EVALUATION OF THE EFFECT OF LOW AND INTERMEDIATE FREQUENCY ELECTROMAGNETIC WAVES ON RADIOSENSITIVITY

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Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Nuclear Medicine (Radiobiology) in the Faculty of Medicine and Health Sciences at Stellenbosch University

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December 2016

Declaration

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

Signature: Date: December 2016

Abstract

The incidence of epidemic Kaposi's sarcoma in HIV/AIDS patients is high due to their compromised immune system. HIV-positive individuals presenting with cancer tend to be more sensitive to ionising radiation and are at a higher risk of developing severe side effects during radiotherapy, and there is a need to develop non-invasive methods to preferentially sensitise cancer cells and reduce therapeutic doses.

Here, the effects of 100 and 1000 Hz electromagnetic fields (EMF) broadcast via an argon plasma ray tube at 50 W on the radiosensitivity of apparently normal Chinese hamster lung fibroblasts (V79) and human malignant melanoma cells (MeWo) were evaluated using the colony forming assay. Pre-exposure of the fibroblasts to both fields had no effect on their radiosensitivity, if X-ray irradiation followed within 2 h or at 6 h. Significant radiosensitisation was observed when X-rays were administered 4 h after EMF exposure. For the MeWo cells, pre-exposure to 100 Hz resulted in a significant radioprotection when irradiation followed within 6 h. However, treatment of these cells with a 1000 Hz field significantly potentiated the effect of X-rays. When cells were irradiated prior to EMF exposure, the V79 cells were marginally protected by the 100 Hz field and sensitised by the 1000 Hz field. In contrast, the melanoma cells were slightly protected by the 1000 Hz field and sensitised by the 100 Hz field.

The survival rate of the normal fibroblasts when treated with 2 Gy, in two fractions of 1 Gy 6 h apart, was similar to those obtained when cells received an acute dose of 2 Gy 6 h prior to or after exposure to both EMF frequencies. On the other hand, the melanoma cells were significantly sensitised when they were either treated with a

combination of X-rays and then 100 Hz EMF 6 h later or with a combination of either of the EMF frequencies and then X-rays 6 h later.

These data suggest that use of electromagnetic fields may sensitise tumours to radiation therapy and reduce normal tissue toxicity. Informed and well-designed combinations of low-medium frequency electromagnetic fields and radiation therapy might be beneficial in the management of cancers, especially epidemic Kaposi's sarcoma.

Opsomming

Die insidensie van epidemiese Kaposi-sarkoom in pasiënte met MIV/VIGS is hoog weens die gekompromitteerde immuunstelsel. MIV-positiewe persone met kanker neig om meer gevoelig te wees vir ioniserende bestraling en loop die hoër risiko om ernstige nuwe-effekte op te doen gedurende bestraling. Daar is dus ‘n behoefte om nie-indringende metodes te ontwikkel wat kankerselle by voorkeur meer kwesbaar maak en daardeur terapeutiese bestralingsdosisse te kan verminder.

Die navorsing illustreer die gevolge van blootstelling aan 100Hz en 1000Hz elektromagnetiese velde (EMV), geproduseer deur ‘n argon-plasmastraalbuis van 50W, op die radiosensitiwiteit van skynbaar normale long-fibroblaste (V79) van Chinese hamsters en mens melanoomselle (MeWo), gemeet deur gebruik te maak van kolonievormende toetse. Fibroblaste, vooraf blootgestel aan 100Hz en 1000Hz elektromagnetiese velde, het geen uitwerking teen bestraling getoon indien die bestraling binne 2 en 6 uur plaasgevind het nie. Aansienlike radiosensitiwiteit is waargeneem toe X-strale aangewend is 4 uur na EMV-blootstelling. MeWo-selle vooraf blootgestel aan 100Hz EMV en binne 6 uur opgevolg met bestraling, het opmerklike sensitisering vir bestraling getoon. Behandeling van hierdie selle met ‘n 1000Hz-veldsterkte het egter die effek van X-strale aansienlik versterk. Toe selle bestraal is voor EMV-blootstelling, is die V79-selle marginaal deur die 100Hz-veld beskerm en gesensitiseer deur die 1000Hz-veld. In teenstelling is die melanoomselle tot ‘n mate beskerm deur die 1000Hz-veld en gesensitiseer deur die 100Hz-veld.

Die oorlewingstempo van die normale fibroblaste behandel met 2Gy, in twee fraksies van 1Gy, ses uur na mekaar, was soortgelyk aan dié toe selle ‘n akute dosis van

2Gy ontvang het ses uur voor of na hul blootstelling aan beide EMV-frekwensies. In teenstelling, is die melanoomselle merkwaardig gesensitiseer toe hul behandel is met ‘n kombinasie van X-strale en dan 100Hz EMV ses uur later, of met ‘n kombinasie van die EMV-frekwensies en X-strale ses uur later.

Hierdie data suggereer dat die gebruik van elektromagnetiese velde tumore gevoelig mag maak vir stralingsterapie, terwyl dit toksisiteit vir normale weefsel verminder. Ingeligte en weldeurdagte kombinasies van laagmediumfrekwensie elektromagnetiese velde en stralingsterapie mag moontlik voordelig wees vir die behandeling van maligniteite, veral epidemiese Kaposi-sarkoom.

Acknowledgements

Firstly, I would like to express my sincere gratitude to my supervisors Prof Akudugu and Dr Serafin who were willing to go out of their way to help me make this thesis a success.

I would also like to thank my father, Mr SD Chinhengo, for teaching me to value education and to be persistent towards this worthy goal.

To my mother, Mrs C Chinhengo, I say thank you for all your support and your prayers.

I am also grateful to all my siblings (Alice, Alec, Esher, Beauty, Abu, Abi, Beaula, Fai, Bule, Fari and Janet) for encouraging me and believing in me.

To my children, Tawananyasha and Tinevimbo, I would like to say thank you for being my reasons to keep pushing. I appreciate and love you a lot.

To my friends and extended family all over the world and my fellow students in the department, I thank you for all your support.

Last, but not the least, financial assistance from the Faculty of Medicine and Health Sciences (Stellenbosch University), the Harry Crossley Foundation, and the South African National Research Foundation (NRF) is acknowledged.

Opinions expressed and the conclusions arrived at in this dissertation are those of the author, and are not necessarily to be attributed to the Faculty of Medicine and Health Sciences (Stellenbosch University), the NRF or the Harry Crossley Foundation.

Dedications

I dedicate this study to my:

- ⇨ parents
- ⇨ siblings
- ⇨ children

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α:	linear coefficient of inactivation after X-ray irradiation.
β:	quadratic coefficient of inactivation after X-ray irradiation.
AIDS:	acquired immune deficiency syndrome.
AKT:	serine-threonine protein kinase.
BAX:	BCL-2-associated X protein.
BCL-2:	B-cell lymphoma-2.
BCL-XL:	B-cell lymphoma-extra large.
CO_2:	carbon dioxide.
DNA:	deoxyribonucleic acid.
egr-1:	early growth response protein 1.
ELF-EMF:	extremely low frequency electromagnetic field(s).
EMF:	electromagnetic field(s).
HAART:	highly active antiretroviral therapy.
HIV:	human immunodeficiency virus.
IGF:	insulin-like growth factor.
IL:	interleukin.
KS:	Kaposi's sarcoma.
LQ:	linear-quadratic.
MEM:	minimum essential medium.
MF:	modifying factor.
mTOR:	mammalian target of rapamycin.
MTT:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
$n_{cell}(t)$:	number of cells seeded in treated culture.
$n_{cell}(u)$:	number of cells seeded in untreated culture.
$n_{col}(t)$:	number of colonies in treated sample.
$n_{col}(u)$:	number of colonies in untreated sample.
p21:	phosphoprotein 21.
p53:	phosphoprotein 53.
PI3K:	phosphoinositide 3-kinase.
RI:	recovery index.

ROS: reactive oxygen species.

RT: radiation therapy.

SEM: standard error of the mean.

SF: surviving fraction.

SF_2: surviving fraction at 2 Gy.

TAT: trans-activator of transcription.

TNF: tumour necrosis factor.

VEGF: vascular endothelial growth factor.

VEGFR: vascular endothelial growth factor receptor.

CHAPTER 1

1.1. Introduction

Kaposi's sarcoma (KS) is a skin cancer caused by human herpes virus 8 (HHV8) that develops from the cells that line lymph or blood vessels (Jacobson *et al*., 2000). KS usually appears as tumours on the skin or on mucosal surfaces such as inside the mouth, but tumours can also develop in other parts of the body. Kaposi's sarcomas can feature as cutaneous lesions with or without internal involvement. The high prevalence of HIV/AIDS in sub-Saharan Africa has resulted in a corresponding rise in the frequency of HIV/AIDS patients presenting with a co-morbidity of Kaposi's sarcoma (epidemic KS). Epidemic KS lesions often rapidly progress to plaques and nodules affecting the upper trunk, face, and oral mucosa. KS is now considered as an "AIDS defining" illness. HIV-positive patients are at a greater risk of cancer than the general population due to a compromised immune system (Kaminuma *et al*., 2010). A weak immune system allows cancer to spread faster in HIV-positive patients than it normally would. Non-epidemic Kaposi's sarcoma is ranked the 6th and 8th most common cancer in South African males and females, respectively (Jemal *et al*., 2012). With the increasing burden of HIV/AIDS, the rank of KS, as a disease entity, could be even higher. There is, therefore, an urgent need to develop novel and effective treatment approaches for Kaposi's sarcoma, especially in HIV/AIDS patients.

The treatment of epidemic Kaposi's sarcoma, as for any other cancer, is largely influenced by factors like disease site and extent, symptoms, and overall patient condition. Epidemic KS may be treated by several treatment modalities. The first

step in the treatment of epidemic KS should be optimal control of the HIV infection, using highly active antiretroviral therapy (HAART) which is known to reduce the severity of KS in AIDS patients (Cattelan *et al*., 2005). HAART alone, however, may not reverse progression of KS and other treatments are necessary e.g. surgery, chemotherapy, radiation therapy and biological therapy. The highest response rate is found with radiation therapy, however, due to their compromised immune system, HIV/AIDS patients may not fully recover from the side effects of radiation therapy as they may have an inadequate capacity to repair the damage induced by radiation (Berson *et al*., 1990). Radiation therapy can also weaken the immune system, and so, patients with HIV/AIDS may not be able to complete the full course of treatment without risking severe side effects, such as life threatening infections. Therefore, alternative treatment modalities are needed for epidemic Kaposi's sarcoma.

In light of the current rise in HIV infection and cancer diagnosis in HIV-positive individuals, many factors and many methods are in use or being investigated to influence radiation sensitivity. One such approach is electromagnetic therapy which involves the use of radio waves and other electromagnetic waves. There has been an increase in research on the interaction between extremely low frequency electromagnetic fields (ELF EMF) and various biological processes since the reported association between childhood leukaemia and nearby electrical transmission and distribution equipment (Lee *et al*., 2015). EMFs are shown to have an adverse effect on cells, but, as with ionising radiation which is known to be carcinogenic and is also used in radiotherapy, EMFs may have an anticancer effect on cancer cells (Verginadis *et al*., 2012). Combination therapy options may lead to a reduction in the amount of radiation delivered to a patient during treatment, thus

reducing normal tissue toxicity. Pioneering studies over half a century ago demonstrated that although radiosensitivity can be altered using modifying agents, a given modifying agent does not always change the sensitivity of different cell lines to radiation exposure in the same way (Goodrich, 1943). The phenomenon has recently been observed whereby dual inhibition of phosphoinositide 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) was found to radiosensitise prostate and breast cancer cells, but acted as a radioprotector in normal prostate cells and mouse gut (Potiron *et al*., 2013; Hamunyela *et al*., 2015; Maleka *et al*., 2015). The main objective of radiotherapy is to kill tumour cells, or stop their proliferation, whilst protecting normal tissue. Due to an increase in the diagnosis of cancer there has been an increased desire to develop novel treatment modalities. Non-ionising electromagnetic fields from extremely low frequency to radiofrequency have been shown to affect living cells even at low intensities. Some of these effects may be applied in medical treatments. The effects of ELF-EMF depend upon frequency, amplitude, and length of exposure, and are also related to the intrinsic susceptibility and responsiveness of different cell types. Although the mechanism of this interaction is still obscure, ELF-EMF can influence cell proliferation, differentiation, cell cycle, apoptosis, DNA replication and protein expression (Lucia, 2016; Restrepo *et al*., 2016). It has also been reported that EMFs in conjunction with chemotherapeutic agents can reverse the resistance of cancer cells (Verginadis *et al*., 2012; Janigro *et al*., 2006), with minimal or no side effects, inhibit disease progression and prolong patient survival (Barbault *et al*., 2009; Kirson *et al*., 2007; Verginadis *et al*., 2012).

It is, therefore, possible that low or medium frequency electromagnetic fields could potentiate the cytotoxic effects of ionising radiation. The objectives of this study were to: (1) test whether the anti-proliferative and anticancer effects of radio frequencies can enhance the effectiveness of radiation therapy; and (2) compare the outcomes of conventional fractionated radiation therapy and the proposed therapeutic approach of combining electromagnetic fields with radiation therapy.

1.2. Literature Review

1.2.1. Epidemiology

Kaposi's sarcoma (KS) was first described by Moritz Kaposi in 1872 (Kaposi, 1872). Since then, four variants of KS have been described, namely, classic/Mediterranean, African/endemic, post transplantation, and epidemic/AIDS-associated. Epidemic KS was first discovered in 1981 in males who had had sex with other males and it is more common in individuals with HIV/AIDS (Beral *et al*., 1990; Krigel and Friedman-Kien, 1990). Epidemic KS is usually marked by the appearance of widespread lesions in different parts of the body, sometimes causing painful swelling of the feet and lower legs. Examples of various types of Kaposi's sarcoma are presented in Figures 1.1 and 1.2 (Hengge *et al*., 2002). Given that Kaposi's sarcoma, and especially epidemic KS, tends to be markedly disseminated and rarely localised, multiple treatment approaches are desirable in order to effectively manage the disease.

Figure1.1: Different forms of KS. **A**: classic KS; **B**: epidemic KS; **C**: immunosuppression KS; **D**: endemic African KS (Hengge *et al*., 2002).

Figure 1.2: Manifestations of epidemic KS. **A**: multiple livid, irregular papules and plaques. **B**: purple-black nodules and tumours on the chest in Christmas tree-like distribution along Blaschko's lines. **C**: mucous membrane involvement with reddish tumours and livid plaques on the upper gum. **D**: well demarcated plaque on the tip of the glans penis (Hengge *et al*., 2002).

1.2.2. Gene Involvement in Epidemic Kaposi's Sarcoma

Kaposi's sarcoma is initially a cytokine-driven proliferative process. Cytokine receptors, including interleukins 1, 2, 6 and 8, and tumour necrosis factor have been demonstrated on KS spindle cells (Cianfrocca and Roenn, 1998; Miles, 1993). Studies have also suggested that the trans-activator of transcription (TAT) gene of the HIV activates the vascular endothelial growth factor receptor 2 on KS cells (Ganju *et al*., 1988; Morini *et al*., 2000; Deregibus *et al*., 2002). Deregibus *et al*. found that, in Kaposi's sarcoma cells, the TAT gene enhances transcription of several anti-apoptotic genes, such as, serine-threonine protein kinase-1 (AKT-1), AKT-2, anti-apoptotic B-cell lymphoma-2 (BCL-2), B-cell lymphoma-extra large (BCL-XL), and insulin-like growth factor 1 (IGF-1). Even though the pro-apoptotic genes of the BCL family, such as BAX and BAD, are upregulated in Kaposi's sarcoma, the overall effect is protection from apoptosis. It is also suggested that the phosphoinositide 3-kinase (PI3K)/AKT pathway is involved in TAT-mediated anti-apoptotic effects (Deregibus *et al*., 2002). The HIV TAT gene product is mitogenic for KS spindle cells *in vitro* and may promote the development and progression of epidemic KS (Ensoli *et al*., 1994; Cianfrocca and Roenn, 1998). These factors can, therefore, lead to treatment resistance in Kaposi's sarcoma, necessitating the development of new and more effective therapeutic approaches.

1.2.3. Treatment Options for Epidemic Kaposi's Sarcoma

1.2.3.1. Radiation Therapy

The highest treatment response rate in Kaposi's sarcoma patients is obtained with radiation therapy. However, due to a compromised immune system HIV/AIDS patients may experience significantly higher level side effects, as they may present with an inadequate capacity to repair radiation-induced damage (Berson *et al*., 1990). Some investigators suggest a single dose of 8 Gy as optimal for all cases of cutaneous KS (Berson *et al*., 1990; Stelzer and Griffin, 1993). Such a high dose may cause high tissue toxicity which HIV/AIDS patients may not recover from. Conventional fractionated radiation therapy (given as 20 Gy in 10 fractions or 40 Gy in 20 fractions) has also been used to treat KS in HIV/AIDS patients, and it was noted that acute toxicity increased with increasing radiation dose (Stelzer and Griffin, 1993). On the other hand, a single dose of 8 Gy causes less normal tissue toxicity than the conventional regimens. Electron beam therapy (given as 4 Gy weekly fractions) has also been used in patients with widespread skin involvement and has been found to be effective in controlling disease (Nobler *et al*., 1987; Cooper, 1991; Escalon and Hagemeister, 2006). As the number of HIV/AIDS patients presenting with KS is on the increase and patients are expected to exhibit lower levels of treatment tolerance compared to their non-AIDS infected counterparts, combining radiation therapy with other modalities might be beneficial for epidemic Kaposi's sarcoma patients.

1.2.3.2. Other Therapies

Other therapeutic endeavours may be summarised as follows (Escalon and Hagemeister, 2006): Surgical excision alone, or in combination with laser therapies can be used to palliate small superficial lesions, but this method may not be as effective in deep bulky lesions. Cryotherapy may be a useful treatment option for small facial lesions less than 1 cm in diameter, but is not ideal for large deep lesions. Topical retinoids which are available commercially and generally well-tolerated may also be used to treat Kaposi's sarcoma, but may cause local erythema and irritation. There has also been a significant level of interest in targeting of the vascular endothelial growth factor (VEGF) and the mammalian target of rapamycin (mTOR) pathways with inhibitors as a regimen for treating Kaposi's sarcoma.

1.2.4. Medical Applications and Biological Effects of Electromagnetic Fields

Electromagnetic fields (EMF) have been used to successfully treat ailments, such as, wounds, bone fractures, and depression (Bassett *et al*., 1981; Bassett, 1985; Artacho-Cordón *et al*., 2013; Cheing *et al*., 2014). There is a significant level of evidence to show that electromagnetic fields, such as electric, magnetic, and radiofrequency (RF) fields, in conjunction with chemotherapeutic agents, can reverse the resistance of cancer cells (Janigro *et al*., 2006; Verginadis *et al*., 2012; Tofani, 2015), as well as, synergistically cause genetic effects (Cho *et al*., 2007; Koyama *et al*., 2008; Markkanen *et al*., 2008). Most entities that interact synergistically with EMFs are mutagens, and thus the synergism between EMFs and X-rays should be

considered more seriously. Electromagnetic fields have been shown to inhibit disease progression and prolong patient survival with minimal or no side effects (Kirson *et al*., 2007; Barbault *et al*., 2009; Verginadis *et al*., 2012).

In vitro studies have also shown that extremely low frequency magnetic fields can affect cell death processes like apoptosis (Simkó *et al*., 1998; Tofani *et al*., 2001; Sarimov *et al*., 2005; Crocetti *et al*., 2013; Tofani, 2015). Magnetic fields penetrate cells unattenuated and can thus interact directly with the DNA in the nucleus and other cell constituents (Blank and Goodman, 2009). There is overwhelming evidence supporting the opinion that exposure to magnetic fields has an effect on cellular functions, such as, transcription, protein synthesis, proliferation, and differentiation. Cellular exposure to magnetic field intensities of 0.38-19 mT has been shown to lead to increased transcription of c-myc and histone H2A (Goodman and Henderson, 1991). This can significantly impact on the net cellular response to the field exposure. While c-myc plays an important role in cell cycle regulation and cell death, histone H2A is central in DNA damage repair.

Although apoptotic cell death has been shown to occur in WiDr cells at field intensities greater than 1.0 mT, tumour regression in nude mice bearing WiDr tumours was evident only at much higher intensities (Tofani *et al*., 2001). Anti-tumour and immune modulatory activity has also been demonstrated in mouse melanoma and hepatocellular carcinoma models for magnetic fields of 0.25 and 0.4 T, respectively (Yamaguchi *et al*., 2006; Nie *et al*., 2013). The retardation of tumour growth by electromagnetic fields appears to be correlated with their capacity to suppress tumour vascularisation (Cameron *et al*., 2014). Acute exposure to field

intensities below 1.0 mT does not exhibit anti-proliferative activity, but results in increased level of reactive oxygen species (ROS) (Morabito *et al*., 2010), which may ultimately mediate cellular responses to other cytotoxic agents like chemotherapeutic drugs and ionising radiation.

Electric fields with intensities of 1.0–1.4 V/cm can alter the cell membrane structure leading to changes in the permeability of ions, such as Ca^{2+}, cause changes in the local pH and temperature, reorganise cytoskeletal components, and disrupt microtubule polymerisation (Kirson *et al*., 2004; Sulpizio *et al*., 2011; Lee *et al*., 2015). Exposing cells to electric fields can also cause modifications in gene expression and free radical production which affects DNA structure and provokes strand-breaks and other chromosomal aberrations, such as micronucleus formation and oxidative response (Kirson *et al*., 2004; Wolf *et al*., 2005; Vijayalaxmi and Prihoda, 2009; Buldak *et al*., 2012; Artacho-Cordón *et al*., 2013; Deng *et al*., 2013; Luukkonen *et al*., 2014; Mattsson and Simkó, 2014; Lee *et al*., 2015; Li *et al*., 2015). In addition, electric fields can physically affect the movement and orientation of electrically charged molecular entities (Singh *et al*., 1997; Menéndez, 1999). EMF exposure can also lead to an increase in the density of phosphorylated receptors on the surfaces of cell membranes (Del Monache *et al*., 2008). The membrane is believed to be the primary site for electromagnetic field interaction (Singh *et al*., 1997).

An extremely low frequency magnetic field of 1.0 mT has been suggested to induce immune cell activation through three different pathways, namely, the classical activation, the alternative activation and the lectin-dependent activation pathways

(Lupke *et al*., 2006). The classical activation pathway includes activation of inflammatory responses, destruction of extracellular matrix and induction of apoptosis. The alternative activation pathway promotes extracellular matrix construction, cell proliferation, resolves inflammation, and angiogenesis. The lectin-dependent activation pathway also initiates inflammation and apoptosis and inhibits cell growth in a way comparable to the classical activation (Lupke *et al*., 2006). All the perturbations exerted by electromagnetic fields ultimately exert anti-proliferative and anticancer effects by influencing cell cycle progression, the rate of cell proliferation, differentiation, tubulin polymerisation, antioxidant activity and apoptosis (Kirson *et al*., 2004; 2007; Wolf *et al*., 2005; Polaniak *et al*., 2010; Zimmerman *et al*., 2013; Artacho-Cordón *et al*., 2013; Butters *et al*., 2014; Lee *et al*., 2015; Ross *et al*., 2015).

Other reports have indicated that EMFs may induce genetic effects without the involvement of free radicals (Ferreira *et al*., 2006; Alcaraz *et al*., 2014; Furtado-Filho *et al*., 2013). EMF exposure has been shown to increase the expression levels of the VEGF receptor, KDR/Flk-1, in normal human umbilical vein endothelial cells (Del Monache *et al*., 2008). Intermittent powerline magnetic field exposure to 5 min ON/30 min OFF cycles at a flux density of 2.3 mT for 6 h also resulted in a significant upregulation of p21 and egr-1 mRNA levels in p53-deficient, but not wild-type cells (Czyz *et al*., 2003). Electromagnetic fields have also been shown to modulate the activity of hormones, antibodies, neurotransmitters and oncogenes at their surface receptor sites (Singh *et al*., 1997). These perturbations of important biomolecules by electromagnetic fields could act as precursors for better response of cancer to other therapies.

The aforementioned therapeutic potential of electromagnetic fields, notwithstanding the application of plasma ray tubes (the so-called Rife Frequency Generator) in the treatment of cancer, largely remains a controversial issue. Over two decades ago, the American Cancer Society discouraged the use of devices, such as the Rife frequency generator for cancer therapy, due to paucity of experimental and scientific evidence (American Cancer Society, 1994). However, the concept of targeting pro-survival genes with characteristic resonant frequencies broadcast from a Rife device to induce cell death was recently demonstrated in a colon cancer cell line (Agulan *et al*., 2015). Also, a significant level of evidence exists for effectively targeting malignancies with cancer-specific radiofrequency electromagnetic fields (Zimmerman *et al*., 2013).

1.2.5. Rationale for Using Electromagnetic Fields for Cancer Therapy

Cellular exposure to a 60 Hz magnetic field can cause DNA single and double strand-breaks (Lai and Singh, 1997a), DNA-protein and DNA-DNA crosslinks (Singh and Lai, 1998), and increased apoptosis (Lai and Singh, 2004). Removal of iron from cellular systems using iron chelators have been shown to eliminate the cytotoxic effects of magnetic fields (Lai and Singh, 1997b). The aforementioned findings led to the proposal that magnetic fields generate free radicals via the Fenton reaction as depicted in Figure 1.3 (Lai and Singh, 2010). In this reaction, the interaction between an EMF and hydrogen peroxide released from mitochondria is catalysed by iron to yield highly reactive species, such as the hydroxyl radical ($OH^{\bullet}$). These species, if not adequately picked up by scavengers, can damage DNA by causing single or

double strand-breaks (Lai and Singh, 2010; Ruediger, 2009). Cancer cells, by virtue of their high rate of proliferation, have a much higher rate of iron intake than their normal counterparts (Lai and Singh, 2010). As the production of free radicals is correlated to iron content, it is conceivable that cancer cells would be more responsive to magnetic field exposure than normal cells. Therefore, the use of electromagnetic fields in cancer therapy might a viable option.

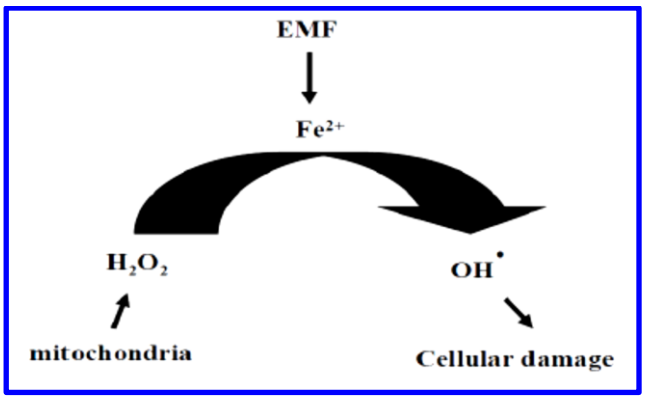

Figure 1.3: The Fenton reaction: conversion of hydrogen peroxide (H_2O_2) into a highly reactive hydroxyl radical ($OH^{\bullet}$) in the presence of iron (Lai and Singh, 2010).

1.3. Problem Statement

Kaposi's sarcoma (KS) is a skin cancer caused by human herpes virus 8 that develops from the cells that line lymph or blood vessels. Currently, the incidence of KS co-morbidity in HIV/AIDS patients is high due to their compromised immune system. HIV-positive individuals presenting with cancer are treated in much the same way as those who do not have HIV through surgery, chemotherapy, radiation therapy and biological therapy. These therapeutic approaches can put HIV-positive cancer patients at a higher risk of developing severe side effects that they may not recover from. Radiotherapy, in particular, has proven to be the most effective regimen for managing Kaposi's sarcoma. However, given that HIV-positive patients by virtue of their compromised immune system tend to experience significantly higher levels of treatment related toxicity, there is an urgent need to develop non-invasive methods to sensitise cancer cells (or tumours) to therapeutic doses of ionising radiation. Such procedures should significantly lower the doses of radiation required to yield a given level of tumour control and, therefore, reduce toxicity to normal tissue.

1.4. Hypothesis

It is hypothesised that *in vitro* exposure of cancer cells to low or intermediate frequencies of electromagnetic waves (e.g. radio-frequency waves) can preferentially increase their radiosensitivity.

1.5. Aims and Objectives

This study aimed to assess the potential therapeutic benefit of combining electromagnetic fields with radiation therapy in an *in vitro* setting, using lung fibroblasts and melanoma cells.

To achieve this specific aim, the study objectives are as follows**:**

1. To determine the radiosensitivity of Chinese hamster lung fibroblasts (V79) and human melanoma cells (MeWo) following acute and fractionated irradiation.
2. Compare cellular response to acute and fractionated X-ray irradiation, in order to establish capacity of cell lines to repair radiation-induced damage.
3. To evaluate the influence of radio-frequency electromagnetic fields (EMF) on radiosensitivity, in order to determine the EMF frequency, sequence, and time interval relative to X-ray irradiation to yield the highest and least radiosensitisation in the melanoma cells (MeWo) and fibroblasts (V79), respectively.
4. To compare results from the best combination of EMF exposure and X-ray irradiation with those from fractionated irradiation.

CHAPTER 2

2. Materials and Methods

2.1. Cell Lines and Culture

V79

The V79 cell line (ATCC® Number: CCL-93™) was established from the lung of a Chinese hamster. These cells have a fibroblast-like morphology and were used to represent normal tissue. The culture was obtained from Flow Laboratories (Irvine, Scotland). The cells were cultivated as monolayers in 75 cm^2 flasks in Minimum Essential Medium (MEM) supplemented with 10% foetal bovine serum, penicillin (100 U/ml), streptomycin (100 µg/ml) and incubated at 37°C in a humidified atmosphere (95% air, 5% CO_2). Cells were used for experiments upon reaching 80-90% confluence.

MeWo

The human melanoma cell line (MeWo) (ATCC® Number: HTB-65™) was kindly provided by F. Zölzer and C. Streffer (University of Essen, Germany). The cells were cultivated as monolayers in 75 cm^2 flasks in minimum essential medium (MEM) supplemented with 20% foetal bovine serum, penicillin (100 U/ml), streptomycin (100 µg/ml) and incubated at 37°C in a humidified atmosphere (95% air, 5% CO_2). Cells were used for experiments upon reaching 80-90% confluence.

2.2. Cell Culture Irradiation and Clonogenic Cell Survival

Exponentially growing cell cultures were trypsinised into single-cell suspensions and seeded in varying numbers into 25 cm^2 tissue culture flasks, dependent on the cell line and the level of absorbed radiation dose. The final volume of culture medium in each flask was 10 ml. Table 2.1 summarises the cell numbers that were plated for each dose and cell line. For each experiment and dose point, triplicate cell culture flasks were prepared.

Table 2.1: Summary of number of cells seeded at each radiation dose of X-rays delivered acutely.

Cell line	0 Gy	1 Gy	2 Gy	4 Gy	6 Gy	8 Gy	10 Gy
V79	300	300	300	1000	4000	6000	7000
MeWo	300	300	500	2000	4000	5000	6000

The cells were left to settle for 3 h (for V79) and 4 h (for MeWo) after which they were irradiated with X-rays. Cell culture irradiation was performed at room temperature (20°C) at a dose rate of 1 Gy/min, using a Faxitron MultiRad 160 X-ray irradiator (Faxitron Bioptics, Tucson, AZ, USA; Figure 2.1). Sham-irradiated cultures (0 Gy cultures) were left on the turntable of the Faxitron X-ray irradiator for 2 min with the X-ray source turned off, and were used as controls. The cell cultures were then left in an incubator at 37°C for 7 and 14 days (for V79 and MeWo, respectively) for colony formation.

Figure 2.1: Photograph of the Faxitron MultiRad 160 X-ray irradiator (door opened) showing cell culture flasks on the turntable.

To terminate cultures, the growth medium was decanted and colonies were washed with phosphate buffered saline, fixed in glacial acetic acid:methanol:water (1:1:8, v/v/v), stained in 0.01% amido black in fixative, washed in tap water, air-dried, and counted using a stereoscopic microscope (Nikon, Japan; Model #: SMZ-1B). Colonies containing at least 50 cells were deemed to have originated from single surviving cells and were scored. Cytotoxicity was assessed on the basis of a surviving fraction (*SF*) which was calculated from the relation: $SF=n_{col}(t)/\{[n_{col}(u)/n_{cell}(u)]\times n_{cell}(t)\}$, where $n_{col}(t)$ and $n_{col}(u)$ denote the number of

colonies counted in treated and untreated samples, respectively. $n_{cell}(t)$ and $n_{cell}(u)$ are the number of cells seeded in treated and untreated cultures, respectively. Three independent experiments were performed for each cell line. To generate clonogenic cell survival curves, the determined mean surviving fractions were fitted to the linear-quadratic (LQ) model of the form:

$$SF = \exp[-\alpha D - \beta D^2] \qquad (1)$$

α and *β* are the linear and quadratic cell inactivation constants, respectively, and *D* is the absorbed dose in Gy. Cellular radiosensitivity was expressed in terms of the surviving fraction at 2 Gy (SF_2).

2.3. Radiation Dose Fractionation Assay

To evaluate the capacity of the cell lines to recover from radiation insult, cells were seeded into 25 cm^2 tissue culture flasks in numbers as depicted in Table 2.2 for subsequent irradiation to 2, 3, and 4 Gy, and the flasks were split into two sets. After allowing cells to attach, one set of flasks received two fractions of 1, 1.5, and 2 Gy 6 h apart, while the other set received 2, 3, and 4 Gy, respectively, as a single fraction. The time interval of 6 h was chosen as it is the optimum period used in the clinic when two fractions are to be administered in a day. Sham-irradiated cultures (0 Gy cultures) were used as controls. For each experiment and dose point, triplicate cell culture flasks were prepared. After colony formation, the cultures were terminated and the colonies were scored to determine the surviving fractions (*SF*) at the total

doses administered. Cellular capacity to recover from radiation damage (repair capacity) was expressed in terms of a recovery index as follows:

$$Recovery\ Index = \frac{SF(fractionated\ dose)}{SF(acute\ dose)} \quad (2).$$

Table 2.2: Summary of number of cells seeded at each radiation dose of X-rays delivered acutely or as a split dose.

Cell line	0 Gy	2 Gy	3 Gy	4 Gy
V79	200	200	300	300
MeWo	400	400	400	400

2.4. Experimental Set-Up for Electromagnetic Field Generation and Exposure

Electromagnetic fields were generated using an EMEM oscillator amplifier, to produce 27.125 MHz fields, square-wave amplitude-modulated at 100 or 1000 Hz, with a peak-to-peak amplitude of 5 V (EMEM Devices Rife Machine, Model #: 1-2012B, Boulder, CO, USA). The modulating frequencies were generated using a GME frequency generator with an output impedance of 50 Ω and a duty cycle of 50% (GME Technology, Model #: SG-10, Pomona, CA, USA). The resulting radiofrequency (RF) was then broadcast via a double bubble argon plasma ray tube (length = 25 cm; external diameter = 6.7 cm). The set-up for EMF exposure of cell cultures through a plasma Rife tube is illustrated in Figure 2.2. A maximum of 24 cell culture flasks could be exposed at a given time, and were stacked in groups of four,

such that the outside dimensions of the volume occupied by the cell culture layers was 11 cm (Width: 2 flasks breadthwise) × 18 cm (Length: 2 flasks lengthwise) × 14 mm (Height: 6 flasks by height). The perpendicular distances from the axis of the plasma tube to the cell culture planes were 10.0, 12.4, 14.8, 17.2, 19.6, and 22.0 cm. Each cell layer was covered with 3.5 mm (10 ml) of culture medium.

To estimate the magnetic and induced electric fields in the cell cultures, the plasma ray tube was assumed to function as an antenna that is transmitting at ~27.12 MHz. Near field magnetic field strengths for this frequency can vary between 0.5 A/m (magnetic flux density of 0.63 μT) and 0.8 A/m (magnetic flux density of 1.0 μT) at a radial distance of 12 cm from the antenna (Mantiply *et al*., 1997). Therefore, by adopting the maximum magnetic flux density of 1.0 μT as the peak flux density in the plane 12 cm from the axis of the plasma tube (Figure 2.2B), the magnetic flux densities in cell culture planes at 10.0 to 22.0 cm were deduced using the inverse-square law.

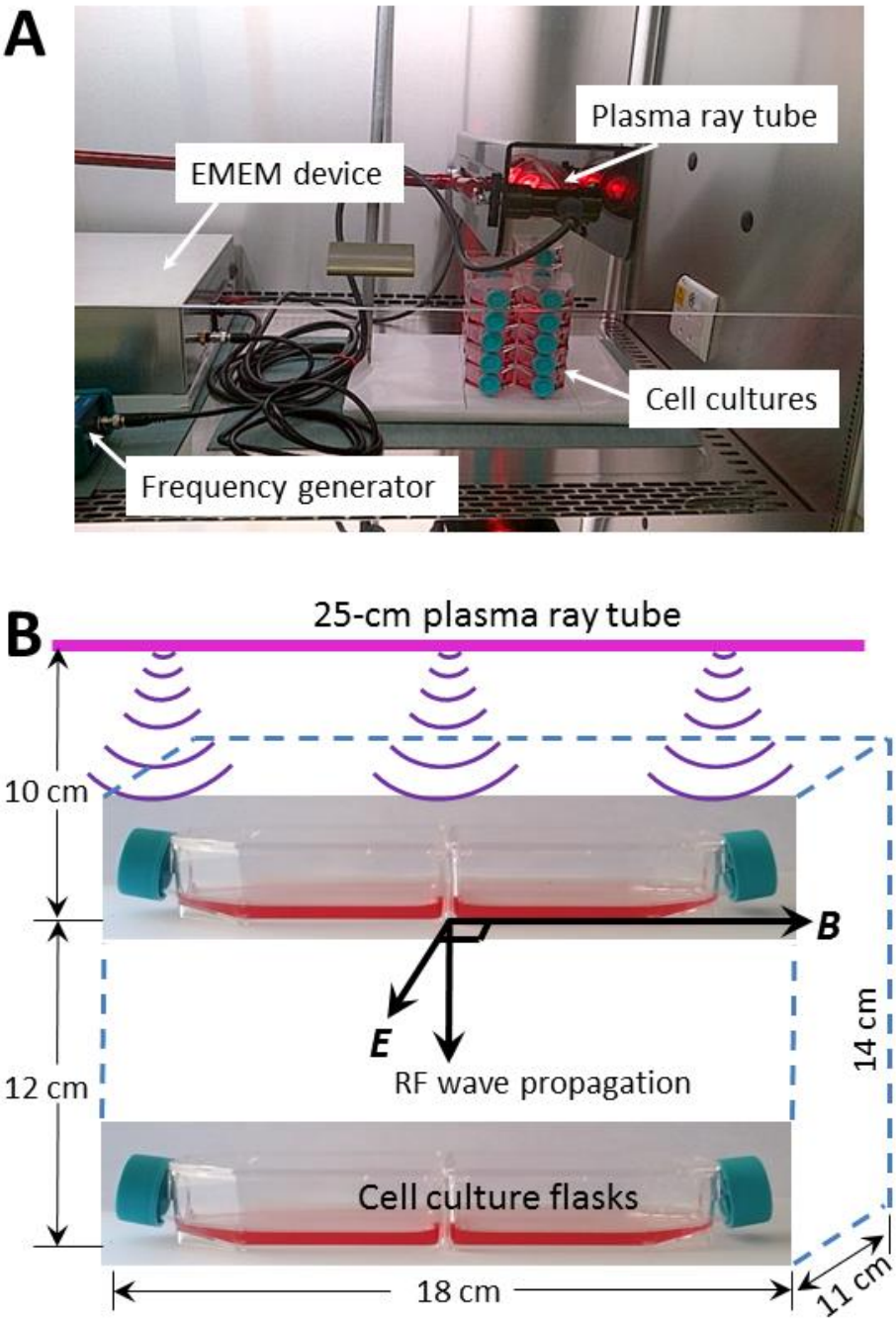

Figure 2.2: (A) Photograph of the electromagnetic field (EMF) exposure system. (B) A 2-dimensional schematic diagram showing the top and bottom cell culture planes of the 2×2×6 flask matrix. In the set-up, the plasma ray tube is centred horizontally above the cell culture flasks, such that the induced magnetic field (*B*) is parallel to the base of a flask and the induced electric field (*E*) in the culture medium is parallel to the width of the flask.

The corresponding induced peak electric fields (V/m) were then calculated as $E_{peak} = 2h\pi fB$ (Bassen *et al*., 1992), where B is the peak value peak magnetic flux density (T), f is the transmitted frequency in (27.125×10^6 Hz), and $2h$ is the depth of the cell culture medium (0.0035 m). Thus, the estimated magnetic flux densities in the cell cultures ranged from 0.30 to 1.44 μT, and the corresponding peak electric fields varied between 0.09 and 0.42 V/m (Table 2.3).

Table 2.3: Estimated peak magnetic flux density (*B*), electric field (*E*), and current density (*J*) induced at a distance (*d*) from the axis of a 25-cm plasma ray tube.

d (cm)	*B* (μT)	*E* (V/m)	*J* (A/m^2)
10.0	1.44	0.42	0.63
12.4	0.94	0.28	0.42
14.8	0.66	0.20	0.30
17.2	0.49	0.15	0.23
19.2	0.39	0.12	0.18
22.0	0.30	0.09	0.14

Using a conductivity (σ) of 1.5 S/m for the cell culture medium (Bassen *et al*., 1992), induced current densities (J) were calculated from the relation $J = \sigma E$. Estimated current densities in cell cultures ranged from 0.14 to 0.63 A/m^2 (Table 2.3). Since the ratio of the depth to the width (0.05 m) of the culture medium in each flask is less than 0.3, estimation of peak electric fields from the magnetic flux densities has an uncertainty of ≤1% (Bassen *et al*., 1992). For sham-EMF exposure (0 Hz), the control samples were treated as described with the plasma ray tube turned off.

To test whether the radial variation in induced magnetic flux density across the cell culture layers had an impact on cell viability, the proportions of seeded cells that eventually form colonies (plating efficiencies) were determined in cell cultures placed at the different radial distances, as in Figure 2.2, for 0, 100, and 1000 Hz exposures. In the current setting, no significant frequency- and location-dependent differences in plating efficiency were observed. For the V79 cells, the plating efficiency at 0 Hz (73 ± 4%) did not differ significantly from those at 100 Hz (82 ± 3%; P = 0.12) and 1000 Hz (73 ± 5%; P = 0.94). Similarly, the plating efficiency for sham exposed MeWo cells (55 ± 4%) was not significantly different from those determined when the cells were exposed to 100 Hz (62 ± 7%; P = 0.30) and 1000 Hz (57 ± 6%; P = 0.82).

2.5. Radiomodulatory Effects of Electromagnetic Frequencies

To investigate the influence of EMF exposure on radiosensitivity, stock cell cultures were trypsinised and 200-500 cells seeded per 25 cm^2 tissue culture flask and left to settle for 3-4 h (depending on the cell line). The cells were subsequently exposed to an electromagnetic field as described under Section 2.4 for 30 min prior to or following an X-irradiation of 2 Gy at time points of 0, 0.5, 1, 2, 4, and 6 h. The final volume of culture medium in each flask was 10 ml. For each experiment and dose point, triplicate cell culture flasks were prepared. Unirradiated cultures with and without electromagnetic field exposure were used as controls for EMF and X-ray treatment, respectively. For sham-EMF exposure, unirradiated cell cultures were treated as described in Figure 2.2 with the plasma ray tube turned off. Three independent experiments were performed for each time point and experimental arm.

Radiosensitivity was expressed in terms of the surviving fraction at 2 Gy. The interaction between EMF and X-rays was expressed as a modifying factor (*MF*), given as the ratio of surviving fractions at 2 Gy in the absence and presence of EMF:

$$MF = \frac{SF(2\ Gy)}{SF(EMF{+}2\ Gy)} \qquad (3).$$

The criteria for inhibition, no effect, and enhancement of radiosensitivity by EMFs are MF<1.0, MF=1.0, and MF>1.0, respectively.

2.6. Statistical Analysis

Statistical analyses were performed using the GraphPad Prism (GraphPad Software, San Diego, CA, USA) computer program. To compare two data sets, the unpaired two-sided *t*-test was used. A *P*-value of less than 0.05 indicates a statistically significant difference between the data sets. Data were presented as the mean (±SEM) from at least 3 independent experiments. For each experiment, 3 replicates were assessed.

CHAPTER 3

3. Results

3.1. Radiosensitivity

The cellular radiosensitivity was determined using the clonogenic cell survival assay. Cell survival data for the V79 and MeWo cell lines were fitted to the linear quadratic model (Equation 1) and are presented in Figure 3.1.

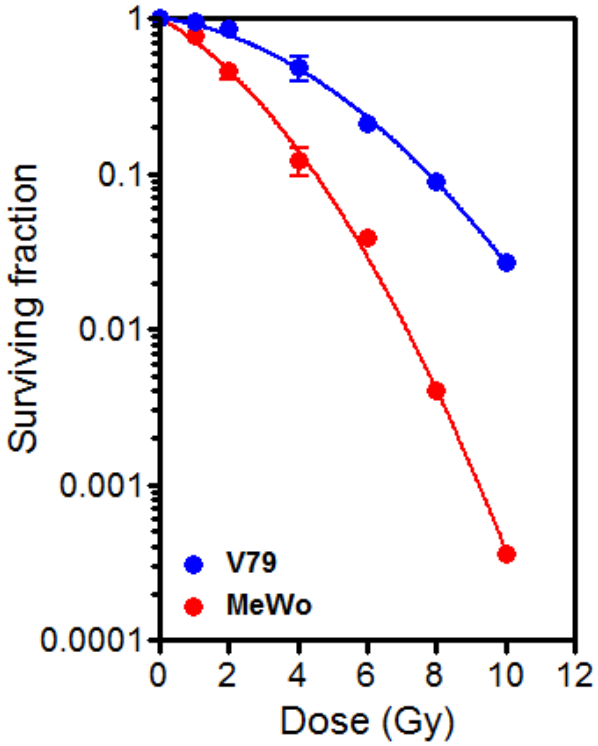

Figure 3.1: Clonogenic survival curve for the V79 and MeWo cell lines after X-ray irradiation. The survival curve was obtained by fitting experimental data to the linear-quadratic model (Equation 1).

From the dose-response curves, it is apparent that the MeWo cell line is more radiosensitive than the V79 cell line. This is consistent with the relatively steeper

survival curve for MeWo than for V79. The linear and quadratic coefficients of cell inactivation (α- and β-coefficients) for the MeWo and V79 cells were found to be 0.29 ± 0.04 Gy^{-1} and 0.050 ± 0.005 Gy^{-2} and 0.07 ± 0.02 Gy^{-1} and 0.029 ± 0.003 Gy^{-2}, respectively (Table 3.1). The larger α-coefficient of the MeWo cells also reflects their higher radiosensitivity relative to the V79 cells. For a direct dose-related comparison, intrinsic cellular radiosensitivity was expressed in terms of the surviving fraction at 2 Gy (SF_2). The V79 cell line was found to be more radioresistant than the MeWo cell line. The SF_2-values emerged as 0.77 ± 0.04 and 0.45 ± 0.04 for V79 and MeWo, respectively, and were significantly different (P = 0.0318).

Table 3.1: Summary of radiobiological parameters for Chinese hamster lung fibroblasts (V79) and human melanoma cells (MeWo). SF_2 denotes the surviving fraction at 2 Gy. α and β are the linear and quadratic coefficients of cell inactivation, respectively. Data are presented as the mean ± SEM from 3 independent experiments.

Cell line	SF_2	α (Gy^{-1})	β (Gy^{-2})
V79	0.77 ± 0.04	0.07 ± 0.02	0.029 ± 0.003
MeWo	0.45 ± 0.04	0.29 ± 0.04	0.050 ± 0.005

3.3. Cellular Response to Radiation Dose Fractionation

The data presented in Figure 3.2 show that the V79 cells had a better survival when they were exposed to 2 and 3 Gy, given in two fractions 6 h apart than when they were irradiated with a single full dose. However, the differences in the rates of

recovery at these doses were not statistically significant (P = 0.1016 for 2 Gy; P = 0.0765 for 3 Gy). At a total dose of 4 Gy, no difference was apparent between cell survival for the fractionated and single dose deliveries (P = 0.2379). The recovery indices (RI) as defined by Equation 2 emerged as 1.17, 1.18 and 1.04 for total doses of 2, 3 and 4 Gy, respectively (Table 3.2). This further proves that the net recovery of these cells from damage induced by the first fraction prior to delivery of the second is minimal.

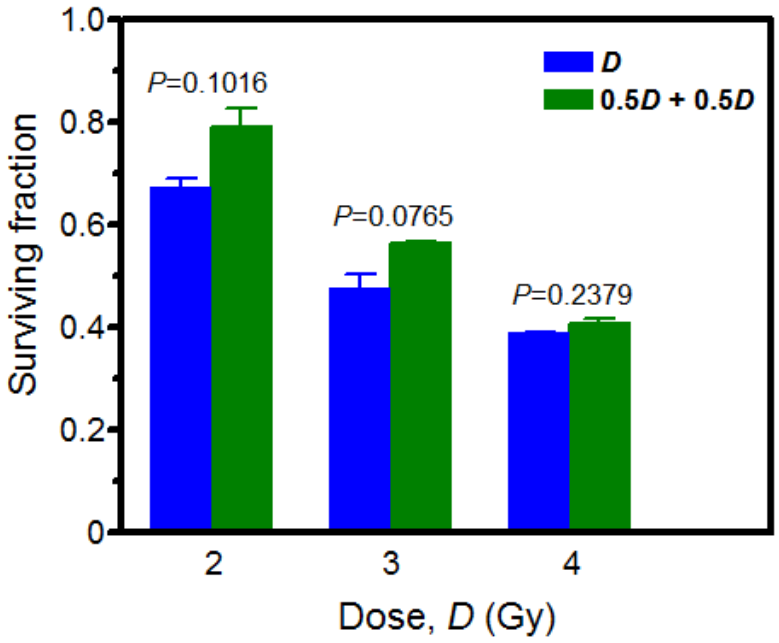

Figure 3.2. Surviving fractions for Chinese hamster lung fibroblasts (V79) following X-ray irradiation, either as acute doses (D Gy) or split doses (0.5D Gy per fraction) given 6 h apart.

Similarly, the melanoma cells (MeWo) show a better survival rate when exposed to split doses, 6 h apart, than for acute irradiation (Figure 3.3). This recovery phenomenon was seen at total doses of 2, 3, and 4 Gy, although the differences in cell survival between split and acute dose delivery were statistically significant for the

higher doses (P = 0.0046 for 3 Gy; P = 0.03 for 4 Gy). The corresponding recovery indices at total doses of 2, 3, and 4 Gy were 1.18, 1.61, and 1.48 (Table 3.2). The relatively larger *RI*-values of the melanoma cells (MeWo) is an indication that these cells recover more efficiently than the lung fibroblasts (V79).

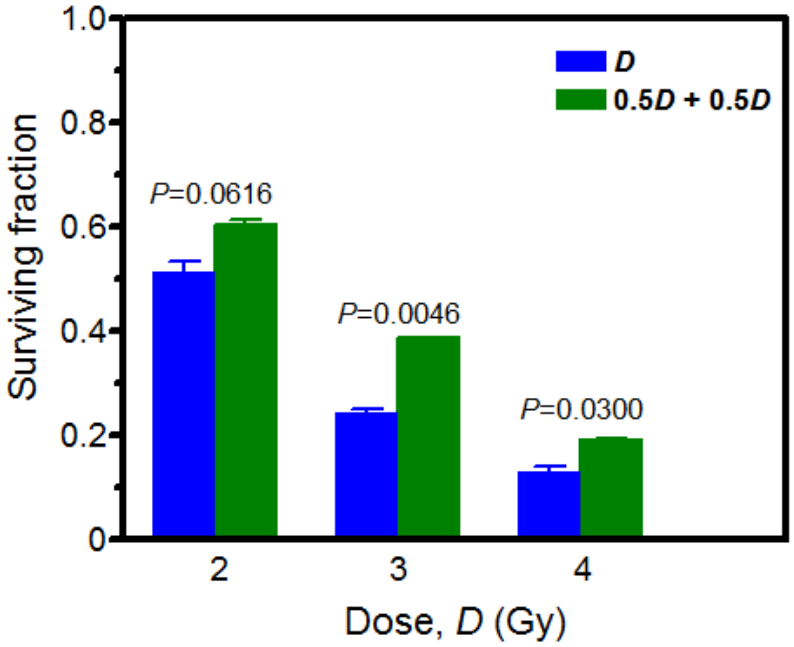

Figure 3.3: Surviving fractions for human melanoma cells (MeWo) following X-ray irradiation, either as acute doses (D Gy) or split doses (0.5D Gy per fraction) given 6 h apart.

Table 3.2: Summary of radiosensitivity and recovery data for Chinese hamster lung fibroblasts (V79) and human melanoma cells (MeWo), following acute and fractionated (two fractions with 6-hour interval) irradiation. *SF* and *RI* denote the surviving fractions and cell recovery indices, respectively (Equation 2).

Cell line	Treatment	*SF* *	*RI* #
V79	2 Gy	0.673 ± 0.014	
	1 Gy + 1 Gy	0.788 ± 0.037	1.17 ± 0.06
	3 Gy	0.475 ± 0.025	
	1.5 Gy + 1.5 Gy	0.562 ± 0.003	1.18 ± 0.06
	4 Gy	0.388 ± 0.001	
	2 Gy + 2 Gy	0.405 ± 0.010	1.04 ± 0.03
MeWo	2 Gy	0.511 ± 0.022	
	1 Gy + 1 Gy	0.602 ± 0.010	1.18 ± 0.05
	3 Gy	0.240 ± 0.010	
	1.5 Gy + 1.5 Gy	0.386 ± 0.001	1.61 ± 0.07
	4 Gy	0.128 ± 0.011	
	2 Gy + 2 Gy	0.190 ± 0.002	1.48 ± 0.13

*Mean ± SEM. #Mean ± error: errors were calculated using appropriate error propagation formulae.

3.3. Modulation of Radiosensitivity by Electromagnetic Fields

Radiosensitivity was expressed in terms of the surviving fraction at 2 Gy. Figure 3.4 shows the relationship between radiosensitivity of the Chinese hamster lung fibroblasts (V79) and the time of X-ray treatment after electromagnetic field (EMF)

exposure. For time intervals of 0−2 h, exposure to fields of 100 and 1000 Hz appeared to have no effect on radiosensitivity, with a modifying factor of ~0.99 as shown in Table 3.3. No effect on radiosensitivity was also apparent when cells were irradiated 6 h after EMF exposure (Figure 3.4). However, the cells were marginally sensitised when X-ray irradiation occurred 4 h after EMF treatment, giving modifying factors of 1.08 ± 0.11 and 1.22 ± 0.22 for the 100 and 1000 Hz fields, respectively.

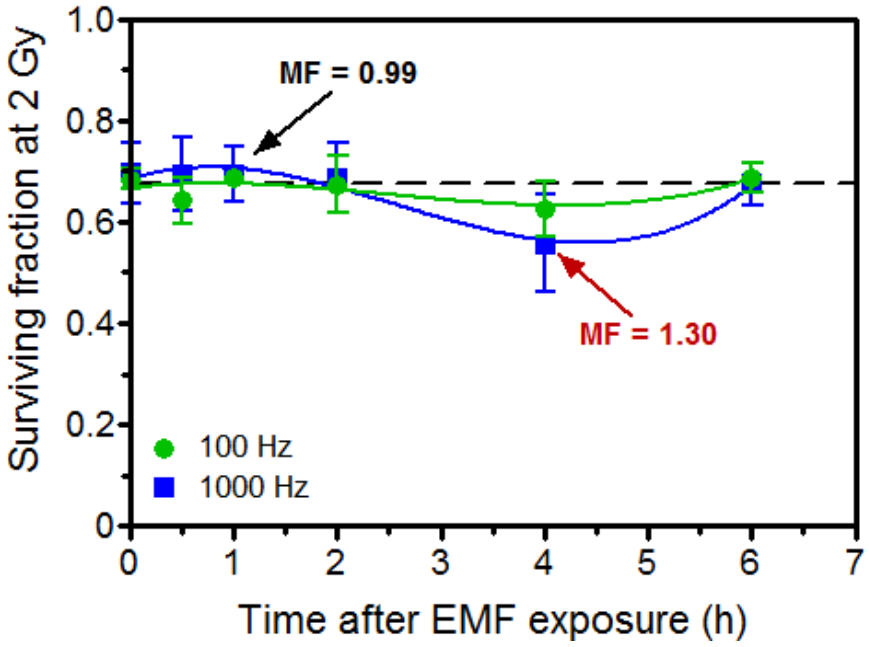

Figure 3.4: Clonogenic cell survival at 2 Gy in Chinese hamster lung fibroblasts (V79), when cells were exposed to a 100 or 1000 Hz electromagnetic field (EMF) prior to X-irradiation, as a function of time between EMF exposure and X-ray treatment. Data points are means ± SEM of 3 independent experiments. Horizontal dashed line represents the surviving fraction at 2 Gy without EMF exposure.

Irradiating V79 cells to 2 Gy prior to exposure to a 100 Hz field yielded a small radioprotection, while a 1000 Hz field exposure resulted in a slight radiosensitisation (Figure 3.5). The corresponding modifying factors ranged from 0.87−0.96 and

1.05−1.13. These effects seemed to be independent of the time interval between X-ray irradiation and EMF exposure.

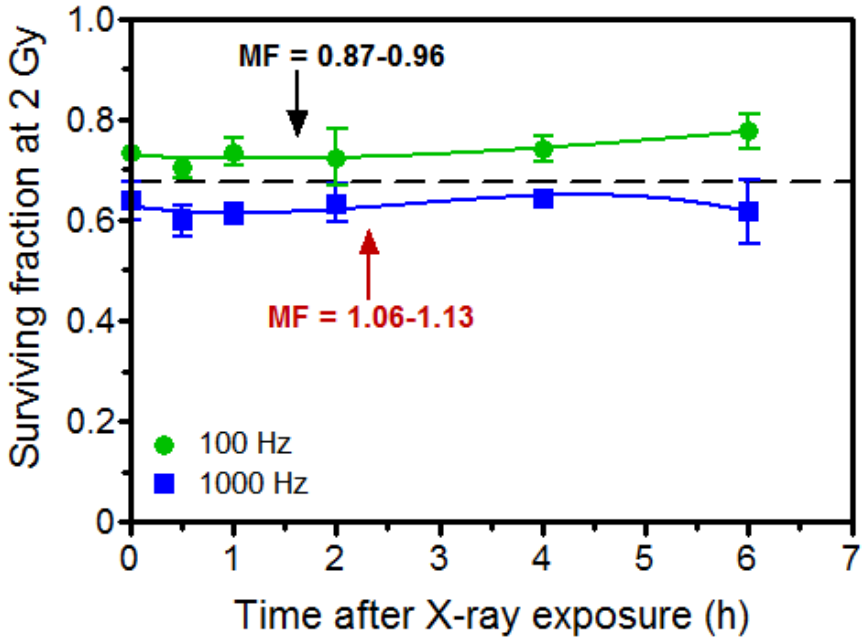

Figure 3.5: Clonogenic cell survival at 2 Gy in Chinese hamster lung fibroblasts (V79), when cells were exposed to X-irradiation prior to a 100 or 1000 Hz electromagnetic field (EMF), as a function of time between X-ray treatment and EMF exposure. Data points are means ± SEM of 3 independent experiments. Horizontal dashed line represents the surviving fraction at 2 Gy without EMF exposure.

Table 3.3: Summary of radiation dose modifying data for Chinese hamster lung fibroblasts (V79) when cells were exposed to EMF (100 and 1000 Hz) prior to or after a 2-Gy X-ray irradiation. MF denotes radiation modifying factor (Equation 3).

Treatment	**Time interval (h)**	***MF*** [#]	
		100 Hz	1000 Hz
EMF before X-rays	0	0.98 ± 0.06	0.97 ± 0.10
	0.5	1.05 ± 0.10	0.98 ± 0.12
	1	0.99 ± 0.06	0.98 ± 0.10
	2	1.01 ± 0.10	0.99 ± 0.11
	4	1.08 ± 0.11	1.22 ± 0.22
	6	0.98 ± 0.07	1.00 ± 0.09
X-rays before EMF	0	0.92 ± 0.05	1.06 ± 0.09
	0.5	0.96 ± 0.06	1.13 ± 0.09
	1	0.92 ± 0.06	1.11 ± 0.07
	2	0.93 ± 0.09	1.07 ± 0.09
	4	0.91 ± 0.06	1.05 ± 0.07
	6	0.87 ± 0.06	1.10 ± 0.13

[#]Mean ± error: errors were calculated using appropriate error propagation formulae.

Data for cell survival at 2 Gy in the human melanoma cells (MeWo), when cells were exposed to either a 100 or 1000 Hz electromagnetic field before being irradiated with X-rays, are presented in Figure 3.6. For all time intervals between EMF and X-ray treatment, pretreatment with a 100 Hz field resulted in significant radioprotection, with modifying factors ranging from 0.68 ± 0.07 to 0.79 ± 0.07. On the contrary, pre-

exposure to a 1000 Hz field yielded significant radiosensitisation, giving modifying factors between 1.35 ± 0.11 and 1.60 ± 0.21. The radiation modifying factors when cells were irradiated at 2 h and 4 h after exposure emerged as 1.60 and 1.52, respectively (Figure 3.6; Table 3.4). When the MeWo cells were irradiated to 2 Gy of X-rays followed by exposure to a 100 Hz EMF, the cells were rendered more radiosensitive, as shown in Figure 3.7, with modifying factors ranging from 1.17−1.33. However, when X-ray exposure was followed by treatment with a 1000 Hz EMF, the cells were less radiosensitive with modifying factors ranging from 0.90-0.95.

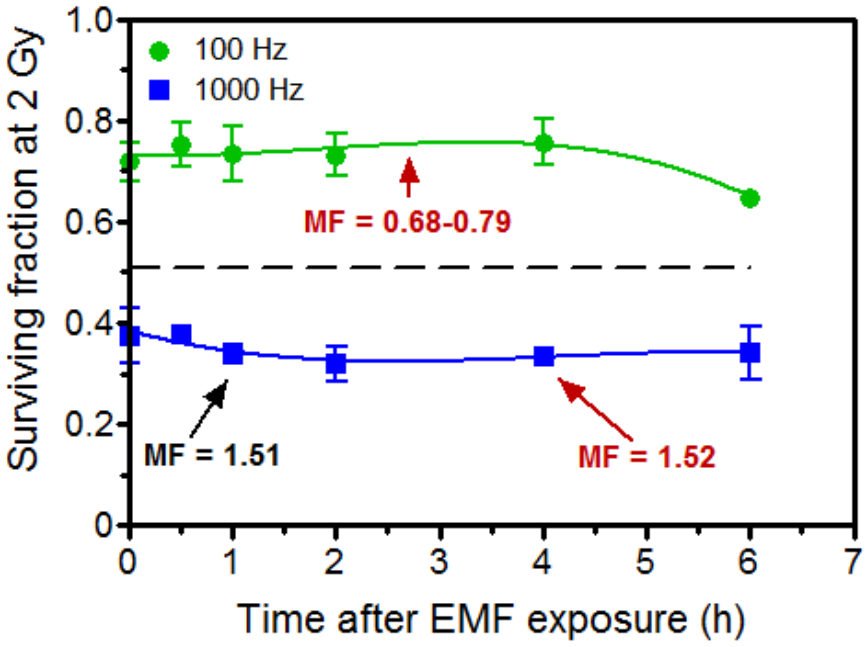

Figure 3.6: Clonogenic cell survival at 2 Gy in human melanoma cells (MeWo), when cells were exposed to a 100 or 1000 Hz electromagnetic field (EMF) prior to X-irradiation, as a function of time between EMF exposure and X-ray treatment. Data points are means ± SEM of 3 independent experiments. Horizontal dashed line represents the surviving fraction at 2 Gy without EMF exposure.

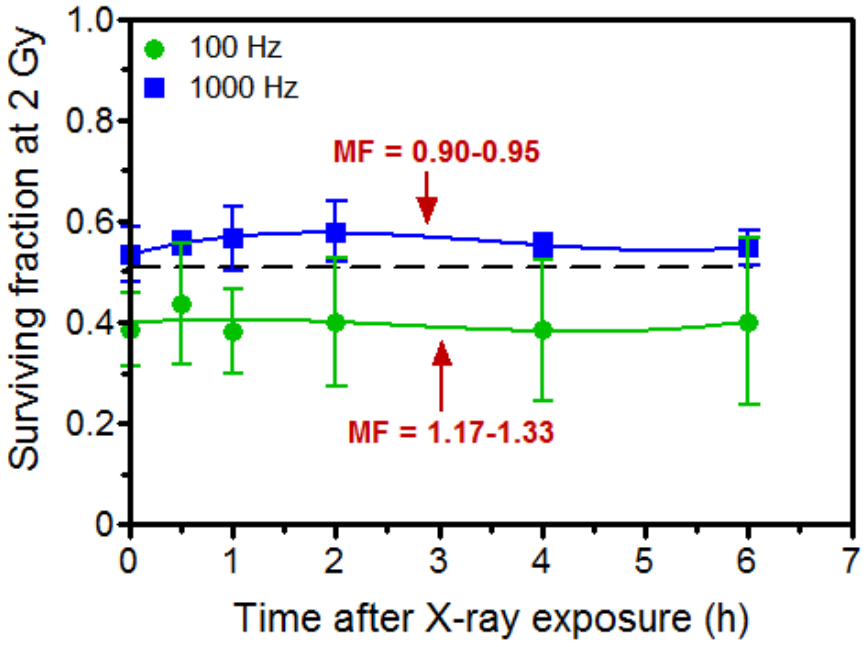

Figure 3.7: Clonogenic cell survival at 2 Gy in human melanoma cells (MeWo), when cells were exposed to X-irradiation prior to a 100 or 1000 Hz electromagnetic field (EMF), as a function of time between X-ray treatment and EMF exposure. Data points are means ± SEM of 3 independent experiments. Horizontal dashed line represents the surviving fraction at 2 Gy without EMF exposure.

Table 3.4: Summary of radiation dose modifying data for human melanoma cells (MeWo) when cells were exposed to EMF (100 and 1000 Hz) prior to or after a 2-Gy X-ray irradiation. MF denotes radiation modifying factor (Equation 3).

Treatment	**Time interval (h)**	***MF*** [#]	
		100 Hz	1000 Hz
EMF before X-rays	0	0.71 ± 0.07	1.36 ± 0.22
	0.5	0.68 ± 0.07	1.35 ± 0.11
	1	0.69 ± 0.08	1.50 ± 0.15
	2	0.70 ± 0.07	1.60 ± 0.21
	4	0.67 ± 0.07	1.52 ± 0.13
	6	0.79 ± 0.07	1.50 ± 0.26
X-rays before EMF	0	1.33 ± 0.27	0.95 ± 0.13
	0.5	1.17 ± 0.33	0.92 ± 0.08
	1	1.33 ± 0.31	0.90 ± 0.12
	2	1.27 ± 0.41	0.88 ± 0.12
	4	1.33 ± 0.49	0.92 ± 0.08
	6	1.27 ± 0.53	0.93 ± 0.10

[#]Mean ± error: errors were calculated using appropriate error propagation formulae.

3.4. Fractionation versus Combination of X-Rays and EMF

To test whether fractionated radiotherapy is superior to combining a single dose of radiation with electromagnetic field exposure, surviving fractions at 2 Gy given in two fractions 6 h apart were compared with those obtained when cells were treated with

a combination of 2 Gy of X-rays and an EMF of 100 Hz or 1000 Hz. The data presented in Figure 3.8 show similar levels of survival when the V79 cells were treated with either a split dose of X-rays or with an acute dose followed by exposure to a 100 Hz EMF 6 h later. Cell survival rates when cells were exposed to EMF (at 100 or 1000 Hz) 6 h prior to a 2 Gy irradiation or primed with a dose of 2 Gy before exposure to 1000 Hz EMF were similar and comparable to that obtained for an acute dose of 2 Gy alone.

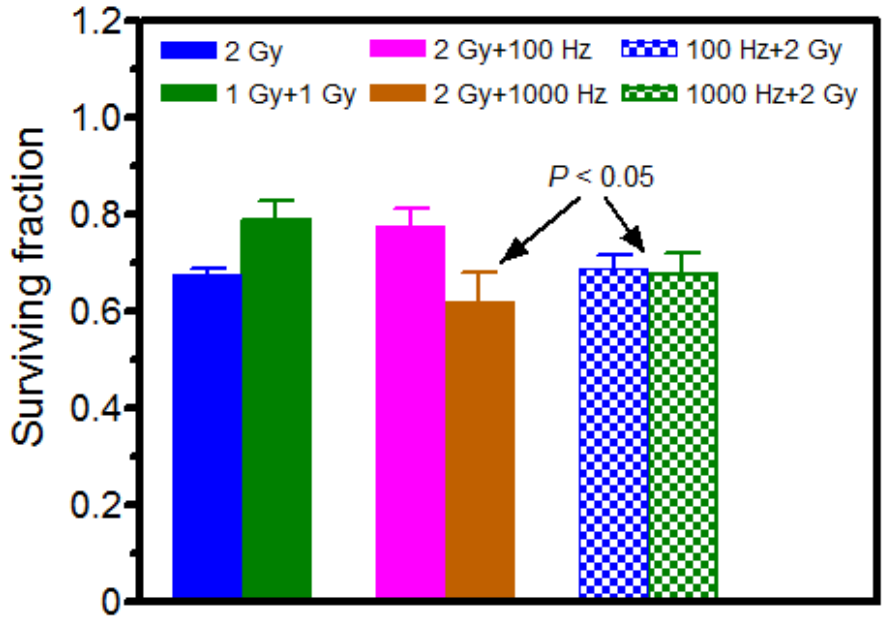

Figure 3.8: Surviving fractions for Chinese hamster lung fibroblasts (V79) following various treatment protocols. Split doses of X-rays (1 Gy per fraction) or an acute dose (2 Gy) and EMF exposure were given 6 h apart.

For the melanoma cell line (MeWo), a 2 Gy irradiation followed by exposure to 1000 Hz EMF or priming with a 100 Hz EMF before a 2 Gy treatment yielded similar levels of cell survival as for the split irradiation (Figure 3.9). However, these cells were sensitised when they were irradiated with 2 Gy and then exposed to a 100 Hz EMF

albeit not significantly (P = 0.2069). Pre-exposure to a 1000 Hz EMF markedly radiosensitised the MeWo cells (P = 0.0195).

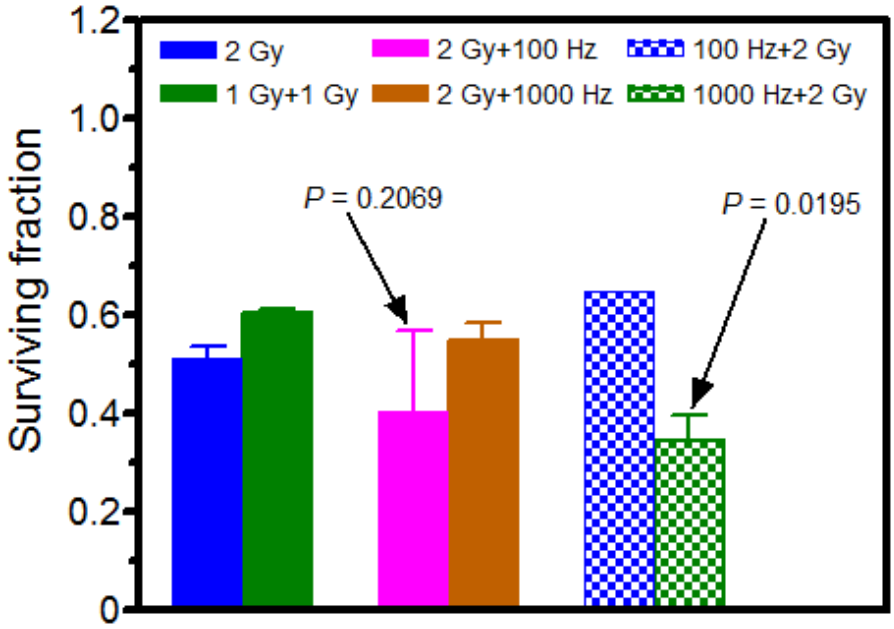

Figure 3.9: Surviving fractions for human melanoma cells (MeWo) following various treatment protocols. Split doses of X-rays (1 Gy per fraction) or an acute dose (2 Gy) and EMF exposure were given 6 h apart.

CHAPTER 4

4. Discussion

Radiation therapy (RT) is considered the first line of treatment for most skin cancers as these malignancies tend to respond very well. Radiation therapy may be used alone or in combination with chemotherapy, surgery or other treatment modalities in skin cancer treatment. Radiation therapy has, however, been discouraged in younger patients because of an increased risk of developing other consequential cancers later in life. The cosmetic side effect of radiation therapy and its carcinogenic consequences have led to RT being underutilised, as dermatologists tend to focus on the consequences rather than the benefits of RT. HIV/AIDS patients with Kaposi's sarcoma (KS) often suffer from disease-related pain and other cosmetic disorders which at times cause them emotional distress (Kirova *et al*., 1998; Donato *et al*., 2013). Although radiation therapy has good palliative outcome, these immunocompromised individuals tend to be at very high risk of treatment-related toxicity. It is, therefore, essential to minimise damage to normal tissue to avoid the associated mental distress. This may be achieved by employing adjuvant approaches that can help to significantly reduce the amount of radiation dose delivered to the patient.

4.1. Intrinsic and Fractionated Radiosensitivity

This study sought to compare the outcomes of a split dose of radiation and an acute dose given in conjunction with electromagnetic fields, using Chinese hamster lung fibroblasts (V79) and human melanoma cells (MeWo). The disparity in intrinsic

radiosensitivity in these cell lines (Figure 3.1) cannot be attributed to differences in the status of the p53 gene, as the gene is mutated in both cell lines (Albino *et al*., 1994; Chaung *et al*., 1997). Radiation-induced cell death is not necessarily dependent on p53 status (Clarke *et al*., 1993). The higher radiosensitivity of MeWo cells relative to V79 cells is, therefore, likely due to activation of other genes upstream of p53 (e.g. ATM) that are responsible for p53-independent cell death in the former cell line (Zhivotovsky and Kroemer, 2004), making the cells more radiosensitive.

The split-dose data presented in Figures 3.2 and 3.3 show that the melanoma cells recover better from radiation-induced damage relative to their V79 counterparts. However, there appears to be no significant difference in the capacity of MeWo and V79 to rejoin DNA double strand-breaks (Gauter *et al*., 2002). The efficiency with which cells rejoin DNA double strand-breaks correlates with their radiosensitivity (Akudugu *et al*., 2004). The finding that the malignant melanoma cells (MeWo) recover to a larger extent than the apparently normal Chinese hamster lung fibroblasts (V79) seems to suggest that conventional fractionated radiation therapy alone might not be very beneficial for patients presenting with lesions like epidemic Kaposi's sarcoma.

4.2. Modulation of Radiosensitivity by Electromagnetic Fields

To partly address the need for alternative treatment strategies for the immunocompromised cohort of cancer patients, the influence of electromagnetic fields on cellular radiosensitivity was evaluated. The current data suggest that

normal tissue and cancerous cells do not respond to all electromagnetic fields in the same way. While pre-exposure of the V79 cells to both 100 and 1000 Hz fields followed by 2 Gy of X-rays appears to have no effect when cells were irradiated within 2 h of EMF exposure, significant radioprotection and radiosensitisation was seen in the MeWo cells for the 100 and 1000 Hz fields over all time points investigated (Figures 3.6 and 3.7). The findings that the apparently normal fibroblasts appeared to be protected by the 1000 Hz field exposure when cells were irradiated 1 h after EMF exposure and that both fields were radiosensitising at an interval of 4 h, suggest that an EMF of 1000 Hz may potentiate tumour radiosensitivity with little or no normal tissue effect if radiation is given within 1 h of EMF exposure.

Interestingly, the 100 Hz field appeared to protect and sensitise pre-irradiated V79 and MeWo cells, respectively (Figures 3.5 and 3.7). This phenomenon, over all time points, indicated that exposing tumour cells to a 100 Hz electromagnetic field within 6 h of administering a fraction of radiation dose might have a significant level of therapeutic benefit. It is currently not clear why these cell lines behave differently when exposed to the two electromagnetic fields, and in a manner dependent on the sequence of EMF exposure and X-irradiation. However, sensitisation of the cells when they are exposed to EMF followed by X-ray could be due to many different mechanisms, including the EMF causing an influx of calcium ions, with an alteration in homeostasis triggering mitotic division (Pinton *et al*., 2008). This process would prompt otherwise dormant cells to start dividing. Actively dividing cells are more prone to radiation-induced cell death than dormant cells, and the net effect will be a low level of cell survival. Also, the observed radiosensitisation may have been

caused by intracellular cascades, such as activation of matrix metalloproteinases by reactive oxygen species (ROS), the concentration of which is known to be increased by exposure to magnetic fields (Lai and Singh, 2010; Morabito *et al*., 2010). ROS act as radiosensitisers. Furthermore, the radiosensitisation can result from calcium-ion overload, which is highly toxic and leads to cell suicide, by activating proteases and phospholipases (Pinton *et al*., 2008; Artacho-Cordón *et al*., 2013). Cells carrying radiation-induced DNA damage can be expected to be more radiosensitive when exposed to EMF, as the electromagnetic field can disorient charged amino acids, resulting in a change in the 3-dimensional structure of proteins and thus disturbing their function (Menéndez, 1999). This could be a reason for the sensitisation seen when cells are exposed to EMF after X-irradiation, since the enzymes responsible for repairing X-ray damage may be rendered non-functional by the subsequent exposure to appropriate resonant frequencies (Agulan *et al*., 2015). This can result in non-repaired damage and ultimate cell death. However, this cannot explain the radiosensitisation seen 6 h after X-irradiation (Figures 3.5 and 3.7), as most all DNA repair should be completed. The radiosensitisation seen when pre-irradiated cells were exposed to EMF may be due to dysregulation of ion channels and alteration of hormones leading to cells adopting different signalling pathways, some of which may trigger cell death (Orrenius *et al*., 1992). Cells become more radiosensitive when more damage is inflicted on them by another form of treatment. These findings can also be attributed to cells being rendered sensitive to cell type specific radiofrequency fields (Zimmerman *et al*., 2013).

4.3. Fractionation versus Combination of X-Rays and EMF

The data summarised in Figure 3.8 indicate that the recovery exhibited in the fractionated irradiation by the V79 cells is paralleled by an enhancement in survival when cells received an acute dose of 2 Gy and were exposed to a 100 Hz EMF 6 h later. Interestingly, the MeWo cells were sensitised in relation to their recovery rate from the split dose when they were similarly treated with a combination X-rays and the 100 Hz EMF (Figure 3.9). This suggests that a 30 min exposure to a 100 Hz EMF 6 h after a 2 Gy fraction may be beneficial to HIV/AIDS with epidemic KS, as radiosensitisation of tumours will likely be accompanied by a reduced normal tissue toxicity. Exposure of the fibroblasts to both frequencies of EMF followed by a 2 Gy irradiation 6 h later had no effect on their intrinsic radiosensitivity at 2 Gy (Figure 3.8). However, the cell survival rates were lower than when cells were treated with split doses. For this treatment protocol, the melanoma cells were significantly sensitised when compared with their intrinsic radiosensitivity and recovery rate (Figure 3.9). It is, thus, conceivable that patients with epidemic KS could greatly benefit from treatment approaches that employ informed and well-designed combinations of radiation therapy and electromagnetic field exposures.

CHAPTER 5

5. Conclusion

There is a great need for the standardisation of electromagnetic field (EMF) therapy so that it may be applied in cancer treatment as it is a non-invasive method that has a potential to replace or enhance other therapeutic modalities, like radiation therapy, surgery and chemotherapy. As the number of HIV/AIDS cases continues to rise, epidemic Kaposi's sarcoma cases are also on the increase. Radiation therapy plays a palliative role, and combining it with EMF therapy may even lead to better treatment outcomes. The data reported here demonstrate that electromagnetic fields appear to have the desirable toxic and protective effects on tumour and normal cells, respectively, if appropriate frequencies are administered at the right times relative to X-irradiation. Electromagnetic fields, therefore, have the potential of being used in conjunction with radiotherapy to reduce the total radiation absorbed dose administered to patients. This can have a significant positive impact on the management of patients with superficial tumours, especially those who are immunocompromised. The use of EMF in combination with radiation therapy may yield better results than conventional fractionated radiation therapy.

Possible Future Avenues

To fully harness the potential of electromagnetic fields (EMF) as potentiators of radiation therapy, the following avenues need to be explored as they may lead to observations other than those reported here:

1. EMF exposure time could be extended to periods longer than 30 min.
2. The time between EMF and X-ray exposures can be increased to more than 6 h.
3. The dose of X-rays used with EMF exposure could also be varied.
4. Use western blotting techniques to evaluate the effect of EMF on gene expression, as this might give an insight into the molecular factors responsible for the pro-survival and pro-death effects seen here.
5. The use of a wider range of human cell lines (normal and malignant).
6. Experimentation on the effect of EMF on radiosensitivity when X-ray irradiation is delivered in more than one fraction.
7. Use of flow cytometry to determine the mode of cell death induced by the combined EMF/X-ray treatment.
8. Investigate possible synergy between chemotherapy drugs (e.g. cisplatin), X-rays, and EMF.
9. Assess cell viability and proliferative state using methods like the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) tetrazolium reduction assay.
10. Monitor superoxide dismutase/glutathione activity following exposure to EMF and X-ray to assess the role of oxidative stress in induced cytotoxicity.

PAPERS FROM THIS THESIS

[1] Chinhengo A, Serafin A, Hamman B, Akudugu J. Electromagnetic fields induce frequency-dependent radioprotection and radiosensitization in *in vitro* cell cultures. *IEEE Transactions on Plasma Science* (submitted; copy attached).

[2] Chinhengo A, Serafin A, Hamman B, Akudugu J. Electromagnetic fields induce frequency-dependent radioprotection and radiosensitization in *in vitro* cell cultures. *60th Academic Year Day, Faculty of Medicine and Health Sciences, University of Stellenbosch*, Tygerberg, South Africa, August 2016 (oral).

[3] Chinhengo A, Serafin A, Akudugu J. Evaluation of the effect of low and intermediate frequency electromagnetic waves on radiosensitivity. *14th International Congress of the International Radiation Protection Association*, Cape Town, South Africa, May 2016 (poster).

[4] Chinhengo A, Serafin A, Akudugu J. Evaluation of the effect of low and intermediate frequency electromagnetic waves on radiosensitivity. *59th Academic Year Day, Faculty of Medicine and Health Sciences, University of Stellenbosch*, Tygerberg, South Africa, August 2015 (oral).

Bibliography

Agulan RTV, Capule EMF, Pobre RF. 2015. Effect of pulsed electromagnetic fields on colon cancer cell lines (HCT 116) through cytotoxicity test. Proceedings of the DLSU Research Congress Vol. 3, De La Salle University, Manila, Philippines.

Akudugu JM, Theron T, Serafin A, Böhm L. 2004. Influence of DNA double-strand break rejoining on clonogenic survival and micronucleus yield in human cell lines. International Journal of Radiation Biology, 80:93-104.

Albino AP, Vidal MJ, McNutt NS, Shea CR, Prieto VG, Nanus DM, Palmer JM, Hayward NK. 1994. Mutation and expression of the p53 gene in human malignant melanoma. Melanoma Research, 4:35-45.

Alcaraz M, Olmos E, Alcaraz-Saura M, Achel DG, Castillo J. 2014. Effect of long-term 50 Hz magnetic field exposure on the micronucleated polychromatic erythrocytes of mice. Electromagnetic Biology and Medicine, 33:51-57.

American Cancer Society. 1994. Questionable methods of cancer management: Electronic devices. CA- A Cancer Journal for Clinicians, 44:115-127.

Artacho-Cordón F, Salinas-Asensio MM, Calvente I, Ríos-Arrabal S, León J, Román-Marinetto E, Olea N, Núñez MI. 2013. Could radiotherapy effectiveness

be enhanced by electromagnetic field treatment? International Journal of Molecular Sciences, 14:14974-14995.

Barbault A, Costa FP, Bottger B, Munden RF, Bomholt F, Kuster N, Pasche B. 2009. Amplitude-modulated electromagnetic fields for the treatment of cancer: Discovery of tumor-specific frequencies and assessment of a novel therapeutic approach. Journal of Experimental Clinical Cancer Research, 28:51. doi: 10.1186/1756-9966-28-51.

Bassen H, Litovitz T, Penafiel M, Meister R. 1992. ELF *in vitro* exposure systems for inducing uniform electric and magnetic fields in cell culture media. Bioelectromagnetics, 13:183-198.

Bassett CA. 1985. The development and application of pulsed electromagnetic fields (PEMFs) for ununited fractures and arthrodeses. Clinics in Plastic Surgery, 12:259-277.

Bassett CAL, Mitchell SN, Gaston SR. 1981. Treatment of ununited tibial diaphyseal fractures with pulsing electromagnetic fields. The Journal of Bone and Joint Surgery, 63:511-523.

Beral V, Peterman TA, Berkelman RL, Jaffe HW. 1990. Kaposi's sarcoma among persons with AIDS: a sexually transmitted infection? Lancet, 335:123-128.

Berson AM, Quivey JM, Harris JW, Wara WM.1990. Radiation therapy for AIDS related Kaposi's sarcoma. International Journal of Radiation Oncology Biology Physics, 19:569-575.

Blank M, Goodman R. 2009. Electromagnetic fields stress living cells. Pathophysiology, 16:71-78.

Buldak RJ, Polaniak R, Buldak L, Korczala KZ, Skonieczna M, Monsiol A, Kukla M, Buldak AD, Birkner E. 2012. Short-term exposure to 50 Hz ELF-EMF alters the cisplatin-induced oxidative response in AT478 murine squamous cell carcinoma cells. Bioelectromagnetics, 33:641-651.

Butters JT, Xavier A. Figueroa XA, Butters BM. 2014. Non-thermal radio frequency stimulation of tubulin polymerization *in vitro*: a potential therapy for cancer treatment. Open Journal of Biophysics, 4:147-168.

Cameron IL, Markov MS, Hardman WE. 2014. Optimization of a therapeutic electromagnetic field (EMF) to retard breast cancer tumor growth and vascularity. Cancer Cell International, 14:125. doi:10.1186/s12935-014-0125-5

Cattelan AM, Calabrò ML, De Rossi A, Aversa SML, Barbierato M, Trevenzoli M, Gasperini P, Zanchetta M, Cadrobbi P, Monfardini S, Chieco-Bianchi L. 2005. Long-term clinical outcome of AIDS-related Kaposi's sarcoma during highly active antiretroviral therapy. International Journal of Oncology, 27:779-785.

Chaung W, Mi L-J, Boorstein RJ. 1997. The p53 status of Chinese hamster V79 cells frequently used for studies on DNA damage and DNA repair. Nucleic Acids Research, 25:992-994.

Cheing GL, Li X, Huang L, Kwan RL, Cheung KK. 2014. Pulsed electromagnetic fields (PEMF) promote early wound healing and myofibroblast proliferation in diabetic rats. Bioelectromagnetics, 35:161-169.

Cho YH, Jeon HK, Chung HW. 2007. Effects of extremely low-frequency electromagnetic fields on delayed chromosomal instability induced by bleomycin in normal human fibroblast cells. Journal of Toxicology and Environmental Health, 70:1252-1258.

Cianfrocca M, Roenn JH. 1998. Epidemic Kaposi's sarcoma. Oncology, 12:1375-81.

Clarke AR, Purdie CA, Harrison DJ, Morris RG, Bird CC, Hooper ML, Wyllie AH. 1993. Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature, 362:849-852.

Cooper A. 1991. Treating HIV-infected children: the surgeon's role. Bulletin of the American College of Surgeons, 76:13-17.

Crocetti S, Beyer C, Schade G, Egli M, Fröhlich J, Franco-Obregón A. 2013. Low intensity and frequency pulsed electromagnetic fields selectively impair breast cancer cell viability. PLoS ONE, 8(9):e72944. doi:10.1371/journal.pone.0072944

Czyz J, Nikolova T, Schuderer J, Kuster N, Wobus AM. 2003. Non-thermal effects of power-line magnetic fields (50 Hz) on gene expression levels of pluripotent embryonic stem cells - the role of tumour suppressor p53. Mutation Research, 557:63-74.

Del Monache S, Alessandro R, Lorio R, Gualtieri G, Colonna R. 2008. Extremely low frequency electromagnetic fields (ELF-EMFs) induce in vitro angiogenesis process in human endothelial cells. Bioelectromagnetics, 29:640-648.

Deng Y, Zhang Y, Jia S, Liu J, Liu Y, Xu W, Liu L. 2013. Effects of aluminum and extremely low frequency electromagnetic radiation on oxidative stress and memory in brain of mice. Biological Trace Element Research, 156:243-252.

Deregibus MC, Cantaluppi V, Doublier S, Brizzi MF, Deambrosis I, Albini A, Camussi G. 2002. HIV-1-Tat protein activates phosphatidylinositol 3-Kinase/ AKT-dependent survival pathways in Kaposi's sarcoma cells. Journal of Biological Chemistry, 277:25196-25202.

Donato V, Guarnaccia R, Dognini J, De Pascalis G, Caruso C, Bellagamba R, Morrone A. 2013. Radiation therapy of HIV-related Kaposi's sarcoma. Anticancer Research, 33:2153-2158.

Ensoli B, Gendelman R, Markham P, Fiorelli V, Colombini S, Raffeld M, Cafaro A, Chang HK, Brady JN, Gallo RC. 1994. Synergy between basic fibroblast growth factor and HIV-1 Tat protein in induction of Kaposi's sarcoma. Nature, 371:674-680.

Escalon MP, Hagemeister FB. 2006. "AIDS-related malignancies." MD Anderson Manual of Medical Oncology. McGraw-Hill: pp 903-910.

Ferreira AR, Knakievicz T, de Bittencourt Pasquali MA, Gelain DP, Dal-Pizzol F, Fernández CER, de Almeida de Salles ÁA, Ferreira HB, Moreira JCF. 2006. Ultra high frequency-electromagnetic field irradiation during pregnancy leads to an increase in erythrocytes micronuclei incidence in rat offspring. Life Sciences, 80:43-50.

Furtado-Filho V, Borba JB, Dallegrave A, Pizzolato TM, Henriques JAP, Moreira JCF, Saffi J. 2013. Effect of 950 MHz UHF electromagnetic radiation on biomarkers of oxidative damage, metabolism of UFA and antioxidants in the livers of young rats of different ages. International Journal of Radiation Biology, 90:159-168.

Ganju RK, Munshi N, Nair BC, Liu ZY, Gill P, Groopman JE. 1988. Human immunodeficiency virus Tat modulates the Flk-1/KDR receptor, mitogen-activated protein kinases, and components of focal adhesion in Kaposi's sarcoma cells. Journal of Virology, 72:6131-6137.

Gauter B, Zlobinskaya O, Webert K-J. 2002. Rejoining of radiation-induced DNA double-strand breaks: pulsed-field electrophoresis analysis of fragment size distributions after incubation for repair. Radiation Research, 157:721-733.

Goodman R, Henderson AS. 1991. Transcription and translation in cells exposed to extremely low frequency electromagnetic fields. Bioelectrochemistry and Bioenergetics, 25:335-355.

Goodrich JP. 1943. Experimental modification of radiosensitivity of embryonic cells. Radiology, 40:179-187.

Hamunyela R, Serafin A, Hamid M, Maleka S, Achel D, Akudugu J. 2015. A cocktail of specific inhibitors of HER-2, PI3K, and mTOR radiosensitises human breast cancer cells. Journal of Cancer Biology and Therapeutics, 1:46-56.

Hengge UR, Ruzicka T, Tyring SK, Stuschke M, Roggendorf M, Schwartz RA, Seeber S. 2002. Update on Kaposi's sarcoma and other HHV8 associated diseases. Part 1: epidemiology, environmental predispositions, clinical manifestations, and therapy. The Lancet Infectious Diseases, 2:281-292.

Jacobson LP, Jenkins FJ, Springer G, Munoz A, Shah KV, Phair J, Zhang ZF, Armenian H. 2000. Interaction of human immunodeficiency virus type 1 and human herpesvirus type 8 infections on the incidence of Kaposi's sarcoma. The Journal of Infectious Diseases, 181:1940-1949.

Janigro D, Perju C, Fazio V, Hallene K, Dini G, Agarwal MK, Cucullo L. 2006. Alternating current electrical stimulation enhanced chemotherapy: a novel strategy to bypass multidrug resistance in tumor cells. BMC Cancer, 6:72. doi: 10.1186/1471-2407-6-72.

Jemal A, Bray F, Forman D, O'Brien M, Ferlay J, Center M, Parkin DM. 2012. Cancer burden in Africa and opportunities for prevention. Cancer, 118:4372-4384.

Kaminuma T, Karasawa K, Hanyu N, Chang T-C, Kuga G, Okano N, Kubo N, Okuma Y, Nagata Y, Maeda Y, Ajisawa A. 2010. Acute adverse effects of radiation therapy on HIV-positive patients in Japan: study of 31 cases at Tokyo Metropolitan Komagome Hospital. Journal of Radiation Research, 51:749-753.

Kaposi M. 1872. Idiopathic multiple pigmented sarcoma of the skin. Archiv für Dermatologie und Syphilis, 4:265-273. [English translation. 1982. CA-A Cancer Journal for Clinicians, 4:342-347].

Kirova YM, Belembaogo E, Frikha H, Haddad E, Calitchi E, Levy E, Piedbois P, Le Bourgeois JP. 1998. Radiotherapy in management of Kaposi's sarcoma: a retrospective study of 643 cases. Radiotherapy and Oncology 46:19-22.

Kirson ED, Dbaly V, Tovaryš F, Vymazal J, Soustiel JF, Itzhaki A, Mordechovich D, Steinberg-Shapira S, Gurvich Z, Schneiderman R, Wasserman Y, Salzberg M, Ryffel B, Goldsher D, Dekel E, Palti Y. 2007. Alternating electric fields arrest cell proliferation in animal tumor models and human brain tumors. Proceedings of the National Academy of Sciences, USA, 104:10152-10157.

Kirson ED, Gurvich Z, Schneiderman R, Dekel E, Itzhaki A, Wasserman Y, Schatzberger R, Palti Y. 2004. Disruption of cancer cell replication by alternating electric fields. Cancer Research, 64:3288-3295.

Koyama S, Sakurai T, Nakahara T, Miyakoshi J. 2008. Extremely low frequency (ELF) magnetic fields enhance chemically induced formation of apurinic/apyrimidinic (AP) sites in A172 cells. International Journal of Radiation Biology, 84:53-59.

Krigel RL, Friedman-Kien AE. 1990. Epidemic Kaposi's sarcoma. Seminars in Oncology, 17:350-360.

Lai H, Singh NP. 1997a. Acute exposure to a 60 Hz magnetic field increases DNA strand breaks in rat brain cells. Bioelectromagnetics, 18:156-165.

Lai H, Singh NP. 1997b. Melatonin and N-tert-butyl-α-phenylnitrone block 60-Hz magnetic field-induced DNA single and double strand breaks in rat brain cells. Journal of Pineal Research, 22:152-162.

Lai H, Singh NP. 2004. Magnetic-field–induced DNA strand breaks in brain cells of the rat. Environmental Health Perspectives, 112:687-694.

Lai HC, Singh NP. 2010. Medical applications of electromagnetic fields. In Electromagnetic Phenomena and Health - A Continuing Controversy? IOP Conference Series: Earth and Environmental Science, IOP Publishing (UK), pp 10:012006.

Lee HC, Hong M, Jung SH, Kim BC, Suh YJ, Ko Y, Lee Y, Lee B, Cho Y, Myung S, Lee J. 2015. Effect of extremely low frequency magnetic fields on cell proliferation and gene expression. Bioelectromagnetics, 36:506-516.

Li B-l, Li W, Bi J-Q, Zhao J-g, Qu Z-W, Lin C, Yang S-L, Meng Q-g, Yue Q. 2015. Effect of long-term pulsed electromagnetic field exposure on hepatic and immunologic functions of rats. Wiener Klinische Wochenschrift, 127:959-962.

Lucia U. 2016. Electromagnetic waves and living cells: a kinetic thermodynamic approach. Physica A, 461:577-585.

Lupke M, Frahm J, Lantow M, Maercker C, Remondini D, Bersani F, Simkó M. 2006. Gene expression analysis of ELF-MF exposed human monocytes indicating the involvement of the alternative activation pathway. BBA-Molecular Cell Research, 1763:402-412.

Luukkonen J, Liimatainen A, Juutilainen J, Naarala J. 2014. Induction of genomic instability, oxidative processes, and mitochondrial activity by 50 Hz magnetic fields in human SH-SY5Y neuroblastoma cells. Mutation Research, 760:33-41.

Mattsson M-O, Simkó M. 2014. Grouping of experimental conditions as an approach to evaluate effects of extremely low-frequency magnetic fields on oxidative response in *in vitro* studies. Frontiers in Public Health, 2:132. doi:10.3389/fpubh.2014.00132

Maleka S, Serafin A, Hamunyela R, Hamid M, Achel D, Akudugu J. 2015. NVP-BEZ235 enhances radiosensitivity of human prostate cancer cells but acts as a radioprotector to normal prostate cells. Journal of Cancer Biology and Therapeutics, 1:38-45.

Mantiply ED, Pohl KR, Poppell SW, Murphy JA. 1997. Summary of measured radiofrequency electric and magnetic fields (10 kHz to 30 GHz) in the general and work environment. Bioelectromagnetics, 18:563-577.

Markkanen A, Juutilainen J, Naarala J. 2008. Pre-exposure to 50 Hz magnetic fields modifies menadione-induced DNA damage response in murine L929 cells. International Journal of Radiation Biology, 84:742-751.

Menéndez RG. 1999. Three molecular mechanisms to explain some biological effects of electromagnetic fields and hypogravity. Medical Hypotheses, 52:239-245.

Miles SA. 1993. Kaposi sarcoma: a cytokine responsive neoplasia. Cancer Treatment and Research, 63:129-140.

Morabito C, Guarnieri S, Fanò G, Mariggiò MA. 2010. Effects of acute and chronic low frequency electromagnetic field exposure on PC12 cells during neuronal differentiation. Cellular Physiology and Biochemistry, 24:947-958.

Morini M, Benelli R, Giungiuglio D, Carlone S, Arena G, Noonan DM, Albini A. 2000. Kaposi's sarcoma cells of different etiologic origin respond to HIV-Tat through the Flk-1/KDR (VEGFR-2): relevance in AIDS-KS pathology. Biochemical and Biophysical Research Communications, 273:267-271.

Nie Y, Chen Y, Mou Y, Weng L, Xu Z, Du Y, Wang W, Hou Y, Wang T. 2013. Low frequency magnetic fields enhance antitumor immune response against mouse H22 hepatocellular carcinoma. PLoS ONE, 8(11):e72411. doi:10.1371/journal.pone.0072411

Nobler MP, Leddy ME, Huh SH. 1987. The impact of palliative irradiation on the management of patients with acquired immune deficiency syndrome. Journal of Clinical Oncology, 5:107-112.

Orrenius S, McCabe Jr MJ, Nicotera P. 1992. Ca^{2+}-dependent mechanisms of cytotoxicity and programmed cell death. Toxicology Letters, 64/65:357-364.

Pinton P, Giorgi C, Siviero R, Zecchini E, Rizzuto R. 2008. Calcium and apoptosis: ER-mitochondria Ca^{2+} transfer in the control of apoptosis. Oncogene, 27:6407-6418.

Polaniak R, Bułdak RJ, Karon M, Birkner K, Kukla M, Korczala KW, Birkner E. 2010. Influence of an extremely low frequency magnetic field (ELF-EMF) on antioxidative vitamin E properties in AT478 murine squamous cell carcinoma culture *in vitro*. International Journal of Toxicology, 29:221-230.

Potiron VA, Abderrahmani R, Clément-Colmou K, Marionneau-Lambot S, Oullier T, Paris F, Supiot S. 2013. Improved functionality of the vasculature during conventionally fractionated radiation therapy of prostate cancer. PLoS One, 8:e84076.

Restrepo AF, Tobar VE, Camargo, RJ, Franco E, Pinedo CR, Gutiérrez O. 2016 (October). Effects of extremely low frequency electromagnetic fields on *in-vitro* cellular cultures HeLa and CHO. *In* Engineering in Medicine and Biology

Society (EMBC), 38th Annual International Conference of the IEEE, pp. 4193-4196.

Ross CL, Siriwardane M, Almeida-Porada G, Porada CD, Brink P, Christ GJ, S. Harrison BS. 2015. The effect of low-frequency electromagnetic field on human bone marrow stem/progenitor cell differentiation. Stem Cell Research, 15:96-108.

Ruediger HW. 2009. Genotoxic effects of radiofrequency electromagnetic fields. Pathophysiology, 16:89-102.

Sarimov R, Markova E, Johansson F, Jenssen D, Belyaev I. 2005. Exposure to ELF magnetic field tuned to Zn inhibits growth of cancer cells. Bioelectromagnetics, 26:631-638.

Simkó M, Kriehuber R, Weiss DG, Luben RA. 1998. Effects of 50 Hz EMF exposure on micronucleus formation and apoptosis in transformed and nontransformed human cell lines. Bioelectromagnetics, 19:85-91.

Singh N, Lai H. 1998. 60 Hz magnetic field exposure induces DNA crosslinks in rat brain cells. Mutation Research, 400:313-320.

Singh N, Mathur R, Behari J. 1997. Electromagnetic fields and cancer. IETE Technical Review, 14:149-151.

Stelzer JK, Griffin TW. 1993. A randomized prospective trial of radiation therapy for AIDS-associated Kaposi's sarcoma. International Journal of Radiation Oncology Biology Physics, 27:1057-1061.

Sulpizio M, Falone S, Amicarelli F, Marchisio M, Di Giuseppe F, Eleuterio E, Di Ilio C, Angelucci S. 2011. Molecular basis underlying the biological effects elicited by extremely low frequency magnetic field (ELF-MF) on neuroblastoma cells. Journal of Cellular Biochemistry, 112:3797-3806.

Tofani S. 2015. Electromagnetic energy as a bridge between atomic and cellular levels in the genetics approach to cancer treatment. Current Topics in Medicinal Chemistry, 15:572-578.

Tofani S, Barone D, Cintarino M, de Santi MM, Ferrara A, Orlassino R, Ossola P, Peroglio F, Rolfo K, Ronchetto F. 2001. Static and ELF magnetic fields induce tumor growth inhibition and apoptosis. Bioelectromagnetics, 22:419-428.

Verginadis I, Velalopoulou A, Karagounis I, Simos I, Peschos D, Karkabounas S, Evangelou A. 2012. Beneficial effects of electromagnetic radiation in cancer. Electromagnetic Radiation, 11:250-268.

Vijayalaxmi, Prihoda TJ. 2008. Genetic damage in mammalian somatic cells exposed to radiofrequency radiation: a meta-analysis of data from 63 publications (1990–2005). Radiation Research, 169:561-574.

Wolf FI, Torsello A, Tedesco B, Fasanella S, Boninsegna A, D'Ascenzo M, Grassi C, Azzena GB, Cittadini A. 2005. 50-Hz extremely low frequency electromagnetic fields enhance cell proliferation and DNA damage: possible involvement of a radox mechanism. Biochimica et Biophysica Acta, 1743:120-129.

Yamaguchi S, Ogiue-Ikeda M, Sekino M, Ueno S. 2006. Effects of pulsed magnetic stimulation on tumour development and immune functions in mice. Bioelectromagnetics, 27:64-72.

Zhivotovsky B, Kroemer G. 2004. Apoptosis and genomic instability. Nature Reviews – Molecular Cell Biology, 5:752-762.

Zimmerman JW, Jimenez H, Pennison MJ, Brezovich I, Morgan D, Mudry A, Costa FP, Barbault A, Pasche B. 2013. Targeted treatment of cancer with radiofrequency electromagnetic fields amplitude-modulated at tumor-specific frequencies. Chinese Journal of Cancer, 32:573-581.

Appendix A1: Original Ethics Exemption

Ethics Letter

24-May-2013

Ethics Reference #: X13/05/001
Title: Novel approaches for breast cancer therapy

Dear Professor John Akudugu,

Thank you for your application to our Health Research Ethics Committee (HREC). The Health research Ethics Committee considers this proposal to be exempt from ethical review.

This letter confirms that this research is now registered and you can proceed with study related activities.

If you have any queries or need further help, please contact the REC Office 0219389207.

Sincerely,

REC Coordinator
Mertrude Davids
Health Research Ethics Committee 2

Appendix A2: Amended Ethics Exemption

Ethics Letter

23-Jul-2015

Ethics Reference #: X13/05/001
Title: Novel approaches for cancer therapy

Dear Prof John Akudugu,

The HREC approved the amendment dated 23 May 2015.

If you have any queries or need further help, please contact the REC Office 219389207.

Sincerely,

REC Coordinator
Mertrude Davids
Health Research Ethics Committee 2

Appendix B: Submitted Manuscript

Electromagnetic Fields Induce Frequency-Dependent Radioprotection and Radiosensitization in *In Vitro* Cell Cultures

Journal:	*IEEE Transactions on Plasma Science*
Manuscript ID	Draft
Manuscript Type:	10 Original Article (Other Topics in Plasma Science)
Date Submitted by the Author:	n/a
Complete List of Authors:	Chinhengo, Angela; Stellenbosch University, Division of Radiobiology, Department of Medical Imaging and Clinical Oncology Serafin, Antonio; Stellenbosch University, Division of Radiobiology, Department of Medical Imaging and Clinical Oncology Hamman, Bianca; Stellenbosch University, Division of Radiobiology, Department of Medical Imaging and Clinical Oncology Akudugu, John; Stellenbosch University, Division of Radiobiology, Department of Medical Imaging and Clinical Oncology
Key Words:	Electromagnetic fields, Biological effects of electromagnetic radiation, Biological effects of radiation, Biological effects of X-rays
Specialty/Area of Expertise:	

Electromagnetic Fields Induce Frequency-Dependent Radioprotection and Radiosensitization in *In Vitro* Cell Cultures

Angela Chinhengo, Antonio Serafin, Bianca Hamman, and John Akudugu

***Abstract*— The incidence of Kaposi's sarcoma co-morbidity in HIV/AIDS patients is high due to their compromised immune system. HIV-positive individuals presenting with cancer tend to be more sensitive to ionizing radiation and are at a higher risk of developing severe side effects during radiotherapy, and there is a need to develop non-invasive methods to sensitize cancer cells and reduce therapeutic doses. Here, the effects of 100 and 1000 Hz electromagnetic fields (EMF) on the radiosensitivity of Chinese hamster lung fibroblasts (V79) and human malignant melanoma cells (MeWo) were evaluated using the colony forming assay. The induced magnetic flux densities in cell cultures ranged from 0.05 to 0.25 μT. Pre-exposure of the fibroblasts to both fields had no effect on their radiosensitivity, if X-ray irradiation followed within 2 h or at 6 h. Significant radiosensitization was observed when X-rays were administered 4 h after EMF exposure. For the MeWo cells, pre-exposure to a 100 Hz field resulted in a significant radioprotection when irradiation followed within 6 h. However, treatment of these cells with a 1000 Hz field significantly potentiated the effect of X-rays. When cells were irradiated prior to EMF exposure, the V79 cells were marginally protected by the 100 Hz field and sensitized by the 1000 Hz field. In contrast, the melanoma cells were slightly protected by the 1000 Hz field and sensitized by the 100 Hz field. These data suggest that informed combination of low-medium frequency electromagnetic fields and radiotherapy might be beneficial in the management of cancers, especially those presented by HIV-positive patients.**

***Index Terms*— Electromagnetic fields, Kaposi's sarcoma, melanoma, radiomodulatory effects, plasma ray tube.**

I. Introduction

KAPOSI'S sarcoma (KS) usually appears as tumors on the skin or on mucosal surfaces, such as the inner lining of the mouth. KS is now considered as an "AIDS defining" illness. HIV-positive patients are at a greater risk of cancer than the general population due to a compromised immune system [1]. Kaposi's sarcoma is ranked the 6th and 8th most common cancer in South African males and females, respectively [2], and can be treated with surgery, chemotherapy, radiotherapy, or biological therapy. Chemotherapy and radiotherapy can also weaken the immune system, and so, people with HIV/AIDS may not be able to get full courses of cancer treatment without risking severe side effects, such as life-threatening infections. The HIV-positive subset of patients also tends to show higher normal tissue toxicity during conventional radiotherapy than their HIV-negative counterparts.

Pioneering studies over half a century ago demonstrated that although radiosensitivity can be altered using modifying agents, a given modifying agent does not always change the sensitivity of different cell lines to radiation exposure in the same way [3]. This phenomenon has been recently observed whereby a dual inhibition of phosphoinositide 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) was found to radiosensitize prostate and breast cancer cells, but acted as a radioprotector in normal prostate cells and mouse gut [4]-[6]. The main objective of radiotherapy is to kill tumor cells, or stop their proliferation, whilst protecting normal tissue. Due to an increase in the diagnosis of cancer there has been an increased desire to develop novel treatment modalities.

In light of the current rise in HIV infection and cancer diagnosis in HIV-positive individuals, combination therapy options may lead to a reduction in the amount of radiation delivered to a patient during treatment, thus reducing normal tissue toxicity. Reduction in radiation dose during radiotherapy is especially of essence for immune compromised patients who are known to be more radiosensitive [1]. It has also been extensively reported that electromagnetic fields (EMF), such as electric, magnetic, and radiofrequency (RF) fields, in conjunction with chemotherapeutic agents can reverse the resistance of cancer cells [7], [8]. These fields have been shown to inhibit disease progression and prolong patient survival with minimal or no side effects [8]-[10]. Other studies have also shown that extremely low frequency magnetic fields can affect cell death processes like apoptosis [11]-[13]. Magnetic fields penetrate cells unattenuated and can thus interact directly with the DNA

This work was supported in part by the National Research Foundation South Africa under Grants 85703 and 92741.

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in the nucleus and other cell constituents [14]. There is overwhelming evidence supporting the opinion that exposure to magnetic fields has an effect on cellular functions, such as, transcription, protein synthesis, proliferation, and differentiation. Cellular exposure to magnetic flux densities of 0.38-19 mT has been shown to lead to increased transcription of c-myc and histone H2A [15]. These field-induced changes in transcription activity can significantly impact the net cellular response. While c-*myc* plays an important role in cell cycle regulation and cell death, histone H2A is central in DNA damage repair. Although apoptotic cell death has been shown to occur in WiDr cells at magnetic flux densities greater than 1.0 mT, tumor regression in nude mice bearing WiDr tumors was evident only at much higher intensities [12]. Anti-tumor and immune modulatory activity has also been demonstrated in a melanoma mouse model for a magnetic flux density of 0.25 T [16]. Acute exposure to flux densities below 1.0 mT does not exhibit anti-proliferative activity, but results in increased level of reactive oxygen species [17], which may ultimately mediate cellular responses to other cytotoxic agents like chemotherapeutic drugs and ionizing radiation. Electromagnetic fields have also been used to successfully treat ailments, such as, wounds, bone fractures, and depression [18]-[19]. Electric fields with intensities ranging from 1.0 to 1.4 V/cm can alter the cell membrane structure leading to changes in the permeability of ions, such as Ca^{2+}, cause changes in the local pH and temperature, reorganizing cytoskeletal components, and disrupting microtubule polymerization [20]. Exposing cells to electric fields can also cause modifications in gene expression and free radical production which affects DNA structure and provokes strand-breaks and other chromosomal aberrations, such as micronucleus formation [20]. In addition, electric fields can physically affect the movement and orientation of electrically charged molecular entities.

An extremely low frequency magnetic field with a flux density of 1.0 mT has been suggested to induce immune cell activation through three different pathways, namely, the classical activation, the alternative activation and the lectin-dependent activation pathways [21]. The classical activation pathway includes activation of inflammatory responses, destruction of extracellular matrix and induction of apoptosis. The alternative activation pathway promotes extracellular matrix construction, cell proliferation, resolves inflammation, and angiogenesis. The lectin-dependent activation pathway also initiates inflammation and apoptosis and inhibits cell growth in a way comparable to the classical activation [21]. All the perturbations exerted by electromagnetic fields ultimately exert anti-proliferative and anticancer effects by influencing cell cycle progression, the rate of cell proliferation, and apoptosis [9], [18], [20].

The aforementioned therapeutic potential of electromagnetic fields, notwithstanding the application of plasma ray tubes (the so-called Rife Frequency Generator) in the treatment of cancer, largely remains a controversial issue. Over two decades ago, the American Cancer Society discouraged the use of devices, such as the Rife frequency generator for cancer therapy, due to paucity of experimental and scientific evidence [22]. However, the concept of targeting pro-survival genes with characteristic resonant frequencies broadcast from a Rife device to induce cell death was recently demonstrated in a colon cancer cell line [23]. Also, a significant level of evidence exists for effectively targeting malignancies with cancer-specific radiofrequency electromagnetic fields [24].

To test whether the anti-proliferative and anticancer effects of frequencies broadcast from a Rife device could potentiate the cytotoxic effects of ionizing radiation, radiomodulatory effects of low or medium frequency electromagnetic fields were evaluated in Chinese hamster lung fibroblasts (V79 cells) and human melanoma cells (MeWo cells). The potential benefit of such a therapeutic approach to immune compromised patients with superficial cancers is discussed.

II. Material and Method

A. Cell Lines and Culture

The V79 cell line was established from the lung of a Chinese hamster and has a fibroblast-like morphology. These cells were used to represent normal tissue. The culture was obtained from Flow Laboratories (Irvine, Scotland). The human melanoma cell line (MeWo) was kindly provided by F. Zölzer and C. Streffer (University of Essen, Germany). The cells were cultivated as monolayers in 75-cm^2 flasks in Minimum Essential Medium (MEM) supplemented with 20% foetal bovine serum (10% for V79 cells), penicillin (100 U/ml), streptomycin (100 µg/ml) and incubated at 37°C in a humidified atmosphere (95% air, 5% CO_2). Cells were used for experiments upon reaching 80 to 90% confluence. For experiments, cell cultures were trypsinized and 200 to 500 cells seeded per 25-cm^2 tissue culture flask, and left to settle for 2 to 4 h (depending on cell line). The cells were subsequently exposed to an electromagnetic field for 30 min prior to or following an X-ray irradiation at time points of 0, 0.5, 1, 2, 4, and 6 h. The final volume of culture medium in each flask was 10 ml.

B. Electromagnetic Field Generation and Exposure

Electromagnetic fields were generated using an EMEM oscillator amplifier, to produce 27.125 MHz fields, square-wave amplitude-modulated at 100 or 1000 Hz, with a peak-to-peak amplitude of 5 V (EMEM Devices Rife Machine, Model #: 1-2012B, Boulder, CO). The modulating frequencies were generated using a GME frequency generator with an output impedance of 50 Ω and a duty cycle of 50% (GME Technology, Model #: SG-10, Pomona, CA). The resulting radiofrequency (RF) was then broadcast via a double bubble argon plasma ray tube (length = 25 cm; external bubble diameter = 6.7 cm). The set-up for EMF exposure of cell cultures through a plasma Rife tube is illustrated in Fig. 1. A maximum of 24 cell culture flasks could be exposed at a given time, and were stacked in groups of four, such that the outside dimensions of the volume occupied by the cell culture layers

was 11 cm (Width: 2 flasks breadthwise) × 18 cm (Length: 2 flasks lengthwise) × 14 cm (Height: 6 flasks by height). The perpendicular distances from the axis of the plasma tube to the cell culture planes were 10.0, 12.4, 14.8, 17.2, 19.6, and 22.0 cm. Each cell layer was covered with 3.5 mm (10 ml) of culture medium.

To estimate the magnetic and induced electric fields in the cell cultures, the plasma ray tube was assumed to function as an antenna that is transmitting at ~27.12 MHz. Near field magnetic field strengths for this frequency can vary between 0.5 A/m (magnetic flux density of 0.63 μT) and 0.8 A/m (magnetic flux density of 1.0 μT) at a radial distance of 12 cm from the antenna [25]. Therefore, by adopting the maximum magnetic flux density of 1.0 μT as the peak flux density in the plane 12 cm from the axis of the plasma tube (Fig. 1B), the magnetic flux densities in cell culture planes at 10.0 to 22.0 cm were deduced using the inverse-square law. The corresponding induced peak electric fields (V/m) were then calculated as $E_{peak} = 2h\pi fB$ [26], where B is the peak value peak magnetic flux density (T), f is the transmitted frequency in (27.125×10^6 Hz), and $2h$ is the depth of the cell culture medium (0.0035 m). Thus, the estimated magnetic flux densities in the cell cultures ranged from 0.30 to 1.44 μT, and the corresponding peak electric fields were 0.09 to 0.42 V/m (Table I). Using a conductivity (σ) of 1.5 S/m for the cell culture medium [26], induced current densities (J) were calculated from the relation $J = \sigma E$. Estimated current densities in cell cultures ranged from 0.14 to 0.63 A/m^2 (Table I). Since the ratio of the depth to the width (0.05 m) of the culture medium in each flask is less than 0.3, estimation of peak electric fields from the magnetic flux densities has an uncertainty of ≤1% [26]. For sham-EMF exposure (0 Hz), the control samples were treated as described with the plasma ray tube turned off.

To test whether the radial variation in induced magnetic flux density across the cell culture layers had an impact on cell viability, the proportions of seeded cells that eventually form colonies (plating efficiencies) were determined in cell cultures placed at the different radial distances, as in Fig. 1, for 0, 100, and 1000 Hz exposures. In the current setting, no significant frequency- and location-dependent differences in plating efficiency were observed. For the V79 cells, the plating efficiency at 0 Hz (73 ± 4%) did not differ significantly from those at 100 Hz (82 ± 3%; $P = 0.12$) and 1000 Hz (73 ± 5%; $P = 0.94$). Similarly, the plating efficiency for sham exposed MeWo cells (55 ± 4%) was not significantly different from those determined when the cells were exposed to 100 Hz (62 ± 7%; $P = 0.30$) and 1000 Hz (57 ± 6%; $P = 0.82$).

C. Cell Culture Irradiation, Clonogenic Cell Survival and Radiomodulatory Effects of Electromagnetic Fields

Pre-prepared monolayer cell cultures were irradiated at room temperature (20°C) at a dose rate of 1 Gy/min, using a Faxitron MultiRad 160 X-ray irradiator (Faxitron Bioptics, Tucson, AZ). Irradiation was performed at various time points relative to electromagnetic field exposure as described above. Sham-irradiated cultures were left on the turntable of the Faxitron X-ray irradiator for 2 min with the X-ray source turned off.

The irradiated and EMF exposed cell cultures were left in an incubator at 37°C for 7 and 14 days (for V79 and MeWo cells, respectively) for colony formation. Colonies were then fixed in glacial acetic acid:methanol:water (1:1:8, v/v/v), stained with 0.01% amido black in fixative, air-dried, and counted. Unirradiated cultures with and without electromagnetic field exposure were used as controls for EMF and X-ray treatment, respectively. Colonies containing at least 50 cells were deemed to have originated from single surviving cells and were scored. Cytotoxicity was assessed on the basis of a surviving fraction (SF) which was calculated from the relation: $SF=n_{col}(t)/\{[n_{col}(u)/n_{cell}(u)]\times n_{cell}(t)\}$, where $n_{col}(t)$ and $n_{col}(u)$ denote the number of colonies counted in treated and untreated samples, respectively. $n_{cell}(t)$ and $n_{cell}(u)$ are the number of cells seeded in treated and untreated cultures, respectively. Three independent experiments were performed for each time point and experimental arm. Radiosensitivity was expressed in terms of the surviving fraction at 2 Gy.

To investigate the influence of EMF exposure on radiosensitivity, the interaction between EMF and X-rays was expressed as a modifying factor (MF), given as the ratio of surviving fraction at 2 Gy in the absence EMF to that in the presence of EMF. The criteria for inhibition, no effect, and enhancement of radiosensitivity by EMF are $MF<1.0$, $MF=1.0$, and $MF>1.0$, respectively.

D. Statistical Analysis

Statistical analyses were performed using the GraphPad Prism (GraphPad Software, San Diego, CA) computer program. To compare two data sets, the unpaired two-sided t-test was used. A P-value of less than 0.05 indicates a statistically significant difference between the data sets. Data were presented as the mean (±SEM) from at least 3 independent experiments. For each experiment, 3 replicates were assessed.

III. Results

Radiosensitivity was expressed in terms of the surviving fraction at 2 Gy. Fig. 2 shows the relationship between radiosensitivity of the Chinese hamster lung fibroblasts (V79) and the time of X-ray treatment after EMF exposure. For time intervals ranging from 0 to 2 h, exposure to fields of 100 and 1000 Hz had no effect on radiosensitivity, with a modifying factor of ~0.99. Also, no effect on radiosensitivity was observed when cells were irradiated 6 h after EMF exposure (Fig. 2). However, the cells were marginally sensitized when X-irradiation occurred 4 h after EMF treatment, giving modifying factors of 1.09 ± 0.09 and 1.30 ± 0.25 for the 100 and 1000 Hz fields, respectively.

Irradiating V79 cells to 2 Gy prior to exposure to a 100 Hz field yielded a small radioprotection, while a 1000 Hz field exposure resulted in a slight radiosensitization (Fig. 3). The

corresponding modifying factors ranged from 0.87 to 0.96 and from 1.06 to 1.13. These effects were independent of the time interval between X-irradiation and EMF exposure.

Data for cell survival at 2 Gy in the human melanoma cells (MeWo), when cells were exposed to either a 100 or 1000 Hz electromagnetic field before being irradiated with X-rays, are presented in Fig. 4. For all time intervals between EMF and X-ray treatment, pre-treatment with a 100 Hz field resulted in significant radioprotection, with modifying factors ranging from 0.68 ± 0.04 to 0.79 ± 0.01. On the contrary, pre-exposure to a 1000 Hz field yielded significant radiosensitization, giving modifying factors between 1.35 ± 0.02 and 1.64 ± 0.19. The radiation modifying factors when cells were irradiated at 2 h and 4 h after EMF exposure emerged as 1.51 and 1.52, respectively (Fig. 4).

When the MeWo cells were irradiated to 2 Gy of X-rays followed by exposure to a 100 Hz EMF, the cells were rendered more radiosensitive, as shown in Fig. 5, with modifying factors ranging from 1.34 to 1.76. However, when X-ray exposure was followed by treatment with a 1000 Hz EMF, the cells were less radiosensitive with modifying factors ranging from 0.90 to 0.94.

IV. Discussion

Electromagnetic fields are known to affect the normal functioning of cells and their effects differ depending on the cell type. In this investigation, the Chinese hamster lung fibroblasts (V79) were used to represent normal tissue, while the human melanoma cells (MeWo) represented tumor cells. The current data suggest that normal tissue and cancerous cells do not respond to all electromagnetic fields in the same way. While pre-exposure of the V79 cells to both 100 and 1000 Hz fields followed by 2 Gy of X-rays had no effect when cells were irradiated within 2 h of EMF exposure, significant radioprotection and radiosensitization was seen in the MeWo cells for the 100 and 1000 Hz fields over all time points investigated (Fig. 2 and Fig. 4). The findings that the fibroblasts were protected by the 1000 Hz field exposure when cells were irradiated 1 h after EMF exposure and that both fields were radiosensitizing at an interval of 4 h, suggest that an EMF of a 1000 Hz may potentiate tumor radiosensitivity with little or no normal tissue effect if radiation is given within 1 h of EMF exposure.

Interestingly, the 100 Hz field protected and sensitized pre-irradiated V79 and MeWo cells, respectively (Fig. 3 and Fig. 5). This phenomenon, over all time points, indicated that exposing tumor cells to a 100 Hz electromagnetic field within 6 h of administering a fraction of radiation dose might have a significant level of therapeutic benefit. It is currently not clear why these cell lines behave differently when exposed to the two electromagnetic fields, and in a manner dependent on the sequence of EMF exposure and X-irradiation. However, sensitization of the cells when they are exposed to EMF followed by X-ray could be due to many different mechanisms, including the EMF causing an influx of calcium ions, with an alteration in homeostasis triggering mitotic division [27]. This process would prompt otherwise dormant cells to start dividing. Actively dividing cells are more prone to radiation-induced cell death than dormant cells, and the net effect will be a low level of cell survival. Also, the observed radiosensitization may have been caused by intracellular cascades, such as activation of matrix metalloproteinases by reactive oxygen species, the concentration of which is known to be increased by exposure to magnetic fields [17], [28]. Reactive oxygen species act as radiosensitizers. Furthermore, the radiosensitization can result from calcium-ion overload, which is highly toxic and leads to cell suicide, by activating proteases and phospholipases [18], [27]. Cells carrying radiation-induced DNA damage can be expected to be more radiosensitive when exposed to EMF, as the electromagnetic field can disorient charged amino acids, resulting in a change in the 3-dimensional structure of proteins and thus disturbing their function [29]. This could be a reason for the sensitization seen when cells are exposed to EMF after X-irradiation, since the enzymes responsible for repairing X-ray damage may be rendered non-functional by the subsequent exposure to appropriate resonant frequencies [23]. This can result in non-repaired damage and ultimate cell death. However, this cannot explain the radiosensitization seen 6 h after X-irradiation (Fig. 3 and Fig. 5), as most all DNA repair should be completed. The radiosensitization seen when pre-irradiated cells were exposed to EMF may be due to dysregulation of ion channels and alteration of hormones leading to cells adopting different signaling pathways, some of which may trigger cell death [30]. Cells become more radiosensitive when more damage is inflicted on them by another form of treatment. These findings can also be attributed to cells being rendered sensitive to cell type-specific radiofrequency fields [24].

In conclusion, the data reported here demonstrate that electromagnetic fields have the desirable toxic and protective effects on tumor and normal cells, respectively, if appropriate frequencies are administered at the right times relative to X-irradiation. Electromagnetic fields, therefore, have the potential of being used in conjunction with radiotherapy to reduce the total radiation absorbed dose administered to patients. This can have a significant positive impact on the management of patients with superficial tumors, especially those who are immune compromised. To fully realize the potential of this therapeutic approach, additional studies involving a broader range of cell lines are required to understand the mechanism underlying the interaction between electromagnetic fields and ionizing radiation.

References

[1] T. Kaminuma, K. Karasawa, N. Hanyu, T.-C. Chang, G. Kuga, N. Okano, N. Kubo, Y. Okuma, Y. Nagata, Y. Maeda, and A. Ajisawa, "Acute adverse effects of radiation therapy on HIV-positive patients in Japan: study of 31 cases at Tokyo Metropolitan Komagome Hospital," *J. Radiat. Res.*, vol. 51, pp. 749-753, 2010.

[2] A. Jemal, F. Bray, D. Forman, M. O'Brien, J. Ferlay, M. Center, and D. M. Parkin, "Cancer burden in Africa and opportunities for prevention," *Cancer*, vol. 118, pp. 4372-4384, 2012.

[3] J. P. Goodrich, "Experimental modification of radiosensitivity of

embryonic cells," *Radiol.*, vol. 40, pp. 179-187, 1943.

[4] V. A. Potiron, R. Abderrahmani, K. Clément-Colmou, S. Marionneau-Lambot, T. Oullier, F. Paris, and S. Supiot. (2013, Dec.). Improved functionality of the vasculature during conventionally fractionated radiation therapy of prostate cancer. *PLoS One* [Online]. 8(12), p. e84076. Available: http://dx.doi.org/10.1371/journal.pone.0084076.

[5] S. Maleka, A. Serafin, R. Hamunyela, M. Hamid, D. Achel, and J. Akudugu, "NVP-BEZ235 enhances radiosensitivity of human prostate cancer cells but acts as a radioprotector to normal prostate cells," *J. Cancer Biol. Therap.*, vol. 1, pp. 38-45, 2015.

[6] R. Hamunyela, A. Serafin, M. Hamid, S. Maleka, D. Achel, and J. Akudugu, "A cocktail of specific inhibitors of HER-2, PI3K, and mTOR radiosensitises human breast cancer cells," *J. Cancer Biol. Therap.*, vol. 1, pp. 46-56, 2015.

[7] D. Janigro, C. Perju, V. Fazio, K. Hallene, G. Dini, M. K. Agarwal, and L. Cucullo, "Alternating current electrical stimulation enhanced chemotherapy: a novel strategy to bypass multidrug resistance in tumor cells," *BMC Cancer*, vol. 6, p. 72, 2006. doi: 10.1186/1471-2407-6-72.

[8] Verginadis, A. Velalopoulou, I. Karagounis, Y. Simos, D. Peschos, S. Karkabounas, and A. Evangelou, "Beneficial effects of electromagnetic radiation in cancer," in *Electromagnetic Radiation*, S. O. Bashir, Ed. Shanghai: InTech, 2012, pp. 249-268.

[9] E. D Kirson, V. Dbalý, F. Tovaryš, J. Vymazal, J. F. Soustiel, A. Itzhaki, D. Mordechovich, S. Steinberg-Shapira, Z. Gurvich, R. Schneiderman, Y. Wasserman, M. Salzberg, B. Ryffel, D. Goldsher, E. Dekel, and Y. Palti, "Alternating electric fields arrest cell proliferation in animal tumor models and human brain tumors," *Proc. Natl. Acad. Sci. U.S.A.*, vol. 104, pp. 10152-10157, 2007.

[10] A. Barbault, F. P. Costa, B. Bottger, R. F. Munden, F. Bomholt, N. Kuster, and B. Pasche, Amplitude-modulated electromagnetic fields for the treatment of cancer: Discovery of tumor-specific frequencies and assessment of a novel therapeutic approach," *J. Exp. Clin. Cancer Res.*, vol. 28, p. 51, 2009. doi: 10.1186/1756-9966-28-51.

[11] M. Simkó, R. Kriehuber, D. G. Weiss, and R. A. Luben, "Effects of 50 Hz EMF exposure on micronucleus formation and apoptosis in transformed and nontransformed human cell lines," *Bioelectromagnetics*, vol. 19, pp. 85-91, 1998.

[12] S. Tofani, D. Barone, M. Cintorino, M. M. de Santi, A. Ferrara, R. Orlassino, P. Ossola, F. Peroglio, K. Rolfo, and F. Ronchetto. "Static and ELF magnetic fields induce tumor growth inhibition and apoptosis," *Bioelectromagnetics*, vol. 22, pp. 419-428, 2001.

[13] R. Sarimov, E. Markova, F. Johansson, D. Jenssen, and I. Belyaev, "Exposure to ELF magnetic field tuned to Zn inhibits growth of cancer cells," *Bioelectromagnetics*, vol. 26, pp. 631-638, 2005.

[14] M. Blank and R. Goodman, "Electromagnetic fields stress living cells," *Pathophysiology*, vol. 16, pp. 71-78, 2009.

[15] R. Goodman and A. S. Henderson, "Transcription and translation in cells exposed to extremely low frequency electromagnetic fields," *Bioelectrochem. Bioenerg.*, vol. 25, pp. 335-355, 1991.

[16] S. Yamaguchi, M. Ogiue-Ikeda, M. Sekino, and S. Ueno, "Effects of pulsed magnetic stimulation on tumour development and immune functions in mice," *Bioelectromagnetics*, vol. 27, pp. 64-72, 2006.

[17] C. Morabito, S. Guarnieri, G. Fanò, and M. A. Mariggiò, "Effects of acute and chronic low frequency electromagnetic field exposure on PC12 cells during neuronal differentiation," *Cell. Physiol. Biochem.*, vol. 24, pp. 947-958, 2010.

[18] F. Artacho-Cordón, M. M. Salinas-Asensio, I. Calvente, S. Ríos-Arrabal, J. León, E. Román-Marinetto, N. Olea, and M. I. Núñez, "Could radiotherapy effectiveness be enhanced by electromagnetic field treatment?," *Int. J. Mol. Sci.*, vol. 14, pp. 14974-14995, 2013.

[19] G. L.-Y. Cheing, X. Li, L. Huang, R. L.-C. Kwan, and K.-K. Cheung, "Pulsed electromagnetic fields (PEMF) promote early wound healing and myofibroblast proliferation in diabetic rats," *Bioelectromagnetics*, vol. 35, pp. 161-169, 2014.

[20] E. D. Kirson, Z. Gurvich, R. Schneiderman, E. Dekel, A. Itzhaki, Y. Wasserman, R. Schatzberger, and Y. Paltiet, "Disruption of cancer cell replication by alternating electric fields," *Cancer Res.*, vol. 64, pp. 3288-3295, 2004.

[21] M. Lupke, J. Frahm, M. Lantow, C. Maercker, D. Remondini, F. Bersani, and M. Simkó, "Gene expression analysis of ELF-MF exposed human monocytes indicating the involvement of the alternative activation pathway," *BBA-Mol. Cell Res.*, vol. 1763, pp. 402-412, 2006.

[22] American Cancer Society, "Questionable methods of cancer management: Electronic devices," *CA Cancer J. Clin.*, vol. 44, pp. 115-127, 1994.

[23] R. T. V. Agulan, E. M.F. Capule, R. F. Pobre. (2015, March). Effect of pulsed electromagnetic fields on colon cancer cell lines (HCT 116) through cytotoxicity test. Presented at the DLSU Research Congress, vol. 3, De La Salle University, Manila, Philippines. [Online]. Available: http://www.dlsu.edu.ph/conferences/dlsu_research_congress/2015/proceedings/FNH/010FNH_Pobre_RF.pdf

[24] W. Zimmerman, H. Jimenez, M. J. Pennison, I. Brezovich, D. Morgan, A. Mudry, F. P. Costa, A. Barbault, and B. Pasche, "Targeted treatment of cancer with radiofrequency electromagnetic fields amplitude-modulated at tumor-specific frequencies," *Chin. J. Cancer*, vol. 32, pp. 573-581, 2013.

[25] E. D. Mantiply, K. R. Pohl, S. W. Poppell, and J. A. Murphy, "Summary of measured radiofrequency electric and magnetic fields (10 kHz to 30 GHz) in the general and work environment," *Bioelectromagnetics*, vol. 18, pp. 563-577, 1997.

[26] H. Bassen, T. Litovitz, M. Penafiel, and R. Meister, "ELF in vitro exposure systems for inducing uniform electric and magnetic fields in cell culture media," *Bioelectromagnetics*, vol. 13, pp. 183-198, 1992.

[27] P. Pinton, C. Giorgi, R. Siviero, E. Zecchini, and R. Rizzuto, "Calcium and apoptosis: ER-mitochondria Ca^{2+} transfer in the control of apoptosis," *Oncogene*, vol. 27, pp. 6407-6418, 2008.

[28] H. C. Lai and N. P. Singh, "Medical applications of electromagnetic fields," in *Electromagnetic Phenomena and Health - A Continuing Controversy?*, IOP Conference Series: Earth and Environmental Science, IOP Publishing (UK), 2010, pp. 10:012006.

[29] R. G. Menéndez, "Three molecular mechanisms to explain some biological effects of electromagnetic fields and hypogravity," *Med. Hypotheses*, vol. 52, pp. 239-245, 1999.

[30] S. Orrenius, M. J. McCabe Jr, and P. Nicotera, "Ca^{2+}-dependent mechanisms of cytotoxicity and programmed cell death," *Toxicol. Lett.*, vols. 64/65, pp. 357-364, 1992.

TABLE I
ESTIMATED PEAK MAGNETIC FLUX DENSITY (*B*), ELECTRIC FIELD STRENGTH (*E*), AND CURRENT DENSITY (*J*) INDUCED AT A DISTANCE (*d*) FROM PLASMA RAY TUBE

d (cm)	*B* (μT)	*E* (V/m)	J (A/m^2)
10.0	1.44	0.42	0.63
12.4	0.94	0.28	0.42
14.8	0.66	0.20	0.30
17.2	0.49	0.15	0.23
19.2	0.39	0.12	0.18
22.0	0.30	0.09	0.14

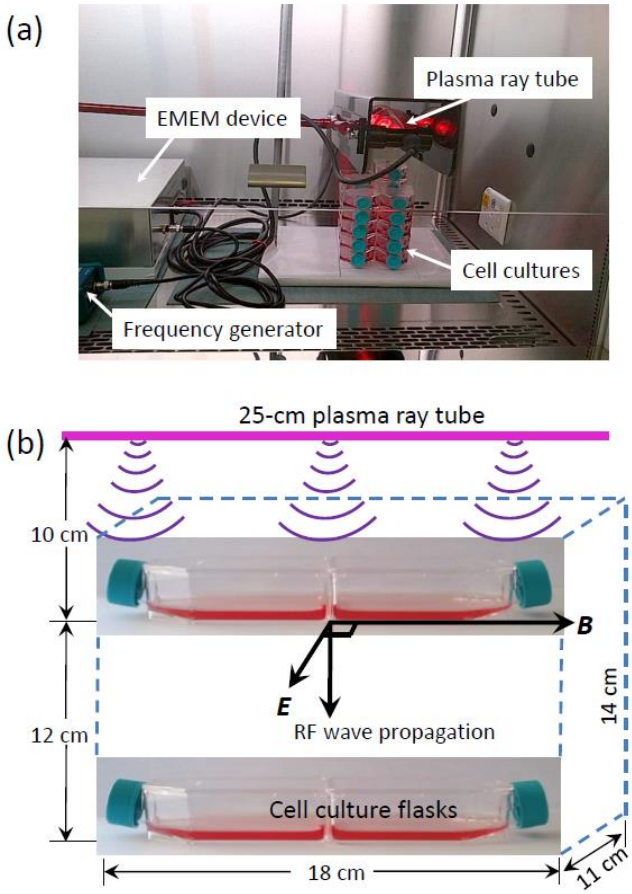

Fig. 1. (a) Photograph of the electromagnetic field (EMF) exposure system. (b) A 2-dimensional schematic diagram showing the top and bottom cell culture planes of the 2×2×6 flask matrix. In the set-up, the plasma ray tube is centred horizontally above the cell culture flasks, such that the induced magnetic field (*B*) is parallel to the base of a flask and the induced electric field (*E*) in the culture medium is parallel to the width of the flask.

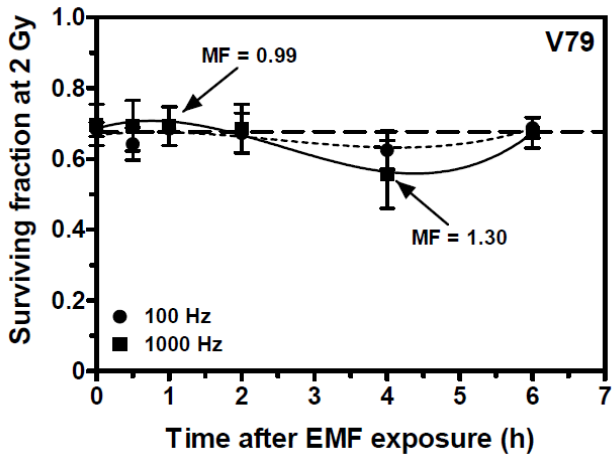

Fig. 2. Clonogenic cell survival at 2 Gy in Chinese hamster lung fibroblasts (V79), when cells were exposed to a 100 or 1000 Hz electromagnetic field (EMF) prior to X-irradiation, as a function of time between EMF exposure and X-ray treatment. Data points are means ± SEM of 3 independent experiments. Horizontal dashed line represents the surviving fraction at 2 Gy without EMF exposure.

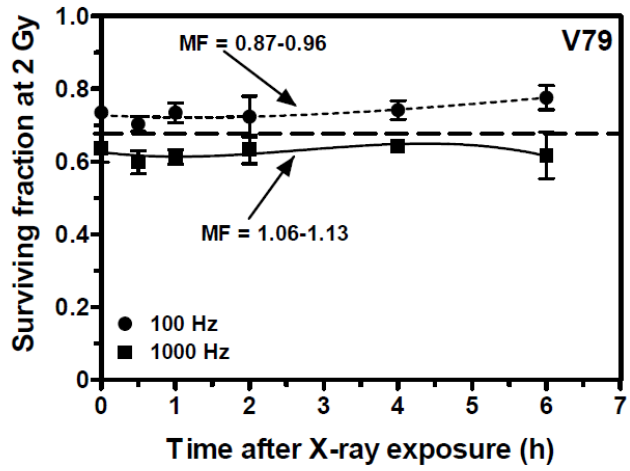

Fig. 3. Clonogenic cell survival at 2 Gy in Chinese hamster lung fibroblasts (V79), when cells were exposed to X-irradiation prior to a 100 or 1000 Hz electromagnetic field (EMF), as a function of time between X-ray treatment and EMF exposure. Data points are means ± SEM of 3 independent experiments. Horizontal dashed line represents the surviving fraction at 2 Gy without EMF exposure.

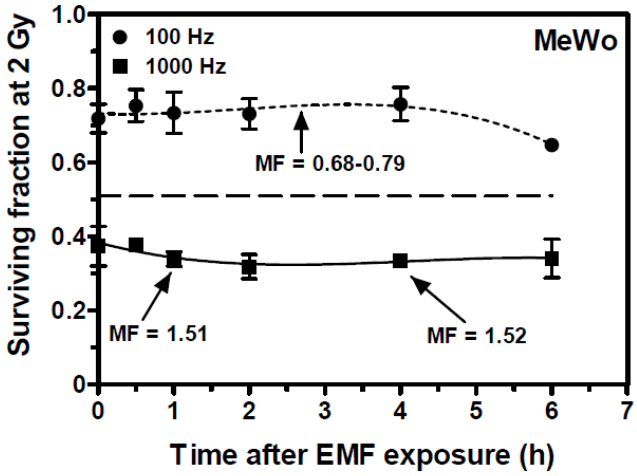

Fig. 4. Clonogenic cell survival at 2 Gy in human melanoma cells (MeWo), when cells were exposed to a 100 or 1000 Hz electromagnetic field (EMF) prior to X-irradiation, as a function of time between EMF exposure and X-ray treatment. Data points are means ± SEM of 3 independent experiments. Horizontal dashed line represents the surviving fraction at 2 Gy without EMF exposure.

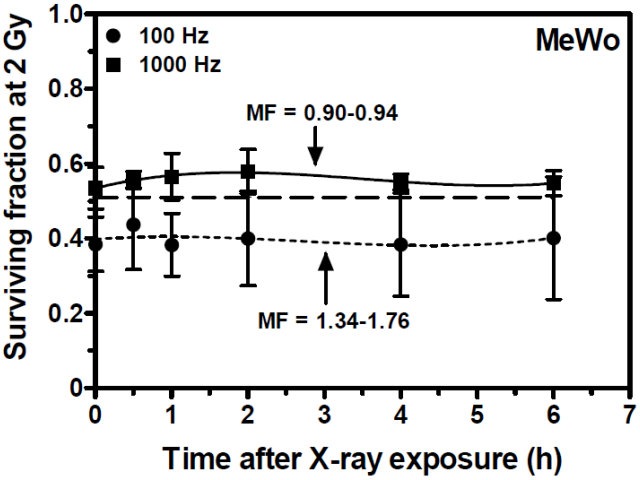

Fig. 5. Clonogenic cell survival at 2 Gy in human melanoma cells (MeWo), when cells were exposed to X-irradiation prior to a 100 or 1000 Hz electromagnetic field (EMF), as a function of time between X-ray treatment and EMF exposure. Data points are means ± SEM of 3 independent experiments. Horizontal dashed line represents the surviving fraction at 2 Gy without EMF exposure.