant consequence which required moment–to-moment management of oesophageal pain due to mucositis, dysmotility and chemotherapy as well as radiation induced malabsorption. Following transplantation gluten intolerance was an unusual complication**.** This biopsy **(Figure 11a and Figure 11b)** demonstrate the features of gluten intolerance. There is a marked mucosal atrophy with flattening of the villi and an increase in the intraepithelial lymphocytes. The CD3 stain in **(Figure 11c and Figure 11d)** highlights a striking increase in intraepithelial T- lymphocytes. Similar symptoms may arise from viral infection such as cytomegaloviral injury on biopsy. (**Figure 12**)

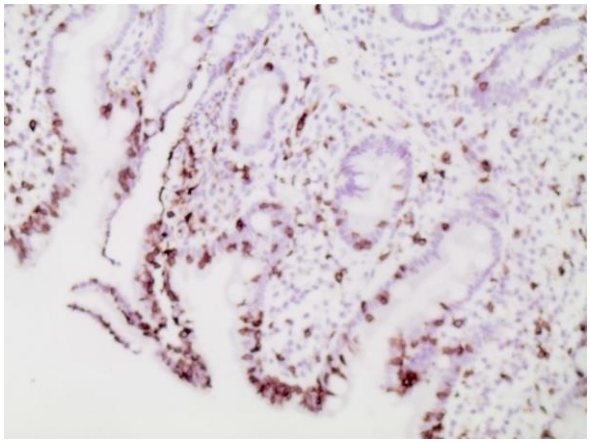
FIGURE 11
PHOTOMICROGRAPHS OF INTESTINAL BIPOSIES IN A CASE OF REFRACTORY MALABSORPTION



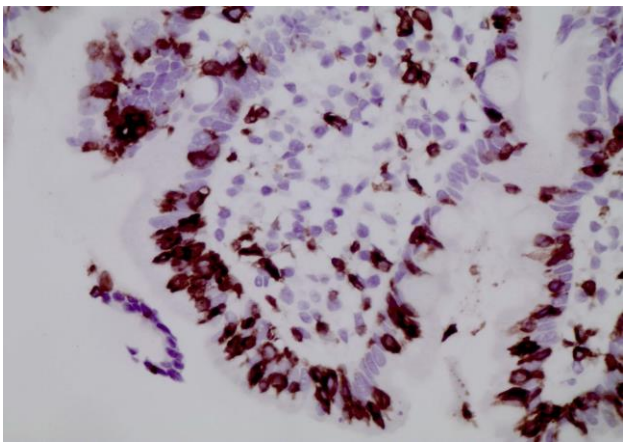
(a)



(b)



(c)

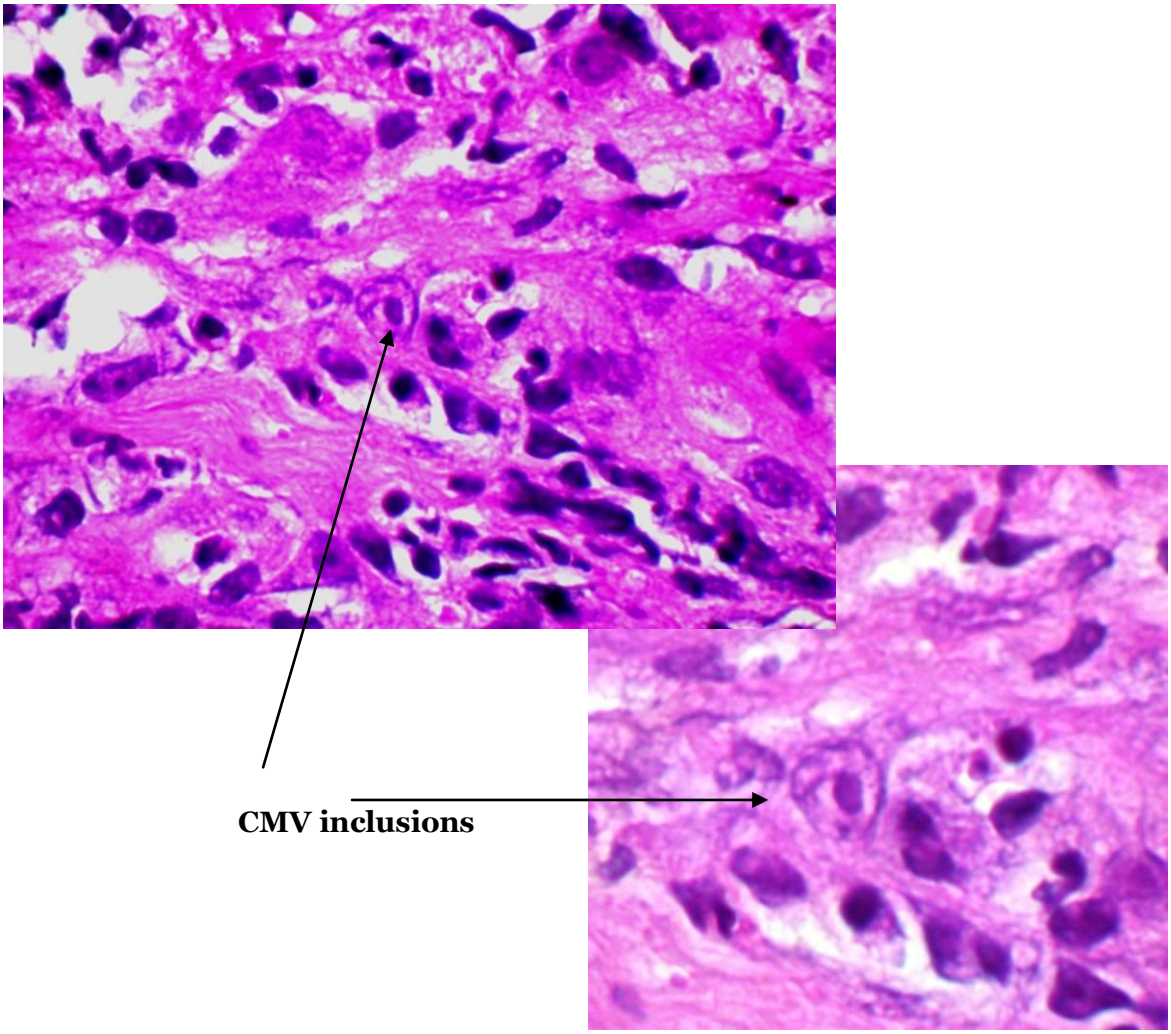


(d)

Courtesy Dr Kathy Taylor – Pathcare

FIGURE 12

PHOTOMICROGRAPHS OF AN ENDOSCOPIC BIOPSY DEMONTRATING CYTOMEGAROVIRUS INCLUSION BODIES

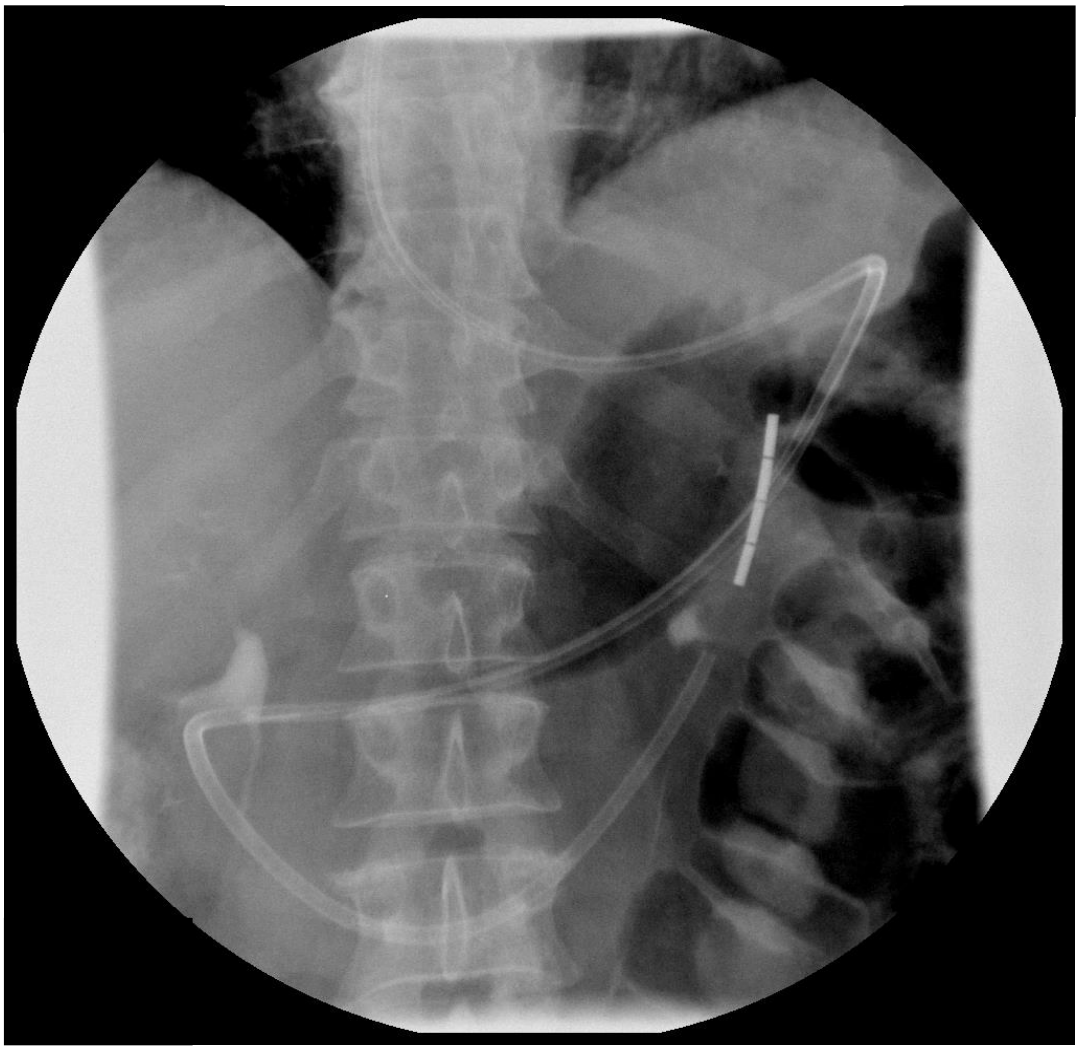


Courtesy Dr Kathy Taylor – Pathcare

Gastroenterological injury resulted in more subtle changes that necessitated individualised or targeted dietary regimens.[8] This segment of the standard protocol incorporated the long experience of enteral nutrition using a fine bore nasojejunal feeding tube that showed that the need for total parenteral alternatives were largely unnecessary.[9] Early on the greater patient acceptance of these tubes placed in the duodenum rather than the stomach was confirmed - careful explanation was provided. This technique was highly cost-effective and represents possibly the largest single unit experience of enteral long term nutritional supplementation. **(Figure 13)**

The use of this route was effective in correcting coeliac changes over a three month period.

FIGURE 13
PLACEMENT OF FINE BORE FEEDING NASO JEJUNAL TUBE (FLUOROSCOPIC IMAGE)



Courtesy Dr Derek Solomon – Tuft and Partners

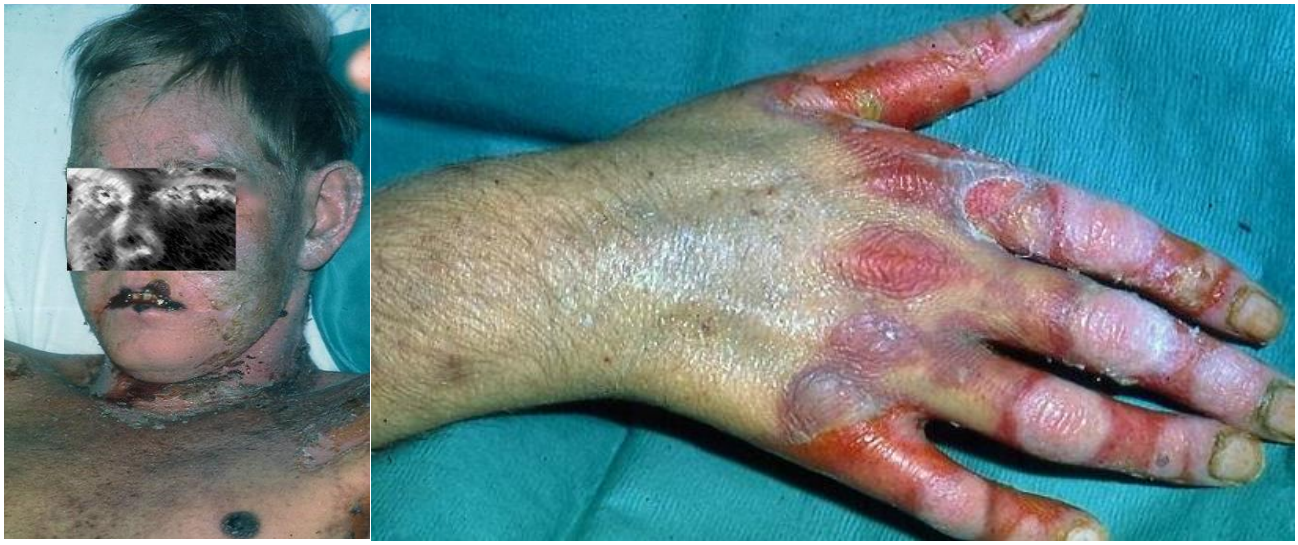
5.2.3 GRAFT-VERSUS-HOST DISEASE

The classical graft-versus-host syndrome, with extensive desquamating dermatitis and enterocolitis giving rise to torrential diarrhoea and cholestasis causing relentless increases in bilirubin, was commonplace. This could be followed by a chronic variant that progressed to the equivalent of scleroderma and destroyed the quality of life.[10] This situation was the frequent scenario on the transplant unit that devastated families as well as medical and nursing staff.

The ominous occurrence of puritic rash on palms and soles accompanied by fulminating diarrhoea which was virtually impossible to control had by far the greatest impact on the post-transplant course. From a nursing stance, this complication created enormous problems with fluid and electrolyte balance and it was characteristically associated with progressive jaundice. This symptom complex, termed acute graft-versus-host disease with extensive skin desquamation resembling whole body burns in extent and severity, was a

destructive and potentially lethal complication. **(Figure 14)** Additionally, the superimposed infection, usually pulmonary, added to both morbidity and mortality. The immense burden of nursing these patients and supporting their families proved both a challenge and underpinned the team's determination to work to abrogate the entity. This pattern was seen in early cases before standard use of the monoclonal Campath® antibody in-the-bag. Once the latter regimen became routine this severe complication virtually disappeared and the incidence decreased to very low levels.[2] This entity was already recognised as one of the most undesirable and not infrequently lethal characteristics of transplantations.

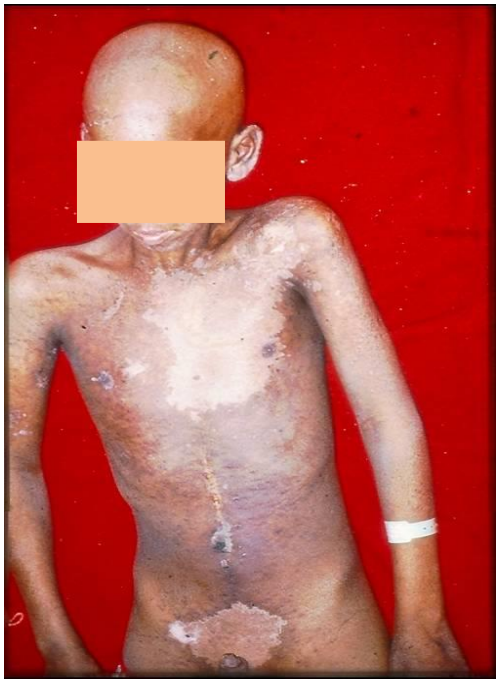
FIGURE 14
ACUTE GRAFT-VERSUS-HOST DISEASE DURING PRE-CAMPATH® DAYS



The acute GVHD could advance over varying periods to severe fibrosis, similar to scleroderma, vastly compromising quality of life. This entity was progressive and almost impossible to reverse so that prevention became a special challenge. It, again, was the trademark of the earliest allografts – prior to *ex vivo* T lymphocyte depletion - and not seen in the Campath® era. [2] **(Figure 15)**

FIGURE 15

CHRONIC GRAFT-VERSUS-HOST DISEASE DURING USE OF UNMANIPULATED STEM CELLS



Many patients had a rash and histopathology characterisation separated allergic drug e.g. cotrimoxazole **(Figure 16)** and viral, frequently due to herpes zoster where painful vesicles occurred once routine post transplant prophylaxis had been discontinued **(Figure 17)** from transplant related graft-versus-host disease which was a significant aspect of this audit.

FIGURE 16
ACUTE ALLERGIC SKIN ERUPTION DUE TO DRUG SENSITIVITY

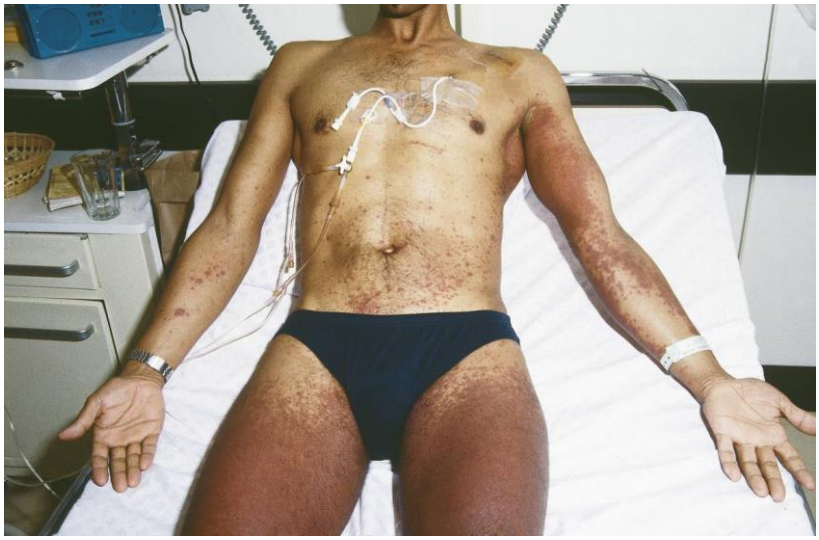
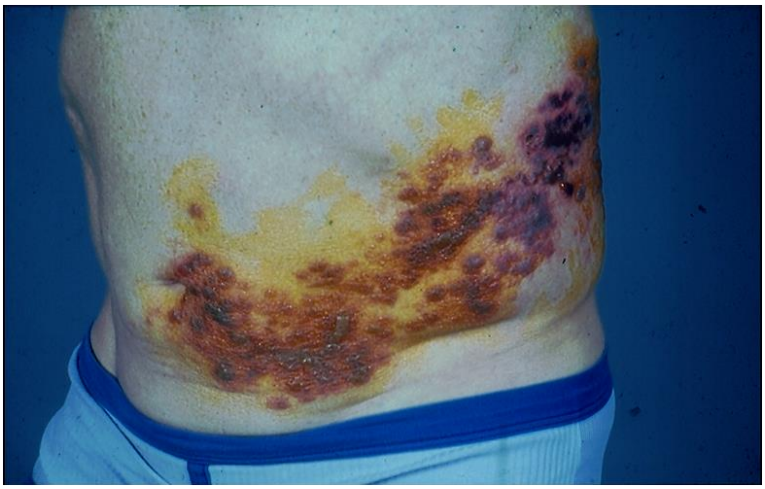


FIGURE 17
EXAMPLE OF A VIRAL RASH DUE TO HERPES ZOSTER



In graft-versus-host disease, grading[11] and staging[12] were combined to dictate management. In Grade I, following biopsy, the erythroderma was treated with steroid creams only in the first two weeks and then reassessed. This entailed ≤25% skin and no systemic involvement. In Grade II, non-response was treated with additional steroid in the first instance and failure at two weeks with cyclosporin A. In the latter, skinlesions were more extensive with liver and GIT also involved. Grade III and IV were not seen in this post Campath® study.

Huge psychosocial problems occurred at all levels and it was a tribute to the trust and fortitude of patients, coupled with the compassionate understanding so evident throughout the multidisciplinary management team, that everybody recognised and rose to meet the challenge. Collectively these sequential events were balanced against the perspective of real benefit. External support and encouragement from others intensively studying this problem – both in experimental laboratory[11] and in dedicated units – enabled our centre to weather a truly stormy period.

5.2.4 ENGRAFTMENT SYNDROME[13]

This was a non infectious syndrome presenting with fever, rash, diarrhoea and often abdominal pain. Less common pulmonary infiltrates and hepatic dysfunction could be present. It could be potentially confused with graft-versus-host disease and was responsive to corticosteroids.[14] This occurred also, and quite typically, with autografts when regeneration of bone marrow took place and could be confused with allergic or infectious cases.

5.2.5 BLEEDING DUE TO THROMBOCYTOPENIA

Bleeding occurred in about 10% of patients due to thrombocytopenia. Where platelet refractoriness was present, the risk could also be reduced by plasma exchange including high-dose gammaglobulin and, where appropriate, elective splenectomy. New options available were rituximab[15] and thrombopoietin agents.[16] Other hazards were primarily diffuse alveolar haemorrhage and this complication needed to be avoided at all cost. It was necessary to ensure that the INR was maintained. Vigilance to emergence of post-transplant immune[17] or thrombotic thrombocytopenic purpura (TTP) [18, 19] was important. Bleeding could also present as GIT or intracranial.

Vaginal bleeding may occur during chemotherapy and particularly after transplantation. All patients were managed on cyclokapron® and mefenamic acid[20] since the combination was believed to be more effective than either alone. Additional benefit appeared to be derived from Nordette® or an equivalent (levonorgestrel/oestrogens).

Where breakthrough vaginal bleeding occurred, the preferred therapeutic approach was to use progestagens in the form of norethisterone 5mg three times a day: this dose could be titrated up to use 10mg six-hourly. An alternative was to replace the Primolut N® with micronised estradiol in the form of estrofem 2mg daily. An additional option, if both the above failed, was the use of an oestrogen-progestogen regimen[21] such as liviferm. Premelle 2.5 was a combination in one tablet of premarin 0.625 mg and 2.5 mg provera. This was not used as a routine in the post-transplant patients.

Prophylactic platelets were given usually at 10 x 10^9/L but with active bleeding, the level was adjusted upwards. There were many variables that could either accelerate or delay platelet recovery. **(Table 7)**

TABLE 7

VARIABLES ASSOCIATED WITH ACCELERATED OR DELAYED PLATELET RECOVERY RESPECTIVELY

Accelerated	**Delayed**
Higher CD34 count	Fever
Higher platelet count at start of myeloablative conditioning	VOD
Graft from HLA identical sibling donors	Use of post-transplant growth factor
Prior stem cell transplant	PBSC transplant
Bone marrow as source for transplant	Prior radiotherapy

5.2.6 HEMORRHAGIC CYSTITIS

This complication could be related to adenoviral infection[22] and also due to thrombocytopenia. Management was multifactorial and difficult[23] usually reflecting drugs used for conditioning such as cyclophosphamide, ifosfamide, busulphan and fludarabine.[24,25] Once bleeding had occurred, intravenous or oral uromixetan was given.[26] If this proved insufficient, it was combined with appropriate hydration and intravesical irrigation with 1L of sorbitol every three hours.[27] ; this was repeated every 24 hours. Local anaesthetic was not needed. Development of any clots within the bladder required immediate evacuation and discontinuation of Cyclokapron®.

The protocol dictated that all patients should maintain platelet counts of above 50 x 10^9/L: only then could cyclokapron® be restarted, provided bleeding had ceased. Premarin 0.625 mg daily could be added orally as empiric therapy. In patients who were refractory to sorbitol, various other options – such as silver nitrate, alum, uromitexan and formalin - were tried.

5.2.7 FEVER

Varying degrees of fever which occurred could be controlled with appropriate antimicrobials and typically improved with engraftment.

Particularly during neutropenia, defined as 0.5 x 10 ^{9}/L, any increase in temperature above baseline was checked and if confirmed within one hour empiric antibiotics were commenced. These have over long periods of time been the subject of detailed results from isolate surveillance. In the absence of any positive cultures from blood, urine or stool, the policy to direct treatment comprised parenteral monotherapy with piperacillin and tazobactam or in combination with amikacin adjusted for any decrease in renal function to maintain therapeutic antimicrobial levels.[28] Failure to achieve temperature resolution predicated escalation at 48 hours to gram-positive cover beginning with intravenous vancomycin at 20-mg/kg/24 hours including appropriate monitoring of plasma levels.[29] In the next stage, again with pyrexia beyond a further 48 hours, the approach was to add a milligram per kilogram of amphotericin B in 200 ml of 5% dextrose water as a continuous 24 hour infusion. This technique was shown to have virtually no side-effects including, particularly, nephrotoxicity.[30]

Culture studies and fungal surveillance were routine while recovery of organisms dictated change in the antibiotic regimen.

Unusually opportunistic atypical infections, tuberculosis or pneumocystis clouded the diagnostic picture. For this reason efforts were made to isolate the offending pathogen with microbiological studies of blood and induced sputum, bronchoalveolar lavage and, in terms of aggressive diagnosis, bronchoscopy with biopsy and brushing may be unavoidable.

Viral infections could be troublesome especially herpes simplex of the oropharynx[31] and reactivation of cytomegalovirus requiring appropriate therapy which should be commenced as soon as possible.[32] Of note, in sub-Saharan Africa, was the challenge of the Epstein Barr virus[33] and in particular retroviral positivity presenting en route to the Acquired Immune Deficiency Syndrome.[34,35]

There was increasing use of fluconazole and a shift towards the greater employment of itraconazole if fungal infections reoccurred or were slow to resolve. In these circumstances long term maintenance may have been necessary. Newer liposomal products or equivalents are under study as are later generation azoles exemplified by voriconazole and posaconazole.[36] Prevention of opportunistic infections extended to the use of immunisation.[37] Early onset of microbial events in patients undergoing bone marrow transplantation was a well-recognized problem. Mechanisms for avoidance led to guidelines from the Centre for Disease Control in Atlanta. These included recommendations which were applicable to children.[38] Screening for cytomegaloviral infections was undertaken using DNA technology[39] by PCR. Strategies for management were evolving with importance placed on the value of ganciclovir, foscarnet and cidofovir. Adenoviral infections were troublesome and until recently had not been treated.

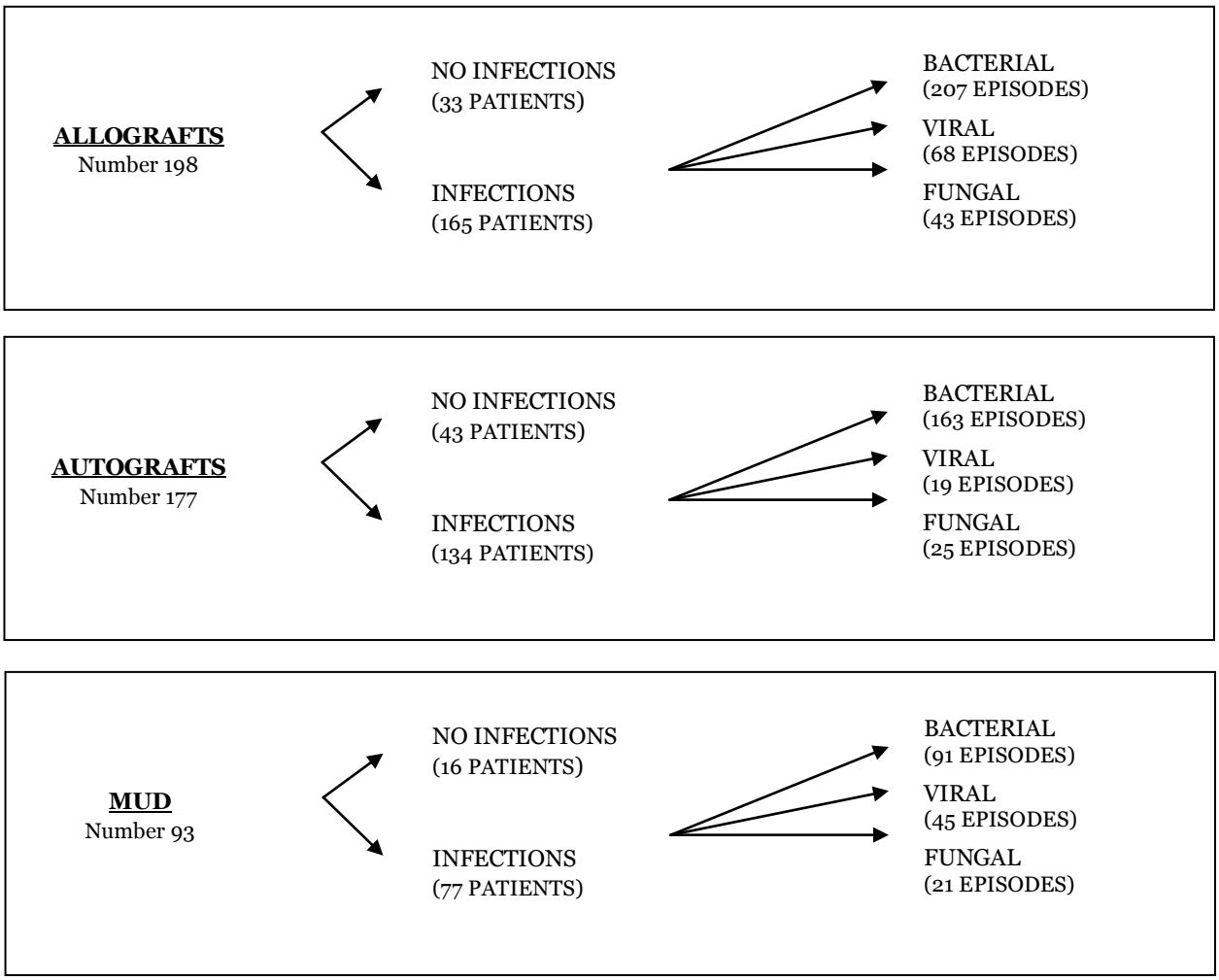
The data in table eight does not necessary apply to the cohort where different immunologic reconstitution may be found with Campath® conditioning and no post – transplant immunosuppression used. **(Table 8)**

TABLE 8
PHASES OF OPPORTUNISTIC INFECTIONS AMONG ALLOGENEIC STEM CELL RECIPIENTS

	Phase 1, Pre-engraftment, <30 days	**Phase II, post-engraftment, 30 – 100 days**	**Phase III, Late phase, > 100 days**
Host immune System defect	Neutropenia, mucositis and Acute graft-versus-host disease	Impaired cellular immunity and acute and chronic graft-versus-host disease	Impaired cellular and humoral immunity and chronic graft-versus-host disease
Device risk	Central line →	→	
Allogeneic Patients	Respiratory and enteric viruses →		
	Herpes simplex virus		
		Cytomegalovirus	
			Varicella-zoster virus
	Facultative Gram-negative bacilli		
		Epstein-Barr virus lymphoproliferative disease	
	Staphylococcus epidermidis		
			Encapsulated bacteria (e.g. pneumococcus)
	Gastrointestinal tract enterococci species		
		All candida species	
	Aspergillus species		Aspergillus species
		Toxoplasma gondii	
		Strongyloides stercoralis	

In the audit cohort, it was found that in 198 allograft patients, 318 episodes of infection occurred, while in the 177 autografts there were 207 episodes. In 93 MUD transplants, 157 episodes were present. It can be seen that infections occurred more frequently in the allografts. In total, in all groups, infectious episodes occurred in 376 (80%) subjects while in 92 (20%), no infections were noted. (**Table 9)**

TABLE 9
INFECTIOUS COMPLICATIONS IN AUDITED GROUP OF PATIENTS



5.2.8 PAIN

The major cause for pain was injury to the mucosal tissues induced by the conditioning regimes. It could occur in any transplant-related phase. Oral mucositis often involving ulcers spreading to the oesophagus caused secondary complications. During mobilization and neutropenia the growth factor used could lead to bone pain and less frequently to headaches. If acute and chronic GVHD was present, desquamation of skin and in the latter, contractures caused pain. Infections such as cellulitis, abscesses as well as herpetic viruses, causing severe neuropathic pain, could be present. Marrow harvesting pain was more severe after anterior than after posterior iliac crest collection. Radiotherapy could cause a painful, inflammatory skin reaction.

During infusion the patient could develop severe abdominal cramps, thoracic pain and headaches due to DMSO toxicity or reaction to Campath®. Urinary bladder irritation caused by certain conditioning drugs (mentioned previously) could occur. In the later phases following BMT, osteolysis, osteopenia and osteoporosis could cause localised and sometimes radiating pain. Bisphosphonates prophylactically, as well as for treatment, could be used.

As pain was a major problem, the guiding principle was to use the World Health Organisation pain control. ladder.[40] It was important to do regular assessments with a rating scale and also assess analgesia efficiency. Appropriate choice of analgesia involved balancing efficacy and avoidance of side-effects. For example, in thrombocytopenia to by-pass use of non-steroidal anti-inflammatory drugs which increases the risk of bleeding, was a crucial protocol consideration. In addition, symptomatic reflux of acidic gastric contents was surprisingly common and responded, in our experience, most efficiently to five or six hourly proton pump inhibition, or to a continuous 24 hour infusion.[41]

In this situation consultation with a gastroenterologist was often needed to define aetiology by means of endoscopy. Haemorrhoids and anal fistulae were not particularly frequent but required prompt intervention using betadine® sitz baths and local pain control with 50% mixture of EMLA cream (lidocaine prilocaine eutectic mixture)[42] and Scheriproct®.[43]

Managing pain was crucial and if mild, started with paracetamol. Moderate pain needed escalation to mild opioids (codeine and tramadol) and if pain was severe, we commenced oral, and progressed to intravenous, titrated doses of strong opioids (morphine). Antidepressants and anticonvulsants could be added.

Late complications appeared after day 100, a few of which will be discussed. Chronic GVHD was incorporated into the GVHD section. (**Table 10)**

TABLE 10

LATE COMPLICATIONS ≥ 100 DAYS

Complication	Test to be performed	Time to complication
OCULAR EFFECTS		
Cataracts	slit lamp examination; Schirmer's test	2-3 years
Kerato-conjuntivitis		2-3 years
SKIN & APPENDAGES		
Chronic GVHD	Clinical examination	No time limit
Dry skin	Skin biopsy	
Hair loss		
Splinting/loss of nails		
Premature greying		
Pruritis		
ORAL & DENTAL		
Mucosal atrophy	Regular examination	No time limit
Erythema		
Lichenoid lesions		

ORAL & DENTAL (continued)		
Hyperkeratinoid lesions		
Salivary gland dysfunction		
Taste disorders		
Dental decay		
THYROID DYSFUNCTION		
Hypothyroid	Thyroid function tests	2-3 years
Hyperthyroid		
FERTILITY & GONODAL DYSFUNCTION		
Azoospermia	Hormonal levels	No time limit
Premature menopause		
NON-INFECTIOUS RESPIRATORY		
BO	Respiratory function tests	1-12 months
COP	Chest X-Ray	
IPS	Bronchoalveolar lavage for histology	120 Days
	High resolution tomography	
CARDIAC		
Cardiac failure	ECG	10 years
	Echocardiograph	
	Pro-PNB	
VASCULAR		
Cerobrovascular	Brain scan	25 years
Cardiovascular	ECHO	
METABOLIC SYNDROME		
Hypertension	Blood pressure measurements	Any time
Diabetes	HbA1C	
Dyslipidaemia	Lipogram	
CHRONIC KIDNEY DISEASE	Renal function	5-10 years
	Urine protein analysis	
	Renal biopsy	
LIVER & IRON OVERLOAD		
Viral hepatitis	Viral screens PCR	10years
Chronic GVHD	Skin biopsy	
Iron overload	Liver enzymes & function	
Cirrhosis	Iron & ferritin	
	Liver biopsy	
MALIGNANCY	Histology	5-10 years

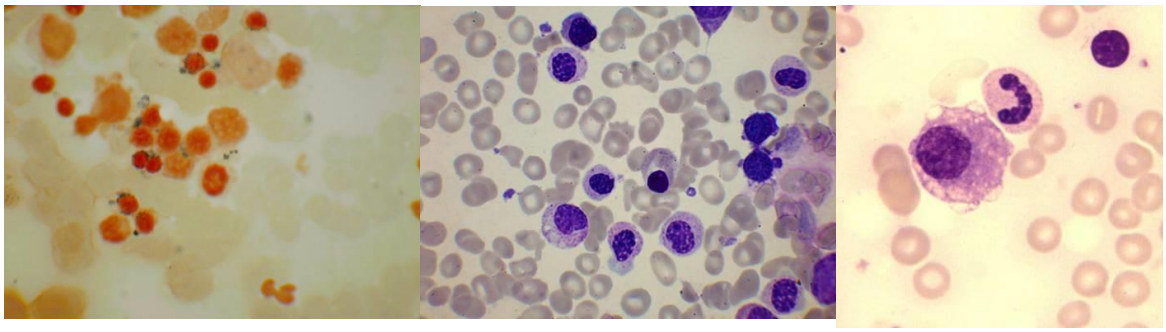
BONE DISEASE		
Osteopaenia	DXA	12-18 Months
Osteoporosis	Biomarkers	

5.2.9 MYELODYSPLASIA

With greater experience and close hands-on contact, a variegated range of individual subtleties emerged scattered among different organ systems. These were sometimes in classical or traditional haematology exemplified by delayed onset of myelodysplasia or pre-leukaemia with cytomorphology showing dysplastic changes in erythrocytes (ringed sideroblasts), granulocytes and megakaryocytes. These features correlated with conventional karyotyping on fluorescent *in situ* hybridization for interphase cytogenetics. **(Figure 18)**

FIGURE 18

PHOTOMICROGRAPHS: POST TRANSPLANT MYELODYSPLASIA

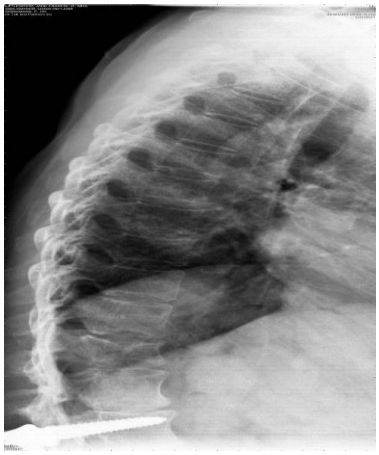


5.2.10 OSTEOPOROSIS

Because premature menopause was present post transplant, it could often lead to bone loss. Minor symptoms and sometimes changes only demonstrable on specific testing in non-haematopoietic tissues were present. Occasionally these symptoms were a slight reduction in maximum effort tolerance and others developed a surprising degree of bone pain and osteoporosis.

Osteopenia could progress rapidly to painful activity. Even minor trauma, such as a fall, could then result in fractures, typically vertebral compression. (**Figure 19)**

FIGURE 19
LATERAL VIEW OF THE SPINE – COMPRESSION FRACTURE IN A PATIENT WITH OSTEOPOROSIS



Courtesy Dr Derek Solomon – Tuft and Partners

5.2.11 MISCELLANEOUS

Minor abnormalities of bowel action and underplayed endocrine disturbances, typically involving thyroid integrity - both hypothyroidism and hyperthyroidism - were sometimes detected. Ocular changes in the form of cataracts occurred frequently following conditioning with radiation. Understandably when these problems occurred in different individuals, varying both in incidence and severity diluted among a large transplant population, a single coordinating influence was necessary for the realisation that, viewed globally, there was cumulative decrease in the quality of life. This point highlights the important concept of survivorship – an opinion needing much better definition and to be addressed in this research investigation taking into account extension beyond bone marrow re-growth.

The onset of complaints of differing intensity located in different organ systems was sometimes overlooked and with their recognition compounded by not only presenting late but typically progressing gradually. Superimposed on this situation were many previously present psychological, psychosocial or even psychiatric disturbances.[3] These could be subtle and, more often than not, correlated with the previous disease, chemotherapy or the multi-organ injuries inflicted by the bone marrow transplant. It became clear that with this substantial dataset, a systematic research study was not only relevant but urgently needed.

5.3 THE PATIENT POPULATION

At commencement of bone marrow transplantation there was a single centre in South Africa. This situation persisted for more than 15 years before a similar programme commenced at the University of Witwatersrand in 1985. The importance of that observation is that all patients - whether private or state and irrespective of where they came from in South Africa or further afield - were sent to our unit in the Groote Schuur Hospital.

In many ways this process heightened the heterogeneity of those cases who were seen and treated. The most striking issue was probably the differences in psychosocial background with nutritional implications. Another notable feature was the relative paucity of children, largely because the viewpoint at the Red Cross War

Memorial Children's Hospital was that these procedures, certainly those for haematological malignancy, had not yet become established as being superior to the available chemotherapy.

For this thesis an audit was done on the 468 transplanted cases to provide data for the acute associations. The late effects involved a group of volunteers of survivors (n=55) available to do tests for the late effects.

5.4 ACUTE ASSOCIATIONS –AUDIT ANALYSIS

Deviation from normal, or at least from pre-transplant values, in the four extra haematopoietic systems chosen for analysis were early events, which were transient, largely predictable and virtually constant features. For this reason they were part of the clinical record and proactively managed as integral to the standard programme and incorporated into this database. Major organs were chosen as sufficient data was available for analysis on them. The results are documented in chapter 7.

5.4.1 NEPHROLOGY

This was closely monitored with serial determinations of urea, creatinine and electrolytes in plasma, weekly or more frequently as needed. When relevant, hydration was documented by means of plasma osmolality or urinary specific gravity. Strict attention was paid to fluid and electrolyte balance and the avoidance of any potentially nephrotoxic agents. Renal status was reviewed daily with a consultant.[44]

5.4.2 CARDIOLOGY

Rested on regular clinical assessment, which was supplemented objectively by 12 lead electrocardiogram[45] and echocardiograph.[46] These referrals were standard care and results were charted serially.

5.4.3 DERMATOLOGY

Involved routine and frequent consultation with a well-trained and experienced specialist with interest in this field.[47] In case of doubt, adequate skin biopsy was obtained for interpretation by a specialist dermatopathologist.[48]

5.4.4 GASTROENTEROLOGY

Required moment-to-moment management and liver function and enzyme estimations done frequently.

5.5 LATE EFFECTS – RESEARCH STUDY

Once sufficient observations had been recorded in consecutive cases, to give confidence to these findings, the Stellenbosch University Bone Marrow Study Group was constituted to provide access to expertise and new technologies that had not been previously available due to cost constraints. These systems were chosen as the specialised units were willing to participate. The subjects consisted of 55 volunteers from long term survivors. Others from the complete data base were not available for various reasons. Results are available in chapter 7

5.5.1 PULMONOLOGY

This centred on the clinical assessment supported by high quality radiology and a selected series of lung function studies.[49,50] Acute changes almost always resolved in the first 14-21 days when they were of bacterial or viral aetiology and in keeping with recovery of the granulocyte count supported by physiotherapy and appropriate protocolised antibiotics. Late consequences defined as non-infectious respiratory tract lesions, [51] emerged as a specific aspect of this research study and necessitated further lung function testing.

5.5.2 IMMUNOLOGY

Both innate and adaptive immunologic reconstitution[52] could be affected by prior disease, treatment and conditioning as well as choice of transplantation.[52] Unfortunately in the early years of this long-running investigation such methodology was not available and, accordingly, a battery of standardised assays could now for the first time be applied to obtain this missing information.[53] It was relevant in that exposure to Campath® monoclonal antibodies lead to impaired immune reconstitution.[35] After transplantation, immunologic reconstitution took place variably between three months and four years.[54,55] In this period a number of infectious complications could arise.[56] These included herpes type 6, cytomegalovirus, which was very common in our patients, adenovirus,[57] hepatitis[58] and influenza.[59] Varicella zoster typically became a problem after withdrawal of acyclovir: if this occurred the trial by Whitley et al, [60] had shown that, albeit in relatively healthy patients, a tapered dose of prednisone with the acyclovir or zeletrix was of further benefit. The use of the current protected environment and locally collected stabilized human serum to provide immunoglobulin may well have been the key to the success of the programme. The whole question of re-immunisation[61] was particularly important. An excellent series of guidelines for re-vaccination was published by the The European Group for Blood and Bone Marrow Tranplant Registry.[62] At one year post-transplant a vaccination schedule as laid out by the group was utilised **(Table 11).**

TABLE 11

SCHEDULE FOR ROUTINE RE-IMMUNISATION

VACCINE	SCHEDULE
23-valent pneumococcal polysaccharide	12 and 24 months
Haemophilus influenza tybe b conjugate	12,14 and 24 months
Varicella zoster virus	Not licensed at present
Infuenza	Yearly lifelong resuming ≥ 6 months post HCST
Tetanus-diphtheria toxoid	12,14 and 24 months
Inactivated polio	12,14 and 24 months
Hepatitis B	12,14 and 24 months
Hepatitis A	Routine administration not recommended.
Meningococcal	Routine administration not recommended
MMR	≥ 24 months if immunocompetent

5.5.3 BONE DISEASE

This was explored to examine any impact on bone mineral density of those patients having undergone transplant with the addition of anti-CD52 monoclonal antibody in-the-bag as opposed to the more traditional preparatory regimens employing cytotoxic agents and steroids typically with post-procedure graft-versus-host prophylaxis.[63,64]

5.6 SUMMARISING COMMENT

The details in each of the inseparable yet consecutive components are specified because they were sequentially tested and, via translational research, transferred to a standard approach as the central activity of this doctoral project. They are the major source of observations making up the study and form the audited or reference database against which, firstly, outcome is defined. Secondly they delineated the cohort that qualified to be entered into the characterisation of late effects in those managed exclusively in this centre designated for transplantation by European and American Registries – including the Centre for International Blood and Marrow Transplant Research.

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CHAPTER 6

OVERALL SURVIVAL BY AGE, PROCEDURE AND DIAGNOSIS

FOCUS

This section details the outcome of every consecutive immunohaematopoietic stem cell transplant carried out by this accredited team between 1995 and 2010. Each was reported to The Centre for the International Blood and Marrow Transplant Research, National Donor Program and the European Group for Blood and Bone Marrow Transplant Registry. The commitment to undertake the audit was given impetus by two feasibility studies, one in children[1] and the other in adults[2]. These audits documented low rates of transplant related mortality, rejection and graft-versus-host disease, with the caution that these findings cannot necessarily be extrapolated to experiences outside academically orientated and supervised facilities. It had the basis for incorporation, after critical statistical analysis, of our data into annual geographical summaries from the EBMT Registry thereby providing stringent external quality control. During a prolonged period of close individualised monitoring of all variables, recognition of underappreciated side-effects accumulated. These symptoms were often accompanied by subtle signs and changes together with supporting laboratory data or other specialised studies that, collectively, signalled departure from normality – adverse impact on survivorship. When these were assembled a pattern emerged in which this cohort, immunosuppressed with Campath® monoclonal antibodies in-the-bag, appeared to differ significantly in a number of aspects from matching groups that used traditional chemotherapy and steroid based preparations including post-transplant regimens for graft-versus-host disease. Accordingly, a study was designed to give greater emphasis to these discrepancies by documenting late effects in lung, patterns of immune reconstitution and, most striking of all, in bone mineral density.

6.1 INTRODUCTION

Two steps preceded the definitive analysis for this study. Initially, the priority was to control the environment or facility in which the grafting was carried out. Secondly, once consolidated over time, to incorporate protocols which generated local results and which could be compared with other centres.

6.2 PATIENTS – GENERATION OF THE DATABASE

Consecutive individuals, (n=468) who were eligible for immunohaematopoietic stem cell transplantation, were registered from April 1995 through December 2010 and their demographic details were captured on a standardised spreadsheet **(Annexure 3).** This opportunity of providing costly and high-technology served two further purposes. It provided a pivotal point for studies emanating from other groups in Africa, making possible national comparisons. Secondly it objectively demonstrated that, with proper selection of correct indications for these procedures, transplant was feasible. It was however appropriate to compete for resources, albeit with some limitations imposed by the state for financial reasons and in the private sector by insurance plans. For the entire cohort, overall survival by Kaplan-Meier analysis split into children, defined as up to 20 years of age and young adults, and the remaining older group above 20 years of age. For each of these groups, outcome was compared by transplantation procedure being sibling allografts, autografts and matched unrelated volunteer donors. Using the latter three categories, a break-down of overall survival by diagnosis was done. Here there were sufficient numbers for only broad subdivision into aplasia, myeloid or lymphoid lineages and miscellaneous entities. Although sufficient data was available in acute myeloid leukaemia (AML) and lymphoma (NHL) the other diseases e.g. myeloma (MM) were not enough to provide survival data. The heterogenecity of the diseases was not taken into account as the numbers for each would then not have been sufficient for analysis. Despite this reality, attempts were made within each to test the possibility that significant differences might be revealed.

It must be emphasised that this is a unique series in which immunosuppression centred on using the Campath® monoclonal antibodies *ex vivo*, in which the graft was exposed to the protein in-the-bag - prior to infusion. The latter universal manipulation made it possible to explore discrepancies that may be attributable to the very specific variation when compared to traditional alternatives using cytotoxic agents, with or without corticosteroids, and extending these into the post- transplant period.[3]

6.3 METHODS

6.3.1 ROUTINE DIAGNOSIS

For patients entered into the transplant programmes following comprehensive clinical assessment, universal definitions were used for accurate diagnosis of congenital and acquired aplasia.[4] The World Health Organisation (WHO) guidelines contained the criteria for acute and chronic leukaemias, Hodgkin and the remaining lymphomas as well as plasma cell neoplasms.[5] This allowed diagnosis to be similar to the rest of the world. Before the WHO guidelines became available, the French-American-British (FAB) classification for acute myeloid leukaemias and the Revised European American categorisation for Lymphomas (REAL) were used.

6.3.2 SPECIAL INVESTIGATIONS

The WHO monograph contains, in each respective section, relevant information on lymph nodes and the bone

marrow as well as extranodal sites.[5] Criteria are provided for diagnosis and classification with inclusion of immunohistochemistry, flow cytometry, karyotyping and molecular genetics. The current study retained these standardised definitions in the interests of uniformity and to make possible exchange of data from this research project with pertinent publications.[6,7]

6.4 PRE-TRANSPLANTATION PHASE

Individuals undergoing these procedures did so in a rigidly standardised environment developed to bring about the best possible return on investment of resources.

The various interlocking components have been developed in the experimental haematology laboratory and subsequently translated into the clinic. Each section has been detailed in chapter four and this substantial infrastructure summarised.

The standardisation of these aspects was particularly challenging yet an indispensable first step in this long running project. Thus the physical plant and uniform protocols exclusively used were each distinct research developments. **(Table 12)**

TABLE 12

COMPONENTS OF THE TRANSPLANT FACILITY

Custom built protected environment
Dedicated nursing and paramedical professional team
Safe central venous access
Actively participating infectious disease, dermatology and pulmonology consultants
Gastroenterologist – dietician – nutritional programme
Policy for transfusion and use of blood products
Counselling and liaison psychiatry
Standardised management and research protocols
Haematology co-ordinator and data manager
Optimal staff and patient information sharing

A homogenous starting population was achieved to systematically explore the non-haematopoietic lesions arising in the course of stem cell transplantation.

6.5 PRIOR TREATMENT EXPOSURE

A major uncontrollable variable is the numerous diagnoses attached to otherwise eligible patients. **(Figure 20)** Whenever possible, standardised regimens were employed.

6.5.1 APLASIA (AA) (n=27) AND FANCONI ANAEMIA (FA) (n=16)

Intensive support included limiting blood and other components as far as possible. Where suitable sibling donors were available, this remained the preferred treatment.[8] In other instances, immunosuppressive regimens including high-dose methylprednisolone and antithymocyte globulin were standard.[9]

6.5.2 ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) (n=40)

Treatment was standardised to the Berlin–Frankfurt-Muenster or BFM protocols for adults and children respectively.[10,11]

6.5.3 ACUTE MYELOID LEUKAEMIA (AML) (n=92) AND MYELODYSPLASIA (MDS) (n=15)

Initially the combination of cytosine arabinoside and anthracycline antibiotic with one or other epipodophyllotoxin was employed.[12] More recently treatment was extended to the British Medical Research Council AML 15 programme. It was unfortunate that in some instances, managed healthcare organisations restricted access to these programmes on advice of local consultants.

Similarly, despite benefit from adding gemtuzumab ozogamicin, used in AML 15, this drug remains controversial and this option was generally unavailable.[13] In the myelodysplastic cases, therapy was in line with international recommendations based on characterisation[14] incorporating molecular genetics[15] but gene-profiling could not be carried out as the laboratory test was not available in South Africa.[16]

6.5.4 CHRONIC MYELOID LEUKAEMIA (CML) (n=72)

Disease control was rapidly achieved with hydroxyurea although, more recently, first-line therapy became imatinib mesylate.[17] Transplantation was offered to fully matched recipients particularly following escalation in disease process after administration of first and second generation tyrosine kinase inhibitors,[18] if cytogenetic abnormalities progressed or if this intervention was a specific patient choice.[19] Transplants were initially , before imatinib became available, done while in chronic phase. No patients were transplanted in blastic phase unless control was achieved by chemotherapy. The numbers in the different categories are not available.

6.5.5 CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL) (n=4)

Individuals received pulse chlorambucil or combinations of rituximab, fludarabine and cyclophosphamide.[20] Latterly, the anti CD52 monoclonal antibody Campath® or alemtuzumab[21] was used. In selected refractory cases allografting remained an option.[22] Patients were mostly transplanted if non responsive to therapy or early relapse, never as first line therapy except individuals who had a p53 deletion/mutation (del 17p13).

6.5.6 MYELOMA (MM) (n=41)

Stratification was by comorbidity[23] and risk factors using guidelines from the international myeloma working group.[24] The VECD regimen comprising vincristine, epirubicin, cyclophosphamide and dexamethasone was the preferred choice. Here the target was greater than 75% reduction in both paraprotein level and plasma cell infiltrate of the bone marrow trephine biopsy to qualify for autologous immunohaematopoietic stem cell grafting. Thalidomide was not approved by local third-party funders during these studies. With negotiation, appropriate cases could obtain bortezomib which was also not routinely available. Alternatives remain pulsed melphalan and methylprednisolone or salvage with dexamethasone and vinorelbine.[25]

6.5.7 HODGKIN (HL) (n=22) AND OTHER LYMPHOMAS (NHL) (n=107)

Current protocols were those used by the European Organisation for Research and Treatment in Cancer (EORTC) [26,27] and the German Hodgkin Lymphoma Study Group.[28,29] Patients proceeded to transplantation on the prevailing criteria. NHL and HL were not divided into different subgroups.

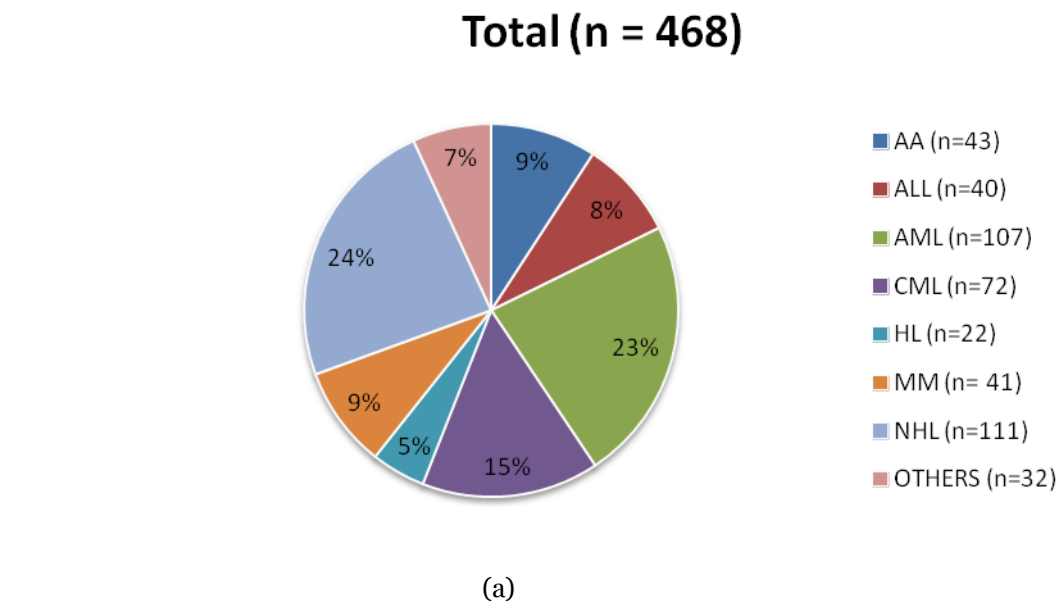
6.5.8 MISCELLANEOUS DIAGNOSES (n=32)

Numbers less than five in a particular category comprised thalassaemia, sickle cell disease, mucopolysaccharidosis and severe combined immunodeficiency disease (SCID). For statistical reasons these were analysed together but efforts made to generate survival by subtype were precluded due to small numbers.

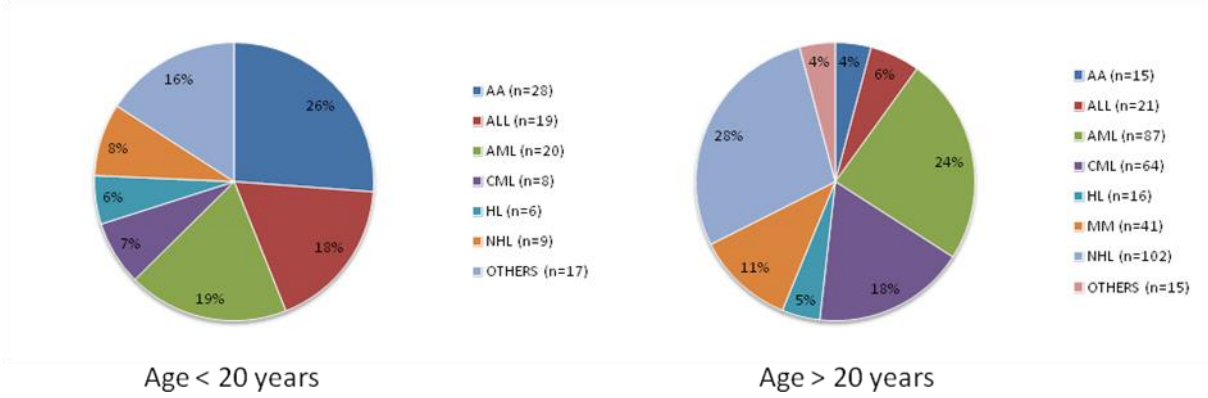
In **Figure 20,** aplastic anaemia included FA while CLL was combined with the NHL group **(Figure 20a)** indicates the total cohort (468), subdivided into **(Figure 20b)** the 107 patients equal or below 20 years and **(Figure 20c)** 361 greater than 20 years old. There were more adults as this is mainly an adult unit and children are transplanted only on referral. Whenever possible, standardised regimens were employed. The diseases for which most transplants were done was AML (23%) and NHL(24%).

FIGURE 20

TRANSPLANT CASES ACCORDING TO DISEASE CATEGORY AND AGE



(a)



(b) (c)

6.6 TRANSPLANTATION PHASE - OVERVIEW

6.6.1 CONDITIONING REGIMENS

Myeloablative options were used and in this study no reduced intensity preparations were employed.

6.6.1.1 RADIOTHERAPY

The transplant procedure was designated day 0 and radiotherapy began with 12 gray fractionated whole-body exposure on days -7, -6 and -5. This was followed by 60 mg per kilogram of cyclophosphamide intravenously on days -4 days -3 and 6 gray fractionated total nodal irradiation on day -2 and day -1.

6.6.1.2 CHEMOTHERAPY

Busulphan combined with cyclophosphamide was originally used in the four-day regimen.[30] Latterly this was replaced by a two-day alternative[31] and, additionally, intravenous agents were used in preference to the oral formulation.[32] In selected individuals where this drug was contraindicated, it was replaced by fludarabine.[33] In over 80% of lymphoma cases the first choice was BEAM preparation. In congenital haematologic disorders fludarabine was combined with antilymphocyte globulin.[34]

6.6.2 MOBILISATION AND QUALITY CONTROL

Stimulatory peptide therapy in the form of granulocyte colony-stimulating factor (Roche) was commenced subcutaneously on day -5 at a flat dose of 300 µg given daily with the last injection at 04h00 on the day of the first large volume apheresis harvest. The circulating CD34 positive population was noted but not specifically used to time these collections.[35]

6.6.3 GRAFT MANIPULATION

6.6.3.1 BONE MARROW

Initially only bone marrow was used. Although no longer routinely used, we continued to accommodate matched-unrelated volunteer programmes when predicated on requirements of the collaborating centres from different parts of the world.[36] This was usually dictated by their donor preference. Specific diseases were not taken into consideration. Here the harvest was modified using the standard apheresis technology, and the 2997 Cobe programme. Once more than 95% of the mononuclear cells had been recovered, the residual blood was re-infused to the donor or discarded: this practice is now obsolete.

The immunohaematopoietic stem and progenitor cell concentrate had the standard *ex vivo* addition of 20 mg of Campath® 1H, then incubated for half an hour at 37°C and infused.[37]

6.6.3.2 PERIPHERAL BLOOD

Collections required a minimum of 6.5 x 10^8 /kg mononuclear cells and greater than 2 x 10^6 /kg CD34 positive population. Long-term colony initiating cells were determined in clonogenic assays.[38,39] These assays were initially done to show how quickly patients would engraft. The cells needed to be incubated for two weeks. Because it was time-consuming and the technologists found it difficult to count the colonies, and by the time they were counted, it was already known whether engraftment had occurred and it was then discontinued. Even in patients who failed to meet the required number of CD34 or mononuclear cells, rapid reconstitution of the bone marrow still occurred.

6.6.4 INFUSION TECHNIQUE

Premedication was given half an hour before the graft containing the monoclonal antibody; this consisted of 100 mg hydrocortisone, 12.5 mg of promethazine hydrochloride intravenously and 500 mg paracetamol orally. Non-invasive cardiovascular and respiratory monitoring was continued until all vital signs were stable. Oxygen desaturation necessitated rate adjustment of the infusion and occurred in less than 5% of the procedures. In approximately 30%, pyrogenic reactions with abdominal discomfort were attributed to the immunoglobulin (Campath®) and treated symptomatically. There were no lasting side-effects. In autografts, transient fever was initially attributed to the presence of DMSO but more recently clarified and ascribed to the presence of contaminating granulocytes.[40]

6.6.5 ALLOGENEIC TRANSPLANTS

In fully histocompatible siblings, immunosuppression was not used. Conversely, in family members and matched-unrelated volunteers, recipients received cyclosporin-A and therapeutic levels of 1500ng/mL on the C 2 assay were maintained.[41] The dose was cut to 50% at six months, 25% at nine months and discontinued at one year. The protocol included 400 ml stabilised human serum weekly[42] in addition to 500 mg valaciclovir twice daily for viral prophylaxis in the initial three months and co-trimoxazole- comprising trimethoprim 80 mg and sulfomethoxazole 400 mg daily- for one year.[43]

6.6.6 AUTOGRAFTS

PBSC was collected from the patient, then cryopreservation was undertaken as previously described.[44] Conditioning was given and the cryopreserved stem cells thawed and reinfused with no further manipulation.

6.7 RESULTS

6.7.1 STATISTICAL ANALYSIS

Statistical analysis was performed using STATA software, version 11 (STATA Corporation, College Station, TX). The difference in frequency was compared using Pearson's chi-square test.[45] Where necessary, means were compared using the Student's test.[46] Patient survival was analysed with the Kaplan-Meier method[47] and the results were compared by means of log-rank statistics.[48] A p-value of <0.05 was considered statistically significant. The median and an interquartile ranges (IQR) between the 1st quantile (Q1) and the 3rd quantile (Q3) were calculated. The median survival was the time it took to reach 50% survival. If more than 50% were alive at the end of the study, then the median survival time could not be defined.

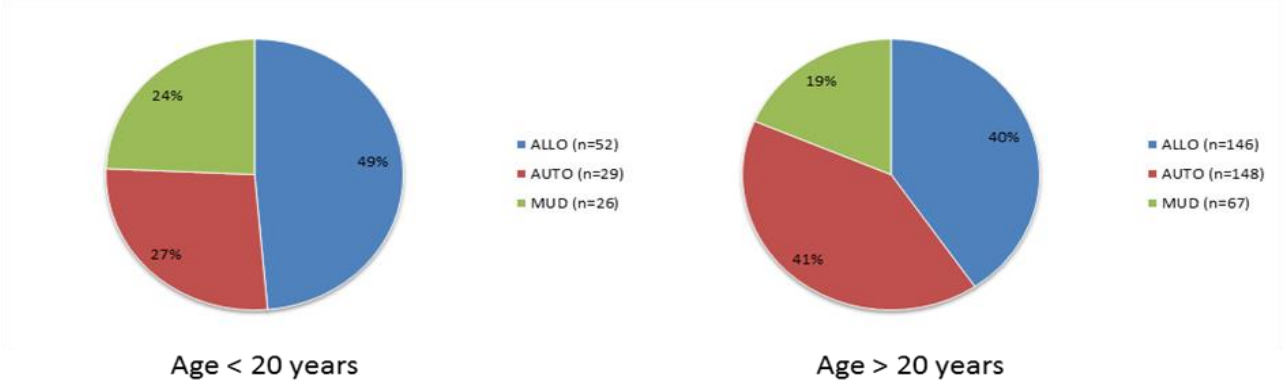
6.7.2 THE DISTRIBUTION OF TRANSPLANT PROCEDURES (ALLOGRAFT AUTOGRAFT AND MATCHED UNRELATED DONOR)

In addition to the total cohort, there were differences between the under and over 20-year-old age groups. In the 198 (42%) that were allografts, 52 (49%) were children and 146 (40%) adults. The autografts 177 (38%) comprised 29 (28%) children and 148 (41%) adults. In the 93 (20%) MUD transplants, 26 (23%) were children and 67 (19%) adults.

Because of various conditions being transplanted, there were differences between adults and children e.g. adults have myeloma while this is rare in children, therefore showing a higher percentage of autografts in the adults. In total 107 (23%) children were transplanted and 361 (77%) adults. The adult transplants were in the

majority as children's procedures were dependent on referral from paediatricians. **(Figure 21)**

FIGURE 21
DISTRIBUTION OF DIFFERENT TYPES OF TRANSPLANTS (ALLOGRAFT, AUTOGRAFT AND MATCHED UNRELATED DONOR)



TRANSPLANT TYPE 468	CHILDREN 107 (23%)	ADULTS 361 (77%)
Allograft 198 (42%)	52 (49%)	146 (40%)
Autograft 177 (38%)	29 (27%)	148 (41%)
Mud 93 (20%)	26 (24%)	67 (19%)

6.7.3 STUDY POPULATION

Table 13 shows the number of female and male patients, children and adults who were transplanted. The median ages for male and females were 39 and 37 respectively. There were far fewer children (as explained in 6.7.2).

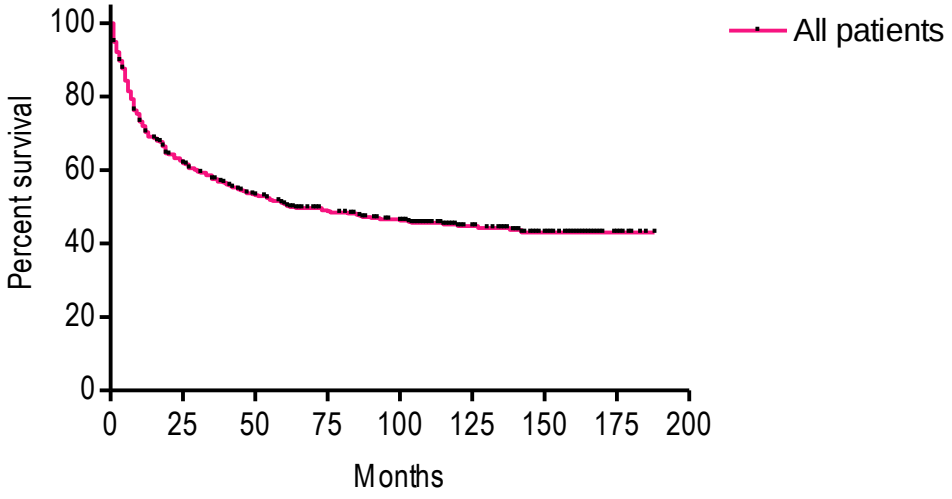
TABLE 13
TOTAL STUDY GROUP DIVIDED INTO SEX AND AGE

TOTAL COHORT 468	CHILDREN 107 (23%)	ADULTS 361 (77%)
Females 195 (42%)	47 (24%)	148 (76%)
Males 273 (58%)	60 (22%)	213 (78%)

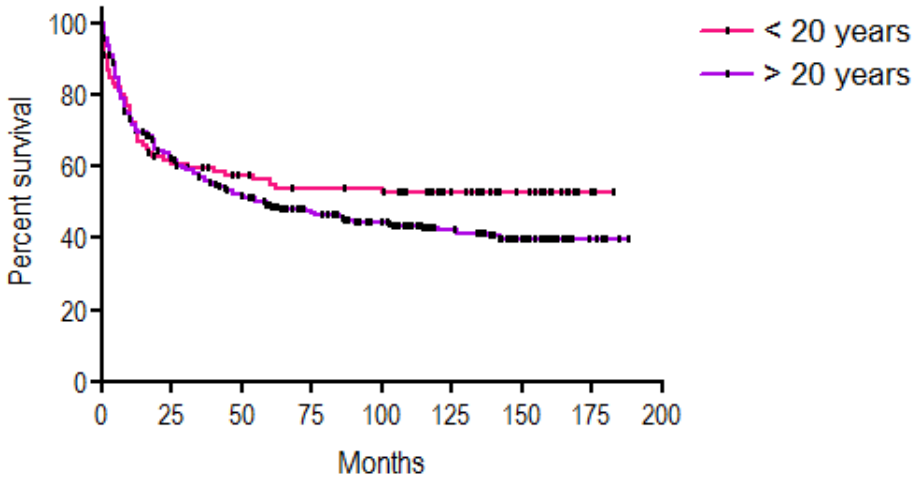
6.7.4 OVERALL SURVIVAL DATA

The total group (468) transplanted had an overall survival rate of 48% at 15 years of follow up **(Figure 22a)** and in **Figure 22b**, the group was subdivided into children and adults showing overall survival rate of 58% and 42% respectively at 15 years follow up. There is no statistical difference in the curves between the age groups (p=0.220)

FIGURE 22
OVERALL SURVIVAL OF TOTAL GROUP AND SUB-DIVIDED INTO AGE DIFFERENCES



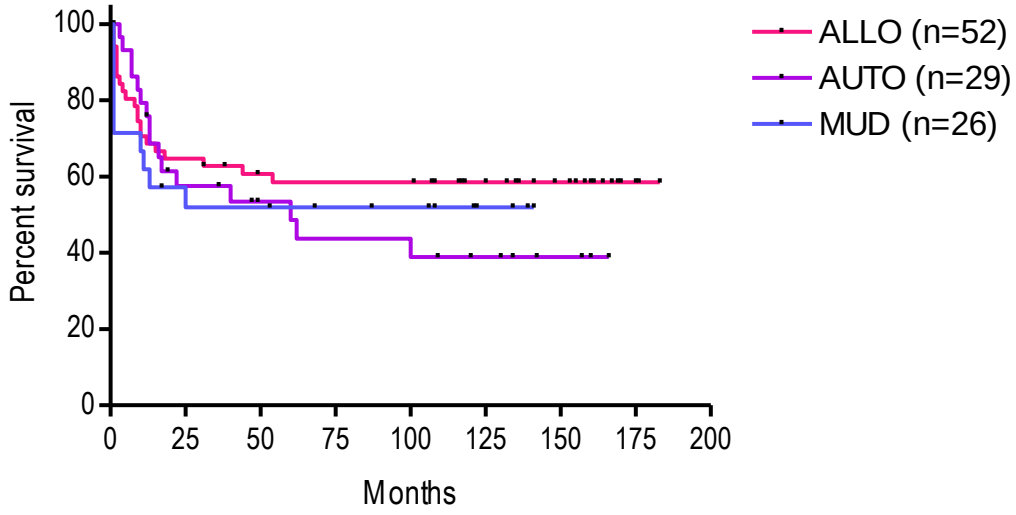
(a)



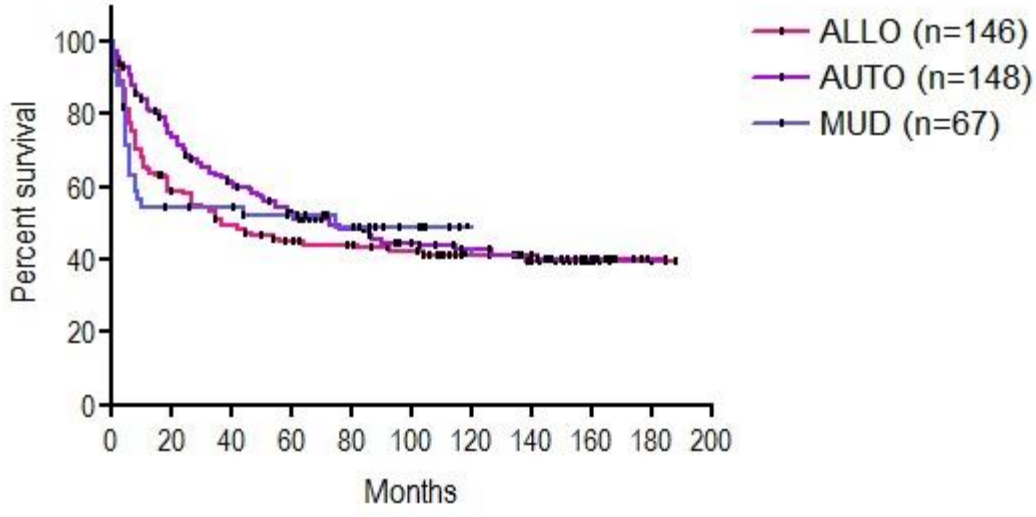
(b)

There was a further split within these two age groups by type of procedure; being allograft from sibling, autograft or volunteer donor. In **Figure 23a** the children showed no statistical difference between the curves (p=0.6151) and overall median survival for ALLO and MUD were undefined but 60 months for AUTO. In **Figure 23b,** the adults' overall median survival for ALLO was 37 months, AUTO 73 months and MUD 75 months. There was no statistical significant difference between the curves (p=0.4637). As MUD transplants started only in 1998, their follow up was shorter at 12 years and for the adults, even shorter at 10 years. This overall survival was difficult to compare with the literature as here individual types of transplants according to disease were usually evaluated separately and not as a group. Different types of transplants in children showed the lowest percentage survivial in autografts. In the adults the results were very similar between the different types.

FIGURE 23
OVERALL SURVIVAL BY AGE AND TRANSPLANT TYPE



Age < 20 years
(a)

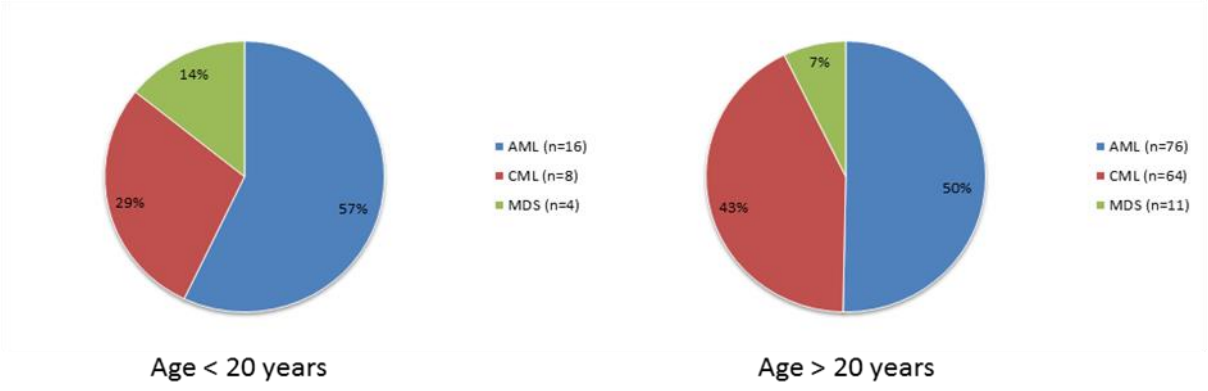


Age > 20 years (b)

Grouped by disease category, it was appreciated that contrasting numbers and variable follow up rendered strict statistical comparison difficult but fortunately survival fell within published reference centre norms. Diseases were not divided into sub-types as the numbers would have been too small to analyse. They were grouped according to lineages. **(Figure 24)** The myeloid group **(Figure 24a)** consisted of AML, CML, MDS while the lymphoid group **(Figure 24b)** comprised ALL, HL, NHL, CLL and MM. In the myeloid lineage for both ages the majority of transplants were for AML. In the lymphoid group most of the children were transplanted for ALL (usually second remission) while for adults it was NHL.

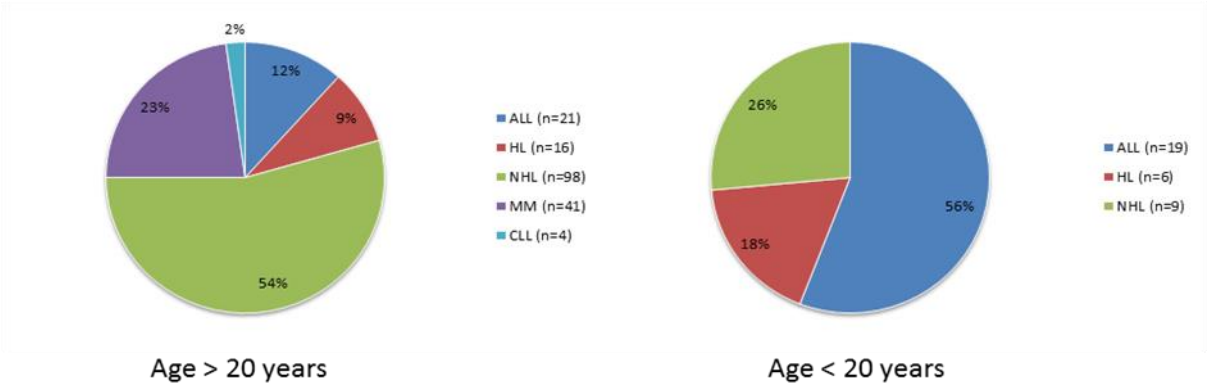
FIGURE 24

DISTRIBUTION OF TRANSPLANTS BY MAJOR DISEASE LINEAGES



MYELOID DISEASE

(a)

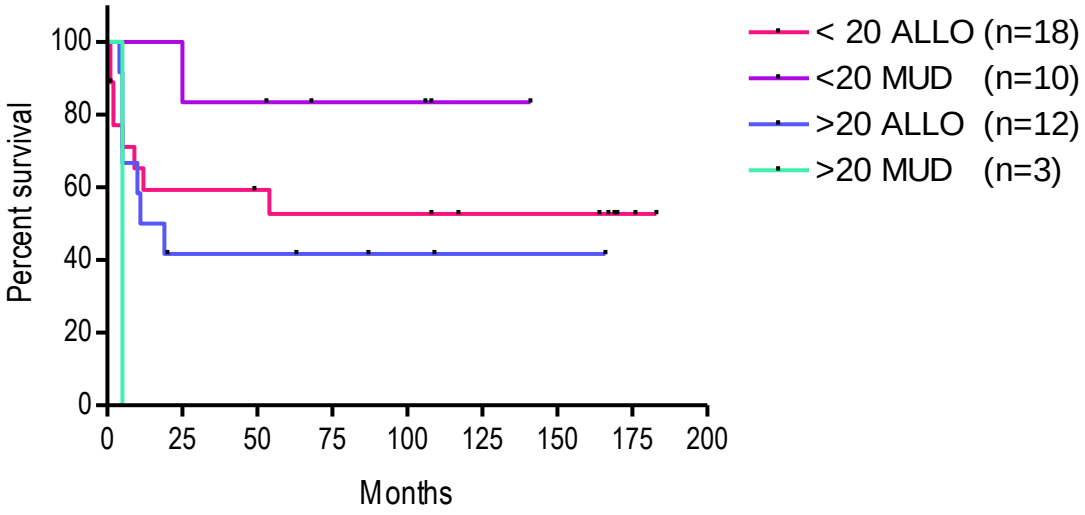


LYMPHOID DISEASE

(b)

Aplasia included AA and FA. The numbers were small and there were no statistical differences between the curves in the under 20 group (p=0.1636) and in over 20 (p=0.3177). In the children the median overall survival time was undefined for both allografts and MUD. In the adults the median overall survival was 15 and five months respectively. **(Figure 25)** The children seemed to have had a better survival (52% - 82%) than the adults (42% ALLO) but it was not possible to analyse MUD as there were only three patients transplanted. In the published literature this is between 80% and 90%; the outcome being dependent on many variables but most importantly being time from diagnosis. Children usually do better than adults, as seen in our group.

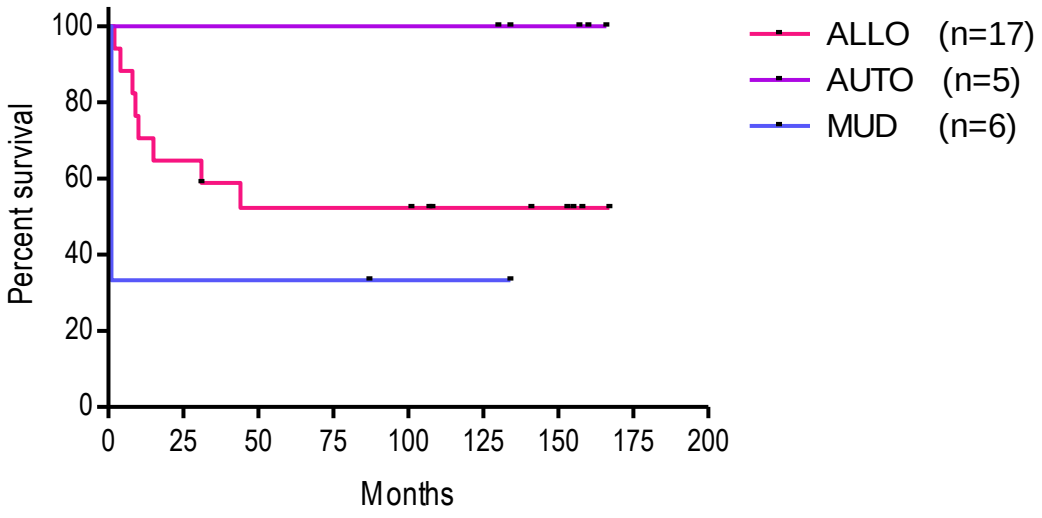
FIGURE 25
OVERALL SURVIVAL BY AGE, TRANSPLANT TYPE AND APLASIA



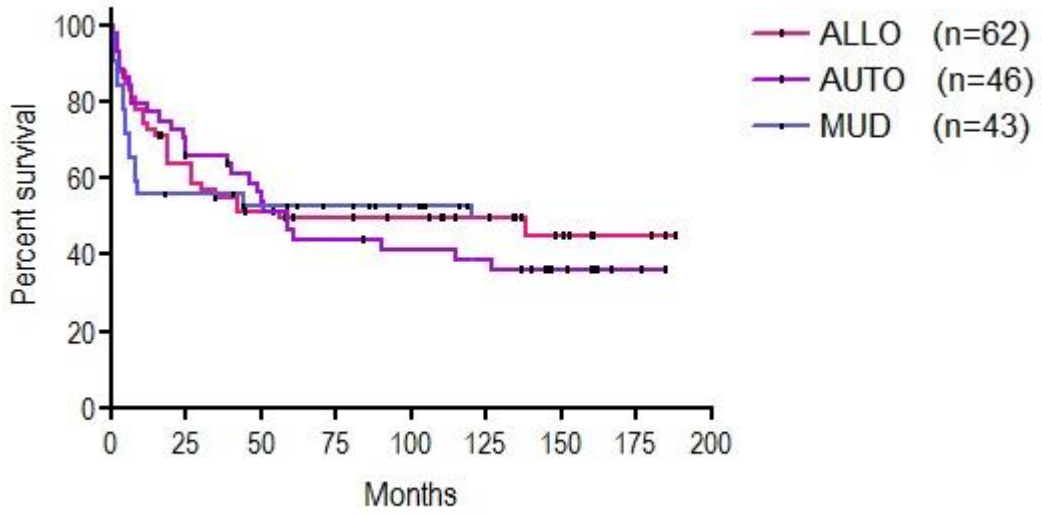
The under 20 age group **(Figure 26a)** again had very few numbers and there was no statistically significant difference between the curves (p=0.496). Median overall survival could not be determined for allografts and autografts but was one month for MUD.

The adults **(Figure 26b)** showed no statistical difference between the curves (p=0.6029). Here for 46% the median overall survival was 56 months for allografts and for autografts 37% at 59 months. For the MUD group it was 56% at 120 months. It seems as if the autografts did worse most probably due to relapse of disease post transplant.

FIGURE 26
OVERALL SURVIVAL BY AGE AND TRANSPLANT TYPE IN MYELOID DISEASES



Age < 20 years
(a)

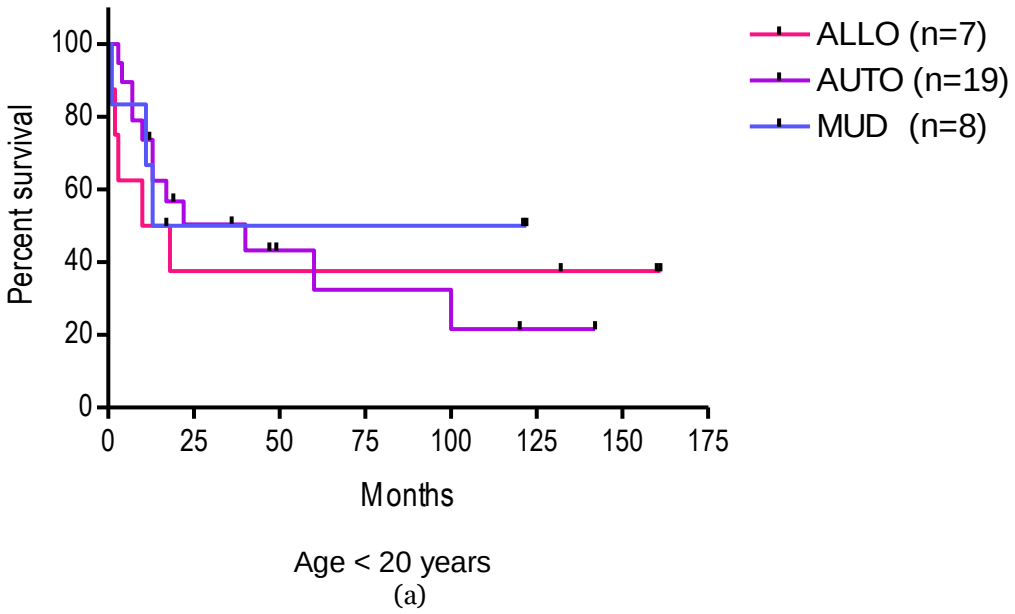


Age > 20 years
(b)

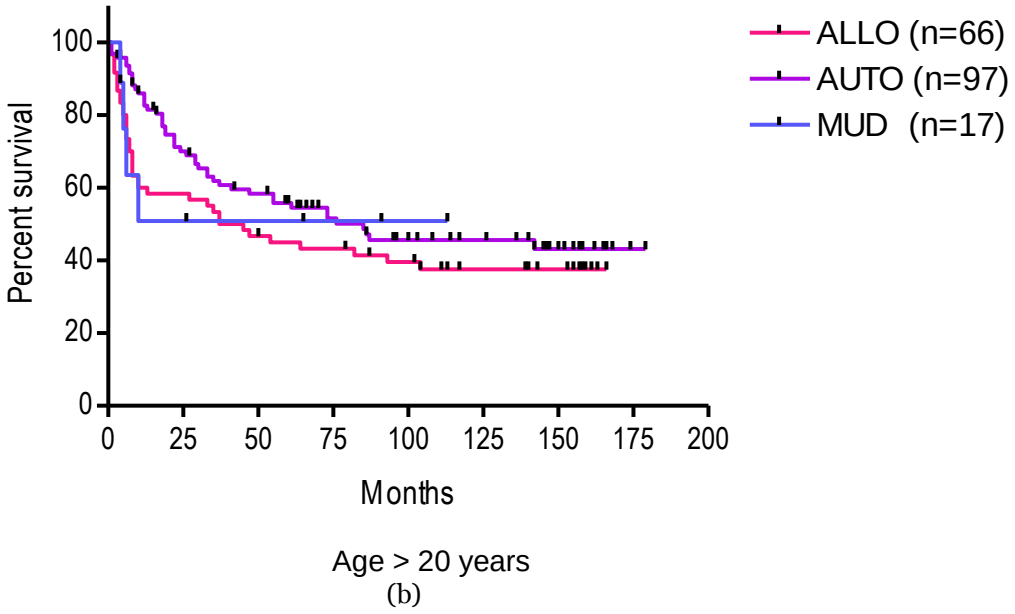
The lymphoid lineages **(Figure 27a)** were also grouped together without taking into account the different types. In the children there was no statistical difference between the curves (p=0.6680). The overall median survival was 14 months for ALLO, 40 months for AUTO and 87 months for MUD. Only 20% of AUTOs were alive at 12 years. This was a poor outcome but was most probably due to the majority being ALL and relapsing from their disease.

In the adults **(Figure 27b)** there was no statistical difference between the curves (p=0.3233). The overall median survival was 41 months for ALLO and 85 months for AUTO. The MUD group was undefined as 50% were still alive. As the majority of the adults in the lymphoid group was NHL and their cure rate regarded as five years post transplant, it appeared as if 42% in this lineage were cured.

FIGURE 27
OVERALL SURVIVAL BY AGE, TRANSPLANT TYPE AND LYMPHOID DISEASES



Age < 20 years
(a)

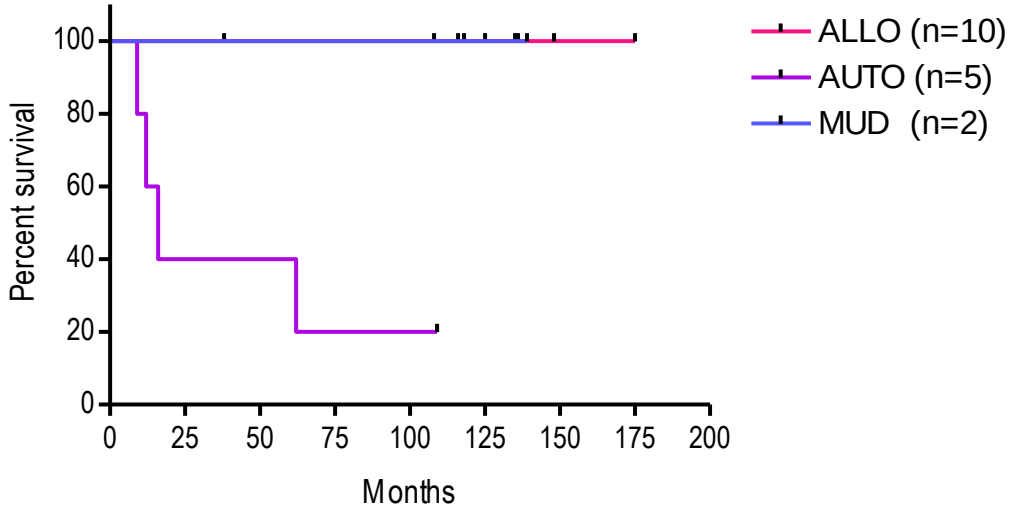


Age > 20 years
(b)

The miscellaneous group constituted numbers less than five in a particular group comprising disease categories such as thalassaemia, sickle cell disease, neuroblastoma and SCID. These were analysed together as statistically efforts made to generate survival by subtype were precluded because of small numbers. **Figure 28a** showed a statistical difference between the curves where children had this procedure (p=0.0032). The overall median survival for ALLO and MUD was undefined while it was 16 months in the AUTO's. **Figure 28b** showed that in the adults no statistical difference was present between the curves (p=0.2598). The median overall survival was 18 months for the ALLO, 61 months for AUTO and 75 months for MUD. These numbers were too small to draw a conclusion about the results.

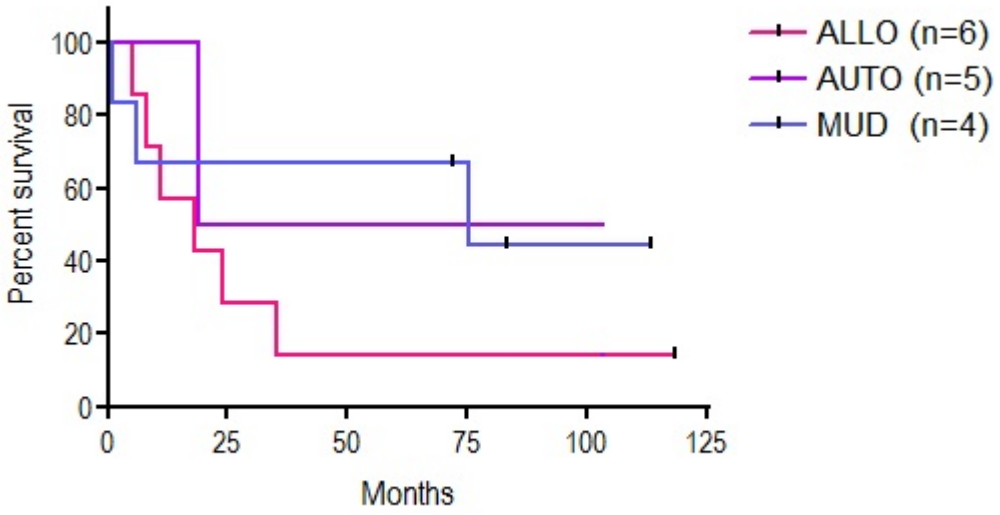
FIGURE 28

OVERALL SURVIVAL BY AGE, TRANSPLANT TYPE AND MISCELLANEOUS DIAGNOSES



Age < 20 years

(a)



Age > 20 years

(b)

6.8 DISCUSSION

The analysis was done to give insight into the overall survival of our transplanted patients. Even though the numbers were small it provided information that we could use for further guidelines. This data however, could not be compared with published literature as they use specific diseases with their sub-categories to provide overall survival data. It would be preferable if future analysis could be conducted on more specific diseases to be able to compare outcome with other centres. This will then provide a better understanding of the success of our programme.

6.9 SUMMARISING COMMENT

From the start of this project in 1972 that captured all demographic data via audit by European, American and CIBMTR registries, the decision to personally document outcome underpinned accountability for every procedure. To do this the most fundamental step was to control the environment in which transplants took place and considerable research development led to the prototype facility at Groote Schuur Hospital upon which all future units in South Africa were based (detailed in chapter 4). From this transplant unit the next research project was to employ absolute uniformity to each case, retaining a constantly reviewed protocol. Overall survival was tested between 1995 – 2006 in two feasibility studies[1,2] and in this chapter brackets age spectrum, choice of procedure and, to the extent statistically possible within above subdivisions, split into diagnosis. The results were limited by numbers. These procedures permeated the private and public sector in subsequent development and became established in both university and private sectors. Survival outcome was reported previously,[1,2] and updated in this chapter. Traditional immunohaematopoietic patient care allowed the final step in this research project of using a now clearly defined cohort to study, in specialised laboratories, changes in non-haematopoietic tissues.

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CHAPTER 7

A SPECTRUM OF POST-TRANSPLANT INJURY TO NON-HAEMATOPOIETIC TISSUES AND ORGANS

FOCUS

This section is the unifying point of the entire research project in that it encapsulates those varying degrees of change from normality that arise outside the blood and bone marrow as having the potential to adversely affect quality of life. They were first noted, then increasingly delineated, during the course of auditing consecutive immunohaematopoietic stem cell transplants carried out by a single designated team during the process of routine reporting to repositories. The latter included the Centre for International Blood and Marrow Transplant Research, National Donor Program and the European Group for Blood and Bone Marrow Transplant. Continued active participation in each was determined by inspections to ensure accuracy of data pertaining to physical facility, staff and protocols collated through the Annual Network Renewal System Complete Survey. This provided a constant source of transparency and accountability. Although initiated over a 22 year period at the University of Cape Town in the Groote Schuur Hospital, changing National resources made it necessary to continue the work in the private sector. Recognition between 1995 and 2010 was achieved as an internationally accredited centre where formal outcome analysis matched international standards. With approval from appropriate Stellenbosch University Committees in 2011, it was possible to embark on the study of non-haematopoietic phenomena in two consequential timeframes – acute associations within three months manifested in kidney, cardiovascular system, skin and gastrointestinal tract accrued from routine post-transplant measurements. Separately are previously underappreciated late effects requiring specialised research technology and reflected in lung function, immune reconstitution and skeletal changes. These problems led to the constitution of a multidisciplinary study group. In the final analysis an additional contribution would be anticipated from wide-ranging effects consequent upon conditioning with the anti CD52 monoclonal antibody as opposed to traditional cytotoxic agents and corticosteroids.

7.1 INTRODUCTION - STUDY DESIGN

Having established a state-of-the-art protected environment and standardised all aspects of management, it could be demonstrated that outcomes matched published literature. This now became a reliable reference point to shift focus from primarily bone marrow recovery to non-haematopoietic sequelae and was given appropriate University Ethics and Research approval. Specifically, the incidence and severity of abnormalities in a number of tissues or organ systems was recorded in some individuals who underwent these procedures over a 15 year period in a privately based - internationally accredited - donor, harvest and transplant centre. This collaborative investigation was designed to capitalise on laboratory diagnostic techniques, some of which had not been previously available due to cost constraints. The two endpoints were to define underappreciated complications which became evident after a year as a basis for proactive intervention where indicated. Secondly, to focus on differences in the approach used locally with the Campath® reagents rather than the more conventional regimens. The study supplied data for the acute associations from an audit, and for the late effects a number of surviving volunteers underwent tests which provided the information.

7.2 PATIENTS - GENERATION OF THE DATABASE

Every individual registered over the 15 year period between 1995 and 2010 (n = 468) was traced.

Of all these volunteers, eight were too ill to undergo testing not necessarily attributable to the transplant or any related complaints. Six chose not to participate but shared residual anxiety about diagnosis and outcome of results that may show abnormalities. In 132, the distances and financial constraints were insurmountable. Fifty-one could not be contacted and the remaining 216 died. Fifty-five individuals comprised the final study cohort. (**Figure 29)**

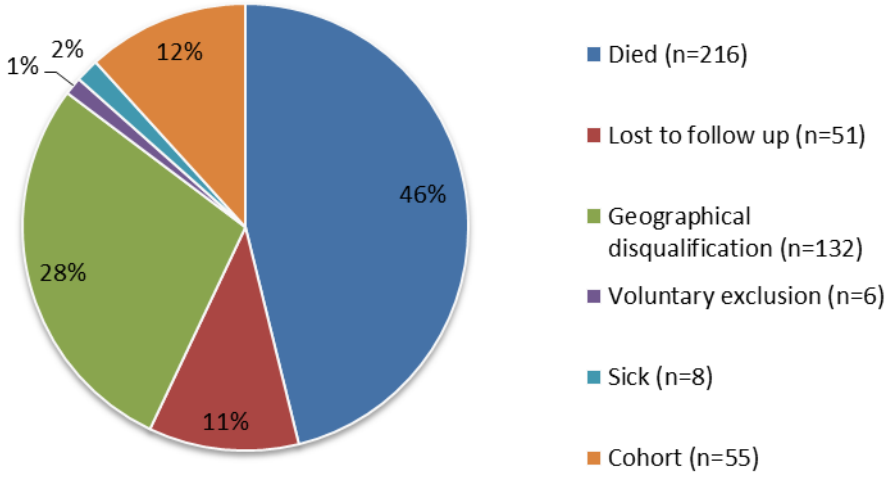
7.2.1 REFINEMENT AND VOLUNTEER PARTICIPATION

Given the research nature of this investigation, the tests were provided at no cost to the patients. Funding for the study was from the Harry Crossley Foundation and from the Haematological Research Trust. The latter was established for research and education. Initial contact was telephonic and after detailed explanation, an extensive informed consent[1] form was provided followed by further discussion in which all questions were clarified. This document was signed and entered into the research record. Additionally, because these studies were carried out in specialised units in Tygerberg Academic Hospital, approval was obtained from the relevant superintendent in charge of research at the hospital (Stellenbosch University Ref: N11/07/242).

Organisationally, all testing was co-ordinated in a single visit by professional and technical staff in the Divisions of Pulmonology, Immunology and Endocrinology within the Department of Internal Medicine at Stellenbosch University and Tygerberg Academic Hospital.

The information which was generated was made available to the patients and then sent back to the referring general practitioners or physicians.

FIGURE 29
DETAILS OF THE STUDY COHORT SELECTED TO DEFINE LATE EFFECTS IN A NUMBER OF ORGANS



	NUMBER	PERCENTAGE
TOTAL	468	100
DIED	216	46
LOST TO FOLLOW UP	51	11
GEOGRAPHICAL DISQUALIFICATION	132	28
ELECTED NOT TO PARTICIPATE	6	1
TOO SICK	8	2
FINAL REGISTERED COHORT	55	12

7.3 METHODS

7.3.1 DEMOGRAPHIC DATA

Demographic data on 468 individuals was collected by using symbols to capture the information. An example is shown on the format used to generate the database e.g. Ia represents bacterial infection with dates of occurrence **(Annexure 3).**

In **Annexure 1** the data is given on the total cohort and for the volunteer group, demographic data was also extracted from this database **(Annexure 2).**

7.3.2 ETHICS APPROVAL

Informed consent was obtained from all the patients. Details on the research project as well as the audit were approved by the Health Ethics Research and the PhD Evaluation Committee; ethics number (N11/07/242).

7.3.3 STANDARD LABORATORY TESTS

In the first three months following transplantation a small number of carefully selected measurements are essential for optimum patient care. These tests were performed regularly during the post transplant period and are listed below. The time interval varied according to the tests done. Abnormalities developing before day 100 are designated as acute associations and find expression in terms of kidney function, cardiovascular symptomatology, skin manifestations and bowel disturbances encompassing nutritional status and hepatic biochemistry.

7.3.3.1 HAEMATOLOGY

Automatically

Full blood count including smear examination (FBC)[2]
Reticulocyte production index[3]
Erythrocyte sedimentation rate (ESR)[4]

Selectively

Bone marrow aspiration[5] and trephine biopsy (BMBX)[6]
Tests for haemolysis including haptoglobin[7]
Metaphase karyotyping[8]
Molecular genetics including short terminal repeat assay[9]
Fluorescent *in situ* hybridisation panels (FISH)[10]
HLA immunogenetic class I and, where appropriate, class II tissue-typing[11]

7.3.3.2 BIOCHEMISTRY

Automatically

Renal function including urea, urate, electrolytes and creatinine[12]
Liver function tests (LFT)[13] and lactic dehydrogenase (LDH)[14]

Selectively

Inflammatory markers including C-reactive protein (CRP)[15] and procalcitonin[16]
Serum protein electrophoresis and quantitative immunoglobulins[17]
Free light chain assay[18]
B2 microglobulin[19]

7.3.3.3 MICROBIOLOGY

Automatically

Gram stain from superficial wounds[20]
Blood cultures from central venous placement sites and peripheral vessels simultaneously[21]
Appropriate samples for fungi[22] and mycobacteria[23]

Selectively

Viral serology[24] and polymerase chain reaction particularly for Ebstein Barr Virus (EBV)[25] and cytomegalovirus (CMV)[26].

7.3.3.4 RADIOLOGY

Automatically

Calibrated chest films in deep inspiration with posterior, anterior (PA) and lateral projections[27]

Selectively

Skeletal survey[28]

High resolution computerised axial tomography[29]

Pulmonary angiography[30]

Positron emission tomography (PET)[31]

7.3.4 SPECIAL INVESTIGATIONS

In contrast to the acute associations where the pertinent information was automatically collected as part of the established protocol and routinely audited, parallel changes in other non-haematopoietic systems were not appreciated at first and only gradually declared themselves over the passage of years in personal follow-up. However, their elucidation posed a quite different problem in that they required access to methodology only available in research facilities and therefore not previously accessible, primarily for financial reasons. These late effects, the reason for this segment of the investigation, were addressed in an expanded evaluation of lung function[32], patterns of immune reconstitution and bone mineral density. This initiative necessitated the establishment of the Stellenbosch University Bone Marrow Transplant Study Group with involvement of colleagues from the three respective disciplines.

The following tests were all done, once only, for the study.

7.3.4.1 BONE DISEASE

Clinical information

Initial assessment

DXA[33]

QCT[34]

Biomarkers

7.3.4.2 PULMONOLOGY

Clinical information

Clinical information was provided to enable interpretation of the results.

Initial assessment

History of respiratory tract disease including sinuses and upper airways

Smoking habits

Chest symptoms especially performance status over time

Examination of lung fields
Baseline radiology – PA and lateral projection in deep inspiration
Review of previous studies[35,36]

Calibrated methodology
A battery of tests for investigation was established by the Pulmonary Unit to evaluate lung function. The results were expressed as percentage of normal predicated by age, height and weight.

Spirometry[37]
Forced expiratory ratio in the first second (FEV1)[38]
Forced vital capacity (FVC)[39]
Forced expiratory flow FER (FEV1/FVC)[39]
Peak expiratory flow rate (PEFR)[40]
Forced expiratory flow at 25 – 75% of forced vital capacity (FEF 25 – 75)

Body plethysmography
Body box[41]
Airway resistance[42]
Total lung capacity (TLC)[43]
Residual volume (RV)[39]
Diffusing capacity for carbon monoxide (DLCO)[39]
Bronchoscopy when indicated[44]

Format for reporting
The interpretation of serial pulmonary function testing results according to the American Thoracic Society/European Respiratory Society (ATS/ERS) 2005[45]
Obstructive – FEV1/FVC < 70% and FVC > 80% of predicted
Obstructive with reduced vital capacity – FEV1/FVC < 70% and FVC < 80% of predicted
Restriction – FEV1/FVC > 70% and FVC < 80% of predicted
FEV1 used to grade the impairment as being obstructive or restrictive
Combination of above was deduced from plethysmography

7.3.4.3 IMMUNOLOGY – EVALUATION OF IMMUNE RECONSTITUTION

Samples uniquely identified were tested and results used, *post hoc,* to interpret subsequently obtained information on symptoms and signs through interaction with patients and referring primary care physicians. Both the innate and adaptive pathways were studied.

7.4 ACUTE ASSOCIATIONS - THE FIRST THREE MONTHS

This audit data, described as early complications, was readily reversible within the first 100 days post procedure and early improvement usually commenced when neutrophil recovery occurred. These findings were in keeping with published outcome[47]

7.4.1 NEPHROLOGY

7.4.1.1 INTRODUCTION

Transplantation almost inevitably exposed recipient kidneys to a range of injuries including fluid and electrolyte imbalance, antimicrobial as well as cytotoxic agents and less frequent occurrences amongst which were graft-versus-host disease and microangiopathy.[48] We took the opportunity to document the incidence and severity of acute kidney injury in this cohort of patients and at the same time, to explore any impact that might arise from the unique immunosuppressive regimen centred on the use of monoclonal antibodies *ex vivo*[49]. An increase in serum creatinine of approximately 1.5 times the baseline figure was accepted as indicating acute kidney injury (AKI).

7.4.1.2 PATIENTS

Between 1995 and 2010 a total number of 468 patients were transplanted in the Mediclinic Centre. Adequate data for assessment of kidney function was available in 76 individuals.

7.4.1.3 METHODS

Derived from the complete biochemical profile, entry to this analysis was determined by serum creatinine level as the index of renal function in kidney injury.[50] It was accepted that estimated glomerular filtration rates were inaccurate in unstable patients. The progression of oliguria to anuria aided in the assessment of AKI. The creatinine level was recorded pre-day of transplant and weekly thereafter for three months. The creatinine measurement was done on a Beckman Coulter.AU and processed with a "Modified kinetic Jaffer method."

7.4.1.4 RESULTS

Forty patients (53%) had normal creatinine levels and this did not change during the course of follow-up. Observation numbers in the table showed a drop particularly, in the later weeks, attributable to the decreasing number of patients attending the clinic. The highest level of creatinine was 969µmol/L. The median creatinine and IQR was used to do the analyses. **(Table 14)** Of this cohort, four died with septicaemia with no alteration in kidney function and a further nine demised under similar circumstances beyond the 12 week observation period. Twenty-seven remained alive and well.

TABLE 14
SEQUENTIAL CREATININE VALUES IN THOSE THAT DID AND DID NOT DEVELOP ACUTE KIDNEY INJURY

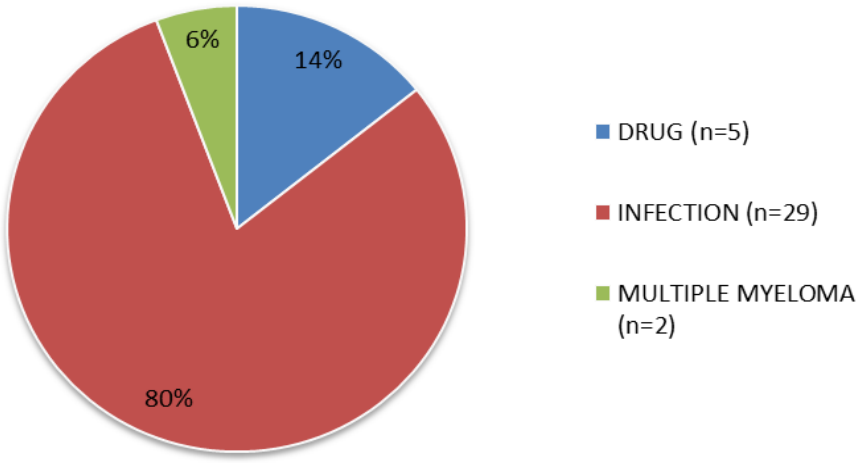
	No injury Median µmol/L **(Obs)**	**Injury Median** µmol/L **(Obs)**	**No injury IQR** µmol/L **Q1;Q3**	**Injury IQR** µmol/L **Q1;Q3**	**No Injury Range** µmol/L **Min;Max**	**Injury Range** µmol/L **Min;Max**
Pre	69 (40)	84.5 (36)	58; 78.5	74.5; 94	38; 103	47; 139
D-O	63 (39)	72 (35)	53; 75	59; 87	41; 118	46; 158
1 week	69.5 (38)	110.5 (36)	57; 84	83.5; 159.5	33; 98	46; 284
2 week	66 (39)	138 (35)	50; 89	82; 192	37; 107	47; 763
3 week	66 (30)	130 (21)	53; 80	96; 160	33; 98	55; 969
4 week	67 (31)	129 (26)	57; 78	90; 159	4; 90	55; 564
5 week	53.5 (10)	129 (9)	51; 57	79; 192	45; 65	52; 212
6 week	77 (7)	175 (7)	53; 85	151; 187	48; 93	131; 283
7 week	62 (5)	124 (7)	53; 63	120; 237	48; 68	79; 712
8 weeks	70 (26)	136 (21)	61; 85	93; 176	41; 118	71; 318
12 weeks	75.5 (22)	107 (19)	63;79	84; 136	44; 94	66; 275

Thirty-six patients (47%) developed AKI, 29 (80%) related to infection, predominantly septicaemia. **(Figure 30)** Of the group, 12 recovered spontaneously within 12 weeks and in a further 11, response occurred after the end of this period making a total of 23 survivors. The remaining 13 patients died at variable times during the three months. Of these, four were dialysed and a further two were excluded from this intervention as they were clinically unstable. All participants underwent myeloablative conditioning that included Campath® *ex vivo* in 24, while the remaining 12 were prepared similarly but without the monoclonal antibody.

AKI was due to infection in 29 (80%) patients. In many instances the infective agent was not identified as patients were started on empiric antibiotics (refer to point 5.2.7). Drugs such as cyclosporin A, conditioning cytotoxics and antibiotics (e.g.Amikacin, Gentamycin, Amphoterecin B) may have been partially responsible for this injury. Underlying disease such as Multiple Myeloma may have contributed to AKI. Fluid and electrolytes played a major part as patients could become dehydrated due to secondary causes such as vomiting and diarrhoea.

FIGURE 30

CAUSES OF KIDNEY INJURY IN THE 36 PATIENTS



Of the 36 patients who had AKI, 24 (67%) were allografts receiving Campath® and 12 (33%) were autografts and had no exposure to the antibody - a relative difference of 50%. **(Table 15a)** In those with AKI there was a slightly higher incidence of AKI in patients with MM, 7 patients (19%). **(Table 15b)** It was not significantly different from the other disease categories.

TABLE 15

DEMOGRAPHICS: AKI AND NORMAL RENAL FUNCTION IN TYPE OF TRANSPLANT AND DISEASE CATEGORY

	Renal Injury (F,M)	No Renal Injury (F,M)	Total
Allograft	24 (8F 16M) (67%)	23 (10F 13M) (58%)	47 (18F 29M) (62%)
Autograft	12 (3F 9M) (33%)	17 (8F 9M) (42%)	29 (11F 18M) (38%)
Total	36 (11F 25M)	40 (18F 22M)	76 (28F 47M)

(a)

No kidney injury

Disease	Frequency (F,M)	Percentage
AA	3 (2F, 1M)	7.5
ALL	5 (2F, 3M)	12.5
AML	15 (6F, 9M)	37.5
CML	4 (1F, 3M)	10
MDS	3 (2F, 1M)	7.5
MM	4 (1F, 3M)	10
NHL	6 (4F, 2M)	15

Kidney injury

Disease	Frequency (F, M)	Percentage
AML	5 (3F, 2M)	13.9
APL	3 (0F, 3M)	8.3
CLL	1 (0F, 1M)	2.8
CML	6 (3F, 3M)	16.7
Fanconi	2 (2F, 0M)	5.6
HL	2 (0F, 2M)	5.6
MDS	3 (0F, 2M)	8.3
MM	7 (3F, 4M)	19.4
Myelofibrosis	1 (0F, 1M)	2.8
NHL	5 (0F, 5M)	13.9
PolycythemiaVera	1 (0F, 1M)	2.8

(b)

When adding the monoclonal antibody, the median creatinine in the allografts (129µmol/L) seemed to be no different from that of the autografts (137µmol/l) where none was added, **(Table 16)** making an assumption that adding Campath® was not additionally nephrotoxic.

TABLE 16

SEQUENTIAL CREATININE MEASUREMENTS FOR KIDNEY INJURY IN ALLOGRAFTS AND AUTOGRAFTS

	ALLO Median µmol/L **(Obs)**	**AUTO Median** µmol/L **(Obs)**	**ALLO IQR** µmol/L **Q1;Q3**	**AUTO IQR** µmol/L **Q1;Q3**	**ALLO Range** µmol/L **Min;Max**	**AUTO Range** µmol/L **Min;Max**
Pre	82 (24)	90 (12)	72; 89	80; 109	47; 108	63; 139
D-O	71 (23)	80.5 (12)	57; 83	65; 89	46; 109	46; 158
Week 1	99.5 (24)	103.5 (12)	80; 134.5	96.5; 200.5	46; 204	60; 259
Week 2	113 (24)	162 (11)	75; 165.5	107; 218	47; 265	75; 763
Week 3	128 (21)	134.5 (8)	95; 159	96.5; 177.5	76; 220	55; 969
Week 4	129 (19)	137 (7)	90; 158	68; 255	74; 282	55; 564
Week 5	148 (9)	124 (2)	66; 210	*	52; 212	119; 129
Week 6	175 (6)	151 (1)	146.5; 235	*	131; 283	151; 151
Week 7	175 (6)	79 (1)	121; 474.5	*	120; 712	79; 79
Week 8	137.5 (16)	108 (5)	108; 184.5	75.5; 206.5	71; 308	74; 249
Week 12	107 (14)	111 (5)	82.5; 124	83.5; 223.5	63; 162	79; 275

*- numbers too small to calculate

7.4.1.5 DISCUSSION

The incidence of AKI in this series was 47% which was comparable with figures in published data.[51] The need for dialysis following AKI was a bad prognostic indicator.[48,50] Assuming the criteria for performing dialysis were comparable with other transplant units then it could be argued, on the basis of patients requiring such intervention, that it did not differ widely between this study population and reference centres – the latter with a frequency between 2 and 5%[51] corresponding to our figure of 5%. The reasons for AKI were multifactorial and using myeloablative regimens, with or without additional Campath®, no differences in AKI were apparent. This observation was in keeping with the suggestion that adding the monoclonal antibody was not additionally nephrotoxic. However, renal injury was less frequent with autologous as opposed to allogeneic bone marrow transplant.[48]

7.4.1.6 CONCLUSION

The incidence of AKI did not differ from those reported in publications[48]. This observation provided endorsement for standards achievable in South African University level programmes.

7.4.2 CARDIOLOGY

7.4.2.1 INTRODUCTION

From the time that various forms of transplantation were introduced into the prototype unit at Groote Schuur Hospital, three patterns of cardiovascular complications were noted. Although not initially appreciated, dose-dependent anthracycline induced toxicity was often present but overlooked upon referral, the model being that of Hodgkin Lymphoma where cumulative doses of anthracycline and radiotherapy increase cardiac complications.[52] Similarly, also underappreciated, was the presence of prior heart disease in the elderly with diffuse B-cell lymphoma and comorbidity of hypertension.[53] Complications due to conditioning regimens are recognised, although these range from 1-26% in various studies. The main cause was cyclophosphamide producing cardiotoxicity.[54] Apart from reduced left ventricular ejection fraction (LVEF) (< 45%) none of the other standard measurements (2-D;M-mode calculations; pulsed wave and colour doppler evaluation) reflected by echocardiography usefully predicted adverse reactions after grafting. On multivariate analysis however, cumulative dose of anthracyclines emerged as a risk factor.[55] Cardiac and cardiovascular complications occurred at a much lower frequency during the first 12 weeks than other post-transplant causes of morbidity and mortality. This has drawn attention to adverse events that occur years or even decades later. With increasing numbers of long-term survivors these side effects are expected to show rising incidence of this problem as had been predicated from the Hodgkin lymphoma data.[56,57]

Cardiac dysfunction could be either systolic or diastolic. Systolic dysfunction was characteristic of dilated cardiac myopathy and a LVEF of less than 43%. Diastolic dysfunction related to abnormal filling and LVEF usually greater than 60%.

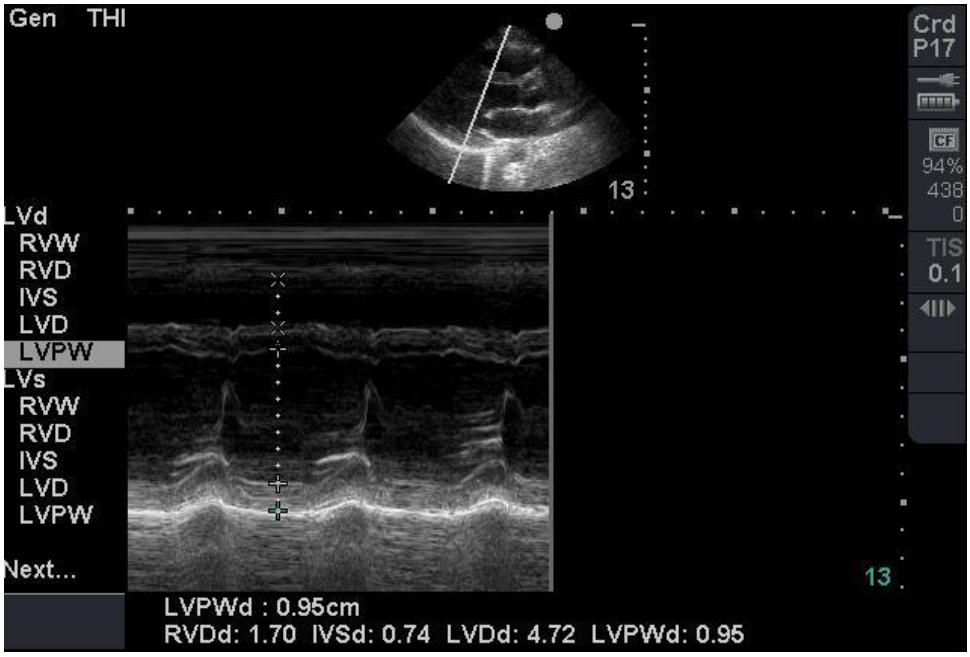
7.4.2.2 PATIENTS

A total of 119 individuals having fully audited data were seen by a single consulting cardiologist with particular expertise in the context of transplantation between 1998 and 2004.

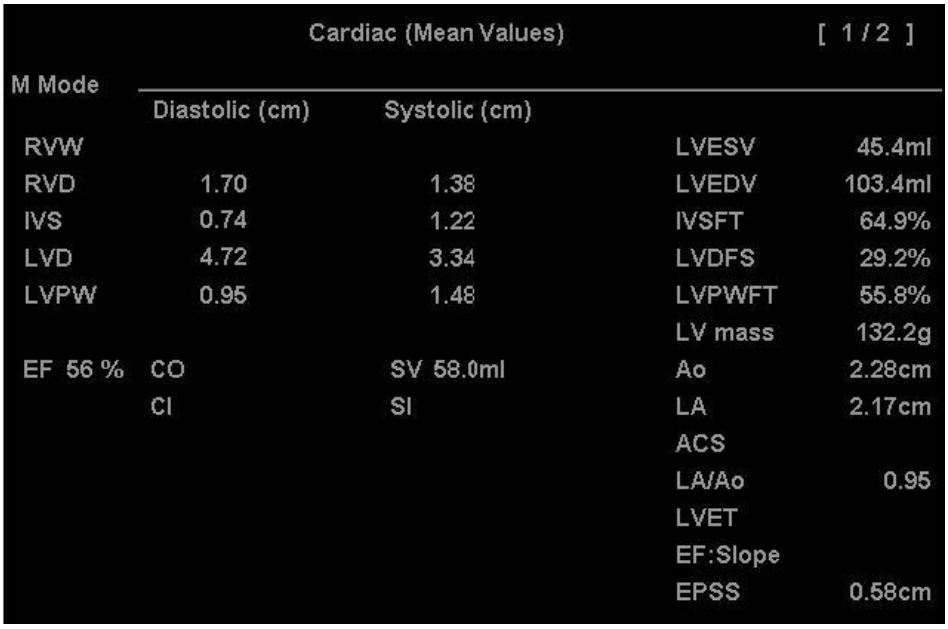
7.4.2.3 METHODS

A carefully taken family and drug history was combined with clinical examination including blood pressure monitoring. **Figures 31a & 31b** show normal pattern M-mode trans-thoracic echocardiogram (ECHO) which assesses cardiac dimensions and left ventricular ejection fraction. The standard 12 lead electrocardiogram with rhythm strip was undertaken **(Figure 32)** where a normal pattern is seen. These procedures were performed to detect abnormalities that might have compromised cardiac function during the post transplant period. To perform the ECG, a Hewlett Page Writer Xli 12 lead ECG Machine (configured to the specifications attached) was used. For the echocardiogram, the Micromax ultrasound system with cardiac imaging applications and P17/5-MHz broadband transducer was utilised. The procedures were done by the same cardiologist.

FIGURE 31
M-MODE TRANS-THORACIC ECHOCARDIOGRAMWITH MEASUREMENTS



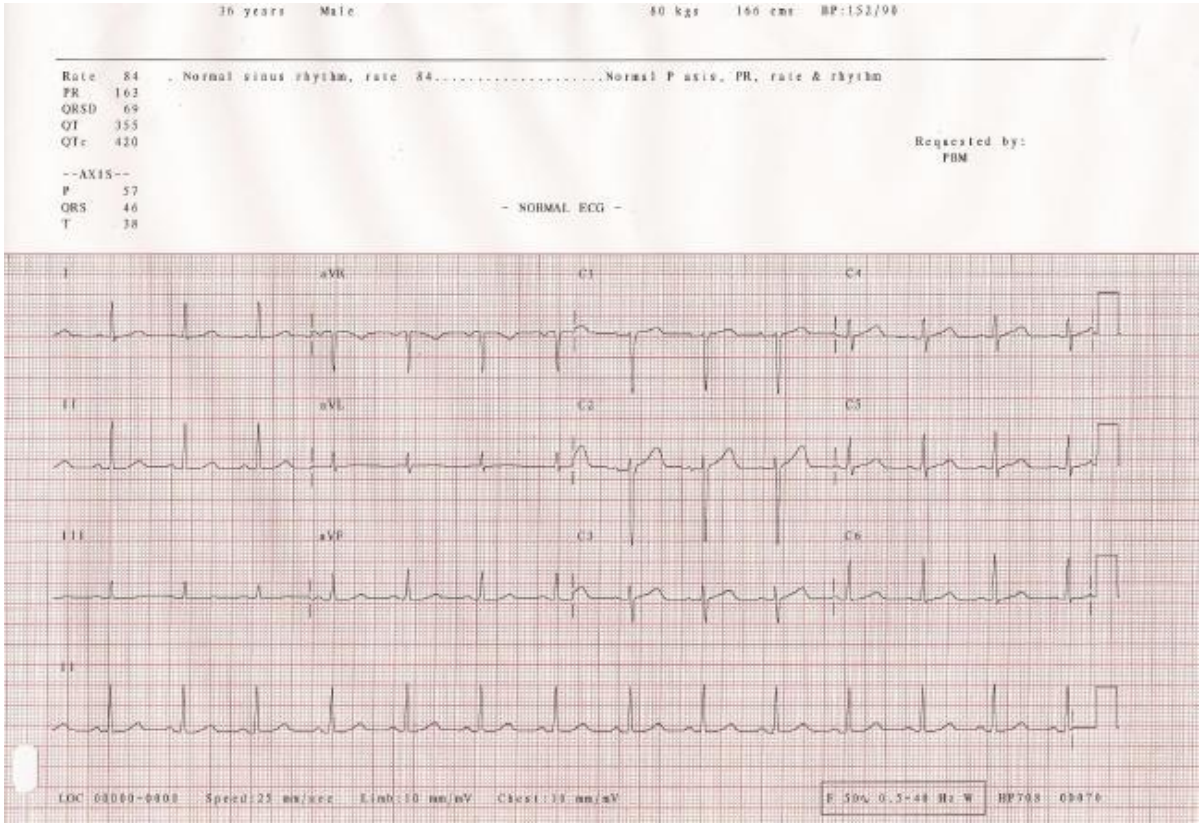
(a)



(b)

Courtesy Dr Phillip Barlow Mills

FIGURE 32
TWELVE LEAD ELECTROCARDIOGRAM WITH RHYTHM STRIP



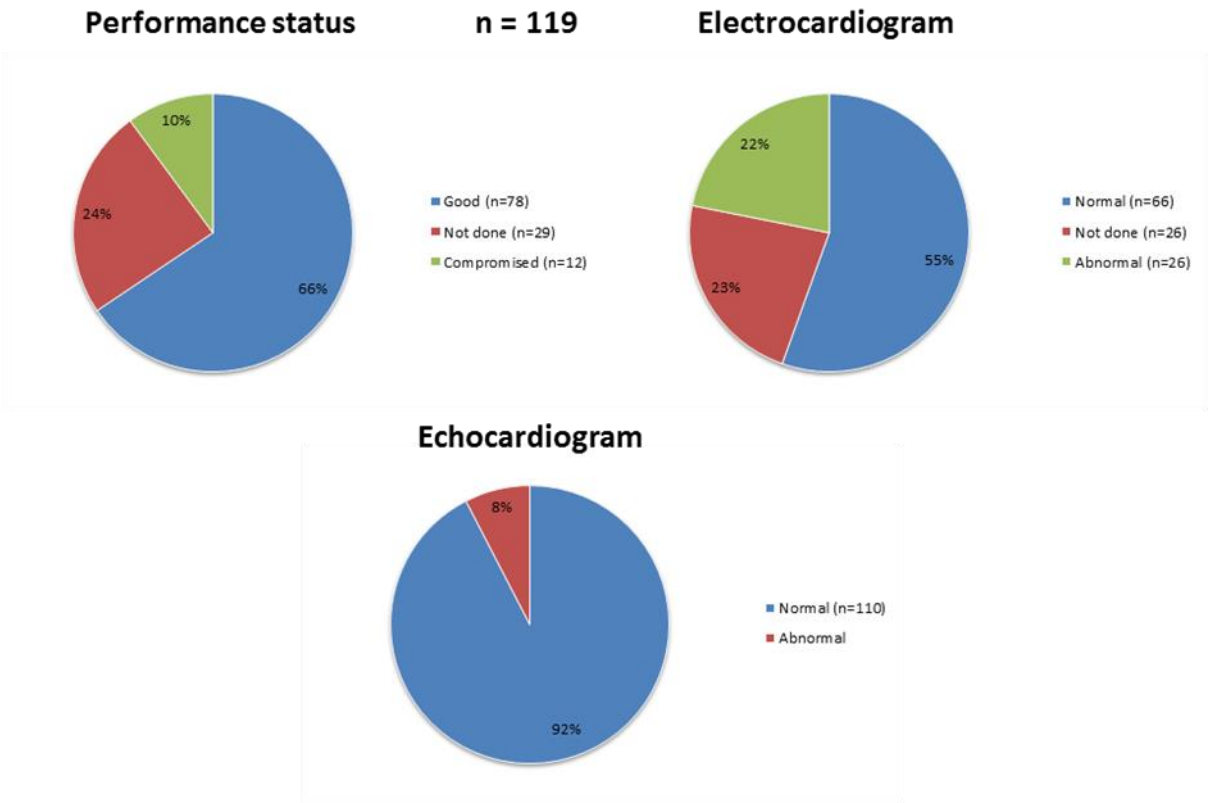
Courtesy Dr Phillip Barlow Mills

Routine laboratory studies included full blood count with attention to haematocrit and screens for hypercoagulability, fasting lipogram, urea and electrolytes, determination of blood sugar and brain natriuretic peptide (Pro-BNP).[57] Inflammatory markers were limited to sensitive C-reactive protein.[58] There was no conclusive data for blood tests on the patients. Assessment was done at completion of chemotherapy where this was part of the protocol. Whenever the subject had a cardiac event during transplantation phase defined practically as three months from the procedure, a full assessment was done. Serial studies were undertaken for the entire duration of long-term patient follow up.[59] Particular attention was given to patients with diabetes[60] and systemic hypertension.[61] Heart failure was noted in terms of the European Society of Cardiology Guidelines[62] or the combined approach from the American College of Cardiology – American Heart Association concurrently with the New York Heart Association Functional Class.[63] Management broadly fell into additional pharmacology for systolic[64] or diastolic subtypes[65] but at all times in the specific care of the consultant cardiologist as an integral member of a multidisciplinary team throughout the entire chemotherapy transplantation and subsequent follow-up course.

7.4.2.4 RESULTS

Performance status was good in 66% of patients: but this was compromised, for a variety of reasons, in 10%. Should a patient commence the transplant procedure with poor performance status, his recovery could be compromised. Of the initially registered cases, electrocardiograms were normal in 66 (55%) individuals and abnormal in a further 26 (22%). Abnormalities were mild such as right or left bundle branch block (RBBB, LBBB) and sinus bradycardia. Echocardiogram (ECHO) was normal in 110 (92%) and abnormal in only 9 (8%). The latter related to small effusions or diastolic dysfunction. All abnormalities were evaluated for their significance. **(Figure 33)** Although not included in this analysis, two illustrations highlight the way in which conventional management can be moderated on the basis of results in a multidisciplinary cardiovascular and haematology combined clinic.

FIGURE 33
NON-INVASIVE CARDIAC ASSESSMENT PRE-TRANSPLANTATION



Case one

An asymptomatic 24-year-old colleague was screened on the basis of prior chemotherapy for leukaemia and shown to have low voltage complexes with a reduced left ventricular ejection fraction of 45% interpreted, as early cardiomyopathy.

She was offered a matched-unrelated volunteer donor transplant for refractory disease after which left ventricular ejection fraction fell to 37% and she developed symptoms of heart failure. She was conventionally treated and remained with a raised pro-BNP and biochemical markers of cardiac injury. Currently she is back to normal and her systolic function recovered, leaving only mild diastolic dysfunction on carefully monitored therapy. This, as with others in the series, cannot be too strongly used to emphasise what can be achieved in the real world setting of cross-disciplinary combined management.

Case two

A 31-year-old male was referred after extensive treatment for refractory diffuse large B-cell lymphoma. He had reduced ejection fraction of 36% and was in symptomatic heart failure. He was considered as having no realistic option for survival without BEAM conditioning and transplantation. There were mild electrocardiographic changes and ECHO showed four chamber dilated cardiomyopathy. Following autologous salvage there was further deterioration in left ventricular ejection fraction of 15% that gradually stabilised between 1998 and 2000 only on medical management. Nevertheless, further decompensation necessitated cardiac transplantation that had a good outcome and he is currently well.

This case again underlines the absolute importance of people embarking on either type of organ transplantation management to be thoroughly conversant with what is reported internationally and particularly in the context of local experience. The emphasis is that, while careful judgement and the dependence on a fully supportive group of co-investigators can often create a medical environment of informed consent, the outcome is not universally futile.

7.4.2.5 DISCUSSION

There are three striking and overriding imperatives.

As has emerged throughout this research project there was the obligation to operate a state of the art transplantation unit. Within this environment, to constantly employ supervised protocols with publication of outcome for comparison with the literature. Even in this setting to offer these high-risk procedures exclusively in the context of a thoroughly committed and compliant cross-disciplinary team that, by definition, is based in transplantation medicine.

There should be interaction with specialists in infectious diseases, pulmonology, haematology and other target organs of which particularly successful outcomes from stem cell grafting is achievable. This was dependent on prior preparation, scrupulous surveillance throughout the procedure and continued maintenance and long-term follow-up. On this basis the logical question is what has been learned from this uniquely African investigation. There seem to be a number of points allowing a clear guideline.

Left ventricular dysfunction, as measured by reduction in left ventricular ejection fraction, predicted for increased problems following transplantation. Even with good recipient performance status and normal cardiac function, outcome could not be predicted with any degree of certainty.

A multidisciplinary team must be prepared, and expertise available, to deal with complexities arising from cardiac toxicities of the transplant procedure. In the presence of comorbidities they were not, in isolation, necessarily sufficient to exclude management in respect of each individual patient. These procedures should never be run on guidelines but on strictly supervised protocols with outcome analysed and reported both locally as well as internationally.

Definitively, and every bit as crucial to lessons learnt, was the fact that all survivors should continue to be managed within the cross disciplinary group for duration of natural life. This approach allowed not only the experience of the group, but also the subtle changes emerging over time to be available for proactive supervision and management thereby extending survivorship to the absolute maximum and very often, with a surprising outcome – in both directions.

7.4.2.6 Concluding summary

The conclusions drawn from this analysis were consistent with findings in the literature. Specifically, there was neither automatic inclusion nor exclusion of different transplant procedures based on the underlying disease but rather on the expertise cultivated in multidisciplinary interaction between cardiovascular disease and haematology – both benign and malignant.

Cardiac consequences occurred at a lower frequency than other post-transplant complications.[55]

There was a high incidence in haematological oncology of myocardial injury but with regular monitoring, this could be managed by intervening early to minimize this risk by refucing exposure to cardiotoxic drugs.

7.4.3 DERMATOLOGY

7.4.3.1 Introduction

Initially, as with other centres worldwide, the use of unmanipulated stem cells from bone marrow resulted in an almost universal occurrence of acute GVHD[66,67] presenting with a rash commencing on palms and the dorsum of hands as erythema. **(Figure 34)** It may then progress to epidural necrosis followed by stripping and subsequently followed by chronic graft-versus-host disease (GVHD).[68] Here the skin had generalised violaceous scaly plagues of the lichenoid variant **(Figure 35)** with very high morbidity and mortality[69]. During the development of the standardised programme cyclosporin A became available and reduced the incidence and severity of these immunologically mediated injuries to the skin, biliary endothelium and enterocytes of the small bowel[70,71] but these features were not entirely abrogated.[72] The addition of monoclonal antibody to the bag then followed which led to a reduction in GVHD.

FIGURE 34

ACUTE GVHD FOLLOWING INFUSION OF UNMANIPULATED STEM CELLS PRESENTING AS A RASH ON PALMS AND DORSUM OF HANDS

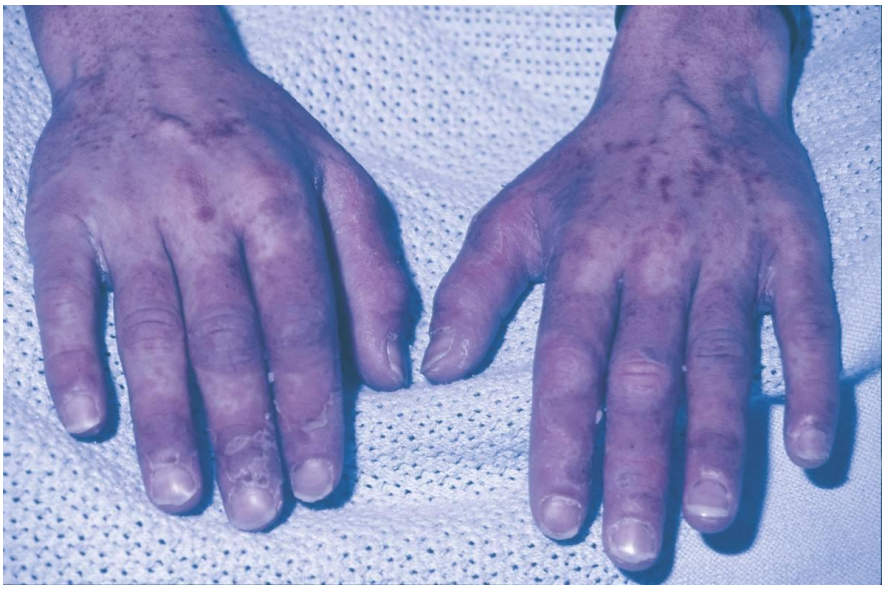
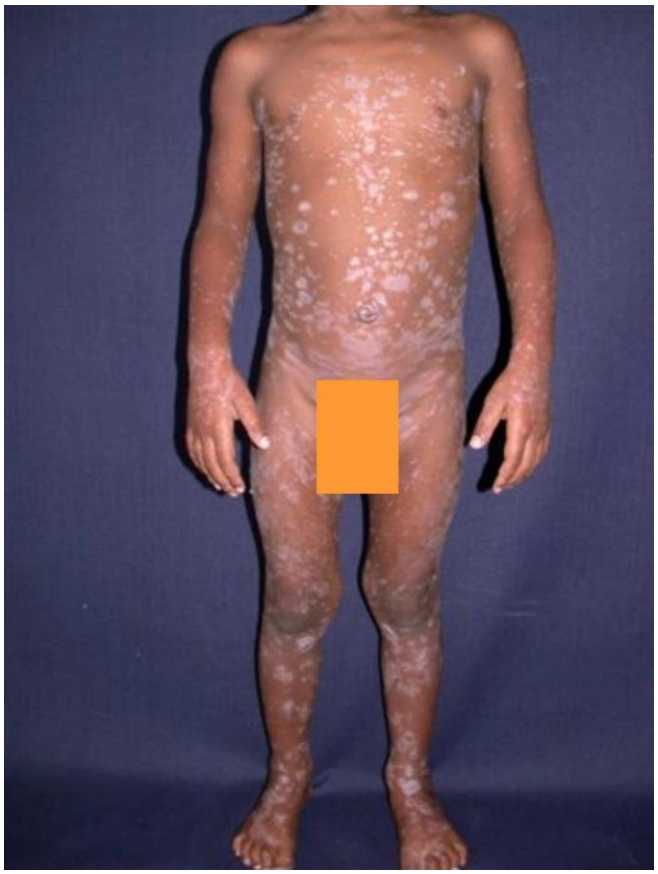


FIGURE 35

CHRONIC GVHD IN PRE-CAMPATH® PERIOD SHOWING VIOLACEOUS SCALY PLAQUES OF LICHENOID VARIANT



Gratifyingly, the safety of this alternative approach, and the generally outstanding results achieved, was later confirmed by the Leiden group,[73] among others. This success brought about the necessary endorsement for us to be encouraged to further characterise this preliminary experience.

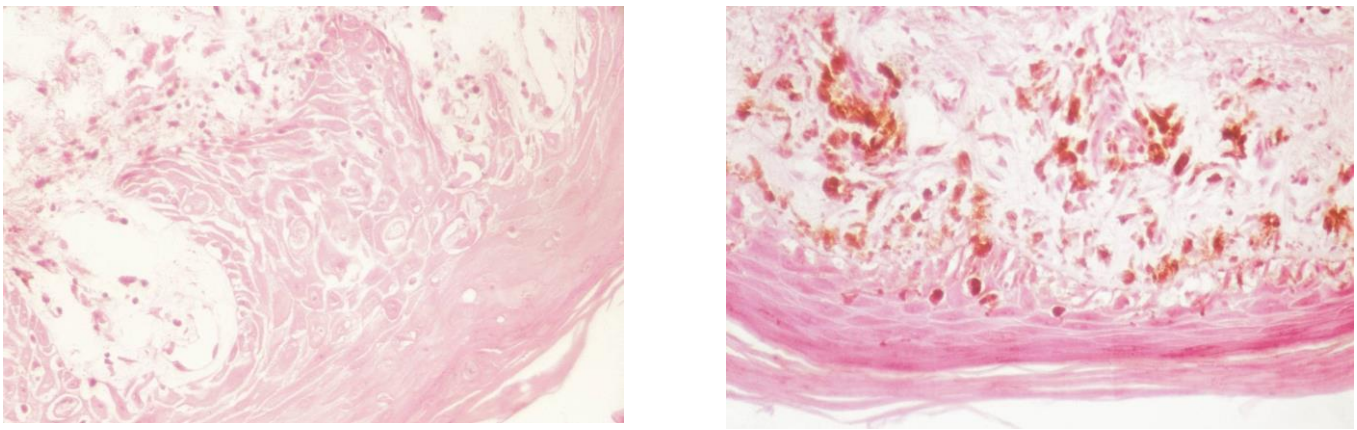
7.4.3.2 PATIENTS

Between 1995 and 2010 a total of 468 patients were transplanted and in all of these, adequate data for evaluating specific abnormalities in the skin, appendages around the eyes and mucous membranes were available.

7.4.3.3 METHODS

Cases were seen daily by the investigator and all cutaneous manifestations were referred to a specialised dermatologist with a research interest in GVHD. The majority of these lesions could be confidently diagnosed as drug hypersensitivity[74] such as rash due to trimethoprim and sulfamethoxazole (Bactrim®) or viral resulting from herpes zoster following discontinuation of prophylactic antiviral therapy and managed accordingly but not discussed any further. Where any doubt existed, photographs supplemented by a skin biopsy which were interpreted by a histopathologist, were required to sustain the diagnosis of GVHD.[75] The definitions for acute and chronic GVHD are provided in **Table 17. Figure 36a** illustrates acute GVHD showing necrotic keratinocytes, vacuolar degeneration, dermal oedema and pigment incontinence and in **Figure 36b,** chronic GVHD showing hyperkeratosis, parakeratosis, scattered necrotic keratinocytes, vacuolar degeneration and pigmentary incontinence.

FIGURE 36
PHOTOMICROGRAPHS OF ACUTE AND CHRONIC GVHD DIAGNOSED BY SKIN BIOPSY



(a) ACUTE (b) CHRONIC

Courtesy Dr Kathy Taylor

Table 17
GVHD DEFINITIONS

Category	Time symptoms develop	Presence of acute GVHD features	Presence of chronic GVHD features
ACUTE GVHD			
Classic	≤ 100 days	Yes	No
Recurrent/Late onset	≥ 100 days	Yes	No
CHRONIC GVHD			
Classic	No time limit	No	Yes
Overlap syndrome	No time limit	Yes	Yes
Forme frust	≤ 100 days	Mild and non-specific	No

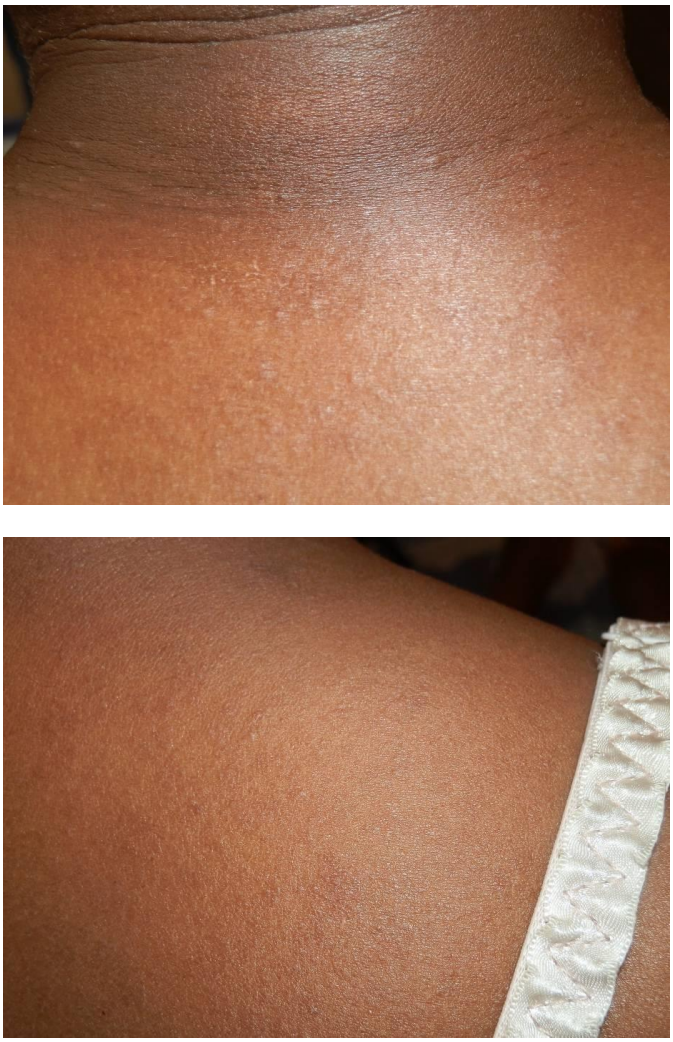
Grading was done according to skin and organ involvement. Grade I only involved skin approximately 25% and Grade II had a combination of skin (25-50%) and mild liver and gut involvement. Grade III and IV are delineated by progressively more severe changes in these 3 organs. *Forme frust* was a mild non-specific rash with no systemic signs.

7.4.3.4 RESULTS

Twenty-eight cases fully met the criteria for the newly emerged variant of GVHD or *forme frust*. The latter had its most striking expression on the reduced extent and severity of the cutaneous injury. Although it was not possible to compare this subgroup to the total earlier pre-Campath® population, experienced medical and nursing personnel were in agreement that it caused less hyperbilirubinaemia. There was less involvement in these two major target organs with the *forme frust* variant than the acute GVHD.

Initially it was difficult to appreciate the significance in comparison to the traditional acute graft-versus-host disease and a number of distinctive features were rapidly confirmed within the study group. GVHD presented with cutaneous manifestations centred on palms and soles. They were florid, aggressive and sometimes explosive lesions. On the other hand those patients exposed to monoclonal antibody showed rashes which extended more slowly and developed usually two or three days later. These rashes were also less centred on the palms and soles. They were invariably less than 20% of the body surface area[76] seldom requiring anything more than topical corticosteroids for reversal.[77] **(Figure 37)** Grade III and IV were never seen. Parental steroid therapy was infrequently required. Other immunosuppressants including cyclosporin A or methotrexate were not needed. In the group of patients with GVHD there was no recognisable chronic graft-versus-host disease except for three cases each of which followed donor lymphocyte infusion. This observation showed the impact of using the monoclonal antibody Campath® *ex vivo*. **(Table 18)**

FIGURE 37
FORME FRUST – A MILDER FORM OF GVHD SEEN WITH CAMPATH® 1H *ex vivo* – REDUCED CUTANEOUS INJURY



Courtesy Dr Ranks Lehloenya

TABLE 18
NUMBER OF GVHD IN THE PERIOD 1998-2010

YEARS	NUMBER	TRANSPLANTS
1998	7	24
1999	7 (1*)	38
2000	4	23
2001	1 (1*)	38
2002	2 (1*)	23
2003	2	19
2004	2	18
2005	1	12
2006	1	14
2007	1	10
2008-2010	0	26
TOTAL	**28**	**245**

*This followed donor lymphocyte infusion leading to chronic GVHD

7.4.3.5 Discussion

The skin, being a vast organ, is constantly bombarded by environmental antigens and may undergo many acute changes, some of which are non-specific and of little relevance. Experience, as a steep learning curve, was needed to crystallise the dominant post-transplantation injury. Once rashes due to drug hypersensitivity and infectious causes were identified and excluded, the complex immunologic syndromes of acute and chronic graft-versus-host disease continued to demand attention.

The striking multisystem presentation of GVHD, relentless progression and often lethal outcome, made it one of the most feared complications, to the extent that on more than one occasion, it threatened to compromise continuation of an entire programme when faced with ethical dilemmas and a range of psychosocial challenges.

It remains a tribute to the pioneers in the field that these daunting experiences were shaped, largely by the ingenuity of transplant team activity, to produce the protocol linking staging and grading.[78,79] The initial experience with GVHD was with immunosuppressive regimens comprising corticosteroids, methotrexate and cyclosporin A.[80,81]

It was rewarding that the cutaneous manifestations were amongst the first to show a reproducible sharp downturn once monoclonal antibody was used. The link of these to the corresponding reduction in the damage to the biliary epithelium and enterocytes lining, primarily the small intestine, was difficult to objectively measure but gratifying paralleled with a striking shift towards increasing manifestation of lesser grades of the chronic graft-versus-host disease.[76,82]

Due to blunting of immunologic process, occurrence of GVHD was reduced. The pathophysiology has been explored by colleagues within the Campath® Users' Group.[49,83] There seems to be a higher occurrence of infections, particularly viral, due to delayed or abnormal lymphocyte reconstitution.[84]

7.4.3.6 Concluding summary

The cardinal point emerges that this innovative exposure to Campath® 1H significantly improved outcome by reducing the incidence and severity of both acute and chronic graft-versus-host disease. Quality of survivorship, in its broadest terms, was further enhanced by psychosocial benefits to the family and staff of the treating transplant team. Significant cost saving, particularly in a resource constrained country translated into greater access in the private as well as public sector. At a more basic scientific level these outcomes were widely replicated within the Campath® Users' Group and added support for continuation with further investigations into the effect of adding the monoclonal antibody to the graft *ex vivo*.

7.4.4 GASTROENTEROLOGY

7.4.4.1 INTRODUCTION

Three interlocking practicalities arose in the bowel and biliary system and were of sufficient magnitude for analysis.

Firstly, a patient-centred group of problems, defined as mucositis with dysphagia resulting in anorexia, nausea, vomiting and diarrhoea,[85, 86] that generally reversed simultaneously with the reappearance of monocytes and neutrophils in the peripheral blood. The relevance that the dramatic dermatologic features may find parallel in the foregut, raised the anticipation of benefit accruing from blunting the graft-versus-host disease as part of the Campath® in-the-bag protocol therapy. This challenge clearly justified audit within the broad spectrum of extra haematopoietic post-transplant acute associations.

Secondly, appreciating the importance of nutrition, at every level, a feasibility study was undertaken over 15 years with two significant end points.[87] The results established that 24 hour continuous fine bore naso-jejunal, as opposed to nasogastric placement, in any individual with absolute indication for supplementation was uniformly effective. A comparison was not done between the two methods.

The need for total parenteral nutrition (TPN) largely became obsolete with major cost saving and significant improvement in quality of life or survivorship. Additionally, major safety considerations emerged without the need for TPN in that vascular thromboembolism and catheter sepsis simply did not occur.[88] Given the still largely controversial status of available publications, this authoritative statement remains a crucial observation impacting on world practice by demonstrating that utilising the intravenous route for total parenteral nutrition was virtually never necessary.

Thirdly, even once these variables had been eliminated, there remained a cohort with non-resolving symptoms at two levels requiring further study in consultation with an experienced gastroenterologist. In one of these, after exhaustion of all conservative approaches, the issue deemed to be best approached by endoscopy. In the second, subtle residual changes in the hepatic biochemistry dictated recourse for further investigation.

7.4.4.2 PATIENTS

Between 1995 and 2010 a total of 468 consecutive individuals were transplanted. Starting in 2000 the multidisciplinary team routinely included expertise from gastroenterology and hepatology applicable to the next 265 procedures. From these 18 cases were identified that remained sufficiently problematic for specialised evaluation. From the combined clinic, three distinctive cohorts were separable.

A domestic analysis of 89 post-transplant cases,[89] characterised by mucositis with dysphagia, accompanied by anorexia, nausea, vomiting and diarrhoea delineated a syndrome restricted to the first three weeks after graft infusion. The condition very strongly stressed the central importance of optimal nutritional management and had two components. The first of these was documentation that adequate caloric and all other requirements could be achieved, exclusively by the enteral route thereby making intravenous access unnecessary. Secondly, that a very high degree of patient acceptability attended continuous supplementation using fine bore naso-jejunal in preference to naso-gastric placement.

There were 18 individuals in whom original symptoms did not resolve and consultation dictated recourse to endoscopy as a basis for further treatment.

A separate cohort of 39 cases, whose dominant clinical problem on routine post-transplant surveillance was unexplained altered liver-function and enzyme tests, had variable outcome and therefore a need for serial follow-up.

7.4.4.3 METHODS

As part of protocol management any delay in delivering optimum daily nutrition necessitated placement, by the interventional radiologist, of a fine bore naso-jejunal – specifically not nasogastric – tube for supplementation. In groups two and three, persistent abnormalities in routine liver function and enzyme tests, which were done twice a week, called for viral serology or polymerase chain reaction to detect CMV, EBV and hepatitis A, B and C. Cases with appropriate indications of diagnostic difficulty were resolved by referral to a gastroenterologist for upper or lower endoscopy.[90,91]

7.4.4.4 RESULTS

In this population the foregut syndrome occurred in 80 (90%) of patients following transplantation. This is in keeping with our earlier experience and that reported in the literature.[85] The combination of chemotherapy with radiotherapy gave rise to more severe symptoms and longer duration although resolution occurred in the majority.[92]

Sixty-seven (75%) of the 89 could not achieve adequate oral intake and required continuous supplementation with a fine bore enteral tube. Somewhat surprisingly, there was no significant difference in weight, at starting conditioning, from those who did, or did not, require enteral feeding.

Placement of enteral tubes had high patient acceptability but were more efficient when nutritional supplements were delivered in the jejunum as opposed to the stomach.[88,93] Importantly, maintenance of nutritional requirements in almost all these patients was sufficient to reserve total parenteral nutrition for those very rare instances where these criteria could not be achieved.[94]

Eighteen of 89 patients (20.2%) with gastrointestinal symptoms were referred to the gastroenterology service. Endoscopy was needed in 10 (55%). Two had upper GI endoscopies and three had colonoscopies alone. In another five combined procedures were done. In four (40%) the procedure was normal. In the eight (44%) who did not have endoscopies a clinical diagnosis was made. **(Table 19)**

TABLE 19
GASTROINTESTINAL SYMPTOMS AND FINDINGS IN PATIENTS REFERRED FOR ENDOSCOPY

SYMPTOMS	COLONOSCOPY	GASTROSCOPY	DIAGNOSIS
Diarrhoea	Normal	ND	IBS Infectious &Bile acid Diarrhoea*
Chonic Constipation	Normal	Normal	IBS Constipation*
Septicaemia	ND	Erosive Oesophagitis Haemorrhagic Gastrites Bleeding Prepyloric Gastric ulcer	
Deranged LFT+ Ascites	ND	ND	Drug induced VOD
Septicaemic	ND	ND	Severe Ileus – Typhlitis
Pain & Diarrhoea	Active Crohn's Disease Right Colonic Disease	ND	Crohns Disease
Deranged LFT	ND	ND	Drug induced liver injury
Dysphagia Dyspepsia N&V		Prepyloric Gastritis	Prepyloric gastritis
Persistent Diarrhoea	Infective Colitis		Infective colitis
Deranged LFT	ND	ND	Liver Bx-Extramedullary Haemopoiesis
Nausea & Vomiting	ND	Mild Corpus Gastritis	Gastritis
Abdominal Pain		Haemorrhagic Gastritis + Erosions	IBS Constipaton*
Deranged LFT			Drug induced Hepatitis
Abdominal Pain	Normal	Erosive Gastritis	Gastritis
Diarrhoea			Bile acid diarrhoea
Abdominal Pain+Diarrhoea	ND	ND	Neutropenic Ileus
Recurrent Diarrhoea	ND	ND	*C.Difficile* Colitis
Deranged LFT	ND	ND	Drug induced liver injury Ileus
Recurrent Diarrhoea Rectal Bleeding	Normal	ND	Internal Haemorrhoids
Deranged LFT	ND	ND	Drug induced Hepatotoxicity CMV+ Hepatitis
Abdominal Pain Diarrhoea	Normal	Gastric erosions Haemorrhagic Gastritis	Gastritis
Epigastric pain	Normal	Oesophageal Candida Multiple Gastric ulcers + erosions	Liver Bx-Siderosis Varicella

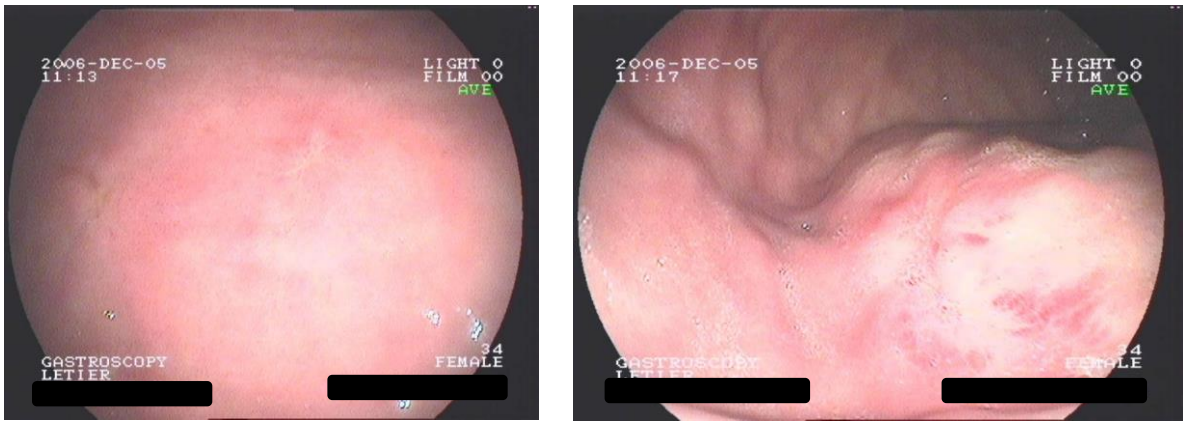
*IBS – irritable bowel syndrome

Normal – no abnormalities

ND – not done

In the stomach and proximal duodenum, no malignancy was encountered. Two persons had gastric erosions **(Figure 38a)** and healing gastric ulcer **(Figure 38b)** present on gastroscopy. One had a pre-pyloric gastric ulcer. **(Figure 39)**

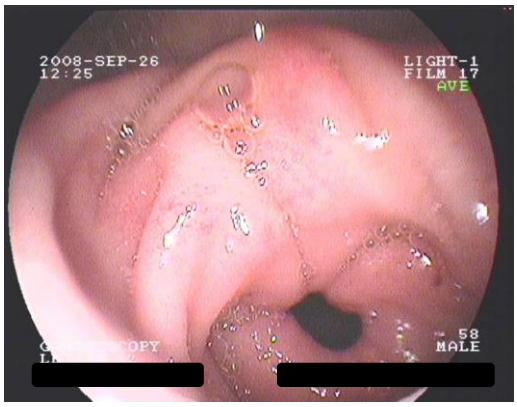
FIGURE 38
ILLUSTRATIVE GASTROSCOPY: (a) GASTRIC EROSIONS (b) HEALING GASTRIC ULCER



(a) (b)

Courtesy of Dr Melvyn Letier

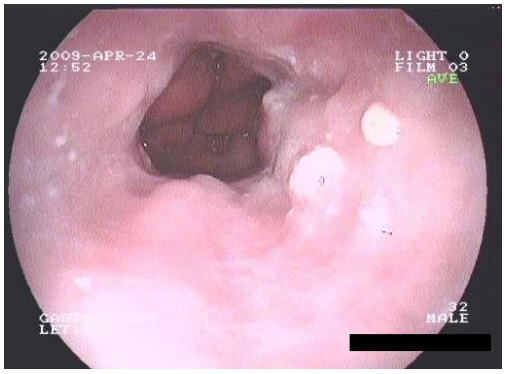
FIGURE 39
ILLUSTRATIVE GASTROSCOPY: PRE-PYLORIC GASTRIC ULCER



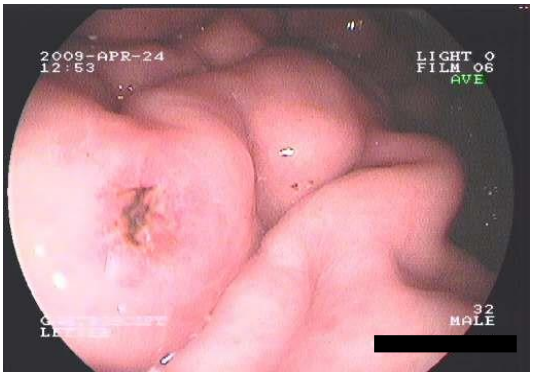
Courtesy of Dr Melvyn Letier

A single individual had viral inclusion bodies with oesophageal candida, **(Figure 40a)** varicella gastroenteritis (**Figure 40b)** and a varicella superficial gastric ulcer. **(Figure 40c)** The virus was confirmed on biopsy and responded to conventional conservative therapy.

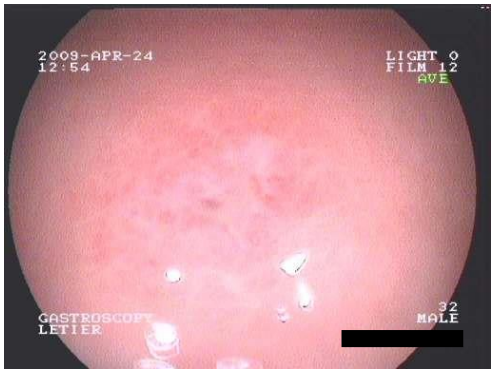
FIGURE 40
GASTROSCOPY AND COLONOSCOPY: MULTIPLE GASTRIC ULCERS, EROSIONS-SUBSEQUENTLY ATTIBUTED TO VARICELLA ZOSTER



(a)



(b)



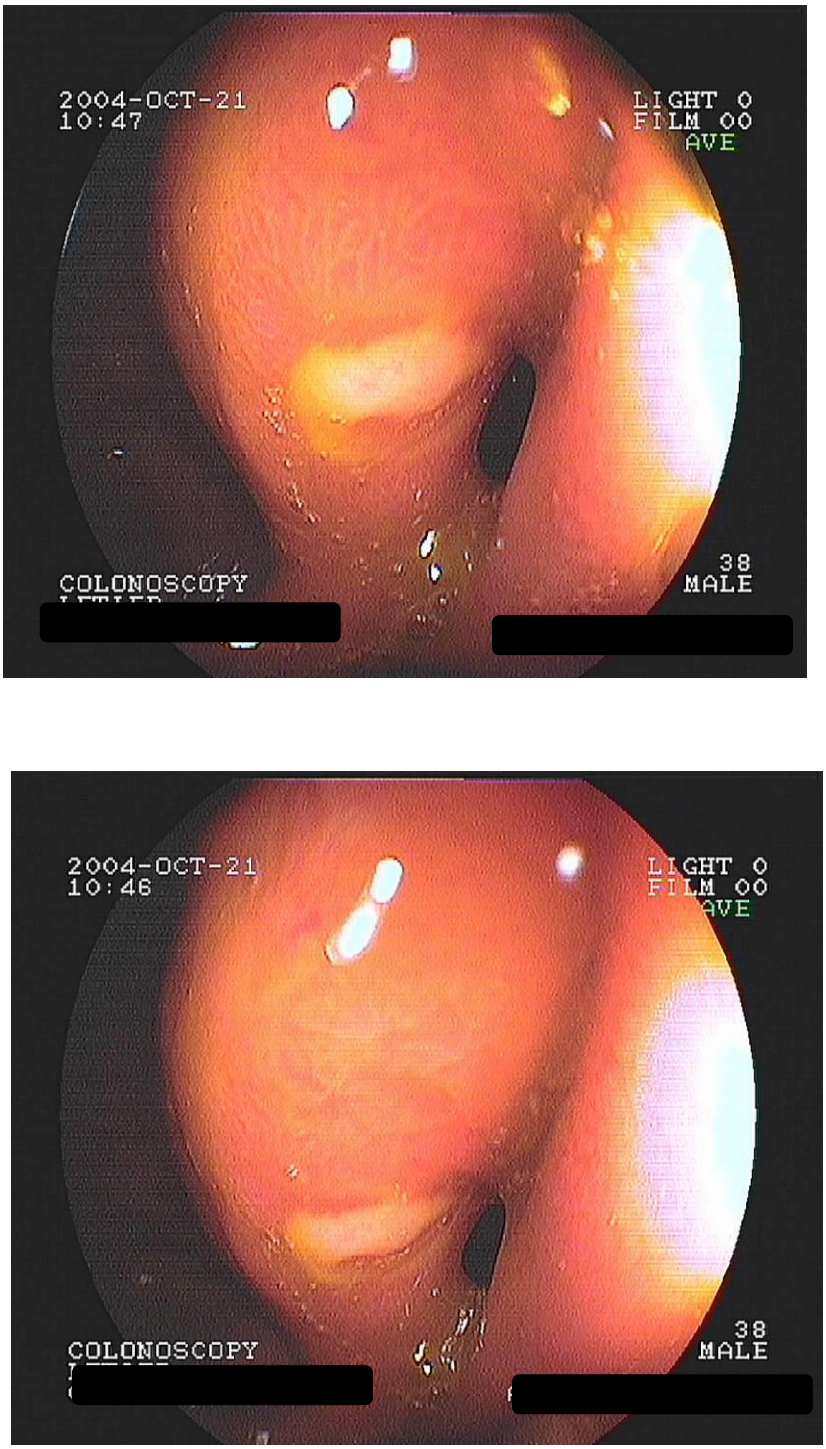
(c)

Courtesy of Dr Melvyn Letier

In the large bowel there was one instance of active Crohn's disease which was proven on biopsy seen at the ileocaecal junction with ulceration, oedema and narrowing **(Figure 41)** and one of infective colitis. In the remainder no additional information was obtained from these procedures.

FIGURE 41

COLONOSCOPY DEMONSTRATING ACTIVE CROHN'S DISEASE



Courtesy of Dr Melvyn Letier

In the separate cohort of 39 individuals, seven died of an unknown cause in the first three weeks. This was clinically ascribed to sepsis.[95] Tabulated data over a 12 week period is presented for bilirubin, alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT). Corresponding data which was available for albumin, lactic dehydrogenase (LDH), Aspartate aminotransferase (AST) and alkaline phosphatase added little to disentangling these liver enzyme and function studies. Variations in the number of observations recorded during this four-week period do not represent loss of patients but non-attendance at the clinic due to personal preference for scheduling.

The highest level of bilirubin noted in any patient was 385 at week six (he had pseudomonas infection). Of the 39 patients, 23 (59%) remained normal and in 16 (41%) abnormal enzymes were present. Seven (44%) of these reversed to normal by week 12 and in only nine (56%) did this fail to reverse. **(Table 20)** The highest median ALT elevation of 51.5 was present at week three and the highest level of 683 was seen in a subject with sepsis, who had received antibiotics and had been hypotensive raising the possibility of hypoxic-ischaemia. Of the 39 patients, 12 (31%) remained normal and of the 27 (69%) with abnormal enzymes 15 (56%) reversed and 12 (44%) did not by three months. Drug induced as well as septic aetiologies all contributed to the liver dysfunction. **(Table 21)** The GGT had the highest median of 195.5 at week two and the highest GGT level was 735 in a patient with likely multifactorial liver insult. Here only 9 (23%) of the 39 patients remained normal with 30 (77%) being abnormal. Of these 17 (57%) reversed by week 12 and 13 (43%) did not. **(Table 22)** Of the 39 individuals with abnormal LFT 9 – 12 patients (23-30%) in terms of bilirubin, ALT and GGT levels, did not revert to normal at three months.

TABLE 20
LIVER FUNCTION – SEQUENTIAL BILIRUBIN LEVELS DURING FIRST TWELVE WEEKS POST TRANSPLANT

Bilirubin µmol/L	**Obs** N	**Median** µmol/L	**IQR** µmol/L	**Range** 2 to 20 µmol/L
Pre	37	10	9 - 14	2 to 28
Tx	38	14	11 -18	6 to 43
Week 1	39	15	12 - 24	6 to 70
Week 2	38	11.5	8 - 19	5 to 65
Week 3	33	13	11 - 24	4 to 101
Week 4	27	15	11 - 19	9 to 85
Week 5	29	17	12 - 23	6 to 132
Week 6	21	17	13 - 26	7 to 385
Week 7	22	16.5	13 - 29	7 to 184
Week 8	20	17	13.5 - 29	11 to 78
Week 9	20	23	12.5 – 37.5	7 to 164
Week 10	16	18.5	11 - 38	6 to 123
Week 11	13	20	11 - 28	8 to 48
Week 12	16	17.5	11 - 25	8 to 59

TABLE 21

LIVER ENZYME –SEQUENTIAL ALT DURING FIRST THREE MONTHS POST TRANSPLANT

ALT U/L	**Obs** N	**Median** U/L	**IQR** U/L	**Range** 10 to 40 U/L
Pre	37	24	17 - 38	8 to 80
Tx	38	29.5	22 - 46	9 to 131
Week 1	39	39	26 - 61	13 to 336
Week 2	38	44	27 - 75	8 to 257
Week 3	34	51.5	26 - 74	7 to 275
Week 4	26	49.5	29 - 100	14 to 287
Week 5	30	39.5	21 - 66	16 to 397
Week 6	22	32	22 - 42	10 to 168
Week 7	21	30	21 - 65	8 to 178
Week 8	22	24.5	20 - 32	14 to 189
Week 9	19	22	17 - 33	13 to 683
Week 10	16	24.5	18 - 36	7 to 66
Week 11	13	33	29 - 45	14 to 77
Week 12	16	27.5	24 - 42	17 to 70

TABLE 22

LIVER ENZYME –SEQUENTIAL GGT MEASUREMENTS IN THE FIRST 100 DAYS POST TRANSPLANT

GGT U/L	**Obs** N	**Median** U/L	**IQR** U/L	**Range** 5 to 50 U/L
Pre	37	36	25 - 54	11 to 387
Tx	38	68	35 - 136	14 to 694
Week 1	39	177	76 - 294	21 to 667
Week 2	38	195.5	105 - 326	34 to 612
Week 3	33	115	57 - 225	14 to 661
Week 4	27	100	56 - 163	30 to 735
Week 5	29	75	39 - 175	22 to 416
Week 6	21	57	38 - 142	19 to 288
Week 7	22	74.5	41 - 144	15 to 336
Week 8	21	56	27 - 113	18 to 323
Week 9	19	64	22 - 123	14 to 357
Week 10	16	79	26.5 – 116.5	14 to 336
Week 11	13	94	26 - 157	16 to 281
Week 12	16	46.5	24.5 – 117.5	11 to 258

7.4.4.5 Discussion

Gastrointestinal complications following bone marrow transplantation are common and referral rates for gastroenterology consultation (GEC) vary widely in different series. Symptoms are fairly uniform and reflected in our experience – persistent nausea and vomiting, abdominal pain, dysphagia and odynophagia, diarrhoea and gastrointestinal bleeding. Gastrointestinal (GI) bleeding occurred in our population to a lesser degree than reported, perhaps due to routine proton pump inhibitor (PPI) prophylaxis.[96] Graft-versus-host disease (GVHD) which was a cause of diarrhoea in the early transplant phase, was not seen at all in this cohort, largely as a result of a specific preconditioning regimen. Of the total 468 patients that underwent BMT, 89 (19%) had gastrointestinal tract (GIT) symptoms and 18 (20.2%) of these were referred for gastroenterology consultation.

Endoscopy was performed in only 6 patients referred for GIT evaluation, since the relatively fragile nature of the post-transplant mucosa increases the risk of endoscopic complications such as tears and perforation. In some publications, fatal endoscopic complication rates of up to 1.8% (much higher than in the immunocompetent patient rates of 0.01 to 0.5%) have been reported.[85] Two deaths occurred and were ascribed to septicaemia and subsequent multi-organ failure unrelated to the GI pathology. In only two patients (11%) referred, one with Crohn's disease and another with oesophageal candidiasis and varicella gastroenteritis, did endoscopy make a profound impact on medical management. In a study of 197 patients post BMT a 40% GEC rate was seen with acute GVHD (46%), gastrointestinal bleeding (25%), dysphagia/ odynophagia (20%), vomiting (29%), diarrhoea (36%) and abdominal pain (13%) being the presenting complaints.[85]

An analysis done showed that in 77% of GEC referrals, a definitive diagnosis was reached and management effected in 54% of cases.[85] In our significantly larger cohort, the impact of GEC was similar with a change in management in nine (50%) of subjects even though the referral rate was much lower than the published data.[85]

Liver function and enzyme derangement post BMT is relatively common and the levels of abnormality in our series were quite mild and did not suggest a specific pattern of injury. It could likely be ascribed to a combination of sepsis, chemotherapy and other drug (antibiotics, etc.) induced hepatotoxicity.[97] In our study, it proved difficult to conclusively ascribe liver dysfunction to drug induced liver injury (DILI), but in the face of a chemotherapeutic conditioning regimen as well as extensive broad spectrum antibiotic therapy and antifungal use for neutropenic fever, it is reasonable to assume that these drugs were responsible.[98]

No liver biopsies were performed due to bleeding risk and recovery of the abnormalities within a reasonable period occurred after discontinuation of treatment with potential offending drugs.

All liver function (LFT) and enzyme tests were documented but monitoring was centred around bilirubin, ALT (representing transaminitis) and GGT (representing cannalicular liver damage). It is well known that the bile transporter "knockout" effect is the primary cause of rapidly escalating bilirubin in septic patients. GVHD is a well described potential source of liver dysfunction[99] but this complication was not observed in any of the patients as a result of a specific Campath® T cell depleted conditioning programme. All patients with liver function derangement were routinely screened for viral infections, including Hepatitis A, B and C, CMV, EBV and Herpes Simplex viruses. Only one patient was found to have a proven varicella zoster infection. Veno-occlusive disease (VOD) was always considered but usually additional signs except elevated LFT and liver enzymes would be present.

7.4.4.6 Concluding summary

The large area of the gastrointestinal tract and exposure to environmental antigens has parallels to the skin and the lung and, in each, short-term or acute associations feature prominently but reverse with myeloid regeneration. In the bowel there remains a subset of patients in whom the lesion extends beyond the first few weeks and necessitates access to specialised investigations including experienced gastroenterology consultation and endoscopy with appropriate biopsy. These non-haematopoietic complications are relatively heterogenous, most have a viral aetiology and with accurate diagnosis, resolve with appropriate treatment. In this series no particular pattern occurred or could be attributed to anything specific including retroviral infection or in consequence of the Campath® conditioning programme. In this way, the complications do not differ in a significant way from experience in the literature.[100]

7.5 LATE EFFECTS - BEYOND ONE YEAR

Increasing the number of transplants successfully performed annually, and the improvement in outcome, remains cure of the primary disease but shifting to survivorship with the expectation of recipients to recover normal health whether this be physical and psychological or social. As a generalisation a number of variables contributed to changing the pattern of clinical manifestations. These included decreasing use of total body irradiation, increasing age of patients and more frequent employment of volunteer donors altering the expression of acute and chronic graft-versus-host disease with nuances between benign and malignant primary disorders.[101]

7.5.1 PULMONOLOGY

7.5.1.1 Introduction

From the earliest days of our embarking on these life-saving interventions, two quite different patterns of outcome were noted. This was in keeping with experience in other centres. Firstly there were acute infectious episodes correlating with neutropenia and having a universally high morbidity and mortality.[101] Reported lung complications developed in parenchyma between 25 and 60% of recipients and account for approximately half of transplant related deaths.[102-104] These observations were not dissimilar to previous reports from our team.[36] With advances in antimicrobial therapy there has been a distinct shift in focus to defining late onset non-infectious pulmonary side effects since these still remain potentially life-threatening.[103,105] Many of these are related to viral infections[106] but not readily distinguished from acute and chronic graft-versus-host disease. At issue is whether the increased risk in our patients on the Campath® in-the-bag program for viral infections is offset by the reduction in the two subtypes of GVHD.

To bring clarity to this issue, these phenomena (GVHD and viral infections) were investigated in our specific setting. In the earlier study of the 78 consecutive patients with sufficient data on 48, none of the measurements used proved to have a predictive role for subsequent complications [35,36] In an extension of those two earlier investigations, this doctoral study included closer examination of the larger cohort of patients. In some cases direct comparison was possible. However, the benefits of function studies in a research setting now added additional expertise imparted by interaction with highly experienced technologists and pulmonologists.

7.5.1.2 PATIENTS

The starting base was a total of 468 patients transplanted between 1995 and 2010 and from these, 55 volunteers were available for this investigation.

Included were 19 cases from the previous pulmonology studies enabling analysis between pre and post-transplant changes over time to be carried out directly. Chest x-rays were performed on 45 individuals because ten of the cohort studied were unable to attend for this procedure.

7.5.1.3 METHODS

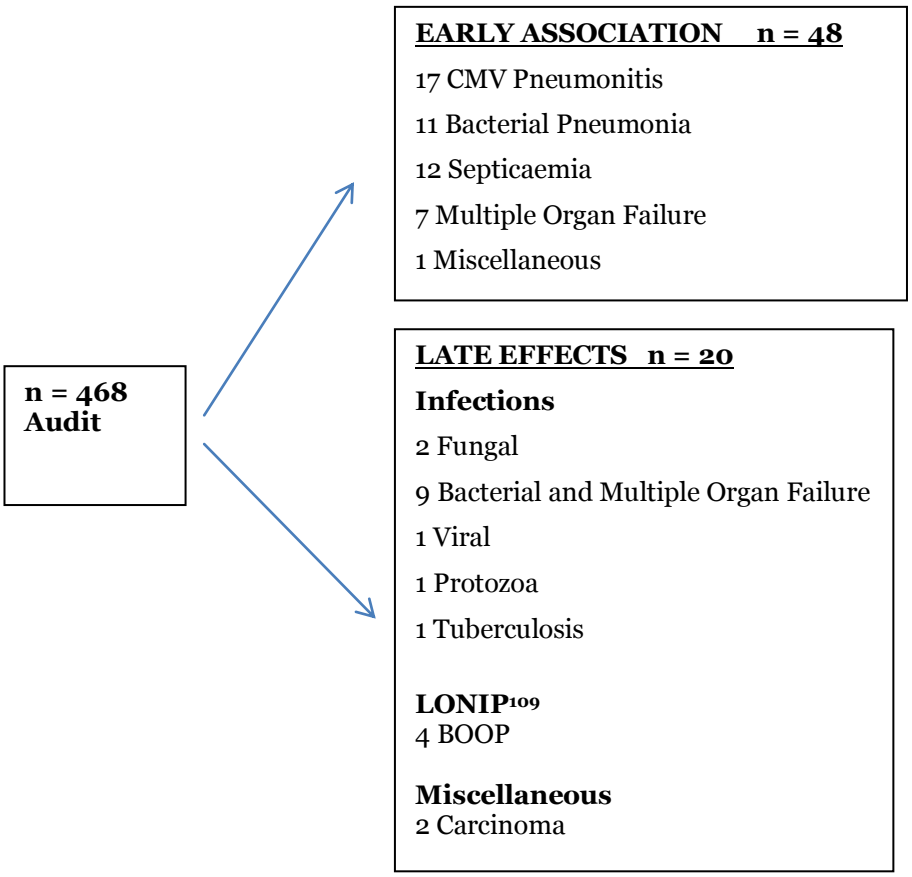
These procedures are all established, calibrated and are detailed in the above section on special investigations and each was appropriately referenced in point 7.3.3. The European Community of Coal and Steel Workers Programme (ECCS) was used to establish the values or percentage prediction as this is the most widely used and validated. This is the actual value compared to predicted based on age, height, gender and race.[107] The tests were all done on the Jaeger Masterlab Version 5.32.0 from Carefusion according to the American Thoracic Society/ European Respiratory Society (ATS/ERS) guidelines 2005. The standard spirometry "flow-volume- Loop" was performed to determine the FVC, $FEV_{1,}$ FEV_1/ FVC. For the diffusion parameters, the "single breath carbon monoxide" test and plethysmography for TLC were done. Chest x-rays posterior, anterior (PA) and lateral views were done using the conventional procedure.[108] Format for reporting was set out in point 7.3.4.2.

7.5.1.4 RESULTS

From the 468 consecutive patients comprising the audit database, 48 were categorised as early associations and a further 20 met the criteria for late effects with only one survivor in each. The early complications, occurring before day 100, were mostly related to sepsis while the majority of late effects were also infectious and most probably due (not confirmed) to a compromised immune system. **(Table 23)**

TABLE 23

RESPIRATORY: EARLY ASSOCIATIONS SEPERATED FROM LATE EFFECTS – AUDIT DATA



(CMV) Cytomegalovirus

(LONIP) Late onset non-infectious pulmonary complications

(BOOP) Bronchiolitis obliterans with organising pneumonia

In the cohort comprising the study done on surviving volunteers (n = 55), all the lung function studies were within the predicted normal range apart from some marginal reduction in diffusing capacity. It was not regarded as being of significance. **(Table 24)**

TABLE 24

SPIROMETRIC DATA AND DIFFUSING CAPACITY IN THE 55 POST-TRANSPLANT PATIENTS

	Median	IQR	Obs	Range
%FVC	108.9	96.1 – 114.4	55	67.3 to 138.3
%FEV1	100.6	88.6 – 109.5	55	43.5 to 135.2
%TLC	102.2	89.7 – 113.9	55	51.8 to 131.3
%TLCO*	73.9	64.5 – 81.5	45	52.2 to 118.8

*10 patients were excluded from TLCO because of technical problems.

Furthermore, of this group, 19 (34%) were available from the previous study making direct comparison available between the two time periods in the same individuals. Here the values are seen to be matched apart from diffusing capacity where a statistically significant (p=0.0004) improvement was noted over 10 years. **(Table 25)** It could be postulated that the pre TLCO was low because it was done immediately after chemotherapy for treatment of their disease. At the time when it was repeated, some of the patients were 10 years post transplant and completely well. Lung function studies were compared by type of transplant and showed no significant difference and were normal in all spirometry and diffusing capacity. **(Table 26)** Similarly, when analysed by disease category comparing myeloid to lymphoid lineages, there was no significant difference with all parameters within normal limits. **(Table 27)** Similarly, comparison between the different conditioning regimens was without any significant effect. **(Table 28)** Chest x-ray (CXR) results were normal in 44 with slight abnormality in one person who was investigated for tuberculosis but not found to be infected.

TABLE 25

SPIROMETRIC DATA AND DIFFUSING CAPACITY FOR 19 PATIENTS PRE AND POST-TRANSPLANT

	Obs	Median	IQR	Range
Pre %FVC	19	107	93 – 133.3	86.0 to 126.1
Post %FVC	19	108.9	103.2 – 114.3	67.3 to 133.7
Pre %FEV1	19	103.8	90.7 – 112.6	61.8 to 126.1
Post %FEV1	19	101.2	95.9 – 111.3	43.5 to 123.9
Pre %TLC	18	95	87.7 - 104	77.6 to 136.9
Post %TLC	19	102.2	94.3 – 113.3	82.3 to 131.3
Pre %TLCO	18	63.8	52 – 71.4	42.8 to 87.4
Post%TLCO	15	76	70.8 – 84.9	57.2 to 95.0

TABLE 26

SPIROMETRY AND DIFFUSING CAPACITY ASSESSMENT FOR ALLOGENEIC, AUTOGRAFTS AND MATCHED UNRELATED TRANSPLANTS

% FVC	Obs	Median	IQR	Range
ALLO	15	112.6	100.3 – 124.6	72.7 to 138.3
AUTO	23	108.9	91.7 – 113.4	76.1 to 133.7
MUD	17	106.3	99 – 112.6	67.8 to 127.4

% FEV1	Obs	Median	IQR	Range
ALLO	15	108.3	101.2 – 112.1	72.0 to 126.5
AUTO	23	93.6	86.6 – 106.9	74.5 to 135.2
MUD	17	98.3	91.3 – 106.8	43.5 to 115.9

% TLC	Obs	Median	IQR	Range
ALLO	15	102.2	94.3 – 115.8	51.8 to 131.1
AUTO	23	104.1	89.5 – 113.3	64.5 to 124.2
MUD	17	100.4	92.1 – 110.8	80.5 to 131.3

% TLCO	Obs	Median	IQR	Range
ALLO	10	80.7	67.8 – 93.5	53.1 to 118.8
AUTO	20	74.1	66.05 - 79	58.5 to 102.5
MUD	15	72.5	61.5 - 81	52.2 to 95.0

TABLE 27

SPIROMETRY AND DIFFUSING CAPACITY ASSESSMENT FRACTIONATED FOR MYELOID AND LYMPHOID PRE-TRANSPLANT MALIGNANCIES

%FVC	Obs	Median	IQR	Range
Lymphoid	22	108.2	91.7 – 113.4	75.9 to 133.7
Myeloid	32	109.05	100.3 – 118.5	67.3 to 138.3

%FEV1	Obs	Median	IQR	Range
Lymphoid	22	92.65	84.8 – 106.2	54.7 to 123.9
Myeloid	32	103.5	95.65 – 110.35	43.5 to 135.2

%TLC	Obs	Median	IQR	Range
Lymphoid	22	99.05	88.5 – 112.7	64.5 to 124.2
Myeloid	32	102.65	92.2 – 116.15	51.8 to 131.3

%TLCO	Obs	Median	IQR	Range
Lymphoid	19	73.5	68.6 – 81.5	58.5 to 102.5
Myeloid	25	74.1	64.1 – 83.8	53.1 to 118.8

TABLE 28

TYPE OF CONDITIONING: CHEMOTHERAPY VS CHEMOTHERAPY PLUS RADIOTHERAPY AND THE EFFECT ON SPIROMETRIC VARIABLES

	Obs	Median	IQR	Range
Chem %FVC	30	110	96.8 – 115.7	72.7 to 138.3
ChemRad %FVC	25	106.3	96.1 – 112.6	67.3 to 127.8
Chem %FEV1	30	96.65	88.3 – 110.9	54.7 to 126.5
ChemRad %FEV1	25	102.6	91.7 – 108.3	43.5 to 135.2
Chem %TLC	30	108.3	89.7 – 116.4	51.8 to 131.1
ChemRad %TLC	25	99.5	91.3 – 109.4	69.3 to 131.3
Chem %TLCO	24	74.5	64.05 – 82.65	53.1 to 118.8
ChemRad %TLCO	21	72.5	67.8 - 81	52.2 to 95.0

7.5.1.5 Discussion

The entire group remained asymptomatic although a single individual had abnormal radiology showing bi-basal subsegmental atelectasis and bilateral pleural effusions despite nothing being found clinically or on sputum microscopy.

In this research setting results were uniformly normal except for a marginal reduction demonstrated exclusively in the diffusion capacity determined by predicted normal values.

7.5.1.6 Concluding summary

In contrast to the acute complications, chronic or inflammatory syndromes encompass a wide range of non-infectious entities that are insidious in onset and progress silently over long periods of time.[109] The most common of these is bronchiolitis obliterans with organising pneumonia (BOOP)[110] with insidious onset over 12 months accompanied by dry cough, progressive dyspnoea and wheezing. Initially obstruction results from deposits of fibrous tissue around the bronchus adding restrictive change. The incidence varies widely and appears to be associated with chronic graft-versus-host disease.[111] It occurs more commonly with peripheral blood as compared to marrow cell grafts source[112] although other risk factors include methotrexate, age, radiotherapy and a number of respiratory viral infections.[113] A variant known as cryptogenic organising pneumonia (COP)[101] also associated with a non-productive cough, shortness of breath and fever involving bronchioles, alveolar ducts and the alveoli themselves becoming filled with granulation tissue. Causes have been ascribed to infections, drugs and radiotherapy.

Thirdly, the idiopathic pneumonia syndrome is more non-specific, encountered earlier in the post-transplant course with the closest relationship being to total or whole-body irradiation and severe chronic graft-versus-host disease, typically the sclerodermatous cutaneous subtype.[114] The absence of these late effects in this cohort may be related to the conditioning regimen and very particularly absence of acute and chronic graft-versus-host disease. No respiratory data is available for comparison with the research subjects (n=55). It appears that they have an improved outcome and better clinical post-transplant course. The latter seems to be as a result of addition of Campath® 1H in-the-bag leading to less GVHD and therefore respiratory

complications are reduced. Although these were long term survivors, they were still prone to develop late complications of the respiratory system. Cytomegalovirus reactivation occurred frequently as with other centres using Campath®.[115] The patients showing positive PCR's for CMV were treated early preventing development of pneumonitis.[115] An earlier study showed that pulmonary side-effects occurred in 30-50% of patients with unmanipulated graft and 16.5% in those where CD 52+ antibody was used as conditioning.[116]

7.5.2 IMMUNOLOGY

7.5.2.1 INTRODUCTION

It is a long accepted generalisation that underlying disease, primary chemotherapy, conditioning and various forms of immunosuppression combine to leave the post-transplant recipient at risk for severe morbidity and potential mortality. Reasons include disease relapse, infections of varying severity from a wide range of environmental pathogens and autoimmune disorders, particularly ominous being acute and chronic graft-versus-host disease.[117] As part of this view are statements that global immune reconstitution is delayed and, when more incisively expressed for functional recovery, is slow and often incomplete. The innate mechanism reconstitutes quickly whereas adaptive B and especially T-cell lymphopoiesis may be compromised for years[118, 119]. Experience with our patients to disentangle relative contributions in the lymphocyte sublineages was precluded primarily for financial reasons.

With this limitation overcome, the opportunity was taken to study, with informed consent and ethics approval, both innate and adaptive *in vitro* immune responses with special emphasis on how this may differ from findings in a first world environment less encumbered by viral infections. Additionally, and a special impact, changes that might reflect the quite different immunosuppressive conditioning used in this cohort with Campath® immunoglobulins in-the-bag[49] rather than *in vivo*. Also in contrast to conventional chemotherapy combined with corticosteroids our population received only monoclonal antibodies known to exert a more generalised effect on cells of the immune system.

7.5.2.2 PATIENTS

Fifty five volunteers were entered into this study.

7.5.2.3 METHODS

The immune parameters evaluated included both the innate[120] as well as adaptive pathways.[121] For the innate segment, the *in vitro* response of the granulocytes to stimuli was defined in the so-called Burst-test.[122] This assay measures the ability of the granulocytes to generate oxygen radicals when stimulated with opsonized E coli bacteria (considered as being physiological), fMLP[123] (a chemotactic factor representing a sub-optimal stimulus) and the positive driver, namely phorbol-myristate acetate (PMA), a potent Protein Kinase C activator.[124]

These outcomes were measured by flow cytometry using a commercial kit (PhagoBurst, Becton Dickinson) where the generation of the oxygen radical is visualized using a dye which fluoresces in the presence of the O2- radical. For comparative purposes, the manufacturer of the kit provides ranges for *E Coli* normal expected responses when using blood samples from healthy subjects. These are as follows:

	WASH	***E. Coli***	**fMLP**	**PMA**
Normal PhagoBurst Results	1-20%	95-100%	1-20%	99-100%

In parallel, the adaptive immune response was evaluated *in vitro* by determining the ability of T cells subsets (CD4+ or CD8+ cells) to generate cytokines once activated using PMA.[125] Two types of cytokines were measured, IFN-γ (a potent TH 1 cytokine able to activate the cellular arm of immunity) and IL 4, the counter-balancing TH 2 derived cytokine (able to drive a more humoral response). These cytokines were determined by making use of specific monoclonal antibodies detecting the intra-cellular cytokines of the surface labeled cells (CD4 or CD8 specific markers) following permeabilisation in order to allow the penetration of the antibodies into the cells. Multi-parameter flow cytometry was used for the analysis.[126]

Although normal ranges are not available, scientific literature suggests that cells from healthy controls, once activated *in vitro*, should display a response at least double that of unstimulated cells. For instance, when the CD4+ T cells are activated in vitro, their IFN- γ production should be at least 2-3 times that produced when the cells are not activated. Similarly, the IL4 output should be 2-3 times compared to the non-activated cells. For ease of comparison between subjects, the ratio of TH1: Th2 cells in each subject was calculated and tabulated.

The flow cytometer (FACSCalibur, Becton Dickinson) was calibrated daily using Autocomp beads to ensure the correct alignment of the laser and also to optimize the compensation of the different fluorochromes to ensure minimal spectral overlap.

7.5.2.4 RESULTS

The data set generated was analysed separately according to several subsets: type of transplant, date (age) of transplant, disease category at diagnosis and pre-transplant conditioning administered**.**

Innate immune response profiling

When the data was tabulated according to the type of transplant, it appeared that the autografts showed more "intact" responses *in vitro* to the physiological stimulus *E.Coli* when compared to the other types of transplant conducted: autografts (median 56.68% versus 34.44% and 42.88% for allo- and matched unrelated grafts respectively). This was the most normal reaction, albeit reduced, when compared to the expected normal ranges. **(Tables 29, 29a, 29b)**

TABLE 29

GRANULOCYTE BURST-TEST ACCORDING TO TYPE OF TRANSPLANT (ALLOGRAFT)

		BURST			
Type	**Subjects**	**Wash**	***E.Coli***	**fMLP**	**PMA**
ALLOTRANSPLANTATION (ALLOGRAFT)	WH 2125	9.23%	19.50%	59.69%	64.39%
	WH 2246	1.88%	31.05%	15.58%	37.68%
	WH 2138	3.45%	56.01%	4.25%	40.07%
	WH 2321	1.89%	34.44%	2.25%	25.89%
	WH 2342	0.69%	39.44%	0.79%	31.50%
	WH 9821	0.66%	23.29%	28.50%	47.53%
	WH 2258	44.73%	37.38%	31.72%	33.11%
	WH 2350	4.93%	55.15%	4.98%	50.90%
	WH 2259	2.08%	36.54%	2.28%	12.90%
	WH 2330	10.20%	60.00%	11.40%	41.40%
	WH 9814	1.32%	69.66%	2.19%	40.63%
	WH 2359	12.45%	12.27%	8.69%	13.37%
	WH 2361	3.00%	2.99%	5.86%	6.99%
	WH 2284	3.90%	3.87%	2.83%	3.16%
	WH 9717	0.70%	1.15%	0.76%	0.68%
	Median	3%	34.44%	4.98%	33.11%
	Maximum	44.73%	69.66%	59.69%	64.39%
	Minimum	0.66%	1.15%	0.76%	0.68%

TABLE 29a
GRANULOCYTE BURST-TEST ACCORDING TO TYPE OF TRANSPLANT (AUTOGRAFT)

BURST					
Type	**Subjects**	**Wash**	***E.Coli***	**fMLP**	**PMA**
AUTOTRANSPLANTATION (AUTOGRAFT)	WHA 9715	3.13%	60.70%	4.41%	51.56%
	WHA 9908	1.86%	61.09%	3.85%	55.55%
	WH 9720	3.71%	58.44%	4.52%	42.55%
	U 2056	0.36%	58.35%	1.47%	49.90%
	U 2044	2.97%	35.64%	5.25%	32.16%
	WH 9704	2.13%	60.31%	2.35%	53.18%
	WHA 9809	1.61%	43.39%	3.24%	34.61%
	U 2065	2.85%	56.68%	2.71%	54.29%
	WHA 2061	3.48%	22.72%	0.49%	44.02%
	WHA 9716	2.62%	9.15%	3.78%	9.58%
	WHA 9909	1.29%	51.96%	2.00%	40.74%
	WHA 9810	6.00%	54.50%	3.50%	61.20%
	U 2046	7.00%	99.80%	9.50%	99.90%
	WHA 9823	2.70%	93.70%	9.60%	95.10%
	U 2037	6.10%	18.10%	6.60%	26.70%
	WHA 9829	10.10%	25.10%	11.60%	29.50%
	WHA 9825	13.30%	44.90%	21.50%	36.50%
	U 2023	10.40%	63.90%	21.10%	34.80%
	WHA 2062	6.38%	14.52%	10.59%	51.97%
	U 2054	46.33%	40.47%	39.72%	49.55%
	U 2047	47.30%	62.43%	36.55%	45.33%
	WHA 2067	46.19%	61.36%	39.98%	44.06%
	WHA 9718	5.60%	57.60%	10.40%	57.90%
	Median	3.71%	56.68%	5.25%	45.33%
	Maximum	47.30%	99.80%	39.98%	99.90%
	Minimum	0.36%	9.15%	0.49%	9.58%

TABLE 29b

GRANULOCYTE BURST-TEST ACCORDING TO TYPE OF TRANSPLANT (MUD)

BURST					
Type	**Subjects**	**Wash**	***E.Coli***	**fMLP**	**PMA**
MATCHED UNRELATED DONOR TRANSPLANTATION	WH 2263	1.71%	31.01%	2.47%	42.63%
	WH 2343	1.90%	68.54%	1.85%	66.18%
	WH 2309	2.27%	32.63%	2.67%	27.37%
	WH 2280	2.29%	73.21%	3.28%	68.60%
	WH 2277	1.26%	61.15%	2.79%	31.16%
	WH 2256	5.99%	22.09%	7.18%	54.93%
	WH 2101	7.29%	10.44%	1.33%	8.98%
	WH 2128	6.06%	12.46%	6.12%	13.44%
	WH 2293	10.16%	18.12%	9.07%	13.68%
	WH 2305	9.30%	39.20%	6.90%	37.30%
	U 9704	5.60%	49.60%	5.00%	53.90%
	WH 2347	13.50%	42.80%	25.10%	48.20%
	WH 2288	4.60%	99.50%	12.80%	99.80%
	WH 2253	3.30%	14.00%	11.00%	40.80%
	WH 2311	3.80%	72.50%	11.30%	73.50%
	WH 2268	6.60%	55.50%	5.60%	62.10%
	WH 2285	6.00%	52.60%	9.00%	63.50%
	Median	5.60%	42.80%	6.12%	48.20%
	Maximum	13.50%	99.50%	25.10%	99.80%
	Minimum	1.26%	10.44%	1.33%	8.98%

Arbitrary times were allocated to determine whether the time from transplantation to recruitment for testing had any effects on the parameters measured. It was decided to regroup the data as recent transplants (0-5 years), intermediate (6-10 years) and older transplants (>10 years). Once again, the *in vitro* innate immune response was determined according to these groupings.

Interestingly, it appears that those patients that were transplanted the longest (>10 years) displayed the more "intact" profile *in vitro* in that their reaction was shown to be most normal to *E.Coli* stimulation: median 58.02% when compared to 37.38% and 46.20% for intermediate and recent transplants respectively. This was however reduced according to normal ranges provided by the manufacturer of the kit. **(Tables 30,30a,30b)**

TABLE 30
GRANULOCYTE BURST-TEST ACCORDING TO PERIOD POST TRANSPLANT (0-5 YRS)

BURST					
Type	**Subjects**	**Wash**	***E.Coli***	**fMLP**	**PMA**
YEARS ABOVE 2003 (0-5 YEARS)	WH 2263	1.71%	31.01%	2.47%	42.63%
	WH 2343	1.90%	68.54%	1.85%	66.18%
	WH 2309	2.27%	32.63%	2.67%	27.37%
	WH 2321	1.89%	34.44%	2.25%	25.89%
	WH 2342	0.69%	39.44%	0.79%	31.50%
	WH 2280	2.29%	73.21%	3.28%	68.60%
	WH 2277	1.26%	61.15%	2.79%	31.16%
	U 2056	0.36%	58.35%	1.47%	49.90%
	U 2044	2.97%	35.64%	5.25%	32.16%
	WH 2350	4.93%	55.15%	4.98%	50.90%
	U 2065	2.85%	56.68%	2.71%	54.29%
	WHA 2061	3.48%	22.72%	0.49%	44.02%
	WH 2330	10.20%	60.00%	11.40%	41.40%
	U 2046	7.00%	99.80%	9.50%	99.90%
	WH 2293	10.16%	18.12%	9.07%	13.68%
	U 2037	6.10%	18.10%	6.60%	26.70%
	WH 2305	9.30%	39.20%	6.90%	37.30%
	U 9704	5.60%	49.60%	5.00%	53.90%
	WH 2347	13.50%	42.80%	25.10%	48.20%
	U 2032	10.40%	63.90%	21.10%	34.80%
	WH 2288	4.60%	99.50%	12.80%	99.80%
	WH 2311	3.80%	72.50%	11.30%	73.50%
	WHA 2062	6.38%	14.52%	10.59%	51.97%
	WH 2359	12.45%	12.27%	8.69%	13.37%
	U 2054	46.33%	40.47%	39.72%	49.55%
	U 2047	47.30%	62.43%	36.55%	45.33%
	WH 2361	3.00%	2.99%	5.86%	6.99%
	WH 2284	3.90%	3.87%	2.83%	3.16%
	WHA 2067	46.19%	61.36%	39.98%	44.06%
	WH 2285	6.00%	52.60%	9.00%	63.50%
	Median	4.77%	46.20%	2.36%	44.04%
	Maximum	47.30%	99.80%	39.98%	99.90%
	Minimum	0.36%	2.99%	0.49%	3.16%

TABLE 30a
GRANULOCYTE BURST-TEST ACCORDING TO PERIOD POST TRANSPLANT (6-10YRS)

BURST					
Type	**Subjects**	**Wash**	***E.Coli***	**fMLP**	**PMA**
1998 - 2002 (6 - 10 YEARS)	WH 2155	9.23%	19.50%	59.69%	64.39%
	WH 2246	1.88%	31.05%	15.58%	37.68%
	WH 2138	3.45%	56.01%	4.25%	40.07%
	WHA 9908	1.86%	61.09%	3.85%	55.55%
	WH 9821	0.66%	23.29%	28.50%	47.53%
	WH 2258	44.73%	37.38%	31.72%	33.11%
	WHA 9809	1.61%	43.39%	3.24%	34.61%
	WH 2259	2.08%	36.54%	2.28%	12.90%
	WH 2256	5.99%	22.09%	7.18%	54.93%
	WH 2101	7.29%	10.44%	1.33%	8.98%
	WH 9814	1.32%	69.66%	2.19%	40.63%
	WHA 9909	1.29%	51.96%	2.00%	40.74%
	WHA 9810	6.00%	54.50%	3.50%	61.20%
	WH 2128	6.06%	12.46%	6.12%	13.44%
	WHA 9823	2.70%	93.70%	9.60%	95.10%
	WHA 9829	10.10%	25.10%	11.60%	29.50%
	WHA 9825	13.30%	44.90%	21.50%	36.50%
	WH 2253	3.30%	14.00%	11.00%	40.80%
	WH 2268	6.60%	55.50%	5.60%	62.10%
	Median	3.45%	37.38%	6.12%	40.63%
	Maximum	44.73%	93.70%	59.69%	95.10%
	Minimum	0.66%	10.44%	1.33%	8.98%

TABLE 30b

GRANULOCYTE BURST-TEST ACCORDING TO PERIOD POST TRANSPLANT (>10YRS)

BURST					
Type	**Subjects**	**Wash**	***E.Coli***	**fMLP**	**PMA**
Years Before 1997 (>10 Years)	WHA 9715	3.13%	60.70%	4.41%	51.56%
	WH 9720	3.71%	58.44%	4.52%	42.55%
	WH 9704	2.13%	60.31%	2.35%	53.18%
	WHA 9716	2.62%	9.15%	3.78%	9.58%
	WH 9717	0.70%	1.15%	0.76%	0.68%
	WHA 9718	5.60%	57.60%	10.40%	57.90%
	Median	2.88%	58.02%	4.10%	47.06%
	Maximum	5.60%	60.70%	10.40%	57.90%
	Minimum	0.70%	1.15%	0.76%	0.68%

The data was analysed according to myeloid and lymphoid lineages at the time of the diagnosis: both these groups had abnormal responses *in vitro* when compared to expected healthy controls as per kit insert. However the lymphoid group displayed better responses in comparison with the myeloid neoplasms where it was severely decreased: 57.14% versus 36.54% for lymphoid and myeloid respectively. This is possibly related to the fact that the myeloid lineage was affected originally and although the peripheral elements normalize with time, their functional potential never seems to fully recover. **(Tables 31, 31a)**

TABLE 31

GRANULOCYTE BURST-TEST ACCORDING TO DIAGNOSTIC LINEAGE (MYELOID)

		BURST			
Type	**Subjects**	**Wash**	***E.Coli***	**fMLP**	**PMA**
MYELOID	WH 2125	9.23%	19.50%	59.69%	64.39%
	WHA 9715	3.13%	60.70%	4.41%	51.56%
	WH 2246	1.88%	31.05%	15.58%	37.68%
	WH 2363	1.71%	31.01%	2.47%	42.63%
	WH 2138	3.45%	56.01%	4.25%	40.07%
	WH 2321	1.89%	34.44%	2.25%	25.89%
	WH 2342	0.69%	39.44%	0.79%	31.50%
	WH 2280	2.29%	73.21%	3.28%	68.60%
	U 2056	0.36%	58.35%	1.47%	49.90%
	WH 2350	4.93%	55.15%	4.98%	50.90%
	WH 2259	2.08%	36.54%	2.28%	12.90%
	WH 2256	5.99%	22.09%	7.18%	54.93%
	WHA 2716	2.62%	9.15%	3.78%	9.58%
	WH 2101	7.29%	10.44%	1.33%	8.98%
	WH 2330	10.20%	60.00%	11.40%	41.40%
	WH 9814	1.32%	69.66%	2.19%	40.63%
	WHA 9909	1.29%	51.96%	2.00%	40.74%
	WHA 9810	6.00%	54.50%	3.50%	61.20%
	WH 2128	6.06%	12.46%	6.12%	13.44%
	WH 2293	10.16%	18.12%	9.07%	13.68%
	WHA 9822	10.10%	25.10%	11.60%	29.50%
	WH 2305	9.30%	39.20%	6.90%	37.30%
	U 9704	5.60%	49.60%	5.00%	53.90%
	WH 2347	13.50%	42.80%	25.10%	48.20%
	WH 2288	4.60%	99.50%	12.80%	99.80%
	WH 2253	3.30%	14.00%	11.00%	40.80%
	WH 2285	3.80%	72.50%	11.30%	73.50%
	U 2062	6.38%	14.52%	10.59%	51.97%
	WH 2359	12.45%	12.27%	8.69%	13.37%
	WH 2361	3.00%	2.99%	5.86%	6.99%
	WH 2284	3.90%	3.87%	2.83%	3.16%
	WH 9717	0.70%	1.15%	0.76%	0.68%
	WH 2268	6.60%	55.50%	5.60%	62.10%
	Median	3.90%	36.54%	5.00%	40.74%
	Maximum	13.50%	99.50%	59.69%	99.80%
	Minimum	0.36%	1.15%	0.76%	0.68%

TABLE 31a

GRANULOCYTE BURST-TEST ACCORDING TO DIAGNOSTIC LINEAGE (LYMPHOID)

BURST					
Type	**Subjects**	**Wash**	***E.Coli***	**fMLP**	**PMA**
LYMPHOID	WH 2343	1.90%	68.54%	1.85%	66.18%
	WHA 9908	1.86%	61.09%	3.85%	55.55%
	WH 2309	2.27%	32.63%	2.67%	27.37%
	WH 9720	3.71%	58.44%	4.52%	42.55%
	WH 2277	1.26%	61.15%	2.79%	31.16%
	WH 9821	0.66%	23.29%	28.50%	47.53%
	WH 2258	44.73%	37.38%	31.72%	33.11%
	U 2044	2.97%	35.64%	5.25%	32.16%
	WH 9704	2.13%	60.31%	2.35%	53.18%
	WHA 9809	1.61%	43.39%	3.24%	34.61%
	U 2065	2.85%	56.68%	2.71%	54.29%
	WHA 2061	3.48%	22.72%	0.49%	44.02%
	U 2046	7.00%	99.80%	9.50%	99.90%
	WHA 9823	2.70%	93.70%	9.60%	95.10%
	U 2037	6.10%	18.10%	6.60%	26.70%
	WHA 9828	13.30%	44.90%	21.50%	36.50%
	U 2032	10.40%	63.90%	21.10%	34.80%
	U 2054	46.33%	40.47%	39.72%	49.55%
	U 2047	47.30%	62.43%	36.55%	45.33%
	U 2067	46.19%	61.36%	39.98%	44.06%
	WHA 9718	5.60%	57.60%	10.40%	57.90%
	WH 2285	6.00%	52.60%	9.00%	63.50%
	Median	3.60%	57.14%	7.80%	44.70%
	Maximum	47.30%	99.80%	39.98%	99.90%
	Minimum	0.66%	18.10%	0.49%	26.70%

When the conditioning regimes for transplantation (chemotherapy vs combination chemotherapy and radiotherapy) were analysed, the combination treatment group was more "intact" (median response of 53.55%) when their innate responses *in vitro* were compared to preparation with chemotherapy alone (median response of 39.44%). However, these were also reduced according to the normal values supplied by the manufacturer of the kit. The patients receiving chemotherapy alone consisted of different regimens according to disease category e.g. BEAM,[127] BU_2[128] or BU and melphalan.[129] The chemo-radiotherapy programme was similar for all the patients: 12 gray fractionated whole body exposure for three days, 60mg/kg

cyclophosphamide for two days and 6 gray fractionated total nodal irradiation for two days.[130] **(Tables 32, 32a)** It implies therefore that the cyclophosphamide plus radiotherapy conditioning had less impact long term on the ability of the granulocytes to respond to a physiological stimulus when compared to the heavier chemotherapeutic agents in combination.

TABLE 32

GRANULOCYTE BURST-TEST ACCORDING TO CONDITIONING REGIMEN (CHEMOTHERAPY)

BURST					
Type	**Subjects**	**Wash**	***E.Coli***	**fMLP**	**PMA**
CHEMOTHERAPY	WH 2125	9.23%	19.50%	59.69%	64.39%
	WH 2363	1.71%	31.01%	2.47%	42.63%
	WH 2343	1.90%	68.54%	1.85%	66.18%
	WHA 9908	1.86%	61.09%	3.85%	55.55%
	WH 9720	3.71%	58.44%	4.52%	42.55%
	WH 2342	0.69%	39.44%	0.79%	31.50%
	WH 9821	0.66%	23.29%	28.50%	47.53%
	WH 2258	44.73%	37.38%	31.72%	33.11%
	U 2056	0.36%	58.35%	1.47%	49.90%
	U 2044	2.97%	35.64%	5.25%	32.16%
	WH 2350	4.93%	55.15%	4.98%	50.90%
	WHA 9809	1.61%	43.39%	3.24%	34.61%
	U 2065	2.85%	56.68%	2.71%	54.29%
	WH 2259	2.08%	36.54%	2.28%	12.90%
	WHA 2061	3.48%	22.72%	0.49%	44.02%
	WHA 9716	2.62%	9.15%	3.78%	9.58%
	WHA 9909	1.29%	51.96%	2.00%	40.74%
	U 2046	7.00%	99.80%	9.50%	99.90%
	WHA 9823	2.70%	93.70%	9.60%	95.10%
	U 2037	6.10%	18.10%	6.60%	26.70%
	WHA 9822	10.10%	25.10%	11.60%	29.50%
	WHA 9828	13.30%	44.90%	21.50%	36.50%
	WH 2347	13.50%	42.80%	25.10%	48.20%
	U 2032	10.40%	36.90%	21.10%	34.80%
	U 2062	6.38%	14.52%	10.59%	51.97%
	WH 2359	12.45%	12.27%	8.69%	13.37%
	U 2054	46.33%	40.47%	39.72%	49.55%
	WH 2361	3.00%	2.99%	5.86%	6.99%
	U 2067	46.19%	61.36%	39.98%	44.06%
	Median	3.48%	39.44%	5.86%	42.63%
	Maximum	46.33%	99.80%	59.69%	99.90%
	Minimum	0.36%	2.99%	2.49%	6.99%

TABLE 32a

GRANULOCYTE BURST-TEST ACCORDING TO CONDITIONING REGIME (CHEMOTHERAPY & RADIOTHERAPY)

BURST					
Type	**Subjects**	**Wash**	***E.Coli***	**fMLP**	**PMA**
CHEMOTHERAPY AND RADIOTHERAPY	WHA 9715	3.13%	60.70%	4.41%	51.56%
	WH 2246	1.88%	31.05%	15.58%	37.68%
	WH 2138	3.45%	56.01%	4.25%	40.07%
	WH 2309	2.27%	32.63%	2.67%	27.37%
	WH 2321	1.89%	34.44%	2.25%	25.89%
	WH 2280	2.29%	73.21%	3.28%	68.60%
	WH 2277	1.26%	61.15%	2.79%	31.16%
	WH 9704	2.13%	60.31%	2.35%	53.18%
	WH 2256	5.99%	22.09%	7.18%	54.93%
	WH 2101	7.29%	20.44%	1.33%	8.98%
	WH 2330	10.20%	60.00%	11.40%	41.40%
	WH 9814	1.32%	69.66%	2.19%	40.63%
	WHA 9810	6.00%	54.50%	3.50%	61.20%
	WH 2128	6.06%	12.46%	6.12%	13.44%
	WH 2293	10.16%	28.12%	9.07%	13.68%
	WH 2305	9.30%	39.20%	6.90%	37.30%
	U 9704	5.60%	49.60%	5.00%	53.90%
	WH 2288	4.60%	99.50%	12.80%	99.80%
	WH 2253	3.30%	14.00%	11.00%	40.80%
	WH 2285	3.80%	72.50%	11.30%	73.50%
	U 2047	47.30%	62.43%	36.55%	45.33%
	WH 2284	3.90%	3.87%	2.83%	3.16%
	WH 9717	0.70%	1.15%	0.76%	0.68%
	WH 2268	6.60%	55.50%	5.60%	62.10%
	WHA 9718	5.60%	57.60%	10.40%	57.90%
	WH 2285	6.00%	52.60%	9.00%	63.50%
	Median	4.25%	53.55%	5.305	41.10%
	Maximum	47.30%	99.50%	36.55%	99.80%
	Minimum	0.70%	1.15%	0.76%	0.68%

Adaptive immune response: CD4+ and CD8+ cell subsets

The data for the adaptative immune response reflected very similar results across all three types of transplants. It appears that the subjects exhibit normal TH 1 and TH 2 cytokine responses of the T cell subset CD4+ helper cells: the stimulated responses when compared to the non-stimulated responses all were greater that 2 as expected.

However it seems that those who received autografts had slightly better cytokine profiles when one compares the ratio of the TH1 versus TH2 cytokine profiles within the CD4+ T cell subset. This corresponds to the earlier observation that the same individuals had better innate responses. **(Tables 33, 33a, 33b)**

TABLE 33

IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD4+ T CELLS IN DIFFERENT TYPES OF TRANSPLANTS (ALLOGRAFT)

CD4+CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	IFN-γ	IL-4	IFN-γ	IL-4	IFN-γ	IL-4
ALLOTRANSPLANTATION (ALLOGRAFT)	WH 2125	1.3%	3.2%	4.1%	4.1%	3.15	1.28
	WH 2246	2.1%	2.5%	8.1%	7.1%	3.86	2.84
	WH 2138	2.6%	2.4%	7.6%	4.7%	2.92	1.96
	WH 2321	0.7%	1.5%	3.9%	2.7%	5.57	1.80
	WH 2342	0.2%	0.5%	2.1%	1.4%	10.50	2.80
	WH 9821	1.6%	2.0%	13.9%	7.1%	8.69	3.55
	WH 2258	0.6%	0.5%	13.7%	7.9%	22.83	15.80
	WH 2350	2.3%	1.2%	10.3%	3.2%	4.48	2.67
	WH 2259	0.5%	0.4%	7.8%	5.3%	15.60	13.25
	WH 2330	0.4%	0.2%	1.9%	0.4%	4.75	2.00
	WH 9814	0.9%	1.1%	13.8%	2.7%	15.33	2.45
	WH 2359	0.5%	1.0%	2.4%	1.5%	4.80	1.50
	WH 2361	0.1%	0.1%	0.7%	0.3%	7.00	3.00
	WH 2284	0.4%	0.4%	10.1%	4.6%	24.59	11.55
	WH 9717	0.3%	0.4%	6.4%	2.7%	19.91	6.38
	Median	0.6%	1.0%	7.6%	3.2%	7.0%	2.8%
	Maximum	2.6%	3.2%	13.9%	7.9%	24.6%	15.8%
	Minimum	0.1%	0.1%	0.7%	0.3%	2.9%	1.3%

TABLE 33a

IN VITRO PRODUCTION IFN-γ AND IL4 WITHIN THE CD4+ T CELLS IN DIFFERENT TYPES OF TRANSPLANTS (AUTOGRAFT)

CD4+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	IFN-γ	IL-4	IFN-γ	IL-4	IFN-γ	IL-4
AUTOTRANSPLANTATION (AUTOGRAFT)	WHA 9715	1.3%	1.8%	9.6%	5.4%	7.38	3.00
	WHA 9908	0.9%	0.6%	9.8%	7.1%	10.89	11.83
	WH 9720	1.2%	0.4%	2.9%	1.4%	2.42	3.50
	U 2056	0.6%	0.6%	15.1%	4.9%	25.17	8.17
	U 2044	1.6%	2.5%	5.0%	4.7%	3.13	1.88
	WH 9704	2.9%	1.5%	6.4%	2.5%	2.21	1.67
	WHA 9809	0.4%	0.5%	3.2%	2.2%	8.00	4.40
	U 2065	2.0%	4.9%	7.6%	18.5%	3.80	3.78
	WHA 2061	1.7%	1.1%	15.9%	11.6%	9.35	10.55
	WHA 9716	0.6%	0.8%	8.2%	5.3%	13.67	6.63
	WHA 9909	2.2%	2.3%	12.8%	7.6%	5.82	3.30
	WHA 9810	0.2%	0.2%	3.0%	0.5%	15.00	2.50
	U 2046	0.1%	1.1%	4.4%	5.3%	44.00	4.82
	WHA 9823	0.3%	0.2%	11.0%	2.3%	36.67	11.50
	U 2037	0.5%	0.5%	4.2%	1.6%	8.40	3.20
	WHA 9829	0.2%	0.6%	4.1%	2.2%	20.50	3.67
	WHA 9825	0.7%	0.9%	8.0%	3.6%	11.43	4.00
	U 2032	0.7%	1.0%	1.5%	0.8%	2.14	0.80
	WHA 2062	0.6%	1.6%	2.6%	2.2%	4.33	1.38
	U 3054	0.9%	0.7%	3.9%	2.5%	4.33	3.57
	U 2047	0.7%	0.5%	5.0%	1.9%	7.14	3.80
	WHA 2067	0.8%	0.6%	3.2%	2.1%	4.00	3.56
	WHA 9718	0.5%	0.9%	3.2%	2.9%	6.4	3.22
	Median	0.7%	0.8%	5.0%	2.5%	7.4%	3.6%
	Maximum	2.9%	4.9%	15.9%	18.5%	44.0%	11.8%
	Minimum	0.1%	0.2%	1.5%	0.5%	2.1%	0.8%

TABLE 33b

IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD4+ T CELLS IN DIFFERENT TYPES OF TRANSPLANTS (MUD)

CD4+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	IFN-γ	IL-4	IFN-γ	IL-4	IFN-γ	IL-4
MATCHED UNRELATED DONOR TRANSPLANTATION	WH 2263	1.1%	1.6%	5.3%	5.1%	4.82	3.19
	WH 2343	2.6%	2.5%	14.0%	10.0%	5.38	4.00
	WH 2309	1.2%	2.5%	2.7%	2.2%	2.25	0.88
	WH 2280	0.5%	0.8%	7.3%	5.2%	14.60	6.50
	WH 2277	0.6%	0.7%	4.2%	4.2%	7.00	6.00
	WH 2256	2.4%	3.3%	6.1%	7.5%	2.54	2.27
	WH 2101	1.5%	1.3%	2.8%	1.6%	1.87	1.23
	WH 2128	0.6%	0.8%	4.8%	2.0%	8.00	2.50
	WH 2293	0.5%	1.0%	3.1%	1.9%	6.20	1.90
	WH 2305	0.1%	0.7%	4.9%	2.4%	49.00	3.43
	U 9704	1.6%	2.3%	10.9%	6.7%	6.81	2.91
	WH 2347	0.5%	1.0%	11.4%	5.5%	22.80	5.50
	WH 2288	0.6%	1.6%	0.8%	2.2%	1.33	1.38
	WH 2253	0.5%	0.5%	2.3%	2.1%	4.60	4.20
	WH 2311	0.2%	0.3%	6.8%	2.5%	34.00	8.33
	WH 2268	0.5%	1.3%	2.3%	1.1%	4.60	0.85
	WH 2285	0.5%	0.4%	4.5%	1.2%	9	3
	Median	0.5%	0.45	4.5%	1.2%	9.0%	3.0%
	Maximum	2.6%	3.3%	14.0%	10.0%	49.0%	8.3%
	Minimum	0.1%	0.3%	0.8%	1.1%	1.3%	0.8%

The type of transplant did not impact negatively on the ability of the T cell CD8+ subset to produce the immune regulatory cytokines. All three subgroups display similar cytokine secretion outputs post activation: however in the case of those who had received allografts, their CD8+ T cell subset had markedly better IFN-γ and IL4 cytokine production implying that their CD8+ cells were more active when stimulated *in vitro* as compared to the other two groups of patients studied. **(Tables 34, 34a, 34b)**

TABLE 34
IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD8+ T CELLS IN DIFFERENT TYPES OF TRANSPLANTS (ALLOGRAFT)

CD8+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	IFN-γ	IL-4	IFN-γ	IL-4	IFN-γ	IL-4
ALLOTRANSPLANTATION (ALLOGRAFT)	WH 2125	1.6%	3.2%	23.5%	16.6%	14.69	5.19
	WH 2246	0.4%	0.6%	9.1%	4.8%	22.75	8.00
	WH 2138	0.2%	0.2%	2.0%	0.9%	10.00	4.50
	WH 2321	0.4%	0.7%	9.6%	3.3%	24.00	4.71
	WH 2342	0.1%	0.4%	12.3%	2.0%	123.00	5.00
	WH 9821	0.8%	1.0%	14.8%	6.1%	18.50	6.10
	WH 2258	0.7%	0.6%	18.0%	8.1%	25.71	13.50
	WH 2350	1.3%	1.0%	12.4%	3.3%	9.54	3.30
	WH 2259	1.1%	1.0%	16.3%	8.0%	14.82	8.00
	WH 2330	0.3%	0.2%	8.4%	1.6%	28.00	8.00
	WH 9814	0.9%	0.8%	22.4%	2.7%	24.89	3.38
	WH 2359	0.6%	0.5%	16.9%	8.6%	28.17	17.20
	WH 2361	1.5%	0.2%	2.5%	1.3%	1.67	6.50
	WH 2284	0.1%	0.3%	22.0%	8.3%	219.50	27.77
	WH 9717	0.3%	0.5%	15.8%	5.4%	52.80	9.96
	Median	0.6%	0.6%	14.8%	4.8%	24.0%	6.5%
	Maximum	1.6%	3.2%	23.5%	16.6%	219.5%	27.8%
	Minimum	0.1%	0.2%	2.0%	0.9%	1.7%	3.3%

TABLE 34a

IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD8+ T CELLS IN DIFFERENT TYPES OF TRANSPLANTS (AUTOGRAFT)

CD8+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	IFN-γ	IL-4	IFN-γ	IL-4	IFN-γ	IL-4
AUTOTRANSPLANTATION (AUTOGRAFT)	WHA 9715	0.9%	0.9%	18.9%	10.1%	21.00	11.22
	WHA 9908	2.1%	0.5%	45.4%	24.9%	21.62	49.80
	WH 9720	0.8%	0.4%	4.8%	0.8%	6.00	2.00
	U 2056	0.6%	0.6%	16.4%	4.5%	27.33	7.50
	U 2044	1.9%	2.7%	9.0%	4.9%	4.74	1.81
	WH 9704	1.6%	0.9%	11.3%	2.2%	7.06	2.44
	WHA 9809	0.5%	0.8%	9.5%	5.1%	19.00	6.38
	U 2065	6.9%	10.5%	12.0%	20.4%	1.74	1.94
	WHA 2061	2.9%	1.8%	19.8%	16.8%	6.83	9.33
	WHA 9716	0.6%	0.5%	11.4%	4.8%	19.00	9.60
	WHA 9909	1.5%	1.9%	20.4%	8.7%	13.60	4.58
	WHA 9810	0.1%	0.2%	6.1%	0.7%	61.00	3.50
	U 2046	0.1%	0.9%	9.3%	9.1%	93.00	10.11
	WHA 9823	0.2%	0.1%	9.9%	1.6%	49.50	16.00
	U 2037	0.5%	0.6%	13.8%	3.2%	27.60	5.33
	WHA 9829	0.2%	0.3%	11.0%	4.4%	55.00	14.67
	WHA 9825	0.3%	0.4%	15.0%	4.7%	50.00	11.75
	U 2023	1.0%	2.1%	4.8%	1.7%	4.80	0.81
	WHA 2062	0.2%	0.8%	6.1%	3.2%	30.50	4.00
	U 2054	0.5%	0.4%	9.0%	2.6%	18.00	6.50
	U 2047	0.4%	0.3%	13.9%	4.1%	34.75	13.67
	WHA 2067	0.5%	0.5%	3.4%	1.8%	6.80	3.60
	WHA 9718	0.3%	0.7%	4.6%	3.6%	15.33	5.14
	Median	0.3%	0.7%	4.6%	3.6%	15.30%	5.10%
	Maximum	6.9%	10.5%	45.4%	24.9%	93.0%	49.8%
	Minimum	0.3%	0.7%	4.6%	3.6%	15.3%	5.1%

TABLE 34b

IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD8+ T CELLS IN DIFFERENT TYPES OF TRANSPLANTS (MUD)

CD8+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	IFN-γ	IL-4	IFN-γ	IL-4	IFN-γ	IL-4
MATCHED UNRELATED DONOR TRANSPLANTATION	WH 2263	0.8%	0.7%	26.0%	12.4%	32.50	17.71
	WH 2343	2.0%	1.0%	26.7%	9.8%	13.35	9.80
	WH 2309	0.5%	0.8%	3.0%	1.9%	6.00	2.38
	WH 2280	0.4%	0.5%	14.8%	7.4%	37.00	14.80
	WH 2277	0.3%	0.4%	8.3%	4.7%	27.67	11.75
	WH 2256	1.0%	1.5%	7.1%	4.4%	7.10	2.93
	WH 2101	1.7%	1.6%	12.9%	5.6%	7.59	3.50
	WH 2128	0.3%	0.4%	11.8%	3.6%	39.33	9.00
	WH 2293	0.2%	0.3%	3.1%	3.3%	15.50	11.00
	WH 2305	0.3%	1.5%	14.6%	4.1%	48.67	2.73
	U 9704	0.9%	1.3%	16.5%	6.5%	18.33	5.00
	WH 2347	0.3%	0.5%	13.3%	4.4%	44.33	8.80
	WH 2288	0.3%	0.9%	1.5%	2.4%	5.00	2.67
	WH 2253	0.1%	0.2%	3.0%	2.5%	30.00	12.50
	WH 2311	0.2%	0.3%	11.2%	2.0%	56.00	6.67
	WH 2268	0.4%	1.0%	7.9%	2.3%	19.75	2.30
	WH 2285	0.4%	0.3%	5.8%	1.3%	14.50	4.33
	Median	0.4%	0.7%	11.2%	4.1%	19.8%	6.7%
	Maximum	2.0%	1.6%	26.7%	12.4%	56.0%	17.7%
	Minimum	0.1%	0.2%	1.5%	1.3%	5.0%	2.3%

The subjects exhibited normal TH 1 and TH 2 cytokine reactions of the T cell subset CD4+ helper cells, irrespective to the date of transplant. **(Tables 35, 35a, 35b)** There are no published data to indicate "normal cytokine profiles". It has been shown that healthy individuals generally have a 2:1 ratio when one compares the response of IFN-γ to IL4. There is some literature which confirms that a ratio of 2:1 or greater is observed in healthy individuals. This ratio may change during bouts of viral disease (influenza for example) when one would expect a greater TH 1 output (IFN-γ production).

TABLE 35
IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD4+ T CELLS ACCORDING TO DATE POST TRANSPLANT (0-5 YEARS)

CD4+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	INFy	IL-4	INFy	IL-4	INFy	IL-4
YEARS ABOVE 2003 (0-5 YEARS)	WH 2263	1.1%	1.6%	5.3%	5.1%	4.82	3.19
	WH 2343	2.6%	2.5%	14.0%	10.0%	5.38	4.00
	WH 2309	1.2%	2.5%	2.7%	2.2%	2.25	0.88
	WH 2321	0.7%	1.5%	3.9%	2.7%	5.57	1.80
	WH 2342	0.2%	0.5%	2.1%	1.4%	10.50	2.80
	WH 2280	0.5%	0.8%	7.3%	5.2%	14.60	6.50
	WH 2277	0.6%	0.7%	4.2%	4.2%	7.00	6.00
	U 2056	0.6%	0.6%	15.1%	4.9%	25.17	8.17
	U 2044	1.6%	2.5%	5.0%	4.7%	3.13	1.88
	WH 2350	2.3%	1.2%	10.3%	3.2%	4.48	2.67
	U 2065	2.0%	4.9%	7.6%	18.5%	3.80	3.78
	WHA 2061	1.7%	1.1%	15.9%	11.6%	9.35	10.55
	WH 2330	0.4%	0.2%	1.9%	0.4%	4.75	2.00
	U 2046	0.1%	1.1%	4.4%	5.3%	44.00	4.82
	WH 2293	0.5%	1.0%	3.1%	1.9%	6.20	1.90
	U 2037	0.5%	0.5%	4.2%	1.6%	8.40	3.20
	WH 2035	0.1%	0.7%	4.9%	2.4%	49.00	3.43
	U 9074	1.6%	2.3%	10.9%	6.7%	6.81	2.91
	WH 2347	0.5%	1.0%	11.4%	5.5%	22.80	5.50
	U 2032	0.7%	1.0%	1.5%	0.8%	2.14	0.80
	WH 2288	0.6%	1.6%	0.8%	2.2%	1.33	1.38
	WH 2311	0.2%	0.3%	6.8%	2.5%	34.00	8.33
	WHA 2062	0.6%	1.6%	2.6%	2.2%	4.33	1.38
	WH 2359	0.5%	1.0%	2.4%	1.5%	4.80	1.50
	U 2054	0.9%	0.7%	3.9%	2.5%	4.33	3.57
	U 2047	0.7%	0.5%	5.0%	1.9%	7.14	3.80
	WH 2361	0.1%	0.1%	0.7%	0.3%	7.00	3.00
	WH 2284	0.4%	0.4%	10.1%	4.6%	24.59	11.55
	WHA 2067	0.8%	0.6%	3.2%	2.1%	4.00	3.56
	WH 2285	0.5%	0.4%	4.5%	1.2%	9	3
	Median	0.6%	1.0%	4.5%	2.5%	6.5%	3.2%
	Maximum	2.6%	4.9%	15.9%	18.5%	49.0%	11.55%
	Minimum	0.1%	0.1%	0.7%	0.3%	1.3%	0.8%

TABLE 35a

IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD4+ T CELLS ACCORDING TO DATE POST TRANSPLANT (6-10 YEARS)

CD4+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	INFy	IL-4	INFy	IL-4	INFy	IL-4
1998 - 2002 (6 - 10 YEARS)	WH 2155	1.3%	3.2%	4.1%	4.1%	3.15	1.28
	WH 2246	2.1%	2.5%	8.1%	7.1%	3.86	2.84
	WH 2138	2.6%	2.4%	7.6%	4.7%	2.92	1.96
	WHA 9908	0.9%	0.6%	9.8%	7.1%	10.89	11.83
	WH 9821	1.6%	2.0%	13.9%	7.1%	8.69	3.55
	WH 2258	0.6%	0.5%	13.7%	7.9%	22.83	15.80
	WHA 9809	0.4%	0.5%	3.2%	2.2%	8.00	4.40
	WH 2259	0.5%	0.4%	7.8%	5.3%	15.60	13.25
	WH 2256	2.4%	3.3%	6.1%	7.5%	2.54	2.27
	WH 2101	1.5%	1.3%	2.8%	1.6%	1.87	1.23
	WH 9814	0.9%	1.1%	13.8%	2.7%	15.33	2.45
	WH 9909	2.2%	2.3%	12.8%	7.6%	5.82	3.30
	WHA 9810	0.2%	0.2%	3.0%	0.5%	15.00	2.50
	WH 2128	0.6%	0.8%	4.8%	2.0%	8.00	2.50
	WHA 9823	0.3%	0.2%	11.0%	2.3%	36.67	11.50
	WHA 9829	0.2%	0.6%	4.1%	2.2%	20.50	3.67
	WHA 9825	0.7%	0.9%	8.0%	3.6%	11.43	4.00
	WH 2253	0.5%	0.5%	2.3%	2.1%	4.60	4.20
	WH 2268	0.5%	1.3%	2.3%	1.1%	4.60	0.85
	Median	0.7%	0.9%	7.6%	3.6%	8.0%	3.3%
	Maximum	2.6%	3.3%	13.9%	7.9%	36.7%	15.8%
	Minimum	0.2%	0.2%	2.3%	0.5%	1.9%	0.8%

TABLE 35b

IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD4+ T CELLS ACCORDING TO DATE POST TRANSPLANT (> 10YEARS)

CD4 + CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	INFy	IL-4	INFy	IL-4	INFy	IL-4
YEARS BEFORE 1997 (>10 YEARS)	WHA 9715	1.3%	1.8%	9.6%	5.4%	7.38	3.00
	WH 9720	1.2%	0.4%	2.9%	1.4%	2.42	3.50
	WH 9704	2.9%	1.5%	6.4%	2.5%	2.21	1.67
	WHA 9716	0.6%	0.8%	8.2%	5.3%	13.67	6.63
	WH 9717	0.3%	0.4%	6.4%	2.7%	19.91	6.38
	WHA 9718	0.5%	0.9%	3.2%	2.9%	6.4%	3.22
	Median	0.9%	0.9%	6.4%	2.8%	4.9%	3.4%
	Maximum	2.9%	1.8%	9.6%	5.4%	19.9%	6.6%
	Minimum	0.3%	0.4%	2.9%	1.4%	6.4%	1.7%

The age of the transplant did not impact on the ability of the T cell CD8+ subset to produce the immune regulatory cytokines. Although all three subgroups displayed similar results post-activation, it appeared that the more recently transplanted individuals had the best CD8 responses *in vitro*, implying that these subjects would be the most protected vis-à-vis viruses or intra-cellular pathogens. Unfortunately we could not correlate the incidence of infections and type of infections within these groups and to compare this to the *in vitro* CD8 response but it would be an interesting exercise to undertake. **(Tables 36, 36a, 36b)**

TABLE 36
IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD8+ T CELLS ACCORDING TO DATE POST TRANSPLANT

CD8+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	INFy	IL-4	INFy	IL-4	INFy	IL-4
YEARS ABOVE 2003 (0-5 YEARS)	WH 2263	0.8%	0.7%	26.0%	12.4%	32.50	17.71
	WH 2343	2.0%	1.0%	26.7%	9.8%	13.35	9.80
	WH 2309	0.5%	0.8%	3.0%	1.9%	6.00	2.38
	WH 2321	0.4%	0.7%	9.6%	3.3%	24.00	4.71
	WH 2342	0.1%	0.4%	12.3%	2.0%	123.00	5.00
	WH 2280	0.4%	0.5%	14.8%	7.4%	37.00	14.80
	WH 2277	0.3%	0.4%	8.3%	4.7%	27.67	11.75
	U 2056	0.6%	0.6%	16.4%	4.5%	27.33	7.50
	U 2044	1.9%	2.7%	9.0%	4.9%	4.74	1.81
	WH 2350	1.3%	1.0%	12.4%	3.3%	9.54	3.30
	U 2065	6.9%	10.5%	12.0%	20.4%	1.74	1.94
	WHA 2061	2.9%	1.8%	19.8%	16.8%	6.83	9.33
	WH 2330	0.3%	0.2%	8.4%	1.6%	28.00	8.00
	U 2046	0.1%	0.9%	9.3%	9.1%	93.00	10.11
	WH 2293	0.2%	0.3%	3.1%	3.3%	15.50	11.00
	U 2037	0.5%	0.6%	13.8%	3.2%	27.60	5.33
	WH 2035	0.3%	1.5%	14.6%	4.1%	48.67	2.73
	U 9704	0.9%	1.3%	16.5%	6.5%	18.33	5.00
	WH 2347	0.3%	0.5%	13.3%	4.4%	44.33	8.80
	U 2032	1.0%	2.1%	4.8%	1.7%	4.80	0.81
	WH 2288	0.3%	0.9%	1.5%	2.4%	5.00	2.67
	WH 2311	0.2%	0.3%	11.2%	2.0%	56.00	6.67
	WHA 2062	0.2%	0.8%	6.1%	3.2%	30.50	4.00
	WH 2359	0.6%	0.5%	16.9%	8.6%	28.17	17.20
	U 2054	0.5%	0.4%	9.0%	2.6%	18.00	6.50
	U 2047	0.4%	0.3%	13.9%	4.1%	34.75	13.67
	WH 2361	1.5%	0.2%	2.5%	1.3%	1.67	6.50
	WH 2284	0.1%	0.3%	22.0%	8.3%	219.50	27.77
	WHA 2067	0.5%	0.5%	3.4%	1.8%	6.80	3.60
	WH 2285	0.4%	0.3%	5.8%	1.3%	14.50	4.33
	Median	0.5%	0.6%	11.6%	3.7%	25.7%	6.5%
	Maximum	6.9%	10.5%	26.7%	20.4%	219.5%	27.767%
	Minimum	0.1%	0.2%	1.5%	1.3%	166.7%	81.0%

TABLE 36a

IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD8+ T CELLS ACCORDING TO DATE POST TRANSPLANT (6-10 YEARS)

CD8+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	INFy	IL-4	INFy	IL-4	INFy	IL-4
1998 - 2002 (6 - 10 YEARS)	WH 2155	1.6%	3.2%	23.5%	16.6%	14.69	5.19
	WH 2246	0.4%	0.6%	9.1%	4.8%	22.75	8.00
	WH 2138	0.2%	0.2%	2.0%	0.9%	10.00	4.50
	WHA 9908	2.1%	0.5%	45.4%	24.9%	21.62	49.80
	WH 9821	0.8%	1.0%	14.8%	6.1%	18.50	6.10
	WH 2258	0.7%	0.6%	18.0%	8.1%	25.71	13.50
	WHA 9809	0.5%	0.8%	9.5%	5.1%	19.00	6.38
	WH 2259	1.1%	1.0%	16.3%	8.0%	14.82	8.00
	WH 2256	1.0%	1.5%	7.1%	4.4%	7.10	2.93
	WH 2101	1.7%	1.6%	12.9%	5.6%	7.59	3.50
	WH 9814	0.9%	0.8%	22.4%	2.7%	24.89	3.38
	WHA 9909	1.5%	1.9%	20.4%	8.7%	13.60	4.58
	WHA 9810	0.1%	0.2%	6.1%	0.7%	61.00	3.50
	WH 2128	0.3%	0.4%	11.8%	3.6%	39.33	9.00
	WHA 9823	0.2%	0.1%	9.9%	1.6%	49.50	16.00
	WHA 9829	0.2%	0.3%	11.0%	4.4%	55.00	14.67
	WHA 9825	0.3%	0.4%	15.0%	4.7%	50.00	11.75
	WH 2253	0.1%	0.2%	3.0%	2.5%	30.00	12.50
	WH 2268	0.4%	1.0%	7.9%	2.3%	19.75	2.30
	Median	0.5%	0.6%	11.8%	4.7%	21.6%	6.4%
	Maximum	2.1%	3.2%	45.4%	24.9%	61.0%	49.8%
	Minimum	0.1%	0.1%	2.0%	0.7%	7.1%	2.3%

TABLE 36b

IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD8+ T CELLS ACCORDING TO DATE POST TRANSPLANT (>10 YEARS)

CD8+ cells							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	INFy	IL-4	INFy	IL-4	INFy	IL-4
YEARS BEFORE 1997 (>10 YEARS)	WHA 9715	0.9%	0.9%	18.9%	10.1%	21.00	11.22
	WH 9720	0.8%	0.4%	4.8%	0.8%	6.00	2.00
	WH 9704	1.6%	0.9%	11.3%	2.2%	7.06	2.44
	WHA 9716	0.6%	0.5%	11.4%	4.8%	19.00	9.60
	WH 9717	0.3%	0.5%	15.8%	5.4%	52.80	9.96
	WHA 9718	0.3%	0.7%	4.6%	3.6%	15.33	5.14
	Median	0.7%	0,6%	11.4%	4.2%	17.2%	7.4%
	Maximum	1.6%	0.9%	18.9%	10.1%	52.8%	11.2%
	Minimum	0.3%	0.4%	4.6%	0.8%	6.0%	2.0%

When the myeloid and lymphoid groupings were compared as far as their CD4+ or CD8+ responses *in vitro*, the subjects exhibited normal TH1 and TH2 cytokine release patterns of the T cell subset CD4+ helper cells. **(Tables 37, 37a)**

TABLE 37

IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD4+ T CELLS ACCORDING TO DIAGNOSIS LINEAGE (MYELOID)

CD4+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	INFy	IL-4	INFy	IL-4	INFy	IL-4
MYELOID	WH 2125	1.3%	3.2%	4.1%	4.1%	3.15	1.28
	WHA 9715	1.3%	1.8%	9.6%	5.4%	7.38	3.00
	WH 2246	2.1%	2.5%	8.1%	7.1%	3.86	2.84
	WH 2363	1.1%	1.6%	5.3%	5.1%	4.82	3.19
	WH 2138	2.6%	2.4%	7.6%	4.7%	2.92	1.96
	WH 2321	0.7%	1.5%	3.9%	2.7%	5.57	1.80
	WH 2342	0.2%	0.5%	2.1%	1.4%	10.50	2.80
	WH 2280	0.5%	0.8%	7.3%	5.2%	14.60	6.50
	U 2056	0.6%	0.6%	15.1%	4.9%	25.17	8.17
	WH 2350	2.3%	1.2%	10.3%	3.2%	4.48	2.67
	WH 2259	13.5%	9.4%	7.8%	5.3%	N/A	N/A
	WH 2256	2.4%	3.3%	6.1%	7.5%	2.54	2.27
	WHA 9716	0.6%	0.8%	8.2%	5.3%	13.67	6.63
	WH 2101	1.5%	1.3%	2.8%	1.6%	1.87	1.23
	WH 2330	0.4%	0.2%	1.9%	0.4%	4.75	2.00
	WH 9814	0.9%	1.1%	13.8%	2.7%	15.33	2.45
	WHA 9909	2.2%	2.3%	12.8%	7.6%	5.82	3.30
	WHA 9810	0.2%	0.2%	3.0%	0.5%	15.00	2.50
	WH 2128	0.6%	0.8%	4.8%	2.0%	8.00	2.50
	WH 2293	0.5%	1.0%	3.1%	1.9%	6.20	1.90
	WHA 9822	0.2%	0.6%	4.1%	2.2%	20.50	3.67
	WH 2305	0.1%	0.7%	4.9%	2.4%	49.00	3.43
	U 9704	1.6%	2.3%	10.9%	6.7%	6.81	2.91
	WH 2347	0.5%	1.0%	11.4%	5.5%	22.80	5.50
	WH 2288	0.6%	1.6%	0.8%	2.2%	1.33	1.38
	WH 2253	0.5%	0.5%	2.3%	2.1%	4.60	4.20
	WH 2285	0.2%	0.3%	6.8%	2.5%	34.00	8.33
	U 2062	0.6%	1.6%	2.6%	2.2%	4.33	1.38
	WH 2359	0.5%	1.0%	2.4%	1.5%	4.80	1.50
	WH 2361	0.1%	0.1%	0.7%	0.3%	7.00	3.00
	WH 2284	0.4%	0.4%	10.1%	4.6%	24.59	11.55
	WH 9717	0.3%	0.4%	6.4%	2.7%	19.91	6.38
	WH 2268	0.5%	1.3%	2.3%	1.1%	4.60	0.85
	Median	0.6%	1.0%	5.3%	2.7%	6.5%	2.8%
	Maximum	13.5%	9.4%	15.1%	7.6%	49.0%	11.6%
	Minimum	0.1%	0.1%	0.7%	0.3%	1.3%	0.8%

TABLE 37a

IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD4+ T CELLS ACCORDING TO DIAGNOSIS LINEAGE (LYMPHOID)

CD4+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	INFy	IL-4	INFy	IL-4	INFy	IL-4
LYMPHOID	WH 2343	2.6%	2.5%	14.0%	10.0%	5.38	4.00
	WHA 9908	0.9%	0.6%	9.8%	7.1%	10.89	11.83
	WH 2309	1.2%	2.5%	2.7%	2.2%	2.25	0.88
	WHA 9720	1.2%	0.4%	2.9%	1.4%	2.42	3.50
	WH 2277	0.6%	0.7%	4.2%	4.2%	7.00	6.00
	WH 9821	1.6%	2.0%	13.9%	7.1%	8.69	3.55
	WH 2258	0.6%	0.5%	13.7%	7.9%	22.83	15.80
	U 2044	1.6%	2.5%	5.0%	4.7%	3.13	1.88
	WH 9704	2.9%	1.5%	6.4%	2.5%	2.21	1.67
	WHA 9809	0.4%	0.5%	3.2%	2.2%	8.00	4.40
	U 2065	2.0%	4.9%	7.6%	18.5%	3.80	3.78
	WHA 2061	1.7%	1.1%	15.9%	11.6%	9.35	10.55
	U 2045	0.1%	1.1%	4.4%	5.3%	44.00	4.82
	WHA 9823	0.3%	0.2%	11.0%	2.3%	36.67	11.50
	U 2037	0.5%	0.5%	4.2%	1.6%	8.40	3.20
	WHA 9828	0.7%	0.9%	8.0%	3.6%	11.43	4.00
	U 2032	0.7%	1.0%	1.5%	0.8%	2.14	0.80
	U 2054	0.9%	0.7%	3.9%	2.5%	4.33	3.57
	U 2047	0.7%	0.5%	5.0%	1.9%	7.14	3.80
	U 2067	0.8%	0.6%	3.2%	2.1%	4.00	3.56
	WHA 9718	0.5%	0.9%	3.2%	2.9%	6.4	3.22
	WH 2285	0.5%	0.4%	4.5%	1.2%	9	3
	Median	0.8%	0.8%	4.8%	2.7%	7.1%	3.7%
	Maximum	2.9%	4.9%	15.9%	18.5%	44.0%	15.8%
	Minimum	0.1%	0.2%	1.5%	0.8%	2.1%	0.8%

There was no negative impact on the ability of the T cell CD8+subset to produce the immune regulatory cytokines. Both disease categories show similar responses post activation although the myeloid group displays a marginally greater IFN-y cytokine profile when CD8+T cells are activated *in vitro*. **(Tables 38, 38a)**

TABLE 38
IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD8+ T CELLS ACCORDING TO DIAGNOSIS LINEAGE (MYELOID)

CD8+ cells							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	INFy	IL-4	INFy	IL-4	INFy	IL-4
MYELOID	WH 2125	1.6%	3.2%	23.5%	16.6%	14.69	5.19
	WHA 9715	0.9%	0.9%	18.9%	10.1%	21.00	11.22
	WH 2246	0.4%	0.6%	9.1%	4.8%	22.75	8.00
	WH 2363	0.8%	0.7%	26.0%	12.4%	32.50	17.71
	WH 2138	0.2%	0.2%	2.0%	0.9%	10.00	4.50
	WH 2321	0.4%	0.7%	9.6%	3.3%	24.00	4.71
	WH 2342	0.1%	0.4%	12.3%	2.0%	123.00	5.00
	WH 2280	0.4%	0.5%	14.8%	7.4%	37.00	14.80
	U 2056	0.6%	0.6%	16.4%	4.5%	27.33	7.50
	WH 2350	1.3%	1.0%	12.4%	3.3%	9.54	3.30
	WH 2259	50.0%	49.0%	16.3%	8.0%	N/A	N/A
	WH 2256	1.0%	1.5%	7.1%	4.4%	7.10	2.93
	WHA 9716	0.6%	0.5%	11.4%	4.8%	19.00	9.60
	WH 2101	1.7%	1.6%	12.9%	5.6%	7.59	3.50
	WH 2330	0.3%	0.2%	8.4%	1.6%	28.00	8.00
	WH 9814	0.9%	0.8%	22.4%	2.7%	24.89	3.38
	WHA 9909	1.5%	1.9%	20.4%	8.7%	13.60	4.58
	WHA 9810	0.1%	0.2%	6.1%	0.7%	61.00	3.50
	WH 2128	0.3%	0.4%	11.8%	3.6%	39.33	9.00
	WH 2293	0.2%	0.3%	3.1%	3.3%	15.50	11.00
	WHA 9822	0.2%	0.3%	11.0%	4.4%	55.00	14.67
	WH 2305	0.3%	1.5%	14.6%	4.1%	48.67	2.73
	U 9704	0.9%	1.3%	16.5%	6.5%	18.33	5.00
	WH 2347	0.3%	0.5%	13.3%	4.4%	44.33	8.80
	WH 2288	0.3%	0.9%	1.5%	2.4%	5.00	2.67
	WH 2253	0.1%	0.2%	3.0%	2.5%	30.00	12.50
	WH 2285	0.2%	0.3%	11.2%	2.0%	56.00	6.67
	U 2062	0.2%	0.8%	6.1%	3.2%	30.50	4.00
	WH 2359	0.6%	0.5%	16.9%	8.6%	28.17	17.20
	WH 2361	1.5%	0.2%	2.5%	1.3%	1.67	6.50
	WH 2284	0.1%	0.3%	22.0%	8.3%	219.50	27.77
	WH 9717	0.3%	0.5%	15.8%	5.4%	52.80	9.96
	WH 2268	0.4%	1.0%	7.9%	2.3%	19.75	2.30
	Median	0.4%	0.6%	12.3%	4.4%	26.1%	6.6%
	Maximum	50.0%	49.0%	26.0%	16.6%	219.5%	27.8%
	Minimum	0.1%	0.2%	1.5%	0.7%	1.7%	2.3%

TABLE 38a

IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD8+ T CELLS ACCORDING TO DIAGNOSIS LINEAGE (LYMPHOID)

CD8+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	INFy	IL-4	INFy	IL-4	INFy	IL-4
LYMPHOID	WH 2343	2.0%	1.0%	26.7%	9.8%	13.35	9.80
	WHA 9908	2.1%	0.5%	45.4%	24.9%	21.62	49.80
	WH 2309	0.5%	0.8%	3.0%	1.9%	6.00	2.38
	WHA 9720	0.8%	0.4%	4.8%	0.8%	6.00	2.00
	WH 2277	0.3%	0.4%	8.3%	4.7%	27.67	11.75
	WH 9821	0.8%	1.0%	14.8%	6.1%	18.50	6.10
	WH 2258	0.7%	0.6%	18.0%	8.1%	25.71	13.50
	U 2044	1.9%	2.7%	9.0%	4.9%	4.74	1.81
	WH 9704	1.6%	0.9%	11.3%	2.2%	7.06	2.44
	WHA 9809	0.5%	0.8%	9.5%	5.1%	19.00	6.38
	U 2065	6.9%	10.5%	12.0%	20.4%	1.74	1.94
	WHA 2061	2.9%	1.8%	19.8%	16.8%	6.83	9.33
	U 2046	0.1%	0.9%	9.3%	9.1%	93.00	10.11
	WHA 9823	0.2%	0.1%	9.9%	1.6%	49.50	16.00
	U 2037	0.5%	0.6%	13.8%	3.2%	27.60	5.33
	WHA 9828	0.3%	0.4%	15.0%	4.7%	50.00	11.75
	U 2032	1.0%	2.1%	4.8%	1.7%	4.80	0.81
	U 2054	0.5%	0.4%	9.0%	2.6%	18.00	6.50
	U 2047	0.4%	0.3%	13.9%	4.1%	34.75	13.67
	U 2067	0.5%	0.5%	3.4%	1.8%	6.80	3.60
	WHA 9718	0.3%	0.7%	4.6%	3.6%	15.33	5.14
	WH 2285	0.4%	0.3%	5.8%	1.3%	14.50	4.33
	Median	0.4%	0.3%	5.8%	1.3%	14.5%	4.3%
	Maximum	6.9%	10.5%	45.4%	24.9%	93.0%	49.8%
	Minimum	0.1%	0.1%	3.0%	0.8%	1.7%	0.8%

Using different conditioning regimens the patients reflected very similar results as they exhibited normal TH1 and TH2 cytokine responses to the T subset CD4+ helper cells. **(Tables 39, 39a)**

TABLE 39

IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD4+ T CELLS ACCORDING TO CONDITIONING REGIMEN (CHEMOTHERAPY)

CD4+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	INFy	IL-4	INFy	IL-4	INFy	IL-4
CHEMOTHERAPY	WH 2125	1.3%	3.2%	4.1%	4.1%	3.15	1.28
	WH 2263	1.1%	1.6%	5.3%	5.1%	4.82	3.19
	WH 2343	2.6%	2.5%	14.0%	10.0%	5.38	4.00
	WHA 9908	0.9%	0.6%	9.8%	7.1%	10.89	11.83
	WHA 9720	1.2%	0.4%	2.9%	1.4%	2.42	3.50
	WH 2342	0.2%	0.5%	2.1%	1.4%	10.50	2.80
	WH 9821	1.6%	2.0%	13.9%	7.1%	8.69	3.55
	WH 2258	0.6%	0.5%	13.7%	7.9%	22.83	15.80
	U 2056	0.6%	0.6%	15.1%	4.9%	25.17	8.17
	U 2044	1.6%	2.5%	5.0%	4.7%	3.13	1.88
	WH 2350	2.3%	1.2%	10.3%	3.2%	4.48	2.67
	WHA 9809	0.4%	0.5%	3.2%	2.2%	8.00	4.40
	U 2065	2.0%	4.9%	7.6%	18.5%	3.80	3.78
	WH 2259	0.5%	0.4%	7.8%	5.3%	15.60	13.25
	WHA 2061	1.7%	1.1%	15.9%	11.6%	9.35	10.55
	WHA 9716	0.6%	0.8%	8.2%	5.3%	13.67	6.63
	WHA 9909	2.2%	2.3%	12.8%	7.6%	5.82	3.30
	U 2046	0.1%	1.1%	4.4%	5.3%	44.00	4.82
	WHA 9823	0.3%	0.2%	11.0%	2.3%	36.67	11.50
	U 2037	0.5%	0.5%	4.2%	1.6%	8.40	3.20
	WHA 9822	0.2%	0.6%	4.1%	2.2%	20.50	3.67
	WHA 2347	0.7%	0.9%	8.0%	3.6%	11.43	4.00
	WH 2347	0.5%	1.0%	11.4%	5.5%	22.80	5.50
	U 2032	0.7%	1.0%	1.5%	0.8%	2.14	0.80
	U 2062	0.6%	1.6%	2.6%	2.2%	4.33	1.38
	WH 2359	0.5%	1.0%	2.4%	1.5%	4.80	1.50
	U 2054	0.9%	0.7%	3.9%	2.5%	4.33	3.57
	WH 2361	0.1%	0.1%	0.7%	0.3%	7.00	3.00
	U 2067	0.8%	0.6%	3.2%	2.1%	4.00	3.56
	Median	0.7%	0.9%	5.3%	4.1%	8.0%	3.6%
	Maximum	2.6%	4.9%	15.9%	18.5%	44.0%	15.8%
	Minimum	0.1%	0.1%	0.7%	0.3%	2.1%	0.8%

TABLE 39a

IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD4+ T CELLS ACCORDING TO CONDITIONING REGIMEN (CHEMOTHERAPY AND RADIOTHERAPY)

CD4+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	INFy	IL-4	INFy	IL-4	INFy	IL-4
CHEMOTHERAPY AND RADIOOTHERAPY	WHA 9715	1.3%	1.8%	9.6%	5.4%	7.38	3.00
	WH 2246	2.1%	2.5%	8.1%	7.1%	3.86	2.84
	WH 3138	2.6%	2.4%	7.6%	4.7%	2.92	1.96
	WH 2309	1.2%	2.5%	2.7%	2.2%	2.25	0.88
	WH 2321	0.7%	1.5%	3.9%	2.7%	5.57	1.80
	WH 2280	0.5%	0.8%	7.3%	5.2%	14.60	6.50
	WH 2277	0.6%	0.7%	4.2%	4.2%	7.00	6.00
	WH 9704	2.9%	1.5%	6.4%	2.5%	2.21	1.67
	WH 2251	2.4%	3.3%	6.1%	7.5%	2.54	2.27
	WH 2101	1.5%	1.3%	2.8%	1.6%	1.87	1.23
	WH 2330	0.4%	0.2%	1.9%	0.4%	4.75	2.00
	WH 9814	0.9%	1.1%	13.8%	2.7%	15.33	2.45
	WHA 9810	0.2%	0.2%	3.0%	0.5%	15.00	2.50
	WH 2128	0.6%	0.8%	4.8%	2.0%	8.00	2.50
	WH 2293	0.5%	1.0%	3.1%	1.9%	6.20	1.90
	WH 2305	0.1%	0.7%	4.9%	2.4%	49.00	3.43
	U 9704	1.6%	2.3%	10.9%	6.7%	6.81	2.91
	WH 2288	0.6%	1.6%	0.8%	2.2%	1.33	1.38
	WH 2253	0.5%	0.5%	2.3%	2.1%	4.60	4.20
	WH 2285	0.2%	0.3%	6.8%	2.5%	34.00	8.33
	U 2047	0.7%	0.5%	5.0%	1.9%	7.14	3.80
	WH 2844	0.4%	0.4%	10.1%	4.6%	24.59	11.55
	WH 9717	0.3%	0.4%	6.4%	2.7%	19.91	6.38
	WH 2268	0.5%	1.3%	2.3%	1.1%	4.60	0.85
	WHA 9718	0.5%	0.9%	3.2%	2.9%	6.4	3.22
	WH 2285	0.5%	0.4%	4.5%	1.2%	9	3
	Median	0.6%	1.0%	4.9%	2.5%	6.6%	2.7%
	Maximum	2.9%	3.3%	13.8%	7.5%	49.0%	11.6%
	Minimum	0.1%	0.2%	0.8%	0.4%	1.3%	0.8%

There was no negative impact on the ability of the T cell CD8+subset to produce the immune regulator cytokines. Both groups displayed similar measurable cytokine production post activation. **(Tables 40, 40a)**

TABLE 40

IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD8+ T CELLS ACCORDING TO CONDITIONING REGIMEN (CHEMOTHERAPY)

CD8+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	INFy	IL-4	IFNy	IL-4	IFNy	IL-4
CHEMOTHERAPY	WH 2125	1.6%	3.2%	23.5%	16.6%	14.69	5.19
	WH 2363	0.8%	0.7%	26.0%	12.4%	32.50	17.71
	WH 2343	2.0%	1.0%	26.7%	9.8%	13.35	9.80
	WHA 9908	2.1%	0.5%	45.4%	24.9%	21.62	49.80
	WHA 9720	0.8%	0.4%	4.8%	0.8%	6.00	2.00
	WH 2342	0.1%	0.4%	12.3%	2.0%	123.00	5.00
	WH 9821	0.8%	1.0%	14.8%	6.1%	18.50	6.10
	WH 2258	0.7%	0.6%	18.0%	8.1%	25.71	13.50
	U 2056	0.6%	0.6%	16.4%	4.5%	27.33	7.50
	U 2044	1.9%	2.7%	9.0%	4.9%	4.74	1.81
	WH 2350	1.3%	1.0%	12.4%	3.3%	9.54	3.30
	WHA 9809	0.5%	0.8%	9.5%	5.1%	19.00	6.38
	U 2065	6.9%	10.5%	12.0%	20.4%	1.74	1.94
	WH 2259	1.1%	1.0%	16.3%	8.0%	14.82	8.00
	WHA 2061	2.9%	1.8%	19.8%	16.8%	6.83	9.33
	WHA 9716	0.6%	0.5%	11.4%	4.8%	19.00	9.60
	WHA 9909	1.5%	1.9%	20.4%	8.7%	13.60	4.58
	U 2046	0.1%	0.9%	9.3%	9.1%	93.00	10.11
	WHA 9823	0.2%	0.1%	9.9%	1.6%	49.50	16.00
	U 2037	0.5%	0.6%	13.8%	3.2%	27.60	5.33
	WHA 9822	0.2%	0.3%	11.0%	4.4%	55.00	14.67
	WHA 9828	0.3%	0.4%	15.0%	4.7%	50.00	11.75
	WH 2347	0.3%	0.5%	13.3%	4.4%	44.33	8.80
	U 2032	1.0%	2.1%	4.8%	1.7%	4.80	0.81
	U 2062	0.2%	0.8%	6.1%	3.2%	30.50	4.00
	WH 2359	0.6%	0.5%	16.9%	8.6%	28.17	17.20
	U 2054	0.5%	0.4%	9.0%	2.6%	18.00	6.50
	WH 2361	1.5%	0.2%	2.5%	1.3%	1.67	6.50
	U 2067	0.5%	0.5%	3.4%	1.8%	6.80	3.60
	Median	0.7%	0.6%	12.4%	4.8%	19.0%	6.5%
	Maximum	6.9%	10.55	45.4%	24.9%	123.0%	49.8%
	Minimum	0.1%	0.1%	2.5%	0.8%	1.7%	0.8%

TABLE 40a

IN VITRO PRODUCTION OF IFN-γ AND IL4 WITHIN THE CD8+ T CELLS ACCORDING TO CONDITIONING REGIMEN (CHEMOTHERAPY AND RADIOTHERAPY)

CD8+ CELLS							
		Unstimulated		Stimulated		Ratio	
Type	Subjects	INFy	IL-4	INFy	IL-4	INFy	IL-4
CHEMOTHERAPY AND RADIOTHERAPY	WHA 9715	0.9%	0.9%	18.9%	10.1%	21.00	11.22
	WH 2246	0.4%	0.6%	9.1%	4.8%	22.75	8.00
	WH 2138	0.2%	0.2%	2.0%	0.9%	10.00	4.50
	WH 2309	0.5%	0.8%	3.0%	1.9%	6.00	2.38
	WH 2321	0.4%	0.7%	9.6%	3.3%	24.00	4.71
	WH 2280	0.4%	0.5%	14.8%	7.4%	37.00	14.80
	WH 2277	0.3%	0.4%	8.3%	4.7%	27.67	11.75
	WH 9704	1.6%	0.9%	11.3%	2.2%	7.06	2.44
	WH 2256	1.0%	1.5%	7.1%	4.4%	7.10	2.93
	WH 2101	1.7%	1.6%	12.9%	5.6%	7.59	3.50
	WH 2330	0.3%	0.2%	8.4%	1.6%	28.00	8.00
	WH 9814	0.9%	0.8%	22.4%	2.7%	24.89	5.20
	WHA 9810	0.1%	0.2%	6.1%	0.7%	61.00	3.50
	WH 2128	0.3%	0.4%	11.8%	3.6%	39.33	9.00
	WH 2293	0.2%	0.3%	3.1%	3.3%	15.50	11.00
	WH 2305	0.3%	1.5%	14.6%	4.1%	48.67	2.73
	U 9704	0.9%	1.3%	16.5%	6.5%	18.33	5.00
	WH 2288	0.3%	0.9%	1.5%	2.4%	5.00	2.67
	WH 2253	0.1%	0.2%	3.0%	2.5%	30.00	12.50
	WH 2285	0.2%	0.3%	11.2%	2.0%	56.00	6.67
	U 2047	0.4%	0.3%	13.9%	4.1%	34.75	13.67
	WH 2284	0.1%	0.3%	22.0%	8.3%	219.50	27.77
	WH 9717	0.3%	0.5%	15.8%	5.4%	52.80	9.96
	WH 2268	0.4%	1.0%	7.9%	2.3%	19.75	2.30
	WHA 9718	0.3%	0.7%	4.6%	3.6%	15.33	5.14
	WH 2285	0.4%	0.3%	5.8%	1.3%	14.50	4.33
	Median	0.4%	0.6%	9.4%	3.4%	23.4%	5.1%
	Maximum	1.7%	1.6%	22.4%	10.1%	219.5%	27.8%
	Minimum	0.1%	0.2%	1.55	0.7%	5.0%	2.3%

7.5.2.5 Discussion

The *in vitro* functional data generated during this study provided some unique findings: to date, most studies following transplant patients have focused on the basic hematology parameters in the blood and such data has only implied that patients have regenerated their peripheral components post transplant. As far as we know, no studies have actually investigated the functionality of such elements. It is apparent from our data that although the subjects showed normal levels of blood elements, the functions of these cells are severely hampered *in vitro*, especially the innate immune cells' responses.

Analysis of the data showed that all three subgroups display a reduced neutrophil activity (innate response) *in vitro:* it is also evident that those who had received autografts had the best, most intact profile when compared to the other patients although these are still considered as impaired when compared to healthy controls. The age of the transplantation had a significant difference between subgroups: the most recently transplanted patients had the best and most "intact" responses (albeit abnormal when compared to healthy controls)

The lymphoid group had better responses to E.Coli stimulation (a physiological stimulus used for the *in vitro* evaluation) in comparison to those who had myeloid diseases. Reduction in the myeloid group could be due to the cells being affected originally, causing them not to recover their function. In the different conditioning regimens both had reduced innate functions *in vitro*. The patients who received combination chemotherapy and radiotherapy seemed more intact than those who had chemotherapy only perhaps implying that the combination of several chemotherapeutic agents has more detrimental effects on the functional potential of neutrophils post transplantation.

When the adaptive responses were evaluated *in vitro* (cytokine production in response to a non-specific stimulus and evaluation of the type of cytokine produced), the subjects who had had autografts showed the best CD4+ cell cytokine production when compared to their counterparts. When regrouped according to age of transplant normal cytokine responses were seen in both CD4+ and CD8+ subsets. No major differences were noted amongst these three groups. Both the myeloid and lymphoid group displayed normal cytokine responses of the T-cell subset CD4+and CD8+ cells. The myeloid category showed marginally better reactivity post activation. There were normal cytokine responses in both CD4+ and CD8+ subsets when various conditioning regimens were used. No differences between the two therapies could be shown.

7.5.2.6 Concluding summary

Studies investigating immune reconstitution post-transplantation are limited and generally relate to episodes of bacteremias including overt neutropaenias in the post-transplantation period. Castagnola and Faraci (2009)[131,132] proposed that irrespective of the magnitude of myeloid suppression, patients should be covered by prophylactic antibacterials to prevent pulmonary complications. To date, there have been no studies undertaken to determine the functionality of the peripheral blood elements in post-transplant patients. Hence we believe that our data contributes new and unique data to the literature: it is the first to analyse both innate and adaptive responses of blood cells from subjects post-transplantation, in a relatively large cohort of 55 subjects who received their transplants more than 10 years ago enrolled in South Africa. Various reports investigating cellular immune deficiency can be found in the literature.[133,134]

The data clearly indicates that irrespective of the type of transplant, age of the graft, disease category and pre-conditioning regimen, this cohort displayed reduced granulocytic functions *in vitro*. Contrastingly, all subjects showed normal cytokine production by CD4+ and CD8+ T cells when these were activated *in vitro* to produce immune regulatory cytokines implying that their lymphoid component is intact post-transplantation. In other words, it appears that the innate response of granulocytic cells never recovers irrespective of time or type of transplant or conditioning regimen. However, the adaptive response seems to be more robust and able to recover fully. It appears that the proposal by Castagnola and Faraci (2009) to prophylactically treat subjects with antimicrobials is supported by our data: patients remain prone to developing bacterial infections (implying that the innate response is hampered) and yet are spared of viral or fungal infections (where the adaptive response is the most important).

There have been individual reports investigating the cellular immune deficiencies following stem cell transplantation: it was reported by Seggewiss and Einsele (2010)[117,135] that cytotoxic activity exhibited by NK cells or CD8+ T cells were severely reduced quantitatively and qualitatively up to 100 days post-transplantation. In this cohort of patients studied, it was evident that such deficiencies were not apparent but this could be due to the fact that these subjects had passed the 100 day period post-transplantation. It is also not clear from this data whether the cytotoxic activity of these cells was reduced: this was not determined. However, the assays conducted, namely the cytokine production post-activation *in vitro*, would imply that cytotoxic activity would be intact compared to healthy subjects.

The granulocyte deficiency deserves some discussion: this assay is extremely sensitive to delay in the processing of samples from blood drawn. All samples were processed within four hours of phlebotomy and the results cannot therefore be explained by loss of functional cells in the sample or the toxic effects of the anti-coagulant used in the tubes. Healthy subjects whose bloods are assayed up to six hours following collection draw display normal responses (data not shown). The results imply that even up to ten years or more post-transplantation, the granulocytes never recover their full functional potential. This would explain the incidence of bacterial episodes affecting predominantly the pulmonary system. Although the subjects display normal haematological parameters on routine analysis, it appears that the granulocytes are defective in their functions

7.5.3 BONE DISEASE

7.5.3.1 INTRODUCTION

At initiation of bone marrow transplantation in South Africa, there was already a worldwide appreciation that, among non-haematopoietic target organs, bone was particularly susceptible to demineralization injury.[136,137] Additionally, the skeleton is not only a very large structural and functional organ that interfaces with haematopoiesis but has distinctive metabolic and hormonal differences existing on a regional basis varying between trabecular and cortical areas that are respectively – also variable - rich in these two components.[138] The impact of solid organ transplantation on rapid bone loss and fracture is impressive but data for bone marrow is scarce[139] although mechanisms have been defined to explain accelerating hazard of osteoporosis secondary to demineralisation associated with underlying illness, chemotherapy, gender and graft-versus-host disease.[140] With other predisposition, including allogeneic as opposed to autologous transplantation, and

often more prominent in the older age group leading to osteoporosis. Longer term follow up suggested benefits from calcium, with addition of vitamin D and bisphosphonate therapy.

It was against this international experience that the opportunity was taken to document corresponding changes in a relatively homogenous population of patients transplanted in an accredited unit and on standardised protocols. Differences present in a wide range of physical activity, calcium and dietary intake, a number of skeletal damaging practices, such as culturally high intake of beer and other alcohol containing toxins described to give rise to alterations in bone mineral density.[141,142] Additionally using immunosuppressive preparation employing monoclonal antibodies in contrast to known damage from conventional chemotherapy and particularly corticosteroids.[143] This is known to influence skeletal integrity. Assessment was thus necessary to establish whether the present regimens also had adverse effects. The study aim was to measure bone mineral density (BMD) employing the gold standard of dual energy x-ray absorptiometry (DXA) and quantitative computer tomography (QCT).

This was linked to obtaining a clinical fracture history and performance of vertebral fracture assessment (VFA) to detect the presence of vertebral fractures. Additionally, by the determination of bone turnover as an independent risk factor for fracture and by measuring serum osteocalcin as a parameter of formation and urine deoxypyridinoline reflecting resorption one hoped to find a correlation with imaging studies. This information was to challenge the clinical impression, coupled with early results, that bone mineral density measured with DXA differed from the same endpoint expressed in quantitative computed tomography (QCT) by directly comparing these two differing technologies.[144]

7.5.3.2 Patients

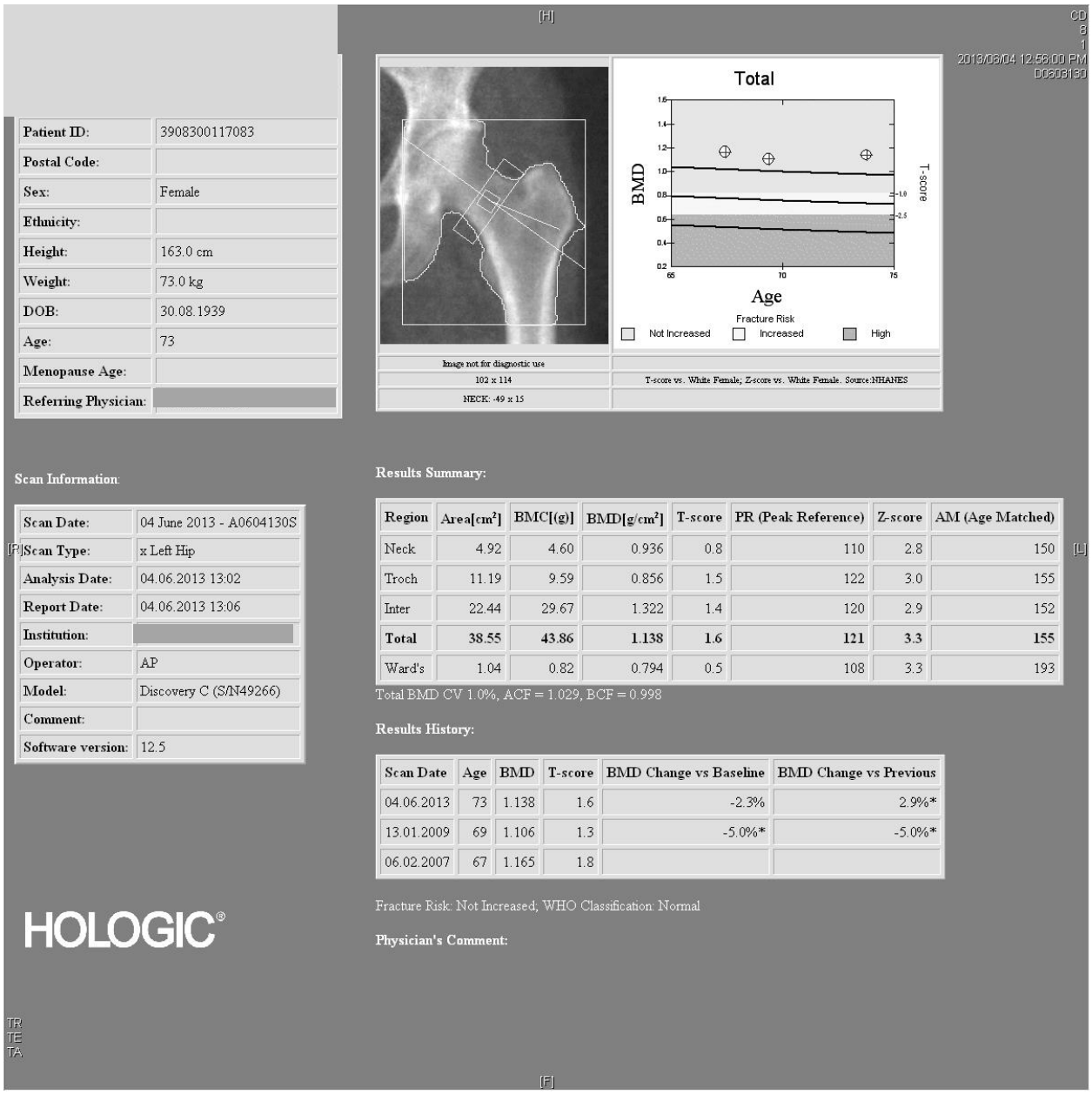
A cohort (n=54) was available for DXA scanning of which 23 were female. Forty three individuals from the same group were evaluated for QCT analysis of which 16 were female. In a separate group derived from the previous study for the DXA scoring, 36 patients were initially available. For the QCT there were correspondingly data on 36 subjects, with results available for 25 pre-procedure, 30 at six months and 20 at one year. For the biochemical studies the starting number was 55 of which 23 (42%) were female.

7.5.3.3 Methods

DXA based bone mineral density and vertebral fracture assessment[145, 146] was carried out on the lumbar spine (L1 - L 4), the total hip and femora, employing a Hologic Discovery DXA machine. The left hip shows normal bone density with a t-score of 1.6. **(Figure 42)** The measurements were subjected to strict quality control, involving the daily scanning of a manufacturer phantom. Values are expressed as t-scores (using the normative NHANES III data for young caucasians)[147] in postmenopausal woman, in men over the age of 50 years and the Z scores in premenopausal woman and men under 50 years. The WHO (1994)[148] classification was employed using t-scores to categorise post-menopausal and subjects over the age of 50 as normal, osteopenic or osteoporotic. In younger individuals, a Z score if decreased or increased by more than 2.0 standard deviations (SD) was regarded as abnormal. Vertebral fracture assessment was performed by manually spiking lumbar and thoracic vertebrae (T 12 - T 6) and having a DXA programme determine the anterior, mid and posterior vertebral heights. This procedure was performed by only one experienced technologist allowing for uniformity.

FIGURE 42

EXAMPLE OF DXA RESULT USING HOLOGIC DISCOVERY DXA MACHINE



Courtesy Dr Derek Solomon

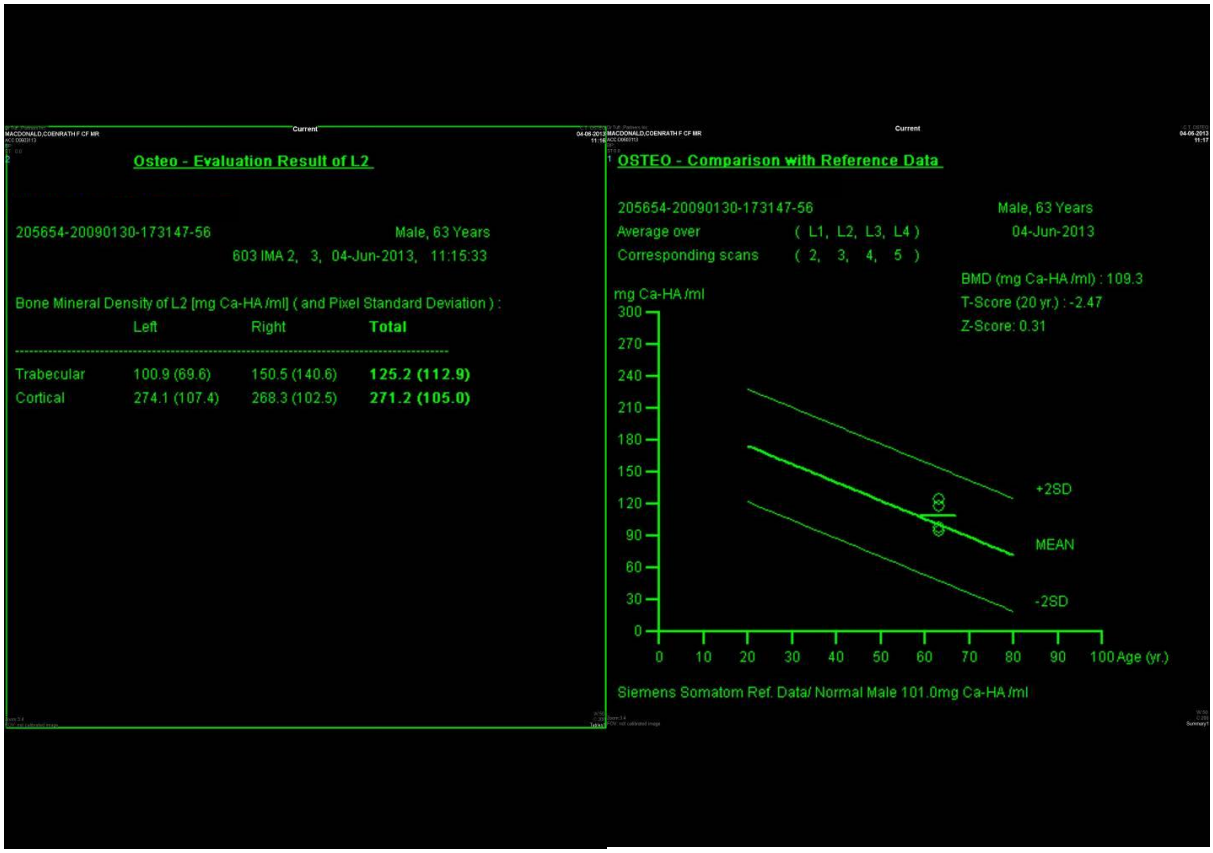
The comment that QCT is not as precise as DXA may stem from earlier studies that preceded the introduction of newer software. To this end the precision of the system in a group of volunteers was tested and found to be in the order of 1 to 2% which is on par with DXA.[138] A CT-Siemens sensation 64 scanner with osteo- package was used. Scanners were calibrated against a stable phantom for each image. The radiation dose was extremely low, less than for a standard posterior anterior chest radiograph. Considering that the studies were performed at intervals of 1 to 2 years this risk was perceived of as minimal and of limited or no clinical

concern. Measurements for trabecular and cortical densities through L2, with a graph showing the trabecular bone densities for L1-4 and the average density indicating a t-score of -2.47 which is just above the osteoporotic threshold. **(Figure 43)**

Quantitative computed tomography (QCT)[149, 150] gives a volumetric measurement of both trabecular and cortical bone with the t-score being calculated from the volume of the trabecular bone density. By analysing the high-resolution axial images of the vertebrae it is possible to visualise directly abnormalities that may account for discrepant readings.[138] This method was chosen to compare with DXA as we had previous results, from an audit, on a group which was studied at six monthly intervals.

FIGURE 43

EXAMPLE OF QCT SCAN – MEASUREMENTS FOR TRABECULAR AND CORTICAL DENSITIES

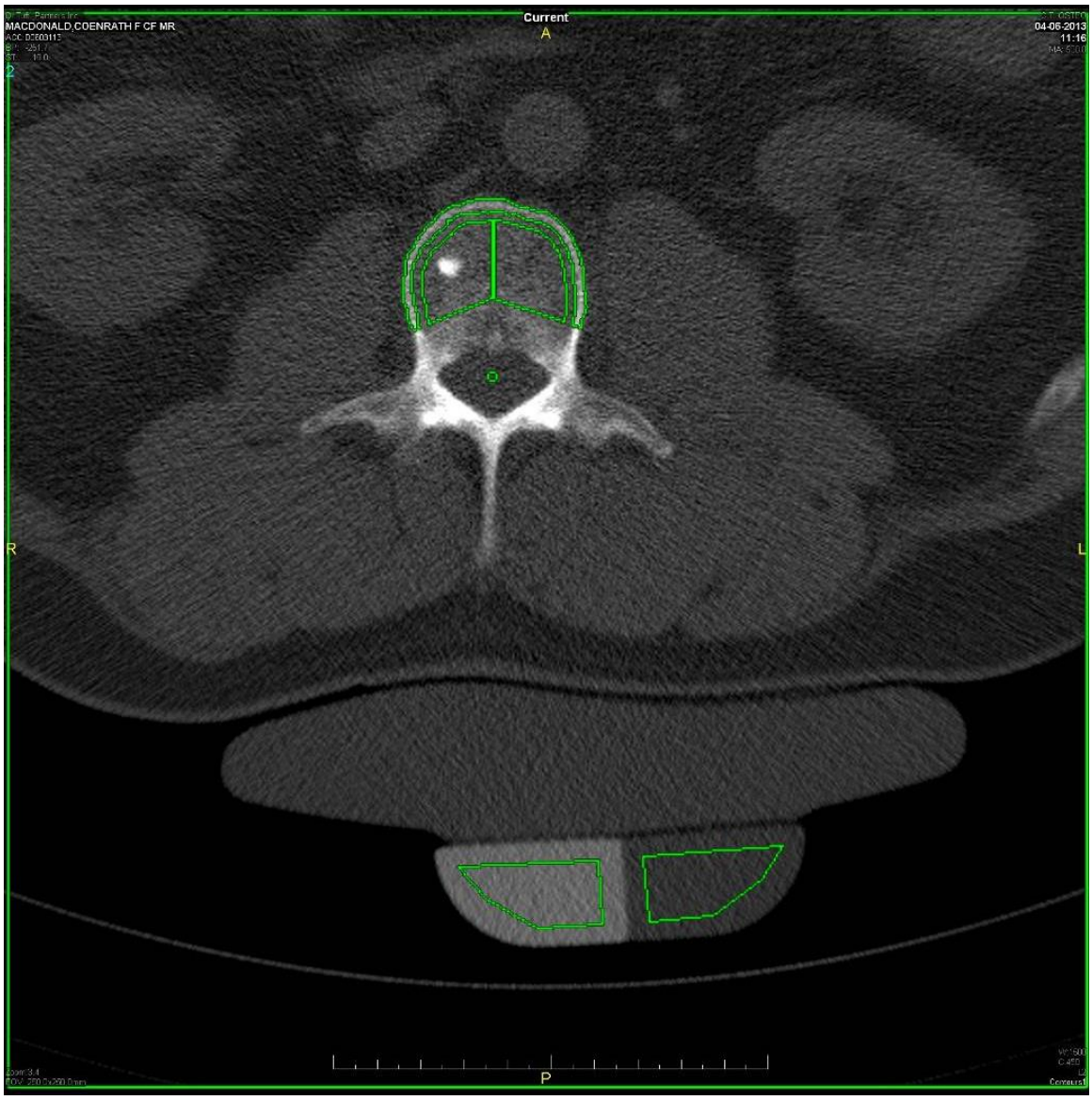


Courtesy Dr Derek Solomon

Figure 44 shows a QCT slice through individual vertebral body with an osteoma in the right half trabecular volume which would give an artificially high density for this reason.[138]

FIGURE 44

QCT SCAN – ABNORMALITY POSSIBLY CAUSING DISCREPANT READING



Courtesy Dr Derek Solomon

Biomarkers were measured to define formation and resorption as an index of bone health[151] employing serum osteocalcin[152] using the liason diasorin assay and deoxypyridinolene[153] in urine respectively – the latter performed on the Immulite 1000 method using chemiluminescence.[153] These findings were correlated with radiological studies. Both platforms were automated and the procedure done by a dedicated technologist.

7.5.3.4 RESULTS

A cohort of patients was available for DXA (n=54) and QCT (n=43). Where bone loss occurred, comparing the same individuals (43), there was a BMD decrease in 9 (21%) for DXA and 30 (70%) with QCT. **(Table 41)**

TABLE 41

BMD RESULTS OF SUBJECTS COMPARING DXA AND QCT

FEMALES < 50 YEARS		
PATIENT	DXA	QCT
WH2359	NORMAL	OSTEOPENIA
WH2256	NORMAL	OSTEOPENIA
WH2309	OSTEOPENIA	OSTEOPENIA
WH9908	OSTEOPENIA	OSTEOPENIA
WHA2061	NORMAL	NOT DONE
WH2321	NORMAL	NOT DONE
U2044	NORMAL	NOT DONE
WH2246	NORMAL	NOT DONE
WH2311	INCREASED	OSTEOPENIA
U2056	NORMAL	NOT DONE
WH2293	NORMAL	NORMAL
WH2253	NORMAL	NORMAL
WH2263	NORMAL	NORMAL
U2062	NORMAL	NORMAL
FEMALES ≥ 50 YEARS		
WH9704	OSTOPENIA	OSTEOPOROTIC
WH2342	OSTOPENIA	OSTEOPENIA
WHA9828	OSTOPENIA	OSTEOPENIA
WHA9809	OSTOPENIA	OSTEOPENIA
WH9720	NORMAL	OSTEOPENIA
WH2101	NORMAL	OSTEOPENIA
WHA9823	INCREASED	NOT DONE
WH2125	INCREASED	NOT DONE
WH2330	NORMAL	NORMAL

MEN < 50 YEARS		
PATIENT	DXA	QCT
U9704	NORMAL	NOT DONE
WH2028	OSTEOPENIA	NOT DONE
WH2277	NORMAL	NORMAL
WH2259	NORMAL	NORMAL
WH2285	NORMAL	NORMAL
WH2138	NORMAL	OSTEOPOROTIC
WH2343	NORMAL	OSTEOPENIA
WHA9829	INCREASED	NOT DONE
WH9814	NORMAL	NORMAL
U2054	NORMAL	OSTEOPENIA
U2037	INCREASED	NORMAL
WHA2067	NORMAL	OSTEOPENIA
WHA9909	INCREASED	OSTEOPENIA
MEN ≥ 50 YEARS		
WH2268	NORMAL	OSTEOPOROTIC
WH2288	NORMAL	OSTEOPOROTIC
WH2280	NORMAL	OSTEOPOROTIC
WHA9718	NORMAL	OSTEOPENIA
WHA9810	NORMAL	OSTEOPENIA
WHA9716	NORMAL	NORMAL
WH2258	NORMAL	NORMAL
U2047	NORMAL	NORMAL
WHA9715	INCREASED	OSTEOPENIA
WH2305	OSTEOPENIA	NOT DONE
WH2347	NORMAL	OSTEOPENIA
WH9821	INCREASED	OSTEOPENIA
U2046	OSTEOPENIA	OSTEOPOROTIC
WH9717	OSTEOPENIA	OSTEOPOROTIC
WH2361	OSTEOPENIA	OSTEOPOROTIC
WH2284	NORMAL	OSTEOPOROTIC
U2032	NORMAL	OSTEOPENIA
WH2350	NORMAL	OSTEOPOROTIC

In a subgroup of 36 individuals on which both DXA and QCT scans could be compared over time, the median shows there was statistical significance (p= 0.0062) in the DXA t-score scan where there was an improvement over time (the reason could not be explained) and in the QCT-t-score there was no significant difference (p=0.2564). **(Table 42)**

TABLE 42

DIFFERENCES IN DXA AND QCT T-SCORES OVER TIME – SIX MONTHLY PROCEDURES

QCT t-score

	Observations	**Median (mg/cm³)**	**IQR**	**Range**
Pre t-score	25	-1.6	-2.4 to -0.8	-2.9 to 0.66
6 month t-score	30	-1.95	-2.57 to -1.2	-3.7 to 1.83
1 year t-score	20	-1.77	-2.7 to -1.275	-4.6 to 2.8

DXA t-score

	Observation	**Median (g/cm²)**	**IQR**	**Range**
Pre t-score	36	-0.65	-1.55 to 0.25	-3.5 to 1.5
6 month t-score	36	-0.55	-1.7 to 0.2	-3.0 to 1.5
1 year t-score	19	-0.3	-1.3 to 0.7	-2.4 to 1.6

In men under the age of 50 years (n=13), DXA showed one decrease at the spine and hip but some had significant increases in BMD**.** The QCT scan (n= 10) recorded normal BMD in five subjects only, osteopenia in four and frank osteoporosis in one. The DXA recorded one subject with osteopenia and no osteoporosis. There was 40% accordance between the scans. The average age, serum osteocalcin - or urine deoxypiridinoline (DPD/creatinine) - did not differ in this group.

In older men above 50 years (n=18) the DXA was normal in 12 and only modestly decreased in the rest. The cut off value, t-score -2.5, was not reached in any individual. With the QCT (n=7) a normal BMD was found in three; osteopenia in another six and osteoporosis in eight (47%) here only 18% showed similarity. The biomarkers between those with normal BMD and those with decreased values did not differ significantly.

Amongst young women less than 50 years (n=14) BMD was normal in 11 and significantly increased in one subject. Two women were osteopenic. In comparison, the QCT (n=9) showed normal BMD in four subjects and osteopenia in five. Here a discrepancy between the scans was 33%.

In women 50 years and above DXA (n=9) was normal in three subjects, significantly increased in two and osteopenic in four. With the QCT (n=7) one had a normal BMD, five had osteopenia and one had osteoporosis. The difference between the scans was 43%.

When diagnosis, type of transplant and conditioning were analysed in those who had DXA showing osteopenia, no significant differences were seen.

With QCT demonstrating osteopenia, diagnosis did not have an impact but in type of transplant, autografts were predominant at 11/20 (55%) and in conditioning, the chemotherapy alone was 13/20 (65%).

No patients had osteoporosis on DXA but 10 (23%) on QCT. Here the myeloid group was 8 (80%), the allografts 6 (60%) and chemotherapy with radiotherapy 7 (70%).

The median age for females was 46 years and for males 55.5 years. DPD and serum osteocalcin values did not differ significantly between age groups or sexes, nor did they differ significantly between those with a normal or abnormal BMD. Biomarkers showed normal results with median levels in all tests being similar between males and females. **(Table 43)**

TABLE 43

BIOMARKERS – RESULTS IN FIFTY-FIVE POST-TRANSPLANT PATIENTS

DPD Creatinine Ratio (nM/mM)	Median	IQR	Min	Max	Normal Range (nM/mM)
Female	4.9	1.75 – 6.1	0.41	8.0	3.0 to 7.4
Male	4.3	3.15 – 5.45	0.24	12.2	2.3 to 5.4

Osteocalcin (ng/ml)	Median	IQR	Min	Max	Normal Range (ng/ml)
Female	13.3	10.8 – 15.6	9.1	21.4	5.4 to 59.1
Male	14.15	11.75 – 16.85	8.1	31.8	4.6 to 65.4

Age (years)	Obs	Median	IQR	Range
All	55	52	37 - 62	26 to 73
Female	23	46	33 - 58	26 to 71
Male	32	55.5	40.5 – 62.5	27 to 73

This population was devoid of any fracture history. With the exception of two vertebral abnormalities/deformities, no clear evidence of a vertebral fracture could be documented.

7.5.3.5 DISCUSSION

The study included 17% elderly women, few over 50 years and hardly any over 70 years. As osteoporosis is a disease affecting the very elderly, this group was not particularly prone to develop this although they were most probably menopausal (not tested) because of myeloablative conditioning. History on the use of bone protective agents e.g. bisphosphonates, calcium and vitamin D was not accurate but it was never used

prophylactically except in the patients with myeloma(n= 3). It is well known that many bone toxic agents (eg. glucocorticosteroids, pre-transplant conditioning therapy) [154] affect patients of all ages and gender. It did not appear to be the case in this study where DXA scan did not have any patient reaching the so called osteoporosis range However QCT scans documented osteoporosis in a number of subjects.

7.5.3.6 Concluding Summary

Unlike conventional post-transplantation chemotherapeutic regimens, the immunotherapy used in the present study of long term survivors, employing a monoclonal antibody against CD52, appeared to be remarkably bone friendly. No fracture history of note and no objective evidence of vertebral fractures assessed quantitatively employing DXA and vertebral fracture assessment (VFA), could be ascertained in this cohort of 54 patients. Moreover, the current gold standard surrogate marker of skeletal strength, DXA-measured bone mineral density (BMD), was quite unremarkable. Very surprising is the fact that not a single subject having DXA had a BMD value in the so-called osteoporosis range (i.e. t-score ≤ 2.5 SD in older subjects or a Z-score ≤ -2.0 SD in those < 50 years). Of particular interest also is the finding of an apparent increase in BMD in no less than 15% (8/54) of the study population. The reason for this remains unclear. The literature shows bone loss after stem cell transplantation to occur frequently due to the conditioning drugs and gonodal dysfunction following radiotherapy.[154]

Biomarkers of bone turnover did not improve insight into the skeletal health of our study population, but this may merely reflect small study numbers.

A poor correlation appeared to exist between the DXA and QCT BMD results, [155] with only 39% concordance. These were available in the vast majority of individuals (80%). However, analysed separately, it is clear that a much better correlation is present in the female population with similar results being reported in more than 63% (10/16) of subjects. The poor correlation therefore appears to exist largely in the male population, where more than 73% of results were discrepant.

No consensus has been reached in establishing which modality (DXA vs QCT) is the more superior. Advantages and disadvantages exist for both methods. [146, 151]

DXA is still the International Gold Standard for measuring BMD. QCT is still a research instrument and it has not yet been validated for predicting fracture risk thus it is not recommended for screening. It seems to be more sensitive and can provide an alternative method as a supplement to DXA. However there is at present no evidence that one is better than the other and more studies are required. To detect and manage osteoporosis, DXA remains the recommended technique for diagnosing and monitoring BMD in South Africa.

7.6 SUMMARISING COMMENT

This chapter included an audit of acute associations that occurred within the first three months and a study for the late effects after one year. It was shown that in nephrology nearly all kidney injuries returned to normal after the first 12 weeks. In dermatology there was a marked reduction in GVHD following the addition of anti-CD52 antibody to the graft ex vivo. This was also reported by other centres. Cardiac complications were negligible, emphasising the need to function within a multidisciplinary team. Gastroenterology immediately post-transplant presented with many side-effects. The nausea, vomiting, anorexia and diarrhoea were mostly related to the high doses of chemotherapy used for the conditioning regimen and worsened with the subsequent neutropenia. The symptoms usually disappeared when there was marrow regeneration. In the few patients where this persisted it was necessary to refer them to a gastroenterologist. In the study using post transplant survivors, no abnormalities were seen in the respiratory group. The chest x-rays and lung function studies were normal. In our cohort, GVHD was not a problem thus reducing respiratory side-effects. Most of these non-infectious complications reported in the literature occur due to GVHD. Immunology indicated that the innate response of granulocytic cells never recovers but the adaptive immune response seems to recover fully. It is unsure whether these results are due to the antibody that was administered ex vivo, as world literature reports reversal of immune status by one year. The bone disease indicated that there were only slight BMD decreases and even increases in some individuals. It is unclear if this could be attributable to the CD52 antibody as unmanipulated transplants have a different outcome. There was a discrepancy between the techniques used to measure BMD with QCT detecting more abnormalities than DXA. This study provided data on complications that can be used as a reference for new research projects on the African continent.

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8. FINAL SUMMARY

It was a challenge to how most lucidly present an evolving series of biological phenomena in which goalposts shifted from the traditional endpoint of haematologic recovery to include events taking place now in non-haematopoietic tissues and encapsulating the concept of survivorship.

In starting a new department with no infrastructure, many obstacles had to be conquered. Negotiations with hospital and university authorities achieved available space and equipment. This culminated in the building of a prototype physical plant in which achievements from international groups could be duplicated. Another impediment was the lack of skills to nurse these patients. This was overcome by arranging international training programmes in established units. Financial constraints were often solved by obtaining sponsorship for special equipment such as cell separators. This unit became the prototype on which all other South African units were built and provided all the training.

During the developmental phase, the source for the transplant procedure changed from bone marrow to peripheral blood stem cells and then cord blood. Initially only allografts were performed, moving to autografts and this was followed by matched unrelated donors that required the establishment of a local Bone Marrow Registry.

For our unit, the most important achievement was when there was the opportunity to collaborate with Professor Herman Waldman and Professor Geoff Hale regarding the use of a monoclonal antibody as a unique immunosuppressant regimen. The anti CD52 antibody was added to the bag and infused - the *ex vivo* approach - leading to diminishing of GVHD and significantly changing quality of life. Thereafter, unmanipulated stem cells were no longer used in our unit.

Data was reported to registries to attain accreditation.

Data collected for the registries stimulated the study on acute associations and late effects in non-haematopoietic tissue. The goal was to establish the influences of the major change in conditioning using monoclonal antibodies of the Campath® series of immunoglobulins. The early complications were analysed by performing an audit extracting data from the patient records. It had advantages as it took less time and the information was readily available but as a negative, this relied on others for the accuracy of the record keeping. It also had fewer financial implications. Biases may have occured at various levels and in this study there was selection bias as only patients with sufficient data for analysis were used. It did not have a control group which would have provided a comparison between unmanipulated and manipulated stem cells, as the question required insight into the difference Campath® made to the outcome.

In the late effects, a volunteer group of post transplant survivors was selected. Specialised tests were done at one point in time. It provided some outcome information but could only be evaluated against published literature. A limitation here could have been a “healthy survivor” effect in that those with major dysfunction could have succumbed and only the healthier subjects who were still alive could be studied. This was

particularly relevant to the respiratory function testing. Again, the main disadvantage was that there was no control group in whom a comparison between the use of unmanipulated grafts and those using Campath® *ex vivo* could be made. An attempt was made to compare outcome in different types of transplants, diseases and conditioning regimens. Most of the results were normal but as there was no control group, no definite conclusion could be made that adding the monoclonal antibody to the graft resulted in these positive outcomes.

The solution would be for all the units in South Africa to create a research study and collaborate with one another, which would then allow comparison, as all the centres do not use Campath® *ex vivo*. The numbers would be increased, diseases could then be categorized and serial studies conducted over a time period. Numerous attempts to achieve such cooperation have unfortunately failed. There would also be financial constraints as State hospitals have fixed budgets and in private facilities, the medical insurance will not pay for research studies. A solution could be sponsorship or research grant requests. Even if no prospective study can be done there would be sufficient data available to do an audit with a control group.

This study showed that our early complications occurred with a similar frequency to published data. In the late effects, most of the results were normal except the immunology where the functional study indicated that the patients had reduced granulocyte function. The immunological evaluation could have been done sequentially to evaluate how long dysfunction persists and thus, when recovery occurs. It would also have been useful to correlate immune function with actual infective episodes and aetiological agents. This would attest to the functional relevance of the specialised immunological tests and delineate the frequency of long-term surveillance. A prospective study with a control group would also be able to prove if the monoclonal antibody could be implicated. Previous studies focusing on haematological parameters implied that immune reconstitution takes place up to one year post transplant. Even if these were healthy individuals, late complications could have occurred as many of them had radiotherapy, known to cause respiratory injury as well as bone loss.

In conclusion, although it was difficult to confirm outcome as related to the Campath® *ex vivo,* it still provided information on the impact of acute and late injury to organ systems. These are judged to solidly underpin accumulation of data supporting a contribution with matching groups from other centres. This could assist in promoting future collaboration in South Africa between transplant teams.

ANNEXURE 1

DEMOGRAPHIC DATA ON ALL PATIENTS TRANSPLANTED AND REPORTED TO REGISTRIES

INITIALS	SEX	AGE (A/C)	DOT	DLS	TYPE	SOURCE	DISEASE	AGE
CC	M	A	12.04.95	28.12.10	ALLO	BM	CML	42
JH	F	A	24.05.95	05.12.98	ALLO	BM	AML	54
BH	F	A	14.06.95	11.01.99	ALLO	BM	NHL	36
YS	F	A	05.07.95	28.12.10	ALLO	BM	AML	35
GP	M	A	16.08.95	15.12.97	ALLO	BM	CML	49
AC	M	C	06.09.95	28.12.10	ALLO	BM	AA	18
JT	M	A	08.11.95	01.06.07	ALLO	BM	CML	24
ME	F	A	06.12.95	28.12.10	ALLO	BM	CML	38
JK	M	A	06.03.96	30.11.96	ALLO	BM	CML	27
GP	F	C	03.04.96	28.12.10	ALLO	BM	FANCONI	10
MS	F	C	15.05.96	28.12.10	ALLO	BM	THALASSAEMIA	7
ET	M	C	31.07.96	24.04.00	ALLO	BM	AML	3
WK	M	A	07.08.96	11.04.01	ALLO	BM	CML	45
RC	M	A	14.08.96	21.08.96	ALLO	BM	CML	48
JS	M	C	28.08.96	13.01.97	ALLO	BM	AML	4
GB	F	A	11.09.96	26.05.05	ALLO	BM	MM	53
RN	F	C	09.10.96	28.12.10	ALLO	PBSC	FANCONI	12
CM	F	C	30.10.96	28.12.10	ALLO	PBSC	FANCONI	6
CB	M	A	04.12.96	18.05.97	ALLO	PBSC	CLL	50
KF	F	C	08.01.97	28.12.10	ALLO	PBSC	FANCONI	11
JD	F	C	15.01.97	28.12.10	ALLO	PBSC	CML	13
BS	M	C	21.01.97	04.04.97	ALLO	PBSC	FANCONI	7
WS	F	A	05.02.97	28.12.10	ALLO	PBSC	NHL	56
VE	M	A	12.02.97	28.12.10	ALLO	PBSC	AA	24
BS	M	C	27.02.97	04.04.97	ALLO	PBSC	FANCONI	7
JK	F	A	12.03.97	15.04.98	ALLO	PBSC	ALL	42
JR	M	C	03.04.97	28.12.10	ALLO	PBSC	AA	11
CW	F	A	23.04.97	05.07.97	ALLO	PBSC	MM	43
KN	M	A	05.05.97	28.12.10	ALLO	PBSC	NHL	37
CT	F	A	25.06.97	01.08.97	ALLO	PBSC	AML	41
PP	M	A	02.07.97	28.12.10	ALLO	PBSC	CML	30
SW	M	C	09.07.97	28.12.10	ALLO	PBSC	HL	12
JN	M	C	10.07.97	14.07.98	ALLO	PBSC	AA	13
BJ	M	A	16.07.97	28.12.10	ALLO	PBSC	CML	49
LK	F	A	23.07.97	28.12.10	ALLO	PBSC	ALL	31
JM	M	C	13.08.97	28.12.10	ALLO	PBSC	ALL	19
TE	M	A	20.08.97	28.12.10	ALLO	PBSC	CML	40
FL	M	A	17.09.97	28.12.10	ALLO	PBSC	NHL	49
PC	M	A	01.10.97	28.12.10	ALLO	PBSC	ALL	34
SN	F	C	23.10.97	28.12.10	ALLO	PBSC	AML	15
AB	M	A	04.11.97	20.07.98	ALLO	PBSC	AML	39
SK	M	A	12.11.97	26.06.99	ALLO	PBSC	AA	34

IS	F	A	26.11.97	28.12.10	ALLO	PBSC	NHL	41
LB	F	C	04.12.97	20.02.98	ALLO	PBSC	GERM CELL	2
DH	M	A	10.12.97	18.11.00	ALLO	PBSC	CML	45
BH	M	A	24.12.97	04.01.98	ALLO	PBSC	MM	59
LW	F	A	31.12.97	28.12.10	ALLO	PBSC	NHL	49
NS	F	C	14.01.98	28.12.10	ALLO	PBSC	MDS-CMML	2
KW	F	A	04.02.98	18.07.98	ALLO	PBSC	HL	32
JN	M	C	10.02.98	14.07.98	ALLO	PBSC	AA	13
KO	M	A	12.02.98	27.06.03	ALLO	PBSC	ALL	33
JP	M	A	12.03.98	28.12.10	ALLO	PBSC	CML	46
LN	F	C	18.03.98	28.12.10	ALLO	PBSC	AML	14
MN	F	A	25.03.98	28.12.10	ALLO	PBSC	AML	34
AR	M	A	08.04.98	28.12.98	ALLO	PBSC	NHL	41
ER	F	A	06.05.98	28.12.10	ALLO	PBSC	CML	40
GW	F	A	20.05.98	09.06.98	ALLO	PBSC	PRA	29
IS	F	A	28.05.98	24.04.99	ALLO	PBSC	AA	50
SL	F	A	10.06.98	23.05.99	ALLO	PBSC	CML	57
PN	M	A	24.06.98	19.07.99	ALLO	PBSC	CML	30
CE	M	A	22.07.98	21.04.99	ALLO	PBSC	ALL	27
GM	M	A	13.08.98	28.12.10	ALLO	PBSC	AML	32
EB	M	A	19.08.98	28.12.10	ALLO	PBSC	CML	55
JC	M	C	21.08.98	28.12.10	ALLO	MUD	HURLERS SYNDROME	2
BK	M	A	16.09.98	12.03.99	ALLO	PBSC	AA	28
JP	M	A	18.11.98	08.07.00	ALLO	PBSC	AML	51
SN	F	C	15.12.98	06.01.99	ALLO	PBSC	AML	12
JB	M	A	17.12.98	03.07.99	ALLO	PBSC	ALL	32
AK	M	A	30.12.98	28.12.10	ALLO	PBSC	NHL	58
DE	M	A	31.12.98	28.12.10	ALLO	PBSC	NHL	28
SN	F	A	06.01.99	02.01.00	ALLO	PBSC	MASTOCYTOSIS	52
NT	M	A	20.01.99	28.09.99	ALLO	PBSC	NHL	42
BO	M	A	03.02.99	28.10.99	ALLO	PBSC	NHL	45
OG	F	A	10.02.99	30.04.99	ALLO	PBSC	AA	94
LS	F	A	17.02.99	27.07.99	ALLO	PBSC	NHL	44
BW	M	A	24.02.99	09.10.99	ALLO	PBSC	NHL	58
DN	M	A	03.03.99	09.09.03	ALLO	PBSC	NHL	29
LB	F	C	17.03.99	28.12.10	MUD	PBSC	FANCONI	9
AB	F	A	10.03.99	26.09.99	ALLO	PBSC	HL	40
IP	M	A	25.03.99	31.08.99	ALLO	PBSC	AA	94
MH	M	C	25.03.99	28.12.10	ALLO	PBSC	CML	19
NS	F	A	31.03.99	11.06.99	ALLO	PBSC	NHL	37
AA	M	A	14.04.99	28.12.10	ALLO	PBSC	MM	42
MZ	M	A	29.04.99	15.12.99	ALLO	PBSC	MM	42
GM	F	A	29.04.99	30.10.99	ALLO	PBSC	NHL	57
AS	F	A	05.05.99	28.12.10	ALLO	PBSC	NHL	53
BJ	M	A	12.05.99	28.12.10	ALLO	PBSC	ALL	44
SC	F	C	19.05.99	28.12.10	MUD	BM	ET	17
PK	M	A	03.06.99	23.11.99	MUD	PBSC	CLL	58
WC	M	C	23.06.99	21.07.99	MUD	PBSC	ALL	16

RR	F	A	07.07.99	28.12.10	ALLO	PBSC	CML	40
JT	M	C	04.08.99	28.12.10	ALLO	PBSC	SICKLE CELL ANAEMIA	10
RS	M	A	25.08.99	10.12.99	ALLO	PBSC	MM	46
AP	F	A	01.09.99	21.03.02	ALLO	PBSC	CML	56
DM	M	C	08.09.99	28.12.10	ALLO	PBSC	SICKLE CELL ANAEMIA	2
CT	F	A	15.09.99	28.12.10	ALLO	PBSC	CML	40
CC	M	C	21.09.99	10.09.00	MUD	PBSC	ALL	17
JS	M	C	22.09.99	28.06.99	ALLO	PBSC	FANCONI	16
JW	M	A	29.09.99	28.12.10	ALLO	PBSC	CML	30
TZ	F	A	06.10.99	28.12.10	ALLO	PBSC	CML	33
NO	M	C	15.10.99	28.12.10	MUD	PBSC	CML	18
OO	M	A	03.11.99	09.11.99	ALLO	PBSC	FANCONI	29
SB	F	A	03.11.99	10.10.00	ALLO	PBSC	AA	25
JM	M	A	10.11.99	13.02.02	ALLO	PBSC	CML	25
RH	M	A	10.11.99	15.08.07	ALLO	PBSC	NHL	50
NY	F	C	08.12.99	28.12.10	ALLO	PBSC	ALL	16
JH	F	A	08.12.99	02.04.02	ALLO	PBSC	CML	44
PF	M	C	23.12.99	17.08.02	ALLO	PBSC	AML	11
SE	F	A	12.01.00	19.12.03	ALLO	PBSC	NHL	25
AH	M	A	26.01.00	06.05.01	ALLO	PBSC	AML	52
EB	F	A	02.02.00	10.12.00	ALLO	PBSC	NHL	37
PD	M	A	02.02.00	24.05.00	ALLO	PBSC	NHL	50
KB	F	A	09.02.00	28.08.10	ALLO	PBSC	AML	31
CR	F	A	16.02.00	26.01.03	ALLO	PBSC	ET	51
BS	F	A	01.03.00	29.04.00	ALLO	PBSC	CML	46
CB	F	A	01.03.00	23.04.00	ALLO	PBSC	NHL	25
NS	F	A	23.03.00	19.11.01	ALLO	PBSC	AML	34
ND	F	C	29.03.00	25.04.00	ALLO	PBSC	FANCONI	6
TC	F	A	17.05.00	29.06.00	MUD	PBSC	MYELOPROLIF	23
ML	F	A	14.06.00	15.12.00	MUD	PBSC	CML	24
DS	M	C	26.07.00	28.12.10	ALLO	PBSC	SCID	11
AM	M	A	02.08.00	16.06.01	ALLO	PBSC	MM	57
CC	M	C	09.08.00	10.09.00	MUD	PBSC	ALL	19
WP	M	A	06.09.00	13.06.03	ALLO	PBSC	ALL	21
MO	F	A	13.09.00	26.01.01	ALLO	PBSC	MM	51
DV	F	C	20.09.00	20.10.00	ALLO	PBSC	FANCONI	4
ND	M	A	20.09.00	24.09.00	MUD	PBSC	NHL	47
LL	M	C	13.10.00	28.12.10	MUD	PBSC	ALL	20
LL	M	C	08.11.00	28.12.10	MUD	PBSC	ALL	20
AB	F	A	29.11.00	09.12.00	MUD	BM	CML	42
SF	M	A	07.12.00	28.12.10	MUD	PBSC	CML	52
CF	F	A	05.01.01	28.12.10	MUD	BM	CML	41
MG	M	A	10.01.01	28.12.10	MUD	PBSC	CML	40
NC	F	C	24.01.01	01.03.02	MUD	PBSC	ALL	17
SF	F	C	25.01.01	11.02.01	MUD	PBSC	AA	8
DH	M	A	07.02.01	06.01.08	ALLO	PBSC	MM	32
KL	M	C	07.02.01	28.12.10	ALLO	PBSC	SICKLE CELL ANAEMIA	16
KD	M	A	17.01.01	23.12.01	ALLO	PBSC	MYELOPROLIF	64

GF	M	A	14.03.01	28.12.10	ALLO	PBSC	ALL	34
ZV	F	C	28.03.01	28.12.10	ALLO	PBSC	FANCONI	7
CM	F	C	04.04.01	28.12.10	ALLO	PBSC	SICKLE CELL ANAEMIA	6
TL	M	C	11.04.01	27.05.01	MUD	PBSC	CML	14
MG	F	A	26.04.01	28.12.10	MUD	BM	AML	44
CW	F	A	16.05.01	28.12.10	ALLO	PBSC	CML	32
CD	M	A	17.05.01	23.05.01	MUD	BM	MDS	56
MM	F	A	23.05.01	28.12.10	ALLO	PBSC	CML	56
JF	M	A	30.05.01	18.09.03	ALLO	PBSC	NHL	39
BR	F	A	04.07.01	28.12.10	MUD	BM	MM	48
WB	M	A	11.07.01	28.12.10	MUD	BM	PNH	29
IZ	M	A	18.07.01	28.12.10	ALLO	PBSC	NHL	44
EG	M	A	08.08.01	29.12.01	ALLO	PBSC	AA	47
NR	F	A	15.08.01	02.09.01	MUD	PBSC	AML	47
PO	M	A	15.08.01	19.03.02	ALLO	PBSC	CML	47
JC	M	A	29.08.01	28.12.10	ALLO	PBSC	ALL	46
JM	M	A	19.09.01	28.12.10	ALLO	PBSC	CML	27
FK	M	A	12.09.01	28.12.10	ALLO	PBSC	MM	54
GG	M	A	19.09.01	13.02.02	MUD	PBSC	MM	38
HW	M	C	24.10.01	16.12.01	ALLO	PBSC	NHL	19
RA	M	A	24.10.01	28.12.10	ALLO	PBSC	CML	37
MT	M	A	07.03.01	14.02.04	ALLO	PBSC	MM	31
PF	M	C	31.10.01	17.08.02	ALLO	PBSC	AML	13
VS	M	A	07.11.01	28.12.10	ALLO	PBSC	AA	53
AP	M	A	07.11.01	15.07.02	ALLO	PBSC	NHL	54
HP	M	A	28.11.01	17.01.05	ALLO	PBSC	MM	59
GC	M	C	12.12.01	28.12.10	MUD	BM	AA	19
KB	M	C	12.12.01	28.12.10	ALLO	PBSC	AML	17
MK	F	C	19.12.01	28.12.10	ALLO	PBSC	SICKLE CELL ANAEMIA	11
PF	F	C	27.12.01	28.12.10	ALLO	PBSC	AA	18
CB	F	C	09.01.02	28.12.10	ALLO	PBSC	AML	19
CS	F	C	23.01.02	19.04.02	ALLO	PBSC	CML	13
AB	M	A	06.02.02	28.12.10	ALLO	PBSC	AML	45
MB	M	C	14.02.02.	28.12.10	MUD	PBSC	AA	14
LC	M	A	27.02.02	03.03.02	ALLO	PBSC	NHL	57
MA	M	A	06.03.02	28.12.10	MUD	PBSC	CML	62
LF	F	A	17.04.02	28.12.10	ALLO	PBSC	MM	51
TS	F	A	24.04.02	28.12.10	MUD	BM	AML	24
JS	M	C	30.04.02	02.11.03	ALLO	PBSC	NHL	11
BM	F	A	30.04.02	13.02.06	ALLO	PBSC	NHL	60
DS	F	A	09.05.02	28.12.10	MUD	BM	AML	36
MN	F	A	19.06.02	28.06.02	ALLO	PBSC	MM	57
DM	M	A	26.06.02	28.12.10	ALLO	PBSC	NHL	48
JK	M	C	17.07.02	28.12.10	ALLO	PBSC	CML	18
JM	M	A	28.08.02	23.03.04	ALLO	PBSC	AMYLOID	50
PB	M	A	11.09.02	06.10.04	ALLO	PBSC	MYELOFIBROSIS	55
CM	M	A	18.09.02	15.01.09	MUD	PBSC	MDS-RARS	61
JK	M	A	20.11.02	17.10.03	MUD	PBSC	HL	35

MC	F	A	04.12.02	20.04.03	MUD	PBSC	CML	47
AN	M	A	04.12.02	15.05.03	MUD	PBSC	AA	28
JI	M	A	11.12.02	29.05.03	MUD	PBSC	MM	42
SK	M	A	28.03.01	28.12.10	ALLO	PBSC	ALL	28
MC	M	A	18.12.02	28.12.10	MUD	PBSC	CML	53
JS	M	A	19.12.02	28.12.02	MUD	PBSC	AML	30
WS	M	A	16.01.03	05.08.03	MUD	PBSC	NHL	45
MH	F	A	29.01.03	13.05.03	ALLO	PBSC	NHL	56
CD	F	A	12.02.03	02.04.03	ALLO	PBSC	MM	47
AF	M	A	05.03.03	11.10.04	ALLO	PBSC	CML	32
JB	F	A	23.04.03	28.12.10	ALLO	PBSC	AML	45
ZR	M	A	24.04.03	31.10.03	ALLO	PBSC	ALL	30
MJ	M	A	30.04.03	16.05.03	ALLO	PBSC	MM	51
PM	M	A	07.05.03	28.12.10	MUD	PBSC	NHL	38
RC	M	A	02.07.03	17.11.03	ALLO	PBSC	NHL	52
AC	M	C	02.07.03	25.09.03	ALLO	PBSC	MDS-RAEB	11
CM	M	A	06.08.03	28.12.10	MUD	PBSC	AML	47
NB	M	C	03.09.03	28.12.10	MUD	PBSC	AA	17
BV	M	A	10.09.03	28.12.10	ALLO	PBSC	NHL	37
AM	M	A	10.09.03	28.12.10	ALLO	PBSC	CML	54
FB	M	A	08.10.03	28.12.10	MUD	PBSC	ALL	31
JK	M	A	15.10.03	17.10.03	MUD	PBSC	NHL	35
JE	M	C	16.11.03	20.01.04	MUD	PBSC	MDS-CMML	1
MP	F	A	10.12.03	19.12.03	MUD	PBSC	MM	52
JE	M	C	18.12.03	20.01.04	MUD	PBSC	MDS-CMML	1
BB	M	A	14.01.04	28.12.10	MUD	PBSC	CML	57
MM	F	C	21.01.04	18.03.04	MUD	PBSC	AML	4
ZR	F	A	18.02.04	03.03.04	MUD	PBSC	CML	32
TN	M	A	18.02.04	23.02.04	ALLO	PBSC	AA	32
LO	F	A	25.02.04	26.07.04	MUD	PBSC	CML	57
GT	M	A	03.03.04	15.11.07	MUD	PBSC	AML	55
LC	F	A	10.03.04	28.12.10	MUD	PBSC	FANCONI	31
AE	F	A	24.03.04	01.04.04	MUD	PBSC	MDS	36
DL	M	A	24.03.04	28.12.10	ALLO	PBSC	HL	22
MT	M	C	05.05.04	11.05.04	MUD	PBSC	ALL	4
MT	M	A	19.05.04	28.12.10	ALLO	PBSC	CML	48
JJ	M	C	27.05.04	30.05.04	MUD	BM	FANCONI	17
NB	F	A	20.08.04	19.10.04	MUD	BM	MDS	60
HW	F	C	15.09.04	08.01.06	ALLO	PBSC	AML	10
DT	M	A	03.11.04	16.08.05	MUD	PBSC	AML	32
DA	M	C	10.11.04	12.05.09	ALLO	PBSC	AA	8
AN	M	A	10.12.04	25.01.05	MUD	BM	CML	38
SG	M	A	15.12.04	28.12.10	MUD	BM	P VERA	37
PJ	M	A	12.01.05	12.01.05	MUD	PBSC	NHL	33
SF	M	A	26.01.05	28.12.10	MUD	PBSC	MDS	60
AN	M	A	25.01.05	25.01.05	MUD	PBSC	CML	38
EB	F	A	13.04.05	26.10.05	ALLO	PBSC	AML	54
LT	F	C	20.04.05	28.12.10	MUD	PBSC	FANCONI	10

CA	F	A	10.05.05	12.06.05	MUD	BM	AML	52
SN	F	A	28.07.05	28.12.10	MUD	BM	ALL	40
NM	F	A	28.09.05	28.12.10	ALLO	BM	AA	23
MH	F	A	12.10.05	28.12.10	MUD	PBSC	CML	28
LT	F	A	09.11.05	28.12.10	MUD	PBSC	AML	27
MS	F	A	30.11.05	05.07.07	ALLO	PBSC	AML	48
BW	M	A	30.12.05	28.12.10	MUD	PBSC	CML	21
GC	F	A	25.01.06	25.03.06	MUD	PBSC	CML	21
MD	M	A	22.02.06	28.12.10	ALLO	PBSC	CML	31
JL	M	A	08.03.06	03.09.06	ALLO	PBSC	NHL	49
PM	M	A	04.05.06	15.10.06	ALLO	PBSC	AML	44
JS	M	A	10.05.06	11.06.09	ALLO	PBSC	MM	48
GN	M	A	31.05.06	30.11.06	ALLO	PBSC	CML	35
ED	F	A	21.06.06	28.12.10	ALLO	PBSC	AML	34
LL	M	C	29.06.06	28.12.10	MUD	PBSC	AA	16
AS	M	C	19.07.06	13.06.07	ALLO	PBSC	AML	9
HM	F	A	16.08.06	05.03.07	MUD	PBSC	AML	43
CB	M	A	13.09.06	05.01.07	ALLO	PBSC	AML	51
LM	F	A	25.10.06	28.12.10	ALLO	PBSC	NHL	44
LC	F	C	01.11.06	28.12.10	ALLO	PBSC	AA	11
FM	M	A	29.11.06	03.06.07	MUD	PBSC	MYELOFIBROSIS	51
DH	M	C	23.03.07	03.02.08	MUD	CORD	WISKOTT-ALDICH	7
SA	F	A	28.03.07	28.12.10	ALLO	PBSC	AML	48
MH	F	A	04.04.07	07.04.07	MUD	CORD	FANCONI	39
CR	F	A	18.04.07	28.12.10	MUD	PBSC	AML	34
SG	M	A	27.07.07	28.12.10	MUD	PBSC	AML	41
JK	F	C	29.08.07	05.07.08	ALLO	PBSC	ALL	6
MK	F	C	10.10.07	28.12.10	ALLO	PBSC	SICKLE CELL ANAEMIA	17
CK	F	A	17.10.07	23.10.07	MUD	PBSC	AML	28
CH	M	A	30.11.07	15.08.08	ALLO	PBSC	CML	50
BB	F	C	05.12.07	07.01.10	MUD	PBSC	AA	14
KS	F	A	24.01.08	28.12.10	ALLO	PBSC	AML	52
AB	F	A	30.01.08	08.03.08	ALLO	PBSC	AML	56
RW	M	A	27.02.08	17.07.08	MUD	PBSC	CLL	63
JK	F	C	19.03.08	05.07.08	ALLO	PBSC	ALL	7
EC	F	A	07.05.08	28.12.10	ALLO	PBSC	CML	54
GB	M	A	23.10.08	28.12.10	MUD	BM	HL	35
JN	M	A	09.07.08	07.04.09	MUD	PBSC	CML	51
JJ	M	A	01.04.09	22.07.09	ALLO	PBSC	AML	43
EB	M	C	21.04.09	28.12.10	ALLO	PBSC	AA	20
CM	M	A	19.06.09	28.12.10	MUD	PBSC	AML	49
JK	M	C	01.07.09	28.12.10	MUD	PBSC	NHL	13
RM	M	A	29.07.09	30.08.09	MUD	CORD	CML	46
KJ	M	A	28.07.09	28.12.10	ALLO	PBSC	MDS	53
WH	M	A	04.08.09	11.10.09	ALLO	PBSC	MDS	59
EE	F	A	18.08.09	28.12.10	ALLO	PBSC	AML	50
JT	M	A	26.08.09	09.05.10	MUD	PBSC	MDS	62
RM	M	A	12.11.09	23.11.09	MUD	PBSC	NHL	55

CG	F	A	28.01.10	16.04.10	MUD	PBSC	AML	35
HL	M	A	09.03.10	26.08.10	MUD	PBSC	AML	59
CG	F	A	20.05.10	31.05.10	MUD	PBSC	AML	25
AJ	M	C	30.06.10	03.08.10	MUD	BM	AML	11
LV	M	A	05.08.10	28.12.10	MUD	BM	NHL	22
MB	F	A	08.09.10	28.12.10	ALLO	PBSC	AML	40
AL	F	C	04.11.10	28.12.10	MUD	PBSC	AA	19
JE	M	A	24.11.10	28.12.10	ALLO	PBSC	AML	58
SK	F	A	1.12.10	28.12.10	MUD	PBSC	MDS	21
ET	F	A	26.07.95	28.12.10	AUTO	PBSC	AML	30
GY	M	A	06.09.95	15.03.97	AUTO	PBSC	MM	48
GS	M	A	27.09.95	13.04.97	AUTO	PBSC	MM	55
JH	M	A	18.10.95	19.11.95	AUTO	PBSC	MM	52
DB	F	A	22.11.95	19.05.99	AUTO	PBSC	NHL	63
SC	F	A	29.11.95	21.08.97	AUTO	PBSC	AML	28
DP	M	C	05.01.96	17.05.04	AUTO	PBSC	ALL	5
HR	M	A	17.01.96	25.01.97	AUTO	PBSC	MM	62
MC	F	A	24.01.96	28.12.10	AUTO	PBSC	NHL	49
GB	M	A	13.03.96	28.12.10	AUTO	PBSC	AML	44
MR	M	A	27.03.96	28.12.10	AUTO	PBSC	AML	28
CD	F	A	11.04.96	03.04.01	AUTO	PBSC	AML	45
RG	F	C	02.05.96	28.09.99	AUTO	PBSC	ALL	6
CT	M	A	12.06.96	26.05.01	AUTO	PBSC	CML	54
HR	F	A	20.06.96	28.12.10	AUTO	PBSC	NHL	30
LP	F	A	26.06.96	28.12.10	AUTO	PBSC	NHL	48
VA	F	A	16.07.96	23.10.99	AUTO	PBSC	CML	40
CM	M	A	01.08.96	04.11.00	AUTO	PBSC	CML	39
AK	F	A	18.09.96	02.05.01	AUTO	PBSC	HL	26
AL	M	A	26.09.96	29.01.97	AUTO	PBSC	AML	41
JN	F	A	02.10.96	08.02.00	AUTO	PBSC	AML	22
KD	F	A	16.10.96	05.12.02	AUTO	PBSC	NHL	53
NW	M	A	11.12.96	28.12.10	AUTO	PBSC	MM	58
PP	M	A	16.01.97	28.12.10	AUTO	PBSC	AML	34
RW	M	A	29.01.97	28.12.10	AUTO	PBSC	ALL	32
SN	F	C	11.02.97	07.05.02	AUTO	PBSC	NEUROBLASTOMA	4
NO	M	C	19.02.97	28.12.10	AUTO	PBSC	CML	16
RS	F	A	26.02.97	05.09.04	AUTO	PBSC	CML	29
IP	F	C	19.03.97	04.05.98	AUTO	PBSC	ALL	5
FM	M	A	20.03.97	28.12.10	AUTO	PBSC	NHL	36
MD	F	A	02.05.97	12.07.99	AUTO	PBSC	NHL	42
JM	F	A	21.05.97	17.09.97	AUTO	PBSC	AML	37
PL	M	A	28.05.97	08.07.99	AUTO	PBSC	CML	23
GJ	F	A	04.06.97	28.12.10	AUTO	PBSC	NHL	38
LW	F	A	12.06.97	17.06.98	AUTO	PBSC	NHL	53
FW	M	A	19.06.97	28.12.10	AUTO	PBSC	AML	47
BE	M	A	03.07.97	28.12.10	AUTO	PBSC	AML	39
SW	M	C	30.07.97	28.12.10	AUTO	PBSC	CML	27
NK	M	A	31.07.97	28.12.10	AUTO	PBSC	AML	58

CB	M	A	06.08.97	28.12.10	AUTO	PBSC	AML	28
CS	M	A	08.10.67	28.12.10	AUTO	PBSC	ALL	40
GD	F	A	15.10.97	14.06.98	AUTO	PBSC	AML	24
VC	F	A	29.10.97	28.12.10	AUTO	PBSC	NHL	54
ST	M	A	21.11.97	28.09.99	AUTO	PBSC	AML	56
DT	F	C	03.12.97	18.04.98	AUTO	PBSC	NHL	9
JM	F	A	18.12.97	09.11.09	AUTO	PBSC	NHL	50
NJ	F	A	07.01.98	11.02.02	AUTO	PBSC	MDS	33
CD	F	C	08.01.98	29.06.99	AUTO	PBSC	ALL	13
SG	M	A	21.01.98	28.12.10	AUTO	PBSC	NHL	28
CC	M	C	05.02.98	23.03.99	AUTO	PBSC	ALL	16
WR	M	C	11.02.98	23.09.98	AUTO	PBSC	ALL	17
AS	F	A	11.03.98	21.03.98	AUTO	PBSC	NHL	59
DV	M	A	18.03.98	14.11.02	AUTO	PBSC	NHL	51
JR	F	A	25.03.98	19.04.98	AUTO	PBSC	CML	44
JU	F	A	01.04.98	28.12.10	AUTO	PBSC	NHL	43
MP	M	A	17.04.98	28.12.10	AUTO	PBSC	AML	55
JK	M	A	22.04.98	02.12.98	AUTO	PBSC	NHL	50
SW	F	A	29.04.98	20.08.98	AUTO	PBSC	AML	64
JM	F	A	03.06.98	28.12.10	AUTO	PBSC	NHL	57
JG	M	A	11.06.98	16.06.98	AUTO	PBSC	AMYLOID	58
DK	M	C	19.06.98	15.11.99	AUTO	PBSC	NEUROBLASTOMA	2
PR	M	A	18.06.98	28.12.10	AUTO	PBSC	NHL	26
WS	M	A	01.07.98	29.06.01	AUTO	PBSC	ALL	22
CT	M	A	16.09.98	28.12.10	AUTO	PBSC	NHL	31
DI	F	A	23.09.98	15.05.08	AUTO	PBSC	MDS	52
PM	F	A	23.09.98	28.12.10	AUTO	PBSC	AML	54
MK	F	A	14.10.98	28.05.00	AUTO	PBSC	HL	21
HO	M	A	21.10.98	28.12.10	AUTO	PBSC	AML	22
IM	F	A	28.10.98	28.12.10	AUTO	PBSC	MM	49
CH	M	C	28.10.98	07.02.99	AUTO	PBSC	HL	19
ES	F	A	04.11.98	28.12.10	AUTO	PBSC	AML	49
MN	M	A	11.11.98	21.12.99	AUTO	PBSC	HL	43
GC	M	A	18.11.98	18.01.06	AUTO	PBSC	NHL	22
DB	F	A	25.11.98	28.12.10	AUTO	PBSC	NHL	35
CW	F	A	09.12.98	23.12.98	AUTO	PBSC	CML	36
KM	M	A	23.12.98	09.02.05	AUTO	PBSC	NHL	42
SB	M	A	13.01.99	03.02.01	AUTO	PBSC	AML	35
NT	M	A	27.01.99	12.03.04	AUTO	PBSC	NHL	39
HM	F	A	03.02.99	28.12.10	AUTO	PBSC	NHL	53
SR	F	C	16.02.99	28.12.10	AUTO	PBSC	ALL	6
AT	M	A	17.02.99	28.11.01	AUTO	PBSC	NHL	62
BR	M	A	24.02.99	19.07.05	AUTO	PBSC	NHL	46
EV	F	A	14.04.99	26.06.03	AUTO	PBSC	AML	42
HS	F	C	21.04.99	28.12.10	AUTO	PBSC	NHL	20
RR	M	A	21.04.99	28.12.10	AUTO	PBSC	AML	39
VM	M	A	26.05.99	15.01.10	AUTO	PBSC	CML	42
FS	M	A	09.06.99	20.06.00	AUTO	PBSC	AML	38

SM	F	A	17.06.99	15.09.99	AUTO	PBSC	CML	41
AB	F	A	30.06.99	29.11.00	AUTO	PBSC	CML	41
MA	F	A	07.07.99	29.04.00	AUTO	PBSC	ALL	37
PF	M	A	14.07.99	28.12.10	AUTO	PBSC	CLL	58
ER	F	A	14.07.99	24.07.99	AUTO	PBSC	EWINGS SARCOMA	22
JB	F	A	21.07.99	18.01.02	AUTO	PBSC	NHL	37
LC	M	C	06.08.99	01.07.00	AUTO	PBSC	ALL	12
JD	M	C	12.08.99	30.08.04	AUTO	PBSC	HL	14
DK	F	A	18.08.99	28.12.10	AUTO	PBSC	NHL	38
AM	M	A	25.08.99	29.11.06	AUTO	PBSC	NHL	39
MR	F	A	01.09.99	10.07.03	AUTO	PBSC	AML	37
HA	M	C	08.09.99	18.06.02	AUTO	PBSC	NHL	20
MN	M	A	18.10.99	16.06.08	AUTO	PBSC	AMYLOID	55
WW	F	C	27.10.99	28.12.10	AUTO	PBSC	AML	14
JK	M	A	17.11.99	21.09.01	AUTO	PBSC	HL	31
BS	M	A	24.11.99	14.12.99	AUTO	PBSC	NHL	48
AX	F	C	09.02.00	28.12.10	AUTO	PBSC	AML	15
LD	M	A	23.02.00	18.07.01	AUTO	PBSC	NHL	95
MR	M	A	23.02.00	22.11.00	AUTO	PBSC	NHL	29
CZ	F	A	31.05.00	28.12.10	AUTO	PBSC	NHL	57
NL	M	A	14.06.00	28.12.10	AUTO	PBSC	NHL	56
GS	M	A	10.08.00	11.09.07	AUTO	PBSC	ALL	43
GW	M	A	16.08.00	06.08.04	AUTO	PBSC	ALL	29
NJ	M	C	07.12.00	28.12.10	AUTO	PBSC	NHL	16
JK	M	A	20.12.00	14.02.03	AUTO	PBSC	AML	61
RL	M	A	14.03.01	28.12.10	AUTO	PBSC	NHL	31
SC	M	A	30.05.01	28.12.10	AUTO	PBSC	NHL	33
AP	F	A	14.06.01	28.12.10	AUTO	PBSC	HL	32
CK	M	A	29.08.01	03.04.03	AUTO	PBSC	MYELOPROLIF	52
SR	M	C	07.11.01	28.12.10	AUTO	PBSC	NEUROBLASTOMA	2
ST	F	A	05.12.01	28.12.10	AUTO	PBSC	NHL	42
DC	M	A	27.02.02	10.09.03	AUTO	PBSC	NHL	51
JS	M	A	15.05.02	14.06.02	AUTO	PBSC	NHL	57
JV	M	A	15.05.02	28.12.10	AUTO	PBSC	AMYLOID	47
PB	M	A	24.07.02	12.09.07	AUTO	PBSC	AML	41
FB	M	A	10.07.02	01.06.04	AUTO	PBSC	HL	24
MM	F	C	07.08.02	29.10.02	AUTO	PBSC	AML	4
DB	F	C	07.08.02	05.09.03	AUTO	PBSC	NEUROBLASTOMA	4
HR	F	A	28.08.02	28.12.10	AUTO	PBSC	HL	22
DE	M	A	09.10.02	20.05.03	AUTO	PBSC	NHL	58
YC	F	A	11.12.02	28.12.10	AUTO	PBSC	NHL	51
NI	F	A	13.01.03	28.12.10	AUTO	PBSC	MM	57
JL	M	C	05.02.03	15.11.03	AUTO	PBSC	NEUROBLASTOMA	3
CJ	M	A	17.03.03	15.09.05	AUTO	PBSC	MM	41
MH	M	A	14.05.03	17.06.06	AUTO	PBSC	NHL	49
AT	F	A	28.05.03	27.12.05	AUTO	PBSC	MM	58
JL	M	A	03.09.03	26.10.03	AUTO	PBSC	AML	51
MB	M	A	20.10.03	28.12.10	AUTO	PBSC	MM	63

AC	M	A	17.12.03	28.12.10	AUTO	PBSC	AML	45
PS	M	A	20.02.04	15.10.05	AUTO	PBSC	MM	44
PJ	M	A	05.05.04	24.01.05	AUTO	PBSC	NHL	42
ML	F	A	14.05.04	02.06.06	AUTO	PBSC	MM	56
LM	M	A	16.02.05	28.12.10	AUTO	PBSC	NHL	28
HF	F	A	23.02.05	05.03.05	AUTO	PBSC	HL	50
CB	M	A	13.04.05	28.12.10	AUTO	PBSC	HL	28
DS	M	A	13.06.05	18.04.06	AUTO	PBSC	MM	40
GO	M	A	14.06.05	28.12.10	AUTO	PBSC	NHL	58
CS	F	A	10.08.05	16.02.06	AUTO	PBSC	NHL	22
RS	M	A	17.08.05	28.12.10	AUTO	PBSC	NHL	60
AD	F	A	07.09.05	28.12.10	AUTO	PBSC	NHL	37
JH	M	A	13.12.05	22.03.06	AUTO	PBSC	MM	48
EF	M	A	22.12.05	28.12.10	AUTO	PBSC	MM	57
IS	M	A	11.01.06	28.12.10	AUTO	PBSC	NHL	50
SW	F	A	04.07.06	28.12.10	AUTO	PBSC	MM	54
MS	M	C	01.11.06	28.12.10	AUTO	PBSC	HL	9
JW	M	A	11.12.06	15.08.07	AUTO	PBSC	NHL	36
DG	F	A	13.12.06	16.12.07	AUTO	PBSC	MM	44
CK	M	C	24.01.07	28.12.10	AUTO	PBSC	HL	16
DH	M	A	20.06.07	28.12.10	AUTO	PBSC	HL	42
AB	M	A	27.06.07	28.12.10	AUTO	PBSC	NHL	39
LA	F	A	19.07.07	25.08.07	AUTO	PBSC	NHL	60
CA	F	A	19.09.07	28.12.10	AUTO	PBSC	AML	40
CE	M	A	19.10.07	13.05.08	AUTO	PBSC	AML	39
MG	M	C	12.12.07	28.12.10	AUTO	PBSC	NHL	14
PS	M	A	09.04.08	18.11.08	AUTO	PBSC	AML	27
IH	F	A	16.04.08	24.04.08	AUTO	PBSC	MM	58
IP	F	A	03.09.08	28.12.10	AUTO	PBSC	NHL	22
NJ	F	A	19.11.08	28.12.10	AUTO	PBSC	AML	27
SG	F	A	18.02.09	20.08.09	AUTO	PBSC	ALL	32
CB	M	A	02.03.09	15.03.09	AUTO	PBSC	NHL	60
KS	M	C	19.05.09	28.12.10	AUTO	PBSC	NHL	19
LS	F	A	27.08.09	28.12.10	AUTO	PBSC	HL	25
NA	M	A	16.09.09	16.10.09	AUTO	PBSC	NHL	44
AS	M	A	23.09.09	28.12.10	AUTO	PBSC	NHL	37
KL	F	C	07.12.09	28.12.10	AUTO	PBSC	HL	13
MB	M	A	11.02.10	28.12.10	AUTO	PBSC	NHL	39
GC	M	A	21.04.10	28.12.10	AUTO	PBSC	MM	54
MK	F	A	18.08.10	28.12.10	AUTO	PBSC	NHL	63
TW	M	A	02.09.10	28.12.10	AUTO	PBSC	MM	55
PB	F	A	08.11.10	28.12.10	AUTO	PBSC	AML	46

ANNEXURE 2

DEMOGRAPHIC DATA ON TRANSPLANTED VOLUNTEERS FOR THE LATE COMPLICATIONS STUDY

NUMBER	DLS	SEX	RACE	DOB	DOT	DISEASE	TYPE
WH2125	20.8.12	F	w	13.2.45	23.5.01	CML	ALLO
WHA9715	27.8.12	M	W	26.3.60	30.7.97	CML	AUTO
WH2263	28.8.12	F	W	16.2.85	13.1.11	PNH	ALLO
WH2246	28.8.12	F	W	26.4.83	9.1.02	AML	ALLO
WH2343	3.9.12	M	W	12.10.76	23.10.08	HD	ALLO
WH2138	3.9.12	M	w	12.5.64	24.10.01	CML	ALLO
WH9908	4.9.12	F	W	2.4.79	21.4.99	NHL	AUTO
WH2309	10.9.12	F	W	1.7.64	28.7.05	ALL	ALLO
WHA2309	10.9.12	F	W	9.6.43	29.10.97	NHL	ALLO
WH2321	11.9.12	F	W	25.3.72	21.6.06	AML	ALLO
WH2342	11.9.12	F	W	21.9.54	7.5.08	CML	ALLO
WH2280	17.9.12	M	W	19.8.56	6.8.03	AML	ALLO
WH2277	17.9.12	M	W	9.3.65	5.7.03	NHL	ALLO
WH9821	18.9.12	M	W	22.12.40	30.12.98	NHL	ALLO
WH2258	18.9.12	M	W	22.8.54	26.6.02	NHL	ALLO
U2044	25.9.12	F	C	11.1.58	7.9.05	NHL	AUTO
U2056	25.9.12	F	W	17.5.67	19.9.07	AML	AUTO
WH9704	1.10.12	F	W	15.6.41	5.2.97	NHL	ALLO
WH2350	1.10.12	M	W	11.10.56	28.7.09	MDS	ALLO
U2065	2.10.12	F	C	7.12.83	27.8.09	HD	AUTO
WHA9809	8.10.12	F	C	22.8.55	1.4.98	NHL	AUTO
WH2259	8.10.12	M	W	29.12.83	17.7.02	CML	ALLO
WHA2061	8.10.12	F	C	30.7.86	3.9.08	NHD	AUTO
WH2256	9.10.12	F	W	1.7.66	9.5.02	AML	ALLO
WH2101	15.10.12	F	W	31.7.60	5.1.01	CML	ALLO
WHA9716	15.10.12	M	W	19.5.39	31.7.97	APL	AUTO
WH9814	16.10.12	M	W	30.5.66	13.8.98	APL	ALLO
WHA9909	16.10.12	M	W	22.1.60	21.4.99	AML	AUTO
WH2128	23.10.12	M	W	24.1.75	11.7.01	PNH	ALLO
WH2293	23.10.12	M	W	13.4.73	10.3.04	FA	ALLO
WH2330	29.10.12	F	W	16.3.62	28.3.07	AML	ALLO
WHA9810	29.10.12	M	W	29.3.43	17.4.98	AML	AUTO
U2037	30.10.12	M	W	11.6.77	16.2.05	NHL	AUTO
WHA9823	30.10.12	F	W	27.6.49	28.10.98	MM	AUTO
WHA9529	5.11.12	M	W	16.1.76	21.10.98	APL	AUTO
WHA9828	5.11.12	F	W	13.2.53	25.11.98	NHL	ALLO
WH2347	5.11.12	M	W	20.1.50	19.6.09	AML	ALLO
WH2305	6.11.12	M	W	20.10.44	26.1.05	MDS	ALLO
U2032	6.11.12	M	W	9.9.40	20.10.03	MM	AUTO
U9704	6.11.12	M	W	16.8.81	20.5.03	CML	ALLO
U2046	12.11.12	M	W	25.8.48	22.12.05	MM	AUTO
WH2288	12.11.12	M	W	21.7.47	14.1.04	CML	ALLO
WH2253	13.11.12	F	W	16.4.78	24.4.02	AML	ALLO

WH2268	19.11.12	M	W	2.3.50	18.12.02	CML	ALLO
WHA9718	19.11.12	M	W	26.3.57	8.10.97	ALL	AUTO
WH2285	21.11.12	M	W	20.2.72	8.10.03	ALL	ALLO
WH2311	23.11.12	F	W	23.2.77	13.10.05	CML	ALLO
U2047	4.2.13	M	W	16.3.55	11.1.06	NHL	AUTO
U2062	4.2.13	F	C	12.9.81	19.11.08	AML	AUTO
WH2361	12.2.13	M	W	2.12.51	24.11.10	AML	ALLO
WH9717	18.2.13	M	W	10.11.47	20.8.97	CML	ALLO
WH2284	18.2.13	M	W	7.1.50	10.9.03	CML	ALLO
U2067	25.2.13	M	W	10.12.71	23.9.09	NHL	AUTO
U2054	5.2.13	M	W	16.7.86	27.6.07	NHL	AUTO
WH2359	4.2.2013	F	W	19.2.70	8.9.2010	AML	ALLO

ANNEXURE 3

KEY TO CAPTURE DEMOGRAPHIC DATA ON ALL TRANSPLANT PATIENTS (n=468)

# - IDENTIFIER					
Number	Initials	Age	DOB	Gender (Depicted by M or F)	Race (Depicted by B, C, A or W)

Δ - DIAGNOSIS							
	a	b	c	d	e	f	g
1. Fanconi							
2. Aplasia							
3. MDS	RA	RARS	RAEB	CMML	Blastic		
4. AML	Secondary	FAB 1	FAB 2	FAB 3	FAB 4	FAB 5	
5. ALL	B lineage	T lineage	Low risk	Intermediate	High risk	Refractory	Relapse
6. CML							
7. CLL	B lineage	T lineage	RAI 1	RAI 2	RAI 3	RAI 4	
8. HL	NLP	NS	MC	LD	CL		
9. NHL	HCL	LL	Follicular	DLBC	Burkitt	T-lineage	Other
10. Myeloma	Durie/Salmon 1	Durie/Salmon 2	Durie/Salmon 3	Durie/Salmon 4			
11. Miscellaneous							

R – TREATMENT						
	a	b	c	d	e	f
1. Fanconi	Androgen	Steroids	NIL			
2. AA	Androgen	Steroids	NIL	ALG	Cyclosporin	
3. MDS	HLL	Flag	CTR IV	CTR V	Erythropoietin	GCSF
4. AML	CTR IV	CTR V	ETI	RA	Other	
5. ALL	Low risk	Intermediate	High			
6. CML	Imatinib	Mini-ICE	Other	Hydrea®		
7. CLL	Chlorambucil	RFC	MFC	Other		
8. HL	ABVD	Cologne	Salvage	Other		
9. NHL	Rituximab	COP	CHOP/CNOP	DHAC	R ICE	Other
10. Myeloma	Melphalan Medrol	Thalidomide	Bortezomib	VECD	Other	
11. Miscellaneous						

T – TRANSPLANT						
	a	b	C	d	e	f
1. Date	Auto	Allo	MUD	Cord	BM	Blood

O - OUTCOME					
	a	b	c	d	e
1. Date	LTFU	Death	CR	PR	Relapse

C - COMPLICATIONS									
	a	b	c	d	e	f	g	h	i
1. Date	Lung	Skeleton	Kidney	Heart	GIT	Skin	Rejection	GVHD	CNS

I – INFECTIONS					
	a	b	c	d	e
1. Date	Bacterial	Viral	Fungal	HIV	Other

EXAMPLE OF SYMBOLS USED TO GENERATE THE DATABASE FOR ANALYSIS

	#	Δ	R	T	O	C	I
001	GB 53 1.3.43 fw	10	a	b c	b c		a
				11.9.98	26.5.05		1.9.96
002	RN 12 13.11.84	1	9b	bf	c		a 16.10.96
				9.10.96	28.10.10		26.10.96 14.11.96

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