

**A PHASE III RANDOMISED TRIAL INVESTIGATING THE BENEFITS
OF THE ADDITION OF ONCOTHERMIA TO CHEMO-RADIATION FOR
CERVICAL CANCER IN HIV-POSITIVE AND -NEGATIVE WOMEN IN
SOUTH AFRICA**

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Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, October 2019

DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(Signature of candidate)

PUBLICATIONS ARISING FROM THIS THESIS

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ABSTRACT

Introduction: Outcomes for locally advanced cervical cancer (LACC) patients in low-to-middle-income countries (LMICs), where HIV infection is prevalent and resources are limited, are poor. Hyperthermia is an effective radiosensitiser, but cost and complexity have prevented its use in LMICs. Modulated electro-hyperthermia (mEHT) challenges the dosing concepts of hyperthermia and is potentially an affordable alternative to conventional hyperthermia.

Methodology: In this Phase III controlled trial, participants with FIGO stages IIB to IIIB LACC were randomised (stratum: HIV status, accounting for age and disease stage) to receive chemoradiotherapy with/without mEHT (twice a week, 55 minutes, 130W). HIV-positive participants with a CD4 count >200 cells/ μ L / on antiretroviral therapy for >6 months were included. Pre- and six months post-treatment 18 F-FDG PET/CT scans were administered. Local disease control (LDC), six month local disease-free survival (LDFS) and disease pattern were assessed. Early toxicity was analysed (CTCAE V4.0 criteria) and quality of life questionnaires were administered. All participants signed an informed consent. The trial was approved by the Human Research Ethics Committee (M120477/M190295) and registered on ClinicalTrials.gov (ID: NCT03332069).

Results: During the recruitment period (January 2014 to November 2017), 271 women were screened and 210 were randomised for trial. On pre-treatment 18 F-FDG PET/CT studies, no significant difference in nodal presentation between HIV-positive and –negative participants was noted and 54 participants in each treatment group presented with extra-pelvic nodal disease. There was no significant difference in treatment toxicity, regardless of HIV status, between groups. In the participants eligible for analysis at six months (mEHT: $n=101$; Control: $n=101$), six month LDFS was significantly higher in the mEHT Group ($n=39[38.6\%]$ *versus* $n=20[19.8\%]$; $p=0.003$), as was LDC ($n=40[45.5\%]$ *versus* $n=20[24.1\%]$; $p=0.003$). In participants with extra-pelvic disease, complete metabolic resolution of all disease was seen in $13[24.1\%]$ mEHT and $3[5.6\%]$ Control Group participants (Chi2, Fisher's exact: $p=0.013$) with no difference seen based on HIV status.

Conclusion: mEHT is a safe, effective and affordable adjunct to chemoradiotherapy for HIV-positive and –negative LACC patients in resource-constrained settings, resulting in improved local control rates and a possible potentiation of the abscopal effect. Long term follow-up is planned to determine the effect on survival rates.

This dissertation is dedicated to my parents:

Hillary and David Minnaar

For their unwavering and unconditional love, support, faith, and belief in me.

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TABLE OF CONTENTS

DECLARATION	i
PUBLICATIONS ARISING FROM THIS THESIS	ii
CONFERENCE PRESENTATIONS	iii
ABSTRACT	vii
ACKNOWLEDGEMENTS	ix
TABLE OF CONTENTS	xi
ABBREVIATIONS	xiv
LIST OF TABLES	xvii
LIST OF FIGURES	xviii
LIST OF SUPPLEMENTARY DOCUMENTS	xix
CHAPTER ONE	1
INTRODUCTION	
1.1. Cervical Cancer	1
1.1.1. Global Incidence	1
1.1.2. Aetiology	1
1.1.3. Diagnosis and Staging	2
1.1.4. Treatment and Follow-up	4
1.1.5. Prognosis for Cervical Cancer	6
1.2. Cervical Cancer in Resource-Constrained Settings	7
1.2.1. Diagnosis and Staging	7
1.2.2. Treatment for Cervical Cancer in Resource-Constrained Settings	7
1.3. Human Immunodeficiency Virus (HIV)	8
1.3.1. Overview	8
1.3.2. Global Incidence	9
1.3.3. Related Immunology	9
1.3.4. Acquired Immune Deficiency Syndrome (AIDS)	10
1.3.5. Radiosensitivity in HIV-Positive Patients	11
1.4. Cervical Cancer in HIV-Positive Patients	12
1.4.1. Treatment of Cervical Cancer in HIV-Positive Patients	12
1.4.2. Prognosis of HIV-Positive Cervical Cancer Patients	12
	xi

1.4.3.	Treatment-Related Toxicity in HIV-Positive Patients	14
1.5.	Ionising Radiation	14
1.5.1.	Principles	14
1.5.2.	Radio-resistance	17
1.5.3.	Abscopal Effect	17
1.6.	Hyperthermia	18
1.6.1.	Overview	18
1.6.2.	Hyperthermia and Chemotherapy	21
1.6.3.	Challenges in Hyperthermia	22
1.6.4.	Hyperthermia and Cervical Cancer	23
1.7.	Modulated Electro-Hyperthermia (mEHT)	27
1.7.1.	Overview	27
1.7.2.	Biophysics	28
1.7.3.	Membrane Alterations	31
1.7.4.	Immune Response	33
1.7.5.	Dose Measurement in mEHT	34
1.7.6.	Clinical Research on mEHT and Cervical Cancer	36
1.7.7.	Modulated Electro-Hyperthermia in Resource-Constrained Settings	36
CHAPTER TWO		38
AIMS AND OBJECTIVES		
2.1.	Aim	38
2.2.	Objectives	38
2.3.	Study Rationale	38
CHAPTER THREE		40
The effect of modulated electro-hyperthermia on local disease control in HIV-positive and -negative cervical cancer women in South Africa: Early results from a Phase III Randomised Controlled Trial		
CHAPTER FOUR		67
Acute Toxicity and Quality Of Life in HIV-Positive and –Negative Patients treated with Modulated Electro-Hyperthermia plus Chemoradiotherapy for Locally Advanced Cervical Cancer		
CHAPTER FIVE		105
Potiation of the abscopal effect by modulated electro-hyperthermia in locally advanced cervical cancer patients		

CHAPTER SIX	123
Defining characteristics of nodal disease on PET/CT scans in HIV-positive and -negative locally advanced cervical cancer patients in South Africa	
CHAPTER SEVEN	146
DISCUSSION	
7.1. Overview	146
7.2. Dosing Calculations in Modulated Electro-Hyperthermia <i>versus</i> Hyperthermia	147
7.3. Depth of Energy Penetration	148
7.4. Adverse Events due to Modulated Electro-Hyperthermia <i>versus</i> Hyperthermia	149
7.5. Effects of Modulated Electro-Hyperthermia in HIV-Positive Participants	150
7.6. Clinical Outcomes: Modulated Electro-Hyperthermia versus Hyperthermia	151
7.7. Disease Pattern and Metastases	155
7.8. HIV Infection and CRT-Related Toxicity	157
7.9. Limitations of the Study	161
CHAPTER EIGHT	163
CONCLUSION	
CHAPTER NINE	165
FUTURE PERSPECTIVES	
9.1. Follow-up and Survival	165
9.2. Cost Effectiveness Analysis	165
9.3. Broadening the Scope of mEHT Use	166
9.4. Immunotherapy	166
9.5. Thermo-sensitivity	167
9.6. Protocol Variations	167
REFERENCES	168
APPENDIX A: Ethics Clearance Certificate	186
APPENDIX B: Patient Information Sheet	187
APPENDIX C: Consent Form	194

ABBREVIATIONS

¹⁸ F-FDG	Fluorodeoxyglucose
3DCRT	Three Dimensional Conformal Radiotherapy
AIDS	Acquired Immune Deficiency Syndrome
APC	Antigen Presenting Cells
ART	Antiretroviral Therapy
ATP	Adenosine Triphosphate
BMI	Body Mass Index
BRCA1	Breast Cancer 2
BT	Brachytherapy
C	Celsius
CD	Cluster of Differentiation
CEM43°C	Cumulative equivalent minutes at 43°C
ChT	Chemotherapy
CI	Confidence Interval
CMR	Complete Metabolic Response
CR	Complete Response
CRT	Chemoradiotherapy
CT	Computed Tomography
CTCAE	Common Toxicity Criteria for Acute Toxicity
DAMPs	Damage-Associated Molecular Patterns
DDHT	Dutch Deep Hyperthermia Trial
DFS	Disease Free Survival
DNA	Deoxyribonucleic Acid
E6	Early protein 6
EBRT	External Beam Radiation
ECM	Extracellular Matrix
EQ	Equivalent dose
ESMO	European Society for Medical Oncology
FIGO	International Federation of Gynaecology and Obstetrics
FITC	Fluorescein Isothiocyanate

GIT	Gastrointestinal Tract
GP	Glycoprotein
GUT	Genitourinary Tract
Gy	Gray
HAART	Highly Active Antiretroviral Therapy
HCC	Hepatocellular Carcinoma
HCRT	Hyperthermia plus chemoradiotherapy
HDR	High Dose Rate
HeLa	Human cervical adenocarcinoma cell line
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
HSP	Heat Shock Proteins
HT	Hyperthermia
HR	Homologous Recombination
IARC	International Agency for Research on Cancer
ICD	Immunogenic Cell Death
ICI	Immune Checkpoint Inhibitors
IL	Interleukin
IMRT	Intensity Modulated Radiotherapy
IR	Ionising Radiation
K ⁺	Potassium Ion
LACC	Locally Advanced Cervical Cancer
LDC	Local Disease Control
LDFS	Local Disease-Free Survival
LMIC	Low-to-Middle-Income Country
mEHT	Modulated Electro-Hyperthermia
mEHCT	Modulated Electro-Hyperthermia plus chemotherapy
MN	Micronucleus
MRI	Magnetic Resonance Imaging
MRN complex	Mre11; Rad50 protein; and Nbs1 protein complex
mV	Millivolt
Na ⁺	Sodium Ion

NHEJ	Non-Homologous End Joining
NK	Natural Killer
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitors
OS	Overall Survival
PD-1	Programmed Cell Death Pathway -1
PET	Positron Emission Tomography
PI	Propidium Iodide
QLQ	Quality of Life Questionnaire
QoL	Quality of Life
RCT	Randomised Controlled Trial
RF	Radiofrequency
RHT	Radiation plus Hyperthermia
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RT	Radiation Therapy; Radiotherapy
SEER	Surveillance, Epidemiology, and End Results
SUV	Standard Uptake Value
UNAIDS	United Nations Programme on HIV and AIDS
UV	Ultra Violet
VEGF	Vascular endothelial growth factor
WHO	World Health Organisation

LIST OF TABLES

CHAPTER ONE

Table 1: Summary of Trials on Triple Therapy (HT plus CRT)	27
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CHAPTER THREE

Table 1: Participant and Treatment Characteristics.....	50
Table 2: Treatment Characteristics.....	51
Table 3: Result of Multivariate Logistic Regression Model to predict LDC (adjusted for HIV status, age, and stage).....	51
Table 4: Result of logistic regression analysis to predict six month LDFS (adjusted for HIV status, age, and stage).....	52
Table 5: Number of patients with nodes visualized in each region on pre-treatment scans.....	54

CHAPTER FOUR

Table 1: Summary of local control outcomes at six months post-treatment in the trial by Minnaar <i>et al.</i> (2019)	97
Table 2: Characteristics of Participants and Treatment.....	97
Table 3: Reasons for Missed or Delayed Cisplatin.....	98
Table 4: Frequency of Grade 3-4 Toxicities in each Treatment Group Analyzed by HIV Status.....	99
Table 5: Regression Analysis for overall Acute Toxicity by System.....	100
Table 6: Significant changes in the QoL results.....	100

CHAPTER FIVE

Table 1: Characteristics of Participants Eligible for Analysis of the Abscopal Effect.....	119
Table 2: Details of the extra-pelvic disease in participants with an *Abscopal Effect....	120

CHAPTER SIX

Table 1: Participant Characteristics.....	138
Table 2: Number of Nodes per Region and the HIV Status of the Participant in which the Nodes were Visualised.....	139
Table 3: Comparison of Means Summary of Nodes per Patient.....	139

CHAPTER SEVEN

Table 1: Summary of Local Disease Control Results in the Reviewed Literature.....	145
---	-----

LIST OF FIGURES

CHAPTER ONE

Figure 1: Conductivity of various tissues at increasing frequencies	29
Figure 2: Permittivity of various tissues at increasing frequencies	29
Figure 3: Virtual part of conductivity for various types of breast tissue calculated	29

CHAPTER THREE

Fig 1. Trial Profile.....	49
Fig 2. Tumour Response as Seen on ^{18}F -FDG PET/CT (PERCIST 1.0) by Treatment Group.....	53
Fig 3. Tumour Response on ^{18}F -FDG PET/CT (PERCIST 1.0) by Treatment Group and HIV Status.....	54

CHAPTER FIVE

Fig 1. Number of Patients with Nodes Visualized by Region.....	121
Fig 2. Frequency of Observed Abscopal Effect in HIV-Positive and HIV-Negative Participants in each Treatment Group.....	122

CHAPTER SIX

Fig 1. Linear Relationship between the SUVmax and Size of the Lymph nodes in HIV-Positive and –Negative Participants.....	140
---	-----

LIST OF SUPPLEMENTARY DOCUMENTS

CHAPTER THREE

S1 Figure:	Reasons for Screening Failure before Randomisation.....	64
S2 Table:	Logistic Regression Model for Prognostic Variables and Six Month Local Disease Free Survival.....	64
S3 Figure:	Patient BMI versus Tumour Response on ¹⁸ F-FDG PET/CT.....	65
S4 Figure:	Average Energy Dose versus Tumour Response on ¹⁸ F-FDG PET/CT.....	65
S5 Table:	Logistic Regression Model of mEHT-Related Prognostic Variables and Six Month Local Disease Free Survival.....	66

CHAPTER FOUR

SUPPL 1:	Hyperthermia Schedule.....	100
SUPPL 2:	Chi-squared frequency tables for grades 3-4 toxicities.....	101
A)	In each Treatment Group	
B)	Analyzed by HIV Status	
SUPPL 3:	Significant improvements in QoL scores based on local control.....	102
SUPPL 4:	Summary of trials with temperature, toxicity, compliance and outcome, arranged by outcome, then temperature.....	102

CHAPTER SIX

Sup 1:	Image–Graphical representation of the maximum diameters of the visualised nodes (centimetres).....	141
Sup 2:	Image–SUVmax of the Visualised Lymph Nodes.....	141
Sup 3:	Image–Maximum SUV visualised on ¹⁸ F-FDG PET/CT images.....	142
Sup 4:	Image–Number of Visualised Lymph Nodes.....	142
Sup 5:	Image–Images with the max number of nodes in the head and neck region.....	143
Sup 6:	Image–Images with the max number of nodes in pelvic region.....	144
Sup 7:	Image–Image with the max SUV and sizes of nodes seen in an HIV-positive participant.....	145

CHAPTER ONE

INTRODUCTION

1.1. Cervical Cancer

1.1.1. Global Incidence

The number of new cervical cancer cases diagnosed worldwide in 2018 was estimated to be 569,847, and the number of deaths from cervical cancer was estimated at 311,365. Cervical cancer is the fourth leading cause of cancer-related death in women globally. The incidence varies considerably between countries, with the highest incidence rates occurring in developing regions. The age standardised rate of incidence in Southern Africa is 43.1, and the age standardised rate of mortality is 20.(Bray *et al.*, 2018) Advances in medicine have allowed for the possibility of complete eradication of cervical cancer(Hall *et al.*, 2019) and in most developed countries the incidence has decreased over the last decade. This decline can be attributed to the introduction of widespread and effective screening programs, an increase in the average socioeconomic levels, a reduction in the Human Immunodeficiency Virus (HIV) infection rates, and national Human Papillomavirus (HPV) vaccination programs.(Bray *et al.*, 2018)

Prophylactic vaccination for HPV is the most effective method of preventing HPV-related cancers(Kessler, 2017) and the implementation of national vaccination programs has the potential to completely eradicate cervical cancer.(Bray *et al.*, 2018) The World Health Organisation (WHO) therefore recommends that all girls globally between the ages of 9 and 14 years should be vaccinated against HPV.(World Health Organisation, 2019) Some low-to-middle-income countries (LMIC) have begun implementing vaccination programs against HPV, however these programs are still not feasible in many resource-constrained settings(Sankaranarayanan, 2015) and cervical cancer remains a source of high morbidity and mortality in resource-constrained settings(Oliver and Chiao, 2017) such as South Africa.

1.1.2. Aetiology

Over 150 HPV strains have been identified,(Hillemanns *et al.*, 2016) 13 of which have been classified as Carcinogenic to Humans (Group 1), and two of which have been classified as Possibly Carcinogenic to Humans (Group 2B).(IARC Working Group on the

Evaluation of Carcinogenic Risks to Humans, 2007) Infection with carcinogenic HPV strains appears essential for the development of cervical cancer, with an estimated worldwide prevalence of HPV in at least 99.7% of cervical cancer cases.(World Health Organisation, 2019) Around 70% of cervical cancers are associated with just two of the carcinogenic HPV strains: HPV types 16 and 18.(World Health Organisation, 2019) Up to 90% of women are estimated to become infected with HPV at some point during their lifetime, although HPV infection only persists in 5%-15% of infected women. Those with persistent HPV infection longer than two years are at a high risk for developing cervical cancer.(Sankaranarayanan, 2015)

The presence of HPV alone does not guarantee the development of cervical cancer.(Walboomers *et al.*, 1999) Cofactors associated with the development of cervical cancer include immunosuppression, parity (higher number of pregnancies), the use of the oral contraceptive pill for more than five years,(Hillemanns *et al.*, 2016; Bray *et al.*, 2018) having a first degree relative with cervical cancer,(Hillemanns *et al.*, 2016) and smoking. Women who smoke may have a two-fold higher risk of developing cervical cancer. This may be linked to the effects of smoking on the immune system and on Deoxyribonucleic Acid (DNA) damage.(Hillemanns *et al.*, 2016; Kabekkodu *et al.*, 2017; Bray *et al.*, 2018) Alcohol consumption is another important risk factor.(Kabekkodu *et al.*, 2017) Because of the causal association between HPV infection and cervical cancer development, research into the early detection and management of cervical cancer supports HPV-based screening starting from the age of 30 years.(Marth *et al.*, 2017)

1.1.3. Diagnosis and Staging

The International Federation of Gynaecology and Obstetrics (FIGO) staging system is commonly used for staging gynaecologic cancers, include cervical cancer. According to the FIGO staging system for cervical cancer, Stage I carcinomas range in size from 7mm or less (Stage IA) to over 4cm (Stage IB2) and the carcinoma must be confined to the cervix. Stage II describes carcinomas that extend beyond the cervix but not into the pelvic wall or as far as the lower third of the vagina. Stage IIB carcinomas extend into the parametrium. Stage III disease extends into the pelvic wall and if there is hydronephrosis or a non-functioning kidney then the disease is classified as stage IIIB. Stage IVA disease describes the infiltration of the adjacent pelvic organs and IVB describes the presence of extra-pelvic, metastatic disease. The European Society for Medical Oncology (ESMO) guidelines for the treatment of cervical cancer group FIGO Stages IB2 to IVA as locally advanced cervical cancer (LACC).(Marth *et al.*, 2017)

Clinical staging is still the standard of care recommendation to determine the FIGO stage of cervical cancer, and it involves a clinical examination in order to determine tumour size, vaginal or parametrial involvement, bladder and/or rectum involvement, and palpable lymph nodes. Chest radiographs and abdomino-pelvic ultrasounds are recommended to identify distant metastases and hydronephrosis.(Marth *et al.*, 2017) Although Computed Tomography (CT), and more recently Positron Emission Tomography (PET) / CT, imaging has been shown to more accurately determine tumour size and the presence of distant disease, clinical staging continues to be prioritised in resource-constrained settings.(Marth *et al.*, 2017) This is most likely due to the high costs and lack of availability of diagnostic imaging techniques in these settings.(Chhabra *et al.*, 2016)

In early stage disease, the differences in accuracy between clinical staging, and staging using imaging techniques such as CT or PET/CT, are comparable.(Shirazi *et al.*, 2014) In locally advanced disease the accuracy of clinical staging decreases. The limitations of clinical staging include inadequate evaluation of the proximal extension of the tumour as well as the invasion of the tumour into the pelvic side wall and parametrium. Accurate lymph node evaluation, especially deep extra-pelvic lymph nodes, is not as accurate with clinical staging.(de la Peña *et al.*, 2008)

Effective staging of a tumour size and invasion can also be obtained using magnetic resonance imaging (MRI),(Shirazi *et al.*, 2014) due to the high soft tissue resolution on MRI images. However MRIs have a low sensitivity and specificity for lymph nodes, especially those smaller than 10mm. Fluorodeoxyglucose (^{18}F -FDG) PET/CT has the ability to detect metabolic changes before anatomical changes occur,(Magné *et al.*, 2008; Hansen *et al.*, 2015) making ^{18}F -FDG PET/CT effective at staging lymph node involvement,(de la Peña *et al.*, 2008) distant metastases,(Morkel *et al.*, 2018) and tumour activity.(Testa *et al.*, 2014) The sensitivity and specificity of lymph node evaluation by ^{18}F -FDG PET/CT is 100% and 99.6% respectively for nodes larger than 5mm.(de la Peña *et al.*, 2008) Morkel *et al.* reported that additional findings on ^{18}F -FDG PET/CT resulted in changes made to the treatment protocols in a significant number of evaluated participants.(Morkel *et al.*, 2018) Fluorodeoxyglucose PET/CT is therefore useful in LACC patients selected for treatment with radiation therapy (RT) as it provides information on nodal involvement and the potential need for extended radiation fields to cover the para-aortic nodes.(Narayan and Lin, 2015) Lymph node status has a significant effect on the patient outcome and this information can be useful in determining prognosis.(Kidd *et al.*, 2012) Fluorodeoxyglucose PET/CT is therefore potentially a less invasive alternative to surgery for staging lymph nodes in patients with LACC.(Narayan and Lin, 2015) However,

a well-documented challenge with the interpretation of ^{18}F -FDG PET/CT scans is the risk of false-positives results in patients with acute infections or inflammatory conditions.(Stadalnik, Bakheet and Powe, 1998) Activated inflammatory cells, such as macrophages, lymphocytes, neutrophil granulocytes, and fibroblasts have been shown to avidly take up FDG(Bental and Deutsch, 1993) resulting in an FDG signal that is higher than the background signal.(Bental and Deutsch, 1993)

1.1.4. Treatment and Follow-up

Treatment of early stages of cervical cancer involves surgery and bilateral lymph node dissection and these cases are usually curable.(Marth *et al.*, 2017) In some patients fertility sparing surgery may even be possible.(Pfaendler and Tewari, 2016) The primary component of the treatment regimen for LACC is RT and unfortunately the treatments are associated with high rates of morbidity(Oliver and Chiao, 2017) and impaired quality of life.(Bjelic-Radisic *et al.*, 2012; Derks *et al.*, 2017)

The international standard of care of treatment for LACC is external beam radiation (EBRT) with high dose rate (HDR) brachytherapy (BT) and a platinum-based chemotherapeutic agent as a radiosensitiser.(Petrelli *et al.*, 2014; Koh *et al.*, 2015; Chuang, Temin and Berek, 2016; Kotzen, 2016; Marth *et al.*, 2017) Extended fields on EBRT to include para-aortic lymph nodes significantly reduces the risk of distant metastases and the rate of para-aortic failure but the effects on overall survival are not yet confirmed.(Sapienza *et al.*, 2017) The addition of BT to the radiation protocols is the only way to introduce the required RT dose to cervical tumours without excessive exposure of the healthy tissues to ionising radiation (IR).(Banerjee and Kamrava, 2014; Kotzen, 2016; Pötter *et al.*, 2018) According to ESMO guidelines and reviews the addition of chemotherapy to RT offers a 10% benefit for patients in stage I and II of disease and a 3% benefit for patients in stage III and IVA. The most commonly prescribed chemotherapy is cisplatin, usually administered weekly during EBRT at a dose of 40mg/m².(Marth *et al.*, 2017) In a review of 14 articles and 2445 participants treated either with chemoradiotherapy (CRT) (n=1217) or RT alone (n=1228), the addition of chemotherapy improved local complete response (CR) rates (+10.2%, $p=0.027$), local regional control (+8.4%, $p=0.001$), and overall survival (+7.5%, $p=0.001$). However the authors report an increase of grade III/IV acute toxicities ($p=0.001$) in the CRT participants.(Datta *et al.*, 2017)

In metastatic or recurrent cervical cancer, management includes administration of a cisplatin-based doublet with topotecan or paclitaxel,(Marth *et al.*, 2017) rather than

cisplatin alone, as most patients who develop metastatic disease have already been treated with cisplatin and radiation.(Koh *et al.*, 2015) In cases where the performance status of the patient is less than 2, the ESMO guidelines recommend palliative chemotherapy with the aim of relieving symptoms and improving quality of life.(Marth *et al.*, 2017)

Both radiotherapy and chemotherapy can cause treatment-related toxicities. High Dose Rate BT treatments are associated with embarrassment, fear, and pain(Dzaka and Maree, 2016) and the adverse events associated with radiotherapy can cause significant physical and psychological discomfort for years after treatment in cervical cancer patients.(Hafiz *et al.*, 2015) Radiation to the pelvis may result in adverse events such as fatigue, pain, bladder dysfunction, lower gastrointestinal tract (GIT) and rectal dysfunction, sexual dysfunction, vaginal symptoms,(Ashing-Giwa and Lim, 2011) and dermatological toxicities. These can range in severity from diarrhoea, cystitis, and desquamation to fistulas, bowel and urethra obstructions, vaginal stenosis, and skin ulceration. Low-grade acute urogenital toxicities occur in up to 40% of patients treated with CRT and severe urinary toxicity occurs in 2% to 5% of patients.(Viswanathan *et al.*, 2014) The most common gynaecologic complications of pelvic radiation are vaginal stenosis (which results in impaired sexual function and may interfere with vaginal examinations), and ovarian failure in premenopausal patients. Gynaecologic toxicities occur in up to 88% of LACC patients treated with CRT.(Viswanathan *et al.*, 2014) The incidence of low grade dermatological toxicities occurs in up to 50% of patients treated with pelvic radiation, and grade 4 skin reactions, which include skin ulceration, haemorrhage, and necrosis, are rare (range: 1%-5%).(Viswanathan *et al.*, 2014) Newer EBRT techniques such as Intensity Modulated Radiotherapy (IMRT), have reduced RT-related toxicity, and may also improve outcomes(Kidd *et al.*, 2010) although these techniques are more costly.(Fernandez-Ots and Crook, 2013)

The addition of cisplatin substantially increases the acute toxicity profile of the treatments for LACC. While there are mixed reports on the extent of the benefits of the addition of cisplatin, the general consensus is that the benefits are worth the cost and toxicity.(Datta *et al.*, 2017) Chemotherapy is associated with adverse events such as upper and lower GIT disturbances, haematological toxicities, renal dysfunction, and central nervous system toxicities such as peripheral neuropathy or ototoxicity.(Viswanathan *et al.*, 2014) Haematological toxicities are associated with platinum based CRT regimens and grade 3 toxicities can occur in 20% to 25% of patients.(Viswanathan *et al.*, 2014) Haematological toxicities can include leucopenia and neutropenia and can induce immunodeficiency as

a result of the depletion of cluster of differentiation 4 (CD4) and 8 (CD8) T-lymphocytes.(Ghebre *et al.*, 2017)

Post-treatment follow-up examinations should be scheduled every three to six months in the first two years post-treatment and every six to twelve months from year three to five post-treatment. The ESMO guidelines also recommend that CT or PET/CT scans should be carried out as clinically indicated for follow-up purposes.(Marth *et al.*, 2017)

1.1.5. Prognosis for Cervical Cancer

The stage of disease at the time of diagnosis plays a large role in the choice of treatment and outcomes.(Marth *et al.*, 2017) According to the Surveillance, Epidemiology, and End Results (SEER) in the United States of America, early stages of cervical cancer are usually treated successfully with a five year survival rate of 91%, however the five year survival rate drops drastically in patients with LACC to 57% and metastatic disease is associated with a 16% five year survival rate.(Surveillance Epidemiology and End Results (SEER) Program, 2017) The poor prognosis of metastatic and recurrent disease has led to investigations into systemic treatments such as anti-vascular endothelial growth factor (VEGF) monoclonal antibody treatments for cervical cancer, but the benefits of these treatments have not yet been confirmed.(Pfaendler and Tewari, 2016)

Fluorodeoxyglucose PET/CT scans have been investigated for their prognostic benefits. Factors such as distant lymph node involvement and metabolic activity of the primary tumour, as seen on the ¹⁸F-FDG PET/CT scans, may be prognostic of poorer outcomes.(Kidd *et al.*, 2012) Para-aortic nodal involvement is associated with poorer survival outcomes, even when extended radiation fields are applied to EBRT.(Chantalat *et al.*, 2015) Other factors which are associated with a poorer prognosis include large tumour volume, advanced FIGO stage of disease, prolonged time to complete radiation protocols, and inadequate radiation doses.(Gichangi *et al.*, 2006)

Chase *et al.* and Fayers and Bottomley independently reported that higher quality of life (QoL) characteristics at baseline assessment can impact on the survival of cervical cancer patients.(Fayers and Bottomley, 2002; Chase *et al.*, 2012) Factors associated with a poor QoL score include persistent gynaecological problems, inadequate social support, poor coping skills, depression, somatization, comorbidities, sleep disturbances, and lower levels of education.(Osann *et al.*, 2014) Good scores on physical state, functioning, and mental health are associated with improved survival.(Ashing-giwa, Lim and Tang, 2010)

1.2. Cervical Cancer in Resource-Constrained Settings

1.2.1. Diagnosis and Staging

The WHO estimated that in 2018, 85% of the cervical cancer cases, and 87% of deaths due to cervical cancer, occurred in LMICs.(Ferlay *et al.*, 2018)

Low-resource settings lack effective screening programs for cervical cancer(Denny and Anorlu, 2012) and in places where screening programs are available, patients do not always have access to the screening interventions. Patients living in rural areas may not have the means to travel to clinics for screening procedures. Awareness and education campaigns are limited and patients may not know when to seek medical advice and what constitutes an abnormal sign or symptom. Lower education and malnutrition, both of which are common in developing countries, are associated with late stages of disease at presentation.(Stewart, Moodley and Walter, 2018)

In a low-resource setting, cervical cancer patients are typically staged clinically, based on a physical (including pelvic and vaginal) examination, a chest radiograph, abdominal ultrasound, and cystoscopy. Further investigations are considered on a case by case basis depending on resources and availability.(Morkel *et al.*, 2018) According to Lagasse *et al.*, in 1980, clinical staging was likely to under-stage an estimated 20%–30% of early stage disease cases compared to surgical staging.(Lagasse *et al.*, 1980) Imaging techniques to accurately stage patients in developing countries are lacking. The guidelines for the staging of cervical cancer, especially in developing countries, therefore continue to recommend clinical staging.(Ghebre *et al.*, 2017)

1.2.2. Treatment for Cervical Cancer in Resource-Constrained Settings

In 2015, Grover *et al.* reported that in Africa, where there are 54 countries, 28 countries do not have radiation equipment.(Grover *et al.*, 2015) In the absence of RT, patients are treated with radical surgery and chemotherapy(Kim *et al.*, 2013) or, if they are able to, they must travel to neighbouring countries. In the countries with radiation equipment, only around 20 have access to BT. Where radiation is available, the planning and delivery techniques are often sub-optimal, with many countries still relying on low energy machines, such as Cobalt⁶⁰, which is associated with higher dermatological toxicities. Three dimensional (3D) conformal planning with CT-based techniques, which are able to reduce the RT dose to healthy tissues, are also not widely available in developing countries.(Simonds, Neugut and Jacobson, 2015) There are often not enough radiation

devices in the countries with access to RT to handle the large volume of patients (Denny and Anorlu, 2012) and this increases the waiting periods to start treatment. Another added complication in LMICs is the limited number of healthcare personnel to care for the population's requirements. (Grover *et al.*, 2015) Many of the public oncology facilities in South Africa can be classified as resource-constrained with too few linear accelerators to handle the high patient volumes in many of the provinces. In low-resource settings, patients are living under extreme socio-economic stress (Denny and Anorlu, 2012) and, as reported by Ashing-giwa *et al.*, mental health is an important prognostic factor for cervical cancer. (Ashing-giwa, Lim and Tang, 2010) Completion of treatment schedules in time may be influenced by socio-economic factors such as transport, work or family obligations, and functioning facilities. HIV infection, which is high in many developing countries, is associated with a higher risk of incomplete treatment schedules and residual tumours. (Gichangi *et al.*, 2006; Ghebre *et al.*, 2017) Limited resources and too few healthcare providers may also result in patients and their symptoms not being well understood which negatively impacts on patient management. One study in a public healthcare facility in South Africa showed that patients receiving BT experienced fear, pain, and humiliation associated with the treatment. The authors recommended that pain management and patient care needed to be optimised. (Dzaka and Maree, 2016) As a result of the complex set of confounding factors and risks associated with late diagnosis, poor socio-economic conditions and sub-optimal treatment, in a resource-constrained setting, cervical cancer was the leading cause of cancer death in South African women in 2014. (Made *et al.*, 2017)

The high burden of cervical cancer on already strained public healthcare systems in Sub-Saharan Africa (Mboumba Bouassa *et al.*, 2017) necessitates the investigation of affordable methods to improve treatment outcomes.

1.3. Human Immunodeficiency Virus (HIV)

1.3.1. Overview

There are two types of HIV (genus: Lentivirus, family: Retroviridae): HIV-1 and HIV-2. The less pathogenic HIV-2 is mostly confined to West Africa while HIV-1 is rapidly evolving and is primarily responsible for the global HIV pandemic. The rapidly evolving nature of the virus poses challenges in prevention strategies involving vaccine development, and in treatment, due to the rapid development of drug resistance. It also enables the virus to

escape detection by the host immune system.(German Advisory Committee Blood Subgroup Assessment of Pathogens Transmissible by Blood, 2016)

Once in the body, the virus requires two receptors on the host cells in order to bind to the cells. The first receptor is the CD4, and the second is a co-receptor that belongs to the chemokine family. These receptors are expressed on the surface of certain T-lymphocytes, macrophages, dendritic cells, and microglial cells in the brain. The virus binds to cells via interaction between the viral surface glycoprotein (gp) 120, and the CD4 molecule. This binding enables the gp120 to bind with the co-receptor as well, triggering the virus to insert the glycoprotein gp41 into the cell membrane and fixing the virus in position on the cell. The viral core is then released into the cytoplasm and the viral ribonucleic acid (RNA) is released and transported to the cell nucleus where it is integrated into the host DNA. This integrated virus is referred to as a provirus. Host enzymes transcribe the proviral DNA and the resulting full-length RNA is transported to the cell membrane for inclusion into new virus particles which will mature, bud through the cell membrane (taking a portion of the cellular lipid bilayer with it), and be released as a mature virus to infect surrounding cells.(Goodsell, 2015; Meulendyke, Croteau and Christine Zink, 2015)

1.3.2. Global Incidence

Infection by HIV and the resulting Acquired Immunodeficiency Syndrome (AIDS) is a global problem, however in LMICs where education, awareness, and effective prevention strategies are limited, infection rates are much higher. The Joint United Nations Programme on HIV and AIDS (UNAIDS) estimated that there were 36.9 million (range: 31.1 million – 43.9 million) people infected with HIV in 2017, of which only 75% were likely aware of their status.(UNAIDS, 2018) Sub-Saharan Africa accounts for 79.1% of the global HIV burden.(Ghebre *et al.*, 2017) The region with the highest infection rates is Eastern and Southern Africa, with an estimated 19.6 million HIV infected people. In comparison, Western and Central Europe and North America combined are estimated to have only 2.2 million HIV-positive people. According to UNAIDS there are 7.1 million people infected with HIV in South Africa.(UNAIDS, 2018)

1.3.3. Related Immunology

The adapted, or acquired, immune response involves the T- and B- lymphocytes. These T- and B- cells have receptors which can bind to specific antigens (proteins on the membrane of an invading pathogen that are unique to the pathogen).(Dimopoulos, Moysi

and Petrovas, 2017) Antigen presenting cells (APC) are able to engulf pathogens, process their antigens and present the antigens to the T-cells, priming the T-cells for a response to all cells containing the antigen. This promotes the adaption of the immune response to new pathogens. The T-cells are divided into CD4 and CD8 cells. The CD4 cells are able to recognise antigens of cells that are foreign, whereas CD8 cells recognise antigens of cells that contain foreign cells.(Brix *et al.*, 2017) After initial infection by HIV-1, the immune system is able to mount a response using CD8 T-cells. During this initial phase of infection, referred to as the acute phase, patients may experience a range of symptoms, including, fever, night sweats, malaise, nausea, myalgia, headaches, generalised lymphadenopathy, hepatosplenomegaly, pharyngitis, and GIT disturbances. However as HIV-1 infects cells containing CD4 receptors, the function of the immune response which is reliant on the CD4 T-cells begins to decrease. Eventually the CD8 cells no longer function adequately and chemotaxis, Interleukin (IL) 1 production, and antigen presentation decreases. This results in an impairment in the host immune system, resulting in an increased risk of infection by invading pathogens.(German Advisory Committee Blood Subgroup Assessment of Pathogens Transmissible by Blood, 2016) Antiretroviral therapy (ART) is able to suppress the replication of HIV, allowing the host immunity to recover. A CD4 count that drops to <200cells/ μ L is an indication for the initiation of ART.(Fox *et al.*, 2011)

The homing theory describes the homing of HIV-infected CD4 cells from the blood to the lymph nodes where they undergo apoptosis, as a possible cause for the decrease in CD4 counts.(Cloyd, Chen and Wang, 2000; Dimopoulos, Moysi and Petrovas, 2017) The accumulation of the infected cells in the lymph nodes correlates with the generalised lymphadenopathy typically seen in HIV-infected patients. Several studies on ^{18}F -FDG PET/CT in HIV-positive patients have demonstrated the importance of taking the HIV status of the patient into account when interpreting the cause of increased FDG-uptake in lymph nodes on PET/CT images.(Iyengar *et al.*, 2003; Scharko *et al.*, 2003; Brust *et al.*, 2006; Sathekge *et al.*, 2010; Davison *et al.*, 2011; Morkel *et al.*, 2018)

1.3.4. Acquired Immune Deficiency Syndrome (AIDS)

Acquired Immunodeficiency Syndrome is the term used to describe the 26 conditions (including opportunistic infections, malignancies, and direct results of the HIV infection, such as encephalopathy or wasting syndrome), caused by the depletion of the host immune system after prolonged HIV infection, as listed by the Centre for Disease Control.(Grover *et al.*, 2018) The median time to developing AIDS after HIV-infection is

ten years, without treatment.(German Advisory Committee Blood Subgroup Assessment of Pathogens Transmissible by Blood, 2016)

1.3.5. Radiosensitivity in HIV-Positive Patients

Research into the radiosensitivity of HIV-positive versus HIV-negative patients is limited. Baeyens *et al.* have previously reported on the increased chromosomal radiosensitivity seen in the lymphocytes of HIV-positive individuals compared to HIV-negative individuals.(Baeyens *et al.*, 2010) Chromosomal radiosensitivity describes the increased frequency of chromosomal aberrations seen after exposure to IR.(Sprung *et al.*, 2005) A paper published in 2016 by Herd *et al.*, showed that in the sample population, cervical cancer patients had a higher systemic chromosomal radiosensitivity, as seen in lymphocyte samples, than healthy controls, with HIV-positive cervical cancer patients being the most radiosensitive.(Herd *et al.*, 2016) Ulrike *et al.* found that HIV-1 treatment with ART regimes containing non-nucleoside reverse transcriptase inhibitors (NNRTI) may increase the chromosomal radiosensitivity of lymphocytes(Ulrike *et al.*, 2015) and Herd *et al.* showed that the administration of ART to HIV infected patients can enhance the chromosomal radiosensitivity of these patients. However prophylactic ART administration to otherwise healthy healthcare workers did not affect their chromosomal radiosensitivity.(Herd *et al.*, 2014)

One study has suggested that this radiosensitivity seen in HIV-positive patients may not be limited to lymphocytes. Formenti *et al.* showed that the fibroblasts from the skin of HIV-positive patients with Kaposi Sarcoma are more sensitive to IR when compared to those of healthy controls.(Formenti *et al.*, 1995)

The link between the chromosomal radiosensitivity of HIV-positive patients and tumour response or RT-related treatment toxicity is still not established and the research on the potential increase in treatment toxicity in HIV-positive patients is limited.(Housri, Yarchoan and Kaushal, 2010) Radiotherapy has been shown to decrease various immunologic parameters, including CD4 counts. This is especially applicable when the radiation field is large and includes a substantial amount of lymphoid tissue or bone marrow.(Housri, Yarchoan and Kaushal, 2010) Human Immunodeficiency Virus infection is associated with neutropenia and, although this can be managed with effective Highly Active Antiretroviral Therapy (HAART) regimens,(Shi *et al.*, 2016) HIV-positive patients treated with cisplatin and RT still have a significantly increased risk of developing leucopenia and other CRT-related toxicities.(Simonds, Neugut and Jacobson, 2015; Vendrell *et al.*, 2018) Myelosuppression experienced by HIV-positive patients also

increases the risk of neutropenia-related bacterial infections and puts the patients at risk of potential reactivation of dormant viral infections including HIV.(Ghebre *et al.*, 2017)

1.4. Cervical Cancer in HIV-Positive Patients

Human Immunodeficiency Virus infection is associated with immunosuppression(Ghebre *et al.*, 2017) and a subsequent increased risk of chronic and persistent HPV infection.(Firnhaber *et al.*, 2009) Persistent HPV infection is associated with an increased risk of developing cervical cancer.(Ghebre *et al.*, 2017) Cervical cancer is the most common AIDS-related malignancy in women and is one of the more important AIDS-defining diseases.(Maiman *et al.*, 1997) Du Toit and Kidd reported on the quality of life of 16 HIV-positive cervical cancer patients compared to 57 HIV-negative cervical cancer patients in South Africa and found that HIV status did not significantly affect the quality of life in cervical cancer patients. However the stage of disease had a significant impact on pain, insomnia, nausea, vomiting, loss of appetite and constipation. In the population sample 7.1% had no formal education, 41.5% were unemployed and 14.2% were pensioners.(Du Toit and Kidd, 2013) While HIV infection may not significantly affect the quality or life of cervical cancer patients, there are many other factors associated with HIV-infection, which impact staging, treatment and clinical outcomes of these patients.

1.4.1. Treatment of Cervical Cancer in HIV-Positive Patients

A CD4 count below 200cells/ μ L is associated with an increased risk of opportunistic infections(Dryden-Peterson *et al.*, 2016) and these patients are excluded from receiving cisplatin. These patients therefore have a higher risk of worse treatment outcomes. In a study in Botswana, authors reported that 10% of HIV-positive cervical cancer patients had a CD4 count below 200cells/ μ L, and a study in a public healthcare facility in South Africa reported a median CD4 count of 341cells/ μ L with a range from 33cells/ μ L to 790cells/ μ L. In the South African study, only 16 out of 36 patients had started ART prior to starting treatment for cervical cancer.(Simonds, Neugut and Jacobson, 2015)

1.4.2. Prognosis of HIV-Positive Cervical Cancer Patients

Although the data on the treatment of LACC in HIV-positive patients is still limited, the body of research on the topic is growing. Several studies have been published from Africa over the last few years which have provided some insight into the response of HIV-positive patients to LACC treatment, and these are described in the following paragraphs-

Mangena *et al.* reported on a retrospective review of 47 HIV-negative and 51 HIV-positive cervical cancer patients with FIGO Stages I through to IV disease who received standard treatments based on recommendations for the stage of disease. Patients infected with HIV were an average of 13 years younger, had higher rates of anaemia, and experienced more treatment interruptions than their HIV-negative counterparts. Only 78% of the HIV-positive patients had a CD4 count above 200cells/ μ L and, although exact numbers are not given, authors state that not all patients were receiving ART at the time of diagnosis and that some patients only started ART at the same time as the oncology treatments. The authors report that the HIV-positive patients had significantly worse survival rates and that HIV-infection was associated with higher rates of treatment interruptions and insufficient radiation.(Mangena *et al.*, 2015)

Ugandan researchers reporting on 802 cancer patients, 316 of which had cervical cancer, found that HIV-infected cancer patients had more than a two-fold higher risk of death than HIV-negative patients. Forty two percent of the cervical cancer patients were HIV-positive and the authors found a more pronounced risk of death in the HIV-positive patients diagnosed and treated before widespread ART rollout programs were initiated.(Coghill *et al.*, 2013) Researchers in Botswana reported on three year survival rates in 231 HIV-positive and 96 HIV-negative cervical cancer patients. The three year survival for the HIV-positive patients was 35% compared to 48% in the HIV-negative patients. In the HIV-positive group, only 81.8% of the participants were receiving ART at the time of diagnosis and 10.4% had a CD4 count below 200cells/ μ L. In an adjusted analysis, HIV infection significantly increased the risk for death among women with cervical cancer (hazard ratio: 1.95; 95% Confidence Interval (CI): 1.20 to 3.17). All stages of cervical cancer were included in the study and the rate of completion of treatment according to prescribed protocols was low.(Dryden-Peterson *et al.*, 2016) Radiation dose, stage of disease, and immune competence are also known to influence treatment outcomes.(Gichangi *et al.*, 2006)

In a study in Kenya on 218 cervical cancer patients treated with EBRT (without BT or chemotherapy), 20% of the patients were HIV-positive. The CD4 count and the number of patients on ART were not reported. In the study, HIV infection was an independent risk factor for treatment interruptions and residual tumours. The authors propose two possible reasons for the increased rates of pelvic control failure in the HIV-positive patients. In their study HIV infection was associated with anaemia, which is a known prognostic factor for tumour control in patients treated with RT. The authors also suggest that HIV infection may alter the cell kinetics of the cancer cells, resulting in a more radioresistant tumour.(Gichangi *et al.*, 2006)

Patients infected with HIV, who are already on ART prior to the diagnosis of cervical cancer, may have better outcomes and less treatment-related toxicity than ART-naïve HIV-positive patients. In a retrospective analysis of 190 patients with FIGO stage IIB cervical cancer patients in South Africa, no difference in survival at five years was seen between the HIV-positive and -negative patients. However in this study only HIV-positive participants receiving ART were included. Due to this strict inclusion criterion, of the 190 participants, only 13 were HIV-positive. In this sample, ART may have positively influenced the survival rates of HIV-positive patients.(Jemu *et al.*, 2018)

1.4.3. Treatment-Related Toxicity in HIV-Positive Patients

In the Kenyan study, HIV infection was associated with a seven fold higher risk of developing multisystem toxicity involving the dermatological, GIT and genitourinary tract (GUT) systems.(Gichangi *et al.*, 2006) In a South African study, Simonds *et al.* reported that HIV status significantly affected the rates of haematological toxicity in patients treated with CRT, and HIV-positive patients were less likely to complete chemotherapy treatment. In this study the completion of RT was not associated with HIV status.(Simonds, Neugut and Jacobson, 2015)

Zambian researchers reported on the acute toxicity of HIV-positive patients (n=60) compared to HIV-negative patients (n=60) treated for cervical cancer (FIGO stage IB2 to IIB) with CRT. The authors concluded that CRT was safely tolerated by the HIV-positive patients in their study, all of whom were receiving ART and were immunocompetent. All patients had a CD4 count of above or equal to 200cells/ μ L which likely contributed to their ability to tolerate cisplatin. Cisplatin was administered at a dose of 80mg/m², three weeks apart. The radiation protocol included both EBRT (using a four field box technique) and HDR BT according to standard of care recommendations for the stage of disease. The only statistically significant difference in toxicity noted between the two groups was acute vomiting (seen more frequently in the HIV-negative patients) and a higher rate of Grade 1 platelet toxicity seen in the HIV-positive patients.(Mdletshe, Munkupa and Lishimpi, 2016)

1.5. Ionising Radiation

1.5.1. Principles

Radiation is the emission or transmission of energy in the form of particles or waves. Ionising radiation has enough energy to remove an electron from an atom or molecule,

resulting in an electrically charged and highly reactive oxygen species (ROS). (Frey *et al.*, 2017) These ROS are the main drivers of radiotherapy-induced cellular damage. (Zhang *et al.*, 2016) Waves with ionising properties are γ -rays, X-rays, and part of the ultra violet (UV)-rays. The main difference between X-rays and γ -rays is that X-rays are produced outside of the atomic nucleus, while γ -rays are produced as a result of nuclear decays. The unit of measurement used to describe IR used in oncology is the Gray (Gy), which is defined as one Joule of energy absorbed per kilogram of matter (1J/kg). The unit does not take into account the biological effects of the absorbed energy and the equivalent dose (EQ) must therefore be calculated to determine the dose required to achieve the desired biological effects. The calculation of the EQ takes into account the tissue type as well as the type of radiation. (Frey *et al.*, 2017)

Ionising radiation used in oncology is referred to as radiotherapy or radiation therapy (RT). The goal of RT is to stop proliferating tumour cells. The damage done to the DNA of malignant cells by RT induces, amongst other things, apoptosis, necrosis, mitotic catastrophe, autophagy, cell cycle arrest, and/or senescence. (Deloch *et al.*, 2016) The required dose is administered in fractions, over several days. Three main dosing regimens are used: “norm”-fractionation (daily doses of around 1.8-2.0Gy), hypofractionation (higher daily doses and reduced overall treatment time), and hyperfractionation (lower daily doses, delivered twice daily). (Frey *et al.*, 2017) Calculations regarding the dose required to damage malignant tissues must take into account the maximum dose that can be administered before causing unacceptable toxicity to healthy tissues as well. Advances in modern RT delivery techniques and planning have resulted in a significant improvement in the focussing of the IR on the tumour and in reducing the exposure of healthy tissues and surrounding organs (organs at risk) to IR, however there is still a degree of exposure of the healthy tissues to IR. (Deloch *et al.*, 2016)

The direct effect of IR refers to the damage resulting when the radiation hits the DNA molecule directly, disrupting the molecular structure. (Desouky, Ding and Zhou, 2015) Breaks in both strands of the DNA (double strand breaks) are the most dangerous for the cell and are the hardest to repair. The indirect effects of IR are largely attributed to the effects of ROS on target tissues. These include damage to cellular membranes and to proteins, such as those required to repair DNA double strand breaks and to replicate RNA for mitosis. (Frey *et al.*, 2017) In the indirect action, the radiation hits the water molecules (which makes up around 70% of the cell), as well as other organic molecules, forming free radicals such as hydroxyl (OH-) and alkoxyl (RO-). (Desouky, Ding and Zhou, 2015) which will cause damage to the DNA.

Deoxyribonucleic acid was first discovered by Swiss doctor Friedrich Miescher during his experiments on the chemical composition of leucocytes in 1869. The young doctor concluded that the (then unknown) precipitate had been extracted from the nucleus of the cells and he therefore named it nuclein.(Dahm *et al.*, 2008) We now know that this double helix shaped substance is the main constituent of the chromosomes which are found in almost every single form of life and are responsible for carrying the genetic code that governs the growth, development, interaction, and evolution of life. Deoxyribonucleic acid is a dynamic molecule, capable of replication, recombination, and repair. The repair of double strand breaks (as may occur from IR) involves either the re-joining of the broken ends (non-homologous end joining) or the homologous recombination of the damaged site using a matching chromosome. Recombination can be risky as mutations resulting from incorrect matching and repairs can have devastating effects. For example the mutation of at least one of these repair proteins, a tumour suppressor gene known as Breast Cancer (BRCA) type 1, is well known for its link to hereditary breast cancer.(Friedberg, 2003)

Most of what we now know in terms of epidemiological data on the effects of IR on DNA and the consequences, comes from the survivors of the Hiroshima and Nagasaki atomic bombs, nuclear fall outs such as Chernobyl, and to some degree from survivors of accidental occupational exposures to IR.(Burgio, Piscitelli and Migliore, 2018)

The non-targeted effects of IR refer to the effects of the IR on tissues not in the radiation field, also known as the Bystander effect. The Bystander effect describes the damage seen in the tissues surrounding the irradiated tissue and it is thought that the damage is caused by the direct cell to cell communication. The release of signal molecules, such as Damage-Associated Molecular Patterns (DAMPs) and other cytokines, may play a role in the response.(Nikitaki *et al.*, 2016; Burgio, Piscitelli and Migliore, 2018; Miousse, Kutanzi and Koturbash, 2018) These effects may be seen as far as 5cm from the irradiated tissue.(Frey *et al.*, 2017)

The immunogenic effect of IR has also been reported. Ionising radiation increases the expression of immunogenic molecules (such as CD80, adhesion molecules, death receptors, stress induced ligands, and endoplasmic reticulum-derived calreticulin), on the surface of tumour cells. The radiation-induced stress-response further leads to the expression of Heat Shock Protein (HSP) 70 on the surface of tumour cells. This HSP is specifically produced by tumour cells and not healthy cells and plays an important role in mounting an immune response at the site when released into the extracellular matrix.(Frey *et al.*, 2017)

1.5.2. Radio-resistance

Various characteristics can render a tissue less susceptible to damage by IR. Reactive Oxygen Species are important mediators of IR-induced cell killing, and low levels of ROS may therefore contribute to radio-resistance.(Frey *et al.*, 2017) Oxygen deprivation (hypoxia) means that the environment or tissue has fewer oxygen molecules and therefore lowered production of ROS. Hypoxic tumours are therefore more radioresistant. Hypoxia is a characteristic condition of solid tumours resulting from the inability of the primitive tumour neo-vasculature that develops via angiogenesis to meet the oxygen demands of the growing malignant tissue.(Elming *et al.*, 2019) Griffin *et al.* reports that oxygen-rich environments exposed to IR can result in up to three times higher cell killing(Griffin *et al.*, 2005) and Akber reports that a small increase in oxygen of only 0.5% improves the state from hypoxic to half way between hypoxia and full oxygenation. (Akber, 2015) The acidification of the tumour as well as the number of cells in the S-phase of cell division further contribute to radio-resistance.(Hurwitz and Stauffer, 2014)

Numerous approaches to act on the characteristically hypoxic conditions and overcome the radioresistant qualities of tumours have been investigated. These include methods to increase oxygen delivery, such as high oxygen content gas breathing (carbogen breathing or hyperbaric oxygen), increasing haemoglobin levels, decreasing oxygen consumption (using medication such as metformin or phenformin), stabilising the blood flow (with medications such as nicotinamide), and increasing the blood flow and oxygen delivery by heating the tumour.(Griffin *et al.*, 2005; Elming *et al.*, 2019) The radiosensitising effect of heating the tumour can be attributed to four main factors: increased perfusion of oxygen to the tumour, inhibition of radiation induced DNA damage repair, sensitising effects of HT on the S-phase of cell division, and the direct effects of heat on cells.(Szasz, Szasz and Szasz, 2001; van der Zee, 2002; van der Zee and González González, 2002; Griffin *et al.*, 2005; Datta *et al.*, 2015)

1.5.3. Abscopal Effect

The term “abscopal effect” was first used in 1953 by R.H. Mole to describe the effects of radiation at a distance (Latin: ab = away, scopus = target).(Brix *et al.*, 2017) In 1979, Slone *et al.* showed that there is an immune component associated with the IR. In their study, a higher dose of IR was required to kill fibrosarcomas in mice who were T-cell deficient, compared to T-cell competent mice. According to their results, the T-cell deficient mice were also more likely to develop metastatic disease.(Slone, Peters and Milas, 1979; Ngwa *et al.*, 2018) Since then, several papers have been published on case

reports of the local application of IR inducing a response at distant sites of disease, resulting in the partial or complete regression of metastases outside of the treated radiation field.(Abuodeh, Venkat and Kim, 2015) It is now generally accepted that the abscopal effect is mediated by the triggering of an immune response by IR. Although the abscopal effect is rare, with only a couple of case reports published per year, the addition of systemic immunotherapies to radiation protocols over the last few years has resulted in an increase in the number of cases of abscopal effects reported and an increased interest in the field.(Brix *et al.*, 2017)

1.6. Hyperthermia

1.6.1. Overview

Hyperthermia (HT) has been around in oncology settings for decades and the clinical value of the addition of HT to treatment modalities has been shown in numerous randomised phase II and III trials. Significant improvements in clinical outcomes have been demonstrated for tumours of the breast, cervix, head and neck, rectum, bladder, oesophagus, lung, melanoma, and soft tissue sarcomas treated with HT in combination with standard therapies.(Datta *et al.*, 2015; Peeken *et al.*, 2017) The potential for HT to reduce healthcare costs has also been demonstrated.(van der Zee and González González, 2002) Hyperthermia in oncology is described as an increase in the temperature of the tumour to between 39°C and 45°C(van der Zee *et al.*, 2008) although the ideal temperature range is still a topic of debate. A review by Dewhirst *et al.*, discusses the biological effects of HT at temperatures ranging from 39–42°C. The authors highlight the inhibition of the repair of DNA double strand breaks, changes in blood perfusion and re-oxygenation, immunomodulation, and the induction of HSPs at moderate temperatures, all of which works synergistically with chemotherapy and RT.(Dewhirst *et al.*, 2005) Hyperthermia induces several steps associated with IR signalling in cells and influences several molecular processes which are involved in sensitising tumour cells to radiation.(Pandita, Pandita and Bhaumik, 2009) Because of the radiosensitising effect of HT on tumours,(Horsman and Overgaard, 2007; Overgaard, 2013; Peeken *et al.*, 2017) HT can be described as a modulator of RT. According to a review by van der Zee *et al.*, the efficacy of RT can be increased by a factor of 1.2 to 5 with the addition of HT.(van der Zee, 2002)

Hyperthermia interferes with protein synthesis,(Fiorentini and Szasz, 2006; Pandita, Pandita and Bhaumik, 2009) and inhibits DNA and RNA synthesis and repair.(Pandita,

Pandita and Bhaumik, 2009; Oei, Vriend, *et al.*, 2015) Hyperthermia induces protein aggregation in the nucleus and changes in the DNA repair foci such as the MRN complex (Mre11; Rad50 protein; and Nbs1 protein complex), which inhibit the DNA double-strand break repair processes. This is synergistic for RT and for certain cytotoxic agents which cause DNA double-strand breaks. By inhibiting the repairs, potentially repairable breaks become lethal to the cells.(Kampinga and Dikomey, 2001; Lepock, 2004; Roti Roti, 2008) The centrioles are particularly sensitive to heat and HT may therefore induce chromosomal aberrations.(Vidair and Dewey, 1991)

Cells in the S-phase of the cell cycle are more likely to be radioresistant. Akber cites a deficiency of molecular oxygen as a contributing factor to the radioresistance of cells in the S-phase.(Akber, 2015) The S-phase of cell division is the longest phase, lasting around half of the cycle time. Based on this an estimated 50% of cells could be expected to be in the more radioresistant S-phase of cell division at any given time. After exposure to higher doses of IR, there is a reduction in the rate of DNA chain elongation which is also associated with radioresistance.(Bernhard *et al.*, 1995)

Studies on HeLa (human cervical adenocarcinoma cell line) showed that after irradiation, an accumulation of cells in the S-phase and a delay in cell division were noted. Cells irradiated in S- and G2-phase showed greater delays in entering the next phase.(Bernhard *et al.*, 1995) Similarly Tsuchida *et al.* showed that in synchronously growing cell populations originating from collected mitotic cells, growth delay is strongly dependent on the cell-cycle phase at which cells were irradiated.(Tsuchida *et al.*, 2015) Karanam *et al.* reported that in G1-phase, double strand break repair of the DNA takes a very short time as only the Non-homologous End Joining (NHEJ) repair pathway is active. However in the S-phase, both NHEJ and homologous recombination (HR) are active, although the HR is used most often, and it takes longer for the cell to choose which repair mechanism to use. This results in a longer time taken overall to repair the damage.(Karanam *et al.*, 2012) Heating inhibits the repair and replication processes in a cell(Pandita, Pandita and Bhaumik, 2009) and the failure of DNA replication and repair in the S-phase of the cell cycle results in mitotic catastrophe.(Griffin *et al.*, 2010) The addition of hyperthermia can therefore sensitise the otherwise more resistant cells in the S-phase of the cell cycle to the damaging effects of IR.(Datta *et al.*, 2015)

At temperatures from 38°C, blood flow increases to the tumour, which increases oxygen and drug delivery to the tumour. This enhanced perfusion results in improved oxygenation in the target tissue, which enhances the effects of ROS induced by IR.(Hurwitz and Stauffer, 2014) Hypoxia is a central causative factor for radio-

resistance.(Peeken *et al.*, 2017) At higher temperatures, healthy tissue can continue to thermoregulate, but the tumour tissue, as a result of limited vascular supply, cannot remove the heat as effectively and the tumour continues to heat up faster than the healthy tissue.(Szasz, Szigeti and Szasz, 2016) However vasoconstriction occurs at higher temperatures and above temperatures of 43°C the oxygen perfusion declines. Moderate HT (39-42°C) therefore appears to be the best temperature range to improve oxygen delivery to the tumour.(Griffin *et al.*, 2010) Despite the healthy tissue's ability to thermoregulate, prolonged exposure to hyperthermic conditions can still result in tissue damage. The selective heating of the tumours is therefore important in order to minimise damage to the surrounding organs and tissues.(Wootton, Hsu and Diederich, 2011)

Increased temperatures result in an increased immune response at the heated site.(Szasz, Szasz and Szasz, 2001; van der Zee, 2002) Hyperthermia has direct actions on the immune system through direct heat-mediated cell killing, and indirect actions by modulating the innate and adaptive immune system.(Frey *et al.*, 2012) The Natural Killer (NK) cell activity is increased and the formation of tumour-specific antigens on the surface of various tumour cells is stimulated.(van der Zee, 2002) In a review of the immunomodulating effects of IR, Frey *et al.* discusses the potential for HT, due to its effects on the immune system, to further enhance the effects of IR on the immune system.(Frey *et al.*, 2017) Exposure to HT plus RT increases the number of necrotic cells seen in murine *in vivo* models more than exposure to RT alone, with the highest number of necrotic cells observed when HT is applied after RT.(Schildkopf *et al.*, 2009) Necrotic cells induced by HT can be immunogenic in that the removal of the debris from the necrotic cells involves inflammation and an immune response.(Hlavata, Solinas and Scartozzi, 2018)

Heat Shock Proteins were first identified in 1961 in response to heat stress to blood proteins in fish.(Pandita, Pandita and Bhaumik, 2009) It is now known that these proteins are produced in response to other cellular stresses as well as heat, such as oxidative stress, IR, chemotherapeutic drugs, and electromagnetic fields.(Andocs, Meggyeshazi, *et al.*, 2015) While HSP70 has cyto-protective properties from within the cell, when released into the extracellular matrix, it is able to signal cells, such as dendritic cells and macrophages, from the immune system to mount a local response. Werthmüller *et al.* observed an increase in HSP70 in the extra-cellular environment in a murine *in vivo* study combining HT plus RT, as well as an increase in the infiltration of dendritic cells and NK cells.(Werthmüller *et al.*, 2016) Extracellular HSP70 has an epitope which acts as a signal for NK cells and leads to enhanced proliferation, migration, and killing activity.(Frey *et al.*, 2017) Schildkopf *et al.* showed that although IR does induce surface expression of

HSP70, a second stimulus, such as heat, is needed in order to release the HSP70 molecule.(Schildkopf *et al.*, 2011; Frey *et al.*, 2017) Heat Shock Protein expression is increased on the cellular membrane of malignant cells in response to mild hyperthermia.(Bukau and Horwich, 1998; Pandita, Pandita and Bhaumik, 2009) This renders the cells more susceptible to lysis by NK cells. Heat Shock Protein 70 also plays a role in the activation of the tumour suppressor gene p53.(Szasz, Szasz and Szasz, 2001; van der Zee, 2002; Schmitt *et al.*, 2007)

Further immunomodulating effects of HT were observed in a study on murine models where one part of a tumour was treated with RT and one part with RT plus HT. The part treated with RT plus HT combined showed an increase in CF68+ macrophages whereas the part treated only with RT showed an infiltration of predominantly CD4+ T cells.(Datta *et al.*, 2015) Datta *et al.* suggests that as a result of these immunomodulating effects of HT, HT has the potential to induce an abscopal effect when combined with IR, which may prove to be of benefit in treating metastatic disease as well as local disease.(Datta *et al.*, 2015)

1.6.2. Hyperthermia and Chemotherapy

Hyperthermia can enhance the effects of chemotherapy by a factor from 1.2 to 10.(van der Zee, 2002) A number of factors are responsible for the ability of increased temperatures to potentiate the cytotoxic effects of chemotherapeutic agents. The thermally increased metabolism means an enhanced reaction rate of the cytotoxic agents and of Adenosine Triphosphate (ATP) depletion(Fiorentini and Szasz, 2006) which increases the cytotoxicity of cytotoxic agents. This has the potential to be of significant benefit to patients in whom the dose of the cytotoxic agents must be lowered, for example as a result of immune suppression, or hepatic or renal insufficiency.(Szasz, Szasz and Szasz, 2001) An increased intracellular drug accumulation, inhibition of DNA repair, enhanced production of free radicals, enhanced sensitisation to the S-phase of cell division, and reversal of drug resistance, further contributes to the chemo-sensitising effect of HT.(Hurwitz and Stauffer, 2014) Cytotoxic agents are less effective during the S-phase of mitosis and generally act on the G- and M₂-phases of mitosis. The action of HT on the S-phase causes cellular damage even during the phases of division in which cytotoxic agents are less effective.(Szasz, Szasz and Szasz, 2001)

The effect of hyperthermia on the chemotherapy depends largely on the type of chemotherapy used. This was shown early on in the investigations into the chemo-sensitising effects of HT in studies. In a study by Mohamed *et al.*, it was shown that

moderate hyperthermia increases the cytotoxicity of docetaxel, irinotecan, and gemcitabine, on mouse fibrosarcomas, while paclitaxel did not show any additional benefit after the addition of HT. Oxaliplatin and docetaxel showed greatest enhancement when the drug dose was high combined with HT.(Mohamed *et al.*, 2003) Some chemotherapeutic agents, such as 5-fluorouracil, methotrexate, and taxanes, have an independent and specific action and may therefore not benefit as much from the addition of HT, while others show a strong potentiation in the presence of heat, such as doxorubicin, cyclophosphamide, ifosfamide, and gemcitabine. Datta *et al.* reports that the synergistic action between chemotherapy and HT is best seen at temperatures of 41–43 °C and combined with cisplatin, carboplatin and bleomycin.(Hurwitz and Stauffer, 2014; Datta *et al.*, 2015) The strongest synergistic action is seen when HT and the chemotherapy are given simultaneously or when HT is administered immediately after the chemotherapy.(Hurwitz and Stauffer, 2014)

1.6.3. Challenges in Hyperthermia

Challenges associated with the real time temperature monitoring, and difficulties incorporating the HT treatments into the clinical workflow, are impeding the widespread adoption of HT into clinical practice.(Mallory *et al.*, 2016) A variety of heating techniques, ranging from radiative technologies (which use microwaves or radiofrequency (RF) waves) to ultrasound and capacitive heating, are available.(Datta *et al.*, 2015) Capacitive devices utilise two electrodes through which an RF current is generated.(Peeken *et al.*, 2017) The HT techniques are not all able to consistently heat tumours. In some instances, authors of studies report categorising patients as “heatable” or “non-heatable”,(Jones, Dewhirst and Vujaskovic, 2003) resulting in the exclusion of certain patients from studies,(Szasz, Szigeti and Szasz, 2016) and ultimately from the benefits of HT. Focussing the energy on the target and selecting the malignant cells are major challenges in HT treatments.(Szasz, Szigeti and Szasz, 2016)

In tumours which are “heatable”, the selection of the tumour, instead of the healthy tissue, is complicated. Various selection techniques have been investigated. One of the methods used is artificial focusing, based on imaging localisation. The principle is similar to that used by IR, whereby electromagnetic waves/fields on geometrically defined points outside of the body are used to focus on the target in the body. One challenge associated with this technique is the development of hot-spots in healthy tissues.(Szasz, Szigeti and Szasz, 2016) The planning systems required in order to achieve this are complex and require CT or MRI images to be obtained beforehand. In multi-antenna systems, the position, power, and phase of each applicator are controlled and electromagnetic and

thermal simulation software is required in order to conform the heat distribution to the patient. While a full three dimensional (3D) patient model is created, the model does not account for the strongly inhomogeneous and dynamic blood flow and tissue perfusion in the patients. In order to monitor the temperature, invasive thermometry is often used. Invasive thermometry is unpleasant for the patient, carries infection risks, and the information obtained from the thermometers is limited to the points within the implanted sites. Invasive temperature monitoring is therefore not ideal, especially for repeated and frequent treatments.(Datta *et al.*, 2015)

Adverse events include hot spot formation and adipose burns. While capacitive coupling heating techniques solve some of the challenges, the depth of heating and the potential for adipose burns resulting from capacitive coupling techniques are well known.(Kok *et al.*, 2018) This is due to the selective electric field-energy absorption of the adipose tissue, especially in deep-seated, pelvic tumours.(D'Ambrosio and Dughiero, 2007) The technical challenges, costs, complexity, and adverse effects of HT have blocked the use and acceptance of HT in developing countries and in resource-constrained settings such as many of the public healthcare facilities in South Africa.

Not all trials on HT have been positive. Some trials have shown no benefit to the addition of HT,(Lutgens *et al.*, 20AD; Emami *et al.*, 1996; Vasanthan *et al.*, 2005; Flameling *et al.*, 2016) while others have raised concerns regarding an increased risk of tumour dissemination following HT.(Sminia *et al.*, 1990; Nathanson *et al.*, 1991; Emami *et al.*, 1996; Vasanthan *et al.*, 2005) The mixed results have been attributed to the lack of standardisation of technique, temperature, and dose control.(Fatehi *et al.*, 2006; Fiorentini and Szasz, 2006)

1.6.4. Hyperthermia and Cervical Cancer

There is evidence that HT may be particularly effective in the treatment of HPV-positive cancers such as cervical cancer. Oncogenic, high risk HPV types produce proteins which interfere with the tumour suppression proteins. For example, early protein 6 (E6), produced by HPV types 16 and 18, binds to the tumour suppressor protein p53, promoting the degradation of the protein via the proteasomal pathway. The degradation of p53 enables the cell to evade the p53-induced cell-cycle arrest (G₂-phase) and apoptosis.(Kabekkodu *et al.*, 2017; The Cancer Genome Atlas Research Network, 2017) Oei *et al.* showed that the treatment of *in vivo* (murine models) and *in vitro* HPV-positive cervical tumour cells with hyperthermia promoted the degradation of E6, preventing the formation of the E6-p53 complex. This resulted in increased levels of p53, restoring the

tumour suppressive functions of p53. The study also found that samples treated with HT alone or combined with RT showed higher concentrations of p53, while the same levels of p53 were not seen in the samples treated with radiation alone. A temperature of 42°C for 60 minutes was required to achieve these results.(Oei, van Leeuwen, *et al.*, 2015)

The body of research on the use of HT for the treatment of cervical cancer is mounting. Numerous phase I and II studies have reported on the safety and efficacy of the addition of HT to treatment regimens.(Datta *et al.*, 2017) Conventional HT techniques have been added to RT protocols and recommended for patients who are unable to receive the prescribed dose of chemotherapy.(Franckena *et al.*, 2009) Combined with chemotherapy, HT can be used to manage local recurrences or residual disease in cervical cancer patients.(Rietbroek *et al.*, 1996; Franckena *et al.*, 2007) Phase II/III studies have investigated the combined use of CRT and HT.(Westermann *et al.*, 2005; Flaming *et al.*, 2016; Harima *et al.*, 2016)

Trials with Hyperthermia and RT vs RT Alone

A Cochrane review of six randomly controlled trials (RCT) assessed the effects of combining HT with RT for the treatment of cervical cancer. The results showed that RT + HT had a significantly higher complete response rate ($p<0.001$) and a significantly reduced local recurrence rate ($p<0.001$) than RT alone. Despite inconsistencies in trial designs and methodology, the authors concluded that the addition of HT appears to improve local tumour control and overall survival.(Lutgens *et al.*, 20AD) One of the trials included in the review was the Dutch Deep Hyperthermia Trial (DDHT) involving 114 participants with stages IIB to IVA cervical cancer. This trial is of particular interest as it had a long follow up (twelve years) and assessed cost effectiveness.

In the DDHT a mean dose of 68Gy was administered to all participants and the study group received weekly HT treatments as well as the RT. Local control rates were 57% (RT) vs 83% (RT + HT), ($p=0.003$); three year Overall Survival (OS) was 27% (RT) vs 51% (RT + HT), ($p=0.009$);(van der Zee *et al.*, 2000; van der Zee and González González, 2002) and twelve year OS was 20% (RT) vs 37% (RT+HT), ($p=0.03$).(Franckena *et al.*, 2008) A cost-saving per year-of-life gained was calculated to be 3956 Euros.(Franckena *et al.*, 2008) The combined treatment of HT plus RT has subsequently been recommended in the Netherlands for patients who are unfit to receive chemotherapy.(Mallory *et al.*, 2016)

Another study in the Netherlands involving 378 cervical cancer participants treated with external beam radiation, HDR BT, and weekly HT sessions, reported a 77% complete remission, 53% local control and 47% five year OS. These results were similar to those of previous studies, including the DDHT. In a multivariate analysis of the study, the number of HT treatments emerged as a predictor of outcome in addition to commonly identified prognostic factors.(Franckena *et al.*, 2009)

Hyperthermia and RT vs Cisplatin and RT

Lutgens *et al.* report on their trial comparing cisplatin versus HT as a radiosensitiser for LACC patients. The trial was closed prematurely due to a lack of difference seen between the groups (87 of 376 planned participants enrolled: n=43 in the RT + Cisplatin group; n=44 in the RT + HT group). The median follow-up time was 7.1 years. Pelvic Recurrence Free Survival (OR: 94; CI: 0.36–2.44) and Overall Survival (OR: 1.04; CI: 0.48–2.23) at five years were comparable between both treatment arms, as was grade 3 radiation-related late toxicity. The results showed that the outcomes were comparable for RT + Cisplatin and RT + HT.(Lutgens *et al.*, 2016)

Triple Therapy: Cisplatin, RT and HT Combined

In a meta-analysis Datta *et al.* reviewed eight articles on the use of HT for the treatment of LACC.(Datta *et al.*, 2016) For the direct comparison of triple therapy versus CRT, only one study was discussed in the review. The study by Chen *et al.* included 120 (stages IIB to IIIB) participants randomised into four groups: RT; CRT; RT + HT; CRT + HT.(Chen, Fan and Luo, 1997) Datta *et al.* reports that the trial showed a significantly better complete response for the triple therapy treatment arm (83.3%) compared to the RT + chemotherapy arm (46.7%) ($p=0.0001$). A pairwise comparison of these results to the results from the rest of the trials in the review showed that triple therapy treatment provides a significant advantage over RT + chemotherapy (OR: 2.65, 95% CI: 1.51–4.87) or RT alone (OR: 5.57, 95% CI: 1.22–23.42).(Datta *et al.*, 2016) Although the trial by Chen *et al.* is the only randomised phase III trial investigating triple therapy treatment discussed in the review by Datta *et al.*, no further information on the trial is available in the literature.

A multicentre Phase I/II study, involving medical centres in Netherlands, Norway and the United States of America, confirmed that the addition of HT to CRT does not increase the treatment-related toxicity. Sixty eight treatment-naïve cervical cancer participants were

treated with EBRT, BT, four doses of cisplatin ($40\text{mg}/\text{m}^2$), and weekly HT sessions. After 538 days, the overall survival was 84%. (Westermann *et al.*, 2005)

Other trials on trimodality therapy include a study in which 15 participants were treated with a five minute infusion of carboplatin before RT treatment. Radiotherapy was administered in 28 fractions of 1.8 Gy doses. The treatment was combined with weekly HT. The treatments were well tolerated and the complete response rate was 93.3%. (Tsuda *et al.*, 2003) A German trial published in 2006 reported that 32 participants with Stage IIIB-IVA cervical cancer were treated with RT, chemotherapy and HT. Participants tolerated the treatments well and the acute and late toxicity was within reasonable and expected levels. At the three-year follow-up, 60% of participants were still alive. (Sreenivasa *et al.*, 2006)

Despite the positive results in studies investigating HT plus RT, and the promising results from triple therapy Phase I/II studies, recent Phase III studies on triple therapy treatment failed to show any benefit. In a multicentre Phase III study in Japan, Harima *et al.* investigated CRT versus CRT plus HT in 101 LACC participants (stages IB to IVA). Survival outcomes were not significantly improved by the addition of HT, however there was a significant difference in local control between the groups when a Chi-squared analysis was applied. Flameling *et al.* registered a study on the use of CRT with/without HT, however the trial was stopped early due to slow recruitment and the early results did not show a difference between the two groups. (Flameling *et al.*, 2016) Table 1 summarises the trials on triple therapy treatment for LACC.

Trials with Hyperthermia Combined with Platinum-Based Chemotherapy for the Treatment of Previously Irradiated Recurrent or Persistent Cervical Cancer

In patients in whom there is local residual disease, or a local recurrence in a previously irradiated area, platinum-based chemotherapy regimens may be prescribed. According to Franckena *et al.* the response rate to treatment in these patients is low. (Franckena *et al.*, 2007) In a study by Franckena *et al.*, 47 LACC patients with local treatment failure after irradiation were treated with weekly cisplatin and HT. The authors reported that there were no unexpected toxicities resulting from the addition of HT and that there was an objective response rate of 55%. Palliation was achieved in 74% of the participants and 19% of participants were eligible for surgery after completing the chemotherapy and hyperthermia regimens. At nine years, two participants remained disease free and two remained disease free until their death (due to other causes). The median survival was eight months and was related to the duration of the disease free period and to the tumour

diameter.(Franckena *et al.*, 2007) Combining their results with results from a similar study by Rietbroek *et al.*,(Rietbroek *et al.*, 1996) an overall response rate of 54% was achieved. The authors concluded that a phase III trial comparing cisplatin alone to cisplatin plus HT for recurrent, previously irradiated cervical carcinoma was not feasible due to the limited number of patients available for such a trial. However, based on their positive results in the phase I/II study, the authors recommend this combined treatment as a standard treatment approach for LACC patients with locally recurrent or residual disease within a previously irradiated area.(Franckena *et al.*, 2007)

Table 1: Summary of Trials on Triple Therapy (HT plus CRT)

	Type	Sample	Survival	Disease control
(Tsuda <i>et al.</i> , 2003)	I	N=15 Post-surgical local recurrences	Not evaluated	CR: n=5 (33.3%) PR: n=9 (60%)
(Westermann <i>et al.</i> , 2005)	I/II	N=68 LACC	84% 18 month survival	CR: n=61 (90%)
(Sreenivasa <i>et al.</i> , 2006)	II	N=32 FIGO IIB-IVA 2	60% 3 year survival	Partial remission: n=24 (75%)
(Harima <i>et al.</i> , 2016)	III	CRT: n=50 CRT+HT: n=51	5 year survival: CRT: 61%; CRT+HT: 71% $p=0.182$	CR: CRT: 78%; CRT+HT: 88%; $p=0.047$
(Flameling <i>et al.</i> , 2016)	III	CRT: n=50 CRT+HT: n=49	5 year survival: CRT: 66% CRT+HT: 71% $p=0.80$	CR: CRT: 76% CRT+HT: 86% $p=0.054$

Abbreviations: CR: Complete Response; PR: Partial Response; LACC: Locally Advanced Cervical Cancer; CRT: Chemoradiotherapy; HT: Hyperthermia

1.7. Modulated Electro-Hyperthermia (mEHT)

1.7.1. Overview

Modulated electro-hyperthermia (mEHT) is a non-invasive local heating technique that uses amplitude modulated radiofrequency waves at a frequency of 13.56MHz in a capacitive-coupled setup to selectively target tumour cells. The principles of capacitive heating are based on the elevated conductivity, permittivity, and current density in malignant tissue compared to healthy tissue.(Szasz, Szasz and Szasz, 2001; Fiorentini and Szasz, 2006) The amplitude modulation promotes the selection of the malignant

tissue further. According to the literature, mEHT enhances the efficacy of conventional hyperthermia by effectively overcoming the previous obstacles associated with hyperthermia and by the addition of the non-thermal effects associated with electromagnetic energy.(Fiorentini and Szasz, 2006)

The mEHT dosing principle is based on the tumour's ability to store a charge and heat up selectively. Once the maximum energy has been absorbed, the excess energy passes through the tumour. The constant forwarding of energy into the system ensures the tumour maintains its charge and temperature during the treatment. The improved selection of the malignant tissue reduces the amount of wasted energy and the energy that is put into the system can therefore be used as a measure of dose. The improved treatment efficiency also means that the power output can be decreased and the risk of adverse events, such as adipose burns, is reduced.(Szasz *et al.*, 2018)

1.7.2. Biophysics

The matrix of all the living tissues is comprised primarily of an aqueous solution containing a variety of electrolytes which are separated by highly polarised surfaces (cellular membrane walls). The biological processes are governed by the electromagnetic forces between highly polarised molecules in a non-homogenous medium.(Andocs, Vincze, *et al.*, 2009)

The differences in permittivity and conductivity between malignant tissue other tissues are well known and are applied in fields of medicine such as diagnostics and treatment.(Scholz and Anderson, 2000) An example is represented graphically in Figure 1 to Figure 3. In this example, different types of freshly excised breast tissue, including malignant samples are tested.(Jossinet, 1998)

Laufer *et al.* cites the higher water concentration, higher sodium concentration and different electrochemical properties in malignant versus healthy cells as contributing factors to the differences in impedance.(Laufer *et al.*, 2010) Tumours utilise primarily anaerobic metabolism which requires higher concentrations of glucose in the extracellular matrix and results in additional lactic acid formation. The extracellular matrix of tumours therefore has a higher glucose and ionic concentration(Fadaka *et al.*, 2017) which in turn decreases the impedance. The increased metabolic rate in tumours resulting from the increased cellular division and activity causes an increase in energy and temperature which further lowers the impedance. The ionic exchange within the tumour is higher than

healthy tissue and the conductivity of malignant tissue is therefore much higher than that of normal tissue. (Dissado *et al.*, 1995)

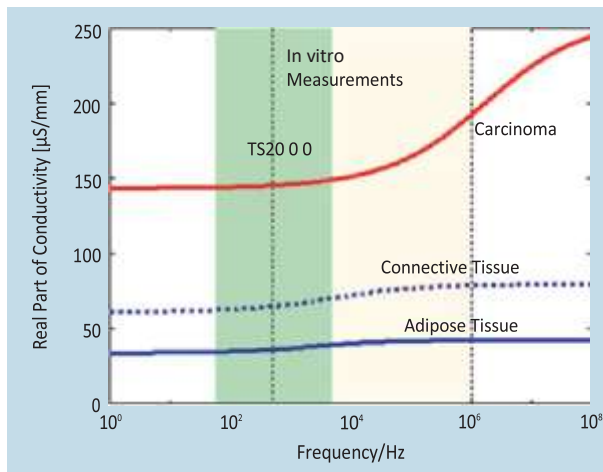


Figure 1: Conductivity of various tissues at increasing frequencies

Real part of conductivity for various types of breast tissue: carcinoma, connective tissue, and adipose tissue.

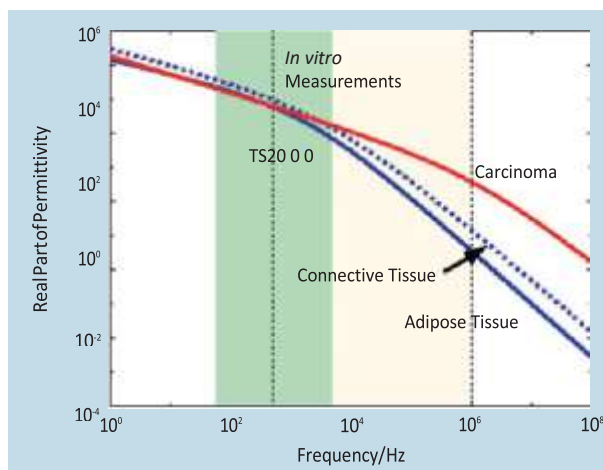


Figure 2: Permittivity of various tissues at increasing frequencies

Real part of permittivity for various types of breast tissue: carcinoma, connective tissue, and adipose tissue.

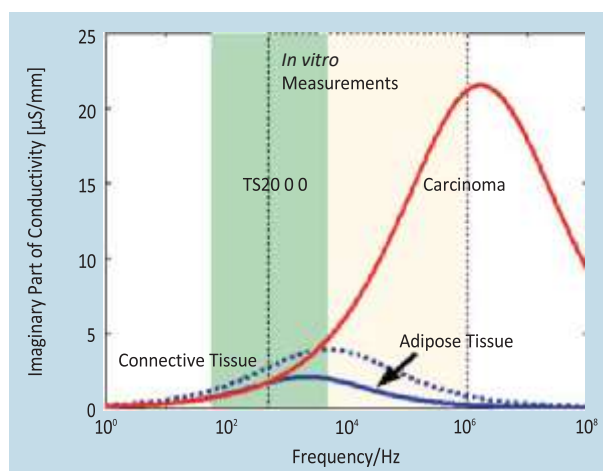


Figure 3: Virtual part of conductivity for various types of breast tissue calculated

Virtual projections of *in vivo* conductivity of various breast tissues based on the actual *in vitro* experiments.

When current is sent through a tumour, the energy absorbed by the tumour at the applied frequency can be described as being proportional to the tumour's conductivity and as the square root of the dielectric constant (relative permittivity of the substance for a frequency of zero) of the tumour.(Fiorentini and Szasz, 2006) The dielectric constant of the extracellular matrix of a tumour is higher than the corresponding healthy tissue. A higher dielectric constant means that the substance breaks down more easily when subjected to intense electric fields.(Smith, Foster and Wolf, 1986) However the dielectric constants within malignant tissue are not homogenous.(Damadian, 1971; Cope, 1975) This contributes to the ability of the current or energy to travel more easily through the malignant tissue than the healthy tissue.(Fiorentini and Szasz, 2006)

Electric Fields

The electric fields associated with cellular processes are in the range of 0–10V/cm, except within cell membranes where they may reach 105V/cm.(Kirson *et al.*, 2007) Based on this, the use of electromagnetic potentials to manipulate cells has been investigated for both medicinal and industrial purposes(Fanning *et al.*, 2003; Garcia *et al.*, 2003; McCaig *et al.*, 2005; Szasz *et al.*, 2018) and a number of research articles have demonstrated the effects of electro-magnetic fields on the biological processes.(Szendro, Vincze and Szasz, 2001b, 2001a; Hegyi, Vincze and Szasz, 2007; Vincze, Szasz and Liboff, 2008) Electro-chemical therapy has even been investigated for its inhibitory and damaging effects on cancer cells.(Kirson *et al.*, 2004, 2006, 2009; Janigro *et al.*, 2006; Barbault *et al.*, 2009; Schroeder, Viglianti and Dewhirst, 2009)

Extracellular heating can be achieved by capacitive coupled electromagnetic field application within a certain frequency range.(Szasz, Szasz and Szasz, 2001) Cell membrane thickness is 4-10nm and the potential is 70-90mV. Therefore the administration of low frequency energy prevents the entry of the energy into the cell and allows the heating of the low millivolt matrix. Provided the frequency is low enough, the fields do not readily penetrate the cell membrane and the bulk of the energy is deposited within the extracellular matrix (ECM).(Vincze, Szasz and Szasz, 2005) Another advantage to the low frequency is the penetration depth. The penetration depth of RF waves decreases as the frequency increases, with excellent penetration at 13.56MHz, decreasing to 17cm at 85MHz, down to 7cm at 220MHz.(Röschmann, 1987) Szasz *et al.* describe in detail the calculations involved in confirming the depth of penetration of mEHT using the capacitive coupled system, where the energy transmitted from one electrode is received by the other, confirming penetration of the entire thickness of the patient.(Szasz *et al.*, 2018)

The mEHT devices use two electrodes (one in an adjustable arm and one fixed electrode in the bed) to generate an electric field. A vacuum has a permittivity of zero, allowing for the free travel of energy between electrodes. The resistance of a substance to the flow of energy, in relation to a vacuum, is the permittivity of the substance and this is affected by the number of free ions in the substance. Free ions have positive and negative charges and these ions, as well as any other dipolar molecules, will align within the tumour on the side of the positive electrode or the negative electrode, based on the ions' charge (positive attracts negative and opposites repel). The tumour develops a positive and negative pole and the difference between the poles gives the tumour a charge (similar to a capacitor), and the ability to store energy which is translated into heat.(Vincze and Szasz, 2018)

The increased heat in the ECM triggers a heat-flow, or thermal-current, from the extra- to intra-cellular regions through the cell membrane. The thermal current carries ions into the cell.(Vincze, Szasz and Szasz, 2005) Sodium ions (Na^+) are the primary ions exchanged. This rapid transfer of Na^+ depolarises and destabilises the membrane and activates Sodium/Potassium (Na^+/K^+) pumps which results in ATP transformation and this further increases the temperature at the cell membrane.(Szasz, Szasz and Szasz, 2001; Fiorentini and Szasz, 2006) The temperature gradients induced by extracellular hyperthermia can be maintained for extended periods of time, provided there is a constant supply of energy delivery.(Szasz, Szasz and Szasz, 2001)

The normal energy flow across the cellular membranes is $20\text{nW}/\text{mm}^2$. However this can be increased to $1500\text{nW}/\text{mm}^2$ with the application of an electric field. Applying an electric field increases the electric potential and the current across the cellular membrane to around $150\text{pA}/\text{mm}^2$ (the increased current is primarily a result of the influx of sodium ions into the cell). Under normal conditions the Na^+ exchange results in a current of around $12\text{pA}/\text{mm}^2$.(Szasz, Szasz and Szasz, 2001; Fiorentini and Szasz, 2006)

1.7.3. Membrane Alterations

The cellular membrane controls the interaction of the electrolytes using a number of ionic exchange mechanisms.(Andocs, Vincze, *et al.*, 2009) Structural alterations to the transmembrane proteins induced by hyperthermic conditions result in changes in the active membrane transport systems, membrane potential, and membrane capacity.(Fiorentini and Szasz, 2006; Papp *et al.*, 2017) This results in changes in the potassium, calcium and sodium ion gradients.

In an attempt to maintain equilibrium, and because the membrane permeability to water is much higher than the permeability to ions, there is a flow of water intracellularly to compensate for the increased intracellular flow of sodium ions. This causes intracellular swelling and increases the intracellular pressure.(Szasz and Vincze, 2006) Since the malignant cells have more rigid membranes than normal cells (due to the increased phospholipid concentration) the membranes are less able to adjust to the increase in pressure and are more likely to be damaged.(Fiorentini and Szasz, 2006) Conventional HT causes direct damage to the cellular membrane as a result of heat.(Nishida, Akagi and Tanaka, 1997; Szasz, Szasz and Szasz, 2001; Fiorentini and Szasz, 2006) Using mEHT, the thermodynamic driving force, or temperature gradient, combined with the applied energy passing through the system, as well as the intracellular swelling, also contribute to the damage of the cellular membrane.(Szasz, Szasz and Szasz, 2001)

Once the cellular membrane is damaged, intracellular HSPs are released into the ECM and the HSPs stimulate macrophages and dendritic cells to secrete cytokines which further activate immunological responses to the area.(van der Zee, 2002; Schmitt *et al.*, 2007)

Membrane rafts

Membrane rafts are sterol- and sphingolipid-enriched domains on the cellular membrane which are responsible for compartmentalising the cellular processes.(Pike, 2006) These highly organised microstructures are thought to be involved in signal transduction and membrane transport and have different electromagnetic properties to the rest of the membrane. These differences in the dielectric constant, permittivity, and conductivity of the rafts increase the sensitivity of membrane rafts to the extracellular heating applied in mEHT.(Andocs, Rehman, *et al.*, 2015; Andocs *et al.*, 2016) Interest in the effect of mEHT on the membrane rafts has grown with numerous studies (*in vitro* and *in silico*) and simulations having been published on the topic.(Vincze, Szasz and Szasz, 2015; Papp *et al.*, 2017) The increased absorption of energy by the membrane rafts appears to speed up the rates of cell death as shown by Andocs *et al.* in a study investigating the differences in rates of apoptosis between cells exposed to mEHT and cells exposed to HT. This showed that mEHT enabled a higher rate of cell death at a lower temperature in than conventional HT.(Andocs, Meggyeshazi, *et al.*, 2015; Andocs, Rehman, *et al.*, 2015)

1.7.4. Immune Response

Heating up the extracellular matrix with low frequency mEHT has only a minor impact on the intracellular environment. The primary action and heating occurs in the ECM and at the membrane.(Papp *et al.*, 2017) As the inside of the cell is not threatened or stressed, it does not respond with the synthesis of intracellular HSPs.(Szasz, Szasz and Szasz, 2001; Tsang *et al.*, 2015) However the extracellular matrix and the cell membrane respond with the expression and release of extracellular HSP(Tsang *et al.*, 2015) which assists in an immune response and tumour destabilisation(Schmitt *et al.*, 2007) and boosts the apoptotic signals, further enhancing the immune reactions.(Fiorentini and Szasz, 2006)

In an *in vivo* (murine) study, Andocs *et al.* showed that cell stress induced by mEHT resulted in the generation of DAMP signals and that the application of mEHT could potentially induce local immunogenic cell death.(Andocs, Meggyeshazi, *et al.*, 2015) In CT26 tumours, Tsang *et al.* showed that mEHT induced significant apoptosis and enhanced the release of HSP70 in the extracellular matrix.(Tsang *et al.*, 2015) In an experiment by Szasz A., the HSP70 distribution in A431 epithelial xenografted nude mice tumour cell samples after HT and after mEHT was measured using immuno-fluorescence microscopic images. The results showed an increased expression of HSP70 on the membranes of the cells exposed to mEHT compared to the cells in the control and in the cells exposed to HT.(Szasz, 2007)

In further investigations by Andocs *et al.*, the different biological responses between mEHT and HT (induced by a traditional hot water bath) were investigated. In both murine models, the temperature and duration of the increased temperature were matched. Morphological changes associated with apoptosis were more prominent in the mEHT-treated cells than in HT-treated cell samples, as seen in DNA fragmentation assays and Giemsa stained cell samples. Flow cytometry analysis of cells subjected to Annexin V-Fluorescein Isothiocyanate (FITC) / propidium iodide (PI) double staining showed an increase in Annexin V-FITC staining in the cells exposed to mEHT, compared to those exposed to HT using hot water. This increase in Annexin V-FITC staining is an indicator of apoptosis. The authors hypothesise that the difference is due to the non-homogenous energy distribution on the cell membrane in the mEHT samples, as a result of the increased energy absorption by the membrane rafts, and that this effect on the cell membrane activates cell death-related signalling pathways during mEHT treatments but not during conventional heat treatments.(Andocs *et al.*, 2016)

1.7.5. Dose Measurement in mEHT

In conventional HT, the dosing concept used is the cumulative equivalent minutes at 43°C (CEM43°C). It was first introduced by Sapareto and Dewey in 1984.(Fatehi, 2000) By the very definition of the dose concept, temperature is the essential and primary measured parameter used to determine the efficiency of the treatment and the temperature must therefore be closely controlled.(Andocs, Rehman, *et al.*, 2015)

The macroscopic temperature is the measurement of the average temperature of all of the microscopic components of the system and each microscopic part will heat differently, based on the energy absorption in that area.(Andocs, Rehman, *et al.*, 2015) Arguments against the insertion of intra-tumoural thermometers cite this as one of the reasons for the inaccuracy of invasive thermo-monitoring techniques: only the temperatures at the point of the thermometers are monitored, and not the overall energy consumption and effect.(Vincze, Szasz and Szasz, 2015; Szasz, Szigeti and Szasz, 2016; Vincze and Szasz, 2018)

Modulated electro-hyperthermia uses a different selection mechanism, based on the different biophysical properties of malignant tissue and the amplitude modulation, to selectively target the cellular membranes of malignant cells. The temperature distribution is therefore different and the energy is absorbed on a molecular level. The effects are reliant on the structural elements of the plasma membrane and membrane rafts.(Andocs, Rehman, *et al.*, 2015)

The temperature of a system, in this case the tumour, would only increase if the energy pumped into the system is more than what is used to convert, or break, chemical bonds. Increasing the energy input further will result in energising of the ions and molecules and the subsequent heating up of the system. The amount of energy that any capacitor can store is limited. Once the tumour has absorbed the maximum energy dose, all the subsequent energy will be sent through the system and will be received by the second electrode.(Szasz, 2007) Using thermo-physics and biophysics calculations, based on the energy dose, the device is then able to estimate the heating of the tumour.(Fiorentini and Szasz, 2006) However these calculations only estimate the temperature and for a more accurate dosing system, a different measurement parameter is needed.

In mEHT, when calculating the dose, the energy output from the first electrode is compared to the energy received by the second electrode. Using algorithms the absorbed energy can be determined.(Vincze, Szasz and Szasz, 2015; Szasz, Szigeti and Szasz,

2016; Szigeti, Szasz and Hegyi, 2016) As long as the energy arrives at the second electrode, the depth of the heating is confirmed, the only difference is likely to be the density of the energy as its distribution widens as it travels from the first to second (larger) electrode.(Szasz *et al.*, 2018) The high Specific Absorption Rate (SAR) following mEHT treatments(Szasz, Szigeti and Szasz, 2016) is achieved by the precise matching of impedance during the treatments which in turn minimizes the reflected energy and maximizes the RF-current.(Szasz *et al.*, 2018) As there is a high treatment efficiency and very little wasted energy, the energy input can be used as a dosing parameter.(Szigeti, Szasz and Hegyi, 2016)

Temperatures of only 40-42°C result in a long lasting increase in blood flow and oxygenation of tumours.(Griffin *et al.*, 2005; Song *et al.*, 2005) The increase in temperature of the tumour during mEHT treatments has been confirmed in animal and human studies(Andocs, Renner, *et al.*, 2009; Szasz, Szasz and Szasz, 2011; Balogh *et al.*, 2016; Lee *et al.*, 2018) and experiments have confirmed the heating of tumours by mEHT(Balogh *et al.*, 2016) as well as the selectivity(Kim, Prasad and Kim, 2017; Prasad *et al.*, 2018) and the safety of the devices.(Fiorentini *et al.*, 2018; Lee *et al.*, 2018; Yoo *et al.*, 2019)

In animal experiments the temperature of HepG2 xenografted tumours in nude mice using conventional HT techniques and mEHT was measured. The temperature was measured intratumourally in both tumours, systemically (rectal), and on the surface of each tumour using a metal-free fibre-optic thermometers. The heating up and cooling down periods were identical. The results of the measurements show the tumour treated with mEHT reached an internal temperature of 42°C whilst the muscle temperature did not exceed 38°C, confirming the selectivity of the heating technique.(Andocs, Renner, *et al.*, 2009)

Renner H. measured the temperature in advanced sarcomas treated with mEHT. The temperatures were measured using CT-guided fluoroptic sensors (positioned and monitored by six interventional radiologists). The maximum temperature measured in the tumour was 44°C, while the surface temperature remained around 32°C. Groenmeyer D. and Sahinbas H. measured the intratumoural temperatures of human breast and brain tumours treated with mEHT and found both tumours reached temperatures of 44°C.(Szasz, Szasz and Szasz, 2011)

In another study, 28 nude mice, divided into four groups, were xenografted with HT29 human colorectal carcinoma cells. Group 1 was untreated, group 2 was treated with

radiative hyperthermia at 42°C, group 3 received mEHT at 42°C, and group 4 received mEHT at 38°C. Cross sectional studies of the tumours after 24 hours showed that mEHT, even at 38°C, resulted in a significantly higher percentage of tumour cell deaths than radiative hyperthermia, demonstrating the effects of electric energy and amplitude modulation on the cells, in the absence of increased temperatures.(Andocs, Renner, *et al.*, 2009)

1.7.6. Clinical Research on mEHT and Cervical Cancer

The randomised controlled trials on mEHT are limited, although several phase I/II studies have proven the safety of mEHT(Fiorentini *et al.*, 2006, 2018; Szasz, 2014; Yoo *et al.*, 2019) and numerous *in vivo* and *in vitro* experiments have shown the effects and heating abilities of mEHT.(Fanning *et al.*, 2003; Andocs, Renner, *et al.*, 2009; Andocs, Meggyeshazi, *et al.*, 2015; Tsang *et al.*, 2015; Andocs *et al.*, 2016) Lee *et al.* investigated mEHT combined with chemotherapy for local recurrent cervical cancer patients who had been previously irradiated. Twenty participants were treated with chemotherapy alone, and 18 were treated with chemotherapy combined with mEHT. Modulated electro-hyperthermia was performed three times per week, beginning at chemotherapy initiation, and participants underwent a total of 36 mEHT sessions. The overall response to treatment was significantly greater in the group of participants who underwent chemotherapy combined with mEHT ($p=0.0461$), and at the evaluation conducted at the last follow-up visit, the response rate remained significantly higher ($p=0.0218$) with a CR of 15% in the chemotherapy group and 50% in the mEHT + chemotherapy group.(Lee *et al.*, 2017) Lee *et al.* also confirmed an increase in temperature by an $+2^{\circ}\text{C}$ in cervical tumours treated with mEHT,(Lee *et al.*, 2018) which is in line with international studies on other HT techniques used for LACC.(McCaffrey, 2005)

1.7.7. Modulated Electro-Hyperthermia in Resource-Constrained Settings

Although the clinical research on mEHT is limited, the mounting literature on mEHT strongly motivates the expansion of the clinical research on the technique. There is a high potential for mEHT to be used as an alternative to conventional HT methods in resource-constrained environments, where complex planning and thermo-monitoring systems are not available. Due to the immunomodulating effects of mEHT, and the potential benefit in low-resource settings, investigations into the effects of mEHT on outcomes of patients in whom outcomes are expected to be worse, such as immunocompromised patients, are also warranted. The overall objective of this research project is to evaluate the theoretical and experimental studies and principles of mEHT, and the clinical effects of mEHT in the

absence of thermo-monitoring systems and in a resource-constrained environment with HIV-positive and -negative patients.

CHAPTER TWO

AIMS AND OBJECTIVES

2.1. Aim

The aim of this research project is to investigate the effects of the addition of modulated-electro hyperthermia to standard chemoradiotherapy protocols for HIV-positive and -negative locally advanced cervical cancer patients in a low-resource setting.

2.2. Objectives

- 1) To determine the effect on local disease control (including tumour and nodal disease in the radiation field) at six months post-treatment as visualised on ¹⁸F-FDG PET/CT scans.
- 2) To determine the effect of the modulated electro-hyperthermia on six month local disease-free survival.
- 3) To evaluate the adverse effects directly attributed to mEHT.
- 4) To evaluate the effect that mEHT may have on the toxicity of chemotherapy and radiotherapy.
- 5) To determine if modulated electro-hyperthermia has an effect on the quality of life of enrolled participants before, and at six weeks and three months after completion of therapy.
- 6) To describe and compare disease patterns as visualised on the six months post-treatment ¹⁸F-FDG PET/CT scans.

2.3. Study Rationale

The African setting is unique in that the numbers of patients and stages of disease are high and the resources and funding are limited. The potential cost per life saved resulting from the potential increase in treatment efficacy by the addition of HT could significantly lower the burden on healthcare facilities in South Africa. Despite the potential benefits, there have to date, been no trials investigating HT or mEHT in Africa.

The high incidence of HIV in Africa compared to developed countries in which HT trials have been conducted also poses a unique situation. The HIV status of a patient affects their immune function and chemotherapy and radiation toxicity. Authors have suggested that HT may be beneficial to patients in whom treatment doses must be lowered due to dose limiting toxicities. Hyperthermia, and the more affordable mEHT,(Kok *et al.*, 2018) may therefore have a role in improving treatment outcomes in HIV-positive patients in whom treatment doses must be lowered.

This trial will be the first phase III randomised controlled study on mEHT combined with CRT. It is also the first study on any HT technique in Africa, in low resource settings, in an African population, and in HIV-positive patients. The results have the potential to significantly enhance the current available treatments in South Africa.

CHAPTER THREE

The effect of modulated electro-hyperthermia on local disease control in HIV-positive and -negative cervical cancer women in South Africa: Early results from a Phase III Randomised Controlled Trial

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Author Contributions

- Minnaar: Protocol design, project planning, conducted the research, gathering and recording data, statistical analysis, interpretation of results, writing of the manuscript.
- Kotzen: Supervisor, senior radiation oncologist, protocol design, planning and prescribing of treatments, overseeing the treatment and follow-ups of trial participants, reviewed manuscript.
- Ayeni: Reviewed ¹⁸F-FDG PET/CT reports, reviewed manuscript.
- Naidoo: Clinical oncologist, screened patients for eligibility, overseeing the administration of treatments and follow-ups of the participants, reviewed the manuscript.
- Tunmer: Radiation oncologist, screened patients for eligibility, overseeing the administration of treatments and follow-ups of the participants, reviewed the manuscript.
- Sharma: Head of Radiation Oncology Department, reviewed manuscript.
- Vangu: Head of Nuclear Medicine Department; oversaw all the ¹⁸F-FDG PET/CT scans and nuclear medicine-related logistics, reviewed manuscript.
- Baeyens: Supervisor, Head of Radiobiology, supervised data collection and data quality control, project planning, management of funding, reviewed manuscript.

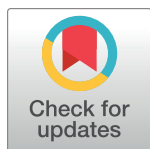
RESEARCH ARTICLE

The effect of modulated electro-hyperthermia on local disease control in HIV-positive and -negative cervical cancer women in South Africa: Early results from a phase III randomised controlled trial

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Abstract

Background

The global burden of cervical cancer remains high with the highest morbidity and mortality rates reported in developing countries. Hyperthermia as a chemo- and radiosensitiser has shown to improve treatment outcomes. This is an analysis of the local control results at six months post-treatment of patients enrolled in an ongoing study investigating the effects of the addition of modulated electro-hyperthermia (mEHT) to chemoradiotherapy for the treatment of HIV-positive and -negative cervical cancer patients in a low-resource setting.

Methods

This ongoing Phase III randomised controlled trial, conducted at a state hospital in Johannesburg, South Africa, was registered with the appropriate ethics committee. After signing an informed consent, participants with FIGO stages IIB to IIIB squamous cell carcinoma of the cervix were randomised to receive chemoradiotherapy with/without mEHT using a secure online random-sampling tool (stratum: HIV status) accounting for age and stage. Reporting physicians were blind to treatment allocation. HIV-positive participants on antiretroviral treatment, or with a CD4 count >200cell/μL were included. mEHT was administered 2/weekly immediately before external beam radiation. The primary end point is local disease control (LDC) and secondary endpoints are toxicity; quality of life analysis; and two year survival. We report on six month LDC, including nodes visualised in the radiation field on ¹⁸F-FDG PET/CT (censored for six month survival), and six month local disease free survival

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(LDFS) (based on intention to treat). Trial status: Recruitment closed (ClinicalTrials.gov: NCT03332069).

Results

271 participants were recruited between January 2014 and November 2017, of which 210 were randomised for trial and 202 were available for analysis at six months post-treatment (mEHT: $n = 101$; Control: $n = 101$). Six month LDFS was higher in the mEHT Group ($n = 39$ [38.6%]), than in the Control Group ($n = 20$ [19.8%]); $p = 0.003$). LDC was also higher in the mEHT Group ($n = 40$ [45.5%]) than the Control Group ($n = 20$ [24.1%]); ($p = 0.003$).

Conclusion

Our results show that mEHT is effective as a chemo-radiosensitiser for cervical cancer, even in high risk a patients and resource-constrained settings.

Introduction

The global burden of cervical cancer remains high, with 570 000 new cases and 311 000 deaths estimated to have occurred in 2018. It is the fourth most common cancer, and the cancer with the fourth highest mortality rate, amongst women. The mortality rate from cervical cancer is around 3 cases per 100,000 in developed countries and around 18 per 100,000 in developing countries.[1]

Current standard of care treatment for locally advanced cervical cancer (LACC), which includes International Federation of Gynaecology and Obstetrics (FIGO) stages IB2 to IVA, is a combination of chemotherapy (most commonly platinum-based), and radiotherapy (RT).[2] The treatment of cervical cancer in developing countries is complicated by limited resources, late stage of disease at presentation,[3] and the high prevalence of Human Immunodeficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS). Out of 54 African countries, 28 do not have access to radiotherapy treatments. Of those with access to RT, only 20 have access to brachytherapy (BT).[4] Where RT is available, the planning and delivery techniques are often sub-optimal, with many countries still relying on low energy machines, such as Cobalt60. There are usually not enough devices or staff to handle the high patient load, resulting in long waiting times for treatment.[5] The lack of radiology support restricts the availability of sophisticated planning techniques which require computed tomography (CT) or magnetic resonance imaging (MRI). Low-resource settings therefore rely on less labour-intensive 2D planning techniques that do not require sophisticated imaging to manage the burden of the high patient volumes. In our facility patients do have access to BT, however imaging techniques are not routinely available for the planning of oncology treatments. Treatment outcomes in low-to-middle-income countries (LMICs) are significantly worse than in less resource-constrained settings with an estimated 87% of cervical cancer-related deaths occurring in LMICs in 2018.[6] There is a scarcity of information on the outcomes and on the confounding prognostic factors unique to LMICs.[7] The protocols and outcome measures used are often sub-optimal in resource-constrained settings and therefore not comparable to international literature. For example in Uganda, 316 cervical cancer patients were evaluated and authors reported that HIV-positive patients (42%) had more than a two-fold higher risk of death than HIV-negative patients. More than two-thirds of the patients that died in the first

year, died in the first 6 months post-treatment and only 32% of the HIV-negative and 35% of the HIV-positive patients were alive at one year post-treatment. While this study did not report on local control, it does highlight the high mortality rates seen in low-resource settings.[8] HIV infection is associated with a higher risk of treatment interruptions and residual disease,[9] a higher risk of not completing the prescribed chemotherapy protocol,[10] and worse survival rates for cervical cancer patients.[11] The high burden of cervical cancer on already strained public health systems in Sub-Saharan Africa necessitates the investigation of feasible chemo-radiosensitisers that are effective, even under such difficult conditions.

Hyperthermia (HT) is well known for its radiosensitising effects and the benefits of its addition to RT for the management of LACC have been described in two meta-analyses.[12,13] Hyperthermia (HT) has been investigated as a chemo- or radiosensitiser in numerous studies on cervical cancer.[14–17] There is evidence that HT may be particularly effective in the treatment of Human Papilloma Virus (HPV) positive cancers as it promotes the degradation of oncogenic proteins.[18] HT as a radiosensitiser has shown to improve survival and local disease control (LDC) in LACC patients,[19] however at least one study has reported worse toxicity, with no improvement in local disease control.[20] As a chemo-sensitiser HT has demonstrated improved outcomes and acceptable toxicity combined with platinum-based cytotoxic agents in the management of residual or recurrent cervical cancer.[14] Phase I/II studies have shown that chemoradiotherapy plus HT for the management of LACC is tolerated well, with no significant increase in treatment related toxicity resulting from the addition of HT.[21,22] Two Phase III trials were published after recruitment for this study began. One was closed early due to slow recruitment.[23] Neither of the trials reported an increase in toxicity, however both trials reported no significant benefit to the addition of HT to chemoradiotherapy. One study used radiative heating,[23] and one study used a capacitive heating device with a power output of 800-1500W.[24] however two phase III trials published after recruitment for this study began, reported no benefit to the addition of HT to chemoradiotherapy.[23,24]

Radiative hyperthermia utilizes external antennas to induce an electromagnetic field using 70 to 120MHz which is coupled into the patient using a water bolus. Complex MRI guided planning systems and thermometry systems have been developed to support these treatments, minimize unwanted hot spot formation, and focus the energy on the tumour. Capacitive heating devices utilize two electrodes to produce an electro-magnetic field by capacitive coupling with the human body, resulting in heating. Both techniques are widely used clinically.[25] Challenges with radiative heating include the higher costs, complexity and sophisticated setting required to administer the treatments. Capacitive heating devices in comparison are more affordable[25] and carry less risk of hot spot formation. Capacitive heating devices frequently make use of intra-tumoral thermometers or locally applied temperature sensors to confirm temperatures, although the invasiveness of the procedures carries certain risks. Challenges associated with capacitive heating involve the effective heating of deep tumours through thick layers of adipose tissue (>2cm).

Hyperthermia faces challenges with acceptance and consistency in results. Szasz *et al* proposed a HT technique in which 13.56MHz capacitive-coupled modulated radiofrequency is applied to selectively target tumour cells based on the differences in the conductivity and permittivity of malignant tissue compared to healthy tissue.[26,27] This selective technique, known as modulated electro-hyperthermia (mEHT), combines the effects of heating and of the electric field to sensitise tumours to treatments.[28] The tumour, having different biophysical properties to healthy tissue, stores a charge thereby heating up selectively. Once the maximum energy has been absorbed, the excess energy passes through the tumour. This can also be described as extracellular heating where the extracellular matrix is heated with minimal impact on the intracellular environment. The device therefore uses the energy (measured in

Kilojoules) that is put into the system, rather than the temperature, as the measured dose. The energy output from the first electrode is compared to the energy received by the second electrode. Using algorithms the absorbed energy can be determined[29–31] and the tumour temperature is calculated. This technique therefore does not require intratumoural or MRI thermo-monitoring systems due to the real-time calculation of temperature by the device based on the energy dose. Confirmation of the heating capacity of the mEHT has been demonstrated.[32,33] The technique is also able to safely heat tumors, regardless of tumour depth or adipose layer thickness.[30] Hyperthermia interferes with protein synthesis,[26,34] and inhibits DNA and RNA synthesis and repair[34,35] by inducing protein aggregation in the nucleus and changes in the DNA repair foci which inhibit the DNA double-strand break repair processes. This is synergistic for RT and for certain cytotoxic agents which cause DNA double-strand breaks.[36] Hypoxia is a central causative factor for radio-resistance.[37] Heating the tumour also results in changes in blood perfusion and re-oxygenation. [38] Enhanced perfusion results in improved oxygenation in the target tissue, which enhances the effects of the Reactive Oxygen Species induced by IR.[39] Increased temperatures result in an increased immune response at the heated site.[27,40] HT has direct actions on the immune system through direct heat-mediated cell killing, and indirect actions by modulating the innate and adaptive immune system.[41] The extra-cellular heating method used by mEHT minimises the stress on the intracellular environment, reducing the risk of thermo-tolerance. Heating up the extracellular matrix with low frequency mEHT has only a minor impact on the intracellular environment and the primary action and heating occurs in the extracellular matrix and at the cellular membrane.[42] According to research conducted by Andocs *et al*, cell stress induced by mEHT results in the generation of DAMP signals and the application of mEHT could potentially induce local immunogenic cell death.[43] mEHT has also been shown to induce apoptosis [44] more readily than HT.[45] Andocs *et al* hypothesise that the energy absorption at the cell membrane results in a non-homogenous energy distribution causing damage which activates the cell death-related signalling pathways during mEHT.[45]

mEHT has shown benefits and safety as a chemo-sensitiser for the treatment of recurrent or residual cervical cancer[17] and the increase in temperature achieved in cervical tumours using mEHT is similar to the increased temperature reported using other HT methods.[46] Locations in which the technique has shown safety include the brain,[47] head and neck,[48] lung,[49] and pancreas,[50] The different dose monitoring system and temperature calculations means that MRI monitoring is not required. This technique is simple to use in comparison to other technologies, making it suitable for investigating as a chemo-radiosensitiser in a low-resource setting.

In South Africa, cervical cancer is the leading cause of cancer death in women with a lifetime risk of 1 in 44 of developing cervical cancer.[51] According to UNAIDS, in 2017 there were 36.9 million people infected with the HIV world-wide with an estimated 7.1 million of those people living in South Africa.[52] The risk of cervical cancer is significantly increased in HIV-positive patients, likely a result of the of HIV-associated immune suppression[53] and the subsequent increased risk of chronic HPV infection.[54] This, combined with constrained resources, makes South Africa a particularly challenging setting in which to test chemo-radiosensitisers.

We report on an analysis of the local control results at six months post-treatment of patients enrolled in an ongoing study investigating the effects of the addition of modulated electro-hyperthermia (mEHT) to chemoradiotherapy for the treatment of LACC patients in a low-resource setting, with specific interest in the effects on the HIV-positive subgroup. Six month local disease-free-survival (LDFS) is also reported in order to account for the participants who did not survive six months post-treatment. To our knowledge, this is the first phase III

randomised controlled trial (RCT) on the trimodality treatment of cervical cancer using this form of HT, the first trial to apply HT twice a week for LACC, the first HT trial in Africa, and the first phase III HT study to include HIV-positive patients in a low-resource setting.

Materials and methods

This a single centre, phase III, randomised controlled (stratum: HIV status; accounting for stage and age) parallel group trial being conducted at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), a public hospital in Gauteng, South Africa, by the Radiation Sciences department of the University of the Witwatersrand. Approval from the Human Research Ethics Committee was obtained (M1704133) on 5th June 2012. According to the requirements of the local ethics board, the trial was registered on the South African National Clinical Trials Register before recruitment was started (ID: 3012). Registration of the trial ClinicalTrials.gov (NCT03332069) was finalised after recruitment began. The authors confirm that all ongoing and related trials for the intervention are registered on ClinicalTrials.gov.

Eligibility

Patients were recruited by the multi-disciplinary team of gynaecology and oncology specialists at the CMJAH (January 2014 to December 2017) after undergoing clinical staging according to FIGO staging criteria,[55] by chest x-ray, ultrasound and physical assessment. Written informed consent was obtained and participants were referred for additional haematological investigations (liver function; urea and creatinine levels; full blood count; pregnancy test with serum β HCG; HIV test; CD4 count if indicated), and a Positron Emission Tomography/Computed Tomography (PET/CT) scan with ^{18}F -Fluorodeoxyglucose (FDG), as part of the screening process.

Eligibility criteria. Primary, treatment-naïve, histologically confirmed invasive squamous or adeno-squamous cell carcinoma of the cervix; FIGO stages IIB (with invasion of the distal half of the parametrium), to IIIB (clinically staged); Eligible for CRT with radical intent; >18years old; Eastern Cooperative Oncology Group (ECOG) score <2; Creatinine clearance >60mL/min; Haemoglobin, platelet count and absolute neutrophil count within normal ranges; HIV positive patients on antiretroviral therapy or with a CD4 count >200cell/ μL ; Ability to understand and the willingness to sign a written informed consent document; Life expectancy >12 months; Patients who underwent emergency RT in the form of brachytherapy for haemostasis prior to enrolment were screened and enrolled provided they met all other eligibility criteria; Body Mass Index (BMI) within normal ranges; Life expectancy of greater than 12 months; Participants with para-aortic and other nodes outside of the radiation field visualised on the ^{18}F -FDG PET/CT scans were included.

Exclusion criteria. Bilateral hydronephrosis; Second primary malignancy; Extra-pelvic visceral disease visualised on the ^{18}F -FDG PET/CT; Vesicovaginal fistula or vesicorectal fistula that required a change in treatment protocols; Abnormal liver function tests; Currently pregnant or breast feeding; Prior hysterectomy; Prior malignancy treated in the preceding two years; Cardiovascular disease (excluding controlled hypertension); Acute or life-threatening infections or medical conditions or life threatening AIDS-defining illnesses other than cervical cancer; Evidence of resistance to antiretroviral therapy in HIV-positive patients; A medical or psychiatric illness that preventing the participant from being able to sign an informed consent or affecting the participant's ability to comply with the protocol stipulations; Carcinoma of the cervical stump; Contra-indications to mEHT (pacemakers, large metallic implants in the treatment area, inability to feel temperature in the treatment area, inability to express or vocalise pain); Any other contraindications to any of the prescribed treatments. Visualisation of para-

aortic lymph nodes on planning CT was also an exclusion criterion, however patients were not 3D planned.

Randomisation and masking

Once participants passed all screening evaluations, participant data was collected and captured by the research co-ordinator using REDCap (Research Electronic Data Capture), an online, secure web based application hosted by the University of the Witwatersrand. The REDCap stratified on-line software generated random-sampling tool was used to randomise participants to receive either CRT alone (Control Group) or combined with mEHT (mEHT Group); stratum: HIV status. Randomisation accounted for age (<30 years; 30–50 years; >50 years), and FIGO stage (IIB; IIIA; IIIB). Participants were informed in a private consultation by the research co-ordinator of their treatment group at treatment verification. The research co-ordinator was not involved in the treatment or clinical evaluations. Double blinding of the participants was not possible due to logistical challenges such as creating a “placebo treatment” on the mEHT device in which the participant would feel heat on the surface but not actually be treated. Clinicians examining the participants during follow-ups were blind to treatment allocation. Physicians reporting on the ^{18}F -FDG PET/CT images were blind to treatment allocation and did not interact with the participants, eliminating the risk of biased reporting.

Treatment

Chemotherapy (CHT). As per the institutional protocol, two doses of cisplatin (80mg/m²) administered 21 days apart were planned during external beam radiation (EBRT), subject to the patient’s ability to receive cisplatin (creatinine clearance >60mL/min; acceptable full blood count results; acceptable ECOG status). All participants who were treated with cisplatin received prehydration with normal saline and electrolytes as well as prophylactic antiemetics intravenously, as per the institutional protocol.

Radiotherapy. A haemoglobin (Hb) level above 10g/dL was required for EBRT and participants were transfused as needed. All participants were prescribed RT in the form of EBRT (50Gy in 25 fractions of 2Gy), and High Dose Rate (HDR) brachytherapy (BT) (3 fractions of 8Gy). The EMBRACE II study aims for an EQD2 dose of >85Gy. However this is not easily achievable without image guided brachytherapy.[56] The European Society for Medical Oncology Guidelines on Cervical Cancer management lists the optimal treatment as 80–90Gy at point A.[2] We planned an intended total equivalent RT dose of 86Gy. Standard planning for EBRT in our facility is 2D planning, and not the more labour-intensive planning techniques that are standard of care internationally. This is due to the limited staff capacity and resources available to treat the high volume of gynaecological oncology patients seen each year and due to the lack of access to imaging techniques, such as MRIs, for planning purposes. Participants for this study were planned using virtual simulation and standard fields were placed but volumes were not contoured as is standard of care for cervical cancer. EBRT was planned using CT guidance and volumes were determined using bony landmarks to encompass the entire pelvis including 2cm margins from the pelvic side wall laterally. The superior border was the midline of L5 and the inferior border extended to 2cm below the tumour. Posterior border encompasses the sacrum to the third sacral vertebra and the anterior border extended to the inferior border of the pubic symphysis. A four field box techniques was used for planning and the dose prescribed was normalized to 95%. The dose to the nodes was the same as the dose to the centre as it was a homogenous dose distribution to the entire field. 15MV or 18MV photon energy linear accelerators (Siemens Pty, Ltd) were used for the four field technique. ^{18}F -FDG PET/CT images were additions to the standard protocols with the sole

purpose of monitoring disease response and the costs of the imaging studies were covered by a research grant. The ^{18}F -FDG PET/CT images were not used to plan treatments. Para-aortic nodes seen on PET/CT images were therefore not targeted with EBRT. While extended fields to include para-aortic nodes significantly decreases the risk of distant metastases and of para-aortic failure, the effects on overall survival are not yet confirmed[57] and extended fields are not standard in a resource-constrained setting such as ours.

HDR BT was given as per the institutional protocol. Patients received 36Gy equivalent (EQD2 –equivalent dose in 2Gy fractions for an alpha-beta ratio of 10), to point A by intracavity BT using standard tandem-ring or tandem-ovoid vaginal applicators (source used: Iridium 192). 2-dimensional calculations were done based on orthogonal x-rays. Chemotherapy was not permitted on the days that BT was given. A 2D technique was used for the brachytherapy, due to lack of availability of imaging techniques. If the tumour was confined to the upper vagina, a ring-tandem applicator was used. Where the tumour extended inferiorly below the upper half of the vagina, a Joss-Flynn ovoid-tandem applicator was used to cover the whole extent of the vagina. The lower part of the vagina was kept within rectal tolerance when treating with a Joss-Flynn ovoid-tandem applicator. Standard IRCU38 bladder and rectal dose points were calculated for BT. More sophisticated BT techniques require sophisticated imaging techniques which are not available in low-resource settings. The first BT was planned to be administered after 10–13 fractions of EBRT with a week between each subsequent BT dose with the aim of completing all radiation treatments within 52 days.

Hyperthermia. Hyperthermia was delivered via a capacitive heating device (Model: EHY2000+; Manufacturer: Oncotherm GmbH, Troisdorf, Germany) which uses 13.56MHz modulated radiofrequency energy. The power was applied using two electrodes, one fixed in the bed and one in an adjustable arm placed over the treatment area. [58] A 30cm diameter applicator with electrode was used to cover as much of the pelvic field as possible. Participants randomised into the mEHT Group received two mEHT treatments (per week at 42.5°C for a minimum of 55 minutes, plus the prescribed CRT. A step-up heating protocol was used starting at a power output of 60W and increasing to the planned power over a period of five minutes. The planned power for the first treatment was 90W, for the second treatment the planned power was 110W, and the third and subsequent treatments were planned for 130W. An average energy dose of 360KJ over ten treatments was planned. mEHT treatments were administered at least 48 hours apart to reduce the risk of developing thermo-tolerance and EBRT was administered within 30 minutes of completing mEHT. mEHT was not administered on the same day as BT or cisplatin.

Follow-up. Participants were examined, and adverse events were documented, weekly on treatment and at six weeks, three months and six months post-treatment. Regular full blood count, renal, and liver function tests were performed. Toxicity was graded according the Common Toxicity Criteria for Adverse Events (CTCAE) version 4 and toxicities worse than Grade 2 were reported to the investigators. The six month follow-up period extended from February 2014 to August 2018 with the final participant completing treatment in January 2018.

Outcome measures

The primary outcome is LDC at six months post-treatment. Secondary outcomes reported in this paper include disease pattern analysis, early toxicity, and quality of life. As the participants were treated with EBRT to the whole pelvis and HT to the pelvis, we considered the entire region that was within the treatment field, assessing local disease control and not only tumour response. LDC was therefore considered a failure if the presence of disease in the radiation field was noted on any one of the evaluation methods post-treatment (Persistent Disease). The

same method of evaluation is described in the protocols of the multicentre (including our centre) study by the AIDS Malignancy Consortium (AMC) evaluating 2D planned EBRT, HDR BT and cisplatin in patients receiving ART (ClinicaTrials.Gov ID:NCT01590017).[59] The results on LDC from this trial are not yet available. Six month LDFS is also reported, taking into account death as a possible risk factor.

All participants were planned to have an ^{18}F -FDG PET/CT scan at six months post-treatment to evaluate local disease response and disease patterns presentation using the PERCIST 1.0 criteria (Complete Metabolic Response [CMR] $\text{SUV} < 2.5$; Partial Metabolic Response [PMR] $> 30\%$ reduction in SUV; Stable Metabolic Disease [SMD] $< 30\%$ reduction in SUV; Progressive Metabolic Disease [PMD] increase in SUV).[60]. 13 participants were unable to have follow-up ^{18}F -FDG PET/CT scans due to poor ECOG status or renal dysfunction and were evaluated by Fine Needle Aspiration (FNA) of palpable nodes ($n = 1$), by CT scan ($n = 3$), and by clinical examination/PAP smear ($n = 9$). Evidence of disease on any of the evaluation methods was regarded as treatment failure. Six month LDFS was analysed according to the intention to treat principle and was considered a failure if there was a disease-related death or failure of LDC. We report on the tumour response determined clinically by examination, and on the level of agreement between tumour response determined by clinical exam and as visualised on ^{18}F -FDG PET/CT images. The early toxicity and quality of life are reported separately. Further papers on late toxicity and two year survival are planned.

Statistics

The sample size was calculated based on the estimated required sample sizes for a two-sample comparison of survivors' functions at two years (statistical power of 90%). We estimated an expected reduction in mortality at two years of 50%, based on survival of 20% in the control group and 40% in the experimental group. The statistical significance is defined as a two-sided $\alpha < 0.05$ for a log-rank test, with a constant Hazard ratio of 0.5693. Initially a 15% screening failure rate was predicted, however the screening failure rate was higher than expected (30%) and recruitment continued until the pre-determined required number of 200 eligible participants were enrolled.

Pearson's Chi squared test and Fisher's exact tests were used to determine the difference in frequencies between groups. T-test was used to compare the difference in means between groups. Logistic regression was used to test if prognostic factors (treatment arm, number of cisplatin doses; age; overall treatment time; RT dose; CD4 count; Body Mass Index [BMI]; number of mEHT treatments; energy dose administered), significantly predicted outcomes. The three stratification factors were used in the multivariate logistic regression model. Two sided p values are reported and p values < 0.05 were considered significant. STATA 13.0 Statistics software program (Stata Corporation, College Station, Texas, USA), was used to analyse the data.

Results

Of 271 screened participants screened between January 2014 and November 2017, 210 were randomised for trial (Fig 1). The most common reasons for omission of the second cycle of cisplatin were renal dysfunction (mEHT: $n = 19$; Control: $n = 11$) and delayed in the administration of the first cycle (mEHT: $n = 10$; Control: $n = 7$), followed by non-compliance, anaemia, vomiting and dehydration, sepsis, poor ECOG, immunosuppression and other, including supply and technical problems. Reasons for all screening failures can be found in S1 Fig. By the stratification to tumour stage, participant age, and HIV status, the numbers of participants in each group were balanced. Four randomised participants did not arrive for treatment and no

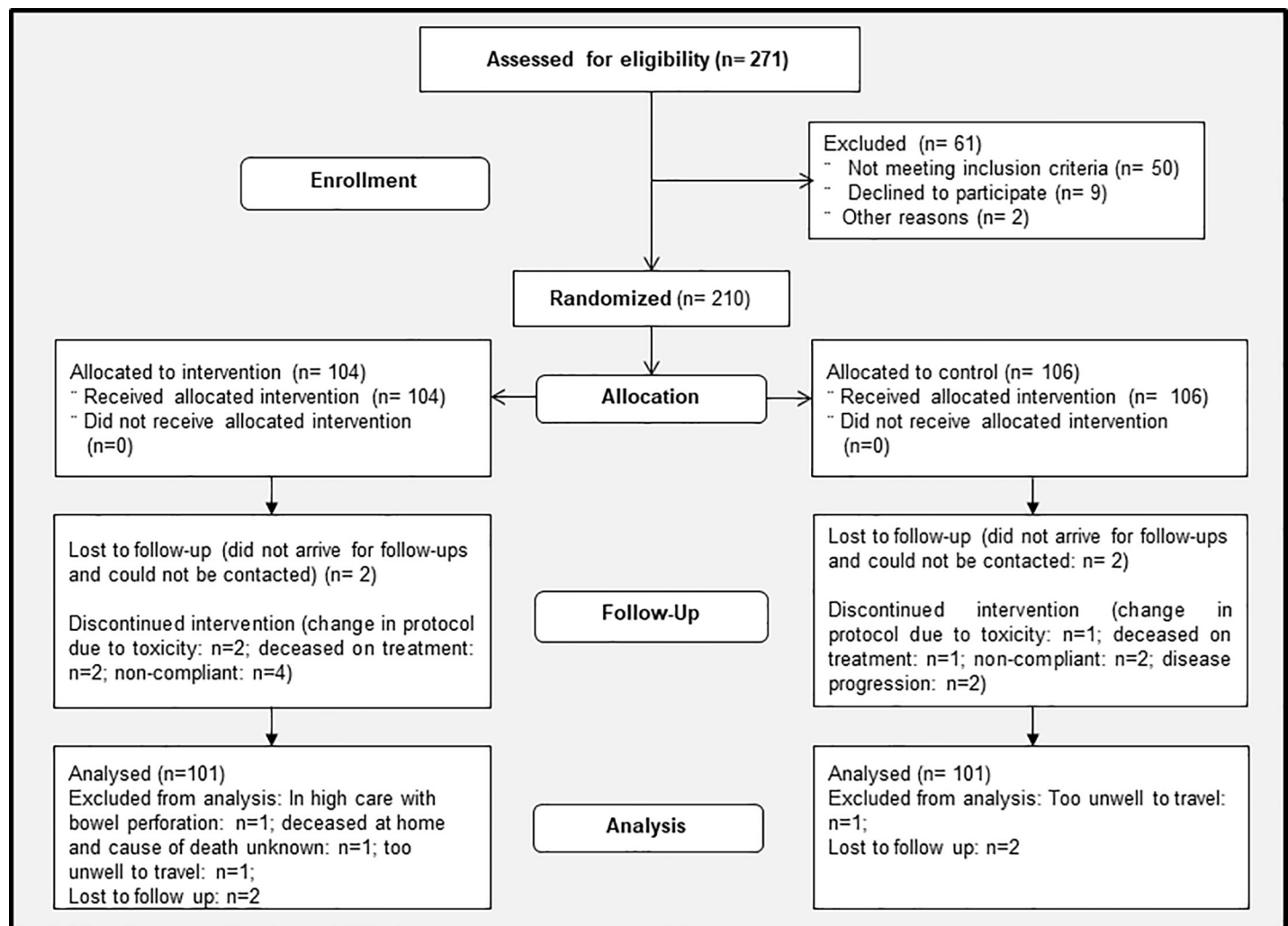


Fig 1. Trial profile: Consort flow diagram. Abbreviations: mEHT: Modulated Electro-Hyperthermia; LDFS: Local Disease Free Survival.

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further data is available on these participants. They were classified as “lost to follow up”. Patient, tumour and treatment characteristics were balanced in both treatment groups with no significant difference in the means or frequencies (Table 1). There were 104 and 102 participants treated in the mEHT and Control Groups respectively. Four participants could not be evaluated for LDC at six months post-treatment (in high care with bowel perforation: n = 1; deceased at home and cause of death unknown: n = 1; too unwell to travel: n = 2). ¹⁸FDG-PET/CT scans were conducted at a median of 186 days after completion of all RT treatments (range 154–610 days).

Treatment characteristics

Treatment characteristics are summarised in Table 2. Participants with a decrease in creatinine clearance to <60mL/min, and in whom measures to improve creatinine clearance failed, or whose CD4 count decreased to <150cells/uL, were not prescribed the planned cisplatin. Mean days to complete all radiation was 41.79 days (range 0–97). Six participants in the mEHT Group and four participants in the Control Group received less than 50Gy EBRT. Three participants died during on treatment (mEHT: n = 1; Control: n = 2), two did not arrive for

Table 1. Participant and treatment characteristics.

		OVERALL	mEHT	Control	P Value
	n	206	104 [50.5%]	102 [49.5%]	
HIV Status	Positive	105 [51%]	51 [49%]	54 [53%]	$p = 575$
	Negative	101 [49%]	53 [51%]	48 [47%]	
FIGO Staging	IIB	75 [36%]	40 [38%]	35 [34%]	$p = 0.825$
	IIIA	2 [1%]	1 [1%]	1 [1%]	
	IIIB	129 [63%]	63 [61%]	66 [65%]	
ECOG	0	10 [5%]	3 [3%]	7 [7%]	$p = 0.184$
	1	196 [95%]	101 [97%]	95 [93%]	
Race	African	191 [93%]	96 [92%]	95 [93%]	$p = 0.035$
	Caucasian	5 [2%]	4 [4%]	1 [1%]	
	Other	10 [5%]	4 [4%]	6 [6%]	
Age [years]	Mean	48.6	48.0	49.2	$p = 0.388$
	SD	10.49	10.77	10.22	
	Range	26–72	26–68	28–72	
BMI	Mean	27.45	27.81	27.08	$p = 0.431$
	SD	6.68	6.95	6.41	
	Range	15–49	15–49	15–41.7	
Hb [g/dL]	Mean	10.95	10.96	10.94	$P = 0.924$
	SD	2.26	2.13	2.39	
	Range	5.2–16.2	5.7–16.2	5.2–15.2	
CD4 count [cells/ μ L] in HIV positive	Mean	550	576.5	524.9	$P = 0.315$
	SD	261.76	251.57	270.97	
	Range	95–1524	148–1204	95–1524	

Abbreviations: mEHT: Modulated electro-hyperthermia; HIV: Human Immunodeficiency Virus; FIGO: International Federation of Gynaecology and Obstetrics; ECOG: Eastern Cooperative Oncology Group; SD: Standard Deviation; BMI: Body Mass Index; Hb: Haemoglobin.

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treatment, and two were moved to palliative care due to progression on treatment. Three participants who missed three or less fractions of EBRT cited transport difficulties as the reasons. There were no significant differences in early toxicities between the two groups. The mean dose of cisplatin received was 1.3. The main reasons for not receiving any cisplatin, a delayed first dose of cisplatin, or the cancellation of the second dose of cisplatin, are renal dysfunction (27 participants in the mEHT Group and 22 participants in the Control Group) and anaemia ($n = 7$ in both treatment groups). Other reasons for not receiving the required dose of cisplatin include leucopenia; dehydration; sepsis; poor ECOG; and a low CD4 count. Three participants in the mEHT Group had a reduction in power during the mEHT treatments by more than 20% due to reported pain at the treatment site.

Local disease control and local disease free survival

In total, 202 participants were eligible for six month LDFS analysis (mEHT: $n = 101$; Control: $n = 101$), of which 171 participants (mEHT: $n = 88$ [87.1%]; Control: $n = 83$ [82.2%]), were alive at six months post-treatment. Complete metabolic response (CMR) of all local disease in the radiation field (LDC) in the participants who survived at six months was significantly associated with mEHT administration with a LDC seen in 40[45.5%] of the mEHT Group participants compared to 20[24.1%] of the Control Group (Pearson's χ^2 : $p = 0.003$). In a multivariate logistic regression model adjusted for HIV status, FIGO stage and age (Table 3),

Table 2. Treatment characteristics.

		OVERALL	mEHT	Control	P Value (t-test)
	n	206	104 [50.5%]	102 [49.5%]	
Total radiation dose	Mean	84.3Gy	84.7Gy	84Gy	$p = 0.624$
	SD	9.55	7.65	11.18	
	Range	2-86Gy	20-86Gy	2-86Gy	
Total external beam radiation	Mean	48.2Gy	49.4Gy	48.0Gy	$p = 0.724$
	SD	8.47	7.74	9.19	
	Range	2-50Gy	20-86Gy	2-50Gy	
Days to complete all RT	Mean	41.79	41.46	42.13	$P = 0.560$
	SD	8.16	5.88	9.98	
	Range	0-97 days	12-65 days	0-97 days	
No of Cisplatin doses	Mean	1.31	1.38	1.25	$p = 0.179$
	SD	0.69	0.67	0.58	
	0	27 [13%]	11 [11%]	16 [16%]	
	1	88 [43%]	43 [41%]	45 [44%]	
	2	91 [44%]	50 [48%]	41 [40%]	
	Mean	2.88	2.89	2.87	$p = 0.778$
	SD	0.55	0.52	0.58	
No of brachytherapy fractions	0	2 [1%]	1 [1%]	1 [1%]	
	1	2 [1%]	0 [0%]	2 [3%]	
	2	4 [2%]	3 [4%]	1 [1%]	
	3	198 [95%]	100 [95%]	98 [95%]	
No of mEHT doses	Mean		9.09		
	SD		2.07		
	Range		1-10		
Average administered (KJ)	Mean		369.32KJ		
	SD		71.06		
	Range		237- 429KJ		

Abbreviations: mEHT: Modulated electro-hyperthermia; SD: Standard Deviation; RT: Radiotherapy; KJ: Kilojoules.

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participants in the mEHT Group were significantly more likely to achieve a LDC than participants in the Control Group (OR: 0.39, 95% CI: 0.20–0.77; $p = 0.006$). Age was also a predictor of LDC (OR: 1.04, 95% CI: 1.00–1.08; $p = 0.040$).

The intention to treat analysis showed a significant association between six month LDFS and the administration of mEHT (six month LDFS in mEHT Group: $n = 39$ [38.6%]; six month LDFS in Control Group: $n = 20$ [19.8%]; Pearson's Chi2: $p = 0.003$). A multivariate logistic regression model for six month LDFS (Table 4) also showed that participants in the

Table 3. Result of Multivariate logistic regression model to predict LDC (adjusted for HIV status, age, and stage).

Variable	Odds Ratio [OR]	P> z	95% Confidence interval [CI] range
Arm	0.39	0.006	0.20–0.77
HIV status	0.91	0.781	0.45–1.83
Age (years)	1.04	0.040	1.00–1.08
Stage (II or III)	1.09	0.604	0.78–1.54

Abbreviations: HIV: Human Immunodeficiency Virus

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mEHT Group were significantly more likely to achieve a six month LDFS than participants in the Control Group (OR: 0.36, 95% CI: 0.19–0.69; $p = 0.002$) and age was also a predictor of six month LDFS (OR: 1.04, 95% CI: 1.00–1.08; $p = 0.031$).

When analysed by HIV status, six month LDFS occurred in $n = 27$ [26.5%] of the HIV positive participants ($n = 102$), and $n = 32$ [32%] of the HIV negative participants ($n = 100$). The association between HIV status and six month LDFS was not significant (Pearson's Chi2: $p = 0.338$). When analysed by HIV status and treatment group, six month LDFS is higher in the HIV-positive and -negative participants treated with mEHT, with the highest six month LDFS seen in the HIV negative mEHT Group. The association between mEHT and six month LDFS was significant in the HIV-negative participants (Pearson's Chi2 $p = 0.006$), but was not significant in the HIV-positive participants (Pearson's Chi2 $p = 0.174$).

Tumour response

CMR of the tumour in the 158 participants (mEHT group: $n = 85$ [HIV-positive: $n = 40$; HIV-negative: $n = 45$]; Control Group: $n = 73$ [HIV-positive: $n = 35$; HIV-negative: $n = 38$]), who were eligible for ^{18}F -FDG PET/CT scans at six months post-treatment was higher in the mEHT Group ($n = 49$ [57.6%]) than in the Control Group ($n = 26$ [35.6%]), with a significant association between the metabolic responses and the administration of mEHT (Fischer's exact: $p = 0.005$). These results are represented graphically in Fig 2. Complete metabolic response was higher in HIV positive participants ($n = 40$ [53.3%]), than the HIV-negative participants ($n = 35$ [42.2%]), but this association was not significant (Fisher's exact: $p = 0.340$).

In an analysis by HIV status and by treatment group, the CMR was higher in the mEHT Group than in the Control Group in both HIV-positive and HIV-negative participants. The association between the CMR rates and mEHT was larger in the HIV-negative participants (mEHT: $n = 25$ [56.5%]; Control: $n = 10$ [26.3%]; Fisher's exact: $p = 0.006$), than in the HIV-positive participants (mEHT: $n = 24$ [60.0%], Control: $n = 16$ [45.7%]; Fisher's exact: $p = 0.196$), as seen in Fig 3.

Prognostic variables

The regression analyses show that age, number of cisplatin doses, treatment time, RT dose, and CD4 count, were not significant predictors of six month LDFS in our study (S1 Table).

In order to assess the efficacy of mEHT further, we considered the effect of BMI[31] (S2 Fig), energy dose (S3 Fig), and number of mEHT treatments on tumour response. Multivariate regression analyses showed that these variables were not a predictor of six month LDFS (S2 Table).

Disease pattern analysis

In the mEHT Group ($n = 95$), there were 15[37.5%] participants with extra-pelvic visceral disease visualised on the post-treatment ^{18}F -FDG PET/CT scans (HIV positive: 9[23%] out of 40;

Table 4. Result of logistic regression analysis to predict six month LDFS (adjusted for HIV status, age, and stage).

Variable	Odds Ratio [OR]	$P > z $	95% Confidence interval [CI] range
Arm	0.36	0.002	0.19–0.69
HIV status	0.91	0.788	0.45–1.83
Age (years)	1.04	0.031	1.00–1.08
Stage (II or III)	1.04	0.830	0.75–1.44

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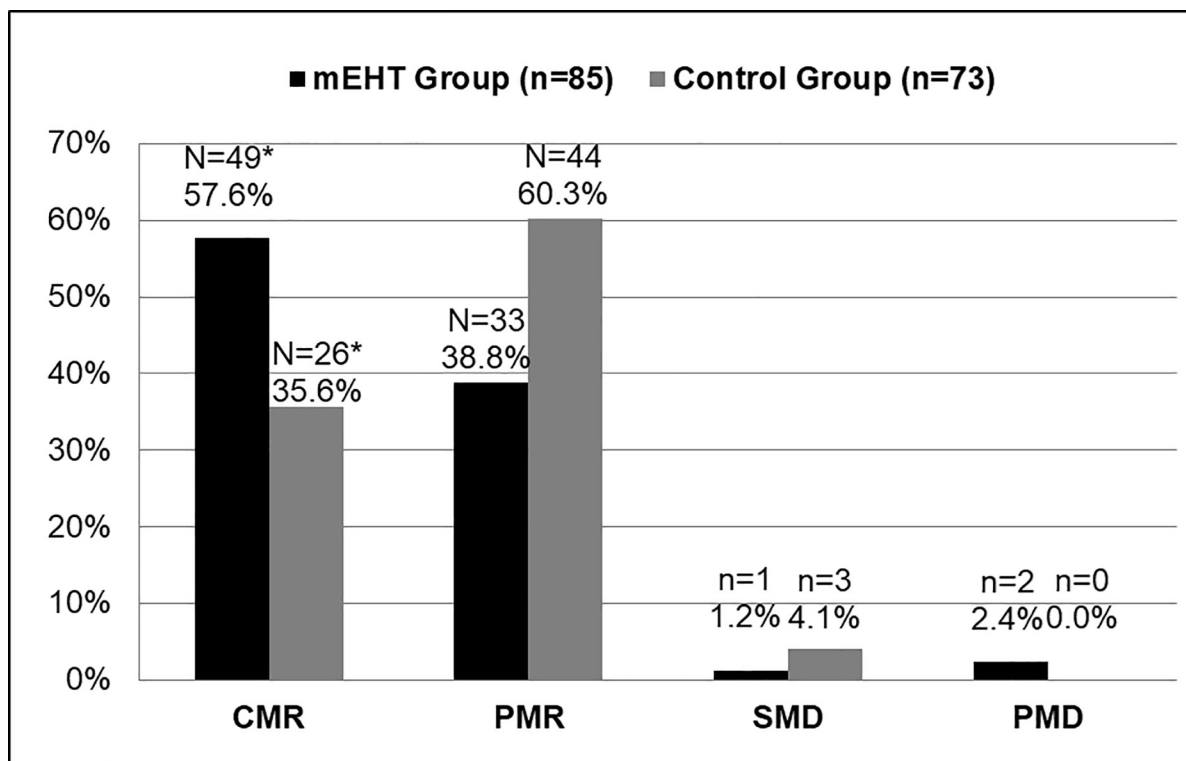


Fig 2. Tumour Response as Seen on ^{18}F -FDG PET/CT (PERCIST 1.0) by Treatment Group. mEHT: Fischer's exact table of association between all four metabolic responses and mEHT: $p = 0.005^*$. Abbreviations: mEHT: Modulated electro-hyperthermia; CMR: Complete Metabolic Response; PMR: Partial Metabolic Response; SMD: Stable Metabolic Disease; PMD: Progressed Metabolic Disease.

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HIV negative: $n = 6$ [13%] out of 45). The Control Group ($n = 73$) had 16 [45.7%] participants with visualised extra-pelvic visceral disease (HIV positive: 7 [20%] out of 35; HIV negative: $n = 9$ [24%] out of 38). The most common site for distant metastasis at six months was lung ($n = 20$), followed by liver ($n = 12$), bone ($n = 9$); soft tissue ($n = 1$), and brain ($n = 1$). Ten participants had multiple sites of distant visceral metastases.

54 Participants (64%) from the mEHT Group and 54 participants (74%) from the Control Group had nodes outside of the radiation field visualised on the pre-treatment ^{18}F -FDG PET/CT scans. The location of the nodes visualised on the pre-treatment scans are listed in Table 5. Para aortic nodes were visualised on the pre-treatment scans in 26 of the mEHT Group participants (25%) and 29 of the Control Group participants (28%). We compared the pre-treatment ^{18}F -FDG PET/CT scans of participants with extra-pelvic nodes to the post-treatment scans of the same participants in order to determine the response, if any, of the disease outside the radiation field. The percentage of participants who showed a systemic response to treatment, with resolution of all metabolically active disease (including extra-pelvic nodes which were not in the RT or mEHT fields, pelvic nodes and the primary tumour), was significantly higher in the mEHT Group: 24.1% ($n = 13$; HIV-positive: $n = 6$; HIV-negative: $n = 7$), versus the Control Group: 5.6% ($n = 3$; HIV-positive: $n = 1$; HIV-negative: $n = 2$) (Chi2, Fisher's exact: $p = 0.007$). One participant in each group did not receive cisplatin. In a multivariate analysis of age, cisplatin doses, total radiation dose and the number of days between the final radiation treatment and the follow-up ^{18}F -FDG PET/CT, none of the variables were indicators of an abscopal effect.

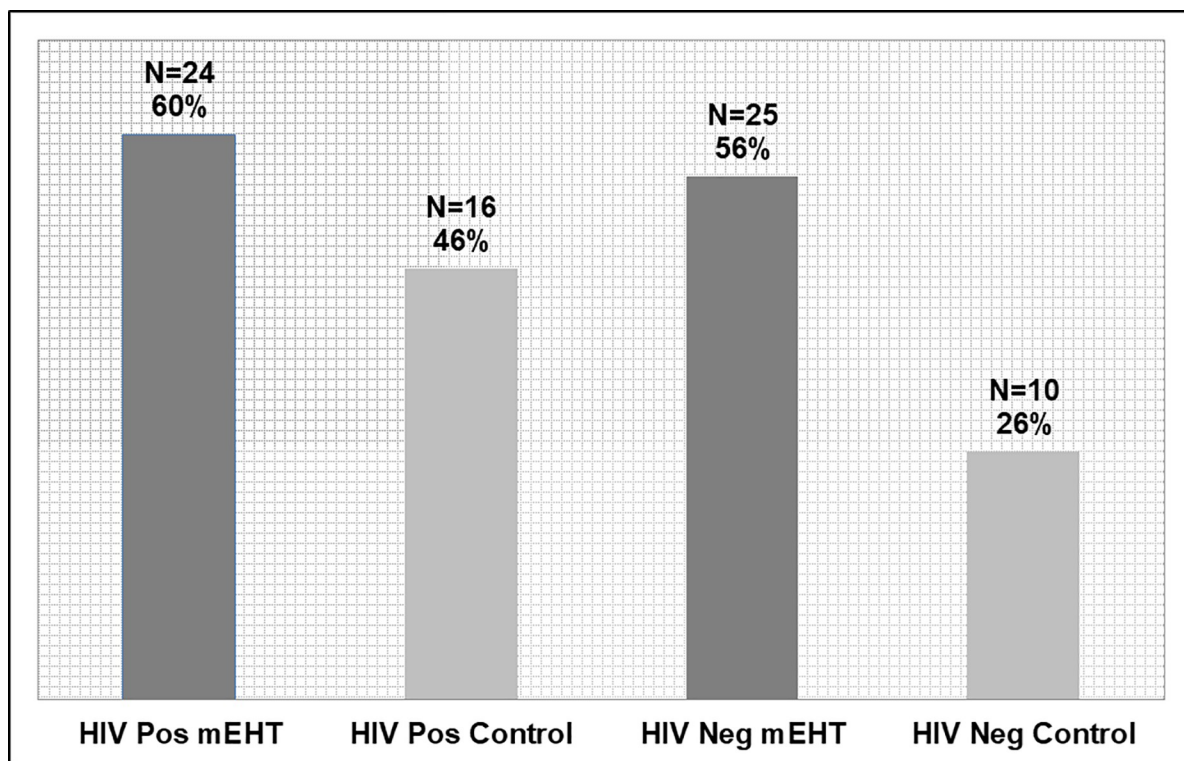


Fig 3. Tumour Response on ^{18}F -FDG PET/CT (PERCIST 1.0) by Treatment Group and HIV Status. Total participants in each subgroup: HIV-Positive mEHT: n = 40; HIV-Positive Control: n = 35; HIV-Negative mEHT: n = 45; HIV-Negative Control: n = 38. Abbreviations: mEHT: Modulated electro-hyperthermia.

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Toxicity

In our study, mEHT did not have any effect on the frequency of CRT-related early toxicities. The administration of mEHT was not associated with an increased risk in RT delays (OR: 1.614; $p = 0.381$; 95% CI: 0.553–4.712). Participant tolerance to mEHT treatments was excellent with 95 out of 104 (91.4%) participants receiving eight or more ($\geq 80\%$) of the planned ten mEHT treatments. The reasons for not receiving more than eight mEHT treatments are adipose burns (n = 2), 1cm blister (n = 1), progressed on treatment (n = 1), moist desquamation resulting in RT and mEHT delay (n = 2), bleeding resulting in RT and mEHT delay (n = 1), did not arrive for RT or mEHT (n = 1), deceased on treatment (n = 1). Two participants in the control group also died during treatment. Eleven (10.6%) mEHT participants reported adverse events: Grade 1–2 adipose tissue burns (n = 10, 9.5%); grade 1 surface burns (n = 2, 2.0%). One participant in the Control Group also reported a subcutaneous burn. A multivariate

Table 5. Number of patients with nodes visualized in each region on pre-treatment scans.

	mEHT (n = 54)		Control (n = 54)	
Head and Neck	30	[56%]	26	[48%]
Thorax	29	[54%]	30	[56%]
Abdomen	32	[59%]	39	[72%]
Pelvis	43	[80%]	46	[85%]

Abbreviations: mEHT: Modulated Electro-Hyperthermia

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analysis showed increased BMI (OR: 1.78; $p = 0.375$; 95% CI: 0.499–6.335), were not predictive of adverse events associated with mEHT treatments.

Quality of life

At three months post treatment, fatigue and pain were significantly reduced in the mEHT Group and there was a significant improvement in social functioning ($p = 0.049$), and emotional functioning ($p = 0.017$) seen in the mEHT Group. There were no significant differences in quality of life between the groups while on treatment.

Discussion

Our results show that the addition of mEHT to chemoradiotherapy protocols for the management of LACC patients, results in a significantly higher six month LDC and six month LDFS, in a resource-constrained setting and in immunocompromised patients. Hyperthermia is a known radiosensitiser[61] and the heating effect of mEHT during the treatments is therefore likely to be the largest contributor to the improved outcomes seen in patients treated with mEHT in our study. Another possible contributing factor is the immunomodulating effect of mEHT described in the literature which may also contribute to the improved outcomes seen even in patients who are immunocompromised.

Due to resource constraints, the standard of care cisplatin schedule (40mg/m² weekly), and standard of care of planning, using at least 3D-conformal radiotherapy (3DCRT) and extended fields (para-aortic), where indicated, was not used. It is not clear how much 3DCRT planning and extended radiation fields would improve the results in the treatment groups. Literature indicates that while extended fields on EBRT to include para-aortic lymph nodes significantly reduces the risk of distant metastases and the rate of para-aortic failure, the effects on overall survival are less significant.[57] Resource-constraints also affected factors such as time to complete treatment (listed as technical problems) and timing of the ¹⁸F-FDG PET/CT scans in some cases (PET/CT down time, source supply problems). The control arm in our group had a lower response than what is generally achieved for LACC patients. Possible reasons include the evaluation of CMR using ¹⁸F-FDG PET/CT studies compared to clinical evaluations of tumour response, a sample taken from a high risk population many of whom may be immunocompromised, and a resource-constrained setting where patient care and available treatments are sub-standard in comparison to less resource-constrained settings. Under-staging of cervical cancer patients is a well-documented challenge in low-resource settings where imaging techniques are not available.[62] There is a high risk that patients in our setting are being under-staged using only clinical staging methods, compared to the patients staged with imaging studies used in less resource-constrained settings and it is possible that many of our patients are in fact in Stage IV, based on the positive lymph nodes seen on pre-treatment ¹⁸F-FDG PET/CT scans. The high rate of screening failure was unexpected and we did not expect that so many of the patients were being under-staged and that there would be so many patients with visceral metastases and bilateral hydronephrosis on PET/CT images. This is an unfortunate challenge in low-resource setting where imaging studies to adequately stage patients are not routinely available.

The reviewed studies on HT for the treatment of cervical cancer, primary or recurrent, use local control, and not local disease control, as an outcome measure and the majority of the studies rely only on the clinical evaluation of local control with only a few using imaging studies.[14–17,20,22–24,63–67] The results from the two Phase III studies on the addition of HT to CRT reported a CR rate of 76% versus 86% ($p = 0.54$)[23] and 77.6% versus 88% ($p = 0.192$)[24] in the CRT versus the CRT plus HT groups respectively. In the Harima study[24] CR was achieved when no tumour was detected on physical examination or MRI, evaluated at one

month post treatment, although the number of patients evaluated using MRI was not reported. A multivariable logistic regression model did show that patients receiving CRT combined with HT in the Harmia study were more likely to have a CR ($p = 0.047$). Whole pelvis radiotherapy was applied however local disease control was not reported. The difference between this study and our study is the type of capacitive heating used and the frequency of HT treatments per week (one per week in the Harmia study and twice per week in our study). In the Harima study the tumour temperature was monitored rectally and vaginally, not intratumorally, compared to the real-time temperature calculations used by the mEHT device. Although 92% of participants in the Harmia study only received four to six HT treatments, the reasons for not completing HT (for example patient concerns or HT adverse events) are not given[24]. The compliance to mEHT treatments in our study was very good indicating good tolerance of the treatment, with more than 90% receiving eight or more mEHT treatments in our study. In the Phase III study by Flameling et al, the results posted did not include methods of evaluation, distribution of stages of disease, or patient characteristics. The study was closed prematurely due to slow recruitment, and was therefore underpowered, making drawing definitive conclusions impossible. In the Flameling study, HT was administered once per week and the primary tumour response was measured three months post-treatment. [23]

Challenges with clinical staging include the inadequate evaluation of the proximal tumour extension or side wall invasion, and inaccurate lymph node evaluation (especially deep pelvic lymph nodes).[68] With a sensitivity and specificity of lymph node evaluation of 100% and 99.6% respectively for nodes larger than 5mm, and the ability to detect metabolic changes before anatomical changes occur,[69,70] ^{18}F -FDG PET/CT is more effective at detecting lymph node involvement,[68] distant metastases,[71] and tumour activity.[72] LDC in our study included lymph nodes within the radiation field as visualised on ^{18}F -FDG PET/CT scans and we report on the loco-regional metabolic response, not the clinical response of the tumour.

The major challenges with the management of advanced cervical tumors are loco-regional control and distant metastases. Image guided adaptive brachytherapy (IGABT) has improved local and pelvic control rates for stage III/IVA disease to between 73% and 86%. Pötter et al report that the improved local and pelvic control compared to historical databases is associated with an overall survival benefit of around 10%.[56] These results are significantly higher than those seen in our study. Due to the differences in RT techniques, resources, staffing, patient characteristics, and confounding factors such as malnutrition and immune suppression, seen in our setting compared to less resource-constrained settings, a direct comparison of outcomes between the settings cannot be accurately made.

In our study, BMI and average energy dose were not predictors of six month LDFS, indicating that the mEHT treatments were able to target tumours regardless of the thickness of the adipose layer and that the required temperature and effect is still achieved in patients in whom the applied power needed to be lowered in order to achieve the prescribed treatment duration. In a phase I/II study investigating the effects of HT combined with CRT for the management of 68 LACC participants, adverse events directly attributed to HT included superficial burns ($n = 12$ [18%]) and subcutaneous fatty necrosis ($n = 5$ [7%]).[22] The number of superficial bones in our study was only two, and each was less than 1cm in diameter.

The significance of the results and benefits of HT for the treatment of LACC in the reviewed literature varies considerably. One possible reason is the lack of a fixed reference point for temperature and dose calculations, and the different heating methods.[73] Various heating techniques and protocols (treatment duration, frequency, and timing) are used which may contribute further to the variation in results. The two phase III trials on CRT plus HT which reported no benefit to the addition of HT, used a protocol of one HT treatment per week plus weekly cisplatin (40mg/m²),[23,24] compared to our protocols of two mEHT treatments per

week plus two doses of cisplatin (80g/m²) three weekly. Franckena *et al* reported that the number of HT treatments emerged as a predictor of outcomes with patients receiving more than three HT treatments showing a higher CR.[74] Our study did not show an association between the number of mEHT treatments and the CR, however the number of participants with three or less mEHT treatments was only three and the majority (91.3%) of participants could tolerate eight or more mEHT treatments. The need for mEHT treatment discontinuation was very low. Although not all participants could receive cisplatin in our study, the number of cisplatin doses was also not a predictor of outcomes.

Some studies have reported an increase in rates of distant metastases in participants treated with radiation plus HT.[20] We have not observed the same trend. In our study we observed the resolution of extra-pelvic nodal disease along with the pelvic disease in some of the participants. This rate was significantly higher in the mEHT Group. This may be due to the abscopal effect being potentiated by the addition of mEHT. A further analysis of these participants is planned.

According to the literature, treatment outcomes for HIV-positive patients are expected to be worse than in HIV-negative patients.[9–11] However, in our study the HIV-positive participants had a higher rate of complete metabolic tumour response to treatment than the HIV-negative participants, although the difference in CMR between the HIV-positive and -negative participants was not significant (53.3% versus 42.2%; Fisher's exact: $p = 0.340$). In a subgroup analysis of treatment groups in HIV-positive versus HIV-negative participants, the difference between the Control and mEHT Groups was larger in the HIV-negative participants. HIV-positive participants in each treatment group had a higher CMR rate at six months. This may suggest either less of an effect of mEHT in the HIV-positive participants, or a higher radiosensitivity of the tumours in HIV-positive participants. There are indications in the literature that HIV-positive patients have a higher chromosomal radiosensitivity[75] and this may contribute to the increased tumour response rates seen in these patients.

The difference between mEHT and other HT techniques is the dosing concept which relies on the energy dose applied to the patient during the treatment and the temperature is calculated based on the energy dose applied. Intratumoural or MRI thermo-monitoring systems are therefore not required, making the technique far simpler and more affordable than other techniques on the market, enabling the integration of the mEHT into the work flow in the resource-constrained setting. Without the need for increased staff to manage the treatments or the need for access to MRIs, theatres, or specialists, the use of the heating technique was viable. Although the device was loaned to the research team for research purposes, should the department be in a position to purchase the device, the cost to purchase the device is currently less than half the price of a BT device and does not require access to imaging techniques that the more advanced BT systems require. While mEHT is very clearly not a replacement for adequate EBRT and BT planning and treatments, it may be viable technique to improve outcomes in a setting where sophisticated RT techniques are not likely to be available for some time to come. A comparison between our results with mEHT and results from the use of mEHT in a less resource-constrained setting would be merited. Although the response rates varied between our study and the literature, the difference in CR between the two groups in our study is 22% and this improvement in outcomes, even in such high risk patients, is a strong indicator of the potential benefit to the use of mEHT.

Conclusion

This phase III RCT provides the first evidence for the use of mEHT to improve treatment outcomes when added to CRT for the treatment of LACC. The results offer evidence of efficacy of

a heating technique that is feasible and can be implemented in a low resource setting in a population sample in whom treatment and management of LACC is particularly challenging. The results indicate that mEHT can be integrated into the multimodal treatment regimens for LACC patients treated with radiotherapy with/without cisplatin, improving outcomes and potentially assisting in alleviating the burden of LACC on healthcare systems. There is also a potential benefit to the addition of mEHT to CRT for the treatment of LACC in less resource-constrained settings. A longer follow up period is required to assess the survival benefits and further papers are planned to report these results. The design of further phase III studies on mEHT as a chemo- or radiosensitiser for the treatment of other malignancies is warranted. The potential abscopal effect observed suggests that further investigations into the use of mEHT combined with immunotherapies are warranted.

Supporting information

S1 Fig. Reasons for screening failure before randomisation. *Abbreviations: VRF: Vesicorectal fistula; PET/CT: Positron Emission Tomography / Computed Tomography; ARV: Antiretroviral* *Bleeding: bleeding that could not be controlled by haemostatic brachytherapy and required admission and a change in protocols before completing the screening process; **Technical: ^{18}F -FDG supply difficulties.
(PDF)

S2 Fig. Patient BMI versus tumour response on ^{18}F -FDG PET/CT. *Abbreviations: BMI: Body Mass Index; CMR: Complete Metabolic Response; PMR: Partial Metabolic Response; SMD: Stable Metabolic Disease; PMD: Progressed Metabolic Disease.*
(PDF)

S3 Fig. Average energy dose versus tumour response on ^{18}F -FDG PET/CT. *Abbreviations: KJ: Kilojoules; CMR: Complete Metabolic Response; PMR: Partial Metabolic Response; SMD: Stable Metabolic Disease; PMD: Progressed Metabolic Disease.*
(PDF)

S1 Table. Logistic regression model for prognostic variables and six month local disease free survival. *Abbreviations: RT: Radiotherapy; HIV: Human Immunodeficiency Virus.*
(DOCX)

S2 Table. Logistic regression model of meht-related prognostic variables and six month local disease free survival. *Abbreviations: mEHT: Modulated electro-hyperthermia; KJ: Kilojoules; BMI: Body Mass Index.*
(DOCX)

S1 Appendix. CONSORT checklist.
(PDF)

S2 Appendix. LACC trial protocol.
(PDF)

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel R, Torre L, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA A J Clin*. 2018; 6(68):394–424.
2. Marth C, Landoni F, Mahner S, McCormack M, Gonzalez-Martin A, Colombo N. Cervical cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. *Ann Oncol*. 2017; 28 (suppl_4):iv72–iv83. <https://doi.org/10.1093/annonc/mdx220> PMID: 28881916
3. Denny L, Anorlu R. Cervical cancer in Africa. *Cancer Epidemiol Biomarkers Prev*. 2012; 21(9):1434–8. <https://doi.org/10.1158/1055-9965.EPI-12-0334> PMID: 22806169
4. Grover S, Xu MJ, Yeager A, Rosman L, Groen RS, Chackungal S, et al. A Systematic Review of Radiotherapy Capacity in Low- and Middle-Income Countries. *Front Oncol*. 2015; 4(380):1–11.
5. Moodley M. Cervical cancer in Southern Africa: the challenges. *South African J Gynaecol Oncol*. 2009; 1(1):11–3.
6. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. Cancer Today (powered by GLOBOCAN 2018) [Internet]. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, et al., editors. IARC CancerBase No. 15. 2018. Available from: <http://publications.iarc.fr/577>
7. Shrestha AD, Neupane D, Vedsted P, Kallestrup P. Cervical Cancer Prevalence, Incidence and Mortality in Low and Middle Income Countries: A Systematic Review. *Asian Pac J Cancer Prev*. 2018; 19 (2):319–24. <https://doi.org/10.22034/APJCP.2018.19.2.319> PMID: 29479954
8. Coghill AE, Newcomb PA, Madeleine MM, Richardson BA, Mutyaba I, Okuku F, et al. Contribution of HIV infection to mortality among cancer patients in Uganda. *Aids*. 2013; 27(18):2933–42. <https://doi.org/10.1097/01.aids.0000433236.55937.cb> PMID: 23921614
9. Gichangi P, Bwayo J, Estambale B, Rogo K, Njuguna E, Ojwang S, et al. HIV impact on acute morbidity and pelvic tumor control following radiotherapy for cervical cancer. *Gynecol Oncol*. 2006; 100(2):405–11. <https://doi.org/10.1016/j.ygyno.2005.10.006> PMID: 16274737
10. Simonds HM, Neugu AI, Jacobson JS. HIV Status and acute hematological toxicity among cervix cancer patients undergoing radical chemoradiation. *Int J Gynecol Cancer*. 2015; 25(5):884–90. <https://doi.org/10.1097/IGC.0000000000000441> PMID: 25853380

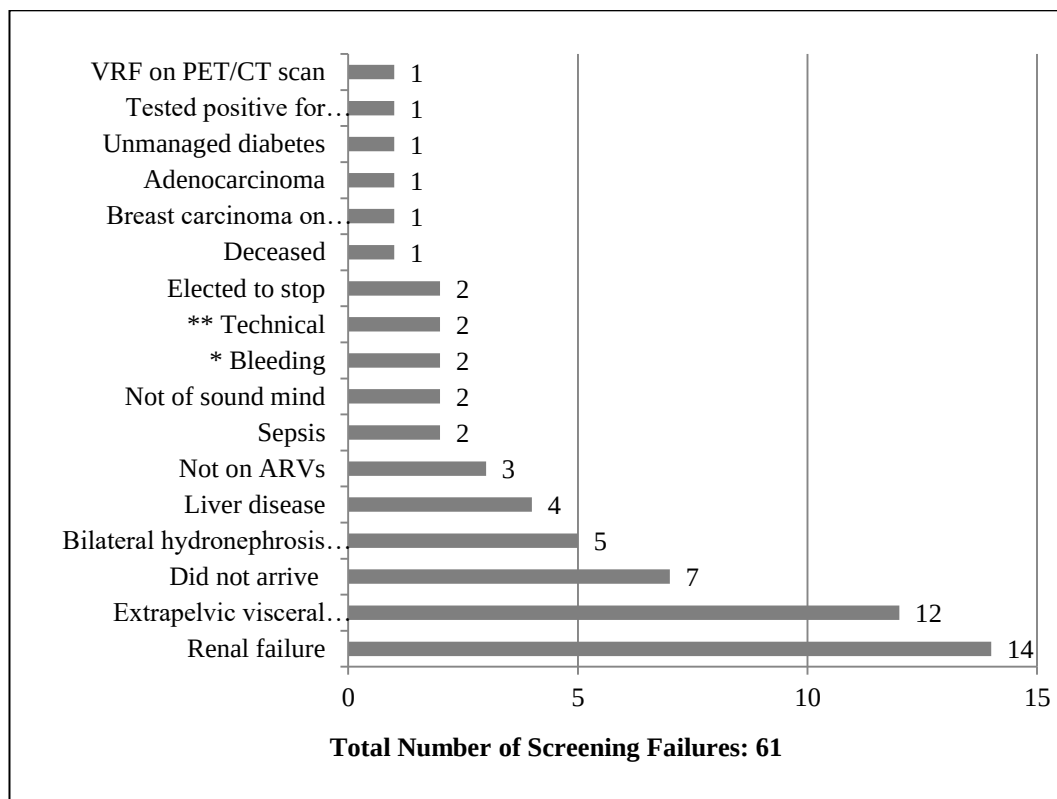
11. Dryden-Peterson S, Bvochora-Nsingo M, Suneja G, Efstathiou JA, Grover S, Chiyapo S, et al. HIV infection and survival among women with cervical cancer. *J Clin Oncol*. 2016; 34(31):3749–57. <https://doi.org/10.1200/JCO.2016.67.9613> PMID: 27573661
12. Lutgens L, Van Der Zee J, Pijls-Johannesma M, De Haas-kock D, Buijsen J, Mastrigt G, et al. Combined use of hyperthermia and radiation therapy for treating locally advanced cervical carcinoma (Review). *Cochrane Database Syst Rev*. 2010;(1):Article No. CD006377. <https://doi.org/10.1002/14651858.CD006377.pub2> PMID: 20091593
13. Datta NR, Rogers S, Klingbiel D, Gómez S, Puric E, Bodis S. Hyperthermia and radiotherapy with or without chemotherapy in locally advanced cervical cancer: a systematic review with conventional and network meta-analyses. *Int J Hyperth*. 2016; 32(7):809–21.
14. Franckena M, de Wit R, Ansink AC, Notenboom A, Canters RAM, Fatehi D, et al. Weekly systemic cisplatin plus locoregional hyperthermia: An effective treatment for patients with recurrent cervical carcinoma in a previously irradiated area. *Int J Hyperth*. 2007; 23(5):443–50.
15. Rietbroek RC, Bakker PJM, Schilthuis MS, Postma AJ, Zum Vörde Sive Vörding PJ, Gonzaléz DG, et al. Feasibility, toxicity, and preliminary results of weekly loco-regional hyperthermia and cisplatin in patients with previously irradiated recurrent cervical carcinoma or locally advanced bladder cancer. *Int J Radiat Oncol Biol Phys Biol Phys*. 1996; 34(4):887–93.
16. de Wit R, van der Zee J, van der Burg M, Kruit W, Logmans A, van Rhooen G, et al. A phase I/II study of combined weekly systemic cisplatin and locoregional hyperthermia in patients with previously irradiated recurrent carcinoma of the uterine cervix. *Br J Cancer*. 1999; 80(9):1387–91. <https://doi.org/10.1038/sj.bjc.6690533> PMID: 10424740
17. Lee S, Lee N, Cho D, Kim J. Treatment outcome analysis of chemotherapy combined with modulated electro-hyperthermia compared with chemotherapy alone for recurrent cervical cancer, following irradiation. *Oncol Lett*. 2017; 14(1):73–8. <https://doi.org/10.3892/ol.2017.6117> PMID: 28693137
18. Oei AL, Van Leeuwen CM, Ten Cate R, Rodermond HM, Buist MR, Stalpers LJA, et al. Hyperthermia selectively targets human papillomavirus in cervical tumors via p53-dependent apoptosis. *Cancer Res*. 2015; 75(23):5120–9. <https://doi.org/10.1158/0008-5472.CAN-15-0816> PMID: 26573798
19. Franckena M, Stalpers LJA, Koper PCM, Wiggendaad RGJ, Hoogendaad WJ, van Dijk JDP, et al. Long-Term Improvement in Treatment Outcome After Radiotherapy and Hyperthermia in Locoregionally Advanced Cervix Cancer: An Update of the Dutch Deep Hyperthermia Trial. *Int J Radiat Oncol Biol Phys*. 2008; 70(4):1176–82. <https://doi.org/10.1016/j.ijrobp.2007.07.2348> PMID: 17881144
20. Vasanthan A, Mitsumori M, Park JH, Zhi-Fan Z, Yu-Bin Z, Oliynychenko P, et al. Regional hyperthermia combined with radiotherapy for uterine cervical cancers: a multi-institutional prospective randomized trial of the International Atomic Energy Agency. *Int J Radiat Oncol Biol Phys*. 2005; 61(1):145–53. <https://doi.org/10.1016/j.ijrobp.2004.04.057> PMID: 15629605
21. Sreenivasa G, Hildebrandt B, Kummel S, Jungnickel K, Cho CH, Tilly W, et al. Radiochemotherapy combined with regional pelvic hyperthermia induces high response and resectability rates in patients with nonresectable cervical cancer > or = FIGO IIB “bulky”. *Int J Radiat Oncol Biol Phys*. 2006; 66(4):1159–67. <https://doi.org/10.1016/j.ijrobp.2006.06.052> PMID: 16979843
22. Westermann AM, Jones EL, Schem B, Steen-banasik EM Van Der, Koper P, Mella O, et al. First Results of Triple-Modality Treatment Combining Radiotherapy, Chemotherapy, and Hyperthermia for the Treatment of Patients with Stage IIB, III, and IVA Cervical Carcinoma. *Cancer*. 2005; 104(4):763–70. <https://doi.org/10.1002/cncr.21128> PMID: 15968685
23. Flameling B, Nordberg T, Ott O, Issels RD, Wust P, Crezee J, et al. An international multicenter phase III study of chemoradiotherapy versus chemoradiotherapy plus hyperthermia for locally advanced cervical cancer. *J Clin Oncol [Internet]*. 2016; 34(Supplement 15):17023. Available from: https://doi.org/10.1200/JCO.2016.34.15_suppl.e17023
24. Harima Y, Ohguri T, Imada H, Sakurai H, Ohno T, Hiraki Y, et al. A multicentre randomised clinical trial of chemoradiotherapy plus hyperthermia versus chemoradiotherapy alone in patients with locally advanced cervical cancer. *Int J Hyperth*. 2016; 32(7):801–8.
25. Kok HP, Navarro F, Strigari L, Cavagnaro M, Crezee J. Locoregional hyperthermia of deep-seated tumours applied with capacitive and radiative systems: a simulation study. *Int J Hyperth*. 2018; 34(6):714–30.
26. Fiorentini G, Szasz A. Hyperthermia today: Electric energy, a new opportunity in cancer treatment. *J Cancer Res Ther*. 2006; 2(2):41–6. PMID: 17998673
27. Szasz A, Szasz O, Szasz N. Electro-hyperthermia: a new paradigm in Cancer Therapy. *Dtsch Zeitschrift für Onkol*. 2001; 33(3):91–9.
28. Szasz O, Szasz A. Heating, Efficacy and Dose of Local Hyperthermia. *Oncothermia J*. 2016; 17:38–47.
29. Szigeti GP, Szasz O, Hegyi G. Personalised Dosing of Hyperthermia. *J Cancer Diagnosis*. 2016; 1(1):1–9.

30. Szasz O, Szigeti GP, Vancsik T, Szasz A. Hyperthermia Dosing and Depth of Effect. *Open J Biophys.* 2018; 8(1):31–48.
31. D'Ambrosio V, Dughiero F. Numerical model for RF capacitive regional deep hyperthermia in pelvic tumors. *Med Biol Eng Comput.* 2007; 45(5):459–66. <https://doi.org/10.1007/s11517-007-0177-y> PMID: 17372778
32. Andocs G, Renner H, Balogh L, Fonyad L, Jakab C, Szasz A. Strong Synergy of Heat and Modulated Electromagnetic Field in Tumor Cell Killing. *Strahlentherapie und Onkol.* 2009; 185(2):120–6.
33. Szasz A, Szasz O, Szasz N. Principles and Practice of Oncothermia. New York: Springer International Publishing; 2011. 194–196 p.
34. Pandita TK, Pandita S, Bhaumik SR. Molecular Parameters of Hyperthermia for Radiosensitization. *Crit Rev Eukaryote Gene Expr.* 2009; 19(3):235–51.
35. Oei AL, Vriend LEM, Crezee J, Franken NAP, Krawczyk PM. Effects of hyperthermia on DNA repair pathways: One treatment to inhibit them all. *Radiat Oncol.* 2015; 10(1):1–13.
36. Szasz O, Szigeti GP, Szasz A. Connections between the Specific Absorption Rate and the Local Temperature. *Open J Biophys.* 2016; 6(3):53–74.
37. Peeken JC, Vaupel P, Combs SE, Combs SE. Integrating Hyperthermia into Modern Radiation Oncology: What Evidence Is Necessary? *Front Oncol.* 2017; 7(Article 132):1–17.
38. Dewhirst MW, Vujaskovic Z, Jones E, Thrall D. Re-setting the biologic rationale for thermal therapy. *Int J Hyperth.* 2005; 21(8):779–90.
39. Hurwitz M, Stauffer P. Hyperthermia, Radiation and Chemotherapy_ The Role of Heat in Multidisciplinary Cancer Care. *Semin Oncol.* 2014; 41(6):714–29. <https://doi.org/10.1053/j.seminoncol.2014.09.014> PMID: 25499632
40. van der Zee J. Heating the patient: a promising approach? *Ann Oncol.* 2002; 13(8):1173–84. <https://doi.org/10.1093/annonc/mdf280> PMID: 12181239
41. Frey B, Rubner Y, Wunderlich R, Weiss E, Pockley AG, Fietkau R, et al. Induction of Abscopal Anti-Tumor Immunity and Immunogenic Tumor Cell Death by Ionizing Irradiation—Implications for Cancer Therapies. *Curr Med Chem.* 2012; 19:1751–64. PMID: 22414083
42. Papp E, Vancsik T, Kiss E, Szasz O. Energy Absorption by the Membrane Rafts in the Modulated Electro-Hyperthermia (mEHT). *Open J Biophys.* 2017; 7:216–29.
43. Andocs G, Meggyeshazi N, Balogh L, Spisak S. Upregulation of heat shock proteins and the promotion of damage-associated molecular pattern signals in a colorectal cancer model by modulated electrohyperthermia. *Cell Stress Chaperones.* 2015; 20(1):37–46. <https://doi.org/10.1007/s12192-014-0523-6> PMID: 24973890
44. Tsang Y, Huang C, Yang K, Chi M, Chiang H, Wang Y, et al. Improving immunological tumor microenvironment using electro-hyperthermia followed by dendritic cell immunotherapy. *Biomed Cent Cancer.* 2015; 15:708.
45. Andocs G, Rehman MU, Zhao Q, Tabuchi Y, Kanamori M, Kondo T. Comparison of biological effects of modulated electro-hyperthermia and conventional heat treatment in human lymphoma U937 cells. *Cell Death Discov.* 2016; 2(16039):1–10.
46. Lee S-Y, Kim J-H, Han Y-H, Cho D-H. The effect of modulated electro-hyperthermia on temperature and blood flow in human cervical carcinoma. *Int J Hyperth.* 2018; 34(7):953–60.
47. Fiorentini G, Patrizia D, Gina T, Carlo M. Phase II clinical study on relapsed malignant gliomastreated with electro-hyperthermia. *Oncothermia J.* 2013; 7(2):2013.
48. Huilgol N, Gupta-dutta S, Sridhar CR. Hyperthermia in the management of head and neck cancer—A single institution study from India. *Oncothermia J.* 2014; 10(2):64–7.
49. Szasz A. Current Status of Oncothermia Therapy for Lung Cancer. *Korean J Thoracic Cardiovasc Surg.* 2014; 47(2):77–93.
50. Dani A, Varkonyi A, Magyar T, Kalden M, Szasz A. Clinical study for advanced pancreas cancer treated by oncothermia Clinical study for advanced pancreas cancer treated by oncothermia. *Oncothermia J.* 2012; 6(3):11–25.
51. National Cancer Registry. Cancer in South Africa 2013: Full Report. Vol. 000. 2014.
52. UNAIDS. Fact Sheet—World AIDS Day 2018 [Internet]. World AIDS Day. 2018 [cited 2019 Jan 7]. p. 1–6. Available from: <http://www.unaids.org>
53. Ghebre RG, Grover S, Xu MJ, Chuang LT, Simonds H. Cervical cancer control in HIV-infected women: Past, present and future. *Gynecol Oncol Reports.* 2017; 21:101–8.
54. Firnhaber C, Zungu K, Levin S, Michelow P, Montaner LJ, McPhail P, et al. Diverse and high prevalence of human papillomavirus associated with a significant high rate of cervical dysplasia in human

- immunodeficiency virus-infected women in Johannesburg, South Africa. *Acta Cytol.* 2009; 53(1):10–7. <https://doi.org/10.1159/000325079> PMID: 19248549
55. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. *Int J Gynecol Obstet.* 2009; 105(2):103–4.
56. Pötter R, Tanderup K, Kirisits C, de Leeuw A, Kirchheiner K, Nout R, et al. The EMBRACE II study: The outcome and prospect of two decades of evolution within the GEC-ESTRO GYN working group and the EMBRACE studies. *Clin Transl Radiat Oncol.* 2018; 9:48–60. <https://doi.org/10.1016/j.ctro.2018.01.001> PMID: 29594251
57. Sapienza LG, Gomes MJL, Calsavara VF, Leitao MM, Baiocchi G. Does para-aortic irradiation reduce the risk of distant metastasis in advanced cervical cancer? A systematic review and meta-analysis of randomized clinical trials. *Gynecol Oncol.* 2017; 144(2):312–7. <https://doi.org/10.1016/j.ygyno.2016.11.044> PMID: 27908530
58. van Leeuwen CM, Oei AL, Chin KWTK, Crezee J, Bel A, Westermann AM, et al. A short time interval between radiotherapy and hyperthermia reduces in-field recurrence and mortality in women with advanced cervical cancer. *Radiat Oncol.* 2017; 12(1):1–8. <https://doi.org/10.1186/s13014-016-0740-5>
59. Einstein MH, Ndlovu N, Lee J, Stier EA, Kotzen J, Garg M, et al. Cisplatin and radiation therapy in HIV-positive women with locally advanced cervical cancer in sub-Saharan Africa: A phase II study of the AIDS malignancy consortium. *Gynecol Oncol.* 2019; 153(1):20–5. <https://doi.org/10.1016/j.ygyno.2019.01.023> PMID: 30773222
60. Hyun JO, Lodge MA, Wahl RL. Practical PERCIST: A Simplified Guide to PET Response Criteria in Solid Tumors 1.0. *Radiology.* 2016; 280(2):576–84. <https://doi.org/10.1148/radiol.2016142043> PMID: 26909647
61. Elming PB, Sørensen BS, Oei AL, Franken NAP, Crezee J, Overgaard J, et al. Hyperthermia: The Optimal Treatment to Overcome Radiation Resistant Hypoxia. *Cancers (Basel).* 2019; 11(60):1–20.
62. Chhabra S, Devi S, Chopra S, Singh A. Staging Issues in Cervical Cancer. *Cancer Surg.* 2016; 01(02):1–5.
63. van der Zee J, Gonzalez D, van Rhoon G et al. Comparison of radiotherapy alone with radiotherapy plus hyperthermia in locally advanced pelvic tumors: A prospective, randomized, multicentre trial. *Lancet.* 2000; 355:1119–25. PMID: 10791373
64. Harima Y, Nagata K, Harima K, Ostapenko V V., Tanaka Y, Sawada S. A randomized clinical trial of radiation therapy versus thermoradiotherapy in stage IIIB cervical carcinoma. *Int J Hyperth.* 2009; 25(5):338–43.
65. Chen H-W, Fan J-J, Luo W. A randomized trial of hyperthermo-radiochemotherapy for uterine cancer. Vol. 24, *Chinese Journal of Clinical Oncology.* 1997. 249–251 p.
66. Datta NR, Bose HK, Kapoor A. Thermoradiotherapy in the management of Carcinoma Cervix (Stage IIIB): A controlled Clinical Study. *Ind Med Gaz.* 1987;(121):68–71.
67. Sharma S, Singhal S, Sandhu AP, Ghoshal S, Gupta BD, Yadav NS. Local thermo-radiotherapy in carcinoma cervix: improved local control versus increased incidence of distant metastasis. *Asia Ocean J Obstet Gynaecol.* 1991; 17(1):5–12.
68. de la Peña MJ, de Vega Fernández VM, Rodríguez MR, Arranz JC, Herráiz Hidalgo L, Moreno EA. Current imaging modalities in the diagnosis of cervical cancer. *Gynecol Oncol.* 2008; 110(Suppl):S49–54. <https://doi.org/10.1016/j.ygyno.2008.05.030> PMID: 18672276
69. Hansen HV, Loft A, Berthelsen AK, Christensen IJ, Høgdall C, Engelholm SA. Survival outcomes in patients with cervical cancer after inclusion of PET/CT in staging procedures. *Eur J Nucl Med Mol Imaging.* 2015; 42(12):1833–9. <https://doi.org/10.1007/s00259-015-3113-7> PMID: 26194714
70. Magné N, Chargari C, Vicenzi L, Gillion N, Messai T, Magné J, et al. New trends in the evaluation and treatment of cervix cancer: The role of FDG PET. *Cancer Treat Rev.* 2008 Dec 1; 34(8):671–81. <https://doi.org/10.1016/j.ctrv.2008.08.003> PMID: 18849119
71. Morkel M, Ellmann A, Warwick J, Simonds H. Evaluating the role of f-18 fluorodeoxyglucose positron emission tomography/computed tomography scanning in the staging of patients with stage IIIB cervical carcinoma and the impact on treatment decisions. *Int J Gynecol Cancer.* 2018; 28(2):379–84. <https://doi.org/10.1097/IGC.0000000000001174> PMID: 29324535
72. Testa AC, Di Legge A, De Blasis I, Cristina Moruzzi M, Bonatti M, Collarino A, et al. Imaging techniques for the evaluation of cervical cancer. *Best Pract Res Clin Obstet Gynaecol.* 2014; 28(5):741–68. <https://doi.org/10.1016/j.bpobgyn.2014.04.009> PMID: 24861248
73. Fatehi D, Van der Zee J, Van der Wal E, Van Wieringen WN, Fatehi D, Van der Zee J, et al. Temperature data analysis for 22 patients with advanced cervical carcinoma treated in Rotterdam using radiotherapy, hyperthermia and chemotherapy: A reference point is needed. *Int J Hyperth.* 2006; 22(4):353–63.

74. Franckena M, Lutgens LC, Koper PC, Kleynen CE, van der Steen-Banasik EM, Jobsen JJ, et al. Radiotherapy and Hyperthermia for Treatment of Primary Locally Advanced Cervix Cancer: Results in 378 Patients. *Int J Radiat Oncol Biol Phys*. 2009; 73(1):242–50. <https://doi.org/10.1016/j.ijrobp.2008.03.072> PMID: [18990505](https://pubmed.ncbi.nlm.nih.gov/18990505/)
75. Herd O, Francies F, Kotzen J, Smith T, Nxumalo Z, Muller X, et al. Chromosomal radiosensitivity of human immunodeficiency virus positive/negative cervical cancer patients in South Africa. *Mol Med Rep*. 2016; 13(1):130–6. <https://doi.org/10.3892/mmr.2015.4504> PMID: [26549042](https://pubmed.ncbi.nlm.nih.gov/26549042/)

SUPPLEMENTARY DOCUMENTS



S1 Figure: Reasons for Screening Failure before Randomisation

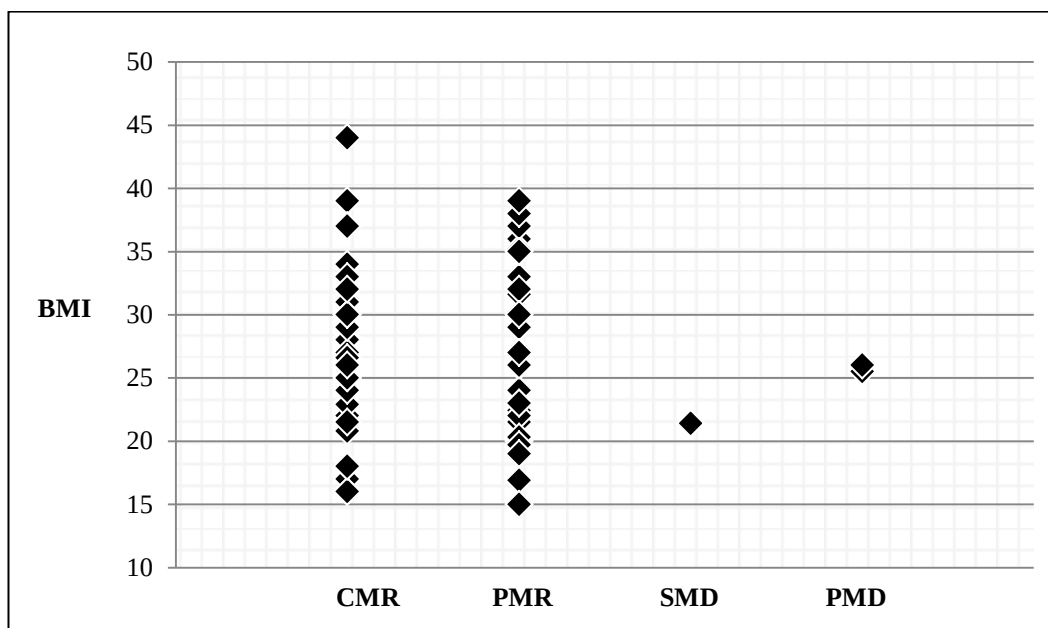
Abbreviations: VRF: Vesicorectal fistula; PET/CT: Positron Emission Tomography / Computed Tomography; ARV: Antiretroviral

*Bleeding: bleeding that could not be controlled by haemostatic brachytherapy and required admission and a change in protocols before completing the screening process; **Technical: ¹⁸F-FDG supply difficulties.

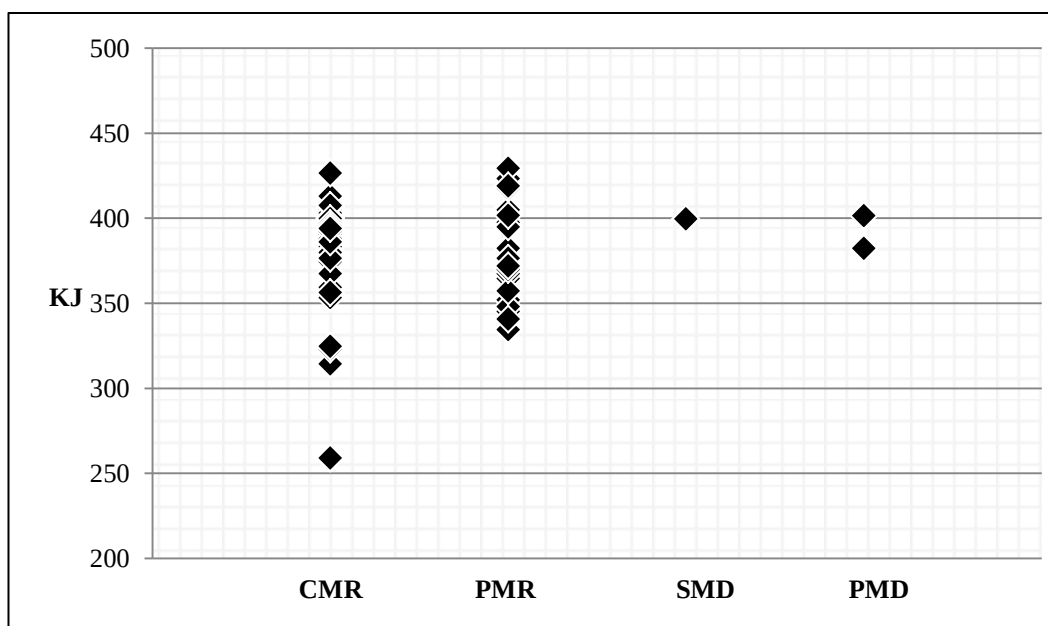
S2 Table: Logistic Regression Model for Prognostic Variables and Six Month Local Disease Free Survival

Prognostic Variable	Odds Ratio [OR]	P> z	95% Confidence interval [CI] range
<i>All Participants (multivariate)</i>			
Age	1.03	0.064	0.993-1.060
Number of cisplatin doses	1.14	0.578	0.72-1.80
Overall treatment time	0.98	0.448	0.93-1.03
Total RT dose	1.05	0.247	0.96-1.15
<i>HIV-positive participants only (univariate)</i>			
CD4 count	1.00	0.279	0.999-1.003

Abbreviations: RT: Radiotherapy; HIV: Human Immunodeficiency Virus



S3 Figure: Patient BMI versus Tumour Response on ^{18}F -FDG PET/CT
 Abbreviations: BMI: Body Mass Index; CMR: Complete Metabolic Response; PMR: Partial Metabolic Response; SMD: Stable Metabolic Disease; PMD: Progressed Metabolic Disease



S4 Figure: Average Energy Dose versus Tumour Response on ^{18}F -FDG PET/CT
 Abbreviations: KJ: Kilojoules; CMR: Complete Metabolic Response; PMR: Partial Metabolic Response; SMD: Stable Metabolic Disease; PMD: Progressed Metabolic Disease

S5 Table: Logistic Regression Model of mEHT-Related Prognostic Variables and Six Month Local Disease Free Survival

Prognostic Variable	Odds Ratio [OR]	P> z	95% Confidence interval [CI] range
No of mEHT treatments	1.165	0.359	0.84-1.62
Energy Dose (KJ)	0.991	0.075	0.98=1.00
BMI	0.995	0.886	0.93-1.06

Abbreviations: mEHT: Modulated electro-hyperthermia; KJ: Kilojoules; BMI: Body Mass Index

CHAPTER FOUR

Acute Toxicity and Quality Of Life in HIV-Positive and –Negative Patients treated with Modulated Electro-Hyperthermia plus Chemoradiotherapy for Locally Advanced Cervical Cancer

Minnaar CA, Kotzen JA, Naidoo T, Tunmer M, Sharma V, Vangu MD, Baeyens A (2019) **Acute Toxicity and Quality Of Life in HIV-Positive and –Negative Patients treated with Modulated Electro-Hyperthermia plus Chemoradiotherapy for Locally Advanced Cervical Cancer**. Submitted to *International Journal of Hyperthermia*, currently under review.

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Author Contributions

- Minnaar: Conducted the research, gathered the data, statistical analysis, interpretation of results, writing of the manuscript.
- Kotzen: Supervisor, senior radiation oncologist, planning and prescribing of treatments, oversaw the treatment and follow-ups of trial participants, reviewed manuscript.
- Naidoo: Clinical oncologist, screened patients for eligibility, oversaw the administration of treatments and follow-ups of the participants, reviewed the manuscript.
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- Sharma: Head of Radiation Oncology Department.
- Vangu: Head of Nuclear Medicine Department; oversaw all the ^{18}F -FDG PET/CT scans and related logistics, reviewed manuscript.
- Baeyens: Supervisor, Head of Radiobiology, supervised data collection and data quality control, project planning, management of funding, reviewed manuscript.

Analysis of the effects of Modulated electro-hyperthermia on the treatment-related toxicity and quality of life of HIV-positive cervical cancer patients

Acute toxicity from mEHT in LACC HIV+ patients

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Abstract

Introduction: HIV infection is associated with increased treatment-related toxicity and worse outcomes in locally advanced cervical cancer patients (LACC), especially in resource-constrained settings. Modulated electro-hyperthermia (mEHT) improves local control (LC) when added to chemoradiotherapy. This analysis investigates whether mEHT adds to the toxicity profile of chemoradiotherapy (CRT) in HIV-positive LACC participants. **Methods:** Local control in a phase III randomized, controlled trial investigating mEHT on LACC patients in South Africa (ethics registration: M120477/M190295), was significantly higher in participants randomized to receive CRT with mEHT compared to CRT alone (stratum: HIV status, accounting for age, stage). Inclusion criteria: signed informed consent; FIGO stages IIB to IIIB squamous cell carcinoma of the cervix; HIV-positive patients: CD4 count >200cell/ μ L/on antiretroviral treatment for >six months; eligible for CRT with radical intent. Recruitment: January 2014 to November 2017 (ClinicalTrials.gov: NCT03332069). Acute toxicity (evaluated using CTCAE v4 criteria) and quality of life (according to EORTC forms) in 206 participants randomized for treatment were evaluated alongside the LC results to determine safety and efficacy in HIV-positive participants. **Results:** Compliance to mEHT treatment was high (97% completed $\geq$ 80% of treatments) with no significant differences in CRT-related toxicity between treatment groups or between HIV-positive and -negative participants. Adverse events attributed to mEHT were minor, even in obese patients, and did not affect CRT compliance. Participants treated with mEHT reported improved fatigue, pain, emotional and cognitive functioning. **Conclusion:** mEHT did not cause unexpected CRT-related toxicities and is a safe and feasible treatment for HIV-positive patients, even in a low-resource setting.

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Key Words

Modulated electro-hyperthermia; Chemoradiotherapy; Cervical cancer; Early toxicity; Quality of Life; HIV

Introduction

The incidence of locally-advanced cervical cancer (LACC) in developed countries has decreased since the introduction of screening and Human Papilloma Virus (HPV) vaccination programs(1,2), yet morbidity and mortality rates remain high in low-resource settings(3). Radiotherapy (RT) with weekly concurrent cisplatin is the standard of care for LACC(4), however in patients who are unable to receive cisplatin, hyperthermia (HT) has shown to be as effective as a radiosensitiser(5). Studies have shown that HT plus RT improves local disease control (LDC) and survival in LACC patients, compared to RT alone, without significant effects on RT-related toxicity (6–10). Hyperthermia has been investigated as a chemo-sensitiser with response rates of more than 50% and acceptable toxicity for the management of recurrent or residual cervical tumors(11,12) and phase II studies have demonstrated the safety of the addition of HT to chemoradiotherapy (CRT)(13–15). Adverse events directly attributed to HT include pain (8%), superficial burns (6% to 18%)(10,13), subcutaneous fatty burns (8% to 12%)(5,10,11,13), and infections after the introduction of interstitial thermometry catheters(10). A retrospective analysis of 420 patients treated with RT and HT reported that 153(36%) patients developed subcutaneous tissue toxicity, ranging from grade 1 to grade 3 in severity, with one patient requiring surgical intervention(16). Acute neurotoxicity following the treatment of deep pelvic tumors with HT is a rare (2.3% incidence) complication which can have an impact on the daily activities of the patient(5,17).

The effect of Human Immunodeficiency Virus (HIV) infection on the treatment of LACC includes increased multi-system RT-related toxicity, treatment interruptions, higher risks of residual disease(18), and lower survival rates(19). Herd *et al* (2016) showed that cervical cancer patients appeared to be more radiosensitive than healthy controls, with HIV-positive cervical cancer patients being the most radiosensitive(20). This follows the work of Baeyens *et al* (2010) on the increased chromosomal radiosensitivity in the lymphocytes of HIV-positive versus HIV-negative individuals(21). Formenti *et al* (1995) showed that the fibroblasts from the skin of HIV-positive Kaposi Sarcoma patients are more sensitive to ionizing radiation (IR) when compared to those of healthy controls(22). Early reports of extreme mucositis in HIV-positive patients(23) alerted physicians to the potential of RT to cause increased toxicity, especially mucosal and cutaneous toxicity, in HIV-positive patients. After a detailed review of the literature, Hoursi *et al* (2010) recommend that HIV-positive patients treated to the head and neck, abdomen, and pelvis, with RT, should be closely monitored for possible mucosal reactions and HIV-positive patients treated with CRT should be closely monitored for hematologic toxicity(24). Possible contributors to the increased radiosensitivity of HIV-positive patients include a systemic glutathione deficiency caused by HIV infection, resulting in the

depletion of radio-protective thiols and increased oxidative stress(25), and the effect of the HIV-1 Tat protein on the cellular capacity of certain cells to repair radiation-induced DNA double strand breaks(24). Information on the use of HT as a radiosensitiser in HIV-positive patients, who may be more likely to experience RT-related toxicity, is not available and therefore requires investigation.

Hyperthermia techniques in use for cervical cancer, such as annular-phased-array or capacitive systems, require patients to be lightly sedated and/or require thermometry probes to be introduced into the vagina, rectum, or intratumorally(10). Alternatively, Magnetic Resonance (MR) guided thermometry may be used(26). This adds to the complexity and costs of the HT treatment and subsequently the investigation of HT in low resource settings has not been feasible.

The biophysical differences between malignant and healthy tissues are well known(27–29). The impedance of malignant tissue is lower, and the conductivity of malignant tissue is therefore higher, than that of normal tissue(30–32), resulting in a higher rate of energy flow through the malignant tissue compared to the healthy tissue(33). Haemmerich *et al* (2010) demonstrated that the average conductivity of tumor tissue from the liver was significantly higher than normal liver tissue over the frequency range from 10Hz (4.11 versus 0.75mS cm⁻¹) to 1MHz (5.33 versus 2.88 mS cm⁻¹)(31). Joines *et al* (1994) reported that differences in permittivity and conductivity between a variety of malignant tissues and their healthy counterparts, range from 6% and 4% respectively in renal tissue, to 233% and 577% respectively in mammary tissue(32). Capacitive heating technologies harness these differences in order to heat up the tumors.

Modulated Electro-Hyperthermia

Modulated electro-hyperthermia is a capacitive heating system which transmits radiofrequency (RF) waves at a frequency of 13.56MHz, between two electrodes. The small electrode is covered by a water bolus in an adjustable arm, and the large electrode is in the bed and is covered by a water mattress. The RF waves undergo amplitude modulation(33–36) which promotes the selection of malignant tissue and potentiates the effects of HT. The modulation, and the selection enhancement of the modulation, are described in detail elsewhere in the literature(36,37).

A high Specific Absorption Rate (SAR) at the tumor is seen during mEHT, indicating high treatment efficiency, allowing for the energy to be used as the dose(34). This technique therefore uses temperature as a consequence of the energy absorption during the

treatment, rather than a dose. Consequently the calculation of the temperature is less important than the calculation of the absorption of the energy. Impedance, being the resistance or opposition to the flow of alternating current in a circuit, is a crucial measured factor in the calculation of the energy dose delivered using mEHT. Impedance matching describes the process of planning the input impedance of an electrical load, or the output impedance of its corresponding signal source, in such a way that the transfer of power can be maximized and/or the reflection of the signal from the load can be minimized. In the case of mEHT, the load is the patient with the tumor. The patient's impedance acts as an electric component in a closed electric circuit, and can therefore be monitored in situ, real time, optimizing the energy delivery by adjusting the matching. The specially developed materials used for the electrodes of the mEHT devices, combined with the highly accurate tuning (varying the circuit resistance so that the load resistance matches the internal resistance of the source), and with the proper compensation of the capacitive factor of the loading impedance (using the serial resonance method), enable the precise matching of impedance during the treatments. This minimizes the reflected (or wasted) energy and maximizes the RF-current, thereby minimizing the applied voltage and a lower, and safer, power output can be used(38).

A lower power is further supported by the selection of the malignant tissue and therefore a lower volume is heated, which requires less energy. Modulated electro-hyperthermia acts on the membrane of the cancer cells(39–41) and does not heat up the complete volume between the electrodes(42–44). These characteristics improve the efficiency of the treatment, minimizing the wasted energy. The induction of an immune response has also been observed after mEHT treatments(45–55) and an increased apoptotic cell death is observed in samples treated with mEHT compared to other heating techniques(41,56,57) which further enhances the treatment efficiency. Modulated electro-hyperthermia has shown to enhance the effects of RT at temperatures below 43°C(44,45,58), with the same amount of apoptotic cell-destruction observed at a temperature 3°C lower than conventional HT(56).

Preclinical studies with cell suspensions and experimental tumors have shown that mEHT is distinctly more effective than water-bath, infrared, or RF-hyperthermia, if adjusted to the same temperature. Yang *et al* (2016) showed mEHT produced a much higher apoptosis rate in Hepatoma (HepG2) cells at 42°C for 30 minutes, than conventional capacitive heating and water-bath heating. In their study on cell suspensions, the apoptosis rate induced by water-bath heating at 46°C was equal to that induced by mEHT at 42°C. The study showed a difference in the responses at the cell membrane to mEHT, compared to other heating techniques, such as increased HSP70 expression and release, increased expression of caspase-3, 8 and 9, and increased levels of calreticulin and E-cadherin in

the cell membrane(41). Modulated electro-hyperthermia induces both thermal and non-thermal effects on cells. In a study by Andocs *et al* (2009), the thermal enhancement ratio (TER) of cell killing was 2.9 in tumors treated with conventional heating and at the low temperature of 38°C in the tumors treated with mEHT, an enhancement ratio of 3.2 was measured, reflecting the non-thermal effects of mEHT. The authors hypothesize that the effects of the electromagnetic field and modulation contribute to the cell killing at the low temperature. Tumors treated with mEHT at 42°C showed a combined therapeutic enhancement of 9.4 from the thermal and non-thermal effects of mEHT(57). This study demonstrates the potentiation of the effects of HT by the addition of amplitude modulation.

The Lab EHY (Oncotherm GMBh, Troisdorf, Germany) is a laboratory model of the mEHT device manufactured with the same specifications as the commercially available device for human treatments. Experiments in murine models using the Lab EHY have effectively demonstrated the technology's ability to heat tumors to within the same temperature ranges as water-bath heating and other capacitive heating devices. All of the murine studies using the Lab EHY measured intratumoral temperatures using a fluorotopic temperature measurement system(45–52,54,55,59).

High resolution temperature mapping techniques have demonstrated a higher temperature increase in tumors compared to healthy tissues in murine models treated with mEHT, which matched the computational measurements. This alludes to a higher energy absorption in the tumor(42), and the selective heating ability of mEHT(43). Murine models showed that the final temperature achieved in the tumor was 42.2°C, while the nearby tissue, body, and bed temperatures were 35.03°C, 30.98°C, and 28.88°C, respectively. The SAR in the tumor region was calculated to be 120.2W/kg, while the SAR towards the skin was only 7.18W/kg(43).

Balogh *et al* (2016) demonstrated the effective heating of a pig liver during six treatments with mEHT. The deep temperature measured in the liver ranged from 40.2°C to 44.8°C(60). Human studies have also demonstrated the ability of mEHT to heat tumors such as sarcomas, abdominal tumors, and breast tumors(61,62),

Lee *et al* (2018) measured the peri-tumor temperatures of cervical tumors in 20 participants during mEHT treatments. The authors reported an average increase in temperature from 36.7 ± 0.2 °C to 37.5 ± 0.5 °C and 38.5 ± 0.8 °C at 30 and 60 minutes respectively, with a maximum temperature of 40.1°C. In the study, the power output was 80W for the first ten minutes, 120W for the next ten minutes and 150W for the final 40 minutes, totaling 133.33W transmitted per hour. Of significance in this study is the finding

that the moderate increase in temperature was sufficient to increase the blood perfusion to the tumors(63). The improved perfusion and oxygen in the tumor is widely agreed to be one of the main radio-sensitizing mechanisms of HT(64–67). Lee *et al* (2018) further suggest that, given the decreased perfusion and heat dissipation in the inner region of tumors, the temperature inside the tumors is likely to be higher than the peri-tumor temperature(63).

The same group investigated mEHT in a randomized controlled study combined with platinum-based chemotherapy for recurrent/residual cervical cancer. The authors applied the same mEHT protocols as in their previous study(63), and reported statistically significant improvements in outcomes of patients treated with chemotherapy combined with mEHT. In this study, treatments were prescribed/administered based on energy dose and not temperature achieved within the tumor and intratumoural temperature measurements were not done, however safety and efficacy was still demonstrated(58).

The specific interaction of mEHT in tumors allows for an effective treatment with less total power in comparison to conventional RF-systems. Modulated electro-hyperthermia is being investigated in a phase III randomized controlled trial (RCT) as a radiosensitizer, combined with cisplatin, for the treatment of LACC in a low-resource setting and in HIV-positive and -negative patients. To date this is the largest RCT on any HT technique combined with CRT for LACC, and the only study on any form of HT in a low-resource setting and in HIV-positive patients. Table 1 summarizes the local control (LC) outcomes at six months post-treatment, as reported by Minnaar *et al* (2019)(68). Two hundred and two participants were eligible for LC analysis (mEHT: $n=101$; Control: $n=101$) at six months post-treatment, of which 171 [mEHT: $n= 88$ (87.1%); Control: $n=83$ (82.2%)], were alive at six months post-treatment. The study did not show any significant difference in treatment related toxicity between the two treatment groups(68).

[Table 1 near here]

Participants who were eligible for evaluation of LC by Fluorodeoxyglucose (^{18}F -FDG) Positron Emission Tomography/Computed Tomography (PET/CT) scans (creatinine clearance $>60\text{mL/min}$, controlled glucose levels, were able to lie still for the duration of the scan), had a second ^{18}F -FDG PET/CT scan at six months post-treatment. Local control was considered to be a failure if any disease was confirmed either by ^{18}F -FDG PET/CT, CT, clinical examination, cytological evaluation, or fine needle aspiration of palpable lymph nodes. Participants treated with mEHT were significantly more likely to achieve six month local disease-free survival (LDFS) (OR: 0.36, 95% CI: 0.19-0.69; $p=0.002$) and the association between HIV status and six month LDFS was not significant

($p=0.338$). The authors reported that the age, number of cisplatin doses, treatment time, RT dose, and CD4 count, were not significant predictors of six month LDFS. In the mEHT group, body mass index (BMI), number of mEHT treatments, and energy dose were not predictors of six month LDFS. In a preliminary report on the first 70 participants in the study to reach two years post-treatment, two year survival was more likely in the participants treated with mEHT (78% vs 65%; $\text{Prob}>\chi^2=0.0334$; adjusted for age), as was two-year progression free survival, adjusted for age (76% vs 61%; $\text{Prob}>\chi^2=0.0317$)(69).

In order to determine the effects of mEHT on CRT-related toxicity in HIV-positive participants, an analysis of the acute toxicity and quality of life of HIV-positive versus HIV-negative participants in the study by Minnaar *et al* (2019) was conducted and is reported in this paper. The effects of characteristics such as obesity, on the frequency of adverse events in the study sample are reported. Quality of life (QoL) characteristics, such as physical well-being, can have an impact on clinical outcomes(70) and given the high morbidity associated with LACC, CRT treatment, and with HIV-infection, QoL in the HIV-positive and –negative participants in each treatment group is also reported here.

Materials and Methods

An ongoing phase III RCT is being conducted at the Charlotte Maxeke Johannesburg Academic Hospital in Gauteng, South Africa, by the Radiation Sciences Department at the University of the Witwatersrand. All work was conducted with the formal approval of the local human subject committees and the trial was registered, as required, on the South African National Clinical Trials Register before recruitment was started (ID:3012), and at ClinicalTrials.gov (NCT03332069). Approval was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (M190295/M120477).

The sample size calculation was based on the estimated required sample sizes for a two-sample comparison of survivors' functions at two years with a minimum sample size of 200 participants. Recruitment began in January 2014 and was closed in November 2017. The outcomes are the LDC at six months post-treatment (reported by Minnaar *et al* (68)) and two year survival. Approval has been obtained to follow participants for five years post-treatment. This analysis includes the 206 participants who arrived for treatment after randomization (mEHT Group: $n=105$; Control Group: $n=101$). Eligibility for the trial, as well as randomization and masking, are described previously(68).

Treatment:

All participants were planned to receive 50Gy external beam radiation (EBRT) in 25 fractions, 24Gy high dose rate (HDR) brachytherapy (BT) in 3 fractions (36Gy equivalent dose in 2Gy fractions for an alpha-beta ratio of 10; source used: Iridium-192), and two doses of cisplatin (80mg/m²) 21 days apart (subject to the participant's ability to receive cisplatin), as per institutional protocol. The EBRT target volume was planned and delivered to the whole pelvis using a four-field box technique on one of four Siemens linear accelerators in the department.

Two mEHT (Model: EHY2000+; Manufacturer: Oncotherm GmbH, Troisdorf, Germany) treatments were administered per week (maximum ten). Modulated electro-hyperthermia was applied immediately before external beam radiation with a maximum of 30 minutes between the completion of mEHT and the completion of EBRT. This protocol was based on logistics and work flow integration in the public hospital. A 30cm electrode was used in the adjustable arm in order to cover as much of the pelvic field as possible. The treatment power was started at 60W and increased using a step-up protocol over five minutes to the desired power of 90W for the first treatment, 105W for the second treatment, and a planned 130W for the third and subsequent treatments (See Suppl. 1: Hyperthermia Schedule). Treatments were planned for a minimum of 55 minutes and a minimum energy dose of 360KJ per treatment. The first treatment is a safety treatment during which the participant is acclimatized to the treatment and the operator is able to identify potential risks. The first treatment is therefore administered at a low power without modulation (which increases the effects of the treatments).

Thermometry:

It is recommended that thermometry be used to evaluate the efficiency of hyperthermia treatments(71,72), however this is associated with challenges such as invasive thermometry to measure intratumoural temperatures or expensive MR-guided thermometry, and the ability to heat uniformly and consistently regardless of tumor and patient characteristics(73). The dose concept of mEHT proposes the measurement of the energy as the dose, rather than temperature, as the energy can be more easily and reliably calculated in mEHT than the achieved temperature in the tumor, provided the SAR of the energy dose is accurate(33,35,38). The mEHT devices display an estimated temperature during the treatment; however the goal of the treatment is not to achieve a maximum temperature, but rather to sensitize tumors to the concomitant treatments and the energy is the dosing parameter. The estimated temperature calculation is based on the SAR, and the mathematics and calculation details are described in detail elsewhere in

the literature(34,35,38,74). This principle makes administering the treatments less complex and less costly and suggests that mEHT could be suitable for low-resource settings.

Outcome Measures:

Adverse events that occurred within 90 days of the start of RT were considered acute RT-related toxicity. The study utilized the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 from the National Cancer Institute (NCI) for the defining of adverse events. The toxicity data was tabulated by treatment arm and HIV status. Thirty nine adverse events from six categories (upper gastrointestinal [GIT]; lower GIT; genitourinary tract [GUT]; dermatologic and subcutaneous; neurological; hematological) at three time points (on treatment, six weeks, and three months post-treatment) were analyzed. The frequency of all toxicities was reported and separate analyses for grade 3-4 toxicities for each system were conducted. Ototoxicity and peripheral neuropathy were grouped together under neurological toxicities, as these are both known to be potential cisplatin-related toxicities(75–77). Adipose tissue burns, presenting as hard, painful, mobile masses within the adipose tissue, were classified according to the CTCAE v4.0 criteria under the heading “Skin and subcutaneous tissue disorders – Other”.

Quality of Life (QoL) was measured at enrolment and again at six weeks, and three months post completion of treatment using the validated Quality of Life Questionnaires (QLQ) C30 and Cx24, from the European Organization for Research and Treatment of Cancer (EORTC). Validated translations were available in English, Afrikaans, isiZulu, Sesotho and isiXhosa.(78) The QLQ-C30 incorporates five functional scales (physical, role, cognitive, emotional, and social), three symptom scales (fatigue, pain, and nausea and vomiting), a global health status, and perceived financial impact of the disease(79). The QLQ Cx24 contains symptoms specific to cervical cancer and its treatment(80). For the literate participants, the QLQ forms were provided in five different languages and the participants completed the forms themselves. For illiterate participants, nurses were asked to administer the forms and translate as needed. Nurses were blinded to the treatment group that the participants were randomized to. The EORTC recommends in their manual that a change of more than 30 points in either direction is considered to be valid. All scoring and reporting were done in accordance with the manual published by the EORTC which is made available with the QLQ forms.

Statistics:

Chi-squared tests were employed to assess observed frequency differences of all toxicities in each treatment arm and by HIV status. Multivariate logistic regression models were used to determine predictors of outcomes such as the development of Grades 3-4 toxicity.

For QoL, items were scored and analyzed according the EORTC scoring manual and were linearly converted to scores from 0 to 100 where a high score represents higher functioning or a higher symptom experience(81). Unpaired t-tests were used to analyze differences in linear scores between the groups at each event. Paired t-tests were used to evaluate the changes in the linear scores between the groups.

Two sided p values are reported and p values <0.05 were considered significant. STATA 13.0 Statistics software program (Stata Corporation, College Station, Texas, USA), was used to analyze the data.

Role of the Funding Sources:

The funding sources did not have any role in protocol design, data collection, analysis or reporting. The corresponding author had full access to all the data and was responsible for the final submission.

Results

The median age was 48.7 (range: 26.5-72.2 years), 63.6% of participants had stage III disease and 51% of participants were HIV-positive. Patient and treatment characteristics were evenly matched in both treatment groups as listed in Table 2.

[Table 2 near here]

Radiotherapy:

The median time to complete RT was 41 days in both treatment groups with a range of 13-65 days in the mEHT Group and 10-97 days in the Control Group. Reasons for taking in excess of 51 days to complete all RT include skin desquamation, bleeding and anemia, vomiting and dehydration, and technical causes (such as machine services and repairs). The frequency of RT delays was significantly associated with HIV status (χ^2 : $p=0.020$)

with 12(11.4%) delays in the HIV-positive participants versus 3(3.0%) delays in the HIV-negative participants (OR: 0.237; $p=0.030$; 95% CI: 0.065-0.868). The association between treatment arm and RT delays was not significant with six out of 105(5.7%) in the mEHT Group versus nine out of 101(8.9%) in the Control Group (χ^2 : $p=0.377$; OR: 1.614; $p=0.381$; 95% CI: 0.553-4.712).

Cisplatin:

Ten (9.5%) and 14(13.9%) of mEHT and Control Group participants respectively did not receive any cisplatin. The mEHT Group received a mean of 1.37 cycles and the Control Group received a mean of 1.28 cycles ($p=0.323$). The most common reasons for omission of the second cycle were renal dysfunction (mEHT: $n=19$; Control: $n=11$), followed by a delay in the administration of the first cycle (mEHT: $n=10$; Control: $n=7$). Table 3 lists the reasons for cancellation/omission of cisplatin.

[Table 3 near here]

In Chi-squared analyses, there were no significant associations between HIV status and number of cisplatin cycles ($p=0.549$) or delays ($p=0.444$), or between treatment arm and number of cisplatin cycles ($p=0.610$) or delays ($p=0.575$). HIV status was not a predictor of cisplatin dose delays (OR: 0.795; $p=0.452$; 95%CI: 0.436-1.448), or cancellations (OR: 0.735; $p=0.474$; 95%CI: 0.316-1.709). Likewise, the treatment group was not a significant predictor of cisplatin dose delays (OR: 1.180; $p=0.588$; 95%CI: 0.648-2.150), or cancellations (OR: 1.504; $p=0.348$; 95%CI: 0.641-3.530).

mEHT-Specific Toxicity:

One hundred and five participants were treated with mEHT of which 102 (97%) were able to receive eight or more ($\geq 80\%$) of the mEHT treatments. One participant had four treatments and two participants had six treatments. Of the three participants who received less than eight mEHT treatments, two had a Body Mass Index (BMI) over 25 and all three reported adipose burns, which was the cause for the reduction in the number of mEHT treatments.

Of the 105 participants treated with mEHT, 17(16.2%) reported adverse events: Grade 1-2 adipose tissue burns ($n=10$, 9.5%); Grade 1 surface burns ($n=2$, 2.0%); and pain ($n=9$, 8.6%). Four (23.5%) of the participants with adverse events had a normal BMI and 13(76.5%) were overweight (BMI 25-29.9) or obese (BMI >30)(82). A multivariate analysis showed that HIV status (OR: 1.47; $p=0.500$; 95% CI: 0.480-4.512), increased

BMI (OR: 1.78; $p=0.375$; 95% CI: 0.499-6.335), and average energy (in Kilojoules) administered (OR: 0.986; $p=0.067$; 95% CI: 0.98-1.00) were not significant predictors of adverse events associated with mEHT treatments.

A significant difference in Grade 1-2 adipose burns between the treatment groups in the on-treatment analysis [mEHT: 10 out of 105(9.5%) compared to Control: 1 out of 101(1%); $p=0.023$] was noted. This difference was not significant at six weeks or three months post-treatment.

Early Toxicity Analyzed by Treatment Group:

The toxicities were analyzed by treatment group in order to determine if the addition of mEHT had any effect on the toxicity of either the chemotherapy or radiotherapy. Chi-squared frequency tables showed a significant association between the number of Grade 1-2 neurological toxicities and the addition of mEHT [mEHT Group: $n=5(5\%)$; Control Group: $n=0(0\%)$; $p=0.030$] at three months post treatment. The neurological toxicities reported were tinnitus ($n=1$; HIV-positive), hearing loss ($n=2$; one HIV-negative and one HIV-positive participant) and peripheral neuropathy ($n=3$; all in HIV-negative participants). However the significance of the association between treatment group and toxicity disappeared when the neurological toxicities were separated into peripheral neuropathy ($p=0.095$) and ototoxicity ($p=174$). Only one participant developed Grade 3-4 neurological toxicity (HIV-positive, in the mEHT Group, treated with cisplatin). There were no other significant associations between the treatment group and Grade 1-2 toxicities or Grade 3-4 toxicities. The results of all of the Chi-squared frequency tables for Grades 3-4 toxicities analyzed a) by treatment group and b) by HIV status are shown in Suppl. 2.

Early Toxicity Analyzed by HIV Status

There were some significant differences in specific symptoms associated with HIV status (diarrhea; creatinine clearance; nausea; vomiting; pigmentation); however no clear pattern of association between the significant toxicities and mEHT treatments could be seen. At six weeks post treatment, HIV-negative participants reported a significantly higher frequency of Grade 1-2 upper GIT symptoms, including nausea and vomiting [HIV-Positive: $n=0(0\%)$; HIV-negative: $n=5(5\%)$; $p=0.020$]. No other significant associations were seen in our sample (see Suppl 2).

Participants were then further analyzed by HIV status within each treatment group in order to identify any effect of mEHT on toxicity in HIV-positive participants. These results are shown in Table 4. In the mEHT Group, there were more Grade 3-4 renal toxicities in

the HIV-positive participants ($n=5$) than in the HIV-negative participants ($n=0$) on treatment ($p=0.027$). In comparison, the Control Group had three HIV-positive (6%) and three HIV-negative participants (6%) who developed Grade 3-4 renal toxicity ($p=0.900$). Eight of the participants with Grade 3-4 renal toxicities were HIV-positive and 3 were HIV-negative ($p=0.137$) and the difference by treatment group overall was not significant (mEHT: $n=5$ [5%]; Control: $n=6$ [6.3%]; $p=0.707$). In the Control Group there were significantly more Grade 1-2 lower GIT toxicities on treatment reported in the HIV-positive participants [$n=28$ (53%) versus $n=15$ (31%); $p=0.028$]. However at six weeks post-treatment there were significantly more Grade 1-2 upper GIT toxicities in the HIV-negative Control Group participants than in the HIV-positive Control Group participants [$n=0$ (0%) versus $n=4$ (8%); $p=0.043$].

[Table 4 near here]

Multivariate Regression Analyses:

In a multivariate regression analysis for the development of early toxicities in each system, HIV status, the number of cisplatin doses, and the treatment group, were tested for predictors of outcomes. These results are shown in Table 5. Seven participants developed Grade 1-2 neurological toxicities, six of which were in the mEHT Group, and the only participant to develop Grade 3-4 neurological toxicities was also in the mEHT Group. The odds of developing a neurological toxicity were therefore almost eight times higher in patients treated with mEHT (OR: 7.98; $p=0.053$; 95%CI: 0.98-65.19), however this was not a significant predictor of neurological toxicity development in the multivariate regression analysis. Cisplatin was however a perfect predictor of the development of neurotoxicity, with all participants reporting neurotoxicity having received cisplatin. HIV-infection and receiving cisplatin were both significant predictors of the development of dermatological or subcutaneous early toxicities. Cisplatin was a significant predictor of renal toxicity (OR: 0.13; $p<0.005$; 95% CI: 0.05-0.33) while HIV-status and the administration of mEHT were not predictors of renal toxicity.

[Table 5 near here]

Quality of life:

There were no statistically significant differences in QLQ scores between the two groups at baseline assessment. At the six week time point the mean change in cognitive function in the mEHT Group was significantly higher than in the Control Group. At three months post treatment, fatigue and pain were significantly reduced in the mEHT Group (Table 6).

[Table 6 near here]

At the three month time point there was an overall improvement in the mEHT group with significant improvements in: social functioning (mEHT: mean: 83.9, SD: 31.6, 95%CI: 77.4-90.4; Control: mean:73.3, SD: 39.4, 95%CI: 64.8-81.8; $p=0.049$), and emotional functioning (mEHT: mean: 83.9, SD: 24.3, 95%CI: 78.9-88.9; Control: mean:73.5, SD: 33.3, 95%CI: 66.3-80.6; $p=0.017$). The mean improvement in social, emotional and physical function at six weeks post-treatment was significantly higher ($p=0.0048$; $p=0.0141$; $p=0.0155$ respectively) in participants who had a complete response. Social function and Emotional function continued to be higher in participants with a complete response at six months post-treatment ($p=0.0074$ and $p=0.0035$ respectively), as was the perception of body image ($p=0.0496$). Suppl. 4 tabulates the significant improvements in QoL alongside the local control at six months post treatment.

Discussion

The cell killing effects of ionizing radiation are enhanced with the addition of mEHT to RT treatment regimens, as shown in preclinical and clinical studies(45,58,68). The results from this report show that this can be achieved safely, even in high risk patients.

In capacitive heating technologies with a higher amount of wasted energy, the energy could not be considered an effective measure of the dose. High levels of energy loss also necessitate higher levels of applied power and these devices require a power output in excess of 600W in order to achieve the deep heating of pelvic tumors. Such high power outputs require extensive cooling mechanisms to reduce the risk of superficial burns(83–85). Several models have shown that RF capacitive heating to deep pelvic tumors is not possible without severe adipose burns(85,86). Harima *et al* did not report any skin or adipose burns using their RF capacitive heating device, at a frequency of 8MHz and a power output of 800W-1500W, in their study on HT combined with RT(9) and CRT(87). However the BMI of the participants is not stated, the reasons for non-compliance to the HT treatments (only 92% could receive 66% or more of the prescribed six HT treatments) is not stated, and the outcomes were not significant in the trial on CRT(87).

In the mEHT devices there is a high amount of treatment efficiency, enabling a lower power output, which, combined with the improved selection, is likely to contribute to the reduction in surface and adipose burns from mEHT treatments(34,88) compared to what is expected from other capacitive technologies(85). Suppl 4 summaries the toxicities,

thermometry methods and treatment outcomes reported using various heating devices, and highlights the variations in temperatures.

Although subcutaneous burns due to mEHT were significantly higher in the mEHT Group on treatment, these resolved by three months post-treatment and the overall dermatological and subcutaneous toxicity was not significantly different between the treatment groups. Subcutaneous adipose tissue is particularly sensitive to the effects of EBRT and exposure to radiation can lead to an alteration of the tissue's developmental potential(89) and fibrosis of the soft tissues is a known complication of EBRT(90). It is therefore not unexpected that one participant in the control group presented with what appeared to be a subcutaneous burn. Our participants reported less subcutaneous toxicity than other studies using HT techniques with highly sophisticated thermometry systems. Additional thermometry to measure the skin temperature and prevent burns is therefore not necessary during mEHT, likely due to the effective cooling mechanisms, lower power output, and selection characteristics of the technology.

The development of neurotoxicity was more frequent in the mEHT Group however cisplatin was a perfect predictor of neurotoxicity in the multivariate regression analysis and was therefore the most likely cause of the development of neurotoxicity. Although peripheral neuropathy is a known but rare side effect of HT to the pelvis(5,17) and we therefore cannot exclude the potential effects of deep heating on the development of peripheral neuropathy.

In our participants the adverse events were less frequent and less severe than what is reported in the literature(5,10,11,13,16,91), our participants could receive more treatments (allowing for up to two treatments per week), and the local control outcomes were significantly improved(68).

HIV infection

In our study, HIV infection was associated with an increased rate of RT delays; however it was not a predictor of RT-related toxicities as reported in the literature(19,92,93). The increased rate of renal toxicity in HIV-positive participants treated with mEHT, and decreased rate of renal toxicity in HIV-negative participants treated with mEHT, compared to the control group, is an unexpected result. Although the multivariate analysis showed cisplatin to be a significant predictor of renal toxicity, while HIV-status and treatment group were not significant predictors of renal toxicity, we still suggest that renal function be closely monitored in HIV-positive patients treated with mEHT to the pelvis, combined with CRT.

All of our participants had either been on ART for longer than six months or had a CD4 count above 200cells/ μ L and this may have contributed to the improved outcomes and less hematological toxicity seen in our HIV-positive participants compared to reports in the literature. We did not observe significant differences in CRT or mEHT-related toxicity between HIV-positive and -negative patients, indicating that mEHT can be safely applied with CRT in HIV-positive patients. We did however observe an increase in upper GIT symptoms, such as vomiting, in the HIV-negative participants, which was also seen in a study analyzing the differences in CRT-related toxicities in HIV-positive (on ART; CD4 count >200cells/ μ L), and HIV-negative cervical cancer patients in Zambia(94).

Heating Depth

The energy is more concentrated nearer to the smaller electrode of capacitive coupled HT devices and according to numerical simulations, capacitive heating is therefore not an effective method of heating deep tumors, especially in the presence of thick adipose layers(85,86).

Although the density of the current is less at the point of absorption (larger electrode), due to the widening of the distribution as it travels from the small to large electrode, the impedance of the complete body-part between the electrodes is also lower (due to the larger diameter). The current density is therefore lower, but the current itself increases by the same factor as a result of the inverse relationship between the impedance and the electrode area. At a constant power, the increasing current will cause a reduction in the applied voltage, which further improves the safety of the mEHT treatment. In order to achieve the maximum absorption, despite the differences in electrode size, the devices are carefully tuned so that the single frequency is able to find an inductive serial to the capacitive electrode (and fat), and the impedance is close to zero in resonance. Using this technique, a higher RF-current can be forwarded with a lower voltage and power(38). This finely tuned selection of the malignant tissue allows for heating of deep tumors even though they are further away from the small electrode. The previously reported improved outcomes in LACC patients treated with mEHT combined with CRT corroborates the thermal effects of mEHT as a radiosensitizer in deep-seated tumors(68).

Temperature and Thermometry

Thermometry in this study was not carried out as recommended with standard RF-capacitive heating or phased array heating techniques(95,96) and although it is not the topic of this report, this does require further discussion. A review of HT techniques used

for the treatment of LACC showed a large variation in both achieved temperatures and outcomes (Suppl 4).

Thermometry is required for dose calculations and to ensure safe and efficient heat delivery to the target volume(83,95). However the measurement of tumor temperatures, and achieving the required temperatures, has proved challenging in cervical and in other tumors(84,91,97). Intratumoral thermometry is a source of treatment toxicity(98), the insertion of interstitial catheters can cause infections(10) and may decrease compliance, and remains a time consuming and costly procedure(98). Although reaching a T90 dose of 39.2°C measured intravaginally using an annular phased-array HT system is achievable as reported by Fatehi *et al* (2006), the authors did not report on the associated outcomes. Fatehi *et al* (2007) reported similar intraluminal (T90 of 38.9°C) and intratumoral temperatures (T90 of 38.3°C), concluding that intraluminal thermometry can provide sufficient information to apply deep hyperthermia to patients with tumors of the rectum, cervix, or bladder(98). However, when referring to Suppl 4, variations in temperatures and outcomes in studies using intraluminal thermometry for cervical cancer treatments are seen.

Radiative heating systems use precise planning and thermometry in order to deliver safe treatments and to heat and measure the temperature in the target volume(96). Radiofrequency capacitive heating systems require thermometry. Modulated electro-hyperthermia applies a different dosing mechanism and thermometry is not applied. According to Fatehi *et al* (2006), comparisons between outcomes from centres using different heating techniques and thermometry are not possible due to the variations in protocols and techniques(99), and this applies to mEHT as well.

Annular phased array heating achieved acceptable temperatures (38.6°C to 39.7°C) in the phase II study by Westermann *et al* (2005) on HT plus CRT, and resulted in similar outcomes (two year survival of 71.6%)(13) to the RCT by Minnaar *et al* (2019) on mEHT (without thermometry) combined with CRT in high risk patients (76% vs 61%; Prob>chi=0.0317)(69). In a Phase II study on HT plus cisplatin for recurrent or residual disease, Franckena *et al* (2009) reported a temperature of 40.4°C in the vagina and a pelvic response rate of 58%(11). This is in line with the results from the RCT on chemotherapy with/without mEHT by Lee *et al* (2017), in which a significantly higher response rate of 61% was seen in the mEHT group(100). Lee *et al* (2018) reported intravaginal temperatures of 38.5°C (similar to Westermann *et al* (2005)(13) but lower than Franckena *et al* (2007)(11)) using mEHT to heat cervical cancer tumors(63). McCaffrey (2005) reported that the study by Westermann *et al* (2005) showed an increase in tumor temperature of 2°C(101), which corresponds to the increase from the

baseline temperature in the study on mEHT by Lee *et al* (2019)(100). These comparisons suggest that the outcomes from mEHT are comparable to other technologies described in the literature.

With such variations in temperature measurements and outcomes, the proposal for a dosing method that does not rely solely on temperature, but rather on the SAR, is appealing, especially in a resource-constrained setting where thermometry techniques cannot be practically integrated into the work flow. However the measurement of temperature in a tumor is still of radiobiological relevance as it is needed in order to predict the equivalent RT dose absorbed in the tumor(102). The combined effects of the addition of HT to RT may be quantified in terms of equivalent dose(44) and the linear–quadratic (LQ) model is used to calculate the equivalent RT dose distributions. Prasad *et al* (2019) calculated the equivalent dose of RT to be increased from 60Gy to 70.3Gy for 40.5 °C; 86.3Gy for 41.6 °C; and 93.6Gy for 42.4°C, after the addition of mEHT(44). While this model incorporates the DNA repair inhibition mechanisms, other mechanisms, such as direct cell killing and reoxygenation, require more sophisticated modelling and planning techniques in order to accurately describe the effects of mEHT on the equivalent RT dose in the tumor(44).

The CEM43 dose calculation provides the time–temperature relationship in equivalent minutes compared to 43°C, measured *in vitro*. In the equation, the variable “R” represents the temperature dependence of the rate of cell death at a given temperature. In mEHT, temperature dependence is associated with the frequency specific dielectric properties of tumors and tissues. However other variations, such as metabolic heat generation and perfusion, should also be taken into account when determining temperature distribution and thermal dose(43). Obtaining these patient-specific properties in clinics remains challenging. Effective thermometry systems and calculations which account for these variables are not yet accessible(73,85), although various sophisticated planning and thermometry techniques are in development. The technique used by Prasad *et al* (2018) is one such example which may prove to be of use for mEHT treatment planning(44).

As the dosing concept of mEHT is based on energy and not temperature, and as it has already been shown that mEHT can mildly heat cervical cancer tumors, and given the severely resource-constrained setting in which the trial took place, additional thermometry was not done for the cervical cancer patients in this trial. The authors acknowledge that this dose measurement technique does not exempt the use of thermometry in future studies on tumors for which temperature measurements have not already confirmed the thermal effects of mEHT.

Treatment Scheduling and Compliance

A time between RT and HT less than 79.2 minutes can reduce the five year in-field recurrence rates(103), however the time interval still appears to be less significant than temperature achieved and duration of treatment(16). The treatment schedules in the literature range between and within trials, making it difficult to draw conclusions between treatment schedules and toxicity. We did not however observe an increase in toxicity after two mEHT treatments per week, compared to studies administering HT once per week, or using different timing schedules. One hundred and two (97.1%) participants in our study received eight or more (80%) mEHT treatments and RT dose and cisplatin dose were not compromised in the study. In the Phase II study by Westermann *et al* (2005), 93% of patients could receive four or five HT treatments(13). In the Dutch Deep Hyperthermia Trial 69% of patients received four or five HT treatments and 12% received no HT. van der Zee *et al* (2002) cites the main reason for not completing the HT regime as patient refusal followed by contra-indications(10). In a review on 420 participants treated with HT for LACC, Franckena *et al* (2019)(16) reported that 299(73%) received all five of the planned HT treatments, and 372(88.6%) received four or more (>80%) of the planned HT treatments. When compared to the literature on HT, patient compliance to mEHT treatments in our study is high.

Quality of Life

These results show an improved functioning and symptom experience associated with the addition of mEHT, and an improved QoL associated with local control. A complete response increases participants' ability to return to normal functioning status sooner, ultimately reducing the social burden of the disease.

Conclusion

The improved local disease control and improved tumor response previously reported(68), combined with the improved QoL factors, high treatment compliance, and low mEHT-related toxicity reported in this paper, supports the selective and sensitizing effect of mEHT on tumors(68,88,100), with a lower power, even in the presence of thick layers of adipose tissue. This report also shows that thermometry is not needed as a safety parameter to prevent subcutaneous damage during mEHT treatments to the pelvis.

Although the maximum power achieved in this study was lower than in the study by Lee *et al* (2018), the step up heating protocol was faster, resulting in a similar 126.9W per

hour transmitted (compared to 133.33W/hour in the study by Lee *et al*). Based on this, on the studies conducted by Lee *et al* (2018) demonstrating heating, increased perfusion and treatment sensitization(63,100), on intratumoral temperature measurements in humans(62), on the numerous animal and *in vitro* studies demonstrating the heating ability of mEHT(45–52,54,55,57,59), and on the improved outcomes reported by Minnaar *et al* (2019)(68) with the addition of mEHT, the authors are satisfied that the mEHT technology does induce a thermal effect sufficient to cause radiosensitization.

To conclude, the addition of mEHT applied at a power of 130W to CRT did not result in any unacceptable treated-related toxicity in our high risk patient sample. The toxicities directly related to mEHT were mild and did not compromise the dose or timing of the cisplatin or RT. Modulated electro-hyperthermia is therefore a safe adjunct to CRT protocols in HIV-positive participants and in a resource-constrained setting.

This report is an important contribution to the field of knowledge in HT and mEHT and provides important safety data for the use of mEHT in high risk populations, in the absence of sophisticated thermometry and planning technologies. We provide evidence of the safety and efficacy of a heating technique that can easily be incorporated into general practices. Further developments of treatment planning systems are likely to improve our understanding of the effects and assist in fine-tuning the protocols in order to optimize the outcome.

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Disclosure of Interest

The authors report no conflict of interest

References

1. Hillemanns P, Soergel P, Hertel H, Jentschke M. Epidemiology and Early Detection of Cervical Cancer. *Oncol Res Treat*. 2016;39(9):501–6.
2. Goodman A. HPV testing as a screen for cervical cancer. *BMJ*. 2015;350:H2372.
3. Oliver NT, Chiao EY. Malignancies in women with HIV infection. *Curr Opin HIV AIDS*. 2017;12(1):69–76.
4. Marth C, Landoni F, Mahner S, McCormack M, Gonzalez-Martin A, Colombo N. Cervical cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. *Ann Oncol*. 2017;28(Suppl 4):iv72–83.
5. Lutgens LCHW, Koper PCM, Jobsen JJ, van der Steen-Banasik EM, Creutzberg CL, van den Berg HA, et al. Radiation therapy combined with hyperthermia versus cisplatin for locally advanced cervical cancer: Results of the randomized RADCHOC trial. *Radiother Oncol*. 2016;120(3):378–82.
6. Lutgens L, Van Der Zee J, Pijls-Johannesma M, De Haas-kock D, Buijsen J, Mastrigt G, et al. Combined use of hyperthermia and radiation therapy for treating locally advanced cervical carcinoma (Review). *Cochrane Database Syst Rev*. 20AD;1(Article No. CD006377).
7. Datta NR, Rogers S, Klingbiel D, Gómez S, Puric E, Bodis S. Hyperthermia and radiotherapy with or without chemotherapy in locally advanced cervical cancer: a systematic review with conventional and network meta-analyses. *Int J Hyperth*. 2016;32(7):809–21.
8. Franckena M, Stalpers LJA, Koper PCM, Wiggendaad RGJ, Hoogendaad WJ, van Dijk JDP, et al. Long-Term Improvement in Treatment Outcome After Radiotherapy and Hyperthermia in Locoregionally Advanced Cervix Cancer: An Update of the Dutch Deep Hyperthermia Trial. *Int J Radiat Oncol Biol Phys*. 2008;70(4):1176–82.
9. Harima Y, Nagata K, Harima K, Ostapenko V V., Tanaka Y, Sawada S. A randomized clinical trial of radiation therapy versus thermoradiotherapy in stage IIIB cervical carcinoma. *Int J Hyperth*. 2001;25(5):338–43.
10. van der Zee J, González González D. The Dutch Deep Hyperthermia trial: Results in cervical cancer. *Int J Hyperth*. 2002;18(1):1–12.
11. Franckena M, de Wit R, Ansink AC, Notenboom A, Canters RAM, Fatehi D, et al. Weekly systemic cisplatin plus locoregional hyperthermia: An effective treatment for patients with recurrent cervical carcinoma in a previously irradiated area. *Int J Hyperth*. 2007;23(5):443–50.
12. Rietbroek RC, Bakker PJM, Schilthuis MS, Postma AJ, Zum Vörde Sive Vörding PJ, Gonzalèz DG, et al. Feasibility, toxicity, and preliminary results of weekly loco-regional hyperthermia and cisplatin in patients with previously irradiated recurrent

- cervical carcinoma or locally advanced bladder cancer. *Int J Radiat Oncol Biol Phys.* 1996;34(4):887–93.
13. Westermann AM, Jones EL, Schem B, Steen-banasik EM Van Der, Koper P, Mella O, et al. First Results of Triple-Modality Treatment Combining Radiotherapy, Chemotherapy, and Hyperthermia for the Treatment of Patients with Stage IIB, III, and IVA Cervical Carcinoma. *Cancer.* 2005;104(4):763–70.
 14. Sreenivasa G, Hildebrandt B, Kummel S, Jungnickel K, Cho CH, Tilly W, et al. Radiochemotherapy combined with regional pelvic hyperthermia induces high response and resectability rates in patients with nonresectable cervical cancer > or =FIGO IIB “bulky”. *Int J Radiat Oncol Biol Phys.* 2006;66(4):1159–67.
 15. Bergs JWJ, Franken NAP, Haveman J, Geijssen ED, Crezee J, van Bree C. Hyperthermia, cisplatin and radiation trimodality treatment: A promising cancer treatment? A review from preclinical studies to clinical application. *Int J Hyperth.* 2007;23(4):329–41.
 16. Franckena M, Fatehi D, Bruijne M De, Canters RAM, Norden Y Van, Willem J, et al. Hyperthermia dose-effect relationship in 420 patients with cervical cancer treated with combined radiotherapy and hyperthermia. *Eur J Cancer.* 2019;45(11):1969–78.
 17. Wielheesen DHM, Smitt PAES, Haveman J, Fatehi D, Zee J Van Der. Incidence of acute peripheral neurotoxicity after deep regional hyperthermia of the pelvis. *Int J Hyperth.* 2009;672436(4):367–75.
 18. Gichangi P, Bwayo J, Estambale B, Rogo K, Njuguna E, Ojwang S, et al. HIV impact on acute morbidity and pelvic tumor control following radiotherapy for cervical cancer. *Gynecol Oncol.* 2006 Feb;100(2):405–11.
 19. Simonds HM, Neugut AI, Jacobson JS. HIV Status and acute hematological toxicity among cervix cancer patients undergoing radical chemoradiation. *Int J Gynecol Cancer.* 2015;25(5):884–90.
 20. Herd O, Francies F, Kotzen J, Smith T, Nxumalo Z, Muller X, et al. Chromosomal radiosensitivity of human immunodeficiency virus positive/negative cervical cancer patients in South Africa. *Mol Med Rep.* 2016;13(1):130–6.
 21. Baeyens A, Slabbert JP, Willem P, Jozela S, Van Der Merwe D, Vral A. Chromosomal radiosensitivity of HIV positive individuals. *Int J Radiat Biol.* 2010;86(7):584–92.
 22. Formenti SC, Chak L, Gill P, Buess EM, Hill CK. Increased radiosensitivity of normal tissue fibroblasts in patients with acquired immunodeficiency syndrome (AIDS) and with kaposi's sarcoma. *Int J Radiat Biol.* 1995;68(4):411–2.
 23. Nisce LZ, Safai B. Radiation therapy of Kaposi's sarcoma in AIDS: Memorial Sloan-Kettering experience. United States: S Karger Publishers Inc; 1985.
 24. Housri N, Yarchoan R, Kaushal A. Radiotherapy for HIV patients: Are special

- precautions necessary? *Cancer*. 2010;116(2):273–83.
25. Vallis KA. Glutathione deficiency and radiosensitivity in AIDS patients. *Lancet*. 1991;337(8746):918–9.
 26. Datta NR, Ordóñez SG, Gaip US, Paulides MM, Crezee H, Gellermann J, et al. Local hyperthermia combined with radiotherapy and- / or chemotherapy : Recent advances and promises for the future. *Cancer Treat Rev*. 2015;41(9):742–53.
 27. Scholz B, Anderson R. On Electrical Impedance Scanning –Principles and Simulations. *Electromedica*. 2000;68:35–44.
 28. Laufer S, Ivorra A, Reuter VE, Rubinsky B, Stephen B. Electrical impedance characterization of normal and cancerous human hepatic tissue. *Physiol Meas*. 2010;31(7):995–1009.
 29. Fadaka A, Ajiboye B, Ojo O, Adewale O, Olayide I, Emuowhochere R. Biology of glucose metabolism in cancer cells. *J Oncol Sci*. 2017;3(2):45–51.
 30. Dissado LA, Alison JM, Hill RM, McRae D., Esrick AM. Dynamic scaling in the dielectric response of excised EMT-6 tumours undergoing hyperthermia. *Phys Med Biol*. 1995;40(6):1067–84.
 31. Haemmerich D, Schutt DJ, Wright AW, Webster JG, David M. Electrical conductivity measurement of excised human metastatic liver tumours before and after thermal ablation. *Physiol Meas*. 2010;30(5):459–66.
 32. Joines WT, Zhang Y, Li C, Jirtle RL. The measured electrical properties of normal and malignant human tissues from 50 to 900 MHz. *Med Phys*. 1994;21(4):547–50.
 33. Fiorentini G, Szasz A. Hyperthermia today: Electric energy, a new opportunity in cancer treatment. *J Cancer Res Ther*. 2006;2(2):41–6.
 34. Szasz O, Szigeti GP, Szasz A. Connections between the Specific Absorption Rate and the Local Temperature. *Open J Biophys*. 2016;6(3):53–74.
 35. Szigeti GP, Szasz O, Hegyi G. Personalised Dosing of Hyperthermia. *J Cancer Diagnosis*. 2016;1(1):1–9.
 36. Szasz O, Andocs G, Meggyeshazi N. Modulation Effect in Oncothermia. *Conf Pap Med*. 2013;2013(Article ID: 398678):1–5.
 37. Vincze G, Szasz A. Similarities of Modulation by Temperature and by Electric Field. *Open J Biophys*. 2018;8(3):95–103.
 38. Szasz O, Szigeti GP, Vancsik T, Szasz A. Hyperthermia Dosing and Depth of Effect. *Open J Biophys*. 2018;8(1):31–48.
 39. Papp E, Vancsik T, Kiss E, Szasz O. Energy Absorption by the Membrane Rafts in the Modulated Electro-Hyperthermia (mEHT). *Open J Biophys*. 2017;7:216–29.
 40. Vincze G, Szasz O, Szasz A. Generalization of the Thermal Dose of Hyperthermia in Oncology. *Open J Biophys*. 2015;5(4):97–114.
 41. Yang K, Huang C, Chi M, Chiang H, Shan Y-. In vitro comparison of conventional hyperthermia and modulated. *Oncotarget*. 2016;7(51):84082–92.

42. Kim JK, Prasad B, Kim S. Temperature mapping and thermal dose calculation in combined radiation therapy and 13.56 MHz radiofrequency hyperthermia for tumor treatment. In: Kessel DH, Hasan T, editors. SPIE Proceedings Vol 10047: Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy XXVI, 1004718. 2017. p. 1–7.
43. Prasad B, Kim S, Cho W, Kim S, Kyung J. Effect of tumor properties on energy absorption, temperature mapping, and thermal dose in 13.56-MHz radiofrequency hyperthermia. *J Therm Biol.* 2018;74:281–9.
44. Prasad B, Kim S, Cho W, Kim JK, Kim YA, Kim S. Quantitative Estimation of the Equivalent Radiation Dose Escalation using Radiofrequency Hyperthermia in Mouse Xenograft Models of Human Lung Cancer. *Sci Rep.* 2019;9(3942):1–10.
45. Kim W, Kim M, Kim H, Lee E, Jeong J, Park I, et al. Role of HIF-1 α in response of tumors to a combination of hyperthermia and radiation in vivo. *Int J Hyperth.* 2018;34(3):276–83.
46. Tsang Y, Huang C, Yang K, Chi M, Chiang H, Wang Y, et al. Improving immunological tumor microenvironment using electro-hyperthermia followed by dendritic cell immunotherapy. *Biomed Cent Cancer.* 2015;15(708):1–11.
47. Vancsik T, Kovago C, Kiss E, Papp E, Forika G, Benyo Z. Modulated electro-hyperthermia induced loco-regional and systemic tumor destruction in colorectal cancer allografts. *J Cancer.* 2018;9(1):41–53.
48. Qin W, Akutsu Y, Andocs G, Suganami A, Hu X, Yusup G. Modulated electro-hyperthermia enhances dendritic cell therapy through an abscopal effect in mice. *Oncol Rep.* 2014;32:2373–9.
49. Meggyeshazi N, Andocs G, Balogh L, Balla P, Kiszner G, Teleki I, et al. DNA fragmentation and programmed cell death by modulated electrohyperthermia. *Strahlentherapie und Onkol.* 2014;190(9):815–22.
50. Andocs G, Meggyeshazi N, Balogh L, Spisak S. Upregulation of heat shock proteins and the promotion of damage-associated molecular pattern signals in a colorectal cancer model by modulated electrohyperthermia. *Cell Stress Chaperones.* 2015;20(1):37–46.
51. Vancsik T, Forika G, Balogh A, Kiss E, Krenacs., Tibor. Modulated electro-hyperthermia induced p53 driven apoptosis and cell cycle arrest additively support doxorubicin chemotherapy of colorectal cancer in vitro. *Cancer Med.* 2019;8(9):4292–303.
52. Jeon T, Yang H, Lee CG, Oh ST, Seo D, Baik IH, et al. Electro-hyperthermia up-regulates tumour suppressor Septin 4 to induce apoptotic cell death in hepatocellular carcinoma. *Int J Hyperth.* 2016;32(6):648–56.
53. Besztercei B, Vancsik T, Benedek A, Major E, Thomas MJ, Schvarcz CA, et al. Stress-Induced , p53-Mediated Tumor Growth Inhibition of Melanoma by

- Modulated Electrohyperthermia in Mouse Models without Major Immunogenic Effects. *Int J Mol Sci.* 2019;20(16):Article 4019.
54. Cha J, Jeon T, Lee CG, Oh ST, Yang H, Choi K, et al. Electro-hyperthermia inhibits glioma tumorigenicity through the induction of E2F1- mediated apoptosis. *Electro-hyperthermia inhibits glioma tumorigenicity through the induction of E2F1-mediated apoptosis. Int J Hyperth.* 2015;31(7):784–92.
 55. Yang W, Han GH, Shin H, Lee E, Cho H, Chay DB, et al. Combined treatment with modulated electro- hyperthermia and an autophagy inhibitor effectively inhibit ovarian and cervical cancer growth. *Int J Hyperth.* 2019;36(1):9–20.
 56. Andocs G, Rehman MU, Zhao Q, Tabuchi Y, Kanamori M, Kondo T. Comparison of biological effects of modulated electro-hyperthermia and conventional heat treatment in human lymphoma U937 cells. *Cell Death Discov.* 2016;2(16039):1–10.
 57. Andocs G, Renner H, Balogh L, Fonyad L, Jakab C, Szasz A. Strong Synergy of Heat and Modulated Electromagnetic Field in Tumor Cell Killing. *Strahlentherapie und Onkol.* 2009;185(2):120–6.
 58. Lee S, Lee N, Cho D, Kim J. Treatment outcome analysis of chemotherapy combined with modulated electro-hyperthermia compared with chemotherapy alone for recurrent cervical cancer, following irradiation. *Oncol Lett.* 2017;14(1):73–8.
 59. Cohen J, Anvari A, Samanta S, Poirier Y, Soman S, Alexander A, et al. Mild hyperthermia as a localized radiosensitizer for deep-seated tumors: investigation in an orthotopic prostate cancer model in mice. *Br J Radiol.* 2019;92(1095):20180759.
 60. Balogh L, Pöstényi Z, Haász K, Gyöngy M. Temperature increase induced by modulated electrohyperthermia (oncothermia ®) in the anesthetized pig liver. *Cancer J.* 2016;12(3):1153–9.
 61. Szasz A, Szasz N, Szasz O. *Oncothermia: Principles and Practices.* Oncothermia: Principles and Practices. Springer Netherlands; 2011. 193–206 p.
 62. Szasz A, Szasz O, Szasz N. *Principles and Practice of Oncothermia.* New York: Springer International Publishing; 2011. 194–196 p.
 63. Lee S-Y, Kim J-H, Han Y-H, CHo D-H. The effect of modulated electro-hyperthermia on temperature and blood flow in human cervical carcinoma. *Int J Hyperth.* 2018;34(7):953–60.
 64. Horsman MR, Overgaard J. Hyperthermia: a Potent Enhancer of Radiotherapy. *Clin Oncol.* 2007;19(6):418–26.
 65. Griffin RJ, Ogawa A, Williams BW, Song CW. Hyperthermic Enhancement of Tumor Radiosensitization Strategies. *Immunol Invest.* 2005;34(3):343–59.
 66. Song CW, Park HJ, Lee CK, Griffin R. Implications of increased tumor blood flow

- and oxygenation caused by mild temperature hyperthermia in tumor treatment. *Int J Hyperth*. 2005 Dec 1;21(8):761–7.
67. Song CW, Shakil A, Osborn JL, Iwata K. Tumour oxygenation is increased by hyperthermia at mild temperatures. *Int J Hyperth*. 2009 Jan 1;25(2):91–5.
 68. Minnaar CA, Kotzen JA, Akinwale O, Id A, Naidoo T, Tunmer M, et al. The effect of modulated electro-hyperthermia on local disease control in HIV-positive and -negative cervical cancer women in South Africa: Early results from a phase III randomised controlled trial. *PLoS One*. 2019;14(6):e0217894.
 69. Minnaar C, Baeyens A, Kotzen J, Vangu M. Survival of cervical cancer patients with or without associated HIV infection and treated with modulated electrohyperthermia combined with chemo-radiotherapy. *Strahlentherapie und Onkol*. 2018;194(5):476.
 70. Chase DM, Huang HQ, Wenzel L, Cella D, McQuellon R, Long HJ, et al. Quality of life and survival in advanced cervical cancer: A Gynecologic Oncology Group study. *Gynecol Oncol*. 2012;125(2):315–9.
 71. van der Zee J, Vujaskovic Z, Kindo M, Sugahara T. The Kadota Fund International Forum 2004 – clinical group consensus. *Int J Hyperth*. 2008;24(2):111–2.
 72. Sapochnik MD, Corry PM, Kapp DS, Myerson RJ, Dewhirst MW, Emami B, et al. RTOG quality assurance guidelines for clinical trials using hyperthermia for deep-seated malignancy. *Int J Radiat Oncol Biol Phys*. 1991;20(5):1109–15.
 73. van Rhoon GC. Is CEM43 still a relevant thermal dose parameter for hyperthermia treatment monitoring? *Int J Hyperth*. 2016;6736:1–13.
 74. Szasz A, Vincze G. Dose concept of oncological hyperthermia: heat-equation considering the cell destruction. *Cancer J*. 2006;2(4):171–81.
 75. Paken J, Govender CD, Pillay M, Sewram V. Cisplatin-Associated Ototoxicity: A Review for the Health Professional. *J Toxicol*. 2016;2016:ID 1809394.
 76. Carozzi VA, Canta A, Chiorazzi A. Chemotherapy-induced peripheral neuropathy: What do we know about mechanisms? *Neurosci Lett*. 2015;596:90–107.
 77. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. *Eur J Pharmac*. 2015;(740):364–78.
 78. Koller M, Aaronson NK, Blazeby J, Bottomley A, Dewolf L, Fayers P, et al. Translation procedures for standardised quality of life questionnaires: The European Organisation for Research and Treatment of Cancer (EORTC) approach. *Eur J Cancer*. 2007;43(12):1810–20.
 79. Fayers P, Bottomley A. Quality of life research within the EORTC — the EORTC QLQ-C30. *Eur J Cancer*. 2002;38(Suppl 4):S125–33.
 80. Aaronson N, Ahmedzai S, Bergman B, Bullinger M, Cull A, Duez N, et al. The European Organisation for Research and Treatment of Cancer QLQ-C30: A quality-of-life instrument for use in international clinical trials in oncology. *J Natl*

- Cancer Inst. 1993;85:365–76.
81. Fayers P, Aaronson N, Bjordal K, Groenvold M, Curran D, Bottomley A. The EORTC QLQ-C30 Scoring Manual (3rd Edition). Brussels; 2001.
 82. The American Cancer Society medical and editorial content team. Normal weight ranges: Body mass index (BMI) [Internet]. 2015 [cited 2019 Jan 2]. Available from: <https://www.cancer.org/cancer/cancer-causes/diet-physical-activity/body-weight-and-cancer-risk/adult-bmi.html>
 83. Kroeze H, van de Kamer J, de Leeuw A, Kikuchi M, Lagendijk J. Treatment planning for capacitive regional hyperthermia. *Int J Hyperth.* 2003;19(1):58–73.
 84. van Haaren PMA, Hulshof MCCM, Kok HP, Oldenburg S. Relation between body size and temperatures during locoregional hyperthermia of oesophageal cancer patients. *Int J Hyperth.* 2008;24(8):663–74.
 85. Kok HP, Crezee J. A comparison of the heating characteristics of capacitive and radiative superficial hyperthermia. *Int J Hyperth.* 2017;33(4):378–86.
 86. D'Ambrosio V, Dughiero F. Numerical model for RF capacitive regional deep hyperthermia in pelvic tumors. *Med Biol Eng Comput.* 2007;45(5):459–66.
 87. Harima Y, Ohguri T, Imada H, Sakurai H, Ohno T, Hiraki Y, et al. A multicentre randomised clinical trial of chemoradiotherapy plus hyperthermia versus chemoradiotherapy alone in patients with locally advanced cervical cancer. *Int J Hyperth.* 2016;32(7):801–8.
 88. Szasz O, Szasz A. Heating , Efficacy and Dose of Local Hyperthermia. *Open J Biophys.* 2016;6:10–8.
 89. Poglio S, Galvani S, Bour S. Adipose Tissue Sensitivity to Radiation Exposure. 2009;44–53.
 90. Shukla L, Morrison WA, Shayan R. Adipose-derived stem cells in radiotherapy injury : a new frontier. 2015;2(January):10–3.
 91. Franckena M, Lutgens LC, Koper PC, Kleynen CE, van der Steen-Banasik EM, Jobsen JJ, et al. Radiotherapy and Hyperthermia for Treatment of Primary Locally Advanced Cervix Cancer: Results in 378 Patients. *Int J Radiat Oncol Biol Phys.* 2009;73(1):242–50.
 92. Vendrell I, Ferreira AR, Abrunhosa-Branquinho AN, Semedo PM, Pulido CF, Jorge M, et al. Chemoradiotherapy completion and neutropenia risk in HIV patients with cervical cancer. *Medicine (Baltimore).* 2018;97(30):e11592.
 93. Levinson KL, Riedel DJ, Ojalvo LS, Chan W, Angarita AM, Fader AN, et al. Gynecologic cancer in HIV-infected women: Treatment and outcomes in a multi-institutional cohort. *Aids.* 2018;32(2):171–7.
 94. Mdletshe S, Munkupa H, Lishimpi K. Acute toxicity in cervical cancer HIV-positive vs . HIV-negative patients treated by radical chemo-radiation in Zambia. *South African J Gynaecol Oncol.* 2016;8(2):37–41.

95. Triantopoulou S, Platoni K, Antypas C, Karaiskos P, Uzunoglou N, Kouloulis V. Quality assurance protocol for superficial and deep hyperthermia systems established by the Hellenic Association of Medical Physicists (HAMP) in cooperation with the Hellenic Society of Oncologic Hyperthermia (HSOH): A study based on European Society for. *J Balkan Union Oncol*. 2018;23(2):494–9.
96. Bruggmoser G, Bauchowitz S, Canters R, Crezee H, Ehmann M, Gellermann J, et al. Quality assurance for clinical studies in regional deep hyperthermia. *Strahlentherapie und Onkol*. 2011;187(10):605–10.
97. Jones EL, Oleson JR, Prosnitz LR, Samulski T V, Vujaskovic Z, Yu D, et al. Randomized Trial of Hyperthermia and Radiation for Superficial Tumors. *J Clin Oncol*. 2005;23(13):3079–85.
98. Fatehi D, Zee J Van Der, Notenboom A, Rhoon GC Van. Comparison of Intratumor and Intraluminal Temperatures During Locoregional Deep Hyperthermia of Pelvic Tumors. *Strahlenthera*. 2007;183(9):479–87.
99. Fatehi D, Van der Zee J, Van der Wal E, Van Wieringen WN, Van Rhoon GC. Temperature data analysis for 22 patients with advanced cervical carcinoma treated in Rotterdam using radiotherapy, hyperthermia and chemotherapy: A reference point is needed. *Int J Hyperth*. 2006;22(4):353–63.
100. Lee SUNY, Lee NARI, Cho DHYU, Kim JSOO. Treatment outcome analysis of chemotherapy combined with modulated electro-hyperthermia compared with chemotherapy alone for recurrent cervical cancer , following irradiation. *Oncol Lett*. 2017;14(1):73–8.
101. McCaffrey P. Warming up to a triple therapy for cervical cancer. *Lancet Oncol*. 2005;6:642.
102. Crezee J, Leeuwen CM Van, Oei AL, Heerden LE Van, Bel A, Stalpers LJA, et al. Biological modelling of the radiation dose escalation effect of regional hyperthermia in cervical cancer. *Radiat Oncol*. 2016;1–9.
103. van Leeuwen CM, Oei AL, Chin KWTK, Crezee J, Bel A, Westermann AM, et al. A short time interval between radiotherapy and hyperthermia reduces in-field recurrence and mortality in women with advanced cervical cancer. *Radiat Oncol*. 2017;12(75):1–8.
104. Vasanthan A, Mitsumori M, Park JH, Zhi-Fan Z, Yu-Bin Z, Oliynychenko P, et al. Regional hyperthermia combined with radiotherapy for uterine cervical cancers: a multi-institutional prospective randomized trial of the International Atomic Energy Agency. *Int J Radiat Oncol Biol Phys*. 2005;61(1):145–53.

Tables

Table 1: Summary of local control outcomes at six months post-treatment in the trial by Minnaar et al (2019)(68)

	mEHT Group	Control Group	p-value
Tumor response on PET/CT	49 out of 85 (58%)	26 out of 73 (36%)	$p=0.005$
LC censored for survival	40 out of 88 (45%)	20 out of 83 (24%)	$p=0.003$
LDFS	39 out of 101 (39%)	20 out of 101 (20%)	$p=0.003$

Abbreviations: PET/CT: Positon Emission Tomography/Computed Tomography; mEHT: Modulated electro-hyperthermia; LC: Local control; LDFS: Local disease free survival

Table 2: Characteristics of Participants and Treatment

		mEHT 105	Control 101	P Value
HIV Status	Positive	52 (50%)	53 (53%)	
	Negative	53 (50%)	48 (48%)	
FIGO Staging	IIB	40 (38%)	35 (35%)	
	III	65 (62%)	66 (65%)	
Race	African	97 (92%)	94 (93%)	
	Caucasian	4 (4%)	1 (1%)	
	Other	4 (4%)	6 (6%)	
Age (years)	Mean	48.1	49.4	$p=0.384$
	SD	10.8	10.3	
	Range	26.5 - 68.9	28.4 - 72.7	
BMI	Mean	27.8	27	$p=0.387$
	SD	6.92	6.48	
	Range	15 - 49	15 - 42	
Total RT dose	Mean	84.7Gy	85.3	$p=0.483$
	SD	7.61Gy	5.69	
	Range	20Gy - 86Gy	30Gy - 86Gy	
Treatment days	Mean	41.6	42.8	$p=0.255$
	SD	5.96	8.81	
	Range	13 - 65	10 - 97	
Number of Cisplatin doses	Mean	1.37	1.28	$p=0.323$
	SD	0.67	0.69	
	0 doses	11 (11%)	14 (14%)	
	1 dose	44 (42%)	45 (45%)	
	2 doses	50 (48%)	42 (42%)	
CD4 count (cells/ μ L)	Mean	586.1	525.1	$p=0.242$
	SD	258.6	272.8	
	Range	148 - 1204	95 - 1524	

No of mEHT doses	Mean	9.26		
	SD	1.65		
	Range	1 - 10		
Average KJ administered during mEHT	Mean	380.18KJ		
	SD	32.07KJ		
	Range	237.1KJ - 454.5KJ		

Abbreviations: BMI: Body Mass Index; FIGO: International Federation of Gynecology and Obstetrics; HIV: Human Immunodeficiency Virus; KJ: Kilojoules; mEHT: Modulated electro-Hyperthermia; RT: Radiation Therapy; SD: Standard Deviation

Table 3: Reasons for Missed or Delayed Cisplatin

	One dose		No Cisplatin	
	mEHT	Control	mEHT	Control
Renal dysfunction	19	11	7	10
Delayed first dose*	10	7		
Leucopenia	0	0		
Noncompliant	2	0	1	1
Anemia	1	2	2	1
Missed dose	1	1		
Vomiting dehydration	1	0		
Deceased	1	1		
Sepsis	0	3		
Technical	0	3		
Too weak	0	1	0	1
Dehydration	0	1		
Low CD4 count			0	1
Total	35	30	10	14

*Reasons for delayed first dose were renal dysfunction (mEHT: $n=1$; Control: $n=1$), leucopenia (mEHT: $n=2$; Control: $n=1$), non-compliant (mEHT: $n=0$; Control: $n=1$), anemia (mEHT: $n=4$; Control: $n=4$), sepsis (mEHT: $n=2$; Control: $n=1$) and supply problems (mEHT: $n=1$; Control: $n=0$).

Table 4: Frequency of Grade 3-4 Toxicities in each Treatment Group Analyzed by HIV Status

	mEHT		p-value	Control		p-value
	HIV-Positive	HIV-Negative		HIV-Positive	HIV-Negative	
<u>On Treatment</u>	<i>n</i> =52	<i>n</i> =53		<i>n</i> =53	<i>n</i> =48	
Hematologic	6 (12%)	6 (11%)	<i>p</i> =0.972	8 (15%)	6 (13%)	<i>p</i> =0.706
Neurological	1 (2%)	0 (0%)	<i>p</i> =0.310	0 (0%)	0 (0%)	
Lower GIT	0 (0%)	1 (2%)	<i>p</i> =0.320	0 (0%)	0 (0%)	
Upper GIT	0 (0%)	1 (2%)	<i>p</i> =0.320	0 (0%)	0 (0%)	
Dermatologic	3 (6%)	1 (2%)	<i>p</i> =0.299	1 (2%)	1 (2%)	<i>p</i> =0.727
Urogenital	3 (6%)	1 (2%)	<i>p</i> =0.299	1 (2%)	2 (4%)	<i>p</i> =0.500
Renal	5 (10%)	0 (0%)	<i>p</i> =0.027	3 (6%)	3 (6%)	<i>p</i> =0.900
Multiple	0 (0%)	0 (0%)		0 (0%)	0 (0%)	
<u>Six Weeks</u>	<i>n</i> =49	<i>n</i> =50		<i>n</i> =48	<i>n</i> =49	
Hematologic	0 (0%)	2 (4%)	<i>p</i> =0.157	3 (6%)	3 (6%)	<i>p</i> =0.979
Neurological	1 (2%)	0 (0%)	<i>p</i> =0.310	0 (0%)	0 (0%)	
Lower GIT	1 (2%)	0 (0%)	<i>p</i> =0.310	0 (0%)	0 (0%)	
Upper GIT	0 (0%)	0 (0%)		0 (0%)	0 (0%)	
Dermatologic	0 (0%)	0 (0%)		1 (2%)	0 (0%)	<i>p</i> =0.310
Urogenital	1 (2%)	0 (0%)	<i>p</i> =0.310	0 (0%)	2 (4%)	<i>p</i> =0.157
Renal	1 (2%)	0 (0%)	<i>p</i> =0.310	0 (0%)	0 (0%)	
Multiple	0 (0%)	0 (0%)		0 (0%)	0 (0%)	
<u>Three Months</u>	<i>n</i> =45	<i>n</i> =48		<i>n</i> =43	<i>n</i> =42	
Hematologic	3 (7%)	0 (0%)	<i>p</i> =0.069	1 (2%)	5 (12%)	<i>p</i> =0.085
Neurological	0 (0%)	0 (0%)		0 (0%)	0 (0%)	
Lower GIT	0 (0%)	1 (2%)	<i>p</i> =0.330	1 (2%)	2 (5%)	<i>p</i> =0.543
Upper GIT	0 (0%)	0 (0%)		1 (2%)	0 (0%)	<i>p</i> =0.320
Dermatologic	1 (2%)	0 (0%)	<i>p</i> =0.299	0 (0%)	0 (0%)	
Urogenital	1 (2%)	0 (0%)	<i>p</i> =0.299	1 (2%)	3 (7%)	<i>p</i> =0.294
Renal	3 (7%)	0 (0%)	<i>p</i> =0.069	1 (2%)	2 (5%)	<i>p</i> =0.543
Multiple	0 (0%)	0 (0%)		0 (0%)	0 (0%)	

Abbreviations: HIV: Human Immunodeficiency Virus; GIT: Gastrointestinal Tract

Table 5: Regression Analysis for overall Acute Toxicity by System

Variable	Odds Ratio [OR]	P> z	95% Confidence interval [CI] range
<i>Neurological</i>			
HIV status	0.82	0.771	0.21-3.20
Cisplatin	<i>All participants with neurological adverse events received cisplatin</i>		
mEHT	7.98	0.053	0.98-65.19
<i>Dermatologic/Subcutaneous</i>			
HIV status	2.61	0.033	1.08-6.29
Cisplatin	3.54	0.016	1.27-9.84
mEHT	1.35	0.485	0.58-3.11
<i>Renal</i>			
HIV status	0.58	0.098	0.30-1.12
Cisplatin	0.13	<0.005	0.05-0.33
mEHT	0.95	0.887	0.50-1.82

Abbreviations: HIV: Human Immunodeficiency Virus; mEHT: Modulated electro-Hyperthermia;

Table 6: Significant changes in the QoL results

	mEHT Group			Control Group			P-value
	Mean change	SD	95% CI	Mean change	SD	95% CI	
<i>Six Weeks</i>							
Cognitive function	13.6	33.1	6.97 to 20.24	3.5	30.6	2.89 to 9.85	<i>p=0.031</i>
<i>Three Months</i>							
Social Functioning	83.9	31.6	77.4-90.4	73.3	39.4	64.8-81.8	<i>p=0.049</i>
Emotional Functioning	83.9	24.3	78.9-88.9	73.5	33.3	66.3-80.6	<i>p=0.017</i>
Fatigue	-13.6	26.0	-18.86 to -8.15	3.0	39.9	11.53 to 5.59	<i>p=0.037</i>
Pain	-21.0	33.0	27.76 to -14.18	-6.7	36.6	-14.62 to 1.05	<i>p=0.007</i>

SD: Standard Deviation

SUPPLEMENTARY MATERIAL

SUPPL 1: Hyperthermia Schedule

Treatment	Start Power	Modulation	2 min	3 min	4 min	5 min	Target power	Time
1	60 W	Off	70 W	80 W	80 W	90 W	90 W	55 minutes
2	60 W	On	80 W	90 W	105 W	105 W	105 W	55 minutes
3	60 W	On	80 W	100 W	115 W	130 W	130 W	55 minutes
4-10	60 W	On	100 W	115 W	130 W	130 W	130 W	55 minutes

SUPPL 2: Chi-squared frequency tables for grades 3-4 toxicities

A) In each Treatment Group

System	On Treatment		p-value	Six Weeks		p-value	Three Months		p-value
	mEHT n=105	Control n=101		mEHT n=99	Control n=97		mEHT n=93	Control n=85	
Hematologic	12 (11%)	14 (14%)	p=0.599	2 (2%)	6 (6%)	p=0.141	3 (3%)	6 (7%)	p=0.244
Neurological	1 (1%)	0 (0%)	p=0.326	1 (1%)	0 (0%)	p=0.321	0 (0%)	0 (0%)	
Lower GIT	1 (1%)	0 (0%)	p=0.326	1 (1%)	0 (0%)	p=0.321	1 (1%)	3 (4%)	p=0.270
Upper GIT	1 (1%)	0 (0%)	p=0.326	0 (0%)	0 (0%)		0 (0%)	1 (1%)	p=0.294
Dermatologic	4 (4%)	2 (2%)	p=0.435	0 (0%)	1 (1%)	p=0.311	1 (1%)	0 (0%)	p=0.338
Urogenital	4 (4%)	3 (3%)	p=0.522	1 (1%)	2 (2%)	p=0.549	1 (1%)	4 (5%)	p=0.143
Renal	5 (5%)	6 (6%)	p=0.707	1 (1%)	0 (0%)	p=0.321	3 (3%)	3 (4%)	p=0.911
Multiple	0 (0%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)	

Abbreviations: HIV: Human Immunodeficiency Virus; GIT: Gastrointestinal Tract

B) Analyzed by HIV Status

System	On Treatment		p-value	Six Weeks		p-value	Three Months		p-value
	HIV-Positive n=105	HIV-Negative n=101		HIV-Positive n=97	HIV-Negative n=99		HIV-Positive n=88	HIV-Negative n=90	
Hematologic	14 (13%)	12 (12%)	p=0.754	3 (3%)	5 (5%)	p=0.489	4 (5%)	5 (6%)	p=0.758
Neurological	1 (1%)	0 (0%)	p=0.326	1 (1%)	0 (0%)	p=0.311	0 (0%)	0 (0%)	
Lower GIT	0 (0%)	1 (1%)	p=0.307	1 (1%)	0 (0%)	p=0.311	1 (1%)	3 (3%)	p=0.323
Upper GIT	0 (0%)	1 (1%)	p=0.307	0 (0%)	0 (0%)		1 (1%)	0 (0%)	p=0.311
Dermatologic	4 (4%)	2 (2%)	p=0.435	1 (1%)	0 (0%)	p=0.311	1 (1%)	0 (0%)	p=0.311
Urogenital	4 (4%)	3 (3%)	p=0.740	1 (1%)	2 (2%)	p=0.573	2 (2%)	3 (3%)	p=0.669
Renal	8 (8%)	3 (3%)	p=0.138	1 (1%)	0 (0%)	p=0.311	4 (5%)	2 (2%)	p=0.391
Multiple	0 (0%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)	

Abbreviations: HIV: Human Immunodeficiency Virus; GIT: Gastrointestinal Tract

SUPPL 3: Significant improvements in QoL scores based on local control

6 weeks	CR	No CR	p-value
<i>Social Function</i> Mean improvement SD [95% CI]	n=57 26% 51.8 -12.9 to 40.4	n=132 3.0% 52.2 -6.0 to 12.0	p=0.0048
<i>Emotional function</i> Mean improvement SD [95% CI]	N=57 28.1% 40.4 12.4 to 38.8	N=132 12.9% 37.9 6.4 to 19.4	p=0.0141
<i>Physical function</i> Mean improvement SD [95% CI]	N=57 11.9% 28.1 4.5 to 19.4	N=132 -0.9% 35.0 -6.9 to 5.2	p=0.0155
6 Months	CR	No CR	p-value
<i>Body Image</i> Mean improvement SD [95% CI]	n=57 -55.6% 38.8 -65.9 to -45.3	N=129 -66.9% 34.7 -72.9 to -60.8	p=0.0496
<i>Social function</i> Mean improvement SD [95% CI]	n=57 -55.1% 41.6 -66.1 to -44.0	N=129 -71.4% 36.0 -77.6 to -65.1	p=0.0074
<i>Emotional function</i> Mean improvement SD [95% CI]	n=57 -51.2% 37.3 -61.2 to -41.3	n=129 -65.9% 28.2 -70.8 to -61.0	p=0.0035

Abbreviations: CR: Complete response; SD: Standard Deviation; CI: Confidence Interval

SUPPL 4: Summary of trials with temperature, toxicity, compliance and outcome, arranged by outcome, then temperature

Trial	Thermo-metry	Toxicity				No of treatments	Survival / local response
		Total	Skin burns	Subcut burns	Other		
(Lutgens et al., 2016) (2016) RT + rHT/CDDP	Not specified	38%	0%	14%	Myopathy: 17%; Neurotoxicity: 7%; periostitis: 2%	5: 98% <4: 2%	rHT = CDDP positive
(van der Zee et al, 2002) (Franckena et al., 2008) (2006) RT +/- rHT	Intraluminal/ Most had no measurements	14%	3%	12%	Some infections due to interstitial thermometry catheters	4-6: 69% <4: 31%	CR p=0.003 3YS p=0.015 12YS p=0.003 positive
(Minnaar et al., 2019) (2019)CRT +/- mEHT	Not done	16%	2%	9.5%	Pain: 9%	8-10: 80% 4-6:20%	LC: p=0.003 2YS: p=0.033 positive
(Lee et al., 2017) (2017)ChT +/- mEHT	+38.5°C Previously reported (Lee et al., 2018)	0%	0%	0%	Hot sensations/ discomfort: 44%	36: 100%	LC: p<0.0218 positive

(Westerman et al., 2005) rHT +CRT	Intraluminal T90: 39.4°C	33%	18%	8%	8%	4 or 5: 93%	CR: 90% 2yr DFS:72% positive
(Franckena et al., 2007) (2019) RT+rHT	Intraluminal T90: 39.8	36%*	0%*	36%*	3% Neurotoxicity*	5:73% 4: 16% <4: 11%	5YS 47% TC 65% positive
(Franckena et al., 2007) (2007) rHT+CDDP	Intravaginal Mean: 40.4°C	11%	0%	11%	0%	95% compliance	RR: 58% MS: 8m positive
(Franckena et al., 2009) (2009) RT+rHT	Intraluminal 40.6°C	NR	NR	NR	NR	5: 74% 4: 15% <4:11%	CR: 77% 5YS: 40% positive
(Harima et al., 2001) (2005) RT +/-cHT*	Intratumoral Intraluminal Tavg: 41.6°C	18%	NR	NR	NR	Median: 5	LC $p>0.05$ 3YS $p>0.05$ negative
(Harima et al., 2001) (2001) RT +/- cHT*	Intratumoral Tmax: 41.8°C Tavg 40.6°C	10%	0%	10%	0%	3	CR $p=0.048$ 3YS $p>0.05$ negative
(Harima et al., 2016) (2016) CRT+/-cHT*	Intravaginal Tmax: 42.2°C Tavg: 41.1°C	0%	0%	0%	0%	4 or 5: 92%	5YDFS, CR (χ^2) $p>0.05$ negative

Abbreviations: CDDP: Cisplatin; ChT: Chemotherapy; cHT: Capacitive Hyperthermia; CR: Complete Response; CRT: Chemoradiotherapy; LC: Local Control; MS: Median Survival; m: Months; NR: Not Reported; rHT: Radiative Hyperthermia; RR: Response Rate; RT: Radiotherapy; Subcut: Subcutaneous; Sx: Symptoms; TC: Tumor Control YDFS: Years Disease Free Survival; YS: Years Survival

**Symptoms were worse with increased temperatures and fatty tissue*

Franckena, M., de Wit, R., et al. (2007) Weekly systemic cisplatin plus locoregional hyperthermia : An effective treatment for patients with recurrent cervical carcinoma in a previously irradiated area, *International Journal of Hyperthermia*, Vol.23, No.5, pp.443–450.

Franckena, M., Stalpers, L. J. A., et al. (2008) Long-Term Improvement in Treatment Outcome After Radiotherapy and Hyperthermia in Locoregionally Advanced Cervix Cancer: An Update of the Dutch Deep Hyperthermia Trial, *International Journal of Radiation Oncology, Biology, Physics*, Vol.70, No.4, pp.1176–1182.

Franckena, M., Lutgens, L. C., et al. (2009) Radiotherapy and Hyperthermia for Treatment of Primary Locally Advanced Cervix Cancer: Results in 378 Patients, *International Journal of Radiation Oncology, Biology, Physics*, Vol.73, No.1, pp.242–250.

Franckena, M., Fatehi, D., et al. (2019) Hyperthermia dose-effect relationship in 420 patients with cervical cancer treated with combined radiotherapy and hyperthermia, *European Journal of Cancer*, Vol.45, No.11, pp.1969–1978.

Harima, Y., Nagata, K., et al. (2001) A randomized clinical trial of radiation therapy versus thermoradiotherapy in stage IIIB cervical carcinoma, *International Journal of Hyperthermia*, Vol.25, No.5, pp.338–343.

Harima, Y., Ohguri, T., et al. (2016) A multicentre randomised clinical trial of chemoradiotherapy plus hyperthermia versus chemoradiotherapy alone in patients with locally advanced cervical cancer, *International Journal of Hyperthermia*, Vol.32, No.7, pp.801–808.

- Lee, S.-Y., Kim, J.-H., et al. (2018) The effect of modulated electro-hyperthermia on temperature and blood flow in human cervical carcinoma, *International Journal of Hyperthermia*, Vol.34, No.7, pp.953–960.
- Lee, S., Lee, N., et al. (2017) Treatment outcome analysis of chemotherapy combined with modulated electro-hyperthermia compared with chemotherapy alone for recurrent cervical cancer, following irradiation, *Oncology Letters*, Vol.14, No.1, pp.73–78.
- Lutgens, L. C. H. W., Koper, P. C. M., et al. (2016) Radiation therapy combined with hyperthermia versus cisplatin for locally advanced cervical cancer: Results of the randomized RADCHOC trial, *Radiotherapy and Oncology*, Vol.120, No.3, pp.378–382.
- Minnaar, C. A., Kotzen, J. A., et al. (2019) The effect of modulated electro-hyperthermia on local disease control in HIV-positive and -negative cervical cancer women in South Africa : Early results from a phase III randomised controlled trial, *PLoS ONE*, Vol.14, No.6, p.e0217894.
- Vasanthan, A., Mitsumori, M., et al. (2005) Regional hyperthermia combined with radiotherapy for uterine cervical cancers: a multi-institutional prospective randomized trial of the International Atomic Energy Agency, *International Journal of Radiation Oncology, Biology, Physics*, Vol.61, No.1, pp.145–153.
- Westermann, A. M., Jones, E. L., et al. (2005) First Results of Triple-Modality Treatment Combining Radiotherapy, Chemotherapy, and Hyperthermia for the Treatment of Patients with Stage IIB, III, and IVA Cervical Carcinoma, *Cancer*, Vol.104, No.4, pp.763–770.
- van der Zee, J. and González González, D. (2002) The Dutch Deep Hyperthermia trial: Results in cervical cancer, *International Journal of Hyperthermia*, Vol.18, No.1, pp.1–12.

CHAPTER FIVE

Potential of the abscopal effect by modulated electro-hyperthermia in locally advanced cervical cancer patients

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Author Contributions

Minnaar: Conducted the research, gathered the data, statistical analysis, interpretation of results, writing of the manuscript.

Kotzen: Supervisor, senior radiation oncologist, planning and prescribing of treatments, oversaw the treatment and follow-ups of trial participants, reviewed manuscript.

Ayeni: Reviewed ^{18}F -FDG PET/CT reports, reviewed manuscript.

Vangu: Head of Nuclear Medicine Department; oversaw all the ^{18}F -FDG PET/CT scans and related logistics, reviewed manuscript.

Baeyens: Supervisor, Head of Radiobiology, supervised data collection and data quality control, project planning, management of funding, reviewed manuscript.

POTENTIATION OF THE ABSCOPAL EFFECT BY MODULATED ELECTRO-HYPERTHERMIA IN LOCALLY ADVANCED CERVICAL CANCER PATIENTS

POTENTIATION OF THE ABSCOPAL EFFECT BY MODULATED ELECTRO-HYPERTHERMIA

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ABSTRACT

BACKGROUND: A Phase III randomized controlled trial investigating the addition of modulated electro-hyperthermia (mEHT) to chemoradiotherapy for locally advanced cervical cancer patients has been conducted in South Africa (Human Research Ethics Committee approval: M1704133; ClinicalTrials.gov ID: NCT03332069). Screening ^{18}F -FDG PET/CT scans were conducted and repeated at six months post-treatment. Significant improvement in local control was reported in the mEHT group and complete metabolic resolution (CMR) of extra-pelvic disease was noted in some participants. We report on an analysis of the participants with CMR of disease outside the radiation field.

METHOD: Participants were included in this analysis if nodes outside the treatment field (FDG-uptake $\text{SUV} > 2.5$) were visualized on pre-treatment scans and if participants were evaluated by ^{18}F -FDG PET/CT scans at six months post-treatment.

RESULTS: 108 participants (mEHT: HIV-positive $n=25$, HIV-negative $n=29$; Control Group: HIV-positive $n=26$, HIV-negative $n=28$) were eligible for analysis. There was a higher CMR of all disease inside and outside the radiation field in the mEHT Group: $n=13[24.1\%]$ than the control group: $n=3[5.6\%]$ (Chi2, Fisher's exact: $p=0.013$) with no significant difference in the extra-pelvic response to treatment between the HIV-positive and -negative participants of each group.

CONCLUSION: The CMR of disease outside the radiation field at six months post-treatment provides evidence of an abscopal effect which was significantly associated with the addition of mEHT to treatment protocols. This finding is important as the combined synergistic use of radiotherapy with mEHT could broaden the scope of radiotherapy to include systemic disease.

KEYWORDS

Modulated electro-hyperthermia; Abscopal effect; Radiotherapy; Cervical Cancer; Immunomodulation

KEY POINTS

In an analysis of 108 locally advanced cervical cancer patients treated with chemoradiotherapy with (n=54) or without (n=54) modulated electro-hyperthermia (mEHT) as a radiosensitiser, a significantly higher complete metabolic resolution of extra-pelvic disease was seen in patient treated with mEHT (n=13[24.1%]) compared to the control group (n=3[5.6%]) (Chi-squared: $p=0.013$). The resolution of disease outside of the radiation field suggests that a systemic response to the disease has been induced by the radiotherapy (abscopal effect) and that this was potentiated by mEHT.

1. INTRODUCTION

The abscopal effect is a systemic response to ionizing radiation (IR) in which non-irradiated lesions respond after irradiation of the primary treatment site.[1,2] It is generally accepted that the abscopal effect is driven by underlying immune mechanisms which are activated by IR.[2–4] One proposed mechanism is the immunogenic cell death (ICD) caused by IR[3] which requires the release of damage associated molecular patterns (DAMPs). These in turn activate dendritic cells and enhance antigen expression and presentation to the immune system. Ionizing radiation has also been shown to enhance the functioning of T-cells.[4]

The frequency of reported abscopal effects in the literature is extremely low with only a handful of published cases per year.[3,4] In a review, Reynders *et al* summarized 23 case reports, one retrospective study, and 13 pre-clinical papers, from the 1970s to 2014. Only one of these involved a primary squamous cell carcinoma of the cervix. The patient (age 69 years) was treated with external beam radiation (EBRT) and brachytherapy (BT) for locally advanced cervical cancer (LACC) and showed a complete response of the para-aortic nodes outside of the radiation field, as well as a complete response of the tumor, on the post-treatment Abdominal and Pelvic Computed Tomography (CT) and Pelvic Magnetic Resonance Imaging (MRI) scans.[5] Reynders *et al* concluded that the abscopal effect is based on anti-tumor immunity and was more common in immunogenic tumor types. Renal cell carcinoma had the most frequently reported cases of the

abscopal effect followed by hepatocellular carcinoma. The abscopal effect was observed at all ages and with a variety of radiotherapy protocols. The preclinical data indicates that some immunomodulatory agents may have potential to act synergistically with IR to induce a systemic response[4] which may explain the increase in the number of reported abscopal effects with the combined treatment of immunotherapies and IR.[6]

The addition of mild hyperthermia to local irradiation has shown to have immunomodulating effects which may result in enhanced tumor regression and an abscopal effect when combined with radiotherapy, as was seen in a liposarcoma patient treated with hyperthermia and radiotherapy.[7] Hyperthermia may directly activate the immune cells present in the tumor and its microenvironment[8] and may further enhance the function of the dendritic cells.[9]

Modulated electro-hyperthermia (mEHT) applies amplitude modulated radiofrequency (13.56MHz), in a capacitive coupling set-up to target and heat malignant tissues, sensitizing them to treatments. The technique exploits the differences in impedance between the malignant and healthy tissue as well as impedance matching technology, to selectively deliver a higher energy dose to the malignant tissues. The increased energy deposition has the net effect of an increase in the thermal energy, and temperature. The biophysics are further described in detail in the literature.[10–12] Preclinical research suggests that mEHT combined with immunotherapies is able to elicit an immune-mediated response[13] which may even extend to untreated tumors. Vancsik *et al* showed that mEHT induced DAMPs in murine models was followed by an invasion of antigen presenting cells (APC) and T-cells at the site of the treated tumor and that when mEHT was administered combined with a T-cell stimulating agent, APC and T-cell invasion was also seen in the untreated tumors of the same murine model.[14] In an *in vivo* study, mEHT combined with dendritic cell therapy elicited a response to untreated tumors in murine squamous cell carcinoma (SCCVII) models.[15] Ionizing radiation has shown to increase the expression of immunogenic molecules such as calreticulin, on the surface of tumor cells and radiation-induced stress-response leads to the expression of Heat Shock Protein (HSP) 70 on cell membranes. This Heat Shock Protein plays an important role in mounting an immune response at the site when released into the extracellular matrix.[16] Yang *et al* reported an increased release of the expressed HSP70 and increased levels of calreticulin after mEHT, compared to other heating methods.[17]

The safety and heating efficacy of mEHT in cervical cancer patients has been demonstrated,[18–20] and the recent report by Minnaar *et al* (2019) showed a significant

improvement in local control of LACC participants treated with chemoradiotherapy (CRT) combined with mEHT in a randomized controlled trial in South Africa.[19] During the local disease control analysis, it was noted that some of the participants with extra-pelvic disease present on the pre-treatment Fluorodeoxyglucose (^{18}F -FDG) Positron Emission Tomography (PET) /CT scans showed a complete metabolic resolution (CMR) of disease outside the treatment field on the post- treatment ^{18}F -FDG PET/CT scans. An analysis of the subset of patients with extra-pelvic disease visualized on the pre-treatment ^{18}F -FDG PET/CT scans was subsequently planned. We present the results of this analysis with the aim of investigating the possibility of an abscopal effect induced by the addition of mEHT to CRT in these participants.

2. METHODS AND MATERIALS

The Randomized controlled trial by Minnaar *et al.*(2019)[19] is being conducted at the Charlotte Maxeke Johannesburg Academic Hospital, a public hospital in Johannesburg, South Africa, by the Radiation Sciences department of the University of the Witwatersrand. The trial was registered on the South African National Clinical Trials Register before recruitment was started (ID: 3012) and approval from the Human Research Ethics Committee was obtained (M704133 and M190295). The trial was also registered at ClinicalTrials.gov (NCT03332069). Enrolment began in January 2014 and was closed in November 2017.

Participants were randomized to receive either CRT alone (Control Group) or combined with mEHT (mEHT Group). Randomization into treatment groups was done using the REDCap on-line computer generated random-sampling tool with stratification according to HIV status and accounting for age and FIGO stage. Physicians reporting on the ^{18}F -FDG PET/CT scans were blinded to treatment allocation and did not interact with the participants, eliminating the risk of biased reporting.

2.1. Eligibility

Eligibility criteria for the trial: Females with International Federation of Gynecology and Obstetrics (FIGO)[21] stages IIB to IIIB primary, treatment naïve, histologically confirmed squamous cell carcinoma of the cervix (diagnosed based on clinical examination, chest radiography, and a pelvic ultrasound) eligible for CRT with radical intent; Signed informed consent; >18years old; Eastern Cooperative Oncology Group (ECOG) score <2; Creatinine clearance >60mL/min. Screening evaluations included full blood count, urea and creatinine levels, liver function, Human Immunodeficiency Virus (HIV) test; and a

CD4 count if necessary. An ^{18}F -FDG PET/CT scan was performed on eligible participants prior to commencement of therapy, as a baseline study against which response to treatment could be measured. Participants with bilateral hydronephrosis, visceral metastases, or fistulas visualized on the ^{18}F -FDG PET/CT scan were excluded from the study. HIV-positive patients were included provided their CD4 count was above 200 cells/ μL and/or they had been on antiretroviral therapy (ART) for more than six months.

Exclusion Criteria for the trial: Bilateral hydronephrosis; Second primary malignancy / or prior malignancy treated in the preceding two years; vesicovaginal fistula or rectovaginal fistula that required a change in treatment protocols; Abnormal liver function tests; Pregnant or breast feeding; Prior hysterectomy; Cardiovascular disease (excluding controlled hypertension); Acute or life-threatening infections or medical conditions; Contraindications to any of the prescribed treatments.

At the time of the analysis all participants were a minimum of six months-post treatment and local disease control data at six months post-treatment was available for all participants.[19] Participants were considered eligible for the sub-analysis presented in this report if: They met all the trial eligibility criteria; the pre-treatment ^{18}F -FDG PET/CT scan showed FDG-avid ($\text{SUV} > 2.5$) nodal disease outside of the pelvic treatment field; and the participants had a post-treatment ^{18}F -FDG PET/CT scan.

2.2. Data management

Participant data was captured using REDCap (Research Electronic Data Capture), an online, secure web based application hosted by the University of the Witwatersrand.

2.3. Treatment

All participants were planned to receive 50Gy in 25 fractions EBRT to the whole pelvis and 24Gy in 3 fractions of high dose rate (HDR) BT (36Gy equivalent dose in 2Gy fractions for an alpha-beta ratio of 10; source used: Iridium-191) and two doses of cisplatin (80mg/m²) administered 21 days apart (subject to the participant's fitness to receive cisplatin), as per institutional protocol. The goal of RT was for participants to receive a total dose of 86Gy equivalent by the combination of EBRT and BT. External beam radiation to the whole pelvis was delivered using a two dimensional four-field-box technique to include the tumor and pelvic nodes. Participants were simulated supine. The superior border of the Anterior-Posterior and Posterior-Anterior (AP-PA) field was mid-L5. The inferior border was either the inferior part of the ischial tuberosity or the

lowest extension of the tumor with at least a 2cm margin, whichever was lower. The lateral borders were 2cm beyond the lateral margins of the bony pelvis. For the lateral fields, the superior and inferior borders were the same as for the AP-PA fields. The anterior border was the mid to anterior third of the symphysis pubis and the posterior border was S2-S3 to include the presacral nodes and possible tumor extension along the uterosacral ligament.

Modulate electro-hyperthermia (Model: EHY2000+; Manufacturer: Oncotherm GmbH, Troisdorf, Germany) was administered twice per week (maximum ten treatments), at a maximum power of 130W, immediately before EBRT (maximum 30 minutes from completion of mEHT to completion of EBRT). Step-up heating protocols were adhered to and mEHT treatments were administered at least 48 hours apart. A 30cm diameter round electrode was used and treatment duration was 55 minutes at the final power output, with a minimum planned energy dose of 360KJs. Details of the technique are described elsewhere in the literature.[11,19,22]

2.4. Outcome Measures

Nodes with FDG-avid disease were grouped by region on the pre-treatment scans: Head and Neck; Thorax; Abdomen (including the upper pelvis outside of the radiation field); and Pelvis (within the radiation field). The standard uptake value (SUV) cut-off was considered to be 2.5 and evaluation of the ^{18}F -FDG PET/CT scans was based on PERSIST 1.0 Criteria. Tumor response was classified as Complete Metabolic Response (CMR); Partial Metabolic Response (PMR); Stable Metabolic Disease (SMD); Progressive Metabolic Disease (PMD).[23] On the follow-up scans each region was scored as: no change; resolved nodes; new nodes. Only the complete metabolic response of all disease (nodes outside of the radiation field, nodes inside the radiation field, and the tumor), as visualized on post-treatment ^{18}F -FDG PET/CT scans, was considered an indicator of the abscopal effect.

2.5. Statistics

The frequency of the observed abscopal effect was compared by group (mEHT or Control) and HIV status (positive or negative) using a Chi-squared frequency table. Paired t-test was used to compare the difference in means between groups and logistic regression was used to test prognostic factors. Two sided p values are reported and p values <0.05 were considered significant. STATA 13.0 Statistics software program (Stata Corporation, College Station, Texas, USA), was used to analyze the data.

2.6. Ethics

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (M120477 and M190295) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

3. RESULTS

3.1. Characteristics

Two hundred and ten participants were randomized for treatment, of which 146 [70%] had FDG-avid nodal disease visualized outside of the radiation field on the pre-treatment ^{18}F -FDG PET/CT scans (mEHT Group: $n=68$ [64%]; Control Group: $n=78$ [75%]). One hundred and eight of these participants survived six months post-treatment and were eligible for the post-treatment ^{18}F -FDG PET/CT scans (mEHT Group: $n=54$ [79%]; Control Group: $n=54$ [69%]) and were therefore included in this analysis. The characteristics, including treatment characteristics, of these 108 participants are listed in Table 1. The number of participants (grouped by treatment group and HIV status) with nodes visualized in each region on the pre-treatment ^{18}F -FDG PET/CT scans are shown in Figure 1. The median number of weeks between the final RT treatment and the follow-up ^{18}F -FDG PET/CT scans was 26.3 in the mEHT Group (Q1: 25.3; Q3: 27.3) and 27 in the Control Group (Q1: 26; Q3: 29).

3.2. Abscopal Effect as visualized on ^{18}F -FDG PET/CT

An abscopal response was only considered if all disease, including the primary tumor, nodes within the radiation field, and all nodes outside of the radiation field showed a complete metabolic response ($\text{SUV} < 2.5$) on the six month post-treatment ^{18}F -FDG PET/CT. Therefore all participants who had an abscopal effect also showed local disease control (a complete metabolic response of the tumor and nodes within the pelvic radiation field). The percentage of participants with complete resolution of all metabolically active disease on six month post-treatment ^{18}F -FDG PET/CT scans was higher in the mEHT group: $n=13$ [24.1%] than in the control group: $n=3$ [5.6%] (Chi-squared: $p=0.013$). There was no significant difference in the response between the HIV-positive ($n=51$) and – negative ($n=57$) groups (HIV-positive: $n=7$ [13%]; HIV-negative: $n=9$ [16%]; Chi-squared:

$p=0.793$) with a close to even split in frequency of abscopal responses observed between the HIV-positive and –negative participants in each treatment group, as seen in Figure 2.

In a multivariate analysis (confidence interval [CI] 95%) of age, cisplatin cycles, total radiation dose and the number of days between the final radiation treatment and the follow-up ^{18}F -FDG PET/CT, none of the variables were indicators of an abscopal effect (Age: OR:1.01, $p=0.692$, CI: .0.96-1.07; Cisplatin cycles: OR: 1.20; $p=0.671$; CI: 0.51-2.83; Days to PET/CT: OR: 1.01; $p=0.283$; CI: 0.99-1.07; Total RT: OR: 0.66; $p=0.316$; CI: 0.30-1.47). In a univariate analysis, the CD4 count of participants was also not predictive on an abscopal effect (OR: 1.00, $p=0.893$, CI: 0.997-1.003). In the participants in whom an abscopal effect was observed, the mean time between the final radiation and the follow-up ^{18}F -FDG PET/CT was 196 days (range 162-266).

3.3. Follow up

One participant had visceral disease on the pre-treatment ^{18}F -FDG PET/CT scan: multiple lung nodules (highest SUV in the left lung of 6.03 and the right lung of 4.38) and a lesion in the T11 vertebra (SUV 9.71). This participant was in the mEHT group. The follow-up ^{18}F -FDG PET/CT scan showed no sign of metabolically active disease. This participant is 18 months post-treatment and is still disease free. Of the participants who showed an abscopal effect, seven out of 13 mEHT participants and two out of three control participants have reached two years post treatment and are still disease free. Two participants in the mEHT group and one in the Control group demised before reaching two years (cause of death: acute renal failure). Four participants in the mEHT group have not yet reached two years post treatment (two are 18 months and two are 12 months post-treatment), however they are still disease free. Table 2 lists all the sites of FDG avid disease seen in the participants in whom an abscopal response was observed, the disease-free survival observed, the viral load, CD4 count, and the HIV status of the participants.

3.4. HIV Status

In order to rule out the effects of HIV on the visualization of nodes, the cases were reviewed with the intention of discarding cases which had nodes known to be visualized in HIV disease. Patients with HIV and high viral load levels may have benign hypermetabolic foci visualized on ^{18}F -FDG PET/CT images, resulting in false positive interpretations of malignancy.[24] Furthermore, Sathekge *et al* showed that the CD4 count of HIV positive participants was inversely proportional to the FDG uptake in the nodes.[25] During acute HIV infection FDG uptake increases in the head and neck lymph

nodes, in mid stage of HIV infection hypermetabolism occurs in cervical, axillary, and inguinal lymph nodes, and an increased FDG uptake occurs in the colon, mesenteric, and ileocecal lymph nodes during late HIV disease.[24] None of our participants were in acute (newly diagnosed) or late stage (no Acquired Immune Deficiency Syndrome-defining illnesses other than cervical carcinoma) of HIV infection. Four of the participants with an abscopal response showed increased FDG uptake in the axillary glands: one was HIV-negative and was therefore still included, three were HIV-positive and all had increased FDG uptake in extra-pelvic nodes other than the axillary nodes. These three participants were therefore still included. Of the seven HIV-positive participants, one had increased FDG uptake in the inguinal nodes however several other extra-pelvic nodes were also visualized and the patient was included.

4. DISCUSSION

The CMR of disease outside the radiation field at six months post-treatment in our sample provides evidence of an abscopal effect. The frequency of the observed abscopal effect was significantly associated with the addition of mEHT. This finding is important as methods to enhance the abscopal effect could broaden the scope of ionizing radiation from a local treatment to a systemic and potentially curative modality for metastatic disease. The abscopal effect was seen equally in HIV-positive and –negative participants in the group treated with mEHT. This suggests that the potentiation of the systemic, immune-mediated response to IR was not inhibited by HIV-infection and could still be possible in such high risk patients.

Reynders *et al* reported that the median time to achieve an abscopal response was five months, ranging from 1 to 24 months.[4] In our study we assessed the abscopal effect as part of the disease response at six months, which corresponds to the findings by Reynders *et al*. In their review Reynders *et al*, report on patients who had received multiple fractions of radiotherapy followed by a reduction in size / metabolic activity of a non-irradiated lesion (partial response). In our report we present only participants who showed a complete metabolic response of all disease, including the primary tumor. This strengthens the probability of an abscopal response in our participants. Reynders *et al* excluded papers in which systemic cytotoxic drugs were administered.[4] We have included participants who were treated with cisplatin as a radiosensitiser, however the administration of cisplatin to participants in the mEHT Group and Control group was evenly matched suggesting that the difference between responses in the two groups was not due to the cisplatin and is associated with the addition of mEHT. Furthermore cisplatin was not a predictor of an abscopal effect in our sample.

The rarity of the abscopal effect documented in the literature suggests that the abscopal effect alone is unlikely to impact clinical regimes and influence treatment choices.[3] Considering their immune components, the combination of radiotherapy with immunotherapies and mEHT may provide an opportunity to boost abscopal response rates. Reynders *et al* reported on four case reports of the abscopal effect using Ipilimumab (one in adenocarcinoma of the lung and three in melanoma patients), one using BCG-vaccination (adenocarcinoma of the lung) combined with IR, and one retrospective study in which 11 out of 21 melanoma patients treated with Ipilimumab followed by palliative radiotherapy showed an abscopal response.[4] The increase in use of immunotherapies combined with IR has resulted in an increase in the reports of abscopal effects. At least ten trials have been registered on ClinicalTrials.gov to investigate the effects, including the abscopal effect, of immunotherapies combined with IR. The lack of reliable biomarkers to predict and confirm the presence of an abscopal effect may impact on the future optimization of protocols to induce an abscopal effect. An important future field of investigation is therefore the development of biomarkers which can reliably predict and quantify the presence of an abscopal effect.

Preclinical data on the synergistic effects of immunomodulating agents and modulated electro-hyperthermia with IR, as well as case reports, and the results of this study, provide strong support for the development of trials on the combined use of IR with mEHT and immunotherapies. Positive results in such trials would broaden the scope of ionizing radiation from local or palliative treatment to a potentially curative modality in metastatic and systemic disease.

COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of Potential Conflict of Interest: The authors declare that they have no conflict of interest.

Research involving Human Participants and/or Animals: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Human Research Ethics Committee approval number: M120477) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

REFERENCES

1. Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: Evolving Considerations for PET Response Criteria in Solid Tumors. *The Journal of Nuclear Medicine*. 2009;50(5):122-151.
2. Brix N, Tiefenthaler A, Anders H, Belka C, Lauber K. Abscopal, immunological effects of radiotherapy: Narrowing the gap between clinical and preclinical experiences. *Immunological Reviews*. 2017;280(1):249-279.
3. Hlavata Z, Solinas C, Scartozzi M. The Abscopal Effect in the Era of Cancer Immunotherapy: a Spontaneous Synergism Boosting Anti-tumor Immunity? *Targeted Oncology*. 2018;13(556):113-123.
4. Reynders K, Illidge T, Siva S, Chang JY, Ruyscher D De. The abscopal effect of local radiotherapy: using immunotherapy to make a rare event clinically relevant. *Cancer Treatment Reviews*. 2018;41(6):503-510.
5. Takaya M, Niibe Y, Tsunoda S, Jobo T, Imai M. Abscopal Effect of Radiation on Toruliform Para-aortic Lymph Node Metastases of Advanced Uterine Cervical Carcinoma – A Case Report. *Anticancer Research*. 2007;504:499-503.
6. Ngwa W, Irabor OC, Schoenfeld JD, Hesser J, Formenti SC. Using immunotherapy to boost the abscopal effect. *Nature Reviews Cancer*. 2018;18(5):313-322.
7. Datta NR, Ordóñez SG, Gaipl US, et al. Local hyperthermia combined with radiotherapy and- / or chemotherapy: Recent advances and promises for the future. *Cancer Treatment Reviews*. 2015;41(9):742-753.
8. Frey B, Weiss E-M, Rubner Y, et al. Old and new facts about hyperthermia-induced modulations of the immune system. *International Journal of Hyperthermia*. 2012;28(6):528-542.
9. Knippertz I, Stein MF, Dörrie J, et al. Mild hyperthermia enhances human monocyte-derived dendritic cell functions and offers potential for applications in vaccination strategies. *International Journal of Hyperthermia*. 2011;27(6):591-603.
10. Fiorentini G, Szasz A. Hyperthermia today: Electric energy, a new opportunity in cancer treatment. *Journal of Cancer Research and Therapy*. 2006;2(2):41-46.
11. Szasz O, Szigeti GP, Szasz A. Connections between the Specific Absorption Rate and the Local Temperature. *Open Journal of Biophysics*. 2016;6(3):53-74.
12. Szigeti GP, Szasz O, Hegyi G. Personalised Dosing of Hyperthermia. *Journal of Cancer Diagnosis*. 2016;1(1):1-9.

13. Tsang Y, Huang C, Yang K, et al. Improving immunological tumor microenvironment using electro-hyperthermia followed by dendritic cell immunotherapy. *Biomed Central Cancer*. 2015;15:708.
14. Vancsik T, Kovago C, Kiss E, Papp E, Forika G, Benyo Z. Modulated electro-hyperthermia induced loco-regional and systemic tumor destruction in colorectal cancer allografts. *Journal of Cancer*. 2018;9(1):41-53.
15. Qin W, Akutsu Y, Andocs G, Suganami A, Hu X, Yusup G. Modulated electro-hyperthermia enhances dendritic cell therapy through an abscopal effect in mice. *Oncology Reports*. 2014;32:2373-2379.
16. Frey B, Rückert M, Deloch L, et al. Immunomodulation by ionizing radiation — impact for design of radio- - immunotherapies and for treatment of inflammatory diseases. *Immunological Reviews*. 2017;280(1):231-248.
17. Yang K, Huang C, Chi M, Chiang H, Shan Y-. In vitro comparison of conventional hyperthermia and modulated. *Oncotarget*. 2016;7(51):84082-84092.
18. Lee S, Lee N, Cho D, Kim J. Treatment outcome analysis of chemotherapy combined with modulated electro-hyperthermia compared with chemotherapy alone for recurrent cervical cancer, following irradiation. *Oncology Letters*. 2017;14(1):73-78.
19. Minnaar CA, Kotzen JA, Akinwale O, et al. The effect of modulated electro-hyperthermia on local disease control in HIV-positive and -negative cervical cancer women in South Africa: Early results from a phase III randomised controlled trial. *PLoS ONE*. 2019;14(6):e0217894.
20. Lee S-Y, Kim J-H, Han Y-H, CHo D-H. The effect of modulated electro-hyperthermia on temperature and blood flow in human cervical carcinoma. *International Journal of Hyperthermia*. 2018;34(7):953-960.
21. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. *International Journal of Gynecology & Obstetrics*. 2009;105(2):103-104.
22. Szasz O, Szasz A. Heating , Efficacy and Dose of Local Hyperthermia. *Open Journal of Biophysics*. 2016;6:10-18.
23. Hyun JO, Lodge MA, Wahl RL. Practical PERCIST: A Simplified Guide to PET Response Criteria in Solid Tumors 1.0. *Radiology*. 2016;280(2):576-584.
24. Davison JM, Subhramaniam RM, Surasi DS, Cooley T, Mercier G, Peller PJ. FDG PET/CT in Patients With HIV. *American Journal of Radiology*. 2011;197(2):284-294.
25. Sathekge M, Maes A, Kgomo M, Wiele C Van De. Fluorodeoxyglucose uptake by lymph nodes of HIV patients is inversely related to CD4 cell count. *Nuclear Medicine Communications*. 2010;31(2):137-140.

TABLES

Table 1: Characteristics of Participants Eligible for Analysis of the Abscopal Effect

		mEHT 54		Control 54		
FIGO Staging	IIB	25	[46%]	22	[41%]	
	III	29	[54%]	32	[59%]	
Race	African	51	[94%]	52	[96%]	
	Caucasian	1	[2%]	0	[0%]	
	Other	2	[4%]	2	[4%]	
Age [years]	Mean	49.3		49.9		$p=0.776$
	SD	9.98		9.99		
	Range	30-68		28-70		
BMI	Mean	28.7		27.0		$p=0.127$
	SD	5.61		6.01		
	Range	18-44		15-39		
Total RT dose (EQD2)	Mean	85.7Gy		86Gy		$p=0.251$
	SD	1.65		0		
	Range	74-86Gy		86Gy		
No of Cisplatin doses	Mean	1.37		1.25		$p=0.321$
	SD	0.69		0.76		
	0 doses	5	[9%]	6	[11%]	
	1 dose	19	[35%]	20	[37%]	
	2 doses	30	[56%]	28	[52%]	
Days between final RT and PET/CT	Mean	188.2		193.4		$p=0.242$
	SD	24.05		22.15		
	Range	154-310		155-266		
CD4 count [cells/μL]	Mean	552.9		543.9		$p=0.089$
	SD	264.15		276.36		
	Range	194-1077		134-1524		
No of mEHT doses	Mean	9.54				
	SD	1.07				
	Range	4-10				
Average KJ administered during mEHT	Mean	382.6KJ				
	SD	29.95				
	Range	259- 427KJ				

*Abbreviations: mEHT: Modulated Electro-Hyperthermia; FIGO: Federation of Gynecology and Obstetrics; SD: Standard Deviation; BMI: Body Mass Index; RT: Radiation Therapy; PET/CT: Positron Emission Tomography / Computed Tomography; KJ: Kilojoules

Table 2: Details of the extra-pelvic disease in participants with an *Abscopal Effect

HIV status	Days to PET	No. of ChT	Description of Extra-pelvic Disease on Pre-treatment PET/CT	Survival
<i><u>mEHT Group</u></i>				
Positive (CD4:863; VL:27)	211	2	Common carotid (SUV 7.5); Para tracheal (SUV 4.41); Axillary (SUV 5.59)	2YDFS
Positive (CD4:194; VL:<20)	163	1	Bilateral jugular digastric IIB (Left SUV 2.62; Right SUV 3.34); Axillary (SUV 4.49); Precarinal (SUV 2.75); Subcarinal (SUV 3.16); Retro-crural (SUV 3.47) Bilateral para-aortic (Left SUV 2.59; Right SUV 3.39)	Survival days: 335
Positive (CD4:905; VL:<20)	200	2	Jugular digastric (SUV 2.6); Hilar (SUV 2.92)	2YDFS
Positive (CD4:845; VL: ND)	190	2	Bilateral supraclavicular (Right SUV 6.04; Left SUV 2.94)	2YDFS
Positive (CD4:456; VL: ND)	192	2	7 Bilateral cervical (highest SUV4.81); Axillary (SUV 4.24); Subcarinal (SUV 2.73); Common iliac (Right SUV 2.93; Left SUV 4.73)	2YDFS
Positive (CD4:284; VL:196)	182	2	Bilateral Jugular Digastric (Right SUV 7.42; Left SUV 2.72); Common iliac (SUV 3.89)	Still DF at 18 month
Negative	185	1	Aorto-pulmonary (SUV 2.75)	2YDFS
Negative	197	1	Para aortic (SUV 4.73)	2YDFS
Negative	185	2	Cervical (Level IIA SUV 5.67; Level IIB SUV 3.49)	2YDFS
Negative	183	0	Bilateral axillary (Left SUV 2.87; Right 2.98)	Survival days: 596
Negative	193	2	Supraclavicular (SUV 7.42); Paratracheal (SUV 20.38); Aorto-pulmonary (SUV 14.72); Multiple bilateral hilar and Peribronchial (Highest SUV Right 17.86 and Left 13.47); Multiple bilateral pulmonary nodules (Highest SUV Right 6.03 and Left 4.38); Lesion on T11 Vertebra (SUV 9.71)	Still DF at 18 month
Negative	224	1	Coelic axis (SUV 7.93)	Still DF at 12 months
Negative	189	2	Aorto-pulmonary (SUV 4.1); Pre-carinal (SUV 3.7)	Still DF at 12 months
<i><u>Control</u></i>				
Positive (CD4:564; VL: ND)	207	0	Para aortic (SUV 3.35); Common iliac (SUV 4.5 0.9)	Survival days: 483
Negative	266	2	Aorto-pulmonary (SUV 2.79); Para aortic (SUV 4.21)	2YDFS
Negative	183	2	Para aortic (SUV 4.81); Paravertebral (SUV 6.56)	2YDFS

Abbreviations: ChT: Chemotherapy; DF: Disease Free; HIV: Human Immunodeficiency Virus; mEHT: Modulated Electro-Hyperthermia; ND: Not Detectable; SUV: Standard Uptake Value; YDFS: Years of Disease Free Survival; VL: Viral Load

*An abscopal response was considered if there was complete metabolic resolution of the extra-pelvic disease and pelvic disease, including the tumor and pelvic nodes, on the post-treatment ^{18}F -FDG PET/CT scans, with an SUV of less than 2.5

FIGURES

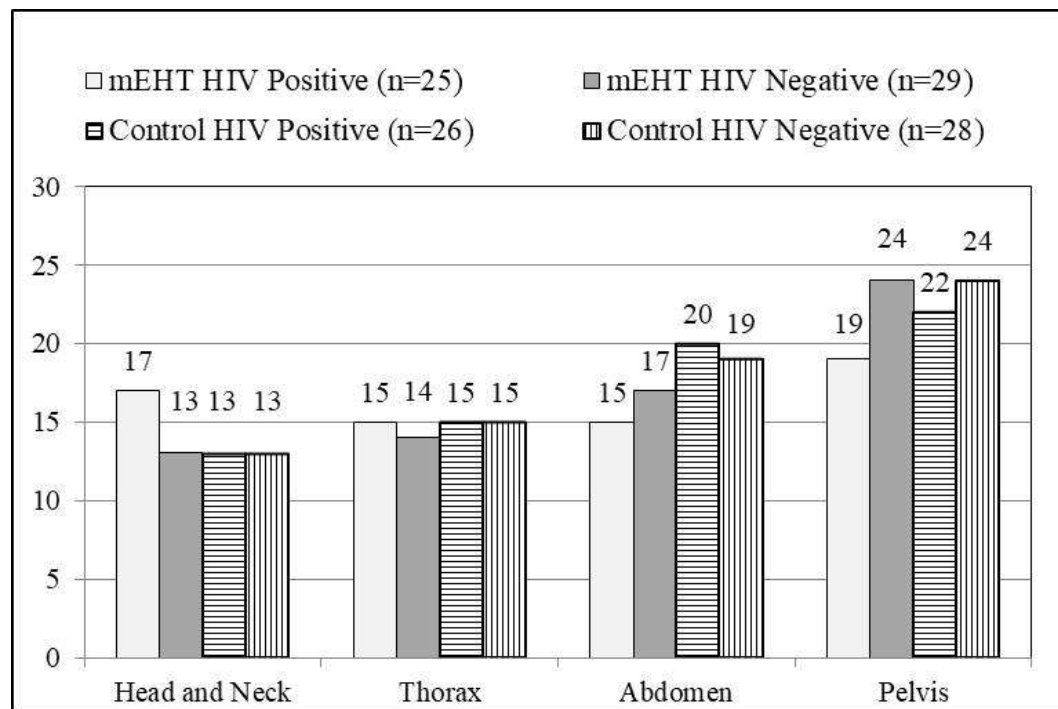


Fig. 1 Number of Patients with Nodes Visualized by Region on Pre-Treatment PET/CT Scans The number of participants with nodes visualized in each region is represented graphically, showing a similar pattern in all participants in each treatment group on the pretreatment ^{18}F FDG PET/CT. Abbreviations: mEHT: Modulated Electro-Hyperthermia; HIV: Human Immunodeficiency Virus

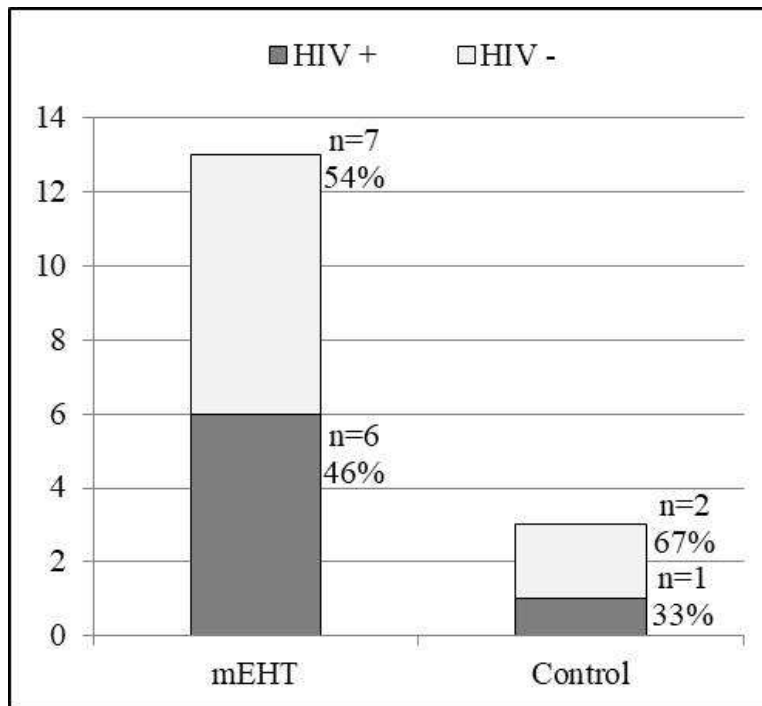


Fig. 2 Frequency of Observed Abscopal Effect in HIV-Positive and HIV-Negative Participants in each Treatment Group A significant difference between the frequency of abscopal effect was noted between the mEHT Group (13 out of 54[24.1%]) and the Control Group (3 out of 54[5.6%])($p=0.013$) There was no significant difference in frequency of the observed abscopal between the HIV-positive and HIV-negative participants *Abbreviations: mEHT: Modulated Electro-Hyperthermia; HIV: Human Immunodeficiency Virus*

CHAPTER SIX

Defining characteristics of nodal disease on PET/CT scans in HIV-positive and -negative locally advanced cervical cancer patients in South Africa

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Author Contributions

Minnaar: Conducted the research, gathered the data, statistical analysis, interpretation of results, writing of the manuscript.

Baeyens: Supervisor, Head of Radiobiology, supervised data collection and data quality control, project planning, management of funding, reviewed manuscript.

Kotzen: Supervisor, senior radiation oncologist, planning and prescribing of treatments, oversaw the treatment and follow-ups of trial participants, reviewed manuscript.

Ayeni: Reviewed ¹⁸F-FDG PET/CT reports, reviewed manuscript.

Vangu: Head of Nuclear Medicine Department; oversaw all the ¹⁸F-FDG PET/CT scans and related logistics, reviewed manuscript.

DEFINING CHARACTERISTICS OF NODAL DISEASE ON PET/CT SCANS IN HIV-POSITIVE AND -NEGATIVE LOCALLY ADVANCED CERVICAL CANCER PATIENTS IN SOUTH AFRICA

NODAL ANALYSIS OF HIV+/- LACC PATIENTS ON PET/CT

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KEYWORDS

¹⁸F-FDG PET/CT; HIV; Locally advanced cervical cancer; Lymph nodes; Disease pattern

ABBREVIATIONS

¹⁸ F-FDG:	¹⁸ Flourine-labelled glucose; 18-fluoro-2-deoxyglucose
AIDS:	Acquired Immunodeficiency Syndrome
ART:	Antiretroviral therapy
CT:	Computed Tomography
FIGO:	International Federation of Gynaecology and Obstetrics
HIV:	Human Immunodeficiency Virus
LACC:	Locally Advanced Cervical Cancer
PET:	Position Emission Tomography
RCT:	Randomised controlled trial
SUV:	Standardised Uptake Value
SUVmax:	Maximum SUV

ABSTRACT

Introduction: Literature reports increased FDG nodal uptake in HIV-positive patients. Our aim is to identify differences in presentation and characteristics of FDG-avid lymph nodes between HIV-positive and HIV-negative locally advanced cervical cancer (LACC) patients in our clinical setting.

Materials and Methods: We evaluated 250 pre-treatment ¹⁸F-FDG PET/CT imaging studies from women screened for a phase III randomised controlled trial investigating modulated electro-hyperthermia as a radiosensitiser (Ethics approval: M120477; ClincialTrials.gov ID: NCT03332069). The number of nodes; size; maximum standardised uptake value (SUVmax); symmetry; and relationship between nodal size and SUVmax uptake, were assessed by region and by HIV status.

Results: 1314 nodes with a SUVmax ≥ 2.5 were visualised. Of 128(51%) HIV-positive participants, 82% were on antiretroviral therapy (ART) and 10 had a CD4 count < 200 cells/ μ L. Overall pattern of presentation and nodal characteristics were similar between HIV-positive and –negative groups and the uniformity in presentation of the nodes draining the cervix strongly suggests these nodes may be attributed to malignancy rather than HIV infection. Novel findings: HIV infection is associated with: >four nodes visualised in the neck, symmetrical inguinal lymph nodes, increased rates of

supraclavicular node visualisation; FDG-avid axillary nodes were more common, but not exclusive, in HIV-positive participants.

Conclusion: ^{18}F -FDG PET/CT is a reliable staging method for LACC in HIV-positive patients who are not in acute stages of HIV infection, have a CD4 count $>200\text{cells}/\mu\text{L}$, and/or are on ART and there is a potential risk of underestimating metastatic spread by attributing increased nodal metabolic activity to HIV infection in these patients.

INTRODUCTION

Position emission tomography (PET) is an imaging modality that uses a tomographic technique to measure the three dimensional distribution of positron emitting labelled radiotracers. ^{18}F Flourine-labelled glucose (18-fluoro-2-deoxyglucose; ^{18}F -FDG), is the most commonly used tracer for PET studies. Most cancers characteristically have increased utilisation of glucose, upregulation of glucose transporters, and consequently an increased uptake of glucose analogue FDG on PET studies. This allows for the non-invasive quantification of tumour metabolic activity, measured by the standardised uptake value (SUV) of FDG by the tumour compared to the FDG uptake of background tissues. The addition of computed tomography (CT) allows the morphological evaluation of the tumour in order to improve the accuracy of the size and localisation, combined with the metabolic measurement on the PET images.[1]

One advantage of ^{18}F -FDG PET/CT is its ability to detect metabolic changes before anatomical changes occur, making it more sensitive to the nodal disease status of patients.[2,3] In the developed world, several studies have assessed the role ^{18}F -FDG PET/CT in the staging and management of patients with locally advanced cervical cancer (LACC), and results from ^{18}F -FDG PET/CT scans can provide important prognostic information for these cervical cancer patients.[4] The staging of cervical cancer is based on the FIGO (International Federation of Gynaecology and Obstetrics) classification, which is a clinical staging system involving a thorough clinical examination and anatomical imaging. In a resource-constrained setting, a chest x-ray, abdominal ultrasound, and cystoscopy are routinely performed on cervical cancer patients with further investigations considered on a case by case basis, and depending on resources and availability.[5]

Morkel *et al* reported that the addition of ^{18}F -FDG PET/CT to the initial screening protocols for FIGO stage IIIB cervical cancer patients revealed relevant disease information that was missed by clinical staging and other imaging techniques. The information gathered from the ^{18}F -FDG PET/CT resulted in a change in treatment

protocols in a significant number of cervical cancer patients.[5] In a study by Kidd *et al*, 53% of 234 cervical cancer patients (FIGO stage IB1–IVA), had FDG-avid lymph nodes on ^{18}F -FDG PET/CT scans, with nodes visualised as high as the supraclavicular region. Lymph node status as visualised on the ^{18}F -FDG PET/CT scans had a significant effect on the patient outcomes and the presence and number of lymph node metastases are known prognostic factors.[6,7] The sensitivity of the PET/CT studies depends on the stage of disease with limited benefit of the use of ^{18}F -FDG PET/CT scans seen in early stages of cervical cancer.[8–10] The typical metastatic pattern for cervical cancer is from the nodes directly draining the cervix (parametrium and paracervix), to the external iliac, internal iliac, obturator, and mesorectum groups (sometimes referred to as level one), to the common iliac and presacral groups (or level two groups), to para-aortic nodes (level three).[11–13]

A well-known challenge regarding the interpretation of ^{18}F -FDG PET/CT scans is the risk of false-positive results in patients with acute infections or inflammatory conditions.[14] Activated inflammatory cells, such as macrophages, lymphocytes, neutrophil granulocytes, and fibroblasts have been shown to avidly take up FDG resulting in an FDG uptake that is higher than the background uptake.[15] The gradual decrease in CD4 cells of Human Immunodeficiency Virus (HIV)-1 infected individuals is associated with a decline in the function of the immune system of the patient. The homing theory suggests that the homing of HIV-infected CD4 cells from the blood to the lymph nodes where they undergo apoptosis, is a possible cause for the decrease in CD4 counts.[16,17] This correlates with the generalised lymphadenopathy typically seen in HIV-infected patients.

Studies have demonstrated the importance of taking the HIV status of the patient into account when interpreting the results of ^{18}F -FDG PET/CT images of cancer patients. Morkel *et al* found a significant inverse relationship between the CD4 count of HIV-positive patients and the mean SUV of their lymph nodes[5] and another study found that HIV-positive patients with high viral load levels may have benign hypermetabolic foci visualised on ^{18}F -FDG PET/CT images.[18] This may result in false positive interpretations of malignancy. Increased FDG uptake of the lymphoid tissue is seen in the head and neck region during acute HIV infection, in the peripheral nodes (including the axillary and inguinal nodes) during chronic infection, and in the abdominal lymph nodes during late stages of HIV infection.[18–20] A detailed study by Brust *et al* showed that healthy HIV-infected individuals with suppressed viral loads, and their HIV-negative counterparts, had little to no FDG uptake in lymph nodes, however HIV-positive patients with high viral loads and advanced HIV infection had increased FDG uptake in the peripheral lymph nodes.[21]

To our knowledge, there are no larger studies investigating the nodal characteristics and distribution patterns of HIV-positive, compared to HIV-negative, LACC patients in a resource-constrained setting. In this paper, we report on results of ^{18}F -FDG PET/CT images from patients enrolled in an ongoing phase III randomised controlled trial (RCT) on the effects of the addition of modulated electro-hyperthermia to chemoradiation protocols in South Africa. We aim to identify differences in the characteristics of positive lymph nodes between HIV-positive and HIV-negative LACC participants. This may assist in more accurate reporting and staging of cervical cancer on ^{18}F -FDG PET/CT studies in geographic areas with a high incidence of HIV infection.

MATERIALS AND METHODS

The data for this analysis are part of a phase III RCT investigating the addition of modulated electro-hyperthermia to chemoradiotherapy for the treatment of locally advanced cervical cancer patients. The trial is being conducted in our local academic hospital by the Radiation Sciences department of the University of the Witwatersrand, in Johannesburg, South Africa. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional/national research committee (Human Research Ethics Committee approval: M120477) and the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The trial was registered on the South African National Clinical Trials Register before recruitment started (ID: 3012) and at ClinicalTrials.gov ID: NCT03332069. Informed consent was obtained from all individual participants included in the study.

Eligibility

As part of the screening evaluation for the trial protocol, potential participants had additional haematological investigations including HIV tests and a CD4 count, and underwent ^{18}F -FDG PET/CT imaging studies before randomisation, but after signing the informed consent.

Patients were considered eligible for screening for the trial, and therefore were eligible for this analysis, if they were >18 years old, with histologically confirmed squamous cell carcinoma of the cervix, FIGO stage IIB (with distal involvement of the parametrium) to IIIB (staged clinically), had a creatinine clearance >60mL/min, reported knowing their HIV-status, did not have any Acquired Immunodeficiency Syndrome (AIDS)-defining illnesses except for cervical cancer, were deemed fit for treatment with radical intent, and had a life expectancy of more than 12 months.

Once the screening process was complete, exclusion criteria for randomisation for the trial based on the screening results included a CD4 count <200cells/ μ L if HIV-positive, visceral metastatic disease, bilateral hydronephrosis or fistulas visualised on ^{18}F -FDG PET/CT scans.

A total of 270 participants were screened, 250 of whom underwent pre-treatment ^{18}F -FDG PET/CT imaging studies, and 210 were deemed eligible for trial and were randomised for treatment. For the purpose of this report we evaluated all 250 of the ^{18}F -FDG PET/CT imaging studies conducted as part of the screening process.

Patient preparation and imaging protocol

Patients were prepared for the ^{18}F -FDG PET/CT scans according to department standard protocols. Plasma glucose was checked prior to the scan and hydration was administered to patients whose plasma glucose levels were 11mmol/L or more in order to lower glucose levels and minimise the interference by high glucose.[1] The PET/CT study was rescheduled if the plasma glucose could not be adequately lowered. Our protocol aimed for an uptake period of +/- one hour between injection and scanning[1] depending on the logistics. Patients were injected with a mean of 378.51 MBq (min: 135.79 MBq; max: 462.5 MBq) of FDG. The mean plasma glucose level of participants was 5.7mmol/L (min: 3.7mmol/L; max: 12.2mmol/L) and there was a mean interval of 66.5 minutes (min: 50; max: 125) between injection time and acquisition of body image.

Scanning protocol begins with a scout image, followed by a low-dose CT image (with intravenous contrast when not contraindicated) of the whole body. This was followed by "wholebody" PET imaging in seven bed positions, 3minutes per bed, starting from mid-thigh to the base of the skull. A diagnostic CT of the chest was then performed. The ^{18}F -FDG PET/CT scans were obtained using a Siemens Biograph™ 40 PET/ CT (Siemens Healthcare GmbH; Erlangen, Germany).

Nodal Analysis

The nodal characteristics assessed were: number of nodes and their largest diameter on CT scans; uptake assessed by measured maximum SUV (SUVmax); symmetry; relationship between nodal size and SUVmax uptake. SUVmax ≥ 2.5 was used as a cut off for active nodes. Comparison of patterns was done between different anatomical regions that included the pelvis, abdomen, thorax, and head and neck. First the characteristics of all of the nodes were analysed depending on whether the node appeared in the HIV-negative or -positive participants (nodal analysis). Then the

participants in each group (HIV-positive and HIV-negative) were analysed and the characteristics of their nodes were compared (participant analysis). A subgroup analysis of specific node groups responsible for the drainage of the cervix and nodes known to be positive in HIV-infection was conducted.

Statistics

Frequencies and percentages were calculated and we used Chi-squared frequency tables, unpaired t-test, linear regression and logistic regression models to measure associations, differences, and odds ratios. STATA 13.0 Statistics software program (Stata Corporation, College Station, Texas, USA), was used to analyse the data.

Funding

This study was funded by the National Research Foundation of South Africa (TP12082710852). The ^{18}F -FDG was supplied at a discounted rate by Radioisotopes SOC Ltd. The funding sources did not play a role in study design, implementation, data collection, data analysis or in the development/review of the manuscript.

RESULTS

The characteristics of the participants are listed in Table 1. The majority of the participants were African (92%), 51% (n=128) were HIV-positive, 119 (82%) of whom were on antiretroviral therapy (ART) at the time of the scan. The median CD4 count was 506.5 cells/ μL (range: 95-1524 cells/ μL) and ten participants had a CD4 count below 200cells/ μL .

Overall Nodal Analysis

In total 2299 nodes were visualised, 1314 of which had a SUVmax ≥ 2.5 and were included in the analysis (HIV-positive: 713[54%]; HIV-negative: 601[46%]). A summary of the number of nodes per region, and of the HIV status of the participant in which the node was visualised, can be seen in Table 2.

SUVmax Analysis by Region: Overall, the mean SUVmax was significantly higher in the pelvis and in the abdomen, as compared to the head and neck region ($p < 0.001$) and thorax ($p < 0.001$), however the difference in SUVmax between the abdominal and pelvic

nodes was not significant ($p=0.397$). The mean SUVmax was highest in the pelvis and this was significantly higher than any other region ($p<0.001$).

SUV and HIV Status: When considering all of the nodes, in a multivariate logistic regression analysis, a higher SUVmax was a predictor of the HIV status of the participants in which the node was visualised (OR: 0.97; $p=0.022$; 95% CI: 0.945-0.996). However when considering the nodes by region, the only region in which the SUVmax of the nodes was predictive of the HIV status of the participants was the abdomen (OR: 0.92; $p=0.006$; 95% CI: 0.86 to 0.98). When considering the SUVmax of all of the visualised lymph nodes, grouped into bins of ten starting from 3, HIV-negative participants were more likely to have a SUVmax of between 3 and 23 and a SUVmax above 23 is associated with an HIV-positive status (Chi-squared: $p=0.028$). The association was however not significant in a Chi-squared analysis of each region. In the HIV positive patients, the relationship between SUVmax and CD4 count was not significant ($p=0.131$; 95%CI: -0.003 to 0.0003).

Size Analysis by Region: The largest nodes were seen in the pelvis and the difference in sizes between the pelvic and the abdominal nodes was significant ($p<0.0001$). The lymph nodes in the abdomen were significantly larger than the nodes of the thorax ($p=0.0047$), and the head and neck ($p<0.0001$).

Size and HIV Status: When considering the size of the nodes (in centimetres), grouped into 0.5cm bins, the only significant finding was that the nodes from the abdomen of HIV-positive participants are more likely to be under 1.49cm (106 out of 146 versus 80 out of 142), and the nodes from HIV-negative participants are more likely to be 1.49cm to 2.49cm (51 out of 145 versus 28 out of 142) (Chi-squared: $p=0.027$). In a logistic regression analysis, lymph node size was not predictive of the HIV status; however, an increased size was significantly associated with lower CD4 counts in the HIV-positive patients (coefficient: -21.34; $p=0.033$; 95%CI -40.94 to -1.75).

Relationship between SUV and Size: The mean size of the visualised nodes was 1.55cm (standard deviation: 0.93; min: 0.5cm; max: 14.2cm chain of nodes) and the mean SUVmax was 6.43 (standard deviation: 4.87; min: 2.5; max: 54.21). There is a moderately strong ($r=0.5$) and significant ($p<0.001$) positive relationship between the overall SUVmax and node diameter. This relationship was significant for each region, regardless of HIV status, when analysed independent of the participants and when analysed per participant (see Fig 1).

Analysis of Lymph Nodes within Participants (Participant Analysis)

In paired t-test analyses; the maximum size of the nodes, the maximum SUVmax of the nodes and the number of nodes overall and by region, were assessed in each participant. There were no significant differences in the numbers of nodes in the HIV-positive compared to the HIV-negative participants ($p>0.05$). These results are summarised in Table 3 and represented graphically in Supplementary images 1, 2 and 4. In order to determine if there was an association between HIV status and a specific size or SUVmax, nodes were organised into bins and frequency tables were generated. No significant association was seen when looking at participants' maximum or mean size or SUVmax. The only significant association between HIV-status and the number of lymph nodes seen in a participant's region was in the head and neck where more than four nodes is more strongly associated with HIV infection (Chi-squared: $p=0.004$).

Sub Group Analysis

A subgroup analysis was conducted of specific lymph node groups known to be positive on ^{18}F -FDG PET/CT in HIV-positive patients in order to determine whether, in our sample, there is a risk of under-staging of the cervical cancer by attributing positive nodes to HIV-infection rather than malignancy.

Head and Neck nodes (known to be positive in early HIV infection): Of specific interest in the head and neck region was the significantly higher number of supraclavicular nodes visualised in the HIV-positive participants (mean: 0.15, standard deviation 0.454, 95%CI 0.069 to 0.228), as compared to the HIV-negative participants (mean: 0.05, standard deviation: 0.217, 95%CI: 0.01 to 0.09) ($p=0.0296$). Posterior cervical nodes were also visualised significantly more ($p=0.0037$) in HIV-positive participants (mean: 0.21, standard deviation 0.053, 95%CI 0.107 to 0.315) than in the HIV-negative participants (mean: 0.04, standard deviation: 0.022, 95%CI: 0.002 to 0.084).

Axillary Nodes (known to be positive in chronic HIV infection): Seven out of 122 (6%) HIV-negative and 29 out of 128 (23%) HIV-positive participants presented with axillary nodes visualised on ^{18}F -FDG PET/CT scans. In a logistic regression analysis, each increase in the number of axillary nodes is associated with a 2.7 fold increase in the odds of the patient being HIV-positive ($p=0.001$) with HIV-positive participants having a maximum of 3 axillary nodes and a mean of 0.37 nodes and HIV-negative participants having a maximum of two and a mean of 0.08 nodes ($p=0.001$). Symmetry was not associated with HIV status (symmetry of axillary nodes in HIV-positive participants: 17 out

of 29, 59%; symmetry of axillary nodes in HIV-negative participants: 3 out of 7, 43%; $p=0.451$).

Inguinal Nodes (known to be positive in chronic HIV infection): The SUVmax and size were not predictors of HIV status in the inguinal lymph nodes. However the number of inguinal lymph nodes and the symmetry was significantly associated with HIV infection (symmetry of inguinal nodes seen in HIV-positive participants: 11 out of 21, 52%; symmetry of inguinal nodes seen in HIV-negative participants: 2 out of 14, 14%; $p=0.022$). The number of nodes was a significant predictor of HIV status ($p=0.047$, 95%CI: 1.01 to 2.83, OR: 1.69) in a logistic regression and there was a significant difference in the mean number of nodes between the two groups with a mean of 0.6 nodes in the HIV-positive participants and a mean of 0.3 nodes in the HIV-negative participants (t-test: $p=0.0417$).

Colic or Mesenteric Nodes (known to be positive in late stage HIV infection): There were no significant associations between HIV status and the number, symmetry, SUVmax or size of the colic, para-colic or mesenteric lymph nodes.

Nodes involved in draining the cervix: Nodes included in this analysis are paracervical, parametrial, presacral, sacral, external and internal iliac, common iliac, hypogastric, and para-aortic nodes. There were no significant relationships between HIV status and SUVmax, size, number, or symmetry in these nodes.

Additional findings: In our sample, aorto-pulmonary nodes were significantly ($p=0.0096$) more frequent in HIV-negative participants (mean: 0.15, standard deviation 0.454, 95%CI 0.01 to 0.08) than in the HIV-positive participants (mean: 0.05, standard deviation: 0.217, 95%CI: 0.08 to 0.22) and abdominal caval nodes had a higher SUVmax in HIV-negative participants (mean: 7.941) than in HIV-positive (mean: 5.685) ($p=0.0430$).

DISCUSSION

The uniformity in presentation of nodes draining the cervix in HIV-positive and HIV-negative LACC participants confirms that visualisation of these nodes on ^{18}F -FDG PET/CT scans may be attributed to malignancy rather than HIV status of the participants in our sample. Overall there are some novel differences between the visualisation of lymph nodes in HIV-positive and HIV-negative participants in our study, which may be of use in deciding the likely cause of the increased FDG uptake in some patients, however

the overall pattern of presentation and nodal characteristics are similar between the two groups.

There is a significant association between a higher SUVmax and a larger diameter of lymph nodes visualised in ^{18}F -FDG PET/CT. The SUVmax and size of the nodes were consistently higher in the pelvis and abdomen than in the thorax and head and neck regions, regardless of HIV status. The number of nodes visualised in the pelvis was higher than in any other region. This is all in line with the literature and the expected route of metastatic spread of cervical cancer.[11–13] In a report by Iyengar *et al* [22] on FDG uptake in 12 patients with acute HIV infection and 11 patients with mid/chronic HIV infection, all patients presented with an FDG uptake that was greater in the cervical and axillary lymph nodes than in the inguinal and iliac chains. The patients in their study were however not all on ART and there was a significant correlation between FDG PET signal and viral load.[22] In our sample of HIV-positive cervical cancer patients, most of which were on ART, the highest SUVmax was still seen in the pelvis and abdomen.

There is a risk of underestimating the extent of lymph node involvement in HIV-positive participants if the increased FDG uptake of a node is incorrectly attributed to HIV infection. We were therefore specifically interested in the differences in lymph node presentation between HIV-positive and HIV-negative participants in our sample.

The visualisation of head and neck nodes is frequently cited in the literature as being associated with acute HIV-infection.[18–20,22] The majority of participants (82%) in our study were on ART for more than six months and the majority of the participants not on ART were aware of their HIV-status and were not likely to be in acute stages of HIV infection. The majority of our participants also had a CD4 count $>200\text{cells}/\mu\text{L}$. Although more head and neck lymph nodes were visualised in the HIV-positive participants in our sample, the differences in the number of head and neck nodes visualised between HIV-positive and HIV-negative participants was not significant. However, more than four nodes in the head and neck region were more likely to be seen in HIV-positive patients. Further histological evaluation of nodes in this region for malignancy in HIV-positive patients may therefore still be warranted.

Kidd *et al*,[6] reported that 53% of 234 LACC patients presented with FDG-avid lymph nodes on ^{18}F -FDG PET/CT scans and in that sample, the nodes were visualised as high as the supraclavicular region. Our sample only included FIGO stages IIB to IIIB patients (staged clinically) and the number of participants with FDG-avid nodes in our study was considerably higher (90%), likely due to a higher proportion of our patients with more advanced stages of disease. We also saw increased FDG uptake in the supraclavicular

nodes which are known to be potential sites of metastatic spread in cervical cancer patients. In our sample however, the number of supraclavicular nodes visualised was significantly higher in the HIV-positive participants, despite the management of the HIV-infection with ART. Based on these findings, further assessment of supraclavicular nodes visualised in HIV-positive cervical cancer patients in order to rule out HIV infection as a cause of the increased FDG-uptake, may be warranted.

While axillary nodes were significantly associated with HIV-infection in our sample, the relationship was not exclusive, with 6% of HIV-negative participants presenting with FDG-avid axillary nodes. Spread to axillary nodes can therefore not be conclusively excluded in HIV-positive immunocompetent patients with chronic HIV-infection who are on ART and have a CD4 count >200 cells/ μ L.

Symmetrical inguinal lymph nodes are more likely to be visualised in HIV-positive participants, even if their HIV infection is managed. This is in line with the literature which has found inguinal nodes to be FDG-avid during chronic HIV infection.

In HIV-positive participants, larger nodes were associated with lower CD4 counts and the only difference in size between HIV-positive and –negative participants was seen in the abdomen where larger nodes were more common in HIV-positive patients. The literature shows a significant association between CD4 count and SUVmax,[16,17] however we did not see the same significant association. The majority of our participants had a CD4 count above 200cells/ μ L and the association may therefore be more relevant to patients with a CD4 count <200 cells/ μ L. Based on our results it appears that the SUVmax in immunocompetent patients is a reliable measurement of metabolic activity in potentially metastatic nodes.

Very few of the participants in this study were in late stage of HIV-infection with only 7.8% of HIV-positive participants presenting with a CD4 count <200 cells/ μ L. The lack of colic or mesenteric nodes visualised in our sample is therefore in line with the literature.[19]

Limitations

Although histology remains the gold standard for a definitive diagnosis of the nature of lymph node pathology, histological sampling of all PET positive lymph nodes is not always ethically feasible.

Conclusion

Our results indicated that ^{18}F -FDG PET/CT is a reliable staging method for LACC regardless of HIV status, provided the patients are not in acute stages of HIV infection, have a CD4 count above 200cells/ μL , and/or are on ART. The results of this study are clinically relevant as this may be of assistance in determining which lymph nodes visualised on ^{18}F -FDG PET/CT are likely to be due to metastatic spread in HIV-positive patients with locally advanced cervical cancer, and in preventing under-staging of HIV-positive LACC patients.

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DISCLOSURE

The authors declare that they have no conflict of interest.

REFERENCES

1. Boellaard R, Delgado-Bolton R, Oyen WJG, et al. FDG PET/CT: EANM procedure guidelines for tumour imaging: version 2.0. *European Journal of Nuclear Medicine and Molecular Imaging*. 2014;42(2):328-354.
2. Hansen HV, Loft A, Berthelsen AK, Christensen IJ, Høgdall C, Engelholm SA. Survival outcomes in patients with cervical cancer after inclusion of PET/CT in staging procedures. *European Journal of Nuclear Medicine and Molecular Imaging*. 2015;42(12):1833-1839.
3. Magné N, Chargari C, Vicenzi L, et al. New trends in the evaluation and treatment of cervix cancer: The role of FDG PET. *Cancer Treatment Reviews*. 2008;34(8):671-681.
4. Sun Y, Lu P, Yu L. The Volume-metabolic Combined Parameters from ^{18}F -FDG PET/CT May Help Predict the Outcomes of Cervical Carcinoma. *Academic Radiology*. 2013;23(5):605-610.
5. Morkel M, Ellmann A, Warwick J, Simonds H. Evaluating the role of f-18

- fluorodeoxyglucose positron emission tomography/computed tomography scanning in the staging of patients with stage IIIB cervical carcinoma and the impact on treatment decisions. *International Journal of Gynecological Cancer*. 2018;28(2):379-384.
6. Kidd EA, El Naqa I, Siegel BA, Dehdashti F, Grigsby PW. FDG-PET-based prognostic nomograms for locally advanced cervical cancer. *Gynecologic Oncology*. 2012;127(1):136-140.
 7. Joo JH, Kim YS, Nam J. Prognostic significance of lymph node ratio in node-positive cervical cancer patients. *Medicine*. 2018;97(30):e11711.
 8. Kidd EA, Siegel BA, Dehdashti F, et al. Lymph Node Staging by Positron Emission Tomography in Cervical Cancer: Relationship to Prognosis. *Journal of Clinical Oncology*. 2010;28(12):2108-2113.
 9. Crivellaro C, Signorelli M, Guerra L, et al. 18F-FDG PET/CT can predict nodal metastases but not recurrence in early stage uterine cervical cancer. *Gynecologic Oncology*. 2012;127(1):131-135.
 10. Driscoll DO, Halpenny D, Johnston C, Sheehy N, Keogan M. 18F-FDG-PET/CT is of limited value in primary staging of early stage cervical cancer. *Abdominal Imaging*. 2015;40(1):127-133.
 11. Silva LP da, Moreira CMM, Amorim MHC, et al. Contextual quality of life of HIV-positive patients with cervical carcinoma at Tygerberg Hospital. *AIDS Care*. 2014;28(1):1-8.
 12. Benedetti-Panici P, Maneschi F, Scambia G, et al. Lymphatic Spread of Cervical Cancer: An Anatomical and Pathological Study Based on 225 Radical Hysterectomies with Systematic Pelvic and Aortic Lymphadenectomy. *Gynecologic Oncology*. 1996;24(0184):19-24.
 13. Höckel M, Horn L, Tetsch E, Eimenkel J. Pattern analysis of regional spread and therapeutic lymph node dissection in cervical cancer based on ontogenetic anatomy. *Gynecologic Oncology*. 2019;125(1):168-174.
 14. Stadalnik RC, Bakheet SM, Powe J. Benign causes of 18-FDG uptake on whole body imaging. *Seminars in Nuclear Medicine*. 1998;28(4):352-358.
 15. Bental M, Deutsch C. Metabolic changes in activated T cells: An NMR study of human peripheral blood lymphocytes. *Magnetic Resonance in Medicine*. 1993;29(3):317-326.
 16. Cloyd MW, Chen JJ, Wang I. How does HIV cause AIDS? The homing theory. *Molecular Medicine Today*. 2000;6(3):108-113.
 17. Dimopoulos Y, Moysi E, Petrovas C. The Lymph Node in HIV Pathogenesis. *Current HIV/AIDS Reports*. 2017;14(4):133-140.
 18. Davison JM, Subhramaniam RM, Surasi DS, Cooley T, Mercier G, Peller PJ. FDG PET/CT in Patients With HIV. *American Journal of Radiology*. 2011;197:284-294.

19. Scharko AM, Perlman SB, Pyzalski RW, Graziano FM, Sosman J, Pauza CD. Whole-body positron emission tomography in patients with HIV-1 infection. *The Lancet*. 2003;362(9388):959-961.
20. Sathekge M, Maes A, Kgomo M, Wiele C Van De. Fluorodeoxyglucose uptake by lymph nodes of HIV patients is inversely related to CD4 cell count. *Nuclear Medicine Communications*. 2010;31(2):137-140.
21. Brust D, Polis M, Davey R, et al. Fluorodeoxyglucose imaging in healthy subjects with HIV infection: impact of disease stage and therapy on pattern of nodal activation. *AIDS*. 2006;20(7):985-993.
22. Iyengar S, Chin B, Margolick JB, Sabundayo BP, Schwartz DH. Anatomical loci of HIV-associated immune activation and association with viraemia. *The Lancet*. 2003;362(9388):945-950.

TABLES

Table 1: Participant Characteristics

		OVERALL		HIV-positive		HIV-negative		P value
		n	[%]	n	[%]	n	[%]	
		250		128	[51%]	122	[49%]	
ECOG Performance Status	0	10	[4%]	5	[3.9%]	5	[4%]	χ^2 : $p=0.382$
	1	238	[95%]	121	[94.5%]	117	[96%]	
	2	2	[1%]	2	[1.6%]	0	[0%]	
Race	African	229	[92%]	123	[96%]	106	[87%]	χ^2 : $p=0.004$
	Caucasian	10	[4%]	0	[0%]	10	[8%]	
	Other	11	[4%]	5	[4%]	6	[5%]	
Age (years)	Median	47.7		43.2		52.5		t-test: $p<0.0001$
	Range	25.9-72.7		26.6-62.4		25.9-72.7		
BMI	Median	26		24		28		t-test: $p=0.0032$
	Range	15-49		5-47		15-49		
CD4 count (cells/μL)	Median	506.5		506.5				
	Range	95-1524		95-1524				

Abbreviations: BMI: Body Mass Index; HIV: Human Immunodeficiency Virus; ECOG: Eastern Cooperative Oncology Group

Characteristics of the participants in the study: ECOG performance was similar in both groups; there were significantly more African HIV-positive participants and more Caucasian HIV-negative participants; the mean age and BMI was significantly higher in the HIV-negative participants.

Table 2: Number of Nodes per Region and the HIV Status of the Participant in which the Nodes were Visualised

	Total	HIV-positive		HIV-negative	
Head and Neck	225	139	[62%]	86	[38%]
Thorax	210	115	[55%]	96	[45%]
Abdomen	293	149	[51%]	144	[49%]
Pelvis	586	310	[53%]	276	[47%]
Total number of nodes	1314	713	[54%]	601	[46%]

Overall there were more nodes visualised in HIV-positive participants, with the largest difference in the number of nodes between HIV-positive and –negative participants seen in the head and neck region.

Table 3: Comparison of Means Summary of Nodes per Patient

	HIV-positive (n=128)		HIV-negative (n=122)		
Head and Neck	n=51	[40%]	n=43	[35%]	p=0.453
	Mean	Std dev	Mean	Std dev	t-test
No nodes per participant	1.09	1.88	0.71	1.31	p=0.0652
SUVmax	4.48	2.05	4.68	2.86	p=0.6869
Diameter	1.17	0.29	1.21	0.36	p=0.5964
Highest SUVmax	5.63	4.07	5.69	4.60	p=0.9442
Maximum Diameter	1.41	0.55	1.34	0.54	p=0.5256
Thorax	n=54	[42%]	n=44	[36%]	p=0.322
	Mean	Std dev	Mean	Std dev	t-test
No nodes per participant	0.90	1.35	0.78	1.31	p=0.4774
SUVmax	5.14	3.09	4.93	3.25	p=0.7473
Diameter	1.42	0.66	1.29	0.47	p=0.2707
Highest SUVmax	5.86	3.65	5.70	3.98	p=0.8300
Maximum Diameter	1.74	1.85	1.49	0.69	p=0.4017
Abdomen	n=68	[53%]	n=62	[51%]	p=0.715
	Mean	Std dev	Mean	Std dev	t-test
No nodes per participant	1.16	1.44	1.18	1.55	p=0.9315
SUVmax	6.65	4.50	7.67	4.38	p=0.1940
Diameter	1.45	0.69	1.55	0.85	p=0.4334
Highest SUVmax	7.85	5.26	9.11	5.35	p=1.1779
Maximum Diameter	1.77	1.21	1.89	1.23	p=0.5763

Pelvis	n=99 Mean	[77%] Std dev	n=98 Mean	[80%] Std dev	p=0.564 t-test
No nodes per participant	2.42	2.06	2.26	1.89	p=0.5248
SUVmax	6.76	4.09	6.79	3.60	p=0.9471
Diameter	1.70	0.73	1.69	0.76	p=0.9218
Highest SUVmax	9.22	6.90	8.90	5.50	p=0.7212
Maximum Diameter	2.27	1.30	2.20	1.34	p=0.7073
Total	n=114 Mean	[89%] Std dev	n=111 Mean	[91%] Std dev	X2:p=0.613 t-test
No nodes per participant	5.57	4.74	4.93	4.54	p=0.2739
SUVmax	3.57	2.44	3.51	2.54	p=0.8676
Diameter	0.88	0.49	0.83	0.48	p=0.4424
Highest SUVmax	9.67	7.05	9.44	5.89	p=0.7911
Maximum Diameter	2.48	1.78	2.32	1.41	p=0.4488

Abbreviations: Std Dev: Standard deviation; SUV: Standardised Uptake Value;

There were no significant differences seen between the number of nodes per patient, maximum SUV uptake and maximum diameter of the visualised lymph nodes between HIV-positive and HIV-negative participants.

FIGURES

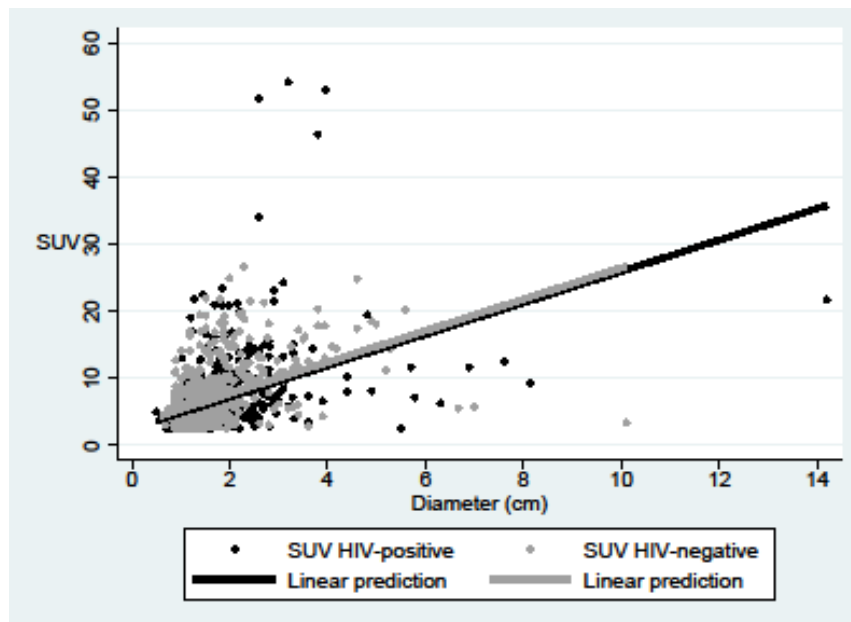
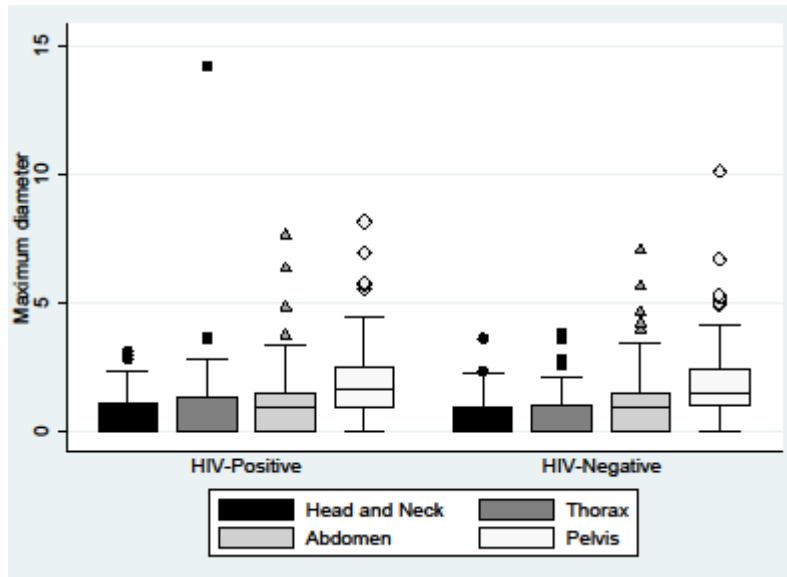


Fig 1 Linear Relationship between the SUVmax and Size of the Lymph nodes in HIV-Positive and –Negative Participants

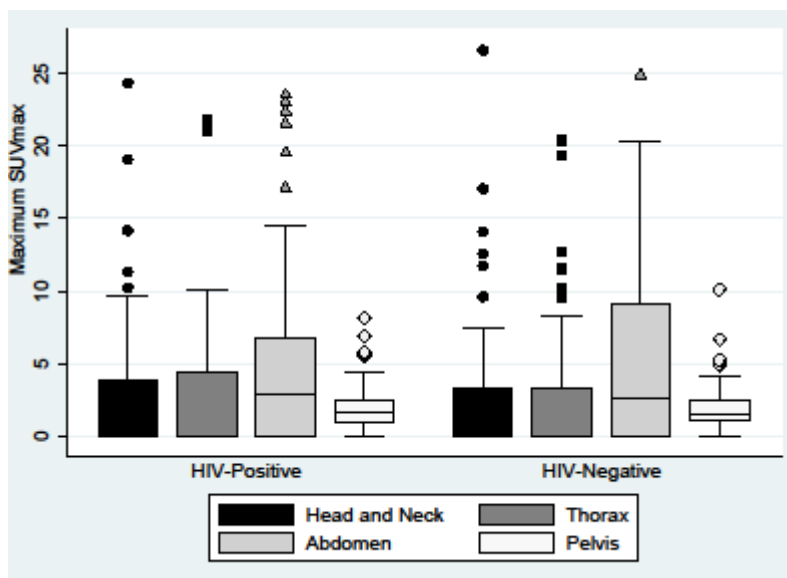
A significant ($p<0.001$) positive association is seen between the SUVmax of the lymph node and the diameter of the lymph node, and this relationship true is independent of HIV status.

SUPPLEMENTARY MATERIAL



Sup 1: Image – Graphical representation of the maximum diameters of the visualised nodes (centimetres)

The largest node visualised was a chain of nodes >14cm in the thorax of an HIV-positive participant. Overall there were no significant differences between the maximum diameters of nodes in each region in HIV-positive participants compared to HIV-negative participants.



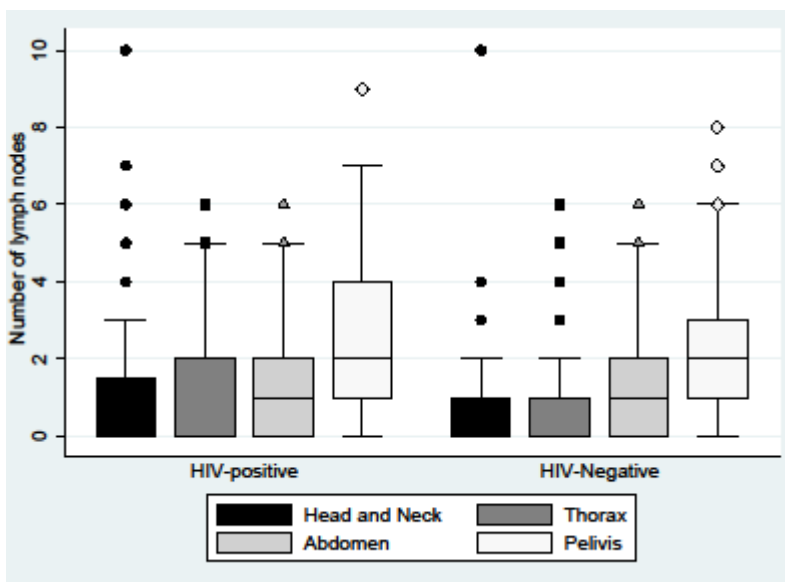
Sup 2: Image – SUVmax of the Visualised Lymph Nodes

The SUVmax of nodes in each region in HIV-positive and -negative participants is not significantly different.



Sup 3: Image – Maximum SUV visualised on ^{18}F -FDG PET/CT images

The maximum SUV measured in a node of an HIV-positive participant was 54.2 in the pelvis of participant 42. This participant had the third highest tumour SUV (62.81) in the HIV-positive group and the fourth highest tumour SUV out of all of the participants. In comparison, the highest SUV measured in a pelvic node in an HIV-negative participant was 25.02 (participant 127) and the maximum node overall visualised in an HIV-negative participant was 26.6 in the head and neck region (participant 28).

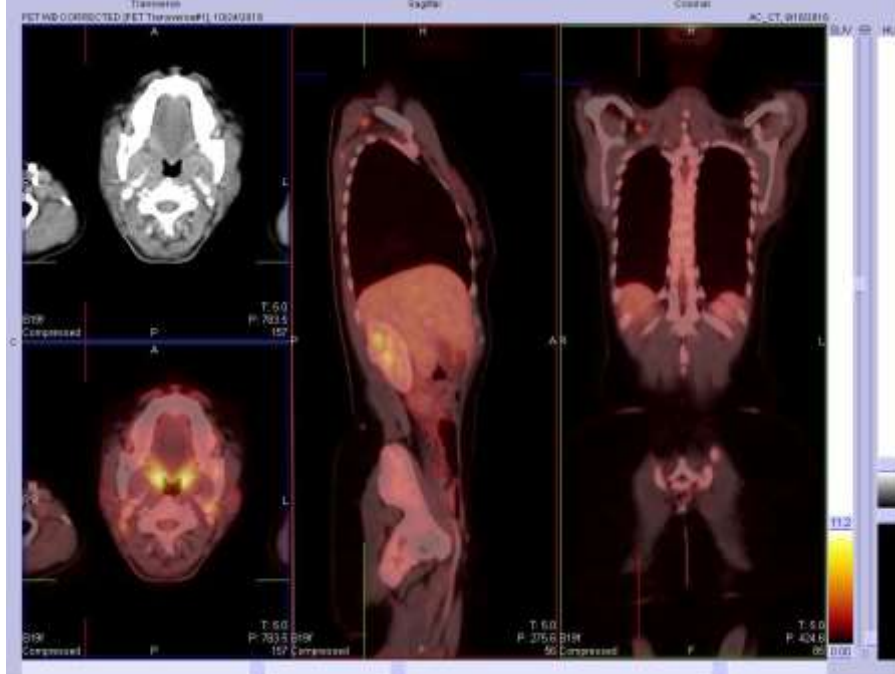


Sup 4: Image – Number of Visualised Lymph Nodes

More HIV-positive participants present with head and neck lymph nodes visualised on PET/CT scans however the mean number of nodes in the pelvis of participants in both groups is higher than the mean number of nodes visualised in any other region.

Sup 5: Image – Images with the max number of nodes in the head and neck region

Side by side comparison of the maximum number of nodes visualised in the head and neck on the ^{18}F -FDG PET/CT scans of the a) HIV-positive and b) HIV-negative participants. Head and neck nodes are not exclusively visualised in HIV-positive participants, although >4 nodes in the region is associated with HIV infection and more supraclavicular and posterior cervical nodes were visualised in HIV-positive patients.



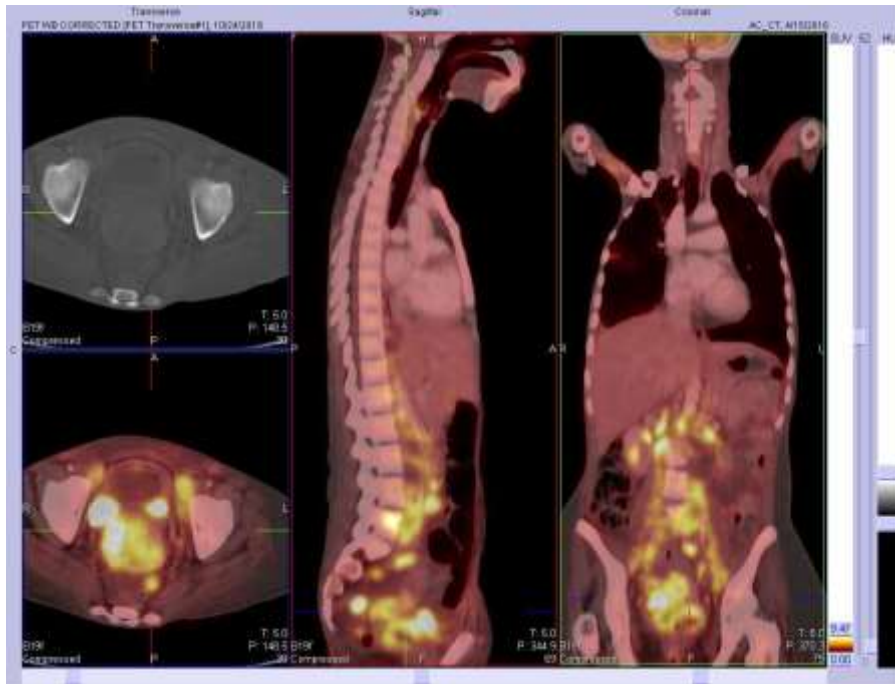
- a) Two HIV-positive participants had 7 nodes visualised in the head and neck region. In this image, participant 167 is shown with a SUVmax in the head and neck of 5.87 and right hand side supraclavicular node (SUV:3.59)



- b) The maximum number of nodes visualised in the head and neck region of an HIV-negative participant was 10 (participant number 215) with a SUVmax of 5.05. This participant has posterior cervical nodes visualised and also had the highest number of abdominal nodes out of all of the HIV-negative participants (n=6).

Sup 6: Image – Images with the max number of nodes in pelvic region

Side by side comparison of the maximum number of nodes visualised in the pelvis on ^{18}F -FDG PET/CT scans of the a) HIV-positive and b) HIV-negative participants.



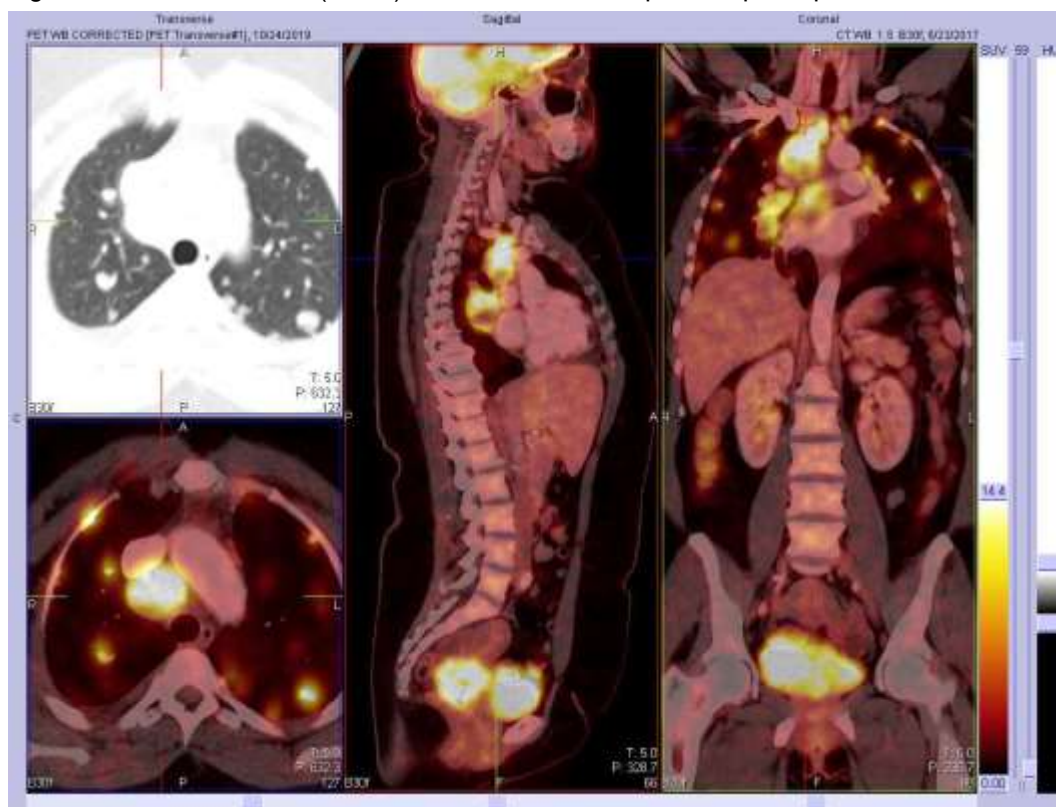
- a) The maximum number of nodes visualised in the pelvis of HIV-positive participants was nine, in participants number 42 and number 114. Participant 114 in this image had a SUVmax of 15.13 in the pelvis, with four nodes visualised in the abdomen (one of which was the largest of all abdominal nodes in the HIV-positive participants: 7.6cm). One node was visualised in the thorax and no nodes were visualised in the head and neck region.



- b) The maximum number of pelvic nodes visualised in an HIV-negative participant was eight, seen in participant 122, with a SUVmax of 6.27 and a tumour SUV of 29.7.

Sup 7: Image – Image with the max SUV and sizes of nodes in an HIV-positive participant.

Participant 237 had the largest head and neck node (3.1cm) and the highest SUV in the neck (24.38) and the largest chain of nodes visualised in the thorax (>14cm) and the highest SUV in the thorax (21.77) out of all of the HIV-positive participants.



CHAPTER SEVEN

DISCUSSION

7.1. Overview

Locally advanced cervical cancer and the treatment of the disease are associated with high rates of morbidity (Oliver and Chiao, 2017) and impaired quality of life. (Bjelic-Radisic *et al.*, 2012; Derks *et al.*, 2017) Treatment for LACC includes external beam radiation, high dose rate brachytherapy (Kotzen, 2016; Marth *et al.*, 2017) and cisplatin as a radiosensitiser. (Marth *et al.*, 2017) The addition of cisplatin substantially increases the toxicity profile of the treatments. Although radiation associated adverse events can be reduced with more advanced treatment and planning techniques, these techniques are expensive and are not available in resource-constrained settings. (Fernandez-Ots and Crook, 2013)

Local disease control and survival outcomes for HIV-positive cancer patients are worse, as are oncology treatment-related adverse events. (Coghill *et al.*, 2013, 2015; Simonds, Neugut and Jacobson, 2015; Ulrike *et al.*, 2015; Dryden-Peterson *et al.*, 2016) The differences in responses to treatment by HIV-positive patients must be taken into account when planning a treatment regimen involving chemotherapy and radiotherapy. (Housri, Yarchoan and Kaushal, 2010) which in itself is challenging in resource-constrained settings. Human Immunodeficiency Virus positive patients may also be more radiosensitive. (Baeyens *et al.*, 2010; Herd *et al.*, 2014, 2016) which may increase the risk of radiation-related adverse events. It is therefore important that any new addition to the radiation treatment protocols is tested for safety in these high-risk, radiosensitive LACC patients.

In resource-constrained settings, follow-up care is limited and improving first-line treatment and minimising treatment-related toxicities are therefore important goals in the management of LACC patients in resource-constrained environments. Expensive and complex radiotherapy techniques, targeted therapies, immunological agents, and complex and expensive hyperthermia devices are not feasible in these settings.

7.2. Dosing Calculations in Modulated Electro-Hyperthermia *versus* Hyperthermia

Modulated electro-hyperthermia is a HT technique that proposes an alternative to the conventional HT dosing calculations and as a result mEHT does not require the thermo-monitoring systems, specialised facilities, and specialised staff that many other HT techniques require. This makes mEHT a potential tool for clinicians in resource-constrained environments to use to improve treatment outcomes without increasing the toxicity profile of the available treatments and without significantly impacting on the limited resources and costs available.

The main difference between mEHT and other HT techniques is the dosing calculation. Conventional HT techniques rely on CEM43 which evaluates the direct necrotic effect of heat. In the CEM43 method, the goal is to reach an average temperature of 43°C in the tumour and the number of necrotic cells at this temperature depends on the tumour volume and exposure time.(van Rhoon, 2016) For this method to be effectively calculated there must be an effective thermo-monitoring system in place. The most common thermo-monitoring methods are real time MR thermo-monitoring during the treatment, or the insertion of multiple probes into the tumour to measure various temperature points. Both thermo-monitoring techniques are expensive and carry risks and HT manufacturers have therefore developed software systems to estimate the temperature in the tumour based on phantom measurements, making the systems more accessible to patients and clinicians. There is strong evidence showing that although temperatures of 43°C have direct tumour killing effects via necrosis, such high temperatures may not result in effective radio-sensitisation and may actually be counterproductive.(Vaupel, Kallinowski and Harburg, 1987; Shen *et al.*, 1994; Song *et al.*, 1995, 2009; Erdmann, Lang and Seebass, 1998) Temperatures higher than or equal to 43°C cause vasoconstriction and a subsequent reduction in circulation and oxygen perfusion to the tumour.(Peeken *et al.*, 2017) This in turn reduces oxygen and drug delivery. The CEM43 method does not take into account the radio-sensitising effects of the HT treatments.(van Rhoon, 2016) In addition, not all tumours are considered to be “heatable” based on the CEM43 method. In one study on 122 participants with superficial tumours (<3cm from the surface), 11(13%) participants were deemed to have “un-heatable” tumours as defined by the CEM43 method, using microwave heating (433MH).(Jones *et al.*, 2005)

The method proposed by Oncotherm, and used in their mEHT devices, is based on the energy (Kilojoules, KJ) applied during the treatment, in much the same way as RT uses J/kg (Gray) and chemotherapies use mg/m2 as a dose. Rather than measuring the outcome, the method measures the input of energy, and the dose limiting factor is patient

tolerability and toxicity.(Szigeti, Szasz and Hegyi, 2016) Heating of the tumours is however still achieved using mEHT and the increased perfusion resulting from the heating has been demonstrated in human and animal studies. In a study by Lee *et al.*, the use of mEHT on cervical tumours resulted in an average 2°C increase in peri-tumour temperature and an increase in blood flow (as measured by a Doppler ultrasound).(Lee *et al.*, 2018) Kim *et al.* showed that mEHT applied for 30 minutes at a temperature of 41°C increased perfusion and tumour oxygenation, enhancing the tumour-killing effect of ionizing radiation, in fibrosarcoma tumours grown subcutaneously in the legs of C3H mice.(Kim *et al.*, 2018)

It is this concept that attracted this research team to mEHT and led to the investigation of the use of mEHT in our resource-constrained setting. Without complex thermo-monitoring systems, the implementation of the mEHT treatments was feasible and mEHT was easily integrated into the work flow in our resource-constrained setting.

7.3. Depth of Energy Penetration

Another common challenge in HT, along with the temperature and dose measurement, is the penetration of thicker layers of adipose tissue without causing adipose burns. Simulations have shown that thicker layers of adipose tissue are contraindications for HT, especially HT techniques using radiofrequency capacitive heating. Numerical models by D'Ambrosio and Dughiero suggest that the ability of radiofrequency capacitive heating to effectively heat tumours in the pelvis through adipose layers thicker than 1.5 to 2cm is not possible without severe adipose burns.(D'Ambrosio and Dughiero, 2007) Kok *et al.* reported that tumour control is correlated to the target temperatures as defined by CEM43 dose measurements. Kok *et al.* used phantom simulations to determine the heating efficacy of capacitive coupled heating versus radiative heating and found that while superficial tumours were easily heated using capacitive coupling, the use of capacitive heating for deep tumours was unreliable.(Kok *et al.*, 2018) The simulation used a 13.56MHz radiofrequency generated capacitive coupling set-up to determine the heating of the deep tumours. Modulated electro-hyperthermia also uses 13.56MHz radiofrequency, however, as described in Chapter Four, factors such as the precise impedance matching and amplitude modulation in the mEHT devices improves the SAR, allowing a lower power output and a safer treatment.

We evaluated the impact of larger adipose layers on treatment outcomes in our study by analysing the incidence of adipose burns in participants who had a Body Mass Index

(BMI) >25 (overweight) and by assessing the frequency of complete response rates of the tumour in this subset of patients. As reported in Chapter Three, overweight participants were equally likely to achieve a complete metabolic response of the tumour and the radio-sensitising effect of mEHT was not impaired in obese patients. Kok *et al.* suggested that effective heating using capacitive methods can only be achieved in thin patients. We can therefore deduce that since the outcomes in our overweight and thin participants were similar in our sample, the overweight participants received an effective energy dose during the treatment.

7.4. Adverse Events due to Modulated Electro-Hyperthermia versus Hyperthermia

Quality of Life and toxicity are reported in Chapter Four. We found that participants who received mEHT experienced significant improvements in cognitive function and a significant reduction in fatigue and pain post-treatment. At three months post-treatment participants in the mEHT groups reported significantly higher social and emotional functioning than in the Control Group. None of the reviewed HT studies on LACC included quality of life assessments in their reports.

In our study, subcutaneous burns due to mEHT were significantly higher in the mEHT Group on treatment (10 out of 105 [9.5%] versus 1 out of 101 [1%]), however these resolved by three months post treatment. In three of these participants the number of mEHT treatments was reduced by 40%. None of the participants had reductions in their RT dose or delays in the RT schedule as a direct result of the mEHT-related adipose burns. Pain was reported in 9[8.6%] and superficial burns in 2[2%] of the mEHT Group participants (Chapter Four).

The participants with adipose burns did not have an increase in RT-related toxicities. Of the participants who had mEHT-related adverse events, three had grade 3 to 4 CRT-related toxicities (one haematological, one lower GIT and one renal). These participants did not experience a significant increase in CRT related toxicities (n=3[17.7%]) compared to the mEHT patients who did not experience mEHT adverse events (28 out of 88, [31.8%]). Kok *et al.* reports that RF capacitive heating has a high risk of inducing adipose burns in thick adipose due to the high power needed to administer the treatment. We used a very low power (130W compared to a range of 800 to 1500W used by other devices) and we showed that adipose layer thickness is not associated with adipose burns using 130W. The reasons for adipose burns are believed to be reduced vascular supply in adipose tissue, resulting in an inability of the tissue to cool down effectively.

Alterations in the flow of current could also result in an increased temperature and an adipose burn. This may happen if there is excessive perspiration, excessive scar tissue, or previous injury at the treatment site. Patients show sensitivities to chemotherapy and radiotherapy and it is possible that sensitivities to mEHT treatments exist which have not yet been identified. Investigations to determine the specific causes of mEHT related sensitivity in patients may be of interest.

In a phase I/II study investigating the effects of HT combined with CRT for the management of 68 LACC participants, adverse events directly attributed to HT included pain (n=5[7%]), superficial burns (n=12[18%]) and subcutaneous fatty necrosis (n=5[7%]). (Westermann *et al.*, 2005) Following this study, a phase III study was designed to investigate long term benefits of the addition of HT to CRT protocols. The study was however closed early due to slow recruit and only 99 participants were randomised. The authors report that there were no unexpected toxicities in either group, although further details are not given. (Flameling *et al.*, 2016) Both of the above mentioned studies used radiative HT. The only completed phase III trial to investigate conventional HT combined with CRT for LACC was conducted in Japan by Harima *et al.* and used a capacitive HT device. The compliance to the HT treatments was low in comparison to our study (92% of participants received only four out of six HT treatments). However the reasons for poor compliance are not given and may be due to HT adverse events, patient concerns, or other reasons not stated. (Harima *et al.*, 2016) The compliance to mEHT treatments in our study was very good indicating good tolerance of the treatment.

Although the frequency of subcutaneous burns was higher in our mEHT Group, the overall dermatological and subcutaneous toxicity was not significantly different between the groups and overall mEHT did not have an effect on the toxicity profile of RT or cisplatin, as reported in Chapter Four, which is in line with the literature. (Westermann *et al.*, 2005; Flameling *et al.*, 2016; Harima *et al.*, 2016)

7.5. Effects of Modulated Electro-Hyperthermia in HIV-Positive Participants

Cervical cancer patients with HIV infection have higher rates of CRT-related adverse events and have worse survival outcomes than HIV-negative patients. (Gichangi *et al.*, 2006; Coghill *et al.*, 2015; Simonds, Neugut and Jacobson, 2015; Dryden-Peterson *et al.*, 2016) The increased systemic radiosensitivity seen in HIV-positive patients (Baeyens *et al.*, 2010; Herd *et al.*, 2014, 2016) may be contributing factors towards the increased rates of radiation-related toxicity. As mEHT is a radiosensitiser, there was a need to

determine the safety of the addition of a radiosensitiser to a sub group of patients (HIV-positive) who are potentially radiosensitive and are already at a higher risk of RT-related toxicities. Modulated electro-hyperthermia purports to be selective with minimal heating of the healthy tissues and reduced risks of superficial and subcutaneous burns. Therefore there should not be an increase in RT-related toxicities of the healthy tissue; however confirmation of safety in high risk HIV-positive patients was still necessary. Chapter Four describes the rates of adverse events in all groups in detail. We did not see significant differences in toxicity between the HIV-positive and –negative participants in the mEHT group, indicating that mEHT does not increase RT-related toxicity in HIV-positive patients.

When considering clinical outcomes, contrary to what the literature predicts, our HIV-positive participants did not have worse rates of local disease control. However in our sample, HIV-positive participants were receiving ART and the majority had CD4 counts above 200cells/ μ L, which is not always the case in the literature reports.

7.6. Clinical Outcomes: Modulated Electro-Hyperthermia versus Hyperthermia

Three trials investigated HT combined with CRT for the treatment of LACC,(Harima *et al.*, 2001; Westermann *et al.*, 2005; Flaming *et al.*, 2016) two meta-analyses have been conducted on RT alone or combined with HT for LACC patients,(Lutgens *et al.*, 20AD; Datta *et al.*, 2017) and four studies have been conducted on chemotherapy with or without HT for local recurrent or residual disease in cervical cancer patients.(Rietbroek *et al.*, 1996; de Wit *et al.*, 1999; Franckena *et al.*, 2007; Lee *et al.*, 2017) The direct comparison of treatment outcomes in our study to the treatment outcomes in the literature is difficult due to the differences in HT techniques and protocols and the differences in measuring tumour response and outcomes between the studies. Table 1 summarises studies on the use of HT for the treatment of cervical cancer, listing the number of participants per study as well as the stages of disease. The type of HT treatments, and treatment protocols as well as the chemotherapy and RT used are also listed.

In our study we applied a protocol of 130W for one hour, at an estimated temperature of 42.5°C based on the calculated temperature coefficient. The mEHT treatments were applied twice per week, immediately before external beam radiation, and not on the same day as cisplatin administration or brachytherapy treatments. We measured LDC using ^{18}F -FDG PET/CT at six months post-treatment. Local disease control for our study

included lymph nodes or disease in the radiation field and our criteria for LDC was strict with any sign of disease in the radiation field resulting in a failure.

Chapter Three discusses our results on LDC and local disease-free survival (LDFS) at six months post-treatment. Two hundred and two participants were eligible for six month LDFS analysis, of which 171 participants were alive at six months post-treatment. Complete metabolic response (CMR) of all local disease in the radiation field in the participants who survived at six months was significantly associated with mEHT administration. Participants in the mEHT Group were significantly more likely to achieve a LDC and six month LDFS than participants in the Control Group.

A study on RT with or without HT by Franckena *et al.* showed that the number of HT treatments emerged as a predictor of outcome with patients who received more than three HT treatments having a higher CR. Seventy percent of patients in the study received five or more HT treatments and 10% received three or less HT treatments. Hyperthermia treatments were administered 0.5-4 hours after RT.(Franckena *et al.*, 2008) Our study did not show an association between the number of mEHT treatments and the CR; however 97% of participants had 8 to 10 mEHT treatments. A further analysis of the combined studies on the effects of the treatment duration, schedule and frequency on the outcomes could help to standardise HT and mEHT treatment protocols.

We reviewed two meta-analyses(Lutgens *et al.*, 20AD; Datta *et al.*, 2016) on the combination of HT and RT for the treatment of LACC. In the meta-analysis of six trials by Datta *et al*, a CR was achieved in 75.6% of patients receiving RT + HT (n=135) compared to 53.8% of patients treated with RT alone (n=132; $p<.001$).(Datta *et al.*, 2016) In the Cochrane review by Lutgens *et al*, six trials were evaluated and the authors found that the combined treatment of HT plus RT resulted in a significantly higher complete response rate, significantly reduced local recurrence rate at 3 years, and a significantly better overall survival at three years.(Lutgens *et al.*, 20AD)

When comparing studies on HT for LACC, ours is the only study using mEHT as a radiosensitiser combined with CRT. In the phase III trial by Harima *et al.* an 8MHz radiofrequency capacitive coupling device was used with a power output of between 800W and 1500W. The HT treatment was applied for 60 minutes, once a week, within 30 minutes after RT. A 4-point micro-thermocouple sensor was placed in the rectum and vagina and the maximum tumour temperature was defined as the maximum temperature between the rectum and vagina. The maximum tumour temperature reading was 42.2°C and the average tumour temperature was 41.1°C. Despite the increase in temperature,

there were no significant differences in survival, DFS or CR rates between the two treatment groups.(Harima *et al.*, 2016) This raises the question of the value of thermo-monitoring and of CEM43 as the only form of dose measurement or measurement of the treatment's ability to affect the tumour and treatment outcomes.

Within the two studies using radiative heating techniques combined with CRT for LACC, one showed no difference in response between the two groups(Flameling *et al.*, 2016) and one did not have a control group, although they showed a 90% complete remission rate in the patient sample which is significantly higher than what is reported elsewhere.(Westermann *et al.*, 2005)

The only study to date investigating mEHT for the treatment of cervical cancer was conducted by Lee *et al.* and investigated mEHT combined with platinum based chemotherapies for the treatment of residual disease or local recurrent disease.

When evaluating outcome measures the majority of studies only assessed tumour control and only used physical examinations and cytology smears to determine response rates. Only a few included pelvic lymph nodes and imaging techniques (CT or MRI) to determine response. Several challenges are associated with clinical staging, such inadequate evaluation of the proximal tumour extension or side wall invasion, and inaccurate lymph node evaluation (especially deep pelvic lymph nodes).(de la Peña *et al.*, 2008) Fluorodeoxyglucose PET/CT is a more effective imaging technique for the detection of lymph node involvement,(de la Peña *et al.*, 2008) distant metastases,(Morkel *et al.*, 2018) and tumour activity.(Magné *et al.*, 2008; Testa *et al.*, 2014; Hansen *et al.*, 2015) In the only complete phase III trial on HT combined with CRT, Harmia *et al.* measured complete response on physical examination or imaging or histopathology studies and the time point was one month post-treatment. The number of patients measured by physical examination compared to imaging studies is not reported.(Harima *et al.*, 2016) Our evaluation of local disease control was strict, with either a complete response or not a complete response (residual disease) reported, and 93.5% of our participants were evaluated using ¹⁸F-FDG PET/CT scans as well as clinical evaluations.

Our local disease control results are considerably better than the results from other triple therapy studies, suggesting that mEHT is a stronger radio-sensitiser than other HT techniques, for LACC participants. The improved local control rates in our study were seen independently of the addition of cisplatin.

Table 1: Summary of Local Disease Control Results in the Reviewed Literature

Reference	Sample and FIGO stage	HT	RT / ChT	Local Control Evaluation	Results
Triple Therapy					
(Westerman <i>et al.</i> , 2005)	Stage LACC; HCRT: n=68; No control	Radiative; 1/week (average 5); concurrent with cisplatin or independent of cisplatin before or after RT; 60 minutes; 40.7°C	EBRT + HDR + Cisplatin	Complete Remission, no further details	Complete remission: 90%
(Flameling <i>et al.</i> , 2016)	Stage LACC CRT: n=50; HCRT: n=49	Radiative heating; 1/week (average 5) concurrent with cisplatin;	EBRT + HDR + Cisplatin	Local control: not specified	CRT: 76%; HCRT: 86% $p=0.54$
(Harima <i>et al.</i> , 2016)	Stage IB-IVA CRT: n=50; HCRT: n=51	8MHz Capacitive heating; 1/week (average 5); Within 30 minutes after RT; 60 minutes; 43°C; 800-1500W	EBRT + HDR + Cisplatin	Tumour only; Examination, MRI(if indicated) Cytology/biopsy	CRT: 77.6%; HCRT: 88% $p=0.192$
HT plus RT					
(Datta, Bose, and Kapoor 1987)	Stage IIIB RT: n=26; RHT: n=27	27MHz capacitive system; 2/week; before RT; 15-20 minutes; 42.5°C; power unknown;	EBRT	Clinical evaluation only	RT: 58% RHT: 74%
(Sharma <i>et al.</i> , 1991)	Stage II-IIIB RT: n=22; RHT: n=20	27MHz capacitive system; 3/week (total 12); before RT; 45 minute; 43°C; Power unknown	EBRT + HDR	Tumour control only;	RT: 30%; RHT: 50%
(van der Zee <i>et al.</i> , 2000)	Stage IIB-IVA RT: n=56; RHT: n=58	Radiative HT; 1/week (total 5); 1-4 hrs after RT; 60 minutes; 42°C; Average 706W;	EBRT + HDR	Tumour only; examination; cytology	RT: 57%; RHT: 83% $p=0.003$
(Harima <i>et al.</i> , 2001)	Stage IIIB RT: n=20; RHT: n=20	8MHz capacitive system; 1/week (total 3); Within 30 minutes of RT; 60 minutes; 40.6°C; 800-1500W;	EBRT + HDR	Tumour only; Examination or MRI (if indicated) Cytology/ biopsy	RT: 50%; RHT: 80% $p=0.048$
(Vasanthan <i>et al.</i> , 2005)	Stage IIB-IVA RT: n=55; RHT: n=55	8MHz Capacitive heating; 1/week (average 5); Immediately after RT; 60 minutes; 38.1-42°C; 450W to >600W	EBRT + HDR	Local control; Examination OR CT/MRI when appropriate (n= unknown)	68.5% No difference $p=0.58$
(Chen, Fan and Luo, 1997)	Stage IIB-IIIB RT n=30; RHT n=39	intravaginal applicators; 2/week (total 6); after RT; 45 minutes; 42°C; Power unknown	EBRT + HDR	Complete Remission	RT: 46%; RHT 60% $p>0.05$

Reference	Sample and FIGO stage	HT	RT / ChT	Local Control Evaluation	Results
HT plus Cisplatin in Previously Irradiated Tumours					
(Franckena <i>et al.</i> , 2007)	Previously irradiated N=47; No control	Radiative HT >400W; vaginal temperature 40.6°C for 60 minutes	Platinum based; 6 cycles	Objective Response (includes PR and CR)	55%
(Rietbroek <i>et al.</i> , 1996)	Previously irradiated N=10; No control	(70MHz four antenna phased array system; 60 minutes	Cisplatin max 12 cycles	Response rate	50%
(de Wit <i>et al.</i> , 1999)	Previously irradiated N=19; No control	Radiative HT >400W; 42°C for 60 min	Platinum based; 6 cycles	Objective Response (PR and CR)	53%
(Lee <i>et al.</i> , 2017)	Previously irradiated CT: n=20; mEHCT: n=18	13.56MHz RF modulated Capacitive heating; 3/week (total 36); 60 minutes; 80-150W	Platinum based; 4-7 cycles	Tumour response	CT: 20%; mEHCT: 50% <i>p</i> =0.0461

Abbreviations: FIGO: International Federation of Gynaecology and Obstetrics; RT: Radiotherapy; ChT: Chemotherapy; HT: Hyperthermia; CRT: Chemoradiotherapy; RHT: Radiotherapy + Hyperthermia; HCRT: Chemoradiotherapy + Hyperthermia; mEHT: Modulated Electro-Hyperthermia; mEHCT: Modulated Electro-Hyperthermia + Chemotherapy; EBRT: External Beam Radiation; HDR: High Dose Rate Brachytherapy; MRI: Magnetic Resonance Imaging; CT: Computed Tomography; LACC: Locally Advanced Cervical Cancer; RF: Radiofrequency.

7.7. Disease Pattern and Metastases

The risk of increasing the rates of distant metastases with the addition of HT has been discussed in the literature with at least one study reporting an increase in rates of distant metastases in LACC participants treated with radiation plus HT. (Vasanthan *et al.*, 2005) Longer follow-ups will provide more information on the risk, if any, of mEHT increasing the risk of distant metastases post-treatment. In our local control results however, we found that disease outside of the radiation field as visualised on pre-treatment ¹⁸F-FDG PET/CT scans, was more likely to resolve in the participants receiving mEHT (13 out of 54, 24.1%) than in the Control Group (3 out of 54, 5.6%). There was no difference in the frequency of complete metabolic resolution of all disease between the HIV-positive and – negative participants. Extra-pelvic disease included lymph nodes visualised as well as a

lesion on T12 and lung metastases in one of the participants. This novel finding which is described in detail in Chapter Five, suggests the potentiation of the abscopal effect by mEHT. This finding may have significant impact on future research and the use of mEHT and RT. An increase in the abscopal response of RT could move RT from a treatment modality that is strictly for local control, to a systemic treatment modality.

In order to confirm that the lymph nodes visualised outside of the radiation field had a higher SUV due to metastatic disease and not due to HIV infection, a thorough comparative analysis of the lymph node characteristics (SUV, size, region, and symmetry) visualised on the pre-treatment ^{18}F -FDG PET/CT scans of HIV-positive and –negative participants was conducted. These results are presented in Chapter Six. We found that the visualisation of four or more lymph nodes in the head and neck region was more likely in HIV-positive participants. The visualisation of axillary lymph nodes was significantly, but not exclusively, associated with HIV infection. In addition we found that the size of the lymph nodes and the SUV of the lymph nodes had a positive linear relationship. The pelvis and abdomen regions were associated with larger lymph nodes with a higher SUV and the pelvis contained the most lymph nodes. The nodes visualised in our sample followed the known pattern of spread of cervical cancer cordially from the regional cervical lymph nodes. Based on our evaluation, the lack of significant differences between HIV-positive and –negative participants may indicate that there is a risk of underestimating the extent of disease spread in HIV-positive participants in whom FDG avid nodes are attributed to HIV-infection rather than metastatic disease. These findings are likely to assist in staging HIV-positive cervical cancer patients, who are on ART and have a CD4 count above 200cells/ μL , more accurately. Based on these findings, the lymph nodes visualised outside of the treatment field and reported on in the analysis of the abscopal effect can confidently be attributed to metastatic disease.

Modulated electro hyperthermia has shown to have enhanced effects on the immune system, compared to conventional HT, and these effects likely contribute to the reported increased rates of abscopal responses seen in patients treated with mEHT in our study (Chapter Five). When compared to other forms of HT, a higher rate of apoptosis, increased HSP70 expression and release, increased expression of caspase-3, 8 and 9, and increased levels of calreticulin and E-cadherin in the cell membrane were seen in samples treated with mEHT.(Yang *et al.*, 2016)

Jeon *et al.* reported on the pathways involved in the suppression of proliferation, long-term colony formation, and the induction of apoptotic cell death by the application of mEHT on human hepatocellular carcinoma (HCC) cell lines in suspension and in murine

models. The study reported a significant inhibition of tumour growth of human HCC xenografts in nude mice. Inhibition of tumour growth was also observed after the treatment of melanoma by mEHT in murine models.(Besztercei *et al.*, 2019)

In a study on glioma cell lines, Cha *et al.* reported that mEHT inhibited glioma cell growth and induced E2F1-mediated apoptosis in suspension models and in murine models.(Cha *et al.*, 2015) Modulated electro-hyperthermia also inhibited cell growth in ovarian and cervical cancer lines in suspension and murine models and the effect was enhanced by the addition of autophagy inhibitors.(Yang *et al.*, 2019) *In vivo* animal studies have demonstrated the induction of apoptosis in HT29 colorectal cancer xenografts and C26 colorectal cancer allografts in mice.(Tsang *et al.*, 2015; Vancsik *et al.*, 2018) Modulated electro-hyperthermia can induce caspase-independent(Meggyeshazi *et al.*, 2014) and -dependent cell death(Vancsik *et al.*, 2019) in colorectal cancer xenografts in mice, and can induce a DAMP-related signal sequence in colorectal cancer xenografts.(Andocs, Meggyeshazi, *et al.*, 2015) The induced immunogenic cell-death by DAMP signals is selective of the cancer-cells as mEHT enhances the production of antigen presenting cells and CD4+ and CD8+ T-cells.(Meggyeshazi *et al.*, 2014; Andocs, Meggyeshazi, *et al.*, 2015; Vancsik *et al.*, 2018)

7.8. HIV Infection and CRT-Related Toxicity

Suggestions in the literature that HIV-infection could result in increased mucosal and dermatological acute toxicities(Housri, Yarchoan and Kaushal, 2010) are based on reports from the early 1980s on the management of HIV-positive Kaposi Sarcoma patients. Antiretroviral therapy has improved since then and the management and understanding of HIV/AIDS has changed. It is not accurate to base current treatment and protocol changes on 30 year old data. In a 2010 review of the literature, Housri *et al.* reported increased GIT, genitourinary (GUT) and cutaneous toxicity in cervical cancer patients treated with RT,(Gichangi *et al.*, 2006; Housri, Yarchoan and Kaushal, 2010) however the authors concluded that there was insufficient evidence to recommend a change in protocols. In the studies reviewed by Housri *et al.*, only a limited number of participants were on ART. Cervical cancer is an AIDS defining malignancy and it is therefore not unexpected that many HIV-positive cervical cancer patients will present with AIDS. Better clarification between the risk of RT-related toxicity in HIV-positive cervical cancer patients who are immunodeficient, versus those who are immunocompetent, would assist in isolating the subset of patients who may be at risk of RT-related toxicities. In a multivariate regression analysis in our study, a positive HIV status increased the

odds of developing dermatologic/subcutaneous early toxicities; however the number of cisplatin doses administered also increased the risk of developing toxicities in the dermatologic/subcutaneous system as well as in the renal and neurological systems. When looking only at grade 3-4 toxicities there was no significant increase in the odds ratio of developing acute toxicities in any system associated with HIV status. Our participants were immunocompetent and were on ART which may have reduced their risk of developing RT-related toxicities as reported in the literature. An interesting finding was the increase in vomiting seen in HIV-negative participants in each group, a finding that was also noted in a study on HIV-positive and –negative cervical cancer patients in Zambia.(Mdletshe, Munkupa and Lishimpi, 2016)

Since the review by Housri *et al*, there have been more papers published on the association between treatment-related toxicity and HIV-positivity in cervical cancer patients. In a study conducted in Cape Town, South Africa, Simonds *et al*. reviewed 213 cervical cancer patients, 36(16.8%) of whom were HIV-positive. The median CD4 count of the HIV-positive patients was 342cells/μL, with a range of 33 to 790cells/μL. Six HIV-positive patients (16.7%) had a CD4 count below 200cells/μ and only sixteen patients (44.4%) began ART prior to starting treatment for their cancer. A higher risk of developing acute haematological toxicity was seen in HIV-positive patients receiving CRT (chemotherapy: cisplatin or carboplatin, depending on renal function) compared to HIV-negative patients, and all patients receiving CRT were more likely to experience haematological toxicities compared to patients treated with RT alone.(Simonds, Neugut and Jacobson, 2015) Vendrell *et al*. confirmed these findings in a report on 61 cervical cancer patients treated with CRT in Europe. Six of the patients (9.8%) were HIV-positive. The authors found that HIV-positive women had a higher risk of developing neutropenia when treated with CRT. This resulted in lower rates of chemotherapy treatment completion, more treatment delays, and more frequent Granulocyte-Colony Stimulating Factor administration. No detrimental impact on the delivery of planned RT was noted. Two (33.3%) of the HIV-positive participants were not receiving ART and were severely immunocompromised.(CD4 counts <100cells/μL).(Vendrell *et al*., 2018)

A recent study by Einstein *et al*. conducted at two sites, one in Harare Zimbabwe and one at the same institution that our study was conducted at, investigated the safety of CRT (using cisplatin) in 40 HIV-positive cervical cancer women on ART. As these women were all on ART for at least four months prior to starting CRT, and the median CD4 count was 424cells/μL (139–1204), the women are assumed to have been at a lower risk of immunodeficiency at the time that they started treatment. This study found that the CRT-related toxicity, and subsequent delays or dose reductions, were comparable to HIV-

negative cervical cancer patients. This suggests that the combination of cisplatin and ART is safe and that these immunocompetent women were not at a higher risk of RT-related toxicities or chemotherapy-related toxicities.(Einstein *et al.*, 2019)

Haematological toxicities as described above can be easily explained by the effects of the HIV virus on the immune system, specifically on the lymphocytes.(Deeks *et al.*, 2015) However the association between HIV infection, systemic (chromosomal) radiosensitivity, the development of local toxicity from RT (such as increased GUT and GIT toxicities), and tumour response, is still not clear. Two theories are described in the literature. One involves a systemic glutathione deficiency caused by HIV infection which results in the depletion of radio-protective thiols and increased oxidative stress,(Vallis, 1991) and the other involves the effects of the HIV-1 Tat protein on the host's ability to repair radiation-induced DNA double strand breaks in certain cells.(Housri, Yarchoan and Kaushal, 2010)

In a retrospective cohort study by Simonds *et al.* on cervical cancer patients in South Africa, HIV-infection was associated with inadequate RT and fewer doses of cisplatin. Importantly this study also showed that a poor response at six weeks was associated with Stage IIIB disease and inadequate radiation. Compared to our sample of HIV-positive patients (n=105), the HIV-positive patients in the study by Simmonds *et al.* in 2012 (n=59) appeared to have a higher risk of being immunocompromised. Forty (77.8%) of the participants were not on ART and the median CD4 count of the HIV- positive patients was 354cells/ μ L (range 33-1249) with only 88.1% of the patients presenting with a CD4 count above 200cells/ μ L.(Simonds *et al.*, 2012) Reasons for inadequate RT in this study included GIT toxicity, neutropenia, and renal failure.

The increased systemic radiosensitivity measured in HIV-positive(Baeyens *et al.*, 2010) and cervical cancer patients(Herd *et al.*, 2016) reported by Baeyens *et al.* and Herd *et al.* respectively does not appear to play a role in the development of acute CRT-related toxicity in our participants. The participants of these two studies, like our participants, were not likely to be immune deficient. Both studies used the micronucleus (MN) assay to assess the systemic radiosensitivity of the circulating lymphocytes extracted from participants and irradiated *in vitro*. Herd *et al.*(Herd *et al.*, 2016) reported that two participants could not be evaluated as the numbers of binucleated cells after exposure to 4Gy or IR were too low. The CD4 counts of the participants and the ART status of the participants were not reported in this study and it is therefore not clear how immunocompromised/immunocompetent these participants were. It is likely that, if the samples produced sufficient lymphocytes to be evaluated, the participants were not extremely immune deficient.

In the study by Baeyens *et al.* all participants who were on ART had been on ART for a minimum of 6 months before entering the study and the CD4 counts were all above 200cells/ μ L. Of the participants not on ART, the lowest CD4 count was 268cells/ μ L. In this study, participants on ART showed enhanced systemic radiosensitivity compared to HIV-positive participants not on ART and all HIV-positive participants showed enhanced systemic radiosensitivity compared to HIV-negative participants.(Baeyens *et al.*, 2010) In our study patients on ART (HIV-positive) did not report higher acute toxicity compared to HIV-negative participants. These results in our large population sample, compared to the literature, provide strong evidence that the chromosomal radiosensitivity seen in immunocompetent HIV-positive participants on ART(Baeyens *et al.*, 2010) is not likely to translate into an increase in CRT-related toxicity compared to HIV-negative participants.

The MN assay is not possible in patients who are immune deficient as it relies on a sufficiently high lymphocyte count for evaluation. An evaluation of the radiosensitivity of immunodeficient patients, using a different technique, alongside reported RT-related toxicity, would be of value.

The design of a study comparing the RT-related toxicity experienced by HIV-positive immunodeficient versus HIV-positive immunocompetent patients warrants consideration and could include an evaluation of systemic radiosensitivity, with genetic profiling if the numbers are large enough, in order to narrow down the cause of the increased treatment related toxicity seen in immunodeficient patients (if any). Long term studies on the effects of HIV-infection on late CRT-related toxicity are also missing. Although Simonds *et al.* reported on a five year follow up of cervical cancer patients, including HIV-positive patients, the late toxicity was not reported and again CD4 count and ART were not standardised variables. Therefore although a lower survival rate was reported, it is unclear what affect the CD4 count and ART administration prior to CRT has on the survival.(Simonds *et al.*, 2019) With the planned five year follow-up of our participants, our data may provide more clarity on the risk of developing late CRT-related toxicity in HIV-positive patients who were immunocompetent at the time of starting treatment for cervical cancer.

We hypothesise therefore that the increased toxicity seen in HIV patients in other studies may be more strongly associated with the immunodeficiency seen in HIV-positive patients who are not on ART and have CD4 counts below 200cells/ μ L, than with the HIV-infection and ARTs. HIV-positive patients on ART without resistance to ART treatment should therefore be able to receive standard treatment.

7.9. Limitations of the Study

The limitations of our study include the high rate of screening failures which increased the costs of screening (FDG costs) and subsequently limited the number of participants that could be enrolled. Of 271 participants screened, 255 had pre-treatment ^{18}F -FDG PET/CT scans and only 210 were randomised for trial. The most common reasons for screening failure were Renal dysfunction: $n=14$, Extra-pelvic visceral disease on pre-treatment ^{18}F -FDG PET/CT scans: $n=12$; and did not arrive for screening evaluations: $n=7$. Other reasons included the detection of fistulas or bilateral hydronephrosis on the ^{18}F -FDG PET/CT scans. The complete list screening failure reasons can be found in Chapter Three.

Due to resource constraints, the institutional protocols do not follow the international standard of care cisplatin schedule (40mg/m² weekly), standard of care of planning, using at least 3D-conformal radiotherapy (3DCRT), and extended RT fields (para-aortic), where indicated. Therefore, while our results are applicable in resource-constrained settings, it is unclear how much 3DCRT planning and extended radiation fields would improve the results in the treatment groups in less resource-constrained settings.

The late stage of disease and longer waiting periods for treatment seen in resource-constrained settings such as ours, likely contributed towards worse outcomes in our sample population. The use of clinical staging without sophisticated imaging techniques resulted in the under-staging of a large number of participants (as seen on the ^{18}F -FDG PET/CT which were conducted for monitoring disease response). It is possible that, if the staging included the ^{18}F -FDG PET/CT results, more participants in our sample would have been up-staged to stage IVA based on the involvement of pelvic nodes and the pelvic organs such as the bladder and rectum. The local disease control rates may be improved in both groups in less constrained settings where staging may be more accurate. Conversely, the possibility that more patients may actually have been in stage IV of disease in our sample may strengthen the value of our results. An analysis of the pre-treatment ^{18}F -FDG PET/CT scans and comparison of their results to the clinical staging would clarify the differences between ^{18}F -FDG PET/CT staging and clinical staging in our sample. Although the results would not change the staging methods in resource-constrained settings in which ^{18}F -FDG PET/CT scans are not routinely available. Resource-constraints also affected factors such as time to complete treatment (listed as technical problems) and timing of the ^{18}F -FDG PET/CT scans in some cases (machine down time, source supply problems).

Double blinding of the patients was not possible due to the nature of the intervention. However measures were taken to ensure that physicians reporting on the ^{18}F -FDG PET/CT scans were blinded and had no interaction with the participants. Clinicians conducting follow-up assessments were also blinded to the treatment group that the participants were in and nurses conducting the QoL were also blinded to the participant's treatment group.

CHAPTER EIGHT

CONCLUSION

Modulated electro-hyperthermia is an effective radiosensitiser when added to chemoradiotherapy protocols for the management of LACC patients, resulting in a significantly higher LDC in patients eligible for evaluation at six months post-treatment. The participants in our study had almost double the chance of local disease free survival at six months. These findings were not significantly affected by the HIV status of participants in our study.

The addition of mEHT to CRT did not significantly increase the toxicity profile of either cisplatin or radiotherapy in our study. Modulated electro-hyperthermia is a safe radiosensitiser for HIV-positive patients who are at risk of higher rates of adverse events and treatment-related toxicities. The evaluation of quality of life showed that mEHT has the potential to improve symptoms such as pain and fatigue and may improve aspects of emotional functioning as well.

The novel finding of the possible potentiation of the abscopal effect during the disease pattern evaluation may be attributed to the effects mEHT on the immune system. This has raised interesting questions regarding the use of mEHT to broaden the scope of RT treatments and regarding the use of mEHT combined with RT and immunotherapy.

The analysis of the ^{18}F -FDG PET/CT scans indicates that the visualisation of the lymph nodes in our sample can be confidently attributed to malignancy rather than HIV infection in the participants in our sample. This analysis also highlights the possibility that HIV-positive cervical cancer patients are at risk of having the extent of disease being underestimated and that careful evaluation of ^{18}F -FDG PET/CT scans is important when staging HIV-positive patients.

This randomised controlled trial provides the first phase III evidence for the use of mEHT as radiosensitiser for the treatment of LACC. The results offer evidence of efficacy of a heating technique that can be implemented in a low-resource setting in a population sample for whom the treatment and management of LACC is particularly challenging.

These results suggest that mEHT has the potential to improve outcomes and to assist in alleviating the burden of LACC on healthcare systems in LMICs. The improved outcomes seen in the study sample treated with mEHT provides a strong motivation for the inclusion of the treatment in standard treatment protocols for locally advanced cervical cancer.

CHAPTER NINE

FUTURE PERSPECTIVES

The results of this trial have provided important insights into the efficacy of mEHT as a radiosensitiser and its effects on the immune system. While these results have answered important research questions on the use of mEHT, the results have also raised new questions and broadened the potential scope of mEHT use combined with treatments such as immunotherapies and radiotherapy. There is the potential for the design of several new research projects for different malignancies based on these results.

9.1. Follow-up and Survival

In oncology, local disease control is an important part of the evaluation of the benefits of a treatment as it may be associated with reduced morbidity and improved quality of life.

The scope of this thesis includes a follow up period of one year post treatment and therefore local disease control is reported. The trial protocols require a follow-up of two years post-treatment and an application for an extension to monitor patient survival and disease status for a further three years post-treatment (totalling five years of follow-ups post-treatment) has been approved. The further follow-up of these participants will provide vital information needed to evaluate the long term effects of mEHT on survival and DFS in the sample group. It is expected that the report on two year DFS will be completed in early 2020 and five year DFS results will be available in early 2023.

9.2. Cost Effectiveness Analysis

The increased costs of oncology treatments have necessitated a careful evaluation of the cost effectiveness and budget impact analysis of treatments. Methods such as cost saving analysis per quality-adjusted-life-year require the use of survival and disease-free survival outcomes.(National Cancer Institute and American Society of Clinical Oncology, 1998; Shih and Halpern, 2008; Sullivan *et al.*, 2014; Nguyen *et al.*, 2018) The assessment of the feasibility of a heating technique in a resource-constrained setting must include the economic impact as well as the clinical benefits. A detailed cost

effectiveness analysis on the economic impact of mEHT in the current study is planned once the two year survival and two year DFS rates are available.

9.3. Broadening the Scope of mEHT Use

The improved outcomes in this study indicate that the efficacy of mEHT is comparable to other HT techniques used in the management of cervical cancer. Several other malignancies, including head and neck, gastrointestinal, sarcoma, glioblastoma, pancreas, and lung, (Datta *et al.*, 2015; Peeken *et al.*, 2017), are known to benefit from the addition of HT and the use of mEHT for the management of these malignancies is therefore motivated, especially in resource-constrained settings.

9.4 Immunotherapy

In a review on the targeted therapies for cervical cancer by Crafton and Salani, the authors reported that preliminary results evaluating the use of targeted therapies such as tyrosine kinase inhibitors, immunotherapy, and vaccine therapy, for the treatment of cervical cancer are promising. (Crafton and Salani, 2016) The two classes of immunotherapy which are currently under investigation for the treatment of cervical cancer are therapeutic vaccines and immune checkpoint inhibitors (ICI). Vaccine therapy aims to elicit an adaptive immune response to a tumour specific antigen. These treatments rely on the cell-mediated immunity against the HPV infected cells with HPV-produced oncoproteins E6 or E7. Immune check point inhibitors are monoclonal antibodies acting against either cytotoxic T lymphocyte-associated antigen 4 (such as ipilimumab and tremelimumab) or components of programmed-cell-death-1 (PD-1) pathway (such as nivolumab, pembrolizumab, and pidilizumab). Two ongoing phase I trials are registered at ClinicalTrials.gov to assess the tolerability and efficacy of ipilimumab and ivolumab for the treatment of resistant or recurrent disease in cervical cancer patients and one phase I study is investigating the addition of ipilimumab to radiation for the primary treatment of stages IB2 to IVA cervical cancer. (Crafton and Salani, 2016) There are sixteen trials registered to investigate the use of immunotherapy agents combined with RT. Two phase II trials are currently registered using pemobrolizumab and HT for the treatment of malignant mesothelioma and abdominal metastases. The reason for investigating the combination of immunotherapy agents and IR is based on the potential of immunotherapy agents to increase the observation of abscopal effect. According to our research, mEHT may potentiate the abscopal effect and

literature has shown that mEHT has strong immune effects. Further studies on the combination of mEHT and immunotherapy agents, with or without IR would provide more information into the relationship of these treatments and the immune system. The high toxicity profile associated with many immunotherapies may be overcome by lowering the dose of the immunotherapy agent and combining it with mEHT and RT.

The identification and validation of biomarkers to indicate an abscopal response would assist in the evaluation and confirmation of the presence and extent of the abscopal effect seen, with or without mEHT.

9.5 Thermo-sensitivity

A challenge in oncology is overcoming the resistance of certain tumours to radiotherapy or certain chemotherapies. While the addition of mEHT significantly improves the chances of achieving local disease control by improving radio-sensitivity in certain tumours, there is still a subset of participants in whom local disease control is not achieved even with the addition of mEHT. This could be due to certain tumours displaying thermo-resistant characteristics as well as radioresistant characteristics. Genetic profiling of these tumours is a potential method of identifying genes associated with thermo-resistance. Evaluation of radiosensitivity in tumour samples pre- and post-treatment, and the correlation of the results with tumour response with or without the addition of mEHT, could provide useful information on any possible link between thermo-resistance and radio-resistance.

9.6 Protocol Variations

The investigation of the use of mEHT in a group of patients who are unable to receive the full dose of cisplatin, versus a group of patients who can receive the full dose of treatment without mEHT, is warranted and the results could reduce the cost and toxicity of treatments available in low resource settings. Hyperthermia has shown to improve the outcomes of patients treated with hypofractionation protocols for recurrent or residual disease in a previously irradiated area and hypofractionation protocols in cervical cancer patients in resource-constrained settings are often applied. The design of a study investigating mEHT combined with hypofractionated RT would certainly be warranted.

REFERENCES

- Abuodeh, Y., Venkat, P. and Kim, S. (2015) Systematic review of case reports on the abscopal effect, *Current Problems in Cancer*, Vol.40, No.1, pp.25–37.
- Akber, S. (2015) Oxygen in S phase of a cell cycle *, *Journal of Radiotherapy in Practice*, Vol.12, No.4, pp.360–362.
- Andocs, G., Vincze, G., et al. (2009) Effect of Curl-Free Potentials on Water, *Electromagnetic Biology and Medicine*, Vol.28, No.2, pp.166–181.
- Andocs, G., Renner, H., et al. (2009) Strong Synergy of Heat and Modulated Electromagnetic Field in Tumor Cell Killing, *Strahlentherapie und Onkologie*, Vol.185, No.2, pp.120–126.
- Andocs, G., Rehman, M. U., et al. (2015) Nanoheating without Artificial Nanoparticles Part II . Experimental Support of the Nanoheating Concept of the Modulated Electro-Hyperthermia Method, Using U937 Cell Suspension Model, *Biology and Medicine*, Vol.7, No.4, pp.1–9.
- Andocs, G., Meggyeshazi, N., et al. (2015) Upregulation of heat shock proteins and the promotion of damage-associated molecular pattern signals in a colorectal cancer model by modulated electrohyperthermia, *Cell Stress and Chaperones*, Vol.20, No.1, pp.37–46.
- Andocs, G., Rehman, M. U., et al. (2016) Comparison of biological effects of modulated electro-hyperthermia and conventional heat treatment in human lymphoma U937 cells, *Cell Death Discovery*, Vol.2, No.16039, pp.1–10.
- Ashing-giwa, K. T., Lim, J. and Tang, J. (2010) Gynecologic Oncology Surviving cervical cancer: Does health-related quality of life influence survival?, *Gynecologic Oncology*, Vol.118, No.1, pp.35–42.
- Ashing-Giwa, K. T. and Lim, J. W. (2011) Health-related quality of life outcomes among cervical cancer survivors: Examining ethnic and linguistic differences, *Cancer Epidemiology*, Vol.35, No.2, pp.194–201.
- Baeyens, A., Slabbert, J. P., et al. (2010) Chromosomal radiosensitivity of HIV positive individuals, *International Journal of Radiation Biology*, Vol.86, No.7, pp.584–592.
- Balogh, L., Pöstényi, Z., et al. (2016) Temperature increase induced by modulated electrohyperthermia (oncothermia ®) in the anesthetized pig liver, *Cancer Journal*, Vol.12, No.3, pp.1153–1159.
- Banerjee, R. and Kamrava, M. (2014) Brachytherapy in the treatment of cervical cancer: A review, *International Journal of Women's Health*, Vol.6, No.1, pp.555–564.
- Barbault, A., Costa, F. P., et al. (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 and Clinical*

Research, Vol.28, No.51, pp.1–10.

Bental, M. and Deutsch, C. (1993) Metabolic changes in activated T cells: An NMR study of human peripheral blood lymphocytes, *Magnetic Resonance in Medicine*, Vol.29, No.3, pp.317–326.

Bernhard, E. J., Maity, A., et al. (1995) Effects of ionizing radiation on cell cycle progression: A review, *Radiation and Environmental and Biophysics*, Vol.32, No.2, pp.79–83.

Besztercei, B., Vancsik, T., et al. (2019) Stress-Induced , p53-Mediated Tumor Growth Inhibition of Melanoma by Modulated Electrohyperthermia in Mouse Models without Major Immunogenic Effects, *International Journal of Molecular Sciences*, Vol.20, No.16, p.Article 4019.

Bjelic-Radasic, V., Jensen, P. T., et al. (2012) Quality of life characteristics in patients with cervical cancer, *European Journal of Cancer*, Vol.48, No.16, pp.3009–3018.

Bray, F., Ferlay, J., et al. (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA: A Journal for Clinicians*, Vol.6, No.68, pp.394–424.

Brix, N., Tiefenthaler, A., et al. (2017) Abscopal, immunological effects of radiotherapy: Narrowing the gap between clinical and preclinical experiences, *Immunological Reviews*, Vol.280, No.1, pp.249–279.

Brust, D., Polis, M., et al. (2006) Fluorodeoxyglucose imaging in healthy subjects with HIV infection: impact of disease stage and therapy on pattern of nodal activation, *AIDS*, Vol.20, No.7, pp.985–993.

Bukau, B. and Horwich, A. L. (1998) The Hsp70 and Hsp60 Chaperone Machines, *Cell*, Vol.92, No.3, pp.351–366.

Burgio, E., Piscitelli, P. and Migliore, L. (2018) Ionizing Radiation and Human Health: Reviewing Models of Exposure and Mechanisms of Cellular Damage. An Epigenetic Perspective, *International Journal of Environmental Research and Public Health*, Vol.15, No.9, p.Article 1971.

Cha, J., Jeon, T., et al. (2015) Electro-hyperthermia inhibits glioma tumorigenicity through the induction of E2F1- mediated apoptosis Electro-hyperthermia inhibits glioma tumorigenicity through the induction of E2F1-mediated apoptosis, *International Journal of Hyperthermia*, Vol.31, No.7, pp.784–792.

Chantalat, E., Vidal, F., et al. (2015) Cervical cancer with paraaortic involvement: Do patients truly benefit from tailored chemoradiation therapy? A retrospective study on 8 French centers, *European Journal of Obstetrics Gynecology and Reproductive Biology*, Vol.193, pp.118–122.

Chase, D. M., Huang, H. Q., et al. (2012) Quality of life and survival in advanced cervical cancer: A Gynecologic Oncology Group study, *Gynecologic*

Oncology, Vol.125, No.2, pp.315–319.

Chen, H.-W., Fan, J.-J. and Luo, W. (1997) A randomized trial of hyperthermo-radiochemotherapy for uterine cancer, *Chinese Journal of Clinical Oncology*, Vol.24, pp.249–251.

Chhabra, S., Devi, S., et al. (2016) Staging Issues in Cervical Cancer, *Cancer Surgery*, Vol.01, No.02, pp.1–5.

Chuang, L. T., Temin, S. and Berek, J. S. (2016) Management and Care of Women With Invasive Cervical Cancer: American Society of Clinical Oncology Resource-Stratified Clinical Practice Guideline Summary, *Journal of Oncology Practice*, Vol.12, No.7, pp.693–696.

Cloyd, M. W., Chen, J. J. and Wang, I. (2000) How does HIV cause AIDS? The homing theory, *Molecular Medicine Today*, Vol.6, No.3, pp.108–113.

Coghill, A. E., Newcomb, P. A., et al. (2013) Contribution of HIV infection to mortality among cancer patients in Uganda, *Aids*, Vol.27, No.18, pp.2933–2942.

Coghill, A. E., Shiels, M. S., et al. (2015) Elevated cancer-specific mortality among HIV-infected patients in the United States, *Journal of Clinical Oncology*, Vol.33, No.21, pp.2376–2383.

Cope, F. W. (1975) A review of the applications of solid state physics concepts to biological systems, *Journal of Biological Physics*, Vol.3, No.1, pp.1–41.

Crafton, S. M. and Salani, R. (2016) Beyond Chemotherapy: An Overview and Review of Targeted Therapy in Cervical Cancer, *Clinical Therapeutics*, Vol.38, No.3, pp.449–458.

D'Ambrosio, V. and Dughiero, F. (2007) Numerical model for RF capacitive regional deep hyperthermia in pelvic tumors, *Medical & Biological Engineering & Computing*, Vol.45, No.5, pp.459–466.

Dahm, R., Avery, O. T., et al. (2008) Discovering DNA : Friedrich Miescher and the early years of nucleic acid research, *Human Genetics*, Vol.122, No.6, pp.565–581.

Damadian, R. (1971) Tumor Detection by Nuclear Magnetic Resonance, *Science*, Vol.171, No.3976, pp.1151–1153.

Datta, N. R., Ordóñez, S. G., et al. (2015) Local hyperthermia combined with radiotherapy and- / or chemotherapy : Recent advances and promises for the future, *Cancer Treatment Reviews*, Vol.41, No.9, pp.742–753.

Datta, N. R., Rogers, S., et al. (2016) Hyperthermia and radiotherapy with or without chemotherapy in locally advanced cervical cancer: a systematic review with conventional and network meta-analyses, *International Journal of Hyperthermia*, Vol.32, No.7, pp.809–821.

Datta, N. R., Stutz, E., et al. (2017) Concurrent chemoradiotherapy vs. radiotherapy alone in locally advanced cervix cancer: A systematic review and meta-analysis, *Gynecologic Oncology*, Vol.145, No.2, pp.374–385.

- Datta, N. R., Bose, H. K. and Kapoor, A. (1987) Thermoradiotherapy in the management of Carcinoma Cervix (Stage IIIB): A controlled Clinical Study., *Indian Medical Gazette*, No.121, pp.68–71.
- Davison, J. M., Subhramaniam, R. M., et al. (2011) FDG PET/CT in Patients With HIV, *American Journal of Radiology*, Vol.197, No.2, pp.284–294.
- de Wit, R., van der Zee, J., et al. (1999) A phase I/II study of combined weekly systemic cisplatin and locoregional hyperthermia in patients with previously irradiated recurrent carcinoma of the uterine cervix, *British Journal of Cancer*, Vol.80, No.9, pp.1387–1391.
- Deeks, S. G., Overbaugh, J., et al. (2015) HIV infection, *Nature Reviews Disease Primers*, Vol.1, p.Article: 15035.
- Deloch, L., Derer, A., et al. (2016) Modern Radiotherapy Concepts and the impact of Radiation on immune Activation, *Frontiers in Oncology*, Vol.6, No.141, pp.1–16.
- Denny, L. and Anorlu, R. (2012) Cervical cancer in Africa, *Cancer Epidemiology Biomarkers and Prevention*, Vol.21, No.9, pp.1434–1438.
- Derks, M., van Lonkhuijzen, L. R. C. W., et al. (2017) Long-Term Morbidity and Quality of Life in Cervical Cancer Survivors: A Multicenter Comparison between Surgery and Radiotherapy as Primary Treatment, *International Journal of Gynecological Cancer*, Vol.27, No.2, pp.350–356.
- Desouky, O., Ding, N. and Zhou, G. (2015) Targeted and non-targeted effects of ionizing radiation, *Journal of Radiation Research and Applied Sciences*, Vol.8, No.2, pp.247–254.
- Dewhirst, M. W., Vujaskovic, Z., et al. (2005) Re-setting the biologic rationale for thermal therapy, *International Journal of Hyperthermia*, Vol.21, No.8, pp.779–790.
- Dimopoulos, Y., Moysi, E. and Petrovas, C. (2017) The Lymph Node in HIV Pathogenesis, *Current HIV/AIDS Reports*, Vol.14, No.4, pp.133–140.
- Dissado, L. A., Alison, J. M., et al. (1995) Dynamic scaling in the dielectric response of excised EMT-6 tumours undergoing hyperthermia, *Physics in Medicine & Biology*, Vol.40, No.6, pp.1067–1084.
- Dryden-Peterson, S., Bvochora-Nsingo, M., et al. (2016) HIV infection and survival among women with cervical cancer, *Journal of Clinical Oncology*, Vol.34, No.31, pp.3749–3757.
- Du Toit, G. C. and Kidd, M. (2013) Contextual quality of life of HIV-positive patients with cervical carcinoma at Tygerberg Hospital, *Southern African Journal of Gynaecological Oncology*, Vol.5, No.2, pp.41–46.
- Dzaka, A. and Maree, J. (2016) Experiences of women receiving high dose rate brachytherapy for cervical cancer at an academic hospital, *Southern African Journal of Gynaecological Oncology*, Vol.8, No.2, pp.42–45.
- Einstein, M. H., Ndlovu, N., et al. (2019) Cisplatin and radiation therapy in HIV-positive women with locally advanced cervical cancer in sub-Saharan Africa : A phase II study of

the AIDS malignancy consortium, *Gynecologic Oncology*, Vol.153, No.1, pp.20–25.

Elming, P. B., Sørensen, B. S., *et al.* (2019) Hyperthermia : The Optimal Treatment to Overcome Radiation Resistant Hypoxia, *Cancers*, Vol.11, No.60, pp.1–20.

Emami, B., Scott, C., *et al.* (1996) Phase III study of interstitial thermoradiotherapy compared with interstitial radiotherapy alone in the treatment of recurrent or persistent human tumors: A prospectively controlled randomized study by the radiation therapy oncology group, *International Journal of Radiation Oncology, Biology, Physics*, Vol.34, No.5, pp.1097–1104.

Erdmann, B., Lang, J. and Seebass, M. (1998) Optimization of Temperature Distributions for Regional Hyperthermia Based on a Nonlinear Heat Transfer Model, *Annals of the New York Academy of Sciences*, Vol.858, No.1, pp.36–46.

Fadaka, A., Ajiboye, B., *et al.* (2017) Biology of glucose metabolism in cancer cells, *Journal of Oncological Science*, Vol.3, No.2, pp.45–51.

Fanning, P. J., Emkey, G., *et al.* (2003) Mechanical Regulation of Mitogen-activated Protein Kinase Signaling in Articular Cartilage *, *The Journal of Biological Chemistry*, Vol.278, No.51, pp.50940–50948.

Fatehi, D. (2000) *Technical Quality of Deep Hyperthermia Using the BSD-2000*.

Fatehi, D., van der Zee, J., *et al.* (2006) Temperature data analysis for 22 patients with advanced cervical carcinoma treated in Rotterdam using radiotherapy, hyperthermia and chemotherapy: A reference point is needed, *International Journal of Hyperthermia*, Vol.22, No.4, pp.353–363.

Fayers, P. and Bottomley, A. (2002) Quality of life research within the EORTC — the EORTC QLQ-C30, *European Journal of Cancer*, Vol.38, No. Supp 4, pp.S125–S133.

Ferlay, J., Soerjomataram, I., *et al.* (2018) *Cancer Today (powered by GLOBOCAN 2018)*, IARC CancerBase No. 15. Edited by J. Ferlay *et al.* Available at: <http://publications.iarc.fr/577>.

Fernandez-Ots, A. and Crook, J. (2013) The role of intensity modulated radiotherapy in gynecological radiotherapy: Present and future, *Reports of Practical Oncology and Radiotherapy*, Vol.18, No.6, pp.363–370.

Fiorentini, G., Giovanis, P., *et al.* (2006) A Phase II Clinical Study on Relapsed Malignant Gliomas Treated with Electro-hyperthermia, *in Vivo*, Vol.20, No.6A, pp.721–724.

Fiorentini, G., Sarti, D., *et al.* (2018) Retrospective observational Clinical Study on Relapsed Malignant Gliomas Treated with Electro-hyperthermia Presented at 35 th ICHS, Guangzhou, 2017, *Oncothermia Journal*, Vol.22, No. February, pp.32–45.

Fiorentini, G. and Szasz, A. (2006) Hyperthermia today: Electric energy, a new opportunity in cancer treatment, *Journal of Cancer Research and Therapy*, Vol.2, No.2, pp.41–46.

Firnhaber, C., Zungu, K., *et al.* (2009) Diverse and high prevalence of human

papillomavirus associated with a significant high rate of cervical dysplasia in human immunodeficiency virus-infected women in Johannesburg, South Africa, *Acta Cytologica*, Vol.53, No.1, pp.10–17.

Flameling, B., Nordberg, T., et al. (2016) An international multicenter phase III study of chemoradiotherapy versus chemoradiotherapy plus hyperthermia for locally advanced cervical cancer, *Journal of Clinical Oncology*, Vol.34, No.S15, p.17023.

Formenti, S. C., Chak, L., et al. (1995) Increased radiosensitivity of normal tissue fibroblasts in patients with acquired immunodeficiency syndrome (AIDS) and with kaposi's sarcoma, *International Journal of Radiation Biology*, Vol.68, No.4, pp.411–412.

Fox, M. P., Sanne, I. M., et al. (2011) Initiating Patients on ART at CD4 Counts above 200 is Associated with Improved Treatment Outcomes in South Africa, *AIDS*, Vol.24, No.13, pp.2041–2050.

Franckena, M., de Wit, R., et al. (2007) Weekly systemic cisplatin plus locoregional hyperthermia : An effective treatment for patients with recurrent cervical carcinoma in a previously irradiated area, *International Journal of Hyperthermia*, Vol.23, No.5, pp.443–450.

Franckena, M., Stalpers, L. J. A., et al. (2008) Long-Term Improvement in Treatment Outcome After Radiotherapy and Hyperthermia in Locoregionally Advanced Cervix Cancer: An Update of the Dutch Deep Hyperthermia Trial, *International Journal of Radiation Oncology, Biology, Physics*, Vol.70, No.4, pp.1176–1182.

Franckena, M., Lutgens, L. C., et al. (2009) Radiotherapy and Hyperthermia for Treatment of Primary Locally Advanced Cervix Cancer: Results in 378 Patients, *International Journal of Radiation Oncology, Biology, Physics*, Vol.73, No.1, pp.242–250.

Frey, B., Rubner, Y., et al. (2012) Induction of Abscopal Anti-Tumor Immunity and Immunogenic Tumor Cell Death by Ionizing Irradiation – Implications for Cancer Therapies, *Current Medicinal Chemistry*, Vol.19, No.12, pp.1751–1764.

Frey, B., Rückert, M., et al. (2017) Immunomodulation by ionizing radiation — impact for design of radio- - immunotherapies and for treatment of inflammatory diseases, *Immunological Reviews*, Vol.280, No.1, pp.231–248.

Friedberg, E. C. (2003) DNA damage and repair, *Nature*, Vol.421, No.4921, pp.436–440.

Garcia, A. M., Szasz, N., et al. (2003) Transport and binding of insulin-like growth factor I through articular cartilage, *Archives of Biochemistry and Biophysics*, Vol.415, No.1, pp.69–79.

German Advisory Committee Blood Subgroup Assessment of Pathogens Transmissible by Blood (2016) Human Immunodeficiency Virus (HIV), *Transfusion Medicine and Hemotherapy*, Vol.43, pp.203–222.

Ghebre, R. G., Grover, S., et al. (2017) Cervical cancer control in HIV-infected women: Past, present and future, *Gynecologic Oncology Reports*, Vol.21, pp.101–108.

Gichangi, P., Bwayo, J., et al. (2006) HIV impact on acute morbidity and pelvic tumor

control following radiotherapy for cervical cancer., *Gynecologic oncology*, Vol.100, No.2, pp.405–411.

Goodsell, D. S. (2015) Illustrations of the HIV Life Cycle, in Torbett, B. E., Goodsell, D. S., and Richman, D. D. (eds) *The Future of HIV-1 Therapeutics: Resistance Is Futile?*, pp.243–252.

Griffin, R. J., Ogawa, A., et al. (2005) Hyperthermic Enhancement of Tumor Radiosensitization Strategies, *Immunological Investigations*, Vol.34, No.3, pp.343–359.

Griffin, R. J., Dings, R. P. M., et al. (2010) Mild temperature hyperthermia and radiation therapy: role of tumor vascular thermotolerance and relevant physiological factors, *International Journal of Hyperthermia*, Vol.26, No.3, pp.256–263.

Grover, S., Xu, M. J., et al. (2015) A Systematic Review of Radiotherapy Capacity in Low- and Middle-Income Countries, *Frontiers in Oncology*, Vol.4, No.380, pp.1–11.

Grover, S., Desir, F., et al. (2018) Reduced Cancer Survival Among Adults With HIV and AIDS-Defining Illnesses Despite No Difference in Cancer Stage at Diagnosis, *Epidemiology*, Vol.79, No.4, pp.421–429.

Hafiz, A., Abbasi, A. N., et al. (2015) Frequency and Severity of Acute Toxicity of Pelvic Radiotherapy for Gynecological Cancer., *Journal Of The College Of Physicians And Surgeons--Pakistan*, Vol.25, No.11, pp.802–806.

Hall, M. T., Simms, K. T., et al. (2019) The projected timeframe until cervical cancer elimination in Australia : a modelling study, *The Lancet Public Health*, Vol.4, No.1, pp.e19–e27.

Hansen, H. V., Loft, A., et al. (2015) Survival outcomes in patients with cervical cancer after inclusion of PET/CT in staging procedures, *European Journal of Nuclear Medicine and Molecular Imaging*, Vol.42, No.12, pp.1833–1839.

Harima, Y., Nagata, K., et al. (2001) A randomized clinical trial of radiation therapy versus thermoradiotherapy in stage IIIB cervical carcinoma, *International Journal of Hyperthermia*, Vol.25, No.5, pp.338–343.

Harima, Y., Ohguri, T., et al. (2016) A multicentre randomised clinical trial of chemoradiotherapy plus hyperthermia versus chemoradiotherapy alone in patients with locally advanced cervical cancer, *International Journal of Hyperthermia*, Vol.32, No.7, pp.801–808.

Hegyi, G., Vincze, G. and Szasz, A. (2007) Axial-Vector Interaction with Bio-Systems, *Electromagnetic Biology and Medicine*, Vol.26, No.2, pp.107–118.

Herd, O., Francies, F., et al. (2014) The Effect of HIV and Antiretroviral Therapy on Chromosomal Radiosensitivity AIDS & Clinical Research, *Journal of AIDS and Clinical Research*, Vol.5, No.12, p.397.

Herd, O., Francies, F., et al. (2016) Chromosomal radiosensitivity of human immunodeficiency virus positive/negative cervical cancer patients in South Africa,

Molecular Medicine Reports, Vol.13, No.1, pp.130–136.

Hillemanns, P., Soergel, P., et al. (2016) Epidemiology and Early Detection of Cervical Cancer, *Oncology Research and Treatment*, Vol.39, No.9, pp.501–506.

Hlavata, Z., Solinas, C. and Scartozzi, M. (2018) The Abscopal Effect in the Era of Cancer Immunotherapy: a Spontaneous Synergism Boosting Anti-tumor Immunity?, *Targeted Oncology*, Vol.13, No.556, pp.113–123.

Horsman, M. R. and Overgaard, J. (2007) Hyperthermia: a Potent Enhancer of Radiotherapy, *Clinical Oncology*, Vol.19, No.6, pp.418–426.

Housri, N., Yarchoan, R. and Kaushal, A. (2010) Radiotherapy for HIV patients: Are special precautions necessary?, *Cancer*, Vol.116, No.2, pp.273–283.

Hurwitz, M. and Stauffer, P. (2014) Hyperthermia, Radiation and Chemotherapy_ The Role of Heat in Multidisciplinary Cancer Care, *Seminars in Oncology*, Vol.41, No.6, pp.714–729.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2007) *Human Papillomaviruses*, IARC Monographs on the evaluation of carcinogenic risks to humans. World Health Organisation. Available at: <http://monographs.iarc.fr>.

Iyengar, S., Chin, B., et al. (2003) Anatomical loci of HIV-associated immune activation and association with viraemia, *The Lancet*, Vol.362, No.9388, pp.945–950.

Janigro, D., Perju, C., et al. (2006) Alternating current electrical stimulation enhanced chemotherapy: a novel strategy to bypass multidrug resistance in tumor cells, *BioMed Central Cancer*, Vol.6, No.72, pp.1–12.

Jemu, M., van Wijk, L., et al. (2018) Tumour and treatment factors influencing the outcome of chemo-radiation in stage IIB cervical cancer: a single institution experience, *South African Journal of Gynaecological Oncology*, Vol.4, No.1, pp.1–6.

Jones, E., Dewhirst, M. and Vujaskovic, Z. (2003) Hyperthermia Improves the Complete Response Rate for Superficial Tumours Treated with Radiation: Results of a Prospective Randomized Trial Testing the Thermal Dose Parameter CEM 43°T90., *International Journal of Radiation Oncology, Biology, Physics*, Vol.57, No.2, pp.S253–S254.

Jones, E. L., Oleson, J. R., et al. (2005) Randomized Trial of Hyperthermia and Radiation for Superficial Tumors, *Journal of Clinical Oncology*, Vol.23, No.13, pp.3079–3085.

Jossinet, J. (1998) The impedivity of freshly excised human breast tissue, *Physiological Measurement*, Vol.19, No.1, pp.61–75.

Kabekkodu, S. P., Chakrabarty, S., et al. (2017) Epigenomics, Pharmacoeepigenomics, and Personalized Medicine in Cervical Cancer, *Public Health Genomics*, Vol.20, No.2, pp.100–115.

Kampinga, H. H. and Dikomey, E. (2001) Hyperthermic radio- sensitization: mode of action and clinical relevance, *International Journal of Radiation Biology*, Vol.77, No.4, pp.399–408.

Karanam, K., Kafri, R., *et al.* (2012) Quantitative Live Cell Imaging Reveals a Gradual Shift between DNA Repair Mechanisms and a Maximal Use of HR in Mid S Phase, *Molecular Cell*, Vol.47, No.2, pp.320–329.

Kessler, T. A. (2017) Cervical Cancer: Prevention and Early Detection, *Seminars in Oncology Nursing*, Vol.33, No.2, pp.172–183.

Kidd, E. A., Siegel, B. A., *et al.* (2010) Clinical Outcomes of Definitive Intensity-Modulated Radiation Therapy With Fluorodeoxyglucose-Positron Emission Tomography Simulation in Patients With Locally Advanced Cervical Cancer, *International Journal of Radiation Oncology Biology Physics*, Vol.77, No.4, pp.1085–1091.

Kidd, E. A., El Naqa, I., *et al.* (2012) FDG-PET-based prognostic nomograms for locally advanced cervical cancer, *Gynecologic Oncology*, Vol.127, No.1, pp.136–140.

Kim, H. S., Sardi, J. E., *et al.* (2013) Efficacy of neoadjuvant chemotherapy in patients with FIGO stage IB1 to IIA cervical cancer: An international collaborative meta-analysis, *European Journal of Surgical Oncology (EJSO)*, Vol.39, No.2, pp.115–124.

Kim, J. K., Prasad, B. and Kim, S. (2017) Temperature mapping and thermal dose calculation in combined radiation therapy and 13.56 MHz radiofrequency hyperthermia for tumor treatment, in Kessel, D. H. and Hasan, T. (eds) *SPIE Proceedings Vol. 10047: Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy XXVI*, 1004718, pp.1–7.

Kim, W., Kim, M., *et al.* (2018) Role of HIF-1 α in response of tumors to a combination of hyperthermia and radiation in vivo, *International Journal of Hyperthermia*, Vol.34, No.3, pp.276–283.

Kirson, E. D., Gurvich, Z., *et al.* (2004) Disruption of Cancer Cell Replication by Alternating Electric Fields, *Cancer Research*, Vol.64, No.9, pp.3288–3295.

Kirson, E. D., Dbalý, V., *et al.* (2006) Treatment of locally advanced solid tumors using alternating electric fields (TTFields) - a translational study, *Cancer Research*, Vol.66, No.8, p.S1233.

Kirson, E. D., Dbalý, V., *et al.* (2007) Alternating electric fields arrest cell proliferation in animal tumor models and human brain tumors, *Proceedings of the National Academy of Sciences of the United States of America*, Vol.104, No.24, pp.10152–10157.

Kirson, E. D., Schneiderman, R. S., *et al.* (2009) Chemotherapeutic treatment efficacy and sensitivity are increased by adjuvant alternating electric fields (TTFields), *BMC Medical Physics*, Vol.9, No.1, pp.1–13.

Koh, W., Greer, B. E., *et al.* (2015) Cervical Cancer , Version 2. 2015 Featured Updates to the NCCN Guidelines, *Journal of the National Comprehensive Cancer Network*, Vol.13, No.4, pp.395–404.

Kok, H. P., Navarro, F., *et al.* (2018) Locoregional hyperthermia of deep-seated tumours applied with capacitive and radiative systems : a simulation study, *International Journal of*

Hyperthermia, Vol.34, No.6, pp.714–730.

Kotzen, J. A. (2016) South African guidelines for cervical brachytherapy, *South African Journal of Gynaecological Oncology*, Vol.8, No.1, pp.4–5.

de la Peña, M. J., de Vega Fernández, V. M., et al. (2008) Current imaging modalities in the diagnosis of cervical cancer, *Gynecologic Oncology*, Vol.110, No.3 Supp2, pp.S49–S54.

Lagasse, L. D., Creasman, W. T., et al. (1980) Results and complications of operative staging in cervical cancer: Experience of the Gynecologic Oncology Group, *Gynecologic Oncology*, Vol.9, No.1, pp.90–98.

Laufer, S., Ivorra, A., et al. (2010) Electrical impedance characterization of normal and cancerous human hepatic tissue, *Physiologic Measurements*, Vol.31, No.7, pp.995–1009.

Lee, S.-Y., Kim, J.-H., et al. (2018) The effect of modulated electro-hyperthermia on temperature and blood flow in human cervical carcinoma, *International Journal of Hyperthermia*, Vol.34, No.7, pp.953–960.

Lee, S., Lee, N., et al. (2017) Treatment outcome analysis of chemotherapy combined with modulated electro-hyperthermia compared with chemotherapy alone for recurrent cervical cancer, following irradiation, *Oncology Letters*, Vol.14, No.1, pp.73–78.

Lepock, J. R. (2004) Role of nuclear protein denaturation and aggregation in thermal radiosensitization, *International Journal of Hyperthermia*, Vol.20, No.2, pp.115–130.

Lutgens, L., van der Zee, J., et al. (20AD) Combined use of hyperthermia and radiation therapy for treating locally advanced cervical carcinoma (Review), *Cochrane Database of Systematic Reviews*, Vol.1, No. Article No. CD006377.

Lutgens, L. C. H. W., Koper, P. C. M., et al. (2016) Radiation therapy combined with hyperthermia versus cisplatin for locally advanced cervical cancer: Results of the randomized RADCHOC trial, *Radiotherapy and Oncology*, Vol.120, No.3, pp.378–382.

Made, F., Wilson, K., et al. (2017) Distribution of cancer mortality rates by province in South Africa, *Cancer Epidemiology*, Vol.51, No. October, pp.56–61.

Magné, N., Chargari, C., et al. (2008) New trends in the evaluation and treatment of cervix cancer: The role of FDG PET, *Cancer Treatment Reviews*, Vol.34, No.8, pp.671–681.

Maiman, M., Fruchter, R. G., et al. (1997) Cervical cancer as an AIDS-defining illness, *Obstetrics and Gynecology*, Vol.89, No.1, pp.76–80.

Mallory, M., Gogineni, E., et al. (2016) Therapeutic hyperthermia: The old, the new, and the upcoming, *Critical Reviews in Oncology/Hematology*, Vol.97, pp.56–64.

Mangena, M., Snyman, L., et al. (2015) The impact of HIV infection on women receiving radiation for cervical cancer, *South African Journal of Gynaecological Oncology*, Vol.7, No.2, pp.44–51.

Marth, C., Landoni, F., et al. (2017) Cervical cancer: ESMO Clinical Practice Guidelines

for diagnosis, treatment and follow-up†, *Annals of Oncology*, Vol.28, No. Suppl 4, pp.iv72–iv83.

Mboumba Bouassa, R.-S., Prazuck, T., et al. (2017) Cervical cancer in sub-Saharan Africa: a preventable noncommunicable disease, *Expert Review of Anti-infective Therapy*, Vol.15, No.6, pp.613–627.

McCaffrey, P. (2005) Warming up to a triple therapy for cervical cancer, *The Lancet Oncology*, Vol.6, p.642.

McCaig, C. D., Rajnicek, A. M., et al. (2005) Controlling Cell Behavior Electrically: Current Views and Future Potential, *Physiological Reviews*, Vol.85, No.3, pp.943–978.

Mdletshe, S., Munkupa, H. and Lishimpi, K. (2016) Acute toxicity in cervical cancer HIV-positive vs . HIV-negative patients treated by radical chemo-radiation in Zambia, *Southern African Journal of Gynaecological Oncology*, Vol.8, No.2, pp.37–41.

Meggyeshazi, N., Andocs, G., et al. (2014) DNA fragmentation and programmed cell death by modulated electrohyperthermia, *Strahlentherapie und Onkologie*, Vol.190, No.9, pp.815–822.

Meulendyke, K. A., Croteau, J. D. and Christine Zink, M. (2015) HIV life cycle, innate immunity, and autophagy in the central nervous system, *Current Opinion in HIV and AIDS*, Vol.9, No.6, pp.565–571.

Miousse, I. R., Kutanzi, K. R. and Koturbash, I. (2018) Effects of Ionizing Radiation on DNA Methylation: From Experimental Biology to Clinical Applications, *International Journal of Radiation Biology*, Vol.93, No.5, pp.501–526.

Mohamed, F., Marchettini, P., et al. (2003) Thermal Enhancement of New Chemotherapeutic Agents at Moderate Hyperthermia, *Annals of Surgical Oncology*, Vol.10, No.4, pp.463–468.

Morkel, M., Ellmann, A., et al. (2018) Evaluating the role of f-18 fluorodeoxyglucose positron emission tomography/computed tomography scanning in the staging of patients with stage IIIB cervical carcinoma and the impact on treatment decisions, *International Journal of Gynecological Cancer*, Vol.28, No.2, pp.379–384.

Narayan, K. and Lin, M. Y. (2015) Staging for cervix cancer: Role of radiology, surgery and clinical assessment, *Best Practice and Research: Clinical Obstetrics and Gynaecology*, Vol.29, No.6, pp.833–844.

Nathanson, S. D., Nelson, L., et al. (1991) Development of lymph node and pulmonary metastases after local irradiation and hyperthermia of footpad melanomas, *Clinical & Experimental Metastasis*, Vol.9, No.4, pp.377–392.

National Cancer Institute and American Society of Clinical Oncology (1998) Integrating economic analysis into cancer clinical trials the National Cancer Institute-American Society of Clinical Oncology Economics Workbook., *Journal of the National Cancer Institute. Monographs*, Vol.24, No.1, pp.1–28.

- Nguyen, T. K., Goodman, C. D., et al. (2018) Evaluation of Health Economics in Radiation Oncology: A Systematic Review, *Radiation Oncology Biology*, Vol.94, No.5, pp.1006–1014.
- Ngwa, W., Irabor, O. C., et al. (2018) Using immunotherapy to boost the abscopal effect, *Nature Reviews Cancer*, Vol.18, No.5, pp.313–322.
- Nikitaki, Z., Mavragani, I., et al. (2016) Systemic mechanisms and effects of ionizing radiation: A new 'old' paradigm of how the bystanders and distant can become the players, *Seminars in Cancer Biology*, Vol.37, No.38, pp.77–95.
- Nishida, T., Akagi, K. and Tanaka, Y. (1997) Correlation between cell killing effect and cell membrane potential after heat treatment: analysis using fluorescent dye and flow cytometry, *International Journal of Hyperthermia*, Vol.13, No.2, pp.227–234.
- Oei, A. L., Vriend, L. E. M., et al. (2015) Effects of hyperthermia on DNA repair pathways: One treatment to inhibit them all, *Radiation Oncology*, Vol.10, No.1, pp.1–13.
- Oei, A. L., van Leeuwen, C. M., et al. (2015) Hyperthermia selectively targets human papillomavirus in cervical tumors via p53-dependent apoptosis, *Cancer Research*, Vol.75, No.23, pp.5120–5129.
- Oliver, N. T. and Chiao, E. Y. (2017) Malignancies in women with HIV infection, *Current Opinion in HIV and AIDS*, Vol.12, No.1, pp.69–76.
- Osann, K., Hsieh, S., et al. (2014) Factors associated with poor quality of life among cervical cancer survivors: Implications for clinical care and clinical trials, *Gynecologic Oncology*, Vol.135, No.2, pp.266–272.
- Overgaard, J. (2013) The heat is (still) on – The past and future of hyperthermic radiation oncology, *Radiotherapy and Oncology*, Vol.109, No.2, pp.185–187.
- Pandita, T. K., Pandita, S. and Bhaumik, S. R. (2009) Molecular Parameters of Hyperthermia for Radiosensitization, *Critical Reviews in Eukaryote Gene Expression*, Vol.19, No.3, pp.235–251.
- Papp, E., Vancsik, T., et al. (2017) Energy Absorption by the Membrane Rafts in the Modulated Electro-Hyperthermia (mEHT), *Open Journal of Biophysics*, Vol.7, pp.216–229.
- Peeken, J. C., Vaupel, P., et al. (2017) Integrating Hyperthermia into Modern Radiation Oncology: What Evidence Is Necessary?, *Frontiers in Oncology*, Vol.7, No. Article 132, pp.1–17.
- Petrelli, F., De Stefani, A., et al. (2014) Radiotherapy with concurrent cisplatin-based doublet or weekly cisplatin for cervical cancer: A systematic review and meta-analysis, *Gynecologic Oncology*, Vol.134, No.1, pp.166–171.
- Pfaendler, K. S. and Tewari, K. S. (2016) Changing paradigms in the systemic treatment of advanced cervical cancer, *American Journal of Obstetrics and Gynecology*, Vol.214, No.1, pp.22–30.
- Pike, L. J. (2006) Rafts defined: a report on the Keystone symposium on lipid rafts and

cell function, *Journal of Lipid Research*, Vol.47, No.7, pp.1597–1598.

Pötter, R., Tanderup, K., et al. (2018) The EMBRACE II study: The outcome and prospect of two decades of evolution within the GEC-ESTRO GYN working group and the EMBRACE studies, *Clinical and Translational Radiation Oncology*, Vol.9, pp.48–60.

Prasad, B., Kim, Subin, et al. (2018) Effect of tumor properties on energy absorption, temperature mapping, and thermal dose in 13.56-MHz radiofrequency hyperthermia, *Journal of Thermal Biology*, Vol.74, pp.281–289.

Rietbroek, R. C., Bakker, P. J. M., et al. (1996) Feasibility, toxicity, and preliminary results of weekly loco-regional hyperthermia and cisplatin in patients with previously irradiated recurrent cervical carcinoma or locally advanced bladder cancer, *International Journal of Radiation Oncology Biology Physics*, Vol.34, No.4, pp.887–893.

Röschmann, P. (1987) Radiofrequency penetration and absorption in the human body: Limitations to high-field whole-body nuclear magnetic resonance imaging, *Medical Physics*, Vol.14, No.6, pp.922–931.

Roti Roti, J. L. (2008) Cellular responses to hyperthermia (40- 46 degrees C): cell killing and molecular events, *International Journal of Hyperthermia*, Vol.24, No.1, pp.3–15.

Sankaranarayanan, R. (2015) HPV vaccination: The most pragmatic cervical cancer primary prevention strategy, *International Journal of Gynecology and Obstetrics*, Vol.131, No. Supp 1, pp.S33–S35.

Sapienza, L. G., Gomes, M. J. L., et al. (2017) Does para-aortic irradiation reduce the risk of distant metastasis in advanced cervical cancer? A systematic review and meta-analysis of randomized clinical trials, *Gynecologic Oncology*, Vol.144, No.2, pp.312–317.

Sathekge, M., Maes, A., et al. (2010) Fluorodeoxyglucose uptake by lymph nodes of HIV patients is inversely related to CD4 cell count, *Nuclear Medicine Communications*, Vol.31, No.2, pp.137–140.

Scharko, A. M., Perlman, S. B., et al. (2003) Whole-body positron emission tomography in patients with HIV-1 infection, *The Lancet*, Vol.362, No.9388, pp.959–961.

Schildkopf, P., Holmer, R., et al. (2009) Hyperthermia in combination with X- irradiation induces inflammatory forms of cell death, *Autoimmunity*, Vol.42, pp.311–313.

Schildkopf, P., Frey, B., et al. (2011) Radiation combined with hyperthermia induces HSP70-dependent maturation of dendritic cells and release of pro-inflammatory cytokines by dendritic cells and macrophages, *Radiotherapy and Oncology*, Vol.101, No.1, pp.109–115.

Schmitt, E., Gehrmann, M., et al. (2007) Intracellular and extracellular functions of heat shock proteins: repercussions in cancer therapy, *Journal of Leukocyte Biology*, Vol.81, No.1, pp.15–27.

Scholz, B. and Anderson, R. (2000) On Electrical Impedance Scanning –Principles and Simulations, *Electromedica*, Vol.68, pp.35–44.

- Schroeder, T., Viglianti, B. L. and Dewhirst, M. W. (2009) Low-Intensity Alternating Electric Fields: A Potentially Safe and Effective Treatment of Cancer, *Onkologie*, Vol.31, No.7, pp.357–358.
- Sharma, S., Singhal, S., et al. (1991) Local thermo-radiotherapy in carcinoma cervix: improved local control versus increased incidence of distant metastasis, *Asia Oceania Journal of Obstetrics and Gynaecology*, Vol.17, No.1, pp.5–12.
- Shen, R.-N., Lu, L., et al. (1994) Influence of elevated temperature on natural killer cell activity, lymphokine-activated killer cell activity and lectin-dependent cytotoxicity of human umbilical cord blood and adult blood cells, *International Journal of Radiation Oncology Biology Physics*, Vol.29, No.4, pp.821–826.
- Shi, X., Sims, M. D., et al. (2016) Neutropenia during HIV Infection: Adverse Consequences and Remedies, *International Reviews in Immunology*, Vol.33, No.6, pp.511–536.
- Shih, Y.-C. T. and Halpern, M. T. (2008) Economic Evaluations of Medical Care Interventions for Cancer Patients: How, Why, and What Does it Mean?, *CA: A Cancer Journal for Clinicians*, Vol.58, No.4, pp.231–244.
- Shirazi, A. S., Razi, T., et al. (2014) Diagnostic accuracy of magnetic resonance imaging versus clinical staging in cervical cancer, *Asian Pacific Journal of Cancer Prevention*, Vol.15, No.14, pp.5729–5732.
- Simonds, H. M., Wright, J. D., et al. (2012) Completion of and early response to chemoradiation among HIV-positive and HIV-negative patients with locally advanced cervical carcinoma in South Africa: A retrospective cohort study, *Cancer*, Vol.118, No.11, pp.2971–2979.
- Simonds, H. M., Botha, M. H., et al. (2019) Five-year overall survival following chemoradiation among HIV-positive and HIV-negative patients with locally advanced cervical carcinoma in a South African cohort, *Gynecologic Oncology*, Vol.151, No.2, pp.215–220.
- Simonds, H. M., Neugut, A. I. and Jacobson, J. S. (2015) HIV Status and acute hematological toxicity among cervix cancer patients undergoing radical chemoradiation, *International Journal of Gynecological Cancer*, Vol.25, No.5, pp.884–890.
- Slone, H. B., Peters, L. J. and Milas, L. (1979) Effect of Host Immune Capability on Radiocurability and Subsequent Transplantability of a Murine Fibrosarcoma2, *JNCI: Journal of the National Cancer Institute*, Vol.63, No.5, pp.1229–1235.
- Sminia, P., Jansen, W., et al. (1990) Incidence of Tumours in the Cervical Region of the Rat after Treatment with Radiation and Hyperthermia, *International Journal of Radiation Biology*, Vol.57, No.2, pp.425–436.
- Smith, S. R., Foster, K. R. and Wolf, G. L. (1986) Dielectric Properties of VX-2 Carcinoma Versus Normal Liver Tissue, *IEEE Transactions on Biomedical*

Engineering, Vol.33, No.5, pp.522–524.

Song, C. W., Choi, I. B., *et al.* (1995) Microvasculature and Perfusion in Normal Tissues and Tumors, in Seegenschmiedt, M. H., Fessenden, P., and Vernon, C. C. (eds) *Thermoradiotherapy and Thermochemotherapy: Biology, Physiology, Physics*, pp.139–156.

Song, C. W., Park, H. J., *et al.* (2005) Implications of increased tumor blood flow and oxygenation caused by mild temperature hyperthermia in tumor treatment, *International Journal of Hyperthermia*, Vol.21, No.8, pp.761–767.

Song, C. W., Shakil, A., *et al.* (2009) Tumour oxygenation is increased by hyperthermia at mild temperatures, *International Journal of Hyperthermia*, Vol.25, No.2, pp.91–95.

Sprung, C. N., Chao, M., *et al.* (2005) Chromosomal Radiosensitivity in Two Cell Lineages Derived from Clinically Radiosensitive Cancer Patients, *Clinical Cancer Research*, Vol.11, No.17, pp.6352–6358.

Sreenivasa, G., Hildebrandt, B., *et al.* (2006) Radiochemotherapy combined with regional pelvic hyperthermia induces high response and resectability rates in patients with nonresectable cervical cancer > or =FIGO IIB 'bulky'. *International journal of radiation oncology, biology, physics*, Vol.66, No.4, pp.1159–1167.

Stadalnik, R. C., Bakheet, S. M. and Powe, J. (1998) Benign causes of 18-FDG uptake on whole body imaging, *Seminars in Nuclear Medicine*, Vol.28, No.4, pp.352–358.

Stewart, T. S., Moodley, J. and Walter, F. M. (2018) Population risk factors for late-stage presentation of cervical cancer in sub-Saharan Africa, *Cancer Epidemiology*, Vol.53, pp.81–92.

Sullivan, S. D., Mauskopf, J. A., *et al.* (2014) Budget impact analysis - Principles of good practice: Report of the ISPOR 2012 budget impact analysis good practice II task force, *Value in Health*, Vol.17, No.1, pp.5–14.

Surveillance Epidemiology and End Results (SEER) Program (2017) *Cancer Stat Facts: Cervical Cancer*, *Cancer Stat Facts*. Available at: www.seer.cancer.gov (Accessed: 19 January 2019).

Szasz, A. (2007) Hyperthermia, a modality in the wings, *Journal of Cancer Research Therapy*, Vol.3, No.1, pp.56–66.

Szasz, A. (2014) Current Status of Oncothermia Therapy for Lung Cancer, *Korean Journal of Thoracic Cardiovascular Surgery*, Vol.47, No.2, pp.77–93.

Szasz, A., Szasz, O. and Szasz, N. (2001) Electro-hyperthermia: a new paradigm in Cancer Therapy, *Deutsche Zeitschrift fur Onkologie*, Vol.33, No.3, pp.91–99.

Szasz, A., Szasz, O. and Szasz, N. (2011) *Principles and Practice of Oncothermia*. Springer International Publishing.

Szasz, A. and Vincze, G. (2006) Dose concept of oncological hyperthermia: heat-equation considering the cell destruction, *Cancer Journal*, Vol.2, No.4, pp.171–181.

- Szasz, O., Szigeti, G. P., et al. (2018) Hyperthermia Dosing and Depth of Effect, *Open Journal of Biophysics*, Vol.8, No.1, pp.31–48.
- Szasz, O., Szigeti, G. P. and Szasz, A. (2016) Connections between the Specific Absorption Rate and the Local Temperature, *Open Journal of Biophysics*, Vol.6, No.3, pp.53–74.
- Szendro, P., Vincze, G. and Szasz, A. (2001a) Bio-response to white noise excitation, *Electro- and Magnetobiology*, Vol.20, No.2, pp.215–229.
- Szendro, P., Vincze, G. and Szasz, A. (2001b) Pink-noise behaviour of biosystems, *European Biophysics Journal*, Vol.30, No.3, pp.227–231.
- Szigeti, G. P., Szasz, O. and Hegyi, G. (2016) Personalised Dosing of Hyperthermia, *Journal of Cancer Diagnosis*, Vol.1, No.1, pp.1–9.
- Testa, A. C., Di Legge, A., et al. (2014) Imaging techniques for the evaluation of cervical cancer, *Best Practice and Research: Clinical Obstetrics and Gynaecology*, Vol.28, No.5, pp.741–768.
- The Cancer Genome Atlas Research Network (2017) Integrated genomic and molecular characterization of cervical cancer, *Nature*, Vol.543, No.7645, pp.378–384.
- Tsang, Y., Huang, C., et al. (2015) Improving immunological tumor microenvironment using electro-hyperthermia followed by dendritic cell immunotherapy, *Biomed Central Cancer*, Vol.15, No.708, pp.1–11.
- Tsuchida, E., Kaida, A., et al. (2015) Effect of X-Irradiation at Different Stages in the Cell Cycle on Individual Cell – Based Kinetics in an Asynchronous Cell Population, *PLoS ONE*, Vol.10, No.6, p.e0128090.
- Tsuda, H., Tanaka, M., et al. (2003) Phase I study of combined radiation, hyperthermia and intra-arterial carboplatin for local recurrence of cervical cancer, *Annals of oncology*, Vol.14, No.2, pp.298–303.
- Ulrike, K., Markus, H., et al. (2015) NNRTI-based antiretroviral therapy may increase risk of radiation induced side effects in HIV-1-infected patients, *Radiotherapy and Oncology*, Vol.116, No.2, pp.323–330.
- UNAIDS (2018) *Fact Sheet - World AIDS Day 2018, World AIDS Day*. Available at: <http://www.unaids.org> (Accessed: 7 January 2019).
- Vallis, K. A. (1991) Glutathione deficiency and radiosensitivity in AIDS patients, *The Lancet*, Vol.337, No.8746, pp.918–919.
- van der Zee, J. (2002) Heating the patient: a promising approach?, *Annals of Oncology*, Vol.13, No.8, pp.1173–1184.
- van der Zee, J., Vujaskovic, Z., et al. (2008) The Kadota Fund International Forum 2004 – clinical group consensus, *International Journal of Hyperthermia*, Vol.24, No.2, pp.111–112.
- van der Zee, J., González González, D., et al. (2000) Comparison of radiotherapy alone with radiotherapy plus hyperthermia in locally advanced pelvic tumors: A prospective,

- randomized, multicentre trial, *Lancet*, Vol.355, No.9210, pp.1119–1125.
- van der Zee, J. and González González, D. (2002) The Dutch Deep Hyperthermia trial: Results in cervical cancer, *International Journal of Hyperthermia*, Vol.18, No.1, pp.1–12.
- van Rhoon, G. C. (2016) Is CEM43 still a relevant thermal dose parameter for hyperthermia treatment monitoring?, *International Journal of Hyperthermia*, Vol.32, No.1, pp.50–62.
- Vancsik, T., Kovago, C., et al. (2018) Modulated electro-hyperthermia induced loco-regional and systemic tumor destruction in colorectal cancer allografts, *Journal of Cancer*, Vol.9, No.1, pp.41–53.
- Vancsik, T., Forika, G., et al. (2019) Modulated electro- hyperthermia induced p53 driven apoptosis and cell cycle arrest additively support doxorubicin chemotherapy of colorectal cancer in vitro, *Cancer Medicine*, Vol.8, No.9, pp.4292–4303.
- Vasanthan, A., Mitsumori, M., et al. (2005) Regional hyperthermia combined with radiotherapy for uterine cervical cancers: a multi-institutional prospective randomized trial of the International Atomic Energy Agency, *International Journal of Radiation Oncology, Biology, Physics*, Vol.61, No.1, pp.145–153.
- Vaupel, P., Kallinowski, F. and Harburg, A. K. (1987) Physiological Effects of Hyperthermia, *Recent Results in Cancer Research*. Edited by C. Streffer, Vol.104, pp.71–109.
- Vendrell, I., Ferreira, A. R., et al. (2018) Chemoradiotherapy completion and neutropenia risk in HIV patients with cervical cancer, *Medicine*, Vol.97, No.30, p.e11592.
- Vidair, C. A. and Dewey, W. C. (1991) Division-associated and division-independent hyperthermic cell death: comparison with other cytotoxic agents, *International Journal of Hyperthermia*, Vol.7, No.1, pp.51–60.
- Vincze, G. and Szasz, A. (2018) Similarities of Modulation by Temperature and by Electric Field, *Open Journal of Biophysics*, Vol.8, No.3, pp.95–103.
- Vincze, G., Szasz, A. and Liboff, A. R. (2008) New theoretical treatment of ion resonance phenomena, *Bioelectromagnetics*, Vol.29, No.5, pp.380–386.
- Vincze, G., Szasz, N. and Szasz, A. (2005) On the thermal noise limit of cellular membranes, *Bioelectromagnetics*, Vol.26, No.1, pp.28–35.
- Vincze, G., Szasz, O. and Szasz, A. (2015) Generalization of the Thermal Dose of Hyperthermia in Oncology, *Open Journal of Biophysics*, Vol.5, No.4, pp.97–114.
- Viswanathan, A. N., Lee, L. J., et al. (2014) Complications of pelvic radiation in patients treated for gynecologic malignancies, *Cancer*, Vol.120, No.24, pp.3870–3883.
- Walboomers, J. M. M., Jacobs, M. V, et al. (1999) Human papillomavirus is a necessary cause of invasive cervical cancer worldwide, *The Journal of Pathology*, Vol.189, No.1, pp.12–19.
- Werthmöller, N., Frey, B., et al. (2016) Combination of ionising radiation with

hyperthermia increases the immunogenic potential of B16-F10 melanoma cells in vitro and in vivo, *International Journal of Hyperthermia*, Vol.32, No.1, pp.23–30.

Westermann, A. M., Jones, E. L., et al. (2005) First Results of Triple-Modality Treatment Combining Radiotherapy, Chemotherapy, and Hyperthermia for the Treatment of Patients with Stage IIB, III, and IVA Cervical Carcinoma, *Cancer*, Vol.104, No.4, pp.763–770.

Wootton, J. H., Hsu, I. J. and Diederich, C. J. (2011) Endocervical ultrasound applicator for integrated hyperthermia and HDR brachytherapy in the treatment of locally advanced cervical carcinoma, *Medical Physics*, Vol.38, No.2, pp.598–611.

World Health Organisation (2019) *Human papillomavirus (HPV) and cervical cancer, Fact Sheets*. Available at: <https://www.who.int> (Accessed: 19 February 2019).

Yang, K., Huang, C., et al. (2016) In vitro comparison of conventional hyperthermia and modulated, *Oncotarget*, Vol.7, No.51, pp.84082–84092.

Yang, W., Han, G. H., et al. (2019) Combined treatment with modulated electro-hyperthermia and an autophagy inhibitor effectively inhibit ovarian and cervical cancer growth, *International Journal of Hyperthermia*, Vol.36, No.1, pp.9–20.

Yoo, H. J., Lim, M. C., et al. (2019) Phase I/II clinical trial of modulated electro-hyperthermia treatment in patients with relapsed, refractory or progressive heavily treated ovarian cancer, *Japanese Journal of Clinical Oncology*, Vol.49, No.9, p.832.838.

Zhang, D., Zhou, T., et al. (2016) Reactive oxygen species formation and bystander effects in gradient irradiation on human breast cancer cells, *Oncotarget*, Vol.7, No.27, pp.41622–41636.

APPENDIX A: Ethics Clearance Certificate



R14/49 Ms C Minnaar

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL) CLEARANCE CERTIFICATE NO. M190295

NAME: Ms C Minnaar
(Principal Investigator)
DEPARTMENT: School of Clinical Medicine
Department of Radiation Sciences
Medical School
University

PROJECT TITLE: A randomised trial investigating the benefits of the addition of Oncothermia to chemo-radiation for cervical cancer in HIV positive and negative women in South Africa

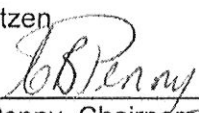
DATE CONSIDERED: Ad hoc

DECISION: Approved unconditionally

CONDITIONS: Renewal of M1704133

SUPERVISOR: Dr K Kotzen

APPROVED BY:

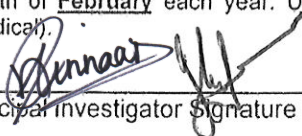

Dr CB Penny, Chairperson, HREC (Medical)

DATE OF APPROVAL: 14/03/2019

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and ONE COPY returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. I agree to submit a yearly progress report. When a funder requires annual re-certification, the application date will be one year after the date of the meeting when the study was initially reviewed. In this case, the study was initially reviewed in February and will therefore reports and re-certification will be due early in the month of February each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).


Principal Investigator Signature

Date

20/3/19

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX B: Patient Information Sheet

Study title: A randomised trial investigating the benefits of the addition of Oncothermia to chemo-radiation for cervical cancer in HIV positive and negative women in South Africa

Principal Investigators: Jeffrey Kotzen, Carrie Minnaar

Institution: University of the Witwatersrand and Charlotte Maxeke Academic Hospital

Information Sheet:

“A randomised trial investigating the benefits of the addition of Oncothermia to chemo-radiation for cervical cancer in HIV positive and negative women in South Africa”.

Dear Prospective Participant

Dr. Jeffrey Kotzen and Carrie Minnaar are conducting a clinical trial at the Charlotte Maxeke Johannesburg Academic Hospital. We are working together with the University of the Witwatersrand Medical School and a small group of doctors and staff members at the radiation oncology department at the Charlotte Maxeke Johannesburg Academic Hospital. Oncology is the treatment of cancer. In this clinical trial we are investigating a type of therapy called hyperthermia. Hyperthermia means hot. In oncology, hyperthermia therapy means to heat up tumours. We will be using a medical device (machine) to heat up tumours. We want to see if this helps the treatments for cervical cancer to work better.

The title of our clinical trial is:

“A randomised trial investigating the benefits of the addition of Oncothermia to chemo-radiation for cervical cancer in HIV positive and negative women in South Africa”.

A clinical trial is a method of testing new types of treatments or therapies. Clinical trials involve participants who voluntarily choose to take part in the clinical trial. This means that it is entirely your choice whether or not to participate in the clinical trial. We are inviting you to take part in our clinical trial. You have been asked because you have advanced cervical cancer and this is the cancer that we will be testing the effects of hyperthermia on.

Before you make your decision, you need to know about the treatments being offered, the risks and the benefits, what will be expected of you and what is expected of us. This document contains all the information that you need to make a decision. You may have more questions after reading this information sheet. Your study doctor and the study investigators will be available to answer all of your questions before you make a decision. Our contact details are at the end of this information sheet and you may contact us with any further questions that you may have relating to this study. It is important that you have all the information you need to make a decision. It is also important that you are kept up to date with any new information that affects you or the clinical trial. It is our responsibility to make sure that you are kept informed of any new information that we get during the trial.

If you decide to take part in the trial, you will be asked to sign a consent form. You will also be given a copy of this information sheet to take home and refer to at a later stage.

Why are we doing this clinical trial?

The purpose of this trial is to see if the normal treatment for advanced cervical cancer can be improved if the tumours are heated before they are treated. If the treatment is improved, we want to see if this helps women with advanced cervical cancer to live longer. This is very important, especially in South Africa, where we have thousands of women treated for cervical cancer every year. The results of this trial will help doctors in the future to decide whether or not heating the tumours is helpful.

How does the trial work?

We will be inviting 236 women between the ages of 18 and 70 who have been diagnosed with advanced cervical cancer to participate in the trial. Each woman who participates will receive the standard treatment for advanced cervical cancer. Standard treatment means the normal treatment that all women with advanced cervical cancer have access to at the Charlotte Maxeke Academic

Hospital. The normal treatment involves three (3) doses of chemotherapy, five weeks of radiation therapy and three doses of brachytherapy. This is the standard treatment for advanced cervical cancer and it is given because it is currently the best way that we know of to treat advanced cervical cancer. These treatments are explained in more detail in the next few pages. It is important to know that these treatments are given to all women with advanced cervical cancer, even if they are not a part of this trial.

Half of the women in the trial will receive only the standard, or normal, treatments. This means that they will not receive anything else that is not standard or normal for women with advanced cervical cancer. Any risks and benefits for these women will be the same as the risks and benefits for women being treated for advanced cervical cancer who are not involved in the clinical trial. This group of women will be known as the control group.

The other half of the women will receive the standard treatments and will also receive the hyperthermia treatments. This group will be known as the treatment group.

Each woman who agrees to participate in the trial will be randomly assigned to either the control group or the treatment group. This means that neither the women nor the study doctor(s) will know which group you will be entered into at the beginning of the trial. This is important because it means that the study doctor(s) cannot control or decide which group a woman will be assigned to and can therefore not affect the results of the study. Only after each woman has been randomly assigned to a group, will the woman and the study doctors will be told which group the woman has been assigned to.

If you agree to be part of the trial, you will be given the standard treatment either with or without hyperthermia. The treatments last about six (6) weeks. After the treatment has been finished, we will ask you to return to us for regular check-ups so that we can see how well the treatment worked and how well you are feeling. We will be checking on you for two years after you have finished your treatment. At the end of the trial we will compare the health of the group of women who did not receive hyperthermia with the group of women who did receive hyperthermia so that we can see which group of women showed the most benefits.

Does your HIV status affect your involvement in the trial?

If you want to participate in the trial we will need to know what your HIV status is. This means that, unless you have already had a recent HIV test, we will need to do an HIV test on you before we start the treatment.

HIV causes damage to the parts of your body that help you to stay healthy. Some of the medicines that are used to treat cancer also cause damage to the parts of your body that help you to stay healthy. This is why it is important to know your HIV status before you are given the medicines to treat your cancer.

If you are HIV positive, some blood will be used to test the viral load and the CD4 count in your body. These two tests will tell us if you are healthy enough to have the medicine for your cancer. If the tests tell us you are not healthy enough for medicine then we will refer you to an HIV clinic where you will be given medicines called antiretroviral treatments, or ARTs, to help you to fight the virus. Once you are on these medicines and your health improves, then you can have the medicine to treat the cancer. You may be asked by the doctors at the HIV clinic to visit them regularly for tests and for medication. In order to be a part of the trial we need your permission to contact the HIV clinic for copies of any tests that they do on you and to find out what medicines they are giving you. This will help us to make sure you stay healthy and to understand the best way to treat you and other HIV positive patients with cervical cancer.

The results of your HIV tests will also be used in the trial and will be made available to the study doctors and people working on the trial. Your HIV test results will be kept strictly confidential, just like the rest of your results.

What treatments will be given?

If you are randomly assigned to the treatment group then you will receive all of the standard treatments (chemotherapy, radiation therapy, brachytherapy) and examinations plus hyperthermia treatments. If you are randomly assigned to the control group then you will receive the standard treatments (chemotherapy, radiation therapy, brachytherapy) without hyperthermia.

Regardless of which group you are assigned to, you will also still receive any other treatments and medications that you may need, such as medicine to stop you from feeling sick and medicine to help manage any pain that you might have.

Chemotherapy:

Chemotherapy is a type of medication that is used to treat cancer. The type of chemotherapy used to treat advanced cervical cancer at the Charlotte Maxeke Academic Hospital is called Cisplatin. Cisplatin is given to you through a needle that is put into a vein. Cisplatin is poisonous to cancer cells and helps the radiation therapy to destroy the cancer cells.

Radiation therapy:

Radiation is an invisible energy that is made by a large machine. The radiation machine can control where the energy goes. It is similar to a microwave, but much bigger and more complicated. Just like a microwave makes energy which cooks food, radiation machines make energy which helps to destroy tumours. You will receive radiation therapy for a few minutes every Monday to Friday for five weeks. The radiation energy will be put into the area of your body where the tumour is.

Brachytherapy:

Brachytherapy is another type of radiation but this type is given inside your body, and not outside your body. This means that it is put inside your vagina. You will be given brachytherapy three times.

Hyperthermia:

If you are assigned to receive hyperthermia, the hyperthermia treatments will be given to you twice a week during the five weeks that you receive radiation therapy. Each treatment lasts only one (1) hour and will be given before your daily dose of radiation therapy. This means that if you are in the hyperthermia group, then on the days that you receive hyperthermia treatments you will be asked to come to the hospital two (2) hours before your radiation appointment. Having hyperthermia does not hurt. You will be asked to lie on a bed and a round device that feels like a pillow full of water will be placed over the area of your body where your cancer is. You will be asked to lie still for an hour and during this time you will feel that your skin will get warm. It is a very comfortable treatment and it should not hurt you at all. If it gets too hot, the doctor or nurse giving you the treatment will turn the temperature down.

What will you need to do to participate in the trial?

You have been given this information sheet because your doctor, after diagnosing and examining you, feels that you are suitable for the clinical trial. If you decide to participate in the trial you will be asked to sign a form that gives us your written permission to include you in the trial. The tests and examinations that will be done are the same tests and examinations that everybody with your kind of cancer has done.

There will however be some things that we will ask you to do that are not part of the standard treatment. These are:

You will be asked to give regular feedback and answer questions on how you are feeling so that we can monitor your health. This will be done by completing questionnaires called Quality of Life forms. You will be asked to complete these forms before your treatment is started, straight after you have finished your treatment, every three months for the first year after your treatment and every six months during the second year after your treatment. These forms can be completed at the hospital with your study doctor or over the phone if you are unable to come to the hospital on that day.

You will be asked to have a CT scan before you start treatment and six (6) months after your treatment has been completed. CT scans are special machines which are able to take pictures of the inside of your body. The pictures that we get from your scans will show us whether your tumours are getting smaller, getting bigger or staying the same size.

Before you receive treatment, the following will be required:

- Physical examination – during the examination your study doctor will check your health and make sure that you are able to participate in the trial.

- Quality of Life form
- Special machines will be used to help the doctors have a look at the cancer inside your body. These machines are called CT scanners, X-ray machines and an ultrasound. Each of these machines gives us a special picture of what is happening inside you and helps us to plan your treatment. These machines do not cause any pain and they are all very safe. You cannot have pictures done with these machines if you are pregnant though as it could cause the baby harm.
- About 20 ml of your blood samples will be taken and tested to make sure that you are healthy enough to participate in the study. Taking this amount of blood is safe and will not harm you at all.
- Some of the blood that is taken will also be used to test you for HIV.
- You will also be asked to do a pregnancy test. The standard treatment for cervical cancer is very dangerous for the babies of pregnant women. It is important to make sure that you are not pregnant before you start the treatment.

During your treatment:

Everyone in the trial will receive the same treatment as all other woman with advanced cervical cancer like yours. During your treatment your doctor will take samples of your blood at least another three (3) times to make sure that the chemotherapy dose that the doctors are giving you is safe for you. The doctors may also examine you to make sure that the radiation therapy and brachytherapy is not causing any serious side effects.

After your treatment is complete:

You will be examined regularly and will have regular blood tests to monitor your health during the two years after you have finished your treatment. You will be asked to come in and see your study doctor every three months during the first year and every six months during the second year after your treatment. Your final appointment will be two years after the treatment has been finished. These appointments are important and will allow your study doctor to make sure that your treatment is working and that you are healthy. You will also be asked to have another CT scan and another PAP smear six (6) months after you have completed treatment and will be asked to have an x-ray one year and two years after you have finished your treatment.

If you are HIV positive then your doctor may also ask you to have another test three months after completing your treatment to check that the HIV is still controlled.

Your Information and Confidentiality

All of the information relating to your health will be recorded in your medical files which are kept at the hospital. The study doctors will have access to your files in order to monitor your health. The study doctors will make copies of your blood tests, quality of life questionnaires, imaging (CT, x-ray and ultrasound) results and other medical information. However once the study doctor has made copies of your reports, your name will be taken off all the reports and replaced with your reference number. In order to participate in the trial you will be asked to give us permission to use your medical records for the clinical trial. We need these records in order to compare your health to everybody else's health on the trial and to see which group of women has the best results after two years.

The study doctor(s) will do everything possible to make sure that your information is protected and kept private. Each woman in the trial will be given a number. Only the study doctors will have the list that shows which person has which number. The file that is made for you for the trial will only have a number on it and your name and personal details will be taken off all the information in the study file. This means that no one other than the study doctors treating you will know who you are.

It is important to know that every effort will be made to make sure that your results are kept confidential. However there may be times when regulatory bodies require the study doctors to release the names of the participants. Regulatory bodies are groups of people who control and monitor all the clinical trials in South Africa in order to make sure that the study doctors and researchers are looking after the participants properly. They may also want to make sure that the study investigators are being truthful about the results of the trial. It is important that all trials are monitored so that all participants are looked after and the results of the trials are accurate. If a regulatory body requests your information from the study doctors and investigators, the regulatory body will also make every effort to maintain your confidentiality. Sometimes the study doctors will

be required to release your information to authorities such as law enforcement agencies. If this happens it will be done in accordance with the law and everyone involved will handle your information with discretion and will work hard to make sure that your information remains private.

You are allowed to talk about your treatment to anyone that you want to. You are not required to keep your treatment or your participation in the trial a secret.

If you participate in the trial, what is your responsibility?

It is your responsibility to ensure that you attend all of your treatment appointments and receive all the treatments prescribed to you. It is important that you understand that the standard treatments are given with your best interest in mind and they are your best chance of managing your cancer. It is therefore important that you make every effort possible to ensure that you attend each treatment appointment in order to make sure that your health is managed properly. This would be the same whether you are participating in the trial or not. It is your responsibility to make sure that you are available to receive your treatment. If you skip too many appointments or are unable to attend your appointments then we may discontinue your participation in the trial. This is because we need results from people who have followed the treatments properly so that at the end of the trial we can make the correct decision about which treatment was best. However if you are unable to attend your treatments you will not in any way be discriminated against. If your participation in the trial is discontinued you will still have access to treatment just like everybody else who is not participating in the trial.

What if you fall pregnant?

The treatments for cervical cancer are very dangerous to the babies of pregnant women and can be harmful to the children of breast feeding mothers. It is therefore your responsibility to make sure that every effort is made to prevent yourself from falling pregnant while you are being treated for cervical cancer. It is also important that you do not breast feed while you are receiving the treatment. If you suspect that you are pregnant you must tell your study doctor straight away so that the study doctors can make sure that your baby is not harmed by the treatments.

Your options for preventing pregnancy include the following:

- Birth control pills
- Female condoms
- Diaphragms
- Birth control injections
- Intrauterine device (IUD)
- Not having sex
- Ensuring that your male partner wears male condoms

What will it cost to participate in the clinical trial?

You will not be asked to pay for any of the treatments that you receive during the trial. The costs for you will only be the costs for transport and travelling to the hospital to receive your standard treatments. You will be reimbursed for the transport costs for any follow-ups that are not part of the standard treatments.

What are the risks of taking part in the clinical trial?

The risks with the type of hyperthermia that we are using are very small. In eight (8) out of one hundred (100) people the skin, where the machine touches your body, turns red for up to half an hour after the treatment. One (1) out of a hundred (100) people will have mild burns where the machine touches the body. This risk is very small and every attempt will be made by your study doctor to minimise this risk. If you do get a burn we will make sure that you burns are treated correctly.

The risks associated with the standard treatments are the same risks that any other person may have when being treated for advanced cervical cancer. These risks include:

Risks associated with Cisplatin:

- Common: Decreased immune function, infections, mouth sores, anaemia, tiredness, weight loss, decreased appetite, nausea, vomiting, diarrhoea, constipation, kidney problems, hair loss, tingling or pain in your fingers and toes, changes in your taste sensations, mouth sores, ringing sound in your ears and a skin rash.
- Uncommon: Allergic reaction, severe infections, bruising and bleeding, changes in your ability to see, some cancers, deafness, heart problems, liver problems,

Risks associated with Radiation Therapy:

Diarrhoea, Constipation, vaginal bleeding, difficulty urinating, skin rashes and pain in the area being treated, vaginal pain, painful sex, decreased sexual function, loss of your pubic hair.

Non-study medications

There is also a risk that you may have side effects to some of the medications that are given to you during the study but are not for the treatment of your cancer. Examples of these medications are medicines which stop you from vomiting, medicines for pain, medicines which stop diarrhoea etc. Mixing certain medications can also be dangerous. It is important that you tell your nurse or doctor all of the medicines that you are taking so that we can make sure that mixing the medicines with the chemotherapy does not make you sick.

While we have listed all the risks that we can think of above, there may be some risks which we are not aware of or do not know about. This clinical trial will help us to identify any risks that we may not yet know of.

What are the benefits to participating in the study?

There are several benefits that you may experience if you participate in the study. However these benefits cannot be guaranteed. If you participate and you are assigned to the treatment group you may experience the benefits of the hyperthermia. Previous clinical trials suggest that having hyperthermia with your normal treatment will improve your overall health and help you to live longer. These benefits cannot be guaranteed.

If you are not assigned to the hyperthermia group, you will still benefit from the close monitoring and care that you will receive whilst participating in the trial.

Can the study doctors stop your participation in the trial?

The study doctors will always have your best interests in mind and may therefore decide to stop your treatment, without your permission, if they feel that the treatment is harming you, is not benefiting you or if you become pregnant. The study doctors may need to stop your participation in the trial if the trial is stopped or cancelled or if you are not able to attend all of your treatment and follow up appointments.

What if you decide not to participate in the trial?

If, after reading through this information sheet, you decide not to participate in the trial, your treatment and healthcare will not in any way be affected. You will still receive the same standard treatment that everyone else with your disease receives. Healthcare is your right and a decision not to participate in this trial will not in any way affect your right to healthcare. It is also your right to choose whether or not to participate in the clinical trial. Your participation is entirely optional.

You also have the right to change your mind once you have been entered into the trial. You can decide to stop participating in the trial at any time for any reason. If you decide to stop participating in the trial you will still receive standard treatment for your cancer. You will still have access to all of the treatments that every other woman with advanced cervical cancer who is not participating in the trial has access to.

What are your other options for treatment?

If you choose not to participate in this clinical trial you can still receive all the normal treatments and care that women with advanced cervical cancer have access to. You also have the right to choose not to have any treatment for your cancer. However these decisions must be discussed with your doctors so that you are sure that you make the right decision for your health.

Thank you for your time and please feel free to contact us if you have any further questions.

Best regards,

Dr. Jeffrey Kotzen (011) 481 2137
Study doctor and Principal Investigator

Carrie Minnaar (072) 1234 292
Co-Investigator

APPENDIX C: Consent Form

MODULATED ELECTRO-HYPERTHERMIA

Consent Form (SIGNED AND KEPT IN PATIENT FILE):

Hospital number: _____ Contact no: _____

I, the undersigned, hereby confirm that:

1. The potential risks and benefits of the modulated electro-hyperthermia treatment been explained to me.
2. I understand that the hyperthermia treatment is to be given in combination with the chemotherapy or radiotherapy and that the hyperthermia is used to increase the efficacy of the chemotherapy and/or radiotherapy.
3. I understand that this treatment is still considered to be experimental in South Africa and that there are no guaranteed outcomes.
4. I understand that due to the experimental nature of the hyperthermia treatment, there are no fees or costs associated with the hyperthermia treatment.
5. Although the risk of potential side effects is very low, I understand that there is a small chance that I may develop erythema (red rash) due to the heat, or superficial burns to the skin.
6. I understand that there are no laboratory tests that will predict the success of the treatment
7. I understand that I must immediately report any unusual symptoms to my treating doctor and be especially aware of itchiness, pain, discomfort etc. at the treatment site.
8. All of my questions have been answered to my satisfaction.

Print Name of Patient

Signature of Patient

Date

Print Name of Witness

Signature of Witness

Date

Print Name of Treating Doctor

Signature of Treating Doctor

Date

