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Maastricht University

Doctoral dissertation submitted to obtain the degree of
Doctor of Doctor of Biomedical Sciences, to be defended by

Jolien Robijns

DOCTORAL DISSERTATION

Photobiomodulation therapy for the prevention and management of acute radiodermatitis in cancer patients

Promoter: Prof. Dr Jeroen Mebis
Co-promoter: Prof. Dr Veerle Somers

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"If you want others to be happy, practice compassion.
If you want to be happy, practice compassion."

Dalai Lama

Promoter	Prof. Dr. Jeroen Mebis	Hasselt University, BE Jessa Hospital, BE
Co-promoter	Prof. dr. Veerle Somers	Hasselt University, BE
Jury	Prof. dr. Marcel Ameloot (Chair)	Hasselt University, BE
	Prof. dr. Niels Hellings	Hasselt University, BE
	Prof. Dr. Ivo Lambrichts	Hasselt University, BE
	Prof. Dr. René-Jean Bensadoun	Centre de Haute Energie, FR
	Dr. Hans-Joachim Laubach	University Hospital Geneva, CH

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ADSC	adipose-derived stem cell
AP-1	activator protein 1
ARD	acute radiodermatitis
ATP	adenosine triphosphate
AUP	Actual Utilization Proportion
BC	breast cancer
BCA	bicinchoninic acid assay
bFGF	basic fibroblast growth factor
BL	basal layer
BMI	Body Mass Index
BMDSC	bone marrow-derived stem cell
cAMP	cyclic adenosine monophosphate
CCL	Chemokine (C-C motif) ligand
CCO	cytochrome c oxidase
cGMP	cyclic guanosine monophosphate
CREB	cAMP response element-binding protein
CTCAE	Common Terminology Criteria for Adverse Events
CTRL	control
CXCL	chemokine (C-X-C motif) ligand
DIBH	deep inspiration breath hold
DMBA	dimethylbenzanthracene
DNA	deoxyribonucleic acid
ECM	extracellular matrix
EGF	epidermal growth factor
EORTC	European Organisation for Research and Treatment of Cancer
H_2O	water
H_2O_2	hydrogen peroxide
Hb	haemoglobin
HBOT	hyperbaric oxygen therapy
HNC	head and neck cancer
ICAM	intercellular adhesion molecule
IFN-γ	interferon gamma

IGF	insulin-like growth factor
IL	interleukin
IMRT	intensity modulated radiotherapy
IPL	intense pulsed light
IR	infrared
LED	light-emitting diode
LPDL	long- pulsed dye laser
LLLT	low-level light therapy
LT	laser therapy
MASCC	Multinational Association of Supportive Care in Cancer
MLCK	myosin light-chain kinase
MLS	Multiwave Locked System
MMP	matrix metalloproteinase
MSC	mesenchymal stem cell
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NICE	National Institute for Health and Care Excellence
NIR	near-infrared
NO	nitric oxide
OLED	organic dot light emitting device
OM	oral mucositis
OUP	Optimal Utilization Proportion
PBMT	photobiomodulation therapy
PBS	phosphate-buffered saline
PDGF	platelet-derived growth factor
PDL	pulsed dye laser
PDT	photodynamic therapy
PKA	protein kinase A
PTV	planned target volume
PTX	pentoxifyline
QLED	quantum dot light emitting device
qRT-PCR	quantitative real-time PCR
RCT	randomised controlled trial
RD	radiation dermatitis
RIF	radiation-induced fibrosis

RISRAS	Radiation Induced Skin Reaction Assessment Scale
ROS	reactive oxygen species
RT	radiotherapy
RTOG	Radiation Therapy Oncology Group
SC	stratum corneum
SCC	squamous cell carcinoma
SD	standard deviation
SG	stratum granulosum
SOD	superoxide dismutase
SP	stratum spinosum
STAT	Skin Toxicity Assessment Tool
TEWL	transepidermal water loss
TGF-β	transforming growth factor beta
TNF-α	tumor necrosis factor alpha
TP	total protein
TRPV	transient receptor potential vanilloid
VEGF	vascular endothelial growth factor
VitE	vitamin E
VCAM	vascular cell adhesion molecule
VVRO	Vereniging Verpleegkundigen Radiotherapie en Oncologie
WALT	World Association for Laser Therapy
WCS	Woundcare Consultant Society
WHO	World Health Organisation

CHAPTER 1

General introduction

1.1 RADIOTHERAPY

Cancer incidence in Belgium was estimated to be around 67,087 in 2015 [1]. Breast and prostate cancer are the most frequently occurring malignancies in females and males with 10,378 and 8,366 cases in 2015, respectively [1]. Radiotherapy (RT) is an important modality in the modern cancer treatment. Approximately 50% of the cancer patients are treated with RT alone or in combination with other modalities (eg. surgery, chemotherapy, immunotherapy and/or hormone therapy) [2].

RT is a locoregional treatment option of which the main goal is to maximise damage to the tumour, while minimising the effect to normal tissues. It can be used in two different settings. It is mainly used with the intent to destroy the tumour and therefore to cure the cancer. In addition, RT can also play a role in palliation therapy in order to reduce symptoms (eg. pain, seizures, obstructions, ulcerations, etc.) associated with advanced tumours. As such, RT can also diminish the effects of the malignancy on the patients' quality of life [3].

1.1.1 Molecular mechanism of radiotherapy

RT uses ionising radiation to damage the DNA of tumour cells, which stops the cell proliferation. When ionising particles interact with a cell, ionisations and excitations are produced in biological macromolecules (e.g., DNA) and water (H_2O) [4].

Based on the site of the interaction, the action of the radiation is classified as either direct or indirect. Direct action of the radiation occurs when the atoms of the DNA are directly ionised, which leads to dysfunctional or inactive DNA. On the other hand, indirect action of ionising radiation refers to the interaction of radiation particles with H_2O, which results in the production of free radicals. These free radicals cause biological damage by breaking chemical bonds in DNA. Eventually, DNA damage, whether direct or indirect, will lead to mitotic cell death of the tumour cells, which occurs after one or more cell divisions. Since the human body consists of 80% water and less than 1% DNA, most of the damage caused by ionising radiation results from indirect action [4].

1.1.2 Radiobiology

Ionising radiation does not make a distinction between the DNA of tumour and normal cells; so normal cells surrounding the tumour are also affected by radiation. The main principle of RT is maximising the dose to the tumour, while minimising the dose to the normal surrounding cells in order to protect them from damage. Therefore, the total dose of RT is divided in several smaller daily doses, also known as fractionation, which relies on the 4 R's of the radiobiology; repair, reoxygenation, redistribution, and repopulation.

1.1.2.1 Repair

Normal cells have generally better mechanisms to detect and repair sub lethal damage than cancer cells, which allows them to survive until the next fraction of RT [5-7].

1.1.2.2 Reoxygenation

Tumours are known to have hypoxic areas due to poor vasculature. Oxygen is important in the formation of free radicals, which are necessary to cause DNA damage during RT. Due to fractionation, the tumour shrinks while the number of blood vessels remains constant, resulting into an increased oxygen tension in the tumour. This implies that the previously hypoxic tumour will reoxygenate and eventually become more susceptible to radiation damage [5-7].

1.1.2.3 Redistribution

Further, radiosensitivity of cells varies considerably as they pass through the cell cycle. The most sensitive phases are M and late G2, followed by late G1 and early S phase, while cells are the most resistant in the mid to late S and early G2 phase. The vast majority of irradiated cells, that harbor remaining DNA damage, die usually after attempting mitosis one or more times, depicted as mitotic catastrophe. Sensitivity of the late G2 phase probably results from the fact that those cells have passed the final cell cycle checkpoint in the early G2. The high radioresistance of the mid to late S phase is due to the large amounts of synthesis enzymes present, which have the ability to rapidly repair DNA. When a single, high dose of RT is given, the surviving cells are likely to be in the resistant phases of the cell cycle. When RT is applied over multiple sessions, the surviving cells will have more time to move into more radiosensitive phases of the cell cycle, which will increase the effectiveness of RT. This phenomenon is called redistribution [5-7].

Moreover, RT will cause delays in the movement of cells through the G1, S, and G2 phases of the cell cycle, via the activation of the DNA damage checkpoints. When these checkpoints are activated cells will have more time to repair DNA damage. In contrast to normal cells, many tumour cells have lost one or more of these checkpoints, which will affect redistribution of tumour cells into more radiosensitive phases of the cell cycle [5-7].

1.1.2.4 Repopulation

During each RT fraction the number of tumour cells is reduced. However, the surviving cancer cells can repopulate the tumour by proliferation. Therefore, any prolongation or interruption of RT might lead to regrowth of the tumour, which will eventually influence the treatment outcome. Especially, squamous cell carcinomas (SCC) of the head and neck show an accelerated regrowth at four to five weeks RT. To minimise this repopulation effect, hyper fractionation and the prevention of treatment interruptions is recommended in these cases [5-7].

1.2.2.5 Side effects

Despite the developing RT techniques in order to minimise the effect of radiation of normal tissues, patients still develop several complications. RT-related side effects that occur most frequently are fatigue, dysphagia, pain, oral mucositis (OM), and skin reactions (also referred to as radiation dermatitis, radiodermatitis (RD) or radiotherapy-induced skin reactions dermatitis) [8].

1.2 THE HUMAN SKIN

1.2.1 Normal physiology of the skin

The largest organ of the human body is the skin, compromising 16% of the total body weight. The skin serves as a physical barrier as it prevents the loss of water and other components of the body to the environment and it protects the body against environmental insults, such as microorganisms or chemical substances. Furthermore, it has also different sensory functions, plays a role in thermoregulation, and synthesizes vitamin D [9-11]. The skin is composed of two main layers, the epidermis and the dermis, as illustrated in figure 1. The epidermis is the superficial layer of the skin. The main cell type of the epidermis is the keratinocyte, which makes the protein keratin. The epidermis is composed of four layers starting at the stratum basale, moving upward trough the stratum spinosum (SP), the stratum granulosum (SG) to the stratum corneum (SC) [9-13].
The basal layer of the epidermis consists mainly of keratinocytes that are dividing or non-dividing, also known as interfollicular epidermal stem cells. Furthermore, also melanocytes, pigment-producing cells, can be found in this layer. When the basal stem cells divide and mature, they move towards the surface of the skin and form prickle cells found in the SP. These are polyhedral cells connected to each other by linking proteins (i.e. desmosomes). In addition, Langerhans cells can be identified in the SP. These are antigen-presenting cells, which have an important task in inflammatory processes [12].

Continuing the movement of the cells towards the upper layer, they become more flat and lose their nuclei. The cells in the SG consist of intracellular granules of keratohyalin and smaller cytoplasmatic lamellated granules (i.e. Odland bodies) that contain lipids. The lipid components of these cells are discharged into the intercellular spaces, which is important for the skin barrier function. The final step in the maturation process of the keratinocytes can be found in SC, also named the horny layer. This layer is made up of corneocytes, which are surrounded by a resistant protein envelope and are filled with water-retaining keratin proteins. The SC can be compared to a brick wall where the corneocytes are considered as the "bricks" and the lipid layers between the cells are the "mortar". This most upper layer provides the physical and water-retaining skin barrier function. In normal healthy skin the superficial cells of the SC are shed through normal desquamation, and are replaced by stem cells from the underlying basal layer. After approximately four weeks the epidermis is totally repopulated with new cells [10, 13].

A basement membrane connects the epidermis to the underlying dermis. The dermis provides structural strength to the skin. It is mainly composed of connective tissue consisting of collagen and elastin fibres produced by fibroblasts. Collagen gives strength and structure to the skin, while elastin maintains the elasticity and flexibility. The fibrous tissue of the dermis also contains blood vessels, glands, nerves, lymph vessels, and hair follicles [10]. Also within hair follicles and sebaceous glands distinct stem cell niches can be found, which are important for the maintenance and renewal of these important appendages (Figure 1) [13].

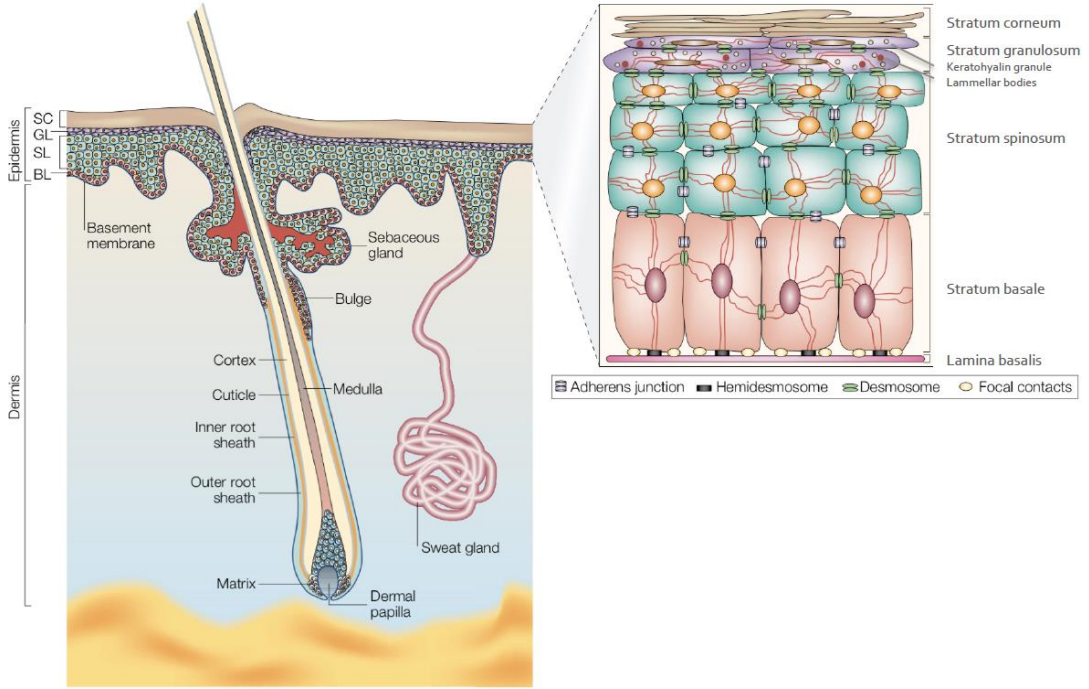

Figure 1: Structure of the skin and its appendages
The adult mammalian epidermis is a stratified squamous epithelium, which provides the skin with its barrier. It consists of an inner layer of proliferative basal cells (keratinocytes) that are separated from the underlying dermis by the lamina basalis. Resting on the lamina basalis is the stratum basale, consisting of rapidly, proliferating interfollicular epidermal stem cells. The stratum basale gives rise to differentiated cell layers of the stratum spinosum (SP), stratum granulosum (SG), and the stratum corneum (SC). Lipids are synthesized in the keratinocytes, stored in the lamellar bodies, and released into the SC, where they form intercellular layers. Filaggrin arises from profilaggrin (contained in keratohyaline granules), contributes to formation of the barrier, and its degradation products are involved in hydration of the SC. Tight junctions seal neighboring cells in the SG, forming the second line barrier. Desmosomes interconnect adjacent keratinocytes and are important for cell–cell cohesion in the nucleated layers. In hair follicles two main subpopulations of stem cells are believed to exist, a subset located within the hair germ just below the bulge that gives rise to the hair shaft and inner root sheath and the quiescent group, which resides in the bulge region that gives rise to the basal outer root sheath keratinocytes. A third niche of skin stem cells can be found in the sebaceous glands. (With permission adapted from Fuchs et al., 2002 [13])

1.2.2 Skin wound healing

Adequate wound healing relies on a complex network of cells (e.g. keratinocytes, fibroblasts, macrophages, endothelial cells, mesenchymal stem cells), cytokines, growth factors and extracellular matrix (ECM) molecules. There are four phases of wound healing: haemostasis, inflammation, proliferation, and tissue remodelling. During haemostatis, a fibrin clot is formed, which serves as a protective layer to the underlying tissue and as a matrix through which cells can migrate. It also acts as a reservoir for growth factors [14-16].

Secondly, in the inflammatory phase, platelets are activated which leads to the production of several mediators. These mediators initiate the wound healing process by attracting neutrophils and monocytes to the wound bed. These cells will phagocytise bacteria and damaged tissue and finally release signalling molecules that initiate the proliferative phase of wound healing [14-16].

The proliferation phase is characterized by the formation of new blood vessels (i.e. angiogenesis) and the reinforcement of the injured tissue. This is driven by the proliferation of fibroblasts, which synthesize granulation tissue and a new ECM by excreting collagen, proteoglycans, and glycoproteins. Simultaneously, re-epithelialization of the epidermis occurs, whereby keratinocytes proliferate and migrate to the wound bed [14-16]. During the remodelling phase, matrix metalloproteinases (MMPs) modify the newly formed granulation tissue by cross-linking the collagen and scar maturation [14-16].

1.3 RADIATION DERMATITIS

Ionising radiation essentially damages the cell's ability to divide and multiply. Skin cells are one of the most radiosensitive cells due to their high proliferation rate. Therefore, one of the most important side effects of RT is radiodermatitis (RD), a cutaneous reaction to the inflicted cellular injury [8]. Radiation-induced skin injuries are deterministic effects, which implies that they occur once the threshold level of exposure has been exceeded. The severity and progression of the skin reactions varies widely between patients depending on both treatment- and patient-related risk factors. RD can occur as an acute (early) effect, developing within the first hours to weeks after radiation exposure, or as a "late" effect (chronic), occurring months or years after the intervention [17].

Up to 95% of the cancer patients treated with RT will develop some degree of skin reaction in the treated area [18]. RD is the most common in patients treated for breast, head and neck, anal and vulvar cancer, in which the irradiated targets are located close to the skin surface. Research has demonstrated that a low source-to-surface distance is inversely correlated with a high skin dose [19].

1.3.1 Clinical appearance

1.3.1.1 Acute radiodermatitis

Early skin effects occur within two to four weeks after the initiation of RT. Acute RD can be graded based on the criteria of the Radiation Therapy Oncology (RTOG) as shown in table 1 [20]. Acute RD starts with red rashes and dry desquamation (grade 1). A grade 2 skin reaction is characterized by a bright erythema combined with patchy moist desquamation located in the skin folds. When RD worsens, confluent moist desquamation outside the skin folds (grade 3) develops. In some rare cases necrosis with, haemorrhage and eventually ulceration can occur (grade 4) [20-23]. Especially grade 2 to 4 RD can be very painful and affects greatly the patients' quality of life. Patients have to cope with problems during their daily life (e.g. washing practices, getting dressed, household activities, hobbies) [24]. In severe cases of acute RD, premature interruption of RT might be necessary which will eventually affect the treatment outcome and overall patient survival [25]. Usually, acute skin reactions heal within a month after completion of RT [17].

Table 1: Radiation Therapy Oncology Group (RTOG) scoring for radiotherapy-induced skin injuries [20]

Onset	RTOG Grade				
	0	**1**	**2**	**3**	**4**
Acute	No change over baseline	Follicular, faint or dull erythema; epilation; dry desquamation; decreased sweating 	Tender or bright erythema; patchy moist desquamation; moderate oedema 	Confluent, moist desquamation, other than skin folds; pitting oedema 	Ulceration; haemorrhage; necrosis 
Chronic	None	Slight atrophy; pigmentation change; some hair loss 	Patchy atrophy; moderate telangiectasia; total hair loss 	Marked atrophy; gross telangiectasia 	Ulceration 

RTOG, Radiation Therapy Oncology Group
Pictures with permission from Claes Stefan – Limburg Oncology Centre

1.3.1.2 Chronic radiodermatitis

Late skin effects can develop months to year after the exposure to RT. The main cutaneous reactions that characterize chronic RD are skin atrophy, fibrosis, pigmentation changes, telangiectasia, necrosis, and secondary malignant skin tumours (Table 1) [26].

Skin atrophy is related to a decreased number and activity of dermal fibroblasts and the reabsorption of collagen, resulting in a fragile and thin skin [27]. Remaining dermal fibroblasts are pathologically activated by growth factors (e.g. transforming growth factor beta, TGF-β) into myofibroblasts. This is typically seen within the context of wound healing. However, in the scenario of chronic RD this happens also even though no active wound is present, resulting in an excessive, unstoppable accumulation of collagen and ECM components leading to skin fibrosis. RT-induced fibrosis (RIF) is clinically characterized by induration, thickening of the dermis, and even a reduced range of motion [28]. Different types of pigmentary changes can be observed: the focal depletion of melanocytes in combination with focal melanocytic hyperactivity due to the underlying chronic inflammatory process results in the typical dyspigmentation. This is clinically seen as a combination of areas with hyperpigmentation in between areas with hypopigmentation [26].

Furthermore, due to the continuous wound healing response within the skin and the resulting neovascularisation, visible telangiectasia are also a typical clinical finding in the late RT skin reaction [26].

Skin areas affected by chronic RD are also at high risk for secondary malignant skin tumours years after RT [27, 29, 30]. It is imperative to have a regular dermatological follow up for patients affected by chronic RD. In contrast to acute RD, late skin reactions are irreversible and progressive, which substantiality affect the patients' quality of life and cosmetic outcome [26].

1.3.2 Pathogenesis

The pathogenesis of RD is rather complex and comprises of a combination of direct radiation tissue injury followed by an inflammatory reaction. RT causes damage to the basal cells of the epidermis, the connective tissue, and vascular components via direct DNA damage or the production of reactive oxygen species (ROS) [27].

1.3.2.1 Acute radiodermatitis

Early effects result from damage to the mitotic ability of the stem cells within the basal layer of the epidermis, which leads to a disruption in the self-renewing property of the skin (Figure 2). The degree to which skin reactions develop depends on the survival of actively proliferating basal cells in the epidermis [29, 31]. In a first phase, an erythematous skin reaction develops caused by an increased vascular permeability and vasodilation. This is followed by an inflammatory response leading to a secondary erythematous reaction. During this inflammatory reaction keratinocytes, fibroblasts, and endothelial cells stimulate resident and circulating immune cells. These irradiated skin cells produce a wide array of cytokines and chemokines (e.g., interleukin (IL)-1α, IL-1β, tumor necrosis factor (TNF)-α, transforming growth factor beta (TGF-β), IL-6, IL-8, C-C motif chemokine ligand (CCL)-4, C-X-C motif chemokine ligand (CXCL)- 10, and CCL2) [32].

These molecules upregulate the expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) on keratinocytes and endothelial cells as well as vascular cell adhesion molecule (VCAM) and E-selectin on endothelial cells [32]. The upregulation of these adhesion molecules plays a major role in the transendothelial migration of circulatory immune cells to the irradiated skin, which is a "hallmark" of radiation-induced skin injury. The inflammatory response that is caused by RT not only occurs immediately but it builds up at each fraction of RT leading to a greater recruitment of immune cells and subsequently more tissue damage (i.e. "fractionated inflammatory insult") [32-34].

At a higher RT dose, the skin tries to compensate the damage by increasing its rate of mitosis in the basal epidermal cell layer. However, as the turnover of new cells is faster than the shedding of the old cells, this leads to a thickened, dry, scaly skin (i.e. dry desquamation). Finally, moist desquamation arises if all the stem cells in the basal layer are destroyed. Consequently, the skin is unable to replace the damaged tissue by new cells, which leads to a broken epidermis, skin blisters filled with a serous exudate, and substantial pain. These different phases of tissue injury will negatively affect the skin's barrier and immune function, leading to an increased risk of infection [18, 27, 29, 30].

Further, RT can also cause damage the stem cell niches of the sebaceous glands and hair follicles in the dermis resulting in dryness and epilation. In some cases, hyperpigmentation can occur when the epidermal melanocytes are stimulated by the ionising radiation [18].

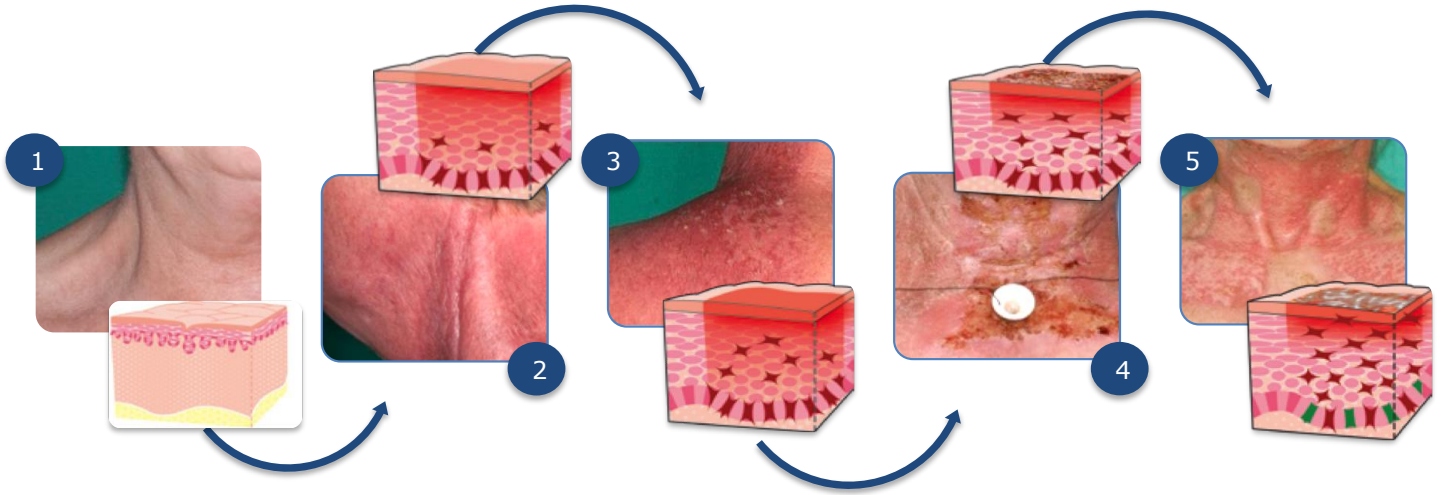

Figure 2: Pathophysiology of acute radiodermatitis
1) Before start of radiotherapy, normal healthy skin is present. 2) Radiotherapy induces an inflammatory skin reaction. 10-14 days after the first radiotherapy session, damaged basal cells migrate to skin surface. 3) As the skin is damaged through further exposure to radiation it tries to compensate by increasing mitotic activity in order to replace the damaged cells. However, if the new cells reproduce faster than the old cells are shed, the skin will become dry and flaky (dry desquamation). 4) As radiotherapy continues the basal layer cannot produce enough new cells to replace the old ones and therefore the outer layer of the epidermis will become broken, oedematous with exudate (moist desquamation). 5) The severity of skin reactions may increase for 7-10 days after radiotherapy has finished. It can take this amount of time for the cells that have been affected by radiotherapy to reach the outer epidermis. Skin will be completely healed after approximately three to four weeks. (With permission adapted from Princess Royal Radiotherapy Review Team, 2011 [31])

1.3.2.2 Chronic radiodermatitis

The underlying mechanism behind chronic skin reactions is based on an extended inflammatory reaction that starts after the first RT session and is prolonged for months to years afterwards. Inflammatory cytokines (e.g. IL-1α, IL-6, TNF-α) are responsible for this reaction. In addition, TGF-β and platelet-derived growth factor (PDGF) are upregulated in irradiated skin. These cytokines enhance tissue fibrosis by activating fibroblasts and inducing synthesis of ECM proteins and metalloproteinases, as well as the formation of telangiectasia. The prolonged inflammatory reaction induces skin atrophy and necrosis via the accumulation and activation of leucocytes at the irradiated area [26].

1.3.3 Risk factors

The risk of developing RD depends on various therapy- and patient-related factors. Treatment-related factors that influence the severity of the skin reactions include the irradiation dose delivered per fraction and the total dose, the duration of exposure, the volume of the treated area, and the combination with other therapies (e.g., chemotherapy and/or targeted therapy). Furthermore, the cumulative effect of radiation implies that tissue damage builds up with every fraction of RT. This results into more severe forms of RD at the end of RT. Patient-related factors include, large breast volume, high body mass index (BMI), overlapping skin folds, the sensitivity of the skin region to sun exposure, smoking and nutritional status, pre-existing skin conditions (e.g., psoriasis), and genetic susceptibility [17, 18, 29, 35, 36].

1.3.4 Assessment of RD

In order to assure an appropriate treatment and monitoring of RD in daily clinical practice, RT-induced skin reactions need to be properly assessed and classified. Several assessment tools have been developed to determine the degree of RD. However, there still is no gold standard up to now. The most widely used grading scales are the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 for the classification of acute RD, Radiation Therapy Oncology Group (RTOG)/European Organisation for Research and Treatment of Cancer (EORTC) grading system, and the Skin Toxicity Assessment Tool (STAT) [20-23].

A limitation of these grading systems is that they only measure the outward signs of the skin reactions and thus gives no indication of how the patients feel. Therefore, the Radiation-Induced Skin Reaction Assessment Scale (RISRAS) can be used to address this problem. The RISRAS consists of a researcher score based on the outward signs of the skin reaction and a patient component based on the patients' personal experience of the skin reactions [37].

All these grading systems lack reliability and validity. Therefore, a variety of objective skin measurement techniques have been developed to determine RT-induced skin changes. The impact of RT on the skin barrier function can be assessed by measuring the transepidermal water loss (TEWL) and the degree of hydration of the SC [38-40].

The TEWL is inversely correlated with the hydration level of the SC. Whereas a normal skin barrier function is related with a low TEWL value and a high hydration level, an impaired skin barrier function is associated with an elevated TEWL and a reduced water content of the SC. Several studies showed that RD is associated with an increase in TEWL and a reduction in the skin hydration level since ionising radiation interferes with the differentiation of the epidermis [41-43]. Furthermore, changes in skin pigmentation and degree of erythema during RT can be assessed by reflectance spectrophotometry [38-40].

1.3.5 Prevention and treatment of RD

1.3.5.1 Acute RD

Management of acute RD is an important task of the RT department. RD may be distressing or painful for the patient, which may affect their general well-being. Therefore, proper management of RD is necessary to improve the patients' quality of life [17, 30].

Up to now, a comprehensive, evidence-based consensus for the prevention and treatment of RD has not been published. As a consequence each RT department uses a different skin care protocol to prevent and/or manage acute RD. Though, the Multinational Association for Supportive Care in Cancer (MASCC) has published some general clinical guidelines [44].

Concerning preventive measures for acute RD the MASCC panel makes a strong recommendation for daily hygiene practices such as gentle washing with water, with or without a mild soap and prohibit the use of deodorants. In order to reduce discomfort and itchiness caused by the skin reactions, the panel makes a strong recommendation for the prophylactic use of potent topical steroids [45, 46]. Next to the preventive measures, the MASCC panel only shows poor evidence for the use of silver sulfadiazine cream in patients with established acute RD. There is insufficient evidence to support or refuse the use of the other agents for the prevention and management of acute RD according to the MASCC guidelines [17, 30, 44, 47]. Therefore, it is necessary to perform more randomised controlled clinical trials (RCT) to investigate the use of other preventive and therapeutic modalities for acute RD [17, 29, 30, 48, 49].

1.3.5.2 Chronic RD

For the management of chronic RD the available scientific data is limited. Hereunder, the treatment options for telangiectasia, fibrosis, and ulceration/necrosis are described.

Telangiectasia

Up to now, even though clinical experience with the treatment of telangiectasias is more than three decades old, there is only limited evidence of three clinical trials investigating the use of pulsed dye laser (PDL) therapy for the management of telangiectasia in the context of chronic RD [44].

In a study by Lanigan et al. eight female breast cancer patients that developed telangiectasia within one year after RT were treated by Candela SPTLIB PDL (585 nm, 450 ys pulse, 7-mm spot, 6 J/cm). All treated patients showed complete clearance of vessels after their PDL treatment [50]. Another study compared the use of long pulsed-dye laser (LPDL) with intense pulsed light (IPL) in a randomised split-lesion trial. Thirteen female patients with telangiectasia underwent three treatments at a 6-week interval. The left or right side of the affected skin area was treated with PDL (V-beam Perfecta, 595 nm) and the other side with IPL (Ellipse Flex). Results showed that both treatment options were effective in reducing telangiectasia. However, LPDL was a more effective treatment option with a 90% vessel clearance while it was 50% in the skin area treated with IPL. In addition, the patient satisfaction was higher for LPDL than IPL and LPDL was associated with a lower pain score. However, the study compared three passes with LPDL with one single pass with the IPL and should hence be taken with caution regarding the comparison of the two techniques [51]. Finally, in a retrospective study by Rossi et al., eleven patients were treated with PDL. There was clinical improvement in all the cases after an average of 4 PDL sessions. The average laser fluence was 4.2 (585 nm platform) and 7.8 (595 nm) J/cm^2 (4–8 J/cm^2). The average percentage of reduced vessels was 72.7 (50–90%). Furthermore, some patients also described an increased sense of confidence and satisfaction after their final PDL session [52]. Based on these data the MASCC panel made a weak recommendation for the use of LPDL for telangiectasia [44].

Fibrosis

The management of RIF is quite difficult. There are several options available ranging from physiotherapy, pharmacotherapy, hyperbaric oxygen, to laser therapy. However, the scientific evidence for these options is limited. In order to avoid a diminished quality of life of patients with RIF, supportive care consisting of pain management, psychological support, and wound care is necessary [26].

Physiotherapy

One of the most important treatment options for RIF is physical therapy, which includes deep massage and a range of motion exercise. These can improve the mobility of the affected area and prevent the development of contractures [26]. In a randomised, prospective study by Bourgeois et al. the use of the LPG technique in twenty women with RIF after RT and surgery for breast cancer was investigated. The LPG technique is a technique of mechanical massage that allows skin mobilization by folding/unfolding. Ten patients underwent LPG treatment three times a week for one month, while the other ten patients were only placed under medical supervision. Results of this study showed that the LPG treatment was able to significantly reduce erythema, pain, pruritus, and the feeling of induration of the skin [53].

Pharmacotherapy

A limited number of studies investigated the use of pentoxifyline (PTX) alone or in combination with tocopherol (vitamin E) to prevent or treat RIF [54-59]. PTX is a methylxanthine derivative that has a multitude of inflammatory effects. It can upregulate polymorphonuclear leukocyte and monocyte phagocytic activity, inhibit TNF-α and TNF-β synthesis, decrease granulocyte–macrophage colony-stimulating factor and interferon gamma (IFN-γ), and inhibit the TGF-β expression [59-61]. Vitamin E (VitE), on the other hand, reduces the ROS concentration [62].

The available data of several small, randomised trials show contrasting results, with little to no benefit over the placebo treatment [57, 58]. On the other hand, Delanian et al. did show a beneficial effect of the combination of PTX with tocopherol in the treatment of RIF. In their latest study, they compared the effects of a long-term (24 to 48 months) versus short-term treatment (6 to 12 months) with PTX and tocopherol in 44 breast cancer patients. Their results showed that a long treatment of PTX-VitE (average 24 months) was necessary to reduce RIF with an average of 68%. There was a rebound effect when patients stopped their treatment before the 12-month period [56]. Larger, RCT's are necessary to confirm these results and to estimate the optimal drug dose and duration.

Hyperbaric oxygen

In chronic RD, hyperbaric oxygen therapy (HBOT) can have beneficial effects by inducing re-epithelialization and reducing pain, edema, erythema, or lymphedema. However, the scientific evidence for the reduction of RIF by HBOT is weak [63-65].

Laser therapy

The use of laser therapy for the management of RIF is new and has recently been introduced in the field. In a study by Tran et al., three Vietnamese children who developed chronic RD after RT for haemangioma were treated with PDL and/or fractional laser in combination with skin grafting. Patients showed softened and repigmented skin with an increase in flexibility after the intervention [66]. These positive results need to be further investigated in larger clinical trials.

Ulceration and necrosis

Standard care

For the management of ulcerations and necrotic wounds in chronic RD, the general wound care guidelines are the most important. These include the application of wound dressings that absorb the wound exudate and protect the wound from environmental damage and bacteria to prevent secondary infections. For patients with very moist wounds, hydrogel or hydrocolloid dressings can be used. These dressings do not adhere to wounds, are absorbent, and can easily be replaced. Studies have shown that these dressings are stimulating the wound healing process and improve the patients' comfort [17, 44]. For infected wounds silver-containing dressings can be used. Chronic ulcerations need to undergo selective and careful debridement in order to clean the wound and stimulate the healing process [17, 44]. In some severe cases, surgical interventions are necessary in which skin-flaps are used [67].

Stem cell therapy

The use of stem cell therapy for the management of chronic RT-induced skin wounds is still under investigation [68, 69]. Preclinical studies in a wide variety of animal models have identified mesenchymal stem cells (MSCs) as promising cell-based agents for stimulating skin regeneration, suppressing inflammation, and thereby reducing fibrosis. Both bone marrow-derived stem cells (BMDSCs) and adipose-derived stem cells (ADSCs) are the most studied stem cell types in this field [70-75]. Data on the use of MSC therapy in humans is limited. Two case report studies demonstrated that the combination of skin grafting and local autologous BMDSC therapy improved the wound healing of radiation cutaneous injuries caused by industrial accidents [76, 77]. Akita et al. showed in several patients with chronic RT-induced wounds that the use of subcutaneously harvested ADSC in combination with artificial dermis and local application of angiogenic factors (e.g. basic fibroblast growth factor, bFGF) resulted in complete skin regeneration [78-80]. The underlying mechanism for the beneficial effects of stem cell therapies in wound healing is still not fully elucidated. It is suggested that is not due to the replacement of the damaged cells by the transplanted cells but probably by the paracrine effects of the stem cells. MSCs have been suggested to stimulate vascularization and regulate the production and secretion of various growth factors and cytokines, which will trigger the host cells to regenerate the damaged tissue [68, 69].

Leukocyte- and platelet-rich fibrin (L-PRF)

Another innovative technique in the management of skin ulcerations is the application of leukocyte- and platelet-rich fibrin (L-PRF), which is an autologous blood-derived product. It consists of a fibrin matrix incorporated with platelets, leucocytes, cytokines, and circulating stem cells [81].

The beneficial effect of L-PRF for wound healing is based on the progressive release of a significant amount of growth factors such as, PDGF, TGF-β, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and insulin-like growth factor (IGF) over a period of at least seven days. Nowadays, L-PRF is mainly used in dentistry to stimulate tissue regeneration and wound healing in oral surgery and implantology [81].

The use of L-PRF for chronic wounds is quite new and therefore the number of clinical trials is limited. In a study by Jørgensen et al. 12 patients with lower extremity chronic wounds underwent six weekly applications with Leucopatch, an autologous, additive-free, platelet-rich fibrin. Of the 13 wounds included in the analysis, four healed completely, while the size of the remaining wounds decreased with 65% [82]. A retrospective study investigated the use of L-PRF in 12 patients with a wide variety of chronic wounds. Eight wounds closed and three wounds reduced in diameter by up to 66% after one up to seven applications. One wound did not reduce in size, although it did reduce in depth. One of these patients had a RT-induced skin ulcer on her breast, which completely healed after two treatment sessions [83]. A recent study by Pinto et al. evaluated the use of L-PRF in refractory leg ulcers of different aetiology in 44 patients. All wounds showed significant improvements after the L-PRF therapy (4-12 weekly applications), with a complete healing response in ulcers smaller than 10 cm^2. No study reported any adverse events [84].

Based on the available data L-PRF could be a safe, efficient, and easy-to use adjuvant therapy for treating bad-healing chronic RT ulcers. However, further clinical research is warranted.

Photobiomodulation therapy (PBMT)

The final and emerging therapy to stimulate the wound healing process is photobiomodulation therapy (PBMT). A case report by Schindl et al. showed that PBMT was able to improve the wound healing process and to increase the vascularisation of radiotherapy-induced skin ulcers in breast cancer patients [85, 86]. Gobbo et al. investigated the use of PBMT in the management of RT-induced anal and genital ulcers in two female patients treated for anal squamous cell carcinoma. The lesions were accompanied with severe itching, pain and bleeding. Furthermore, the patients' quality of life was significantly impaired. Complete healing of the lesions and symptoms was achieved after six PBMT sessions over two weeks [87].

1.4 PHOTOBIOMODULATION THERAPY

Photobiomodulation therapy (PBMT), also known as low-level light therapy (LLLT), has been discovered in 1965 by Endre Mester. PBMT is defined by the World Association for Laser Therapy (WALT) as a light therapy that elicits non-thermal, photophysical and photochemical events at various biological scales leading to physiological changes in the target cells or tissues. It uses visible red and/or (near)-infrared ((N)IR) low-powered light produced by laser diodes and/or light-emitting diodes (LEDs) [88, 89]. The main functions of PBMT are stimulating wound healing, reducing inflammation, and relieving pain [90-96]. The number of applications of PBMT varies widely ranging from dermatology, neurology, physiotherapy to dentistry. Thereby, it is a non-invasive, non-carcinogenic, and non-traumatic procedure that has very few (if any) side effects [97].

1.4.1 PBMT parameters

The choice of the correct PBMT parameters are crucial for the effectiveness of this treatment method [90, 91, 98-100.] The dosimetry of PBMT is determined by several parameters, which can be subdivided into two groups, the irradiation parameters, "the medicine" (including the wavelength, power density, energy density, pulse structure) and the treatment parameters, "the dose" (including timing, treatment schedule, anatomical location) (see Table 2). Concerning the wavelength, PBMT mainly uses a wavelength that falls into an optical window of IR or NIR light between 600–1000 nm (Figure 3). Tissue penetration is maximized in this range, as tissue scattering is higher at shorter wavelengths and the principal tissue chromophores (haemoglobin and melanin) have high absorption bands wavelengths below 600 nm [98, 101].

Furthermore, at wavelengths above 1000 nm, water is absorbing many photons, reducing their availability for specific chromophores. Wavelengths between 600-700 nm (red light) are chosen for treating superficial tissue, and wavelengths between 780-1000 nm (NIR light) are chosen for deeper-seated tissues, because of their deeper penetration into tissue [98, 101].

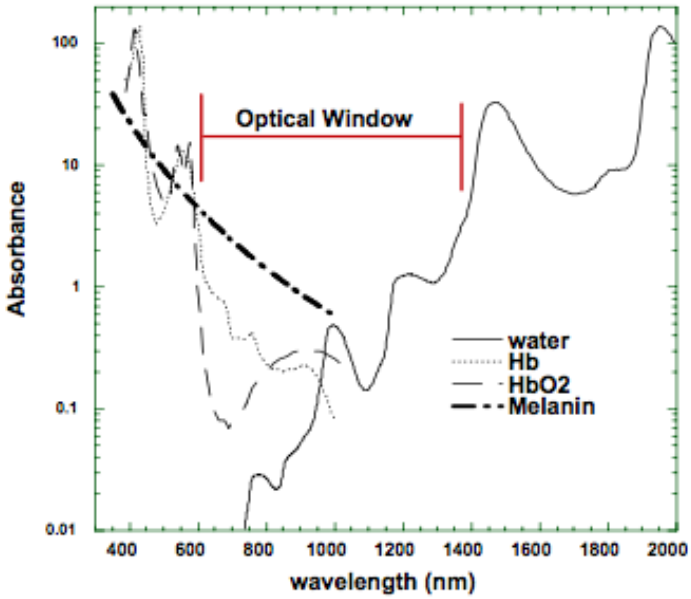

Figure 3: Optical window of infrared (IR) and near-infrared (NIR) light absorption in tissue
Between 600-1000 nm the penetration of IR and NIR light in tissue is maximized due to low absorption by tissue chromophores (e.g. Hemoglobin (Hb), water and Melanin). (With permission from Chung et.al, 2012 [98]).

Table 2: Photobiomodulation (PBM) parameters to be reported in clinical studies (With permission from Zecha et al., 2016 [99])

Irradiation parameters	Unit of measurement	Explanation
Wavelength	Nanometer (nm)	Light consists of packets of electromagnetic energy that also have a wave-like property. Wavelength is expressed in nanometers (nm). PBMT devices are typically within the range of 600–1000 nm.
Power	Watt (W)	Energy (J) per second (s), peak and average when pulsed (see pulsed beam).
Aperture diameter	Centimetre squared (cm^2)	The area of the light source tip.
Beam area	Centimetre squared (cm^2)	The surface area of the beam on the patient.
Irradiance (power density, intensity)	Power/Area (W/cm^2)	Power (W) ÷ beam area (cm^2).
(Radiant) Energy	Joules (J)	Power (W) × time (s).
Irradiation time	Seconds (s)	How long each treatment is applied at each location.
Dosage (fluence, or energy density)	Joules per centimetre squared (J/cm^2)	Energy (J) ÷ beam area (cm^2), or power (W) ÷ beam area (cm^2) × time (s).
Operating mode	Continuous wave (CW), switched CW, pulsed, Q-switched	The continuity of the production of the output beam may be continuous or pulsed.
Pulse structure	Peak Power (W) Pulse Frequency (Hz) Pulse Width (s) Duty Cycle (%)	In case the beam is pulsed, the power is reflected by the average power, which is calculated as follows: peak power (W) x pulse width (s) x pulse frequency (Hz). Duty cycle is the fraction of time in which the pulse is on.
Coherence	Coherence length depends on spectral bandwidth	Coherent light produces laser speckle, which has been postulated to play a role in the PBM interaction with cells and subcellular organelles.
Polarization	Polarized or not, or linear	Polarized light may have different effects than otherwise identical non-polarized light.

Table 2 (continued)

Treatment parameters	Explanation
Physical relationship to the organ	Applicable when there is more than one manner to approach the organ. For example, intra-oral device versus extra-oral device.
Anatomical location	The anatomical site that was exposed to the light beam. If multiple locations were treated, all need to be described.
Application technique	The way in which the PBM device is placed in contact with the irradiated area. (e.g. Skin contact, contact with pressure, interstitial fiberoptic)
Timing	Time of the treatment session relative to the cancer treatment.
Treatment schedule	The frequency of treatments per day/week and the total number of treatments.

There is scientific evidence that suggests that the effectiveness of PBMT varies greatly on both the irradiance and the energy density (or irradiation time) used. PBMT seems to have a biphasic dose response, also known as the "Arndt-Schulz law", which indicates that the most optimal PBMT parameters depend on the type of application (Figure 4). This means that when PBMT is applied with a very low irradiance or a too short irradiation time, no response is recorded. Only when one of these two parameters is increased and the threshold has been reached, biostimulation can occur efficiently. On the other hand, a negative therapeutic outcome can occur when the irradiance is too high or the treatment time is longer than the optimal value, also referred to bio inhibition [98, 102]. As such, it is important that when PBMT is applied, the irradiation and treatment parameters need to lay in the therapeutic window in order to achieve a positive clinical outcome [97, 102].

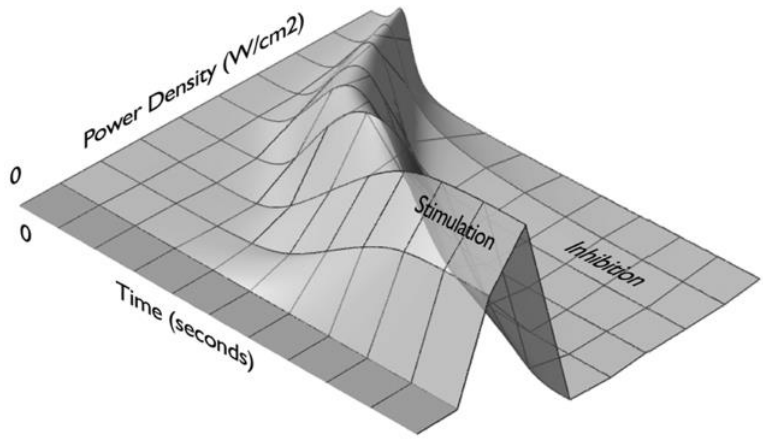

Figure 4: Three-dimensional model of the Arndt-Schulz curve. This curve illustrates that either irradiance or irradiation time (fluence) can have biphasic dose response effects in PBMT (With permission from Chung, 2012 [98])**.**

1.4.2 Molecular mechanism of PBMT

The biochemical mechanism associated with PBMT is not yet fully elucidated and may vary among different cell types and tissue conditions. The proposed underlying mechanism behind the effects of PBMT is believed to start with the absorption of the red and NIR light by chromophores in the mitochondria of the exposed cells, as shown in figure 5 [97].

1.4.2.1 Chromophores

Cytochrome c oxidase

The crucial chromophore in the cellular response to PBM is cytochrome c oxidase (CCO). This enzyme of the electron transport chain makes the transfer of electrons from cytochrome c to oxygen possible. Scientific evidence demonstrates that PBMT is able to stimulate CCO, resulting into an increased reduction of oxygen and thereby the mitochondrial membrane potential (MMP) will increase that drives the adenosine triphosphate (ATP) production. ATP is the substrate for cyclic adenyl cyclase, and therefore ATP determines the cyclic adenosine monophosphate (cAMP) level. cAMP is an important second messenger, regulating a wide variety of intracellular signalling pathways. Additionally, the increase in ATP will also stimulate the activity of the Na^+/K^+ ATPase. This ion pump is necessary to keep the intracellular potassium concentration high and the intracellular sodium concentration low, which is essential for the propagation of action potentials [97].

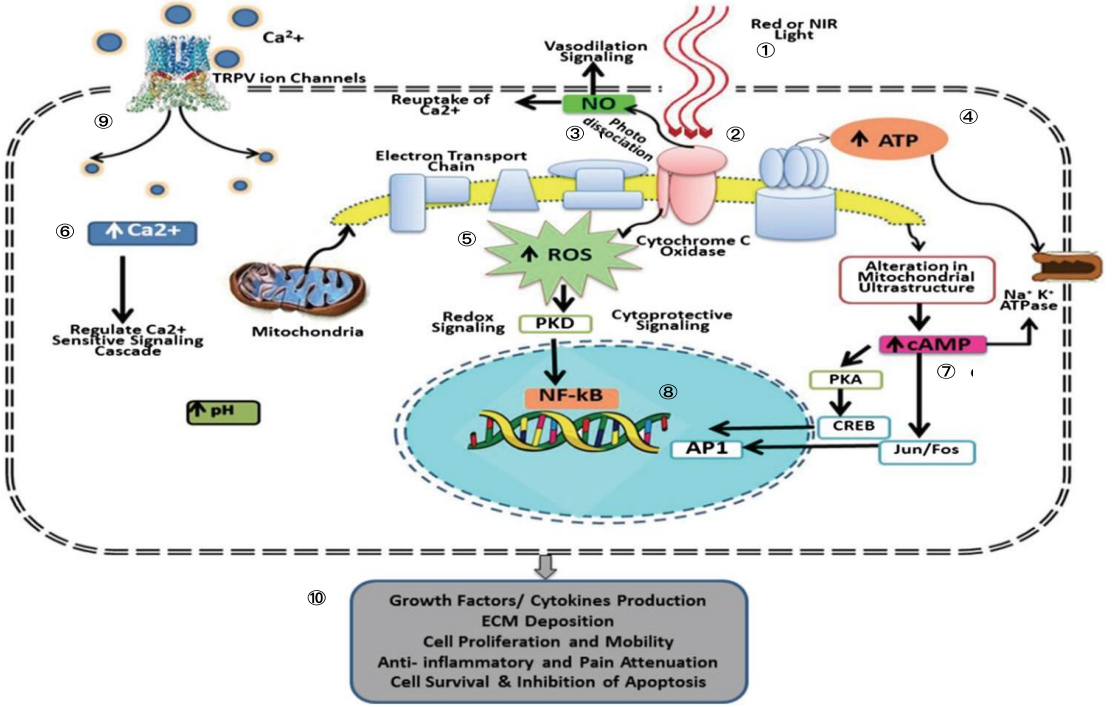

Figure 5: Molecular and intracellular mechanisms of photobiomodulation therapy
Photons (1) are initially absorbed by mitochondrial chromophore (photoacceptor, cytochrome c oxidase (CCO)) (2). Photon absorption leads to dissociation of inhibitory nitric oxide (NO) from CCO (3), leading to increased enzyme activity and ATP production (4) and a short burst of reactive oxygen species (ROS) (5), which in turn cause changes in the cellular redox potential, Ca^{2+} ions (6), and cyclic adenosine monophosphate (cAMP)(7), and induce several transcription factors (NF-kB, AP-1, etc.) (8). After photon absorption, light-sensitive ion channels can be activated, allowing Ca^{2+} ions to enter the cell (9). A photosignal transduction and amplification chain induced by red or near-infrared (NIR) light leads to an increase in growth factor production, cell proliferation, cellular mobility, adhesion, and extracellular matrix deposition (10). *AP1, activator protein 1; ATP, adenosine triphosphate; CREB, cAMP response element-binding protein; ECM,* extracellular matrix; *NF-kB, nuclear factor kappa B; NIR, near-infrared; PKA, protein kinase A; PKD, protein kinase D; TRPV, transient receptor potential vanilloid.* (With permission from Hamblin et al., 2018 [97]).

Besides ATP and cAMP, the increase in MMP also leads to a shift in the overall cell redox potential in the direction of oxidation resulting into an increase in the production of reactive oxygen species (ROS). ROS can have two very distinct functions in the cell depending on their concentration level. As such, a low level of ROS during a short exposure time is beneficial, whereas a chronic long-term exposure of a high ROS concentration is detrimental for the cell. The main molecule responsible for these contrasting effects of ROS is hydrogen peroxide (H_2O_2). There are different endogenous sources of H_2O_2 such as various oxidases as well as the mitochondria. In the mitochondria, superoxide is transformed into H_2O_2 by the enzyme superoxide dismutase (SOD). At low concentrations (1-10 nM), H_2O_2 acts as a redox-signalling molecule for maintaining the normal physiological health of the cells, which is also known as "oxidative eustress". Whereas, at high H_2O_2 concentrations, oxidative stress will lead to a disruption in redox signalling pathway and eventually cause oxidative damage to biomolecules, this refers to "oxidative distress" (Figure 6) [103].

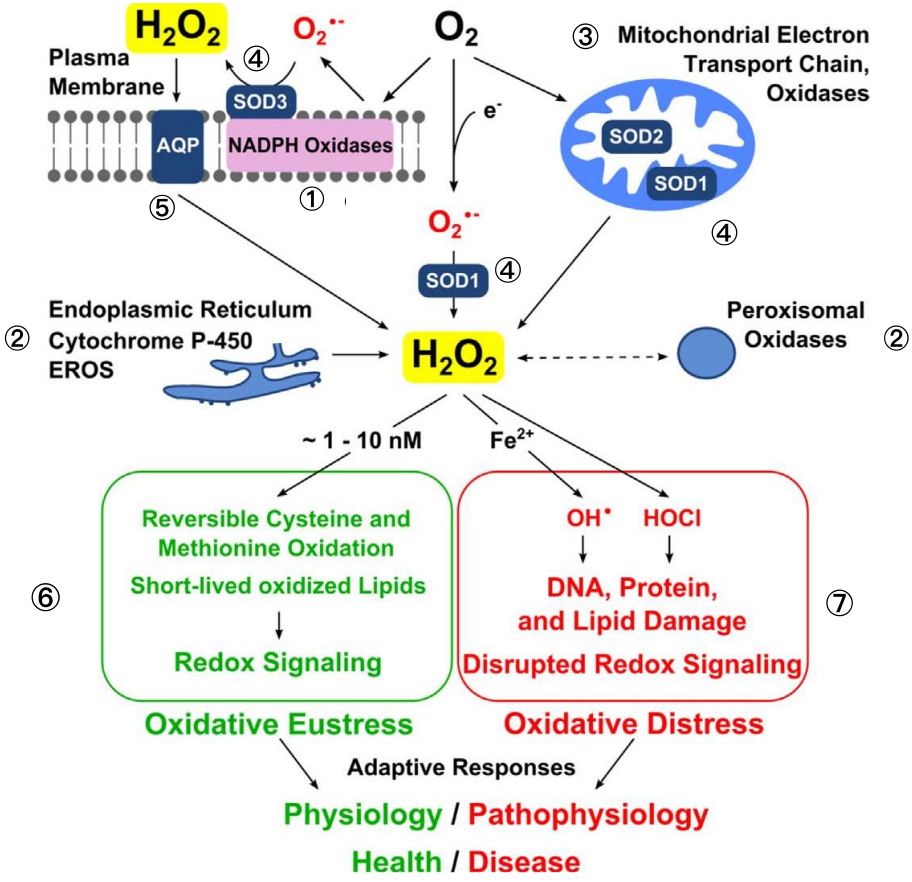

Figure 6: Role of hydrogen peroxide in oxidative stress
Top: Endogenous H_2O_2 sources include NADPH oxidases (1) and other oxidases (membrane-bound or free) (2) as well as the mitochondria (3). The superoxide anion radical is converted to hydrogen peroxide by the three superoxide dismutases (SODs 1,2,3) (4). Hydrogen peroxide diffusion across membranes occurs by some aquaporins (AQP), known as peroxiporins (5). Bottom: In green, redox signaling comprises oxidative eustress (physiological oxidative stress) (6). In red, excessive oxidative stress leads to oxidative damage of biomolecules and disrupted redox signaling, oxidative distress (7).
DNA, deoxyribonucleic acid; NADPH, Nicotinamide adenine dinucleotide phosphate. (With permission from Sies et al., 2017 [103]).

Finally, also the nitric oxide (NO) levels are changed by an increase in MMP. In stressed cells, mitochondria produce NO, which binds to CCO and displaces oxygen. This binding leads to the inhibition of cellular respiration followed by a decrease in the ATP production and an increase in oxidative stress. During oxidative stress, the antioxidant defence mechanism of the body cannot counteract the high levels of ROS. Several studies demonstrated that PBMT can reduce the oxidative stress in cells by modifying the NO release and/or production in the mitochondria [98].

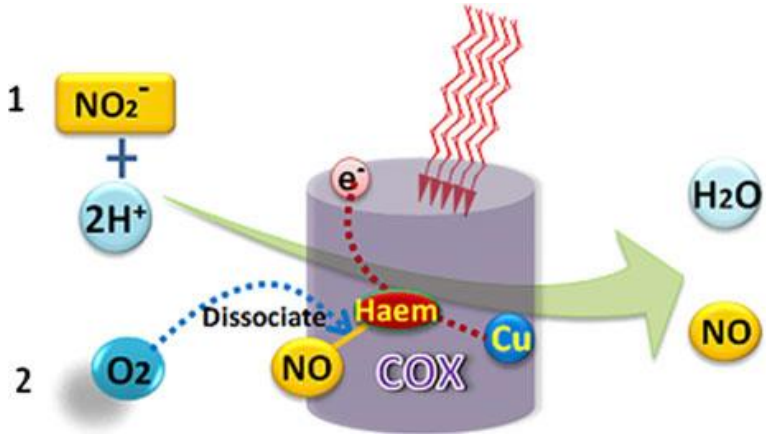

Figure 7: Two possible pathways of NO release by cytochrome c oxidase (CCO)
Pathway 1 shows that CCO can act as a nitrite reductase enzyme. Pathway 2 shows the possible photodissociation of NO from CCO. *CCO, cytochrome c oxidase; Haem, haemoglobin*; *NO, nitric oxide.* (With permission from Chung et al., 2012 [98]).

When PMBT is applied it can lead to dissociation of NO from its binding to CCO leading to a restored cellular respiration followed by an increase in ATP production and a reduction in oxidative stress [104]. Secondly, PBMT can stimulate the nitrite reductase activity of CCO (a one-electron reduction of nitrite gives NO), which leads to an increase in NO production[104]. Finally, a small rise in NO can also be caused by the dissociation of NO from intracellular stores (e.g. nitrosothiols) or from haemoglobin or myoglobin (Figure 7) [99, 102, 105-109].

Light-sensitive ion channels

Another hypothesis, which is currently under investigation, is the effect of PBMT on light-sensitive ion channels. The most important channels are the "transient receptor potential" (TRP) channels. Currently, there are up to 50 different TRP isoforms identified, divided into seven subfamilies. TRP channels can be activated by different stimuli such as, light, heat, cold, sound, etc. One class of TRP channels is mainly mentioned in PBMT research, namely the "vanilloids" TRP channels (TRPV), which are highly selective Ca^{2+} channels. There is evidence that PBMT can activate TRP channels both directly and indirectly. PBMT can induce a decrease in the pH of the cell. This cellular alkalization can facilitate the Ca^{2+} influx via the TRPV channels in an indirect way. On the other hand, PBMT can also increase the Ca^{2+} concentration in the cell by directly activating the TRPV channels (Figure 8) [97].

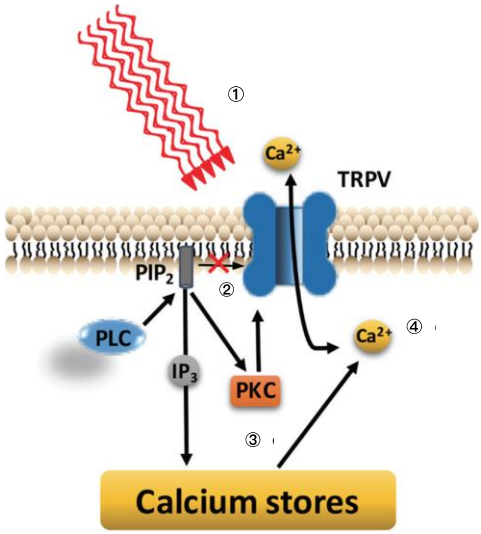

Figure 8: Possible mechanism of PBMT and transient receptor potential channels for calcium signaling
PBMT can directly activate transient receptor potential vanilloids (TRPV) channels, which are Ca^{2+}channels (1). PBMT can also induce a decrease in pH leading to an indirect influx of Ca^{2+} via TRPV channels (2). PBMT can also induce the release of Ca^{2+} from intracellular stores (3). Ca^{2+} is an important signalling molecule in the cell (4). *IP_3, inositol trisphosphate; PIP_2, phosphatidylinositol biphosphate; PKC, protein kinase C; PLC, phospholipase C.* (With permission from Hamblin et al., 2018 [97])

1.4.2.2 Signalling pathways

After the initial absorption of the photons by the chromophores in the target cells, the signalling molecules, cAMP, ROS, and Ca^{2+} will activate a wide variety of intracellular pathways resulting into the activation of transcription factors. These transcription factors play a major role in regulating gene expression [97].

Cyclic AMP

The production of cyclic AMP (cAMP) is upregulated after PBMT, caused by the direct increase of mitochondrial ATP. cAMP will activate the cAMP-dependent protein kinase A (PKA). PKA phosphorylates and activates the cAMP-response element binding protein (CREB), which then binds to the CRE domain on the DNA in the cell nucleus, which will activate several genes. In addition, cAMP will also upregulate the production of the activator protein 1 (AP-1) transcription factor via the Jun/Fos pathway [110, 111].

Reactive oxygen species

As described above, ROS can have both beneficial and detrimental effects depending on the concentration of the intracellular ROS. At low levels ROS acts as a redox-signalling molecule involved in regulating several mitochondrial-signalling pathways. In addition, ROS production results in the activation of the transcription factor, nuclear factor kappa B (NF-κB). NF-κB acts as a redox-sensor regulating the expression of various genes [112].

Nitric oxide

PBMT also upregulates the production of NO by two different pathways, as mentioned earlier. NO can have several beneficial effects on the target cells. NO is a known dilator of blood and lymph vessels, by stimulating guanylate cyclase to form cyclic-GMP (c-GMP). cGMP activates protein kinase G, which results in the re-uptake of Ca^{2+} in the sarcoplasmic reticulum of the smooth muscle cell and opening of calcium-activated potassium channels. The decrease in cytosolic Ca^{2+} prevents the myosin light-chain kinase (MLCK) from phosphorylating the myosin molecule, leading to relaxation of the smooth muscles of the blood and lymph vessels. In addition, NO can act as a signalling molecule in different cellular pathways [105, 113].

1.4.3 PBMT and wound healing

Several studies have demonstrated that PBMT is able to improve the skin's wound healing process by modifying different cellular processes [15, 16, 90, 91, 101]. As described earlier, PBMT stimulates the production of several transcription factors (NF-κB, AP-1, etc.), which are involved in protein synthesis, ECM deposition, cell migration, proliferation, anti-inflammation, cell survival, and inhibition of apoptosis. *In vitro* and *in vivo* studies have demonstrated that PBMT affects each phase of the wound healing process. PBMT is able to upregulate phagocytosis, enhance angiogenesis, downregulate inflammatory mediators, increase the proliferation and migration of keratinocytes and fibroblasts, and increase the collagen synthesis [14, 90, 91, 114-117].

Furthermore, PBMT increases the production of collagen and granulation tissue, improves tensile strength, and causes faster epithelisation of the wound bed [117-125]. Finally, it has been shown that PBMT can modulate the production of several growth factors (basic fibroblast growth factor (bFGF), VEGF, TGF-β, and cytokines (IL-1, IL-8, TNF-α) [126, 127].

1.4.4 PBMT and acute RD

Schindl et al. was the first to study the clinical effect of PBMT on RD in patients. The study showed that PBMT was effective in the induction of wound healing in RT-induced skin ulcers after mastectomy in a small group of breast cancer patients [85, 128, 129].

More recently two studies evaluated the efficacy of LED in the prevention of RD [128, 129]. LED is another type of PBMT that has approximately the same characteristics as laser diodes but it uses non-coherent light. In a study by DeLand et al., LED treatment significantly reduced the incidence and the severity of RD in breast cancer patients [129]. On the other hand, Fife et al. did not find a significantly reduced incidence or severity of RD after LED treatment in breast cancer patients. These conflicting results may be attributed to a variety of factors (e.g., type radiation technique, non-blinded vs. blinded scoring of skin reactions, set–up of the LED treatment) [128].

Based on these preliminary data, our study group investigated the efficacy of PBMT as a treatment for RD in breast cancer patients (DERMIS trial). During this prospective study (August 2013- April 2014), two successive groups of breast cancer patients undergoing identical RT regimen (33 daily fractions of 2 Gy) post-lumpectomy were compared. The control group (CTRL group, N=41) received the institutional skin care protocol, while the experimental group (LT group, N=38) was treated with this protocol plus biweekly with PBMT (6 sessions) starting at fraction 20 of RT. PBMT was delivered to the patients by a diode laser in the infrared range (808 ± 5 nm - 905 ± 5 nm) with a fixed energy density (4 J/cm^2). The severity of RD was evaluated according to the criteria of the RTOG [130].

Before the start of PBMT, at a RT dose of 40 Gray (Gy) the distribution of the RTOG grades was comparable between both groups, with most of the patients presenting RTOG grade 1. At the end of RT (dose 66 Gy), the severity of RD was significantly different between the two groups, with more presence of grade 2 in the CTRL group when compared to the laser treated group. Furthermore, there was a significant intensification of the skin reactions in the CTRL group, while it remained stable in the LT group (Figure 9) [130].

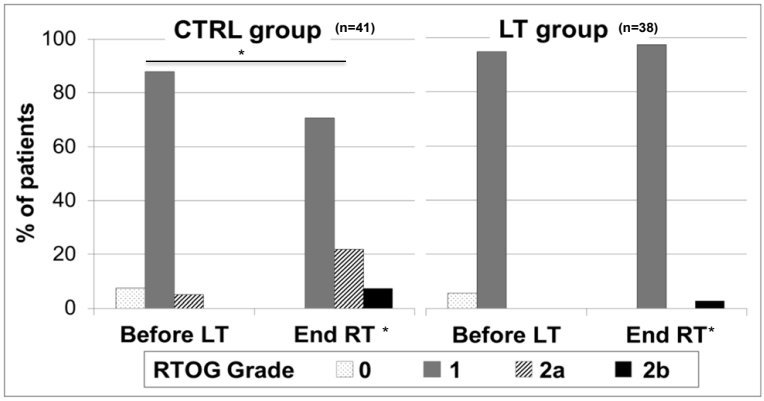

Figure 9: RTOG classification (DERMIS trial)
Severity of radiation dermatitis (RTOG grades) for the control group and the group that received MLS® laser therapy (LT) before the start of LT and at the end of radiotherapy (RT). *Significant difference within the control group between the two time points and between the two groups at the end of RT ($p<.0.05$; χ^2 or Fisher's exact tests, two-tailed). *RTOG, Radiation Therapy Oncology Group* (With permission from Censabella et al, 2017 [130])

1.5 AIM OF THE PROJECT

The positive results of our previous DERMIS trial laid the foundation for further research on the clinical implementation of PBMT for the prevention and management of acute RD in cancer patients.

For this research project we aimed to investigate the efficacy of PBMT in the prevention and management of acute radiodermatitis in cancer patients.

To **evaluate this, we formulated two study objectives:**

- Objective 1: Investigate the effectiveness of PBMT in the prevention and management of acute RD in breast cancer patients (Chapter 2-4)
- Objective 2: Investigate the effectiveness of PBMT in the prevention and management of acute RD in head and neck cancer patients (Chapter 5)

PART 1

TRANSDERMIS TRIAL

PHOTOBIOMODULATION THERAPY AND ACUTE RADIATION DERMATITIS IN BREAST CANCER PATIENTS

CHAPTER 2

Prevention of Acute Radiodermatitis by Photobiomodulation: A Randomized, Placebo-Controlled Trial in Breast Cancer Patients (TRANSDERMIS trial)

Robijns J, *Censabella S, Claes S, Pannekoeke L, Bussé L, Colson D, Kaminski I, Bulens P, Maes A, Noé L, Brosens M, Timmermans A, Lambrichts I, Somers V, Mebis J.*

Lasers in Surgery and Medicine, 2018. 50(7): p. 763-771.

2.1 ABSTRACT

Objective

Acute radiodermatitis (RD) is a distressing and painful skin reaction that occurs in 95% of the patients undergoing radiotherapy (RT). The aim of this study was to evaluate the effectiveness of photobiomodulation therapy (PBMT) in the prevention of acute RD in breast cancer (BC) patients undergoing RT.

Methods

This study was a randomised, placebo-controlled trial including 120 BC patients that underwent an identical RT regimen post-lumpectomy. Patients were randomly assigned to the laser therapy (LT) or placebo group, with 60 patients in each group. Laser or placebo treatments were applied two days a week, immediately after the RT session, starting at the first day of RT. PBMT was delivered using a class IV MLS® M6 laser that combines two synchronized laser diodes in the infrared range (808-905 nm) with a fixed energy density (4 J/cm^2). Skin reactions were scored based on the criteria of the Radiation Therapy Oncology Group (RTOG) and the Radiation-Induced Skin Reaction Assessment Scale (RISRAS). The patients completed the Skindex-16 questionnaire to evaluate their quality of life. All the measurements were collected at the first day, at a RT dose of 40 Gray (Gy), and at the end of RT (total dose 66 Gy).

Results

At a RT dose of 40 Gy, there was no significant difference between the groups in the distribution of RTOG grades. However, at the end of RT the severity of the skin reactions significantly differed between the two groups (P=0.004), with a larger percentage of patients experiencing RTOG grade 2 or higher (e.g. moist desquamation) in the placebo group (30% vs. 6.7%, for the placebo and laser group, resp.). The objective RISRAS score confirmed these results. In addition, the Skindex-16 and RISRAS subjective score demonstrated that the patients' quality of life was significantly better in the LT than in the control group.

Conclusions

The results of this trial show that PBMT is an effective tool to prevent the development of grade 2 acute RD or higher in BC patients. In addition, it also reduces the patients' symptoms related to RD.

2.2 INTRODUCTION

About 90% of the breast cancer (BC) patients undergo radiotherapy (RT) during their cancer treatment [131]. In approximately 95% of the patients, RT can lead to acute skin reactions, also known as radiodermatitis (RD) [33]. Ionising radiation induces an inflammatory skin reaction, followed by damage to stem cells within the basal layer of the epidermis, which leads to a disruption in the self-renewing property of the skin [17]. Acute skin reactions start approximately two weeks after the first RT session with erythema, which can progress into dry or eventually moist desquamation [17].

The severity of RD depends of various intrinsic (e.g. breast volume, comorbidities, genetic susceptibly) and extrinsic (e.g. RT dose, fractionation regimen, use of radio sensitizers) factors [35].

RD is a distressing and a possibly painful side effect. It affects the patients' quality of life, as they have to cope with problems during their daily life (e.g. washing practices, getting dressed, household activities, hobbies) [132]. In some rare cases of severe skin reactions, the radiotherapist needs to adjust the fractionation regimen or interrupt RT, which will eventually affect the treatment outcome and patient survival [133].

Concerning the prevention and treatment of RD, the Multinational Association of Supportive Care in Cancer (MASCC) developed skin care guidelines in 2013. However, the evidence supporting the effectiveness of these preventive and/or treatment options is still weak and there is no comprehensive, evidence-based consensus [44, 134].

Photobiomodulation therapy (PBMT) uses non-ionising light sources such as laser diodes and light-emitting diodes (LEDs) in the visible and near-infrared spectrum (600-1000 nm) [135, 136]. The light is absorbed in the cells by endogenous chromophores resulting in non-thermal, photophysical and photochemical events at various biological scales. Although, the underlying mechanism of PBMT is still unclear, several studies suggest that PBMT is able to stimulate wound healing and reduce inflammation, oedema, and pain [102, 137-140].

In the last 20 years, the use of PBMT in the supportive care of cancer patients is increasing for several cancer-therapy related side effects (e.g. oral mucositis, lymphedema, neuropathy, RD) [141]. Concerning safety issues on the use of PBMT in cancer patients, the results of *in vitro* and *in vivo* studies investigating the effect of PBMT on the proliferation rate of cancer cells are reassuring [142-146].
Schindl et al. introduced the use of PBMT for the management of acute RD in the late 1990s. In a case report study they showed a beneficial effect of PBMT for the treatment of RT-induced skin ulcers in BC patients after a mastectomy [85, 86, 147].

Recently, our study group performed a prospective trial with 79 BC patients, in which PBMT was started once the first skin reactions already developed. The results of this study demonstrated a beneficial effect of PBMT for the management of RD [130].

The aim of the current study was to evaluate the efficacy of PBMT for the prevention of RD in BC patients undergoing RT with respect to the severity of RD. Secondly, the effect on the patient's quality of life was assessed.

2.3 MATERIALS AND METHODS

2.3.1 Study design and setting

This was a prospective, placebo-controlled, randomised controlled trial to evaluate the effectiveness of PBMT in BC patients undergoing RT. Patients were divided into two groups: a control group receiving placebo treatments and a laser therapy (LT) group receiving PBMT. This was a single centre study and all patients were treated at the RT department of the Limburg Oncology Centre (Jessa Hospital, Hasselt, Belgium) between April 2015 and June 2017. Both the ethics committees of the Jessa Hospital and the University of Hasselt approved the study (B243201524443). The study was registered at ClinicalTrials.gov (NCT02443493).

2.3.2 Study population

Patients were eligible for inclusion if they were diagnosed with primary BC, underwent lumpectomy, and were scheduled to undergo a RT regimen consisting of 25 fractions of 2 Gray (Gy) to the whole breast and 8 fractions (2 Gy/fraction) to the tumour region (total RT dose 66 Gy). Exclusion criteria were previous irradiation to the same breast, mastectomy, metastatic disease, concomitant chemotherapy, and infection of the to-be-irradiated zone. Patients were recruited at the RT department of the Jessa Hospital (Hasselt, Belgium) during the CT-simulation session, approximately two weeks before start of the RT. All participants gave written informed consent before start of the study.

2.3.3 Randomisation

Eligible patients were stratified based on their planning target volume (PTV) into three groups: small (<450 cc), medium (450-800 cc), and large breasts (>800cc) [148]. This was followed by a random allocation (1:1) of the patients to the LT or control group. Patients were allocated based on a block randomisation process, with a block size of four by using a computer-generated random number list prepared by a researcher who was not clinically involved in this trial. Only the laser operator knew the allocation of the patients in the groups.

2.3.4 Interventions

Radiotherapy

RT was planned using the EclipseTM treatment planning system (version 11.0, Varian Medical System, Palo Alto, CA). Patients received a standard RT regimen consisting of a dose of 50 Grays (Gy) of 25 daily fractions (2 Gy/ fraction, 5 fractions/week) to the whole breast followed by an 8-fraction boost of 16 Gy to the tumour bed over a period of 6 to 7 weeks (total dose of 66 Gy). Patients were treated in a supine position with their arms supported above their head. Irradiation to the whole breast was delivered by applying two tangential photon (half) beams set up isocentrically using a 6 MV or a 6+15 MV linear accelerator (Clinac® DHX, Varian Medical Systems, Palo Alto, CA). The boost treatment was delivered through a two-field conformal photon (4-15 MV) or a one-field vertical electron (6-15 MeV) beam. Segmented fields were used where required in order to reduce hot spots. Deep Inspiration Breath-Hold (DIBH) was used for a selected group of patients in order to reduce the mean heart dose.

Topical skin care treatment

Each patient received the institutional standard skin care. This included the application of a topical, hydroactive colloid gel (Flamigel®, Flen Pharma, Kontich, Belgium) on the irradiated zone (3x/day), starting at the first day of RT. Patients that developed painful skin reactions and/or moist desquamation, received a foam, absorbent, self-adhesive silicone dressing on the irradiated zone (Mepilex®, Mölnlycke Health Care, Gothenburg, Sweden).

PBMT

Patients in the LT group received 14 sessions of PBMT (2x/week), starting at the first day of RT. PBMT was provided by a trained operator using a class IV MLS® M6 laser (ASA Srl, Vicenza, Italy). This laser device is commercially available and built in compliance with EC/EU rules, which received FDA approval and is CE certified.

The device combines two laser diodes of two different wavelengths, peak power, and emission mode. The first one is a laser diode emitting at 905 (± 5) nm in pulsed mode (peak radiant power 25W, duty cycle of 50 % independently of the repetition rate). The second one emits in continuous mode at 808 (± 5) nm (peak radiant power 1.1 W). The two laser beams work simultaneously and synchronously with coincident propagation axes (average radiant power 3.3 W, aperture diameter 5 cm, beam spot size at target 19.625 cm^2, power density at target 0.168 W/cm^2). The energy density (fluence) was set at 4 J/cm^2 based on earlier recommendations[149]. The treatment time varied according to the to-be-treated surface area in order to keep this fluence constant (for a spot size of 19,625cm^2 a radiation exposure time of 467,27 sec was necessary). More specific PBMT parameters can be found in table 3.

Patients in the control group received sham treatments in which the laser device was switched off, but still made the same sound as an active laser. Patients in both groups wore safety glasses and eye shields to prevent eye damage and to blind them during the laser or sham sessions.

Table 3: Photobiomodulation parameters (TRANSDERMIS trial)

PBMT parameters			
Device information	Manufacturer	ASA srl	
	Model Identifier	MLS® laser M6	
	Year Produced	2012	
	Number of Emitters	3	
	Emitter Type	IR laser diodes	
	Spatial distribution of emitters	Three emitters spaced 2 cm apart in a triangle pattern	
	Beam Delivery System	Scanning head (five pre-settled directions)	
Irradiation parameters		**Laser diode 1**	**Laser diode 2**
	Center wavelength	808 nm	905 nm
	Spectral bandwidth	±5 nm	±5 nm
	Operating mode	Continuous pulsed wave mode	
	Peak radiant power	1.1 W	25 W
	Average radiant power	3.3 W	
	Maximum frequency (frequency range)		90kHz (1-2000 Hz)
	Pulse on duration Duty cycle		100-ns single pulse width 50 %
	Aperture diameter	5 cm	
	Irradiance at aperture	0.168 W/cm^2	
	Beam divergence at 60%	42.8 mrad	59.2 mrad
	Beam profile	Two laser beams work simultaneously and synchronously with coincident propagation axes	
Treatment parameters	Beam spot size at target area	19.625 cm^2	
	Irradiance at target	0.168 W/cm^2	
	Radiant exposure (fluence)	4 J/cm^2	
	Number of points irradiated	Whole breast, inframammary fold, axilla (depending on the site of radiodermatitis)	
	Exposure duration	Whole breast ± 420-720 s Inframammary fold ± 103 s Axilla ±68 s	
	Application technique	5 cm above skin	
	Timing	After the RT session	
	Frequency of treatment sessions	Biweekly from the first until the last day of RT over a period of 7 weeks (14 sessions in total)	

IR, infrared; MLS, Multiwave Locked System; PBMT, photobiomodulation therapy; RD, radiodermatitis; RT, radiotherapy

2.3.5 Outcome measures

Skin reaction evaluation

The primary outcome measure was the degree of RD at the end of RT. The criteria of the Radiation Therapy Oncology Group/European Organisation for Research and Treatment of Cancer (RTOG/EORTC [20]) and the Radiation-Induced Skin Reaction Assessment Scale (RISRAS [150]) were used to evaluate the skin reactions by two experienced RT nurses. The RISRAS consists of a health professional score (0-24) based on the outward signs of the skin reaction and a patient score (0-12) based on the patients' personal experience of the skin reactions (pain, burning sensation, itchiness, and quality of life). Both subscale scores were summed up to become a total score (a higher score indicated a greater skin toxicity).

Quality of life

The quality of life of the patients was assessed by using the Skindex-16 [151]. This is a validated, 16-item self-assessment questionnaire that measures to what extent the patients' life is affected by their skin condition. Each item on the scale is rated from 0 (Never Bothered) to 6 (Always Bothered). The Skindex-16 is divided in three subscales: symptoms, emotions and functioning. The total score is the average of the three subscales scores (range: 0-100) and a higher score is correlated with a lower quality of life.

Measurement collection schedule

All the previously described measurements were collected on three time points: at the first day, at a RT dose of 40 Gy, and at the last day of RT (66 Gy).

2.3.6 Sample size

Based on preliminary data, a decrease of the incidence of moist desquamation (RTOG grade 2 or higher) of 17.5% in the LT group was expected. Therefore, a sample size of 60 patients in each group was needed to detect such a difference with a two-sided t-test with a power of 80% and a significance level of 0.05.

2.3.7 Statistical analysis

Differences in patient- and therapy-related characteristics between both groups were analysed by means of chi-square tests (χ^2), Fisher's exact tests, Student t-tests, or Mann-Whitney U-tests, as appropriate. Ordinal data (RTOG) were analysed by means of χ^2 or Fisher's exact tests. Continuous data (RISRAS and Skindex-16) were analysed by mixed analyses of variance (ANOVAs) with time (between the RT dose of 40 Gy and 66 Gy) as within-subject factor and groups (control vs. LT group) as between-subject factor. The level of statistical significance for all analyses was set assuming a significance level of 5% ($p<0.05$, two-tailed). SPSS 24.0 (IBM, Chicago, IL) was used for all analyses.

2.4 RESULTS

2.4.1 Patient characteristics

Between April 2015 and June 2017, a total of 754 patients were screened on eligibility, 139 of them were randomised into the placebo or LT group. During follow-up 19 patients (10 and 9 in the control and LT group, resp.) were lost due to different reasons of which change of RT regimen change was the most frequent one (63%). Eventually a total of 120 patients, 60 patients in each group, were included in the present analysis, as shown in the patient flow chart (Figure 10). Patient- and treatment-related characteristics are shown in table 4-5. Statistical analysis revealed that there were no significant differences between the two groups with respect to any of these characteristics. Therefore, both groups were perfectly comparable with respect to extrinsic and intrinsic risk factors for RD.

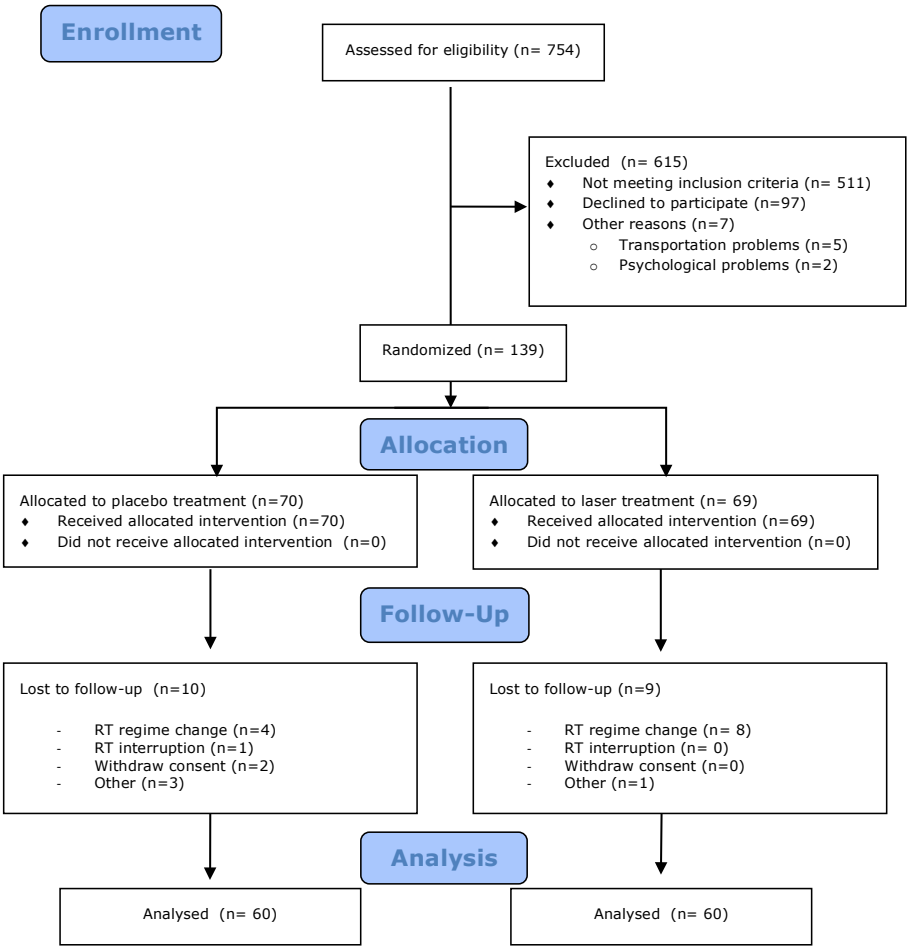

Figure 10: CONSORT flow chart of the TRANSDERMIS trial
RT, radiotherapy

Table 4: Baseline demographic patient characteristics (TRANSDERMIS trial)

	Control group (n=60)		**LT group** (n=60)		
	Mean ± SD		Mean ± SD		p[a]
Age (years)	56.92 (10.34)		56.52 (10.54)		0.88
Body Mass Index (BMI)	25.03 (4.47)		25.27 (3.87)		0.63
	n	%	n	%	P[b]
WHO weight classification					0.63
Underweight (BMI < 18.50)	1	1.7	0	0	
Normal (BMI 18.50-24.99)	33	55	32	53.3	
Overweight (BMI 25-29.99)	19	31.7	23	38.3	
Obese (BMI ≥ 30)	7	11.7	5	8.3	
WHO skin type classification[c]					0.98
Melano-compromised	13	21.7	7	20.0	
Melano-competent	44	73.3	22	75.0	
Melano-protected	3	5	2	5.0	
Smoking status					0.75
Never smoked	36	60.0	40	66.7	
Former smoker	14	23.3	12	20.0	
Current smoker	10	16.7	8	13.3	
Alcohol consumption (drinks/week)					0.19
0-1	25	41.7	36	60.0	
1-3	23	38.3	15	25.0	
3-10	11	18.3	8	13.3	
10-20	0	0	1	1.7	
>20	1	1.7	0	0	
Menopausal status					0.14
Pre-menopausal	27	45.0	36	60.0	
Post-menopausal	33	55.0	24	40.0	
Comorbidities					
Skin disease	12	20	10	16.7	0.81
Hypertension	16	26.7	16	26.7	>0.99
Hypercholesterolemia	8	13.3	14	23.3	0.16
Diabetes mellitus	5	8.3	4	6.7	>0.99
Rheumatoid arthritis	3	5.0	5	8.3	0.49
Cardiovascular disease	3	5.0	3	5.0	>0.99

BMI, Body Mass Index; LT, laser therapy; SD, standard deviation; WHO, World Health Organisation;
[a] Student t-test or Mann Whitney u-test, as appropriate (two-tailed)
[b] Chi-square tests, or Fisher's exact tests, as appropriate (two-tailed).
[c] WHO skin type classification is based on Fitzpatrick's phototype scale: melano-compromised (Fitzpatrick's skin type I-II), melano-competent (skin type III-IV), and melano- protected (skin type V-VI).

Table 5: Disease and therapy- related characteristics (TRANSDERMIS trial)

	Control group (n=60)		**LT group** (n=60)		
Characteristic	n	%	n	%	p[a]
Disease-related					
Tumour type					0.85
Ductal carcinoma in situ	6	10	7	11.7	
Invasive ductal adenocarcinoma	48	80	48	80	
Invasive lobular adenocarcinoma	5	8.3	5	8.3	
Missing	1	1.7	0	0	
Tumour stage					0.30
0	1	1.7	1	1.7	
I	18	30	12	40	
II	33	55	17	55	
III	6	10	2	3.3	
Missing	2	3.3	0	0	
Other cancer therapy					
Chemotherapy prior to RT	46	76.6	44	73.3	0.83
Hormone therapy	44	73.3	46	76.7	0.58
Trastuzumab	12	20.0	15	25.0	0.50

Table 5 (continued)

	Control group (n=60)		**LT group** (n=60)		
Characteristic	n	%	n	%	p[a]
RT-related					
Energy level					0.19
6 MV	43	71.7	50	83.3	
6 MV + 15 MV	17	28.3	10	16.7	
Boost type					0.86
Photons	31	51.7	29	48.3	
Electrons	29	48.3	31	51.7	
DIBH[b]	17	28.3	11	18.3	0.28
No. of segmented fields					0.07
0	4	6.7	5	8.3	
1	23	38.3	22	36.7	
2	18	30.0	29	48.3	
3	10	16.7	4	6.7	
4	4	6.7	0	0	
5	1	1.7	0	0	
					p[c]
Breast PTV (cm^3)[d]	796.27± 439,67		742.55 ± 353.92		0.67
Maximum dose (%)[e]	106.73 ± 1.08		106.79 ± 0.97		0.81

DIBH, deep inspiration breath hold; LT, laser therapy; MV, megavolt; No, number; PTV, planning target volume; RT, radiotherapy; SD, standard deviation

[a] Chi-square tests, or Fisher's exact tests, Student-test or Mann Whitney U-test, as appropriate (two-tailed).

[b] DIBH was used when the patients matched the following criteria: ≤ 70 years with left-sided breast cancer and lymph node metastases; >70 years undergoing chemotherapy; patients with left-sided BC without lymph node metastasis but with a MHD ≥35 Gy. DIBH was applied using the Varian Real-Time Position Management (RPM) Gating system (Varian Medical System, Palo Alto, CA).

[c] Wilcoxon Mann-Whitney U-test (two-tailed).

[d] Radiotherapy target volume that consists of the macroscopic primary tumour, the surrounding microscopic tumour spread and a margin to account for patient- and/or organ movement, shape changes of the tumour and daily setup variations. PTV was measured via treatment planning system by contouring manually each slice of breast tissue on planning CT.

[e] Maximum received irradiation dose (expressed in percentage of prescribed dose).

2.4.2 Skin reaction evaluation

At a RT dose of 40 Gy, almost all patients in both groups developed some degree of RD. As such, there was no significant difference between the two groups at this time point (P= .562). However, as RT progressed, the degree of skin reactions worsened in the control group (P= .008), while in the LT group the skin reactions stabilised (P= .204). As such at the end of RT there was a significant difference in degree of RD between the control and LT group (P= .004), with a higher percentage of patients presenting RD grade 2 or worse in the control group (30% vs. 6.7% in the control and LT group, resp.). In the control group there were even two patients that presented the most severe form of RD (grade 3), while in the LT group no patient developed this grade (Figure 11).

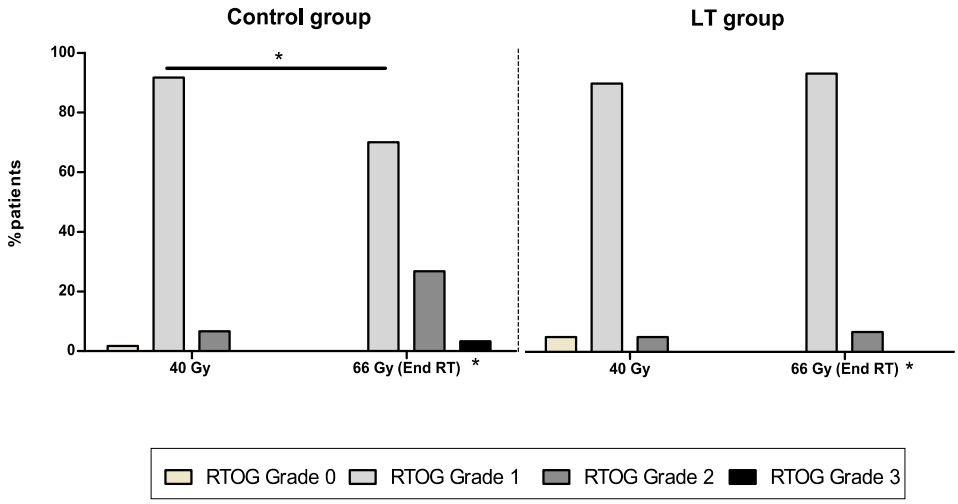

Figure 11: Severity of acute radiodermatitis expressed in RTOG grades (TRANSDERMIS trial)

RTOG grades of the control and LT group at a RT dose of 40 Gy and at the end of RT (66 Gy) *Significant difference within the control group between the two time points and between the two groups at the end of RT ($p<.0.05$; χ^2 or Fisher's exact tests, two-tailed)**.** *RT, radiotherapy*; *RTOG: Radiation Therapy Oncology Group (Grade 0: no change; grade 1: follicular, dull, or faint erythema, dry desquamation; grade 2: tender or bright erythema, patchy moist desquamation; grade 3: confluent moist desquamation other than skin folds).*

Regarding the RISRAS, the mixed 2×2 ANOVAs revealed no significant main group effect for all the scores. However, the main effect of time and the group by time interaction was significant for the all the RISRAS scores (Ps<.05). As shown in figure 12, the subjective RISRAS score decreased in the LT group, while it remained constant in the control group during RT. The increase of both the objective and total score between the RT dose of 40 Gy and the end of RT was more pronounced in the control than in the LT group (Figure 12).

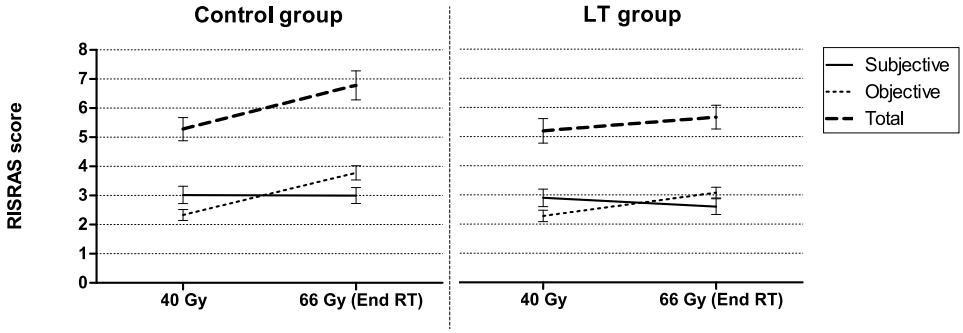

Figure 12: Severity of acute radiodermatitis expressed in RISRAS scores (TRANSDERMIS trial)
Average subjective, objective and total RISRAS scores of the control and LT group at a RT dose of 40 Gy and the end of RT (66 Gy). Data are shown as means (± SEM) and higher scores indicate a more severe skin reaction. *Gy, Gray; LT, laser therapy; RISRAS: Radiotherapy-Induced Skin Reaction Assessment Scale; RT, Radiotherapy; SEM, standard error of measurement.*

2.4.3 Quality of life

Figure 13 demonstrates the progression of the quality of life of the patients during RT. There was a significant main time effect for the Symptom and Emotions subscale and the total Skindex-16 scale (Ps<0.05), but not for the Functioning subscale (P= .704). In addition, for all the subscales and the total scale the main group effect (Ps<0.05) and time by group interaction were significant (Ps<0.05). As shown in figure 13, the Emotions and Symptoms subscale scores decreased more prominently in the LT group than in the control group. While, the functioning subscale score increased in the control group, it decreased in the LT group. In overall, there was a decrease in the total Skindex-16 score in the LT group, while it remained constant in the control group.

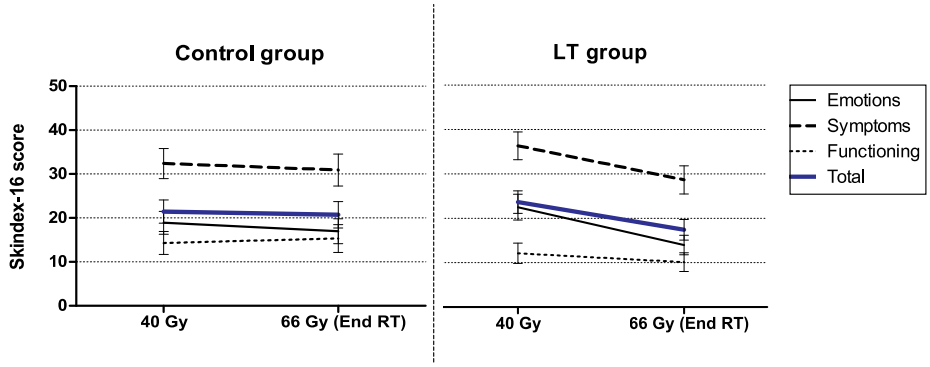

Figure 13: Quality of life scores (TRANSDERMIS trial)
Symptoms, Emotions, Functioning and total score of the control and LT group at a RT dose of 40 Gy and the end of RT (66 Gy). Data are shown as means (± SEM) and higher scores indicate a diminished patients' quality of life. *Gy, Gray; LT, laser therapy; RT, Radiotherapy; SEM, standard error of measurement.*

2.4.4 Comparison with the DERMIS trial

There was no significant difference in the RTOG scores between the LT group of the TRANSDERMIS trial and the LT group of the previous reported DERMIS trial (Ps>0.3 at a dose of 40 Gy and at the end of RT). In both groups most of the patients presented RD grade 1 at the end of RT and only a minority of patients developed grade 2 skin reactions (6.7% and 2.6% in the TRANSDERMIS and DERMIS trial, resp.) [130].

2.5 DISCUSSION

Results of this trial show that PBMT is able to prevent the development of severe acute skin reactions and it seems to provide symptomatic relief during RT.

Theses results are in line with our previous pilot trial (DERMIS trial), in which PBMT was started during RT (i.e. at fraction 20 of RT, dose of 40Gy) [130]. This indicates that starting with PBMT at the first day of RT does not provide an advantage compared to starting with PBMT once the patient already established RTOG grade 1. However, this can mean a more practical benefit for both the patient and the laser therapist, by reducing the number of PBM sessions that are necessary to deliver a positive effect.

Other studies investigating the use of PBMT in the management of acute RD are limited. There were three studies that evaluated the use of LED-PBMT for acute RD in BC patients. The study by DeLand et al. treated 19 BC patients undergoing intensity-modulated radiation treatments (IMRT) with LED-PBMT (590 nm, standard 100-pulse, 250 milliseconds per pulse at a fluence of 0.15 J/cm^2) on a daily basis and compared the grade of RD with a retrospective control group of 28 patients. The incidence of severe skin reactions (grade 2 or higher) at the end of RT in de LED group was 5.3%. In the control group of Deland the incidence of severe skin reactions was higher than in our study (85.7% vs. 26.7%, resp.). This dissimilarity may be due to differences in standard skin care used in both studies [129].

Fife et al. compared the degree of skin reactions of a LED-PBMT treated group (n=18) with a placebo group (n=15). Patients in the PBMT group received LED-PBMT (same parameters as Deland et.al.) before and after each three-dimensional conformal RT session, while the placebo group received sham LED treatments. This study showed that still 66.6% of the LED-PBMT patients developed grade 2 RD, while in the placebo group also 66.6% of the patients presented RD grade 2 or higher [128]. The contrasting results of both LED studies may be caused by a variety of factors such as the type of RT technique, non-blinded vs. blinded scoring of skin reactions, and set–up of the LED treatment.

More recently, Strouthos et al. treated 25 BC patients with LED-PBMT (660-850 nm, peak radiant power 1390mW, average power density 44.6mW/cm^2, 250 ms per pulse at a fluence of 0.15 J/cm^2) twice weekly from the start of RT prior to their RT session and compared the skin reaction results with 45 control patients that only received the standard skin care. Their results showed that in the LED-PBMT group 12% of the BC patients demonstrated grade 2 RD, while in the control group 44.4% of the patients developed RD grade 2 or higher[152]. Bay et al. performed another study concerning the use of PBMT for wound healing purposes. They exposed the left or right side of the buttock of 20 healthy volunteers to PBMT (830/590, 109mW/cm^2, 65 J/cm^2 per treatment) or placebo (595nm, 0.19mW/cm^2, 0.13 J/cm^2 per treatment) during 5 daily sessions after ablative fractional laser-assisted photodynamic therapy (PDT), to investigate the effectiveness of PBM in the reduction of inflammatory skin reactions. Results of this study did not show any benefit of PBMT in the reduction of PDT-induced skin reactions[153].

All together, these results demonstrate that PBMT is only an effective treatment option for wound healing, when the appropriate irradiation and treatment parameters are applied[99, 154]. Not only for the treatment of acute RD, but also for chronic (i.e. late) RD (e.g. telangiectasia's, fibrosis, and ulceration/necrosis) the application of laser therapy (PBMT or pulsed dye laser, PDL) seems to be effective [52, 66, 85, 86, 155].

A few limitations of the present study need to be addressed. The RTOG grading system as well as the researcher component of the RISRAS scale lack objectivity. Over the past few years, a variety of objective skin measurement techniques have been reported and will increase the objectivity of the study results. Most of these techniques have been developed to measure the degree of erythema and the skin barrier function (e.g. transepidermal water loss and skin hydration measurements) [39-41, 43]. Another important limitation might be the patient population, which was confined to breast cancer patients that underwent a lumpectomy and a standard RT regimen of 33 fractions. More clinical trials in a broader patient population with different cancer types and RT regimens need to be conducted, which will increase the generalizability of the study results. Finally, a multicentre trial will allow us to include a larger number of participants of clinical centres at different geographic locations. This will be necessary for validation of this promising treatment technique for RD.

2.6 CONCLUSION

This was the first randomised, placebo-controlled clinical trial demonstrating that a twice-weekly treatment with PBM starting from the first day of RT in breast cancer patients can prevent the development of moist desquamation (RTOG grade 2 or higher). In addition, PBMT also seems to improve the patients' quality of life during RT. Future (multi)centre trials are necessary to confirm these positive results in a larger patient population with a broader range of cancer types and at different clinical centres. This will increase the general applicability of PBMT in supportive care of cancer patients.

CHAPTER 3

Biophysical skin measurements to evaluate the effectiveness of photobiomodulation therapy in the prevention of acute radiation dermatitis in breast cancer patients

***Robijns J**, Censabella S, Claes S, Pannekoeke L, Bussé L, Colson D, Kaminski I, Lodewijckx J, Bulens P, Maes A, Noé L, Brosens M, Timmermans A, Lambrichts I, Somers V, Mebis J.*

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3.1 ABSTRACT

Purpose

The purpose of this study was to evaluate objectively the effectiveness of photobiomodulation therapy (PBMT) for the prevention of acute radiation dermatitis (ARD) by using biophysical skin measurements.

Methods

A randomised, placebo-controlled trial with 120 breast cancer patients who underwent an identical radiotherapy (RT) regimen post-lumpectomy was performed (TRANSDERMIS trial). Patients were randomised to receive PBM (808 nm CW/905 nm pulsed, 168 mW/cm2, spot size 19.6 cm2, fluence 4 J/cm2) or placebo treatments from the first day of RT (2x/week). Biophysical skin measurements were collected to assess the skin pigmentation and barrier function. Measurements were collected at the first day of RT, a RT dose of 40 Gray (Gy), and the end of RT (66 Gy).

Results

The incidence of moist desquamation was significantly higher in the control than in the PBMT group at the end of RT (30 vs. 7%, respectively, odds ratio= 6, p=0.004). The biophysical skin measures showed that the mean percentage change from the baseline transepidermal water loss (TEWL), erythema and melanin value was significantly higher in the control than in the PBMT group at the end of RT (Ps<0.05). Logistic regression analysis revealed that the risk on moist desquamation was significantly increased in patients with a large (>800cc) breast volume (odds ratio= 4, p=0.017).

Conclusions

This is the first randomised controlled trial demonstrating by objective measurements that PBMT is effective in reducing the incidence of moist desquamation in breast cancer patients undergoing RT. Additionally, a large breast volume is an important risk factor for the development of moist desquamation.

3.2 INTRODUCTION

Acute radiation dermatitis (ARD) is a severe side effect occurring in about 90-95% of the cancer patients undergoing radiotherapy (RT) [29]. This is a cutaneous reaction that is caused by direct damage of ionising radiation, which manifests 2-4 weeks after the first RT session [134].

In normal healthy skin, the superficial cells of the epidermis (i.e. upper skin layer) are shed through normal desquamation and replaced by stem cells from the underlying basal layer. From the first RT dose, stem cells within the basal layer of the epidermis are destroyed leading to a disruption in the self-renewing property of the skin. During RT, this process continues which will negatively affect the skin barrier function and the wound healing process. This ultimately results into changes of the skin structure and vasculature, clinically characterized by erythema, dryness, flaking skin, pruritus, folliculitis (i.e. skin rash), and hyperpigmentation. Due to the compromised skin barrier function and cutaneous immune system, the skin will become more susceptible for water loss, chemical substances, allergens, ultraviolet radiation (UV), and infections [156, 157].

Clinically ARD is evaluated by the criteria of the Radiation Therapy Oncology Group (RTOG) into 3 grades: mild erythema and dry desquamation (grade 1), bright erythema and moist desquamation in skin folds (grade 2), and confluent moist desquamation (grade 3). However, this grading system lacks objectivity [20].
A variety of biophysical skin techniques are available to measure the skin pigmentation, hydration, pH, blood flow, and sebum level to investigate the underlying physiological mechanism of ARD [158].

Up to now, the evidence for a general consensus on the prevention and management of ARD is limited. Nevertheless, the Multinational Association of Supportive Care in Cancer (MASCC) developed skin care guidelines concerning the prevention and treatment of RD in 2013. Still, many RT centers develop their own skin care protocol [44].

Photobiomodulation therapy (PBMT) is the application of visible and/or (near-) infrared light at a low power on tissue to stimulate the wound healing process and reduce inflammation and pain [97]. There is evidence that PBMT could be used as a new preventive and therapeutic tool in the management of ARD [85, 128, 129, 152]. Recently, our research group performed two clinical trials in which we demonstrated that PBMT is able to prevent the development of ARD grade 2 or higher in breast cancer patients by clinically evaluating the skin reactions by the RTOG grading [130, 159].

In this project, we evaluated the effectiveness of PBMT in the prevention of ARD in breast patients by objectively assessing the skin hydration, trans epidermal water loss (TEWL), and pigmentation.

3.3 MATERIALS AND METHODS

3.3.1 Study design and setting

This was a secondary analysis of the TRANSDERMIS trial, a monocentric, prospective, placebo-controlled, randomised controlled trial (RCT) [159], to evaluate objectively the effectiveness of PBMT in breast cancer (BC) patients undergoing RT. Female patients with unilateral BC who were treated at the RT department of the Limburg Oncology Centre (Jessa Hospital, Hasselt, Belgium) were screened on eligibility between April 2015 and June 2017. The study was approved by the ethics committees of the Jessa Hospital and the University of Hasselt (B243201524443) and was conducted according to the Declaration of Helsinki. The study was registered at ClinicalTrials.gov (NCT02443493).

3.3.2 Study population

To be eligible for the study, patients needed to fulfil the following criteria: female, diagnosed with primary unilateral BC, underwent lumpectomy, scheduled to undergo a RT regimen consisting of 25 fractions of 2 Gray (Gy) to the whole breast and 8 fractions of 2 Gy to the tumour region (total RT dose 66 Gy). Patients were excluded when they met the following criteria: irradiation to the same breast in the past, hypofractionated RT, mastectomy, metastatic disease, concomitant chemotherapy, and infection of the to-be-irradiated zone. Eligible patients were recruited during the CT-simulation session, approximately two weeks before the start of the RT. Written informed consent of all patients was collected before study participation.

3.3.3 Randomisation and blinding

The planning target volume (PTV) of the eligible patients was used to stratify them into three groups: small (<450 cc), medium (450-800 cc), and large breasts (>800cc) [148]. Patients were randomly assigned to the control or PBMT group in a 1:1 ratio based on a computer-generated random number list, which was held by a researcher who was not involved in the clinical part of the study. Allocation was concealed to the PBM operator until the first treatment session. Both the participating patient and the outcome assessor were blinded until the last treatment session.

3.3.4 Interventions

Radiotherapy

The EclipseTM treatment planning system was used to plan the RT sessions (version 11.0, Varian Medical System, Palo Alto, CA). The standard RT regimen consisted of 25 daily fractions (2 Gy/ fraction, 5 fractions/week) to the whole breast followed by boost of 8 fractions (2 Gy/ fraction, 5 fractions/week) to the tumour bed during a period of 6 to 7 weeks (total RT dose of 66 Gy). The whole breast was irradiated with two tangential photon (half) beams set up isocentrically using a 6 MV or a 6+15 MV linear accelerator (Clinac® DHX, Varian Medical Systems, Palo Alto, CA) and the tumour region with a two-field conformal photon (4-15 MV) or a one-field vertical electron (6-15 MeV) beam. A selected group of patients were irradiated using the Deep Inspiration Breath-Hold (DIBH) in order to reduce the mean heart dose (MHD).

Topical skin care treatment

Each patient was individually advised to follow the general skin care guidelines (e.g. wear loose fit clothing, gentle washing with or without mild soap, patting dry with a soft towel instead of rubbing). Further, the patients were instructed to apply a topical, hydroactive colloid gel (Flamigel®, Flen Pharma, Kontich, Belgium) on the irradiated zone (3x/day), starting at the first day of RT. Foam, absorbent, self-adhesive silicone dressings (Mepilex®, Mölnlycke Health Care, Gothenburg, Sweden) were used in the case of painful skin reactions and/or moist desquamation.

PBMT

PBMT was applied from the first until the last day of RT (2x/week, 14 sessions) by a trained operator using the class IV MLS® M6 laser (ASA Srl, Vicenza, Italy), as described previously [159]. This device is commercially available, built in compliance with EC/EU rules, received FDA approval, and is CE certified. It consists of two laser diodes with different wavelengths (808-905 nm), peak powers (1.1-25W), and emission modes (continuous and pulsed). Both diodes work simultaneously and synchronously with coincident propagation axes (average radiant power 3.3 W). The energy density (fluence) was set at 4 J/cm^2 based on earlier recommendations and on our clinical experience [130, 149]. During the PBMT sessions the whole irradiated area was treated (whole breast, inframammary fold, and axilla). The complete list of PBMT parameters can be found in table 3. The PBMT parameters were selected based on the successful results of our previous trial (DERMIS trial) [160] and on the guidelines of Zecha et al [154].

During the sham treatments of the control group, the PBM device did not emit light but made the same sound as an active device. All patients, independently of their treatment group, wore safety glasses and eye shields to avoid any perceived risk of eye damage and to blind them during the PBM or sham sessions.

3.3.5 Outcome measures

Patient data

Clinical information regarding the patient's personal, disease- and treatment-related characteristics was collected via patient questionnaires and the patient's medical charts.

RTOG grading

Clinically the severity of ARD was evaluated by the criteria of the Radiation Therapy Oncology Group/European Organisation for Research and Treatment of Cancer (RTOG/EORTC [20]). Two experienced RT nurses performed this in a blinded manner.

Objective skin measurements

In order to assess the impact of RT on the skin barrier function the transepidermal water loss (TEWL) and the skin hydration level were determined. TEWL was measured by the Tewameter® TM300 (Courage-Khazaka, Cologne, Germany), according to the guidelines published, both by the standardization group of the European Contact Dermatitis Society [161] and by the European group on Efficacy Measurements of Cosmetics and Other topical products [162].

The skin hydration was measured with the Corneometer® (Courage-Khazaka, Cologne, Germany) according to Heinrich et al. [163].

A reflectance spectrophotometer, Mexameter® MX18 (Courage-Khazaka, Cologne, Germany), was used to measure the pigmentation of the skin (e.g. melanin and erythema) as previously described by Clarys et al. [164].

All four measurements (e.g. TEWL, hydration, erythema, and melanin) were taken at the four quadrants of each breast (irradiated and non-irradiated), with three measurements per quadrant (Figure 14). The average values of these measurements were taken as a value for the whole breast. The measurements were carried out after a 30-min acclimatization period at room temperature (20–22°C) and 40–60% humidity. The final objective measurements were described as percentages in order to calculate deviations from pre-treatment baseline values, also termed as indexes. Therefore, the following formula was used:

$$\left[\left(\frac{\text{Obj. measure irradiated breast at indicated time}/\text{Obj. measure control breast at indicated time}}{\text{Obj. measure irradiated breast at baseline}/\text{Obj. measure control breast at baseline}}\right)-1\right] x\ 100\%$$

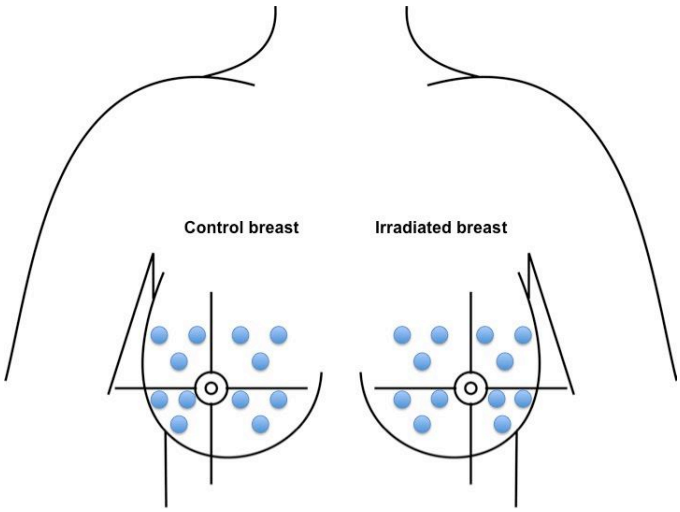

Figure 14: Objective skin measurement points
Overview of the measured points in the irradiated and control breast

Measurement collection schedule.

All the previously described measurements were collected on three time points: at the first day of RT, at a RT dose of 40 Gy, and at the last day of RT (66 Gy).

3.3.6 Statistical analysis

Differences in patient- and therapy-related characteristics between both groups were analysed by means of chi-square tests ($\chi 2$), Fisher's exact tests, Student t-tests, or Mann-Whitney U-tests, as appropriate. RTOG scores were analysed by means of $\chi 2$ or Fisher's exact tests, as appropriate. The objective skin measurements at each time point were analysed by Mann-Whitney U-tests. Longitudinal analysis of the biophysical skin measurements was performed by mixed analyses of variance (ANOVAs) with time (between the RT dose of 40Gy and 66 Gy) as within subject factor and group (control vs. PBMT group) as between-subject factor. To determine the risk on moist desquamation, univariate and multivariate logistic regressions with, as predictor variables, age, group (with the control group as reference group), whether patients had prior chemotherapy or not, breast size (based on the PTV), and the objective skin measurements was performed. The level of statistical significance for all analyses was set assuming a significance level of 5% ($P<0.05$, two-tailed). SPSS 24.0 (IBM, Chicago, IL) was used for all analyses.

3.4 RESULTS

3.4.1 Patient characteristics

A total of 139 patients were randomised into the placebo or PBMT group between April 2015 and June 2017. During the course of RT, 2 patients of the control group withdrew their consent. Further 17 patients were excluded due to a RT regimen change or a RT interruption (5 and 8 in the control and PBMT, resp.). For the final analysis data of 120 patients, 60 patients in each group, was used (Figure 10). Both groups were matched for all the patient- and treatment-related characteristics (Table 4-5).

3.4.2 Clinical evaluation of ARD

Patients' RT-induced skin reactions were evaluated by the criteria of the RTOG, as shown in Figure 11. Our results demonstrated that the incidence of moist desquamation (ARD grade 2 or higher) was significantly lower in the PBMT group in comparison with the control group at the end of RT ($p=0.004$). This was confirmed by the univariate logistic regression analysis demonstrating that patients only receiving the standard skin care were six times more likely to develop moist desquamation in comparison with patients that also were treated with PBMT ($p=0.003$, 95% CI [OR]: 1.881-19.82). Further, the risk on moist desquamation rose with an increasing breast volume. As such, patients with large breasts (>800 cc) had a four times higher risk to develop moist desquamation than patients with small breast volumes ($p=0.017$, 95% CI [OR]: 1.290-12.936).

3.4.3 Objective evaluation of ARD

Erythema

The mixed 2X2 ANOVAs revealed a significant main time effect and group by time interaction (Ps<0.05) for the erythema index. However, the main group effect was not significant. As depicted in figure 15a, the degree of erythema in both groups increased during the course of RT. At the RT dose of 40 Gy, the percentage change in erythema from baseline did not significantly differ between the control and the PBMT group. However, at the end of RT, the percentage change from baseline in erythema was significantly higher in the control group in comparison with the PBMT group (p=0.016).

Pigmentation

Concerning the melanin index, there was both a significant main time effect and group by time interaction (Ps<0.05), but no significant main group effect. Figure 15b demonstrates that the degree of pigmentation increased during the progression of RT in both groups. The increase in pigmentation started off slowly, with no significant difference in percentage change over baseline in melanin between the two groups at the RT dose of 40 Gy. Towards the end of RT, the melanin index was significantly higher in the control than in the PBMT group (p=0.019).

Hydration

The mixed 2x2 ANOVAS revealed a significant main time and group effect (Ps<0.05), but no significant group by time interaction for the skin moisture level. As shown in figure 15c, during the course of RT, the skin hydration level decreased in both groups in comparison with the baseline values. The skin hydration level was significantly lower at the RT dose of 40 Gy in the PBMT group in comparison with the control group (p=0.036). However, at the end of RT both groups showed a comparable skin moisture index.

Transepidermal water loss

Regarding the TEWL, there was a significant main time and group effect (Ps<0,05), but no significant group by time interaction. The TEWL decreased in comparison with the baseline value in both the control and PBMT group at the RT dose of 40 Gy, to a comparable level (Figure 15d). Towards the end of RT, the TEWL level increased in both groups, although the final TEWL index was significantly lower in the PBMT group in comparison with the control group (p=0.05).

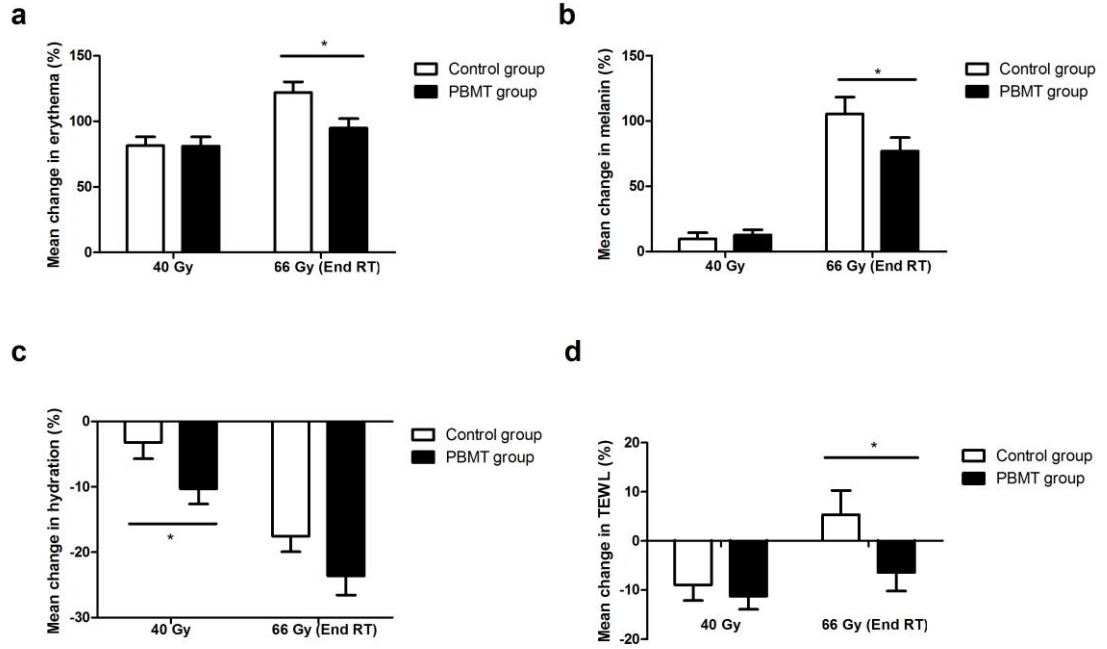

Figure 15: Objective assessment of acute radiodermatitis
Biophysical evaluation of the skin pigmentation (erythema, a and melanin, b) and barrier function (hydration, c and TEWL, d)**.** Data are shown as mean percentages change from baseline (± SEM). *Significant difference between the two groups at the indicated time point ($P < 0.05$; Mann-Whitney U-test, two-tailed). *PBMT, photobiomodulation therapy; RT, radiotherapy; TEWL, transepidermal water loss.*

3.5 DISCUSSION

Results of this trial showed that PBMT is an effective tool to prevent the development of moist desquamation. This was confirmed by objectively evaluating the skin's biophysical condition. Our results demonstrated that PBMT was able to reduce the increase of the skin's pigmentation level and improve the skin barrier function. Additionally, the main risk factor for the development of severe ARD is the breast volume, which implies that patients with large breasts (>800 cc) have an increased risk on moist desquamation.

The erythema index progressively increased during RT in both treatment arms. These findings are in line with previous studies [38, 40, 165, 166]. This increase in erythema is caused by the RT-induced inflammatory reaction leading to vasodilation and leaking of the blood vessels [39, 167, 168]. However, the increase was significantly lower in the PBMT than in the control group at the end of RT. This proves that PBMT is able to reduce the degree of erythema. These results are consistent with earlier *in vivo* studies and clinical trials on various erythematous skin disorders (e.g. acne vulgaris, UV-damage, laser resurfacing wounds, burn wounds) [123, 140, 169, 170]. The anti-inflammatory effect of PBMT, correlated with a decrease in inflammatory cytokine production, might explain this observation [140, 171].

Further, our results also showed a significant increase in skin pigmentation in both groups during the course of RT. This is explained by post-inflammatory hyperpigmentation (PIH) after the RT-induced skin reaction [39, 168]. PIH is caused by the stimulation of melanocytes due to an inflammatory skin reaction leading to an increased melanin production and transport to the surrounding keratinocytes. Remarkably, our results demonstrated that at the end of RT the increase in melanin content of the skin was significantly lower in the PBMT than in the control group. As such, PBMT was able to stabilise the hyperpigmentation reaction of the patients' skin during RT. Several *in vitro* studies showed that PBMT can inhibit the melanin synthesis in human melanocytes cultures [172]. Also clinical trials demonstrated that PBMT is able to reduce hyperpigmentation in numerous skin conditions (e.g. acne vulgaris, photoaging, melasma) [173-175].

In healthy skin, a low TEWL and a high hydration value correlate with a good barrier function [12]. Ionising radiation deregulates the cellular function and causes apoptosis of the epidermal cells, resulting into an affected skin barrier function, correlated with a high TEWL and a low skin moisture level [39, 41-43, 168]. The findings in our control group are in line with these studies. However, in the PBMT group both the TEWL and hydration index were significantly decreased at the end of RT. The epidermal thickening effect might explain these conflicting results. This effect is characterized by epidermal hyperproliferation leading to a thickened stratum corneum (outermost layer of the epidermis) caused by repetitive exposure to external stimuli. The thickening of the stratum corneum improves the skin barrier function and thereby it is correlated with a decrease in TEWL [43, 176, 177].

Several studies, both *in vitro* and *in vivo*, have demonstrated that PBMT can stimulate the proliferation of several types of cells, including keratinocytes. PBMT seems to be able to stimulate the epidermal thickening effect in the skin caused by RT and thereby it can improve the skin barrier function [90, 118, 119, 178, 179].

The results of the logistic regression analysis demonstrated that patients who were treated with standard skin care had a 6 time higher risk to develop moist desquamation in comparison with the patients treated with PBMT. This implies that the preventive application of PBMT can seriously lower the severity of the RT-induced skin reactions, as previously published by our study group [159]. Further, our results showed that patients with large breasts developed more severe skin reactions. These findings are consistent with earlier published studies [35, 180, 181].

The main limitation of the study was the enrolled patient population, which was confined to breast cancer patients post-lumpectomy, who underwent a standard fractionated RT regimen. In the future, more clinical trials in a broader patient population with different cancer types and RT regimens need to be conducted, which will increase the generalizability of the study results.

3.6 CONCLUSION

This is the first RCT demonstrating by an objective approach that the preventive application of PBMT is effective in reducing the incidence of moist desquamation in breast cancer patients. The biophysical skin measurements showed that PBMT is able stabilise the degree of pigmentation (both erythema and melanin) and improve the skin barrier function during the course of RT. Interestingly; patients with a large breast volume have an increased risk on moist desquamation. In conclusion, we can state that PBMT is an effective tool to prevent the development of severe ARD in breast cancer patients. Further, screening patients on breast volume before the start of RT, can allow the radiotherapist to optimize the skin management during the course of RT.

CHAPTER 4

Monitoring of inflammatory mediators in acute radiodermatitis patients undergoing photobiomodulation therapy

Robijns J, *Censabella S, Claes S, Pannekoeke L, Bussé L, Colson D, Kaminski I, Lodewijckx J, Bulens P, Maes A, Noé L, Brosens M, Timmermans A, Lambrichts I, Hellings N, Somers V, Mebis J*

In preparation

4.1 ABSTRACT

Purpose

The purpose of this study was to investigate the effect of photobiomodulation therapy (PBMT) on the inflammatory prolife of acute radiodermatitis (ARD) skin lesions in breast cancer (BC) patients.

Methods

In total 120 BC patients treated with identical radiotherapy (RT) regimen post-lumpectomy were randomised to receive PBM or placebo treatments from the first day of RT (2x/week). Skin toxicity was evaluated by the Radiation Therapy Oncology Criteria (RTOG) and the Radiotherapy-induce Skin Reaction Assesment Scale (RISRAS) at the end of RT. Skin tape samples were collected via tape stripping on the first and last day of RT. Four inflammatory mediators (IL-1β, IL-6, IL-8, and TNF-α) were measured in the stratum corneum (SC) samples by a multiplex assay.

Results

In about 80% of the human skin tape samples (n=96) measurable levels of cytokines were detected. During the progression of RT the level of IL-1β, IL-6, and IL-8 significantly increased. No significant change in TNF-α was detected.

The levels of all the measured cytokines were positively correlated with each other. Moreover, there was a strong positive association between the severity of ARD and the level of IL-6, IL-8, and TNF-α. Regarding the effect of PBMT on the analysed cytokines, only the increase in IL-1β was significantly down regulated by PBMT at the end of RT.

Conclusions

This is the first clinical trial that showed that level of several inflammatory mediators is increased in human samples of ARD lesions. Additionally, the level of inflammatory cytokines is positively associated with severity of ARD. More interesting, our results suggest that PBMT can downregulate the inflammatory reaction by modulating the level of IL-1β. Future research is necessary to investigate the underlying mechanism of PBMT on the inflammatory skin reactions induced by RT.

4.2 INTRODUCTION

RD is an inflammatory skin disorder induced by ionising radiation. RT does not only negatively affect the proliferative capacity of the epidermal stem cells, but it also modulates the cell communication leading to skin damage and eventually a disturbed skin barrier function. Cytokines are the main molecules that regulate the communication both locally between cells and tissues and distantly between organs [32].

Cytokine production is initiated after the first radiation session. Thereby, ionising radiation stimulates the activation of inflammatory cells and the expression of adhesion molecules on keratinocytes and endothelial cells leading to an increased vascular permeability. This eventually results into an increased transport of immune cells from the circulation to the inflamed tissue, which is the ''hallmark'' of RT-induced skin injury. The production of cytokines will continue for months or even years after the first RT session, which implies that they affect each stage of the acute and eventually chronic RT-induced cutaneous reaction [32].

The profile of inflammatory mediators in the skin lesions might give more insight into the underlying mechanism of RD, offering potential biomarkers or targets for more personalised therapy. Furthermore, it could provide us information regarding the effectiveness of a specific therapy [32]. Clinical data concerning the skin's inflammatory profile of patients that suffer from RD is non-existing. This lack of data is due to invasive nature of many procedures to collect skin samples, ranging from skin biopsies, microdialysis, suction-blister technique, microneedles, to electroporation [182-190].

In contrast, tape stripping, the removal of the stratum corneum (SC) using adhesive tapes, is a non-invasive, rapid, and patient-friendly method. It has been used to study the penetration and the reservoir behaviour of topically applied or exogenous substances, the physiology of the SC, epidermal wound healing, and the excretion of endogenous substances [191-194].

PBMT is an emerging therapy to prevent and heal RT-induced skin reactions [159]. One of the main beneficial effects of PBMT is the anti-inflammatory effect. *In vitro* and *in vivo* studies demonstrate that PBMT is able to alter the levels of various cytokines and inflammatory mediators [140].

In the current study we used the tape stripping method to evaluate the effect of PBMT on the inflammatory prolife of skin lesions of acute RD patients. As a control, the level of inflammatory cytokines of RT patients receiving only standard skin care was measured.

4.3 MATERIALS AND METHODS

4.3.1 Study design and population

This is a tertiary analysis of the TRANSDERMIS trial, described in chapter 2. In summary, BC patients undergoing RT post-lumpectomy at the Jessa Hospital (Hasselt, Belgium) were enrolled after they had signed the informed consent. Accordingly, patients were randomised into a control group, which received a placebo treatment, or a PBMT group, which received PBMT. The study was approved by the ethics committees of the Jessa Hospital and the University of Hasselt (B243201524443) and was conducted according to the Declaration of Helsinki. The study was registered at ClinicalTrials.gov (NCT02443493).

4.3.2 Interventions

Radiotherapy

All patients underwent the same RT protocol consisting of 25 fractions of 2 Gy to the whole breast followed by a 8 fraction boost of 2 Gy to the tumour bed. For more specific details concerning the RT regimen, we refer to paragraph 2.3.4.

Topical skin care treatment

Patients were advised to follow the institutional skin care guidelines and instructed to apply a topical, hydroactive colloid gel (Flamigel®, Flen Pharma, Kontich, Belgium) on the irradiated zone (3x/day), starting at the first day of RT. The emollient was applied after the collection of the skin tape samples. Wound dressings (Mepilex®, Mölnlycke Health Care, Gothenburg, Sweden) were used in the case of painful skin reactions and/or moist desquamation.

PBMT

PBMT sessions started at the first day of RT and were applied two times a week until the last day of RT. For more specific details on PBMT parameters, see table 3 and paragraph 2.3.4. Patients in the placebo group underwent sham PBM sessions in which the laser device was switched of. All patients were blinded folded and wore safety glasses, in order to prevent eye damage.

4.3.3 Skin tape stripping

Tape stripping of the SC

Skin tape stripping was performed according to a previously described protocol [193, 194]. In short, adhesive D-squame® tapes (Standard sampling discs, 22-mm diameter, Cuderm, Dallas, TX, USA) were applied to the skin using gloved hands to avoid cross contamination of skin proteins. The adhesive side of the tape was placed directly on the skin and pressed for 10 seconds on the skin site with a constant pressure by using a pressure applicator (225g/cm^2). Afterwards, the tape was removed from the skin site using blunt forceps at a constant removal velocity. In total six D-squame® tape samples were collected by sequential tape stripping of the same skin site on the irradiated breast, before the first and at the last RT session.

Sample preparation and analysis

Extraction of the skin tape samples was performed using sterilized phosphate-buffered saline (PBS) and sonication at 40 kHz for 15 minutes (Branson 5800, Danbury, USA). After centrifugation, the tape extracts of the six skin samples of each breast were pooled and stored at -80 °C until analysis. The analysis was performed using a micro bicinchoninic acid assay (micro BCA protein assay kit, Thermofisher Scientific, Waltham, USA) to measure the total amount of proteins present in the skin samples.

Samples were analyzed on four cytokines (IL-1β, IL-6, IL-8 and TNF-α) by using the MESO QuickPlex SQ 120 assay (MSD, Rockville, Md., USA). All panels used human antibodies. For the analysis, 50 µl of the SC extract and a calibrator (provided by MSD) were incubated overnight on the sealed plate at 2-8°C.

Reading was done after washing with PBS-Tween 20 and after adding the provided reading buffer.

All cytokine results were expressed as interleukin levels (in picograms) per microgram (µg) of total protein (TP) recovered from each tape.

4.3.5 Clinical outcome measures

Patients skin reactions were evaluated using the RTOG criteria and RISRAS score at the end of RT, as described in paragraph 2.3.5.

4.3.6 Statistics

Ordinal data (RTOG) were analysed by means of χ^2 or Fisher's exact tests. The paired t-test was used to analyse the within group differences of the cytokine levels between the first and last RT session. Longitudinal analysis of the RISRAS scores and cytokine profiles was performed by mixed analyses of variance (ANOVAs) with time as within subject factor and group (control vs. PBMT group) as between-subject factor. Spearman's rank correlation coefficient was used to perform correlation analysis of the individual immunomodulators and the RD grading scales. The level of statistical significance for all analyses was set assuming a significance level of 5% ($P<0.05$, two-tailed). SPSS 24.0 (IBM, Chicago, IL) was used for all analyses.

4.4 RESULTS

4.4.1 Clinical scoring of acute RD

Patients in the PBMT group developed less severe skin reactions in comparison with the control patients at the end of RT, based on the RTOG criteria ($p<0.05$; Figure 11). Moreover, figure 12 demonstrates that the RISRAS scores increased significantly in both groups. However, the overall increase was significantly lower in the PBMT group in comparison with the control group, indicated by a significant time and group by time interaction ($Ps<0.05$).

4.4.2 Inflammatory profile

In total 120 patients completed the study and their skin tape samples were analysed. Approximately 80% of the samples (n=49 and n=47 for the control and PBMT group, resp.) were above the detection limit of the assay.

The mixed 2X2 ANOVA of the IL-1β concentrations demonstrated a significant time ($p<0.01$) and group by time interaction ($p=0.02$). However, there was no significant group effect ($p=0.08$). As shown in figure 16A, the level of IL-1β increased along the progression of RT, with a steeper increase in the control group.

The level of inflammatory mediators, IL-6 and IL-8, increased towards the end of RT, indicated by a significant time effect ($Ps<0.01$). No significant group, nor group by time interaction ($Ps>0.1$) was identified for these two cytokines.

As depicted in figure 16B, the level of IL-8 increased significantly in both groups between the two time points ($Ps<0.01$). On the other hand, the increase in the concentration of IL-6 was not significant in the PBMT group ($p=0.23$), while it was significant in the control group ($p=0.05$) at the end of RT (Figure 16C).

Concerning the profile of TNF-α, there was no significant group, time, and group by time interaction ($Ps>0.1$). Figure 16D, demonstrates that the level of TNF-α remained stable in both groups.

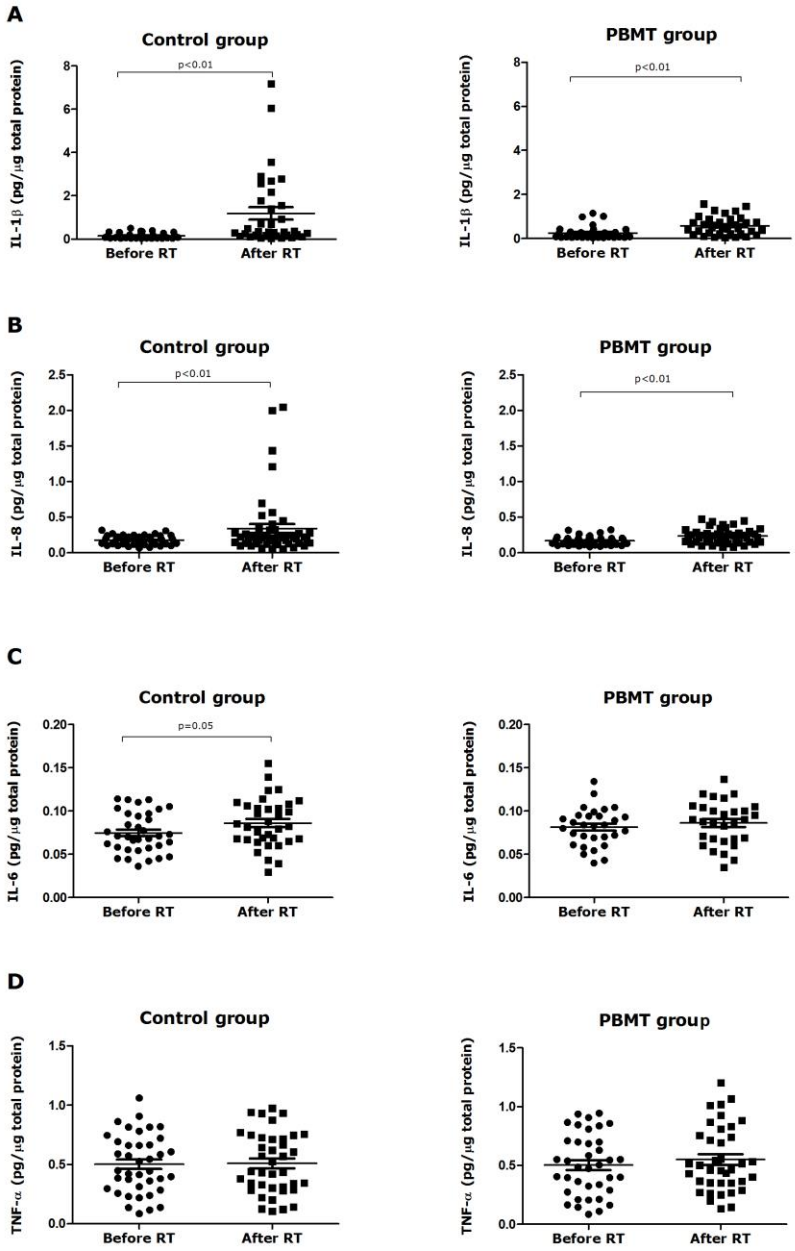

Figure 16: Inflammatory profile of stratum corneum cytokines
Normalized concentrations of IL-1β (a), IL-8 (b), IL-6 (c) and TNF-α (d) in the control and PBMT group before and after RT. Data are shown as mean (± SEM). *Significant difference between the time points within each group (P < 0.01; paired t-test, two-tailed). *IL, interleukin; PBMT, photobiomodulation therapy; RT, radiotherapy; TNF, tumour necrosis factor.*

4.4.3 Correlations

A correlation analysis was performed to investigate if there was a relationship between the various inflammatory mediators and the severity of RD (Table 6).

There was a moderate to strong significant positive correlation between the following cytokines after the RT: IL-1β and IL-8, IL-8 and IL-6, IL-8 and TNF-α, and IL-6 and TNF-α (Ps<0.001).

Furthermore, the RISRAS score at the end of RT correlated strongly with the level of the inflammatory molecules IL-8 and TNF-α measured before the first RT session (Ps<0.001). There was also a positive relationship between the concentration of IL-8, IL-6, and TNF-α measured at the end of RT and the RISRAS score. However, there was no significant correlation between the measured inflammatory mediators and the RTOG score at the end of RT, not even in a subgroup analysis of patients with RTOG grade $\geq$2 ARD (data not shown).

Table 6: Spearman rho correlations between inflammatory mediators, RTOG grading, and RISAS scale

Spearman rho	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1) IL-1β before RT									
2) IL-1β after RT	.160								
3) IL-8 before RT	-.027	.064							
4) IL-8 after RT	-.037	.406**	.610**						
5) IL-6 before RT	-.005	.141	.251*	.213					
6) IL-6 after RT	.028	.229	.421**	.462**	.475**				
7) TNF-α before RT	-.109	.117	.824**	.679**	.232	.522**			
8) TNF-α after RT	-.170	.021	.707**	.682**	.315*	.514**	.822**		
9) RTOG score after RT	-.057	-.202	.044	.029	-.031	-.064	-.019	.071	
10) RISRAS score after RT	-.109	.117	.824**	.679**	.232	.522**	1.000**	.822**	-.019

* Correlation is significant at p<0.05 (two-tailed)

** Correlation is significant at p<0.01 (two-tailed)

IL, interleukin; RISRAS, Radiotherapy-Induced Skin Reaction Assessment Scale; RT, radiotherapy; RTOG, Radiation Therapy Oncology Group; TNF, tumor necrosis factor.

4.5 Discussion

This was the first clinical study investigating the inflammatory profile of the cytokines in acute RD lesions. In overall the levels of IL-1β, IL-8, and IL-6 increased along the progression of RT. No significant change in the level of TNF-α was detected at the end of RT. Furthermore, our results not only demonstrate that RT stimulates the cytokine production, but they also show a positive association between the cytokine (IL-6, IL-8 and TNF-α) concentration after RT and the severity of ARD. Additionally, the levels of IL-8 and TNF-α before RT were positively associated with the RISRAS score.

These findings are in line with previous *in vitro* and *in vivo* studies demonstrating that inflammatory cytokines are involved in the pathogenesis of RD [32, 195-197]. The most commonly described cytokines in relation to acute RD are IL-1β, TNF-α, IL-6, and IL-8 [32].

IL-1β and TNF-α are key mediators of the acute-phase inflammatory reaction. In addition, they are also involved in the development of chronic RT-induced skin reactions as they can stimulate the proliferation of fibroblasts and induce the synthesis of matrix metalloproteases (MMP) and collagen, thereby enhancing tissue fibrosis [198-201]. Further, they can also induce the production of IL-6, an acute inflammatory cytokine, which has both pro- and anti-inflammatory effects. IL-6 can also downregulate the production of IL-1β and TNF-α [202].

Scientific evidence shows that RT can modulate the expression of IL-6 in different human cell types such as fibroblasts, keratinocytes, and epithelial cells [197]. IL-8 differs from the other cytokines, because it works as a chemotactic cytokine that can attract other immune cells to the site of inflammation. The production of IL-8 can be stimulated by RT but also by other cytokines such as IL-1 and TNF-α [203]. All these proteins are activated by the NF-kβ signaling pathway, which is also related to several other cell functions, such as cell viability, migration, and proliferation [32].

Up to now, there were no clinical studies investigating the local cytokine profile of acute RD lesions by the skin tape stripping method. However, there are some clinical trials that used this technique in other inflammatory skin reactions. Studies by Perkins, De Jongh and, Amarbayasgalan et al. investigated the level of IL-8 in different skin conditions such as diaper rash, surfactant exposure, and atopic dermatitis. The average level in of IL-8 ranged from 0.05 to 1 pg/µg TP, which is in line with our findings (0.14-0.34 pg/µg TP)[191-193, 204]. Perkins et al. also studied the concentration of cytokine TNF-α in patients with compromised scalp conditions and found measurable levels between 0.025-0.2pg/µg TP, which is also comparable to our results (0.42- 0.63 pg/µg TP) [194]. None of these available studies investigated the level of neither IL-1β nor IL-6.

To date, only two clinical trials explored the profile of inflammatory mediators in plasma samples of RT patients. A study by Sepah et al. showed that the plasma levels of IL-1β and IL-6 of BC undergoing RT increased [205]. De Sanctis et al. demonstrated that the level of IL-1β, IL-2, IL-6, and TNF-α in plasma samples of BC patients was significantly upregulated four weeks after RT. No significant change in the concentration of IL-8 was detected. In addition, they identified a positive correlation between the measured plasma cytokine levels and the severity of acute RD [206].

Up to now, no clinical trials have studied the biologic modulation of inflammatory mediators by PBMT in the prevention of acute RD. As such, this is the first study that demonstrates that PBMT was able to significantly reduce the increase of IL-1β induced by RT in ARD lesions. These results corroborate our clinical findings that PBMT can significantly reduce the severity of acute RD by diminishing the RT-induced inflammatory reaction [159]. A previous study by Janko et al., investigated the role of the IL-1 pathway in a RD mouse model that was deficient for IL-1. Results demonstrated that especially IL-1β is an important cytokine in the development of RD. Mice lacking this cytokine developed less severe inflammation and pathological skin changes [198]. No significant effects of PBMT on IL-8, IL-6 and TNF-α were found.

As this is the first time that the effect of PBMT on inflammatory profile in RT-induced skin lesions is tested, conformational studies are needed to verify our findings. However, there are numerous studies that demonstrate an anti-inflammatory effect of PBMT in both *in vitro* and *in vivo* conditions by modulating the level of pro- and anti-inflammatory cytokines [123, 207-212]. Several studies on wound healing models showed that PBMT is able to modulate level of different cytokines such as IL-1β, IL-6, TNF- α and enhance the wound healing process by up regulating MMPs and collagen synthesis [123, 213-215].

Clinical studies investigating the effect of PBMT on the cytokine level in acute wounds are scarce due to the invasive nature of most skin sampling techniques. However, recently a study by Ruh et al. evaluated effect of PBMT on pressure ulcer healing in human diabetic patients by analyzing the gene expression of inflammatory mediators IL-6, TNF-α, VEGF, and TGF-β. They collected ulcer border tissue obtained via biopsy from eight patients and analysed the samples by quantitative real-time PCR (qRT-PCR). Results demonstrated that PBMT improved the wound healing process with an increase in VEFG and TGF-β, and a reduction in TNF-α. No significant effects on IL-6 were demonstrated [216].

Study limitations

One of the main limitations of the present study was the sampling technique. Skin tape stripping is an easy and fast method to collect human material, but the measured level of inflammatory mediators remains low (0.02-7 pg/µg total protein). These results are comparable to other studies investigating this sampling technique [191-194, 204, 217]. Although, the measured levels of inflammatory mediators still remain highly variable between and within studies. This is due to a variety factors ranging from the type of tape, experimental conditions, position within the SC, the anatomical sampling location, and the type of skin condition (healthy vs. diseased) [218]. Therefore, comparing our data with other clinical trials that performed this technique is not straightforward. Nevertheless, this sampling method is the most convenient way to collect skin samples in patients with acute RD in comparison with other invasive techniques.

Future perspectives

As this was the first time that human skin tape samples of ARD were analysed on cytokine levels, we cannot compare our results with previous studies. Therefore, further research is warranted to investigate the effect of PBMT the inflammatory profile in human RD samples. Future trials should focus on applying a standardized sampling technique and study a larger patient population.

4.6 Conclusion

This study was the first attempt to analyse the effect of PBMT on the inflammatory profile of acute RD skin lesions in BC patients. Results demonstrated that the skin tape stripping method is a simple, minimally invasive, painless, method to measure the local cytokine levels relevant to RD. The severity of acute RD was positively correlated with the cytokines IL-6, IL-8, and TNF-α. These cytokines could serve as biomarkers to gather more information on treatment efficacy and improve personalised care for RT patients. Most important, this trial demonstrated that PBMT can effectively reduce the severity of ARD. This was supported by the finding that PBMT was able to downregulate the increase of IL-1β, which is correlated to its anti-inflammatory effect. Although, further research is necessary to elucidate the underlying mechanism of PBMT on the inflammatory profile of RT-induced skin reactions.

PART 2

DERMISHEAD trial

PHOTOBIOMODULATION THERAPY AND ACUTE RADIATION DERMATITIS IN HEAD AND NECK CANCER PATIENTS

CHAPTER 5

Photobiomodulation therapy for the prevention of acute radiation dermatitis in head and neck cancer patients (DERMISHEAD trial)

Robijns J, *Censabella S, Claes S, Pannekoeke L, Bussé L, Colson D, Kaminski I, Lodewijckx J, Broux V, Bulens P, Maes A, Noé L, Brosens M, Timmermans A, Lambrichts I, Somers V, Mebis J*

In preparation

5.1 ABSTRACT

Purpose

The purpose of this study was to investigate the effectiveness of photobiomodulation therapy (PBMT) for the prevention of acute radiation dermatitis (ARD) in head and neck cancer (HNC) patients.

Methods

This was a preliminary analysis of a randomised, placebo-controlled trial with 34 HNC patients who underwent radiotherapy (RT) with or without the concomitant chemotherapy (DERMISHEAD trial). Patients were randomised to receive PBM or placebo treatments from the first day of RT (2x/week). The severity of skin reactions was assessed by the criteria of the Radiation Therapy Oncology Group (RTOG) and by the Radiotherapy-Induced Skin Reaction Assessment Scale (RISRAS). Quality of life was evaluated using the Skindex-16 scale.

Results

PBMT was able to significantly reduce the incidence of moist desquamation with 40% at the end of RT. In addition, the patient's quality of life considerably improved during the course of RT in the PBMT group, while it aggravated in the control group.

Conclusions

These preliminary results of the first RCT in HNC patients showed that PBMT is an effective method to prevent the development of severe acute RD. Moreover, it can effectively reduce the impact of acute RD on the patient's daily life.

5.2 INTRODUCTION

In 2015, 2663 new patients were diagnosed with head and neck cancer (HNC) in Belgium [219]. Head and neck cancer comprises a heterogeneous group of tumours arising from the pharynx, larynx, sinuses, salivary glands, and oral cavity. The optimal management of HNC requires a multidisciplinary approach. Radiotherapy (RT) plays an important role in the management of HNC next to surgery, chemotherapy and/or targeted therapy. The most severe side effects associated with RT of the head and neck region are oral mucositis (OM) due to direct damage of the irradiated mucosa and acute skin reactions, also known as acute radiodermatitis (ARD). Almost all HNC patients will develop some degree of ARD during RT, ranging from mild erythema, dry desquamation to eventually moist desquamation [220].

The risk on severe ARD (i.e. moist desquamation) depends mainly on RT parameters (e.g. dose per fraction, total dose, volume of the irradiated area, fractionation regime). Also the combination of chemotherapy or targeted therapy with RT makes the skin cells more susceptible to DNA damage leading to more severe skin reactions. Furthermore, also patient-related risk factors (e.g. genetics, skin type, comorbidities, obesity, nutritional and smoking status) play a role in determining the risk on ARD [221].

Acute RT-induced skin reactions are associated with itchiness, discomfort, burning sensation and pain, in overall affecting the patient's quality of life [17]. In rare cases of extreme ARD, the treatment protocol needs to be delayed or even interrupted, hereby compromising treatment outcome [25].

Concerning the prevention and management of ARD, there is still no general standardised protocol due to the low availability of evidence-based research. There are a wide variety of topical and oral agents but the scientific efficacy is still missing [30]. The most recent guidelines on the prevention and management of ARD) were published in 2013 by the expert panel of the Multinational Association for Supportive Care in Cancer (MASCC) [44]. Therefore, it is still necessary to continuously evaluate new potential prevention options for RD in order the improve the supportive care of head and neck cancer patients [48, 49].

Photobiomodulation therapy (PBMT) implies the application of visible and/or (near)-infrared light produced by laser diodes or light-emitting diodes (LEDs) on tissue to stimulate wound healing, reduce inflammation, and diminish pain [99, 154]. Schindl et al. was the first to investigate the use of PBMT in the management of ARD in breast cancer patients [85]. Our research team was the first to demonstrate in a RCT with 120 breast cancer (BC) patients that PBMT is able to significantly reduce the incidence of moist desquamation and improve the patient's quality of life (TRANSDERMIS trial) [159].

The aim this project was to evaluate the efficacy of PBMT in the prevention of ARD in HNC patients. Secondarily, the patients' quality of life was evaluated.

5.3 MATERIAL AND METHODS

5.3.1 Study design and setting

This was a preliminary analysis of a monocentric, prospective, placebo-controlled RCT[159], to evaluate the effectiveness of PBMT in HNC patients undergoing RT. All patients were treated at the RT department of the Limburg Oncology Centre (Jessa Hospital, Hasselt, Belgium) between January 2016 and May 2018. The study was approved by the ethics committees of the Jessa Hospital and the University of Hasselt (B243201526141) and was conducted according to the Declaration of Helsinki. The study was registered at ClinicalTrials.gov (NCT02738268).

5.3.2 Study population

Patients were eligible for enrolment in the study when they were treated with RT with a minimal dose of 60 Gy alone or in combination with chemotherapy and/or surgery. Exclusion criteria were previous irradiation in the head-neck area, metastatic disease, the use of targeted-therapy, and wound infections in the irradiated area. Additionally, patients with medical, psychological, or social conditions that would interfere with the participation in the study or evaluation of the results were also excluded. All participants had to sign the written informed consent before the start of the study.

5.3.3 Randomisation and blinding

Eligible patients were stratified based on their treatment regime (i.e. RT alone or in combination with chemotherapy). This was followed by a random allocation (1:1) of the patients to the PBMT or control group. Patients were allocated based on a block randomisation process, with a block size of 4 by using a computer-generated random number list prepared by a researcher who was not clinically involved in this trial. Only the laser operator knew the allocation of the patients in the groups.

5.3.4 Interventions

Radiotherapy

The RT plan was prepared using a 3D-planning system (Eclipse™, version 11.0, Varian Medical System, Palo Alto, CA) [222]. All patients received standard RT with the RapidArc® (Varian Medical System, Palo Alto, CA), an advanced technology to deliver intensity modulated radiation (IMRT). The linear accelerator consisted of 2 arcs, delivering photons of 6-15 Mv. All participants also received a simultaneous integrated boost (SIB-IMRT) on the specific tumour region. This boost was delivered by either two-field conformational photon beams (6-15 MV) or a one-field vertical electron beam (6-15 MeV).

Topical skin care treatment

All patients received the institutional standard skin care, which encompassed the application of a topical, hydroactive colloid gel (Flamigel®, Flen Pharma, Kontich, Belgium) on the irradiated zone (3x/day), starting at the first day of RT. In case of a painful skin reaction and/or moist desquamation, a foam, absorbent, self-adhesive silicone dressings (Mepilex®, Mölnlycke Health Care, Gothenburg, Sweden) was used. Additionally, the RT nurses advised the patient to follow the general skin care guidelines (e.g. no tie, no electric shaving, no aftershave, gentle washing with or without mild soap, patting dry with a soft towel instead of rubbing).

PBMT

PBMT was applied from the first until the last day of RT (2x/week, 14 sessions) by a trained operator using the class IV MLS® M6 laser (ASA Srl, Vicenza, Italy), as described previously [159]. This device is commercially available, built in compliance with EC/EU rules, received FDA approval, and is CE certified. It consists of two laser diodes with different wavelengths (808-905 nm), peak powers (1.1-25W), and emission modes (continuous and pulsed). Both diodes work simultaneously and synchronously with coincident propagation axes (average radiant power 3.3 W). The energy density (fluence) was set at 4 J/cm^2 based on earlier recommendations and on our clinical experience [130, 149]. The complete list of PBMT parameters can be found in table 7. During the sham treatments of the control group, the PBM device did not emit light but made the same sound as an active device. All patients, independently of their treatment group, wore safety glasses and eye shields to prevent eye damage and to blind them during the PBM or sham sessions.

Table 7: Photobiomodulation parameters (DERMISHEAD trial)

PBMT parameters			
Device information	Manufacturer	ASA srl	
	Model Identifier	MLS® laser M6	
	Year Produced	2012	
	Number of Emitters	1	
	Emitter Type	IR laser diodes	
	Beam Delivery System	Handpiece	
Irradiation parameters		**Laser diode 1**	**Laser diode 2**
	Center wavelength	808 nm	905 nm
	Spectral bandwidth	±5 nm	±5 nm
	Operating mode	Continuous pulsed wave mode	
	Peak radiant power	1.1 W	25 W
	Average radiant power	3.3 W	
	Maximum frequency (frequency range)		90kHz (1-2000 Hz)
	Pulse on duration Duty cycle		100-ns single pulse width 50 %
	Aperture diameter	2 cm	
	Irradiance at aperture	0.168 W/cm^2	
	Beam divergence at 60%	42.8 mrad	59.2 mrad
	Beam profile	Two laser beams work simultaneously and synchronously with coincident propagation axes	
Treatment parameters	Beam spot size at target area	3.14 cm^2	
	Irradiance at target	0.168 W/cm^2	
	Radiant exposure (fluence)	4 J/cm^2	
	Number of points irradiated	Head and (bilateral) neck region	
	Exposure duration	± 300-600 s	
	Application technique	5 cm above skin with manual device	
	Timing	After the RT session	
	Frequency of treatment sessions	Biweekly from the first until the last day of RT over a period of 7 weeks (14 sessions in total)	

IR, infrared; MLS, Multiwave Locked System; PBMT, photobiomodulation therapy; RD, radiodermatitis; RT, radiotherapy

5.3.5 Outcome measures

Patient data

Patient's personal, disease- and treatment-related characteristics were collected via patient questionnaires and the patient's medical charts in order to out rule possible risk factors.

Skin toxicity grading

Two different grading systems were used to score the severity of the skin reactions. The National Cancer Institute-Common Terminology Criteria for Adverse Events version 4.03 (NCI-CTCAE v4.03) is the most common used grading system for RD in head and neck cancer patients [223]. In addition, the Radiation-Induced Skin Reaction Assessment Scale (RISRAS) was used, which encompasses a researcher ("objective") and a patient ("subjective") score[37, 150]. Two experienced RT nurses evaluated the skin toxicity in a blinded manner.

Quality of life

The patient's quality of life of the patients was assessed by using the Skindex-16 [151]. This is a validated, 16-item self-assessment questionnaire that measures to what extent the patients' life is affected by their skin condition. Each item on the scale is rated from 0 (Never Bothered) to 6 (Always Bothered). The Skindex-16 is divided in three subscales: symptoms, emotions and functioning. The total score is the average of the three subscales scores (range: 0-100) and a higher score is correlated with a lower quality of life.

Measurement collection schedule

All the previously described measurements were collected on three time points: at the first day of RT, at a RT dose of 40 Gy, and at the last day of RT (60-70 Gy).

5.3.6 Statistical analysis

Differences in patient- and therapy-related characteristics between both groups were analysed by means of chi-square tests ($\chi 2$), Fisher's exact tests, Student t-tests, or Mann-Whitney U-tests, as appropriate. RTOG scores were analysed by means of $\chi 2$ or Fisher's exact tests, as appropriate. Longitudinal analysis of the RISRAS and Skindex-16 scores were performed by mixed analyses of variance (ANOVAs) with time (between the RT dose of 40Gy and 60-70 Gy) as within subject factor and group (control vs. PBMT group) as between-subject factor. The level of statistical significance for all analyses was set assuming a significance level of 5% ($P<0.05$, two-tailed). SPSS 24.0 (IBM, Chicago, IL) was used for all analyses.

5.4 RESULTS

5.4.1 Patient data

A total of 165 HNC patients were assessed on eligibility between January 2016 April 2018. Eventually 38 patients were enrolled in the study and randomised into the PBMT or control group. In total 4 patients were lost because they withdrew their informed consent. As such, the preliminary analysis was performed on 34 patients, with 17 patients in each treatment group (Figure 17). Concerning the demographical and treatment-related data, there were no significant differences between the two groups (Table 8-9).

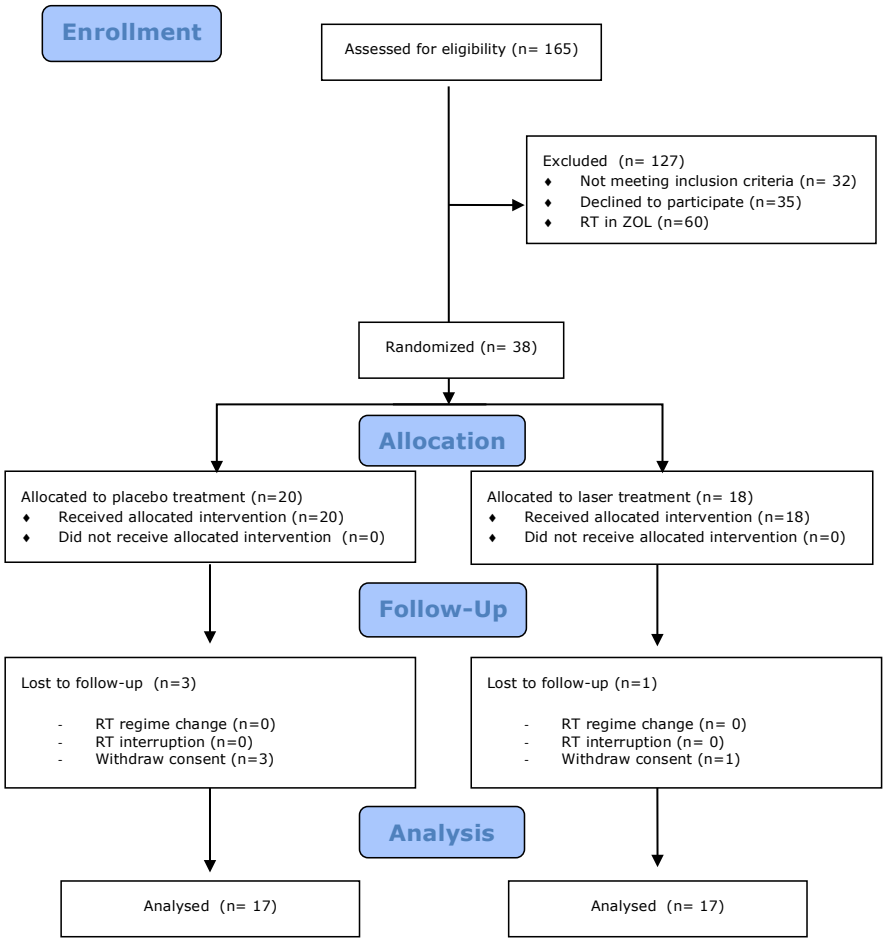

Figure 17: CONSORT flow chart of the DERMISHEAD trial
RT, radiotherapy, ZOL, Ziekenhuis Oost-Limburg

Table 8. Baseline demographic patient characteristics (DERMISHEAD trial)

	Control group (n=17)		**PBMT group** (n=17)		
	Mean ± SD		Mean ± SD		p[a]
Age (years)	62.47 (12.60)		64.65 (9.82)		0.77
Body Mass Index (BMI)	25.41 (4.30)		26.14 (6.50)		0.78
	n	%	n	%	P[b]
Sex					0.18
Male	16	94.1	12	70.6	
Female	1	5.9	5	29.4	
WHO skin type classification[c]					0.29
Melano-compromised	0	0	2	12.5	
Melano-competent	15	88.2	13	81.2	
Melano-protected	2	11.8	1	6.3	
Smoking status					0.10
Never smoked	0	0	4	23.5	
Former smoker	15	88.2	11	64.7	
Current smoker	2	11.8	2	11.8	
Pack years					0.18
0	0	0	4	23.5	
<30	3	17.6	1	6.0	
30-39 years	5	29.4	6	35.3	
40-49 years	5	29.4	3	17.6	
> 50 years	4	23.5	2	11.8	
Missing	0	0	1	5.8	
Number of cigarettes					0.14
0	0	0	4	23.5	
<10	0	0	2	11.8	
10-19	6	35.2	3	17.6	
20-29	7	41.2	5	29.4	
30-39	1	5.9	0	0	
>40	1	5.9	2	11.8	
Missing	2	11.8	1	5.9	
Alcohol consumption (drinks/week)					0.81
0-1	3	17.6	5	29.4	
1-3	3	17.6	3	17.6	
3-10	5	29.4	5	29.4	
10-20	5	29.4	4	23.6	
>20	1	6.0	0	0	

BMI, Body Mass Index; PBMT, photobiomodulation therapy; SD, standard deviation; WHO, World Health Organisation;
[a] Student t-test or Mann Whitney u-test, as appropriate (two-tailed)
[b] Chi-square tests, or Fisher's exact tests, as appropriate (two-tailed).
[c] WHO skin type classification is based on Fitzpatrick's phototype scale: melano- compromised (Fitzpatrick's skin type I-II), melano-competent (skin type III-IV), and melano- protected (skin type V-VI).

Table 9. Disease and therapy- related characteristics (DERMISHEAD trial)

	Control group (n=17)		**PBMT group** (n=17)		
Characteristic	n	%	n	%	p[a]
Disease-related					
Tumour site					0.47
Oropharynx (HPV+)	4 (1)	23.5	7 (3)	41.2	
Larynx	8	47.1	3	17.6	
Oral cavity	3	17.6	4	23.5	
Salivary gland	1	5.9	2	11.8	
CUP	1	5.9	1	5.9	
T-stage					0.57
x	1	5.9	0	0	
1	5	29.4	2	11.8	
2	7	41.2	9	52.9	
3	2	11.8	3	17.6	
4	2	11.8	3	11.8	
N-stage					0.52
0	9	52.9	8	41.2	
1	2	17.6	4	35.3	
2	4	23.5	4	23.5	
3	1	5.9	0	0	
Treatment-related					
Pre-treatment surgical dissection	10	58.8	9	52.9	>.99
Concomitant chemotherapy	4	23.5	4	23.5	>.99
Maximum RT dose					0.4
60	7	41.2	4	23.5	
66	1	5.9	3	17.6	
70	9	52.9	10	58.8	
Neck lymph nodes irradiated	12	75	16	94.1	0.18

HPV, Human Papilloma Virus; PBMT, photobiomodulation therapy; RT, radiotherapy; SD, standard deviation; WHO, World Health Organisation

[a] Chi-square tests, or Fisher's exact tests, as appropriate (two-tailed).

5.4.2 Skin reaction evaluation

At the RT dose of 40 Gy there was no significant difference in skin toxicity between the control and PBMT group (p=0.72). As demonstrated in figure 18, most of the patients developed a grade 1 skin reaction (70.6% vs. 82.4%, in the control and PBMT group, resp.). Towards the end of RT, the number of severe skin reactions (RTOG grade 2-3) increased significantly (p= 0.046) in the control group. On the contrary, in the PBMT group the development of ARD remained stable (p=0.30). As such, there was a significant difference in skin toxicity between the two groups at the end of RT, with a higher percentage of patients presenting moist desquamation in the control group (70.6% vs. 29.4%, in the control and PBMT group, resp., p=0.038).

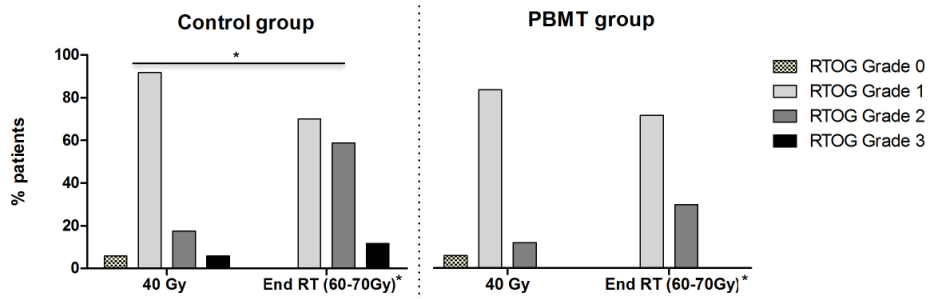

Figure 18: Severity of acute radiodermatitis expressed in RTOG grades (DERMISHEAD trial)
RTOG grades for the control and PBMT group at a RT dose of 40 Gy and at the end of RT (60-70 Gy). *Significant difference within the control group between the two time points and between the two groups at the end of RT ($p<0.05$; χ^2 or Fisher's exact tests, two-tailed). *Gy, Gray; PBMT, photobiomodulation therapy; RT, radiotherapy; RTOG: Radiation Therapy Oncology Group (Grade 0: no change; grade 1: follicular, dull, or faint erythema, dry desquamation; grade 2: tender or bright erythema, patchy moist desquamation; grade 3: confluent moist desquamation other than skin folds).*

The 2x2 mixed ANOVAs on all the RISRAS scores demonstrated a significant main time effect (Ps<0.05). However, there was no significant main group, nor a group by time interaction for all the scores (Ps>0.1). Figure 19, demonstrates that the objective and total RISRAS score increased in both groups towards the end of RT. On the other hand, the subjective score remained stable in the PBMT group, while it increased in the control group.

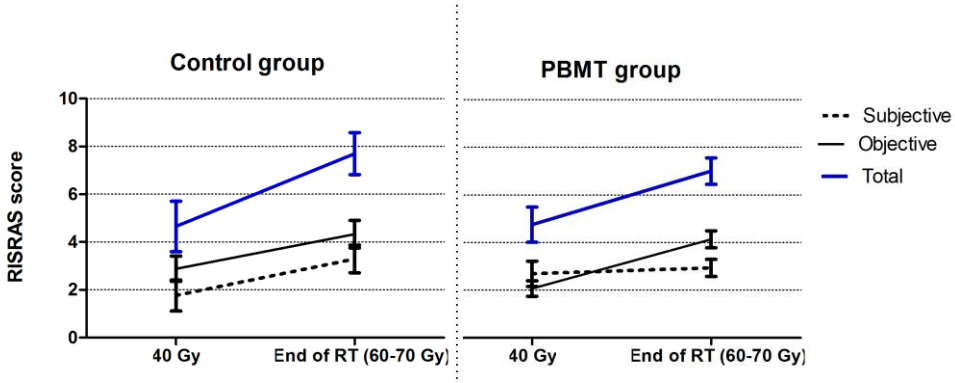

Figure 19: Severity of acute radiodermatitis expressed in RISRAS scores (DERMISHEAD trial)
Average subjective, objective and total RISRAS scores of the control and PBMT group at a RT dose of 40 Gy and the end of RT (60-70 Gy). Data are shown as means (± SEM) and higher scores indicate a more severe skin reaction. *Gy, Gray; PBMT, photobiomodulation therapy; RISRAS: Radiotherapy-Induced Skin Reaction Assessment Scale; RT, Radiotherapy; SEM, standard error of measurement.*

5.4.3 Quality of life

The analysis of Skindex-16 scores by 2X2 mixed ANOVAs demonstrated no significant main time and group effects (Ps>0.2). Although, there was a significant group by time interaction for the symptoms, emotions and total Skindex-16 score (Ps<0.05). As depicted in figure 20, all the Skindex-16 scores increased in the control group, whereas they decreased in the PBMT group towards the end of RT.

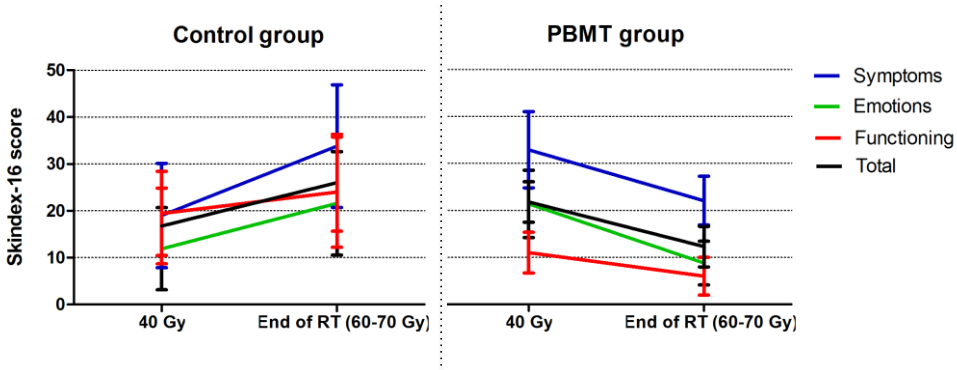

Figure 20: Quality of life scores (DERMISHEAD trial)
Symptoms, Emotions and Functional subscale scores and total scores of the control and PBMT group at a RT dose of 40 Gy and at the end of RT (60-70 Gy). Data are shown as means (± SEM) and higher scores indicate a diminished patients' quality of life. *Gy, Gray; PBMT, photobiomodulation therapy; RT, Radiotherapy; SEM, standard error of measurement.*

5.5 DISCUSSION

After the successful results of the TRANSDERMIS trial, in which PBMT was tested in BC undergoing RT, we set up the current DERMISHEAD trial to investigate if this new treatment strategy could be as effective in HNC patients. To our knowledge this is the first clinical trial that demonstrates that PBMT can effectively reduce the severity of acute RD in HNC patients. More specifically, the incidence of moist desquamation was significantly diminished with 40% by PBMT at the end of RT. The overall RISRAS score increased in both groups, though no significant group differences were detected. This might be due to current low sample size. However, the quality of life of patients that underwent PBMT significantly improved during the course of RT, indicated by a decrease in all the Skindex-16 scores.

To date, no other clinical trials investigated the use of PBMT in HNC patients. In overall the number of studies on PBMT and acute RD are limited with contrasting results [128, 129, 152]. Results of the current study are comparable to those of the earlier described TRANSDERMIS trial, as both studies demonstrate a lower incidence of moist desquamation after PBMT. An interesting difference between the two trials is that the percentage decrease in severe acute RD was higher in the DERMISHEAD trial in comparison with the TRANSDERMIS trial (40% vs. 23 %, resp.). This difference can be rationalised by the fact that in total more HNC patients developed moist desquamation than BC patients (50% vs. 18%). A possible explanation for this finding can be related to the difference in treatment parameters between the two trials.

Firstly, HNC patients received in overall a higher median total RT dose (70 vs. 66 Gy, resp.). Furthermore, about 24% of the HNC patients received concomitant CT, which is an important risk factor for acute RD [224]. Also life style factors, such as smoking and drinking alcohol, might play a role in the development of acute RD. Our results demonstrate that the percentage of patients consuming more than three units alcohol per week was considerably higher in the DERMISHEAD than in the TRANSDERMIS trial (72 vs. 15 %, resp.). The same accounts for the number of patients who were former or are active smokers (77 vs. 33%, resp.).

Other clinical trials investigating the incidence of moist desquamation in HNC patients even demonstrated higher percentages of moist desquamation [225]. For example, a recent study by Iacovelli et al. showed that 78% of the HNC patients presented grade 2 or higher RD after a 7 week IMRT period with a median dose of 70 Gy. All patients received Xonrid®, a topical water-based gel as pre-emptive treatment for RD [166]. In the trial by Tao et al., 73 % of the HNC patients developed moist desquamation after RT (total dose 70 Gy) in combination with Cetuximab. Skin care consisted of a regenerating agent, which is an alternative wound healing approach using innovative-engineered biopolymers [226]. These contrasting numbers on severe acute RD are due to differences in the treatment protocol (e.g. total RT dose, use of concomitant therapies, volume of treated area, etc.) and the standard skin care used [17].

Study limitations

Currently, the main limitation of this study is the sample size. Based on preliminary data a total of 60 patients is needed to detect significant differences between the two groups. Based on the patient flow chart approximately 50% of the eligible patients received RT at Ziekenhuis Oost-Limburg (ZOL), Genk-Belgium. Therefore, we decided to set up a multicentre trial together with ZOL to increase the patient population. Currently, we are still recruiting patients in order to achieve the estimated sample size.

5.6 CONCLUSION

A preliminary data analysis of the DERMISHEAD trial on 34 HNC patients proved that PBMT significantly reduces the severity of acute RD. Thereby; it improves the patients' quality of life during their RT course. A future multicentre trial with ZOL, Genk is necessary to achieve the estimated sample size.

CHAPTER 6

General discussion and future perspectives

6.1 GENERAL DISCUSSION

In Belgium, the estimated number of new invasive tumour diagnoses will rise from 67,087 to 79,140, from 2015 until 2025 [227]. RT will remain an important corner stone in the multidisciplinary approach to treat cancer. In 2015, the percentage of new cancer patients that require at least one course of RT in Belgium was estimated to be 53%, also referred to as the Optimal Utilization Proportion (OUP) [228]. This implies that a total of 35,556 cancer patients needed to undergo RT as part of their cancer treatment. A recent study by Lievens et al., demonstrated that the Actual Utilization Proportion (AUP) in Belgium between 2009 and 2010, was 37%, which was lower than the determined OUP [95]. However, the AUP varies among the different cancer types (77 and 72% for BC and HNC, resp.), with BC and HNC approaching the OUP (86 and 83 %, resp.) [229].

One of the most disturbing and quality of life-impairing side effects of RT is ARD, occurring in about 95% of the cancer patients undergoing RT. The most severe forms of ARD are seen in patients with breast, head and neck, rectal, or gynaecological cancer, because in these cases the skin is one of the main targets. Up to now, there are a large variety of preventive and therapeutic options for ARD. However, there is still no widely accepted, standardised protocol for this indication, due to the lacking scientific evidence. Therefore each RT unit develops their own treatment protocol, based on their clinical experiences [156].

Since the introduction of PBMT in 1967 by Andre Mester, a wide plethora of indications, characterized by tissue damage, inflammation and pain, have been investigated. In oncology the use of PBMT is mainly known for the prevention and management of OM, which has been taken up in the guidelines of the MASCC/ISOO, ESTRO and recently by the National Institute for Health and Care Excellence (NICE) [154, 230]. Based on the success story of PBMT and OM, our research group started investigating the application of PBMT for the management of ARD. Results of our DERMIS trial, a prospective pilot trial in BC patients, demonstrated that PBMT was able to prevent the aggravation of ARD once the first skin reactions already developed [130].

The aim of this project was to evaluate the effectiveness of PBMT in the prevention and management of ARD in BC and HNC patients.

In overall, both the TRANSDERMIS and DERMISHEAD trial proved that when PBMT was applied twice weekly from the first day of RT in combination with the institutional skin care protocol, the incidence of severe ARD, more specifically moist desquamation, was significantly reduced (Chapter 2 and 5). This finding was supported by an improved quality of life of patients undergoing PBMT. These were the two first RCTs in BC and HNC patients that were able to demonstrate these beneficial effects of laser diode based PBMT in ARD.

Grade 2-3 skin reactions are very sensitive and painful for the patient. Further, they typically demonstrate an increased risk on bleeding and bacterial infections. This type of skin toxicity requires a more extensive skin care protocol performed by a RT or home nurse (e.g. cleaning the wounds, application of topical agents and/or wound dressings) [44]. By applying PBMT, the comfort of the patients during and after RT will improve, which implies a better quality of life. In addition the risk on open bacterial infections and bleeding will drop. As such, the number of RT interruptions due to ARD will decrease, leading to eventually an overall better treatment outcome. Furthermore, the necessary skin care products and workload of the nurses to treat the skin reactions will be diminished.

Another innovative part of the TRANSDERMIS trial, is that is the first RCT that was able to demonstrate the effectiveness of PBMT in management ARD by an objective approach (Chapter 3). Evaluating the skin toxicity by the RTOG criteria and RISRAS scoring scale, might be biased by interobserver variation, therefore we used biophysical skin measurements to objectively assess ARD.

Our results demonstrate that PBMT was able to reduce the increase in pigmentation during the course of RT. The skin erythema level was stabilised by the PBMT-induced anti-inflammatory effect [140]. Further, patients that underwent PBMT showed less PIH, explained by the suppressing effect of PBMT on the melanin production [175]. Skin pigmentation changes extensively affect the cosmetic outcome, leading to a diminished quality of life. As such, the use of PBMT can improve the patients' self-esteem and body-image during and after RT [231, 232].

ARD is characterized by a poor skin barrier function, which is a correlated with an increase in the TEWL and decrease in skin hydration [41, 42, 165]. In the TRANSDERMIS trial PBMT seemed to stimulate the epidermal thickening effect of the skin, resulting in the hyperproliferation of the epidermis and thickening of the SC. This was reflected by a significant decrease in the TEWL index resulting into an improved skin barrier function. This result supports the earlier described finding that patients undergoing PBMT develop less moist desquamation. Further, a better skin barrier function is correlated with an improved protection against environmental insults, which means that patients undergoing PBMT will have a lower risk on possible bacterial wound infections [12].

The significantly reduced incidence of moist desquamation and thereby the improved skin barrier function demonstrated by a decrease in TEWL, could be explained by the effect of PBMT on skin stem cells. Stem cells have a high self-renewal potential and can differentiate into a variety of cell types [233]. Countless studies have demonstrated that different niches of stem cells can be activated by PBMT, reflecting into an increase in cell migration, differentiation, proliferation, viability, and protein expression [97, 234]. As such also skin stem cells can be stimulated by PBMT as demonstrated by Liao et al. They showed that the application of a Helium-Neon laser (632.8 nm, 25mW, 2J/cm^2) on human epidermal stem cells *in vitro* resulted in a significant increase in proliferation and migration [235]. Further, Khan et al. demonstrated that PBMT (810 nm) stimulated the expansion of epithelial colony forming units from the skin and mucosa, in a dose-dependent manner (1-3 J/cm^2) [236].

Moreover, in vivo studies using stem cell transplantation treatments showed that PBMT is able to enhance the wound healing process by stimulating wound closure and the skin regeneration [97, 237]. Park et al. demonstrated that the application of PBMT to ADSCs in culture prior to transplantation, enhanced vascularization and tissue regeneration in the wound bed of a mouse model. In a study by Kim et al. PBMT was applied directly on the wound bed after ADSC transplantation in a mouse model. They reported an improvement in survival of the ADSCs and an increase in the secretion of growth factors in the wound bed [238].

Although, PBMT did not seem to affect the skin hydration level, as demonstrated by a decrease in moisture level in both treatment groups. As such, hydrating emollients still need to be enclosed in the standard skin care protocol of patients undergoing RT to keep the irradiated skin fully hydrated [134].

In the TRANSDERMIS trial a first attempt was made to evaluate the skin cytokine profile in acute RD lesions (Chapter 4). Results demonstrated the overall production of inflammatory mediators in the skin lesions increased towards the end of RT. However, no other clinical trials investigated this in humans, evidence from *in vitro* and *in vivo* studies support these findings [32]. Interestingly, the severity of acute RD was positively correlated with the increase in production of IL-6, IL-8 and TNF-α. As such, these cytokines could serve as biomarkers of ARD and might be useful for investigating the course of the skin reactions and develop a personalised management plan.

The most important finding of this translational research is that our results suggest that PBMT could effectively reduce the production of IL-1β. This cytokine is the most important cytokine in the development of both acute and chronic RD. As previously demonstrated in an *in vivo* model, an IL-1 deficient mouse model developed less severe inflammation and pathological skin changes during RT [32, 198, 199]. This finding subscribes our earlier described clinical results in chapter 2 and 3 suggesting that PBMT is able to diminish the RT-induced inflammatory reaction. As this was the first study demonstrating these findings in human skin samples of BC undergoing RT, future studies are needed to confirm our results in a larger and broader patient population.

6.2 LIMITATIONS

Our clinical trials were not without limitations, which will be discussed point by point below.

Sample size

For the TRANSDERMIS trial we were able to include the estimated patient population of 120 BC patients. On the other hand, for the DERMISHEAD trial the anticipated sample size of 60 was not reached by the end of April 2018, as the patient count for the preliminary analysis was 34. The low recruitment rate was addressed to several reasons: radiotherapy at another RT department (47%), patient refusals (28%), or patients not meeting inclusion criteria (25%). Up to half of the patients were lost due to RT at Ziekenhuis Oost-Limburg (ZOL), Genk, Belgium. Therefore, we decided to set up a multicentre trial in collaboration with ZOL in order to increase the sample size.

Despite this small sample size the preliminary results of the trial, were comparable to those of the TRANSDERMIS trial, demonstrating a significant beneficial effect of PBMT in the prevention of ARD. Currently, the DERMISHEAD trial is still actively recruiting patients. As such, we expect that the final results will even be stronger when we reach the anticipated patient sample.

Blinding

Regarding allocation concealment, in our clinical trials we were only able to blind the patient and the outcome assessor. Due to the fact that the laser device we were using, produced a visible red light when it was active, we were not able to blind the laser operator during the placebo and PBMT sessions. However, the risk on bias in our trials was reduced because the laser operator was not actively involved in the outcome assessments. In order to implement double-blinded RCTs and reduce the risk on bias, an adjusted laser device is needed. The new device needs to have following specifications: an invisible laser beam, a disguised laser diode glow, and a sealed randomisation-coding system [239]. Future clinical trials should use certain devices in order to improve the clinical validity of PBMT.

Topical skin care

A wide range of topical skin care products is available on the market to prevent and manage RD. However, the effectiveness of a lot of these products remains still unclear due to a limited amount of scientific evidence [44]. Therefore, many RT centres develop their own protocol.

The basis of our institutional skin care protocol consists of the daily application (3x/day) of a topical agent, a hydroactive colloid gel (Flamigel®, Flen Pharma NV, Kontich, Belgium), on the irradiated zone starting at the first day of RT and continuing during the full course of RT. This topical skin care product was chosen above the oil-in-water emulsion containing 5% dexpanthenol (Bepanthol® Cream, Bayer AG, Leverkusen, Germany) based on evidence of two of our own clinical trials. Censabella et al. demonstrated that the application of the hydroactive colloid gel (n=202) delayed the onset and reduced the incidence of moist desquamation in breast cancer patients in comparison with dexpanthenol (n=131) [180, 240]. However, depending on the severity grade of ARD and the patient symptoms the skin care protocol is adjusted with other topical products and/or wound dressings to reduce pain and improve the patient's comfort during and after RT. Table 10 shows the skin care protocol developed in our institute in collaboration with the "Vereniging Verpleegkundigen Radiotherapie en Oncologie" (VVRO) and the Woundcare Consultant Society (WCS) [241].

Table 10: Institutional standard skin care protocol during and after radiotherapy

Basic preventive measures	RTOG grade 1 -2 Erythema - dry desquamation	RTOG grade 2 - 3 Moist desquamation	After radiotherapy

• Mild, pH-**neutral soap** (& shampoo in case of cranial irradiation)
• **Pat dry** (instead of rubbing), soft towel
• **Avoid friction from clothing**, tight fitted or synthetic cloths (bra…)
• Only use perfume and **alcohol-free skin care products**
• Use an **electric razor** instead of wet shaving
• **Tape is not recommended**, since tape removal can peel of the sensitive skin in the irradiated area.
• **No swimming**
• **No sun bathing**
	• **Moisturizing cream:**
	  • Hydroactive colloid gel (Flamigel®) : 3x/day (basic)
	  • Cicaplast Baume B5 (in case of dryness or itchiness)
	• **Protective dressing**
	  • Mepilex® / Mepilex® Lite
	  • Can be used in combination with moisturizing cream (application time between treatments at least 30 min.)
	• **Inflammatory skin reaction** (pain, burning, itchiness)
	  • Topical steroids: Hydrocortisone 1%
		• **Clean the wound**
		  • Lukewarm water
		  • Wound irrigation solution (Prontosan®/Flamirins®)
		• **Alginogel with antibacterial enzymes** (Flaminal® Hydro)
		• **Absorbing dressing**:
		  • Mepilex®
		• **Pain medication** (if needed)
		• **Bacterial culture** (in case of wound infection)
			• Continue skin treatment for 2 weeks (based on the degree of skin reaction)
			• Contact radiotherapy department if skin reactions aggravate
			• After wound closure, use a moisturizer 3x/day
			• No sun bathing during 3 months

Adapted with permission from Stefan Claes and the Vereniging Verpleegkundigen Radiotherapie en Oncologie (2016) [241]
RTOG, Radiation Therapy Oncology Group

As such, also the use of topical steroids, which was the only strongly recommended topical agent by the MASCC skin toxicity study group, is used at our institute in case of an inflammatory reaction characterized by itchiness, pain, and oedema [44]. The MASCC panel based their recommendation on five clinical trials of which the study by Miller et al. was the most convincing one. This was a phase III double blind, randomised trial with 176 patients. Patients were randomly divided in two study groups; one group applied 0.01% mometasone furoate (MMF), while the other group applied a placebo cream on a daily basis. Based on the CTCAE scoring there was no significant difference between the two study groups. Although, MMF significantly diminished ARD related symptoms such as pruritus, burning, and, redness based on the STAT [242].

PBMT parameters

The correct application of PBMT parameters is crucial to achieve the wanted effect on the target tissue. Therefore, future studies should include light measurements before, during and after the trial to ensure that the intended parameters are applied. As such, spectrometers could be used to analyse spectral characteristics (i.e. light intensity, polarization) of laser light at a certain wavelength. Further, an optical power meter can be used to measure the power (W) and irradiance (W/cm^2) based on the size of the sensor or on beam area values, but they do not provide spectral information. A more reliable method to measure the spatial distribution of light and irradiance, are thermopiles or beam profilers [100]. Eventually, precise measurements and reporting of PBMT parameters is essential to fully understand the potential beneficial biological mechanisms and clinical effect of PBMT.

PBMT devices

PBMT can be applied by using a wide array of devices within the red and NIR wavelength range of 600–1000 nm. In our study setting the Multi-wave Locked system (MLS®) technology was chosen due to its advantage characteristics in comparison with conventional laser diodes. It makes use of two wavelengths in the NIR range, which have a larger penetration depth into skin than red light (2-3 mm vs. 1-2 mm, for NIR and red light, resp.). NIR light penetrates the skin up to the basal layer of the epidermis where the interfollicular stem cells reside. As such, it can stimulate the proliferation and differentiation of these stem cells to promote wound healing [97].

Furthermore, the MLS® technology combines and synchronizes two different emission modes, a continuous and a pulsed one. The continuous mode has an anti-inflammatory and anti-edemic effect, while the pulsed mode has an analgesic effect. The synchronization of the two different emission modes makes the energy transfer towards the target cells more efficient in comparison with a single emission mode. As such the anti-inflammatory and analgesic effect of the MLS® technology reinforce each other [243].

Momanu investigated the difference in anti-inflammatory and analgesic effects between a conventional laser diode (830 nm, 100mW) and MLS® technology in patients with inflamed serous bursae (n=40). Results demonstrated that pain score and thermal reaction was significantly lower in the patients treated with MLS® technology (n=20) in comparison with a classical laser diode (n=20) after 10 sessions [243].

In the past, the most PBM sessions were performed using laser diodes. Scientists thought that the specific characteristics of laser light, such as monochromaticity and coherence, were essential to trigger the PBM effects. The negative aspect to laser diode based PBMT is that these devices are quite expensive and can only be used by trained clinicians [97]. There is increasing evidence that also other light sources can be used in PBMT. As such, also noncoherent light sources such as LEDs are being used in different clinical trials and procedures. These devices are much cheaper and easier to use, even by the patient himself [244].

A recent improvement in LED based devices is the development of organic or quantum dot light emitting devices (OLEDs or QLEDS), which are self-emissive devices. They can be introduced into bandages or clothing. Thereby they offer a variety of advantages including emitting light from a large, flexible surface and easy fit into the 3D contouring of the human body. However, these devices still have to tackle certain issues such as hygiene, handling with moist wounds, minimizing toxicity, and providing sufficient output power [245-247].

Although, there still rests the remaining question if LED based PBMT is as effective as laser diodes. Therefore, future clinical trials should be performed to investigate the difference between laser and LED-based PBMT [97].

Preconditioning of the skin

Recent literature suggests that PBMT can be used to precondition the skin before the true insult, such as UV-damage or in this case even RT. Preconditioning implies that the application of visible and NIR light to healthy skin cells can prepare them for future damage [97, 248]. A clinical trial by Barolet et al. demonstrated that the application of PBMT before UV exposure resulted in a significant protection against UV-B induced erythema [170]. Another study of the same research group demonstrated that PBMT could also reduce the degree of PIH when applied 7 days before a traumatic insults such as CO_2 laser. The underlying mechanism of this photoprevention effect is still unclear [249]. However, *in vitro* studies propose that this effect can be explained by the p53-regulated inhibition of UV-B induced apoptosis and pigment metabolism by pre-PBMT exposure [250]. Precondition patients with PBMT before the actual start of the RT sessions, may represent a new method to prevent the distressing and painful RT-induced skin reactions.

Follow up period

As previously described, acute RT-induced skin reactions resolve two to three weeks after the last RT session. Even in some patients chronic RD develops after a period of 6 months up to a year. However, in our trials we were not able to evaluate all the patient's skin reactions during a follow-up period. As such we were not able to investigate if the skin healed faster and if patients developed less chronic skin reactions after PBMT. In future clinical trials, an extended follow-up period should be included.

Skin tape stripping and analysis

The skin sampling technique is a rather new method and the number of clinical studies is limited [191-194, 204, 217, 251, 252]. To date, it had never been employed In ARD patients.

The measured cytokine concentrations are highly variable within and between patients. This variety is due to various factors related to the skin tape stripping technique (e.g. type of tape, anatomical location, pressure used, skin condition) and the handling of the samples (freezing parameters, freeze-thaw cycles, extraction method). Therefore a correct and standardized tape stripping method is necessary to achieve reliable results [218].

The use of the multiplex analysis was much more sensitive and allowed us to detect a broad range of inflammatory mediators in a single skin tape sample in comparison with the standard analyzing technique such as the enzyme-linked immunosorbent assay (ELISA) [251].

PBMT and cancer

The question that rises by many clinicians is if the use of PBMT is safe in cancer patients, due to its stimulatory effects on the proliferation and differentiation of on target cells [253]. *In vitro* studies investigating the effect of PBMT on various cancer cell lines show contrasting results [254-257]. *In vivo* animal studies demonstrating that PBMT can stimulate tumour growth are very rare. For example, in a study with a hamster cheek pouch model, squamous cell carcinomas (SCC) were chemically induced by dimethylbenzanthracene (DMBA). PBMT (660 nm, 424 mW/cm^2, 56.4 J/cm^2) was applied on the tumour area every other day for four weeks. Results demonstrated that the severity of the SCC worsened, resulting in a poorer progression [258].

Further, a study by Myakishev-Rempel et al. investigated the effect of PBMT on a standard nonmelanoma mouse skin cancer model. They applied PBMT (670 nm, 8 mW/cm^2, 5 J/cm$^{2)}$ on the full body twice a day for 37 consecutive days. No significant changes in tumour growth were detected during the daily measurements [259].

Another study with a melanoma mouse model compared the effect of a low (660 nm, 2500 mW/cm^2, 105 J/cm^2) and a high PBMT dose (660 nm, 2500 mW/cm^2, 1050 J/cm^2). The mice were treated once a day for three days. Low dose PBMT did not influence the tumour size, while the high dose did significantly increase the tumour volume, the number of blood vessels, and cell abnormalities [260].

A recent study by Barasch et al., investigated the effect of PBMT in an orthotopic mouse model with a human SCC of the oral cavity. Animals were divided into four groups: control, RT only, PBMT only or PBMT + RT. They compared the effects of different PBMT (650 nm vs. 650 + 880 nm, 3 vs. 6 J/cm^2) and RT parameters (5 daily RT doses of 4 Gy vs. 1 RT dose of 15 Gy) on the tumour behaviour. There were no significant differences between the RT only and the RT + PBMT group concerning tumour progression and overall survival [261]. Finally, Ottaviani et al. analysed the behaviour of cancer cells both on *in vitro* and *in vivo* level after laser therapy. Three different laser protocols were applied once a day for four consecutive days: (1) 660 nm, 50 mW/cm^2, 3 J/cm^2; (2) 800 nm, 200 mW/cm^2, 6 J/cm^2; (3) 970 nm, 200 mW/cm^2, 6 J/cm^2. PBMT did significantly increase the cell metabolism and proliferation of the mouse B16F10 melanoma cells *in vitro*. On the contrary, the application of PBMT in xenograft melanoma and orthotopic oral carcinogenesis mouse models resulted in a reduced tumour growth and invasiveness [262].

Moreover, recent human clinical trials demonstrate the safety of PBMT in cancer patients. A RCT by Antunes et al. investigated the overall, disease-free and progression-free survival of HNC patients undergoing concurrent chemoradiation therapy and who were treated with PBMT (660 nm, 100mW, 4 J/cm^2) for the prevention of OM. Patients undergoing PBMT had a statistically significant better complete response to the treatment and demonstrated an increase in progression-free and overall survival after a median follow-up of 41.3 months (range 0.7–101.9) [263]. Further, a retrospective analysis on 152 advanced oral SCC patients examined the outcome of cancer therapy and the incidence of tumor recurrence after PBMT (660 nm, 40 mW, 10 J/cm^2) for the prevention of OM. Results demonstrated that prophylactic PBMT did not influence treatment outcome of the primary cancer, recurrence or new primary tumors, or survival in advanced OSCC patients [264].

Together, results of the studies addressed above indicate that effect of PBMT on cancer cell cultures is not representative of an *in vivo* tumour setting. Further, the carcinogenic effect of PBMT mainly depends of the parameters applied. Current literature suggests that PBMT using a low fluence (1–6 J/cm^2) in the red of NIR light spectrum is safe and effective for application in cancer patients [99]. Additionally, some studies even suggest that PBMT can be used to (in)directly attack cancer by three possible mechanisms: direct killing of cancer cells by high dose PBMT [265], selectively destroying cancer cells based on metabolic differences [266], and stimulating anti-tumour immunity [262]. Although, we still have to perform large clinical trials in which different PBMT parameters are used in a wide variety cancer patients with a long follow up period of at least five years to conclude that PBMT does not negatively affect cancer progression and overall survival.

6.3 FUTURE PERSPECTIVES

To address the limitations of our clinical trials and PBMT research on ARD in general, future research is needed.

As the available clinical trials on ARD and PBMT are using a wide variety of parameters, the most optimal irradiation (wavelength, fluence, irradiance) and treatment parameters (number of sessions, timing of the sessions) need to be determined. Together with the International working group on "Light in Oncology" (iGloB) we are striving to develop guidelines with a standardized PBMT protocol for ARD. Once these guidelines are established, they need to be tested in large international multicentric trials with different types of cancer patients undergoing RT. Besides BC and HNC patients, patients undergoing RT for gynecological or intestinal cancer are also prone to develop skin toxicity in the genital and perianal area and they may also benefit from PBMT [267].

In order to assure that the exact PBMT parameters will be applied, light measurements should be performed on a regular basis before, during and after a clinical trial. Moreover, the effect of preconditioning patients with PBMT before RT should be investigated. Future clinical trials should also include a longer follow-up period of at least 5 years to fully exclude potential cancer stimulating effects of PBMT. Another remaining question is to define the true clinical difference between laser diode and LED-based PBMT.

Furthermore, in the field of PBMT and oncology, there are still a lot of topics that need to be investigated more in depth. As such, our research group together with the iGloB strives to broaden the application of PBMT in the supportive care of cancer patients. Current scientific evidence suggests that PBMT besides OM and ARD is a promising option for the management of a wide variety of cancer therapy-related side effects: ranging from lymphoedema, peripheral neuropathy, alopecia, hand-foot syndrome, osteonecrosis of the jaw, xerostomia/hyposalivation, dysphagia, dysgeusia, trismus to voice changes [154, 268]. Currently, we are running a pilot trial on chemotherapy-induced peripheral neuropathy (CIPN) in BC patients undergoing taxanes.

All together, we strive to implement PBMT in the daily hospital setting to manage all cancer-therapy related side effects and especially ARD. This will eventually reduce the incidence, duration and severity of these devastating effects. Thereby, the patient will experience less pain and discomfort during and after their cancer therapy, which enables them to perform their daily activities. This will eventually result into an improved patient's quality of life. Further, the treatment compliance of the patient will increase, resulting in an improved success rate of the cancer therapy. Thus patient care will advance, which will ultimately result into an increased patient survival.

6.4 GENERAL CONCLUSION

The positive results of this research project laid a fundamental basis for the application of PBMT in the prevention and management of ARD in BC and HNC patients. In the future, we will implement further research in the domain of PBMT and supportive care in cancer patients in our PBMT expertise centre. This will be the first centre that will combine patient care with a research and training unit. As such, we strive to the application of PBMT for each cancer patient in Belgium, and eventually worldwide.

I hope that the reader of this thesis is as confident as the author that this is only the beginning of many other studies on PBMT in the supportive care of cancer patients. To conclude, I would like to state with the following saying of the founding father of PBMT, Endre Mester:

"Laser is a solution which is looking for a new problem".

SUMMARY

In 2015, 67,078 persons were diagnosed with cancer. Approximately half of them need to undergo radiotherapy. Up to 90% of the radiotherapy patients will develop a skin reaction at the treated area, also known as radiodermatitis (RD). RD can be graded from red rashes and dry desquamation, patchy/confluent moist desquamation, to ulceration. RD may be distressing and painful for the patient, which may affect their general quality of life. Furthermore, when the skin reactions evolve towards more severe forms, it might be necessary to change the treatment protocol or even interrupt RT, hereby compromising treatment outcome. Currently, a wide variety of strategies are available to manage RD ranging from creams, gels to wound dressings. However, the evidence supporting the effectiveness of these treatment methods is weak and there is no comprehensive, evidence-based consensus for the treatment of RD. Moreover, there is still no therapy to prevent RD.

Photobiomodulation therapy (PBMT) is a non-invasive treatment used to stimulate wound healing, reduce inflammation, and pain. It uses laser or light-emitting diodes in the visible and/or near-infrared spectrum. PBMT is applied in a variety of medical domains ranging from dermatology, physiotherapy, neurology to dentistry. It already has been proven to be a very efficient treatment technique for the prevention and management of oral mucositis (i.e. inflammation of mucosal lining of the mouth), a known side effect of chemotherapy. The first pilot study of our research group demonstrated that PBMT is able to prevent the aggravation of established RD in breast cancer patients.

The aim of this doctoral thesis was to investigate the effectiveness of PBMT in the prevention of acute RD in cancer patients.

Results of this project demonstrated that PBMT was able to significantly reduce the severity of radiotherapy-induced skin reactions in both breast and head and neck cancer patients. As such, patients undergoing laser therapy developed considerably less moist wounds. This was accompanied with an improved patient's quality of life. Furthermore, biophysical skin measurements showed that the skin discolouration was reduced, while the skin barrier function improved after PBMT. The analysis of the inflammatory profile of the skin lesions demonstrated that PBMT could reduce the production of pro- inflammatory mediators, suggesting an underlying anti-inflammatory effect. In short, PBMT makes the cancer treatment for each patient undergoing radiotherapy more bearable. In the future we hope to offer PBMT to each patient who suffers from RD.

SAMENVATTING

In 2015, werden in België 67.087 nieuwe diagnoses van kanker geregistreerd. Ongeveer de helft van de kankerpatiënten dienen radiotherapie te ondergaan gedurende hun behandeling. Patiënten kunnen hierbij nevenwerkingen ondervinden zoals een pijnlijke ontsteking van huid, ook wel bekend als radiodermitis. Ongeveer 90-95% van de patiënten die radiotherapie ondergaan, zullen een bepaalde graad van radiodermitis ontwikkelen. We onderscheiden vier graden van radiodermitis, gaande van rode uitslag, droge huid, vochtige open wonden tot diepe ulcera. Radiodermitis is een storende en pijnlijke huidreactie, welke een negatieve invloed heeft op de kwaliteit van leven van de patiënt. Wanneer de huidreacties te ernstig worden, is men soms genoodzaakt om het bestralingsregime te veranderen of stop te zetten, hetgeen nefast is voor de kankerbehandeling. Huidige behandelingsmethodes bestaan uit crèmes, olies, gels, en wondverbanden. Tot op heden is er echter nog onvoldoende wetenschappelijk bewijs voor een algemeen aanvaarde behandeling voor radiodermitis. Daarnaast is er ook nog geen therapie die kan gebruikt worden om de huidreacties te voorkomen.

Fotobiomodulatie therapie is een niet-invasieve behandelingsmethode die wordt gebruikt om de wondheling te stimuleren, ontsteking te onderdrukken, en de pijn te verminderen. Hierbij wordt er gebruik gemaakt van rood en/of infrarood licht dat geproduceerd wordt door een laser of LED bron.

Fotobiomodulatie therapie wordt reeds gebruikt in verschillende klinische domeinen gaande van tandheelkunde, fysiotherapie, neurologie, en dermatologie. Deze behandelingsvorm is daarnaast ook zeer effectief voor de behandeling van orale mucositis (ontsteking van het mondslijmvlies), een gekende nevenwerking van chemotherapie. Een eerste pilootstudie van onze onderzoeksgroep toonde aan dat fotobiomodulatie therapie de verergering van reeds ontstane huidreacties bij borstkanker patiënten, die radiotherapie ondergingen, kon voorkomen.

Het doel van het huidige doctoraatsproject was onderzoeken of fotobiomodulatie therapie het ontstaan van radiodermitis bij kankerpatiënten kan voorkomen.

Resultaten van dit onderzoek toonden aan dat fotobiomodulatie therapie de ernst van de huidreacties ten gevolge van radiotherapie aanzienlijk kan verminderen bij zowel borst als hoofd-hals kanker patiënten. Zo ontstonden er beduidend minder vochtige wonden bij de patiënten die lasertherapie kregen. Dit ging gepaard met een verbeterde kwaliteit van leven. Verder toonden we aan door middel van fysieke metingen dat de huid minder snel verkleurde en dat de beschermende functie van de huid verbeterd was na de fotobiomodulatie therapie. Een analyse van de ontstekingsmoleculen in huid van patiënten toonden aan dat lasertherapie de productie van deze moleculen kon verminderen, hetgeen correleert met het onderliggende anti-inflammatoire effect van de therapie. Kortom, met behulp van fotobiomodulatie therapie kunnen we de kankerbehandeling van de patiënt meer verdraagbaar maken. In de toekomst streven we er naar om deze vooruitstrevende therapie te kunnen aanbieden aan iedere patiënt die last heeft van radiodermitis.

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CURRICULUM VITAE

ROBIJNS Jolien

Place of birth: Tongeren

Date of birth: 08/02/1991

E-mail: jolienrobijns@hotmail.com

EDUCATION

2015-2017	Teacher education program CVO limo, Diepenbeek, Belgium	*Cum laude*
2012-2014	Master biomedical science Clinical molecular sciences Hasselt University, Hasselt, Belgium	*Magna cum laude*
2009-2012	Bachelor biomedical sciences Hasselt University, Hasselt, Belgium	*Magna cum laude*
2003-2009	Science-Mathematics viio, Tongeren, Belgium	*Magna cum laude*

PROFESSIONAL EXPERIENCE

Current position

2014-2018	PhD student Limburg Clinical Research Program (LCRP) Hasselt University, Hasselt, Belgium Jessa Hospital, Hasselt Belgium Project: "Photobiomodulation therapy for the prevention and management of acute radiodermatitis in cancer patients"

Internships

Nov. 2013-June 2014	Senior internship biomedical sciences: "Low-level laser therapy as a treatment option for radiodermatitis in breast cancer patients" under supervision of Prof. Dr. Jeroen Mebis. Jessa Hospital, Hasselt, Belgium
Feb.– April 2013	Junior internship biomedical sciences: "Cholesterol receptors and transporters in de - and remyelination" under supervision of dr. Vanmierlo Tim. BIOMED, Diepenbeek, Belgium
April – June 2012	Bachelor internship biomedical sciences: "SM22 as a potential new biomarker for the detection of transmural gut injury during intestinal ischemia" under supervision of dr. Schellekens Dirk and dr. Reisinger Kostan. University of Maastricht – department general surgery, Maastricht, The Netherlands

CERTIFCATES

Good Clinical Practice (GCP)
FormaliS
January 2016

Radiation protection
FANC

Laboratory animal science: experimental leader
Felasa C

ACADEMIC AWARDS

Second prize for best oral presentation: " Low-level laser therapy as a tool for the management of radiodermatitis: a pilot study in breast cancer patients". 17th annual Belgian Society of Medical Oncology Symposium, Diegem, Belgium, 7/3/2015

Best of ESLD Laser and EBD Medicine for Breast Cancer Patients Session Award: "Prevention of acute radiodermatitis by photobiomodulation: preliminary results of a randomized, placebo-controlled trial in breast cancer patients". ASLMS conference, San Diego, US, 7-9/4/2017

Third prize for best oral presentation: "Photobiomodulation therapy for the prevention of acute radiation dermatitis: preliminary results of a randomized, placebo-controlled trial in breast cancer patients". 20th annual BSMO meeting, Thermae Palace, Ostend, Belgium, 24/02/2018

Young Investigator Award: "Photobiomodulation therapy prevents severe acute radiodermatitis: a randomized, placebo-controlled trial in breast cancer patients with clinical and objective outcome measures." MASCC/ISOO annual conference, Vienna, Austria, 28-30/06/2018

GRANTS

ASLMS travel grant: ASLMS conference, San Diego, US, April 2017

FWO travel grant: ESLD Ordinary General Meeting, Geneva, Switzerland, September 2017

FWO travel grant: ESTRO conference, Barcelona, Spain, April 2018

Kom op Tegen Kanker project grant: postdoctoral research on "Photobiomodulation for the prevention and management of acute radiodermatitis in cancer patients" October 2017-October 2021

MEMBERSHIPS

Member of the European Society for Lasers and Energy Based Devices (ESLD) since June 2016

Member of the Multinational Association of Supportive Care in Cancer (MASCC) since January 2018

Member of the International working group on "Light in Oncology" (iGloB) since January 2018

Member of the European SocieTy for Radiotherapy & Oncology (ESTRO) since January 2018

Member of the Belgian Laser Group (BLG) since February 2018

SCIENTIFIC PUBLICATIONS

Censabella S, Claes S, **Robijns J**, Bulens, Mebis J. Photobiomodulation for the management of radiation dermatitis: the DERMIS trial, a pilot study of MLS® laser therapy in breast cancer patients. Support Care Cancer. 2016 Sep;24(9):3925-33.

Robijns J, Censabella S, Bulens P, Maes A, Mebis J. 2017 "The use of low-level light therapy in supportive care for patients with breast cancer: review of the literature". Lasers Med Sci. 2017 Jan;32(1):229-242.

Robijns J, Censabella S, Bulens P, Maes A, Mebis J. Letter regarding "Same strategy for pitfalls of radiotherapy in different anatomical districts. Lasers Med Sci. 2017 May;32(4):965-966.

Robijns J, Censabella S, Bulens P, Maes A, Noé L, Brosens M, Van den Bergh L, Claes S, Mebis J. The role of photobiomodulation therapy in the care of cancer patients: review of the literature. Belg J Med Oncol 2017; 11 (8):364-374.

Robijns J, Laubach HJ. Acute and chronic radiodermatitis: clinical signs, pathophysiology, risk factors and management options. Journal of the Egyptian Women's Dermatologic Society 2018, 15:2–9.

Laubach HJ, **Robijns J**. Laser and light therapy for treatment of radiation dermatitis. Hautarzt. 2018 Jan;69(1):5-9.

Robijns J, Censabella S, Claes S, Pannekoeke L, Bussé L, Colson D, Kaminski I, Bulens P, Maes A, Noé L, Brosens M, Timmermans A, Lambrichts I, Somers V, Mebis J. Prevention of acute radiodermatitis by photobiomodulation: A randomized, placebo-controlled trial in breast cancer patients (TRANSDERMIS trial). Lasers Surg Med. 2018. 50(7): p.763-771.

Robijns J, Censabella S, Claes S, Pannekoeke L, Bussé L, Colson D, Kaminski I, Lodewijckx J, Bulens P, Maes A, Noé L, Brosens M, Timmermans A, Lambrichts I, Somers V, Mebis J. Biophysical skin measurements to evaluate the effectiveness of photobiomodulation therapy in the prevention of acute radiation dermatitis in breast cancer patients. Support Care Cancer, 2018. Epub ahead of print

ORAL PRESENTATIONS

Robijns J, Censabella S, Claes S, Bulens P, Mebis J. "Low-level laser therapy as a tool for the management of radiodermatitis: a pilot study in breast cancer patients". 17th Annual Belgian Society of Medical Oncology Meeting, Diegem, Belgium, 07/03/2015

Censabella S, Claes S, **Robijns J**, Bulens P, Mebis J. "Low level laser therapy for the management of radiation dermatitis: final results of the dermis trial, a pilot study in breast cancer patients". MASCC/ISOO Symposium, Copenhagen, Denmark, 25-27/06/2015

Robijns J, Mebis J, Censabella S, Bulens P, Maes A, Claes S, Bussé L. Low-level laser therapy for the prevention and management of radiodermatitis in cancer patients. Journées Parisiennes du laser 2016, Paris, France, 3-4/06/2016

Robijns J, Censabella S, Claes S, Pannekoeke L, Bussé L, Colson D, Kaminski I, Bulens P, Maes A, Noé L, Brosens M, Timmermans A, Hellings N, Lambrichts I, Somers V, Mebis J. Prevention of acute radiodermatitis by photobiomodulation: preliminary results of a randomized, placebo-controlled trial in breast cancer patients. ASLMS conference, San Diego, US, 7-9/04/2017

Robijns J, Censabella S, Holvoet L, Noé L, Brosens M, Maes A, Bulens P, Luyten D, Joosens E, Hellings N, Lambrichts I, Somers V, Mebis J. The use of photobiomodulation therapy for the management of oral mucositis in cancer patients other than head and neck cancer: two retrospective analyses. ASLMS conference, San Diego, US, 7-9/04/2017

Robijns J, Censabella S, Claes S, Pannekoeke L, Bussé L, Colson D, Kaminski I, Bulens P, Maes A, Noé L, Brosens M, Timmermans A, Lambrichts I, Somers V, Mebis J. Latest research about red light therapy for prevention of acute radiation dermatitis. Oral presentation at the Ordinary General Meeting of the European Society for Lasers and Energy based Devices, Geneva, Switzerland, 13/09/2017

Robijns J, Censabella S, Claes S, Pannekoeke L, Bussé L, Colson D, Kaminski I, Bulens P, Maes A, Noé L, Brosens M, Timmermans A, Lambrichts I, Somers V, Mebis J. Photobiomodulation therapy for the prevention and management of radiodermatitis in breast cancer patients. Laser symposium of the Belgian Dermatology Days, Brussels, Belgium, 23/02/18

Robijns J, Censabella S, Claes S, Pannekoeke L, Bussé L, Colson D, Kaminski I, Bulens P, Maes A, Noé L, Brosens M, Timmermans A, Lambrichts I, Somers V, Mebis J. Photobiomodulation therapy for the prevention of acute radiation dermatitis: preliminary results of a randomized, placebo-controlled trial in breast cancer patients. 20th annual meeting of the Belgian Society of Medical Oncology, Ostend, Belgium, 24/02/18

Robijns J, Censabella S, Claes S, Pannekoeke L, Bussé L, Colson D, Kaminski I, Bulens P, Maes A, Noé L, Brosens M, Timmermans A, Lambrichts I, Somers V, Mebis J. Photobiomodulation prevents acute radiodermatitis: final results of a RCT in breast cancer patients. 37th ESTRO congress, Barcelona, Spain, 21/04/18

Robijns J, Censabella S, Claes S, Pannekoeke L, Bussé L, Colson D, Kaminski I, Bulens P, Maes A, Noé L, Brosens M, Timmermans A, Lambrichts I, Somers V, Mebis J. Photobiomodulation therapy prevents severe acute radiodermatitis: a randomized, placebo-controlled trial in breast cancer patients with clinical and objective outcome measures. MASCC/ISOO annual meeting, Vienna, Austria, 28/06/2018

Robijns J, Censabella S, Claes S, Pannekoeke L, Bussé L, Colson D, Kaminski I, Bulens P, Maes A, Noé L, Brosens M, Timmermans A, Lambrichts I, Somers V, Mebis J. Photobiomodulation therapy in the prevention of acute radiodermatitis: a randomized, controlled trial in breast cancer patients (TRANSDERMIS trial) WALT meeting, Nice, France, 3-5/10/2018

Robijns J, Censabella S, Claes S, Pannekoeke L, Bussé L, Colson D, Kaminski I, Broux V, Lodewijckx J, , Bulens P, Maes A, Noé L, Brosens M, Timmermans A, Lambrichts I, Somers V, Mebis J. Photobiomodulation therapy for the prevention of acute radiation dermatitis in head and neck cancer patients: preliminary results of a randomized controlled trial (DERMISHEAD trial). WALT meeting, Nice, France, 3-5/10/2018

POSTER PRESENTATIONS

Censabella S, Claes S, **Robijns J**, Bulens P., Mebis J. Low- level laser therapy for the management of radiation dermatitis: preliminary results of a pilot study in breast cancer patients. ESMO conference, Madrid, Spain, 26-30/09/2014

Robijns J, Censabella S, Claes S, Bulens P, Mebis J. Low- level laser therapy for the management of radiation dermatitis: preliminary results of a pilot study in breast cancer patients. ESMO conference, Copenhagen, Denmark, 7-11/10/2016

OTHER PRESENTATIONS

Minisymposium 'Het maligne melanoom': Nieuwe aanpak radiodermitis. Jessa Hospital, Hasselt, Belgium, 18/02/2016

PhD-Symposium Medisch-wetenschappelijk onderzoek in de Limburgse ziekenhuizen: de weg naar een betere zorginnovatie: Low-level lasertherapie voor preventie en behandeling van radiodermatitis bij kankerpatiënten. Ziekenhuis Oost-Limburg, Genk, Belgium, 19/03/2016

"Een wetenschappelijke carrière - wat Biomedici dienen te weten...": Low-level lasertherapie voor de preventie van radiodermatitis in kankerpatiënten. Hasselt University, Diepenbeek, Belgium, 01/06/2016

Infosession Master clinical Biomedical Sciences: "Een doctoraatsstudent BMW aan het woord". Hasselt University, Diepenbeek, Belgium, 11/04/2018

"Wetenschapper aan het woord". Hasselt University, Diepenbeek, Belgium, 04/06/2018

PXL Health Care Congress: Research on the use of photobiomodulation therapy for the management of acute radiodermatitis in cancer patients. PXL dept. Healthcare, Hasselt, Belgium, 05/06/2018

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"Success is not the key to happiness.
Happiness is the key to success.
If you love what you are doing, you will be successful."

Albert Schweitzer