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Maastricht University

Faculty of Medicine and Life Sciences
School for Life Sciences
Master of Biomedical Sciences

Masterthesis

The use of photobiomodulation therapy for the management of chemotherapy-induced peripheral neuropathy: a pilot trial

Joy Lodewijckx

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Clinical Molecular Sciences

SUPERVISOR :
Prof. dr. Niels HELLINGS

SUPERVISOR :
Prof. Dr. Jeroen MEBIS

MENTOR :
Mevrouw Jolien ROBIJNS

2017
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Acknowledgment

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Joy Lodewijckx

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List of abbreviations

Activator Protein-1	AP1
Adenosine Triphosphate	ATP
Breast Cancer	BC
Brief Pain Index	BPI
Center of Statistics of Hasselt University	CenStat
Chemotherapy	CT
Chemotherapy-Induced Peripheral Neuropathy	CIPN
Clinical Neurological Examination	CNE
cyclic Adenosine Monophosphate	cAMP
Cytochrome C oxidase	Cox
Diabetic Neuropathy Examination	DNE
Electromyography	EMG
Evaluation Database to Guide Effectiveness	EDGE
Functional Assessment of Cancer Therapy/ Gynaecologic Oncology Group taxane scale	FACT/GOG-Taxane
Limburg Clinical Research Program	LCRP
Low-Level Laser Therapy	LLLT
Michigan Neuropathy Screening Instrument	MNSI
Microtubule-Targeting Agents	MTAs
modified Total Neuropathy Score	mTNS
Nerve Conduction Studies	NCS
Neuropathy Impairment Score	NIS
Nitric Oxide	NO
Nonsteroidal Anti-Inflammatory Drugs	NSAIDs
Nuclear Factor kappa B	NFkB
Numerical Rating Scale	NRS
Photobiomodulation Therapy	PBMT
Reactive Oxygen Species	ROS
revised Neuropathy Disability Score	NDS
Taxane-Induced Neuropathy	TIN
Timed Up and Go test	TUG
Toronto Clinical Scoring System	TCSS
Visual Analogue Scale	VAS

Abstract

Introduction: Taxanes, microtubule-targeting agents (MTAs), are one of the most used chemotherapeutic agents in breast cancer (BC) patients despite the side effects. Chemotherapy-induced peripheral neuropathy (CIPN) is one of these common side effects. This complication involves paresthesia, numbness and/or burning pain in the distal limbs. Photobiomodulation therapy (PBMT) is based on the application of (near)-infrared light on tissue to stimulate cell repair processes. Previous research has demonstrated that it can promote functional recovery of peripheral nerves. We hypothesize that PBMT is an effective treatment strategy to prevent sensory symptoms associated with CIPN leading to an improved patient's quality of life.

Material & methods: A prospective, randomized, placebo-controlled study with 7 BC patients that underwent taxane treatment was performed at the Limburg Oncology Centre (Jessa Hospital, Hasselt Belgium). Patients were randomized to receive PBM or placebo treatments twice-weekly starting at first until the last week of their chemotherapy (CT). The modified Total Neuropathy Score (mTNS) was used to evaluate the severity of CIPN. The Functional Assessment of Cancer Therapy/ Gynaecologic Oncology Group Taxane scale (FACT/GOG-Taxane) was used to evaluate the quality of life.

Results: No significance in mTNS scores was experienced at the end of CT between both groups in upper- and lower limbs (Ps>0.2). In addition, no significant difference was found between the placebo- and PBMT group in quality of life (Ps>0.2).

Discussion & conclusions: According to results found during this study, PBMT did not prevent the emergence of CIPN. This can be explained by the small sample size. Nonetheless, based on patients' experience, there seem to be a tendency in the prevention of CIPN with PBMT. In order verify this, a follow-up study is needed to increase the sample size.

Introduction

Breast cancer (BC) is one of the most common cancers in women worldwide with 1.7 million new cases per year (1). In Belgium alone, more than 10 000 women were diagnosed with BC in 2015 (2). Although, there has been an improvement in diagnosing and treating BC, it remains a main cause of death in women, both in developing and in industrialized countries (3). Currently, BC is treated with systemic therapy, radiation therapy, surgery or a combination of those. Systemic- and radiation therapy can be given adjuvant or neoadjuvant to surgery, depending on the type of tumour (4). A commonly used chemotherapeutic agent for patients with early-stage and metastatic BC are the taxanes (e.g. paclitaxel and docetaxel) (5). Taxanes, which are microtubule-targeting agents (MTAs), will interfere with the normal formation and function of microtubules and the mitotic spindle by binding to the β-tubulin subunit. This results in the stabilization of the microtubules and disruption of microtubule function, causing cells to arrest in metaphase and undergo apoptosis (5, 6).

Peripheral neuropathy is a common side effect of taxane therapy. Taxanes can cause neuronal damage to axons, the myelin sheet, and dorsal root ganglia (7). Since the numbers of cancer survivors rises, more attention is being paid to the long term unwanted effects patients may experience as a result of their treatment and the impact these side effects can have on their quality of life (8). The incidence for taxane-induced neuropathy (TIN) in BC patients lays around 81% (9). Yet, the precise incidence and prevalence vary depending on several factors ranging from the type of chemotherapeutic agent, the combination with other systemic therapies, and the study set up. Sensory neurons are particularly affected, while motor, autonomic or CNS involvement is rare. Sensory neurons allow the perception of touch, pain, temperature, position, and vibration. Therefore, TIN is associated with symmetrically symptoms like paresthesia, numbness, burning pain, loss of temperature sensation, and loss of tendon reflexes typically appearing in distal extremities, indicating increased vulnerability of neurons with the longest axons (5, 10). Symptoms of TIN can already develop within 24-72 hours following the first dose of taxanes. In milder cases, chemotherapy-induced peripheral neuropathy (CIPN) is completely reversible after discontinuing or ending chemotherapy (CT). In more severe cases, the neuropathy can be irreversible leading to symptoms as mild foot drop, decreased tendon reflexes or distal sensory deficits (11). Risk factors for the development of TIN include age, dose intensity, cumulative dose, duration of therapy, administration of other neurotoxic agents, and pre-existing conditions such as diabetes and alcohol abuse (12).

The mechanism of TIN is not completely understood. It is likely that, since microtubules also play a critical role in intracellular transport, taxane affected microtubules cannot provide adequate fast axonal transport. Similarly, LaPointe *et al.* found that inhibition of fast axonal transport may contribute significantly to the neurotoxicity induced by MTAs (10). This view is supported by the fact that that taxanes induce conformational changes in the carboxy terminus of tubulin, influencing the regulation of kinesin-1 ATPase activity, which is a motor protein involved in intracellular transport along microtubules (10, 13, 14).

In clinical practice, TIN is poorly diagnosed and under-treated although it has a high health impact. It will impair patients' daily activities because of comorbidities such as psychological distress, fall risk and poor sleep quality resulting in a significant decrease in quality of life (15). For cancer survivors, this TIN is a constant reminder of the cancer disease and its treatments. (5, 16). Lastly, CIPN represents a heavy economic burden with an average of $17,344 per year per patient in the USA (17).

TIN is currently treated with symptom management and includes neuropathic pain management and nutritional supplements (11). Medications such as narcotic analgesics, anticonvulsants, nonsteroidal anti-inflammatory drugs (NSAIDs), and tricyclic antidepressants have been investigated to manage TIN-related symptoms with limited success. In addition, these medications frequently come with adverse effects (18). Neuropathy causes muscle mass atrophy leading to a decrease in muscular strength. To overcome this, physical therapy can be given to the patient (19). However, intense strength training and aerobic exercise can overload the already weakened patient. Another strategy to manage TIN is with CT dose delays and/or reductions. Yet, this will affect treatment outcome and overall survival of the patient (5). Despite the variety of tested substances, there are no established agents recommended for the prevention of TIN (7).

Photobiomodulation therapy

Photobiomodulation therapy (PBMT) or low-level laser therapy (LLLT) has proven its efficiency in general medicine for already more than 40 years (dermatology, physiotherapy, neurology, and dentistry) (20). PBMT uses red or (near)-infrared laser light with the purpose of promoting tissue repair, decreasing inflammation, and reducing (neuropathic) pain (21). Since the last 20 years, PBMT is becoming a new treatment modality in supportive care of cancer patients (22, 23). As demonstrated by our research group, PBMT is an effective preventive and therapeutic tool for oral mucositis and acute radiodermatitis, two devastating side effects of chemo-and radiotherapy (22, 24).

The principle chromophores for PBMT are located inside the mitochondria, making cells with a large number of mitochondria such as muscle cells and neurons, particularly responsive to light (25). The most valid hypothesis in the mechanism of action of PBMT is that cytochrome C oxidase (Cox) of the electron transport chain will act as the primary photo-acceptor for the laser light. The absorption of photons by Cox leads to quickening of electron transfer reactions and automatically an increased production of adenosine triphosphate (ATP) (26, 27). ATP is the substrate for adenylcyclase, and therefore the ATP level controls the level of cyclic adenosine monophosphate (cAMP), which will also increase (27). This leads to an increased activity of activator protein-1 (AP1), promoting cell survival (21). Furthermore, PBMT will cause a shift in overall cell redox potential in direction of greater oxidation, resulting in the production of a low controlled amount of reactive oxygen species (ROS). This will activate the redox-sensitive nuclear factor kappa B (NFkB), also promoting cell survival by an increased production of cytokines, chemokines and, growth factors (21, 28). Furthermore, the activity of Cox is inhibited by nitric oxide (NO). It is proposed that PBMT can photo-dissociate NO from Cox and reverse the mitochondrial inhibition of respiration and thus may increase the respiration rate (29). Since unhealthy or hypoxic cells are more likely to have inhibitory concentrations of NO, this could also explain why PBMT seems to have greater effects in diseased or damaged cells compared to healthy cells. (25). The mechanism of action of PBMT is illustrated in figure 1. However, the complete molecular mechanism behind this laser therapy is not completely clear yet.

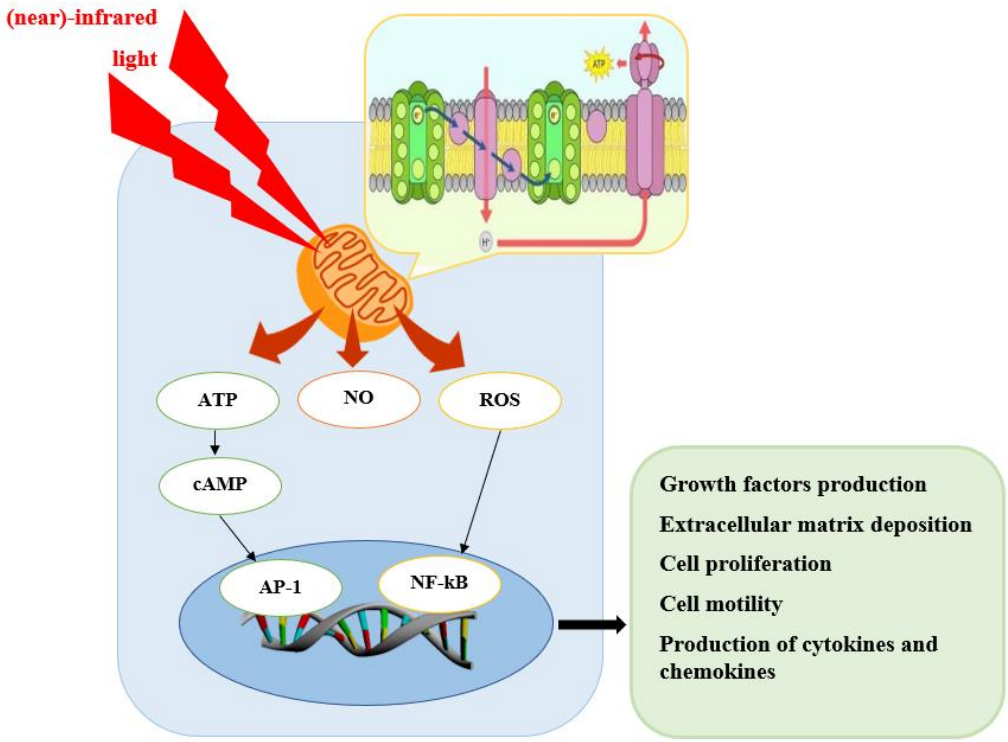

Fig. 1: Mechanism of action of photobiomodulation therapy (PBMT) Red or (near)-infrared laser light will act on the cytochrome C oxidase (Cox) of the electron transport chain leading to an increased production of adenosine triphosphate (ATP), cyclic adenosine monophosphate (cAMP), reactive oxygen species (ROS), and nitric oxide (NO). These will act on the transcription factors nuclear factor kappa B (NFkB) and activator protein-1 (AP1), promoting cell survival. Figure adapted from Hamblin *et al.* (27)

Preliminary data

Animal studies have been done to investigate the effectiveness of PBMT in CIPN. The most relevant one is the study by Hsieh *et al.*, who investigated the effect of PBMT in rats with oxaliplatin-induced neuropathy. Each rat received a total of four doses of oxaliplatin. Next, PBMT was applied for 12 consecutive days to the skin surface directly above sites where the sciatic nerve passes. Relieved cold and mechanical allodynia was observed during this study (30). Furthermore, research with beneficial results has been done concerning PBMT in patients with diabetic neuropathy (18, 31-33). The aim of the study by Khamseh *et al.* was to determine the effectiveness of PBMT in diabetic distal symmetric polyneuropathy. Subjects showed a statistically and clinically significant increase in nerve conduction velocity in all patients after 10 PBMT sessions (18). The same laser device will be used during this study. There is little published data on the effectiveness of PBMT in CIPN in humans. To our knowledge, three studies have been conducted investigating this effect (table 1). All show beneficial results in the management of CIPN with PBMT.

Table 1: Comparison of research undertaken to study the effect of photobiomodulation therapy (PBMT) on chemotherapy-induced peripheral neuropathy (CIPN) (34-36)

Research group	Sample size	Type of chemotherapy	Laser protocol	Result
Yamada *et al. (34)*	34	Taxanes	N/A	Lower score on Brief Pain Index (BPI) questionnaire
Argenta *et al. (35)*	68	N/A	3 times/week for 6 weeks	Reduction in modified total neuropathy score (mTNS) score
Hsieh *et al. (36)*	17	Platinum	3 times/week for 4 weeks	Neurotoxicity symptoms improved

Up to now, there was no study that investigated the use of PBMT in the prevention of CIPN. The purpose of this study was to evaluate effectiveness of PBMT to prevent sensory symptoms associated with TIN and thereby preventing the worsening of the patients' quality of life during and after taxane therapy.

Added value

In this project we will try to develop a novel technique for the treatment of CT-induced side effects, more specifically CIPN. Results of this project will lead to a reduction in the incidence, duration and severity of these complications. Furthermore, it will reduce patient discomfort during and after CT, resulting in an improved quality of life. In addition, the CT compliance of the patient will increase, resulting in an improved success rate. Thereby patient care will advance, which will ultimately result into an increased patient survival.

Materials and methods

Study design and setting

This project was part of the Limburg Clinical Research Program (LCRP), a collaboration between Hasselt University and the two largest hospitals (Jessa and ZOL) in Limburg to strengthen translational research in several medical domains. This was a prospective, randomized, placebo-controlled, double armed study that included BC patients who were planned to undergo taxane treatment at Limburg Oncology Center at the Jessa Hospital (Hasselt, Belgium). A duration time of eight months was needed to complete this study (November 2017 till June 2018). The study was registered at ClinicalTrials.gov (NCT03391271).

Study population

Written informed consent was obtained from all study participants before enrolment. Exclusion criteria were pre-existing neuropathy, medication use, metastasis, an alcohol addiction, and the presence of cardio- respiratory or musculoskeletal disease. Patients were invited to participate in this study during their standard doctor's appointment at the oncology department. Patients were considered for inclusion if they were diagnosed with BC stage 0-3A and were planned to undergo at least three cycles of taxane treatment. These inclusion and exclusion criteria are listed in table 2.

Table 2: Inclusion- and exclusion criteria

Inclusion criteria	Exclusion criteria
• Diagnosed with non-invasive (stage 0) or invasive (stage 1, 2 and 3A) breast adenocarcinoma • Age 18 years or above • Are planned to undergo at least 3 cycles of taxane treatment (i.e. paclitaxel or docetaxel) • Have no documented or observable psychiatric or neurological disorders that might interfere with study participation (e.g., dementia or psychosis). • Signed informed consent	• Have a history of neuropathy before taxane treatment due to other medical conditions: diabetes, peripheral vascular disease, HIV infection, significant degenerative or familial neurologic disorder, nerve compression injuries (i.e., carpal tunnel syndrome, brachial plexopathy, spinal stenosis, or spinal nerve root compression) • Taking stable doses of medication on prescription or dietary supplements for peripheral neuropathy. Related medications are: gabapentin, pregabalin, nortriptyline, amitriptyline, duloxetine, venlafaxine; lidocaine, opioid tramadol and other narcotics; NSAIDs; glutamine, glutathione, Patients need to agree not to initiate a new therapy two weeks before and during the study period. • Metastatic disease • Have a diagnosis of ethanol addiction or dependence within the past 10 years. • Severe or unstable cardio- respiratory or musculoskeletal disease

Randomization

Each recruited participant was randomly assigned to the control or PBMT group in a 1:1 ratio (fig. 2). Patients were allocated based on a block randomization 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.

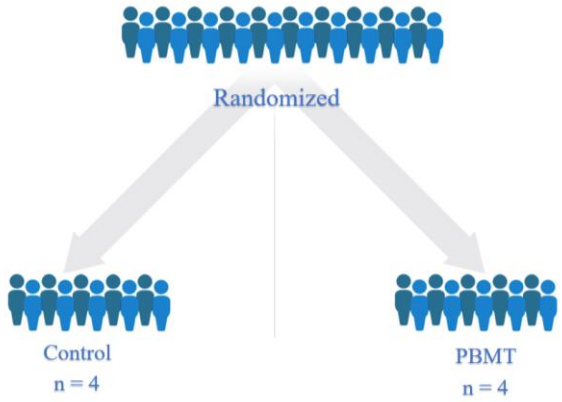

Fig. 2 Study design: The breast cancer patients are divided randomly into two groups, a control group and photobiomodulation therapy (PBMT) group. Each group contains 4 participants.

Ethics

The study was conducted at Jessa hospital Hasselt, in accordance with the Declaration of Helsinki and Guidelines for Good Clinical Practice. The research protocol was reviewed and approved by the Ethical Committee Jessa Hospital and the Ethical committee university Hasselt (B243201733877).

Intervention

The laser device used in this study is a Multiwave Locked System® (M6 laser, ASA srl, Arcugnano (VI), Italy). During PBMT, a wavelength range of 800 till 1000 nm is used since penetration through tissue is maximal at this range. The second parameter to take into account is the power. According to a study by Khamseh *et al*., who investigated the effect of PBMT in diabetic neuropathy, beneficial results were seen with a power density of 528 mW (18). In consequence, the same power was used for this research project. The third is the fluence. A previous study by our own research group on PBMT in radiodermatitis have shown positive results with an energy density of 4 J/cm² (24). The fourth is the irradiance or radiant flux received by a surface per unit area. This was 168 mW/cm² for the laser used in this research. The fifth parameter and last is the delivery method. In general, the frequency of PBMT is between 2-3 sessions a week. For practical reasons, a frequency of twice a week was obtained in this study, as long as the patients received CT (21).

The PBMT-group received these laser sessions starting at first until the last week of CT (9-12 weeks depending on the type of CT). The control group underwent twice-weekly sham laser sessions starting at first until the last week of CT (9-12 weeks depending on the type of CT). The laser device was placed on the same manner and for the same period of time on the identified body points, but the device was not switched on. Patients wore laser-protective goggles both before and throughout treatment for safety and to maintain blinding. An overview of all the parameters can be found in supplementary table 1.

Each patient was lasered at 13 points of the lower extremities including; six paravertebral points (L4-S1); five points along the sciatic nerve (femoral triangle, popliteal fossa, neck of the fibula, malleolus lateralis and, malleolus medialis); and plantar- and dorsal surface of the feet. Likewise, each patients was lasered at 10 points of the upper extremities were the medial-, radial-, and ulnar nerve passes. (supplementary figure 1).

Outcome measures

To find an answer to our previously stated research questions, data was collected using validated questionnaires and quantitative tests to measure the amount of neuropathy on 4 determined time points (Table 3).

Patient data

To detect personal risk factors for TIN, a questionnaire was given to all the patients for the collection of demographics (age, weight, height, comorbidities, alcohol use, smoking habits). Further, patients' medical file was used to collect data regarding the patients' disease and treatment characteristics.

The modified total neuropathy score

The modified Total Neuropathy Score (mTNS) is a composite scale that includes a patient report of sensory and motor symptoms, pin sensibility, quantitative vibration thresholds, strength, and deep tendon reflexes. To test sensory and pin sensibility, a cotton swab and monofilament were used respectively in both legs and arms in a distal to proximal direction. Similarly, a tuning fork was used to assess the patient's vibration sensitivity threshold in bone structures in upper and lower extremities. To investigate strength, guidelines for administer neurological tests were performed. Lastly, the deep tendon reflexes were tested using a reflex hammer. If the reflex was absent, the patient was asked to perform the Jendrassik maneuver. All tests were based on Neurological Examination Made Easy (37). The higher the mTNS score, the more severe the peripheral neuropathy (supplementary table 2). The mTNS is a clinically applicable, sensitive screening tool for CIPN (38).

Pain evaluation

A visual analogue scale (VAS) was used to evaluate the patients' pain level due to TIN. The patients were asked to indicate their subjective experience of pain. Each score is associated with a certain pain score, in which 0 = 'no pain' and 10 = 'the most pain sensation imaginable'. VAS is a reliable and valid tool for the quantification of perceived pain.

Timed Up and Go

The Timed Up and Go test (TUG) was used to assess the patients' mobility and requires both static and dynamic balance (39). During this test, the time was measured each patient needed to rise from a chair, walk three meters, turn around, walk back to the chair, and sit down. The TUG is highly recommend by the Evaluation Database to Guide Effectiveness (EDGE) Oncology Section Task Force on Breast Cancer Outcomes: Chemotherapy Induced Peripheral Neuropathy (CIPN) to measure balance and mobility in breast cancer patients (40).

6 minute walk test

The 6 minute walk test measures the distance the patient can walk quickly on a flat, hard surface in six minutes and reflects their ability to perform daily physical activities. This test is easy to use and is also highly recommended by the EDGE Oncology Section Task Force on Breast Cancer Outcomes: Chemotherapy Induced Peripheral Neuropathy (CIPN) to measure breast cancer patients' mobility (40).

Quality of life

The Functional Assessment of Cancer Therapy/ Gynaecologic Oncology Group Taxane scale (FACT/GOG-Taxane) is a patient questionnaire to test the quality of life of the patients with TIN and can be divided into five components: physical wellbeing, social/family wellbeing, emotional wellbeing, functional wellbeing, and additional concerns specific for taxane treated patients. The higher the score, the better the quality of life (41).

Patients' satisfaction with the therapeutic intervention

The patients were asked to evaluate their global satisfaction with the laser/sham therapy using a numerical rating scale (NRS) from 0 (minimum score) to 10 (maximum score).

Table 3: Individual schedule

	Baseline	Treatment		Follow-up
Time point	**Before start of chemotherapy**	**Week 6 of chemotherapy**	**End of chemotherapy**	**3 weeks after end of chemotherapy**
Informed consent	x			
Demographics	x			
mTNS	x	x	x	x
FACT/GOG-Taxane	x	x	x	x
VAS	x	x	x	x
TUG	x	x	x	x
6 minute walk test	x	x	x	x
Patients' satisfaction			x	x

Sample size

The sample size in this study was based on the calculations of the study by Argenta *et al.* (35). They expected a decrease in mTNS score of 30% in the PBMT-group and therefore a sample size of 30 patients in each arm is needed to detect such a difference with 80% power (using a two-tailed t-test with a significance level of 0.05). Due to time issues we were not capable to include this amount of patients.

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, Mann-Whitney U-tests, independent samples T-test, or Wilcoxon signed rank test, as appropriate. Continuous data were analysed by mixed analyses of variance (ANOVAs) with time as within-subject factor and groups (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.

Results

Patient characteristics

Between December 2017 and March 2018, a total of 32 patients were screened on eligibility, 24 of them were excluded. A total of 17 patients refused to participate due to several reasons with the transport problem as the most frequent one, followed by too time-consuming, anxiety attacks when being blindfolded, or were not even with the study design. 7 Patients did not met the inclusion criteria and 1 patient was lost during the follow-up because of discontinuation of CT treatment. Eventually 7 patients were randomized into the placebo or PBMT group as show in the patient flow chart (Fig. 3).
Patient compliance to the treatment was high with all patients receiving > 90% of scheduled treatments. No complications were observed among patients treated with PBM.

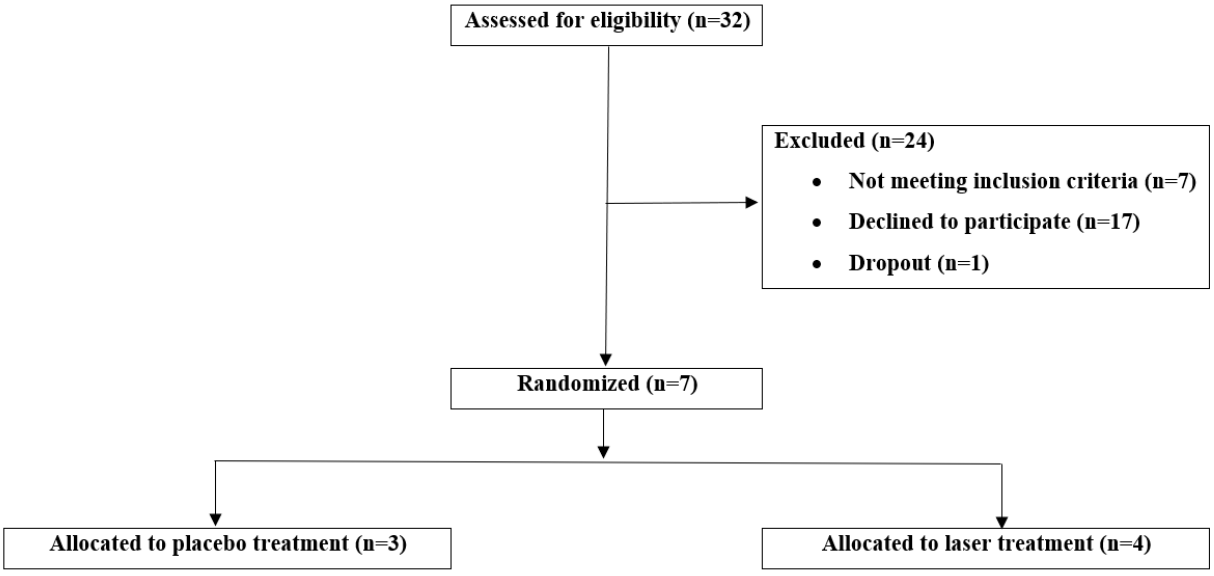

Fig. 3. Patients flow chart: patient flow through the trial.

Patient- and treatment-related characteristics are included in Tables 4 and 5. The mean age of the patients was comparable between the control (56.33 years) and PBMT group (54.50 years). No patient had an in situ cancer type and all patients were in a stage II or higher. Statistical analysis revealed that there were no significant differences between the two groups with respect to all of the patients' characteristics. Therefore, both groups were perfectly comparable.

Table 4: Baseline demographic patient characteristics

	Control group (n=3)	PBMT group (n=4)	
	Mean ± SD	**Mean ± SD**	**P**
Age (years)	56.33 (8.50)	54.50 (7.51)	0.77[a]
Body Mass Index (BMI)	24.27 (1.99)	28.47 (4.11)	0.17[a]
Length (m)	1.68 (0.03)	1.67 (0.08)	0.90[a]
Weight (kg)	68.17 (3.62)	79.00 (9.41)	0.12[a]
Tumour size (cm)	3.27 (1.03)	3.85 (2.50)	1.00[b]

BMI, Body Mass Index; PBMT, photobiomodulation therapy; SD, standard deviation.

[a]*Independent samples T-test (two-tailed).*

[b]*Wilcoxon Mann-Witney U-test (two-tailed).*

Table 5: Disease and Therapy-Related Characteristics

Characteristic	**Control group (n=3)**	**PBMT group (n=4)**	**P[a]**
Tumour type			0.35
Invasive ductal adenocarcinoma	2	3	
Invasive lobular adenocarcinoma	1	0	
Missing	0	1	
Tumour stage			0.53
II	2	2	
III	1	2	
Tumour location			0.37
Left	1	3	
Right	2	1	
Positive lymph nodes			0.57
Yes	3	3	
No	0	1	
Timing chemotherapy			0.63
Neoadjuvant	1	2	
Adjuvant	2	2	
Type of chemotherapy			0.37
Paclitaxel (80 mg/m²)	1	3	
Docetaxel (100 mg/m²)	2	1	
Frequency of chemotherapy			
Every week	1	3	0.37
Every 3 weeks	2	1	
Other cancer therapy			
Hormone therapy	2	1	0.46
Targeted therapy	2	0	0.14
Comorbidities			
Hypertension	0	1	0.35
Diabetes Mellitus	0	1	0.35

Continued on next page

PBMT, photobiomodulation therapy

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

Table 5: Continued from previous page

Characteristic	Control group (n=3)	PBMT group (n=4)	P[a]
Smoking status			0.63
Never	2	2	
Former	1	2	
Alcohol units a week			0.65
0	2	2	
1-3	0	1	
4-10	1	1	
Food supplements			0.29
Yes	3	2	
No	0	2	

PBMT, photobiomodulation therapy

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

Primary endpoint

The primary endpoint was the difference in mTNS score between the control group and the PBMT group at the end of CT. A distinction was made between the upper- and the lower limbs (fig. 4). No significant difference was found in mean mTNS score of the upper limbs at the end of CT between the control group and the PBMT group (5.2 vs. 3.1 resp., $p = 0.20$). Similarly, no significant difference was found between mean mTNS score of the lower limbs at the end of CT between the control group and the PBMT group (5.0 vs. 3.3 resp., $p = 0.27$). The 2 x 2 mixed ANOVA revealed no significant main group effect and group by time interaction for the mTNS score in upper- ($Ps>0.4$) and lower limbs ($Ps>0.8$). However, the main effect of time was significant for mTNS score in the upper- ($p = 0.004$) and the lower limbs ($p = 0.04$). Wilcoxon signed rank test revealed no significance in upper- and lower limbs ($Ps>0.1$) within the control group. Likewise, no significance difference was found in upper- and lower limbs ($Ps>0.05$) within the PBMT group. A detailed list of mean subtest values of the mTNS score at the end of CT can be found in the supplementary information.

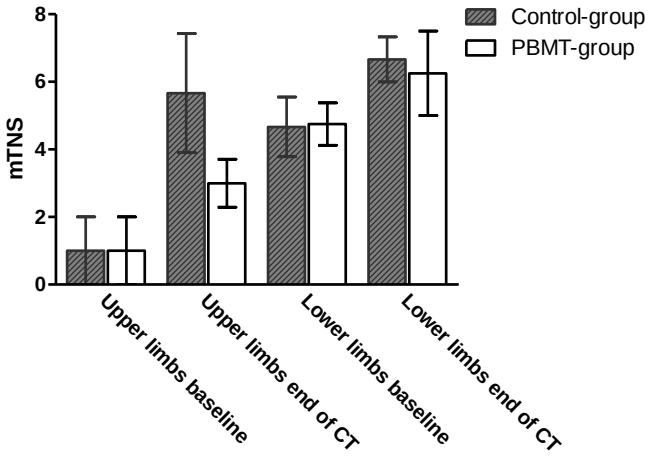

Fig. 4. modified Total Neuropathy Score (mTNS): Comparison in mTNS score at baseline and end of chemotherapy (CT) in upper- and lower limbs between the control en photobiomodulation therapy (PBMT) group. Wilcoxon Mann-Witney U-test (two-tailed) revealed no significance at the end of chemotherapy in upper- and ($p = 0.20$) lower limbs ($p = 0.27$) between the control and PBMT group.

Fig. 5 shows the difference in mTNS score at the end of CT and during the follow up visit. Only 5 people, 2 in the control group and 3 in the PBMT group, were examined approximately three weeks after the end of CT. No significance was found between the control group and the PBMT group in mean mTNS score during their follow up visit in upper- and lower limbs ($p = 0.82$ vs. $p = 0.75$, resp.). The main group ($p = 0.59$ vs. $p = 0.76$) and time effect ($p = 0.69$ vs. $p = 0.13$), and group by time interaction ($p = 0.94$ vs. $p = 0.79$) were all not significant in upper- and lower limbs, respectively. Wilcoxon signed rank test revealed no significant difference in upper- ($p = 0.79$) and lower limbs ($p = 0.16$) in the PBMT group between the end of CT and the follow up visit.

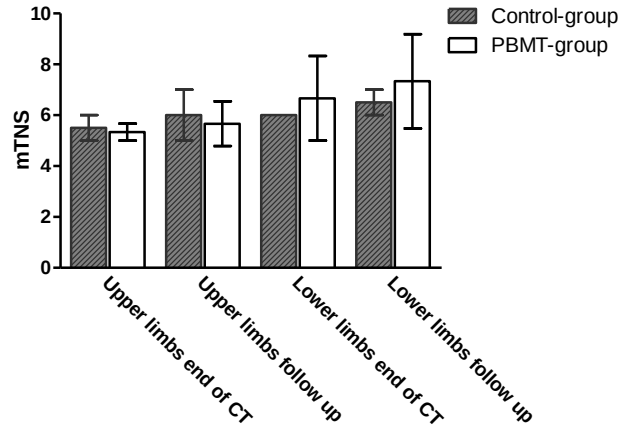

Fig. 5. modified Total Neuropathy Score (mTNS): Comparison in mTNS score at the end of chemotherapy (CT) and follow up period in upper- and lower limbs between the control group en photobiomodulation therapy (PBMT) group. Wilcoxon signed rank test (n=3) revealed no significance between the end of CT and follow up visit in the PBMT-group for both, upper- ($p = 0.79$) and lower limbs ($p = 0.16$).

Secondary outcomes

Pain evaluation

As shown in fig. 6, the 2 x 2 mixed ANOVA of the pain score showed no significance for the main time effect (p = 0.40). Similarly, the main group effect and group and time interaction were not significant (p = 0.99 and p = 0.75, resp.).

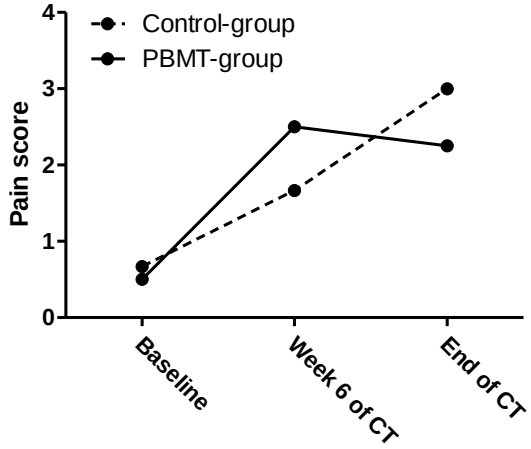

Fig. 6. Visual Analogue Scale (VAS): Comparsison of patients' subjective experience of pain between the control group and the photobiomodulation therapy (PBMT) group. Each score is associated with a certain pain score, in which 0 = 'no pain' and 10 = 'the most pain sensation imaginable'.

Timed Up and Go

The TUG test showed no significance for main time effect (p = 0.74) with the 2 x 2 mixed ANOVA analysis. In addition, the main group effect and group and time interaction were not significant (p = 0.46 and p = 0.54, resp.).

6 minute walk test

No significant difference was found for the main time effect of the 6 minute walking test using 2 x 2 mixed ANOVA (p = 0.27). Similarly, the main group effect and group and time interaction were not significant (p = 0.82 and p = 0.61, resp.).

Quality of life

The subscale scores (physical wellbeing, social/family wellbeing, emotional wellbeing, functional wellbeing, and neurotoxicity) and total score of FACT are presented in fig. 7. A higher score indicates a better quality of life. Regarding the physical wellbeing, the 2 x 2 mixed ANOVA revealed no significance in main time- and group effect and in the group and time interaction ($p = 0.06$, $p = 0.34$, and $p = 0.65$, resp.). Similarly, the 2 x 2 mixed ANOVA of social and family wellbeing presented no significant main time effect ($p = 0.22$), main group effect ($p = 0.67$) and time x group interaction ($p = 0.09$).

In panel C and D of fig. 7 the emotional and functional wellbeing of the patients are illustrated. Despite there is no significant effect group ($p = 0.79$ and $p = 0.67$) and time and group interaction ($p = 0.82$ and $p = 0.99$) respectively, both subscales seems to score higher in the PBMT group. Furthermore, 2 x 2 mixed ANOVA analyses revealed a significant main time effect in both subscales ($p = 0.04$ and $p = 0.009$, resp.).

Panel E of fig. 7 indicates the 11-item patient self-report neurotoxicity tool that describes the CIPN symptom severity and functional consequences. Higher scores indicate a less severe neurotoxicity. A decreased score during time on this subscale is observed in both groups. However, this is not significantly presented by the main time effect of the 2 x 2 mixed ANOVA analyses ($p = 0.08$). In addition, no significance was found for the main group effect ($p = 0.79$) and time x group interaction ($p = 0.74$).

In conclusion, results of the 2 x 2 mixed ANOVA total FACT score presented a non-significant main time effect ($p = 0.36$), main group effect ($p = 0.27$), and time x group interaction ($p = 0.93$).

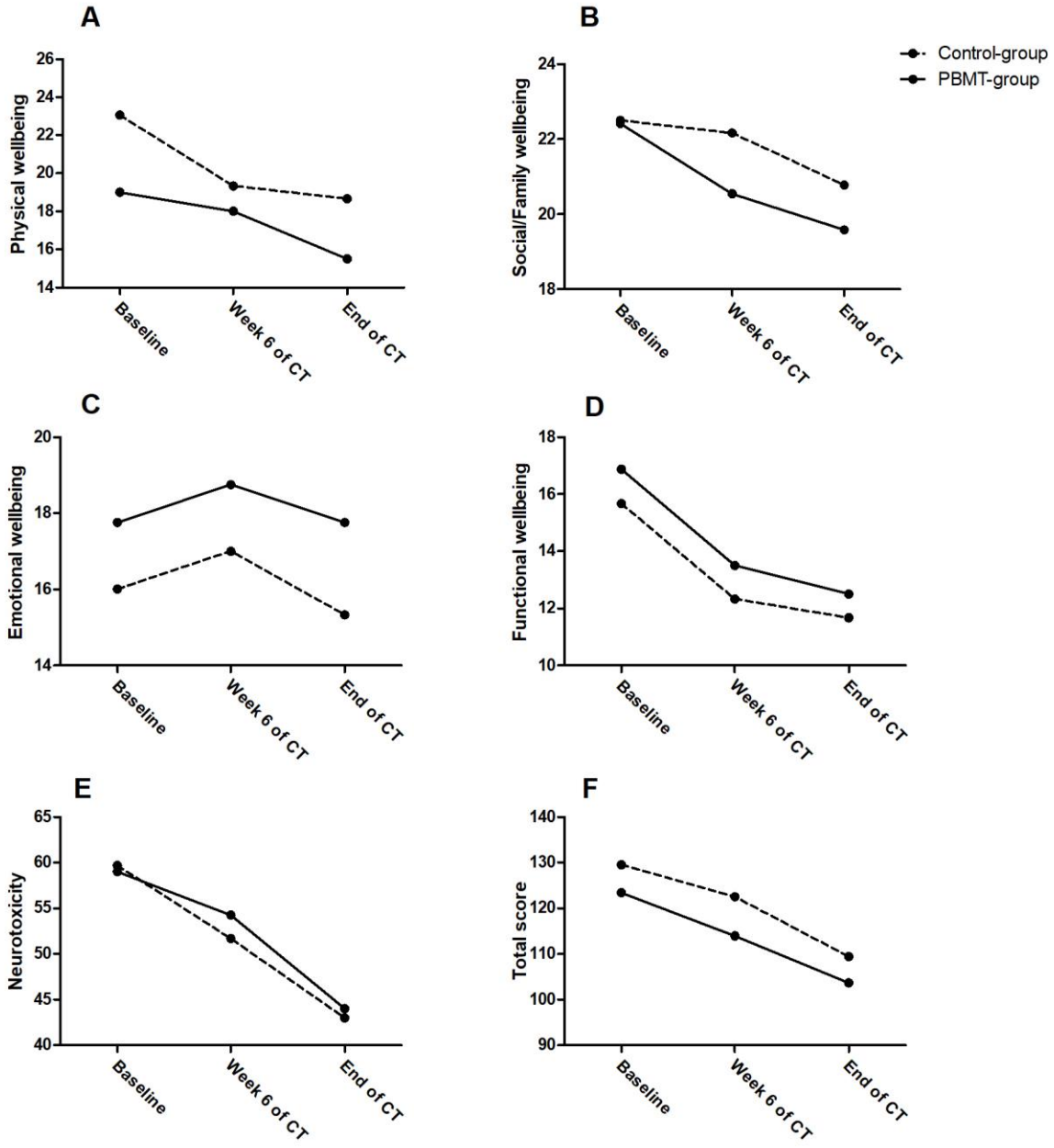

Fig. 7. Functional Assessment of Cancer Therapy/ Gynecologic Oncology Group Taxane scale (FACT/GOG-Taxane): Comparsison of five components (physical wellbeing, social/family wellbeing, emotional wellbeing, functional wellbeing, and neurotoxicity) and a total score of FACT between the control group and the photobiomodulation therapy (PBMT) group.

Patients' satisfaction with the therapeutic intervention

As shown in table 6, no significance was found for the global satisfaction with the treatment and recommendation to other people at the end of CT.

Table 6. Patients' satisfaction with the treatment of the control and PBMT group.

	Control group (n=3)	PBMT group (n=4)	
	Mean ± SD	**Mean ± SD**	**P[a]**
Satisfaction	7.00 (5.20)	9.50 (1.00)	0.66
Recommendation	10.00 (0.00)	9.50 (1.00)	0.39

PBMT, photobiomodulation therapy; SD, standard deviation.

[a]*Wilcoxon Mann-Witney U-test (two-tailed).*

Discussion

Summary and interpretation of the results

Preliminary results of the present study did not demonstrate that the preventive application of PBMT during taxane treatment was associated with a lower mTNS score at the end of CT in comparison with the control group. However, the sensory and motor symptoms subscales of the upper limbs reached almost significance with lower scores in the PBMT group ($p = 0.07$). In addition, all the secondary outcomes measures, *i.e.* quality of life, pain score, mobility tests, and satisfaction with the therapeutic intervention, did not significantly differ between the PBMT- and the placebo group. The main reason for the minor differences between the two study groups, is the small sample size. As determined by sample size calculations, a number of 30 patients in each arm is needed to detect a difference in mTNS score with a power of 80%. In the current analysis only 7 patients were included which is not enough for making strong conclusions.

During the follow up visit, patients allocated to the PBMT group claim to observe an increase in mTNS sensory symptoms after the end of the laser therapy. Although no significance was found to support this, these symptoms indicate the beneficial effect of PBMT for the prevention of taxane-induced peripheral neuropathy. Furthermore, three weeks after the end of CT no significance was found in quality of life, pain score or mobility tests between the placebo- and the PBMT group (data not shown).

PBMT for peripheral neuropathy

Up to now, no others study investigated the effectiveness of PBMT in the prevention of CIPN in BC undergoing taxane treatment. Previous studies that evaluated the effectiveness of PBMT for the management of CIPN are limited. Yamada *et al.* treated 34 BC patients with TIN by an 830 nm GaAIAs Diode laser. The effect of the laser treatment was evaluated using the Brief Pain Index (BPI) questionnaire before and after laser therapy. No other peripheral neuropathy detection methods were used. The laser frequency was not mentioned. This study concluded that PBMT could be used to treat CIPN in an effective and safe manner (34). Furthermore, Argenta *et al.* performed a randomized, double-blinded, sham-controlled, cross-over trial with 70 patients with various cancer types and who were treated with taxanes or platinum-based CT. CIPN was evaluated using the mTNS score and patients were lasered 3-times weekly for 6 weeks (35). The laser parameters (power, wavelength, number of treatment zones, and duration per zone) were determined using a proprietary algorithm driven by the symptom input of the patients' reported symptoms. Results demonstrated that PBMT with or without physiotherapy could significantly reduce the neuropathic symptoms generated during neurotoxic CT.

Hsieh *et al.* lasered 17 gastrointestinal cancer patients with oxaliplatin-induced peripheral neuropathy with a 780 nm GaAIAs diode laser, 3 times per week for 4 weeks. They used the quantitative sensation of touch detection and quantitative cold-triggered pain withdrawal latency (described below) to evaluate the effectiveness of PBMT. Patients demonstrated a significant improvement in both tests after laser therapy (36).

Moreover, PBMT has also been considered as a treatment modality in diabetic distal symmetric neuropathy (18, 31-33). Khamseh *et al.* investigated the effectiveness of PBMT for this condition. Hereby 17 patients with type 2 diabetes were examined using the Michigan Neuropathy Screening Instrument (MNSI) and an nerve conduction study (NCS, described below) before enrolment in the study. A 808-905 nm Multiwave Locked System, MIX5 laser device was used 3 times a week for a total of 10 treatment sessions. At the end of the study, patients showed a significant improvement in nerve conduction velocity (18). Likewise, Kumar *et al.* determined the effect of PBMT on 19 diabetic peripheral neuropathy patients using the 660-850 nm Thor laser. To evaluate the effectiveness of PBMT they used the MNSI, vibration perception threshold and a pain score. During this study, a significant reduction in all the scoring systems was observed (33). However, diabetic peripheral neuropathy arises from a different pathophysiology leading to possibly distinctive outcomes when treated with PBMT. In addition, not all laser parameters were mentioned in these studies and the frequency of laser treatment varied. With exception of the study by Argenta *et al.*, none of the mentioned studies worked with a control group and with a relatively small sample size. In addition, all of these trials comprised a pre-post interventional design with an already existing peripheral neuropathy. In the current study, we investigated the effectiveness of PBMT for the prevention of peripheral neuropathy.

Studies have demonstrated the effect of PBMT in Schwann and nerve cell cultures. Result of these studies suggest that laser therapy can induce Schwann cell proliferation and can affect the nerve cell metabolism (42, 43). According to the study of Hsieh *et al.* PBMT might prevent oxaliplatin-induced peripheral neuropathy by modulating the expression of nerve growth factor and substance P, a neuropeptide involved in transmission of noxious stimuli (30). Lastly, PBMT was shown to induce nerve cell activation, have a positive effect on metabolism of the nerve cells, and to stimulate nerve sprouting processes (44).

PBMT for other cancer therapy related side effects

Our own research group profoundly investigated the effectiveness of PBMT for two main side effects of chemo- and radiotherapy, oral mucositis and acute radiation dermatitis (22, 24). Oral mucositis is a common dose-limiting side effect of many kinds of CT and radiotherapy. It starts as acute inflammation and can lead to an life-threatening stage as a result of physical obstruction of food and water intake (45). Acute radiation dermatitis is a gradually but painful developing radiotherapy-induced skin toxicity occurring in 90% of treated BC patients. It can lead to septic complications or discontinuation of the radiotherapy (46). Results of our own trials have shown that PBMT is able to prevent the development of severe acute skin and mucous reactions as a result of radio- and/or chemotherapy (22, 24).

Furthermore, our research group is currently investigating the effect of PBMT for palmar-plantar erythrodysesthesia or hand-foot syndrome (unpublished data). This condition is caused by the administration of CT with a microvasculature toxicity (e.g. capecitabine) and is characterized with severe skin changes. This can increase the risk for infection and impair the patients' quality of life tremendously resulting in a limited use of potentially effective therapy. By applying PBMT at the affected area, we expect to improve the symptoms associated with this condition. This is supported by the results found by Latifyan *et al.* (47). During this study 32 patients' hands and foots were lasered hemilateraly. 75% Of the patients reported a decreased pain suggesting the effectiveness of PBMT. However, the applications of PBMT for cancer therapy-induced side effects are limitless. A graphical summary of the different kinds of cancer therapy complications that could possibly be treated with PBMT is represented in supplementary figure fig. 2 (48).

Photobiomodulation therapy and cancer

An prerequisite for agents used to prevent cancer therapy-related complications is that it does not adversely affect tumour risk, tumour behaviour, or tumour response to the treatment. The question that rises by many clinicians is if the use of PBMT is safe in cancer patients, due to its stimulatory effects on proliferation and differentiation of target cells (48, 49). Some cell culture studies indicate a growth in cancer cells and increased aggressiveness when stimulated by PBMT though modulation of the Akt/mTOR/CyclinD1 signalling pathway (50, 51). However, these results are contradictive with many others (52, 53).

Wikramanayake *et al.* investigated whether PBMT would provide local protection to cancer cells in a rat model (54). Shay chloroleukemic cells were subcutaneously injected into rat pups who were then randomly divided into four groups. Group I only received cancer cell injection, group II received cancer cell injection and CT, group III received cancer cell injection and PBMT, and group IV received cancer cell injection, CT and PBMT. Afterwards, blood samples were analysed for signs of leukemia. As expected, pups of group I and III were not protected for leukemia whereas 20% of group II and even 22% of group IV was protected against leukemia. Despite there was no significance between group II and IV, there seems to be a trend that PBMT can have a beneficial effect on cancer treatment (48). This beneficial effect can be possibly explained by the Warburg effect, in which the mitochondria of cancer cells shift their metabolism from oxidative phosphorylation to aerobic glycolysis which consumes much less oxygen. Tumour cells are forced to this change due to their rapid growth exceeding the development of sufficient blood supply and thereby become tolerant to chronic hypoxia. Since PBMT will act on the mitochondria, cancer cells and normal cells may behave differently to laser therapy. Normally, cancer cells contain a low amount of ATP. PBMT can give a greater boost of ATP to the tumour cells, resulting in a higher response to pro-apoptotic stimuli. In contrast, PBMT will only induce an adequate supply of ATP in healthy cells. In addition, in healthy tissue a controlled amount of ROS will be produced that could induce protective mechanisms against the damaging effects of radio- or chemotherapy (supplementary fig. 3). However, this favourable scenario remains a hypothesis at the moment (48).

The synergistic effect of CT and PBMT was also observed during the phase III trial of Antunes *et al.* (55). During this placebo-controlled study, the progression-free survival in patients with head and neck cancer undergoing radio- and chemotherapy together with PBMT to prevent oral mucositis was investigated. A longer progression-free survival was observed in the PBMT group compared to the placebo group. However, PBMT was not applied directly to the tumour site and thus these results cannot be attributed to the Warburg effect. Brandao *et al.* examined the outcome of cancer therapy and incidence of tumour recurrence in patients with locally advanced oral squamous cell carcinoma and treated with PBMT for oral mucositis. Results of this retrospective study show that PBMT did not impact treatment outcomes nor overall survival (56).

Another possible way in how PBMT can attack cancer cells involves the direct effect of the light on the tumour cells themselves. PBMT follows a biphasic dose-response curve in which insufficient power density or too short a time will have no effect whereas too much power density or time may have inhibitory effects (57). Normally, an optimal balance needs to be found to obtain beneficial effects in the management of cancer therapy-related side effects. However, due to this biphasic nature of PBMT, tumour cells itself can be overdosed with laser light resulting apoptosis (48, 58). Unfortunately, this will also affect the healthy tissue.

The contradictive findings on the effect of PBMT on tumour cells can be explained by the assumption that not all cell types respond in the same manner to the laser protocol because of different cell content of endogenous porphyrins and cytochromes. These endogenous porphyrins and cytochromes are acclaimed to play a major role as laser light receptors (26). Furthermore, Ottaviani *et al.* demonstrated that cell cultures can react differently to laser light compared to in vivo (52). When cancer cell lines were exposed to various laser protocols, the net effect was a significant increase in cell metabolism and proliferation. Whereas the same cells were lasered in an in vivo experiment, the opposite effect was observed. This reduced tumour progression could be explained by the recruitment of type I interferon secreting immune cells to fight the cancer. In addition, PBMT caused a reduction in angiogenesis.

Limitations

Limitations could impact or influence the interpretation of the findings. As our study was only designed to be an initial exploration in the effectiveness of PBMT for the prevention of CIPN, it has several limitations that must be acknowledged.

Sample size

Since only seven patients were included in the current study, the risk is high that the observations will be due to chance or that the true effect of PBMT for the prevention of CIPN could not be detected. To overcome this type of bias, more time is needed to complete this research project and reach the estimated sample size of 60 patients. Additionally, a collaboration with other research institutions/hospitals (e.g. Ziekenhuis Oost-Limburg, Genk, Belgium) can be set up in order to achieve the appropriate cohort size.

Blinding

In the current study, only the patients were blinded to avoid the placebo effect. Since the researcher was not blinded during the peripheral neuropathy scoring procedure, researcher bias cannot be excluded. This could have led to an over-estimation of the effect of PBMT for the prevention of CIPN. To avoid this kind of bias, a future double-blinded study can be set up with an extra study nurse applying PBMT.

Scoring systems

In this study the mTNS score was chosen as primary outcome to evaluate the amount of peripheral neuropathy, based on the study of Argenta *et al.* (35). This neuropathy score consists of six subscales in which the vibration threshold seems to have the highest sensitivity in establishing peripheral neuropathy. However, this sensitivity could not be significantly confirmed (supplementary table 3-4). Furthermore, the outcome of the mTNS score highly depends on the researcher administer the test. Evaluating neurotoxicity with quality of life tests such as FACT/GOG-Taxane can cause inevitable bias due to other side effects of taxane therapy. In addition, these scoring systems lack objectivity.

Alternative scoring systems

Diverse validated tests exist to evaluated peripheral neuropathy. Many are developed to test diabetic neuropathy. Since they are very similar to our used mTNS score, the revised neuropathy disability score (NDS), diabetic neuropathy examination (DNE), clinical neurological examination (CNE), the neuropathy impairment score (NIS), MNSI, and the Toronto clinical scoring system (TCSS) will not be discussed within this thesis (59).

Electrodiagnostic testing

Electromyography (EMG) and NCS are commonly used diagnostic procedures in clinical practices and are used to detect nerve and muscle dysfunction. During EMG, a needle electrode is inserted into a muscle to record the electrical activity. In addition, surface electrodes are applied to the skin to measure the nerve conduction (60, 61). Due to the high costs and invasive and painful nature of this procedure, EMG was not used during this project.

Quantitative touch-detection threshold

Quantitative sensation of touch detection was used in the study of Hsieh *et al.* to evaluated CIPN after PBMT (36). Von Frey monofilaments with weight from 0.008 g to 300 g were used to measure touch at the bases of the plantar and palmar sides of each toe and finger tips. Hereby they used a gradual increase in pressure until a withdrawal reflex response was observed (30). Since this type of monofilaments is not used in daily clinical practices to evaluate CIPN, we chose not to work with these during this project.

Quantitative cold-triggered pain withdrawal latency

Besides quantitative touch-detection, Hsieh *et al.* also used a 10°C hand immersion test to evaluate the effectiveness of PBMT to treat CIPN (36). Patients were asked to immerse their hands in a water reservoir until they could no longer tolerate the pain. The latency was recorded. Due to practical and ethical reasons, the quantitative cold-triggered pain withdrawal test was not used during this project.

Skin biopsy

Small fibre conduction cannot be measured with an EMG. A routine test to evaluate small fiber sensory neuropathy is a skin biopsy (62). This technique involves a 3 mm punch biopsy of skin from the affected area. Via immunohistochemical or immunofluorescent staining, identification and counting of intraepidermal nerve fibres can be determined (63). Due to the high invasiveness of this procedure, skin biopsies were not taken in this project.

Conclusion and synthesis

Although we cannot state that PBMT can prevent CIPN, we increased the scientific knowledge on the application of PBMT for the management of CIPN within this project. During this study, preliminary data is generated that forms a basis for further research. When the beneficial effect of PBMT for the prevention of CIPN is proven, standardized protocols can be set up. Since PBMT is a non-invasive treatment method that is easy to use, it can be broadly implemented in any Belgian hospital with an oncology department.

In the future, a multicentre study can be conducted to increase sample size and to validate our findings in a population that differs from the original study population. Afterwards, contacts with the industry can be made to implement the use of PBMT for CIPN in every Belgian hospital. In addition, health care costs can be saved on symptom management and extra hospital visits to monitor CIPN.

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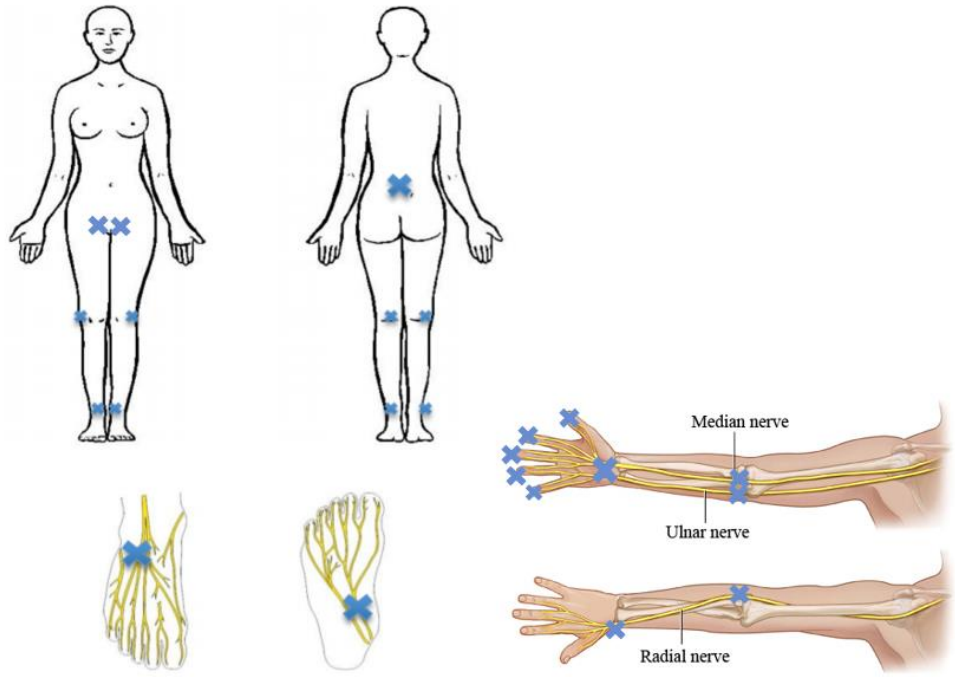
Supplemental information

Supplementary table 1: Photobiomodulation therapy (PBMT) parameters

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
	Wavelength	808 nm	905 nm
	Power	1.1 W	25 W
	Operating mode	Continuous	Pulsed
	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/cm²	
	Beam profile	Two laser beams work simultaneously and synchronously with coincident propagation axes	
Treatment parameters	Beam spot size at target area	3,14 cm2	
	Radiant exposure (fluence)	4 J/cm2	
	Number of points irradiated	- six paravertebral points (L4-S1) -five points along the sciatic nerve in each lower extremity • Femoral triangle • Popliteal fossa • Neck of the fibula • Malleolus lateralis • Malleolus medialis - plantar surface of the feet - dorsal surface of the feet	
	Exposure duration	23,8 seconds per application point of 3,14 cm²	
	Application technique	1 cm above the skin	
	Number and frequency of treatment sessions	2 times/week for 9-12 weeks (depending on the number of CTx cycles)	



Supplementary fig. 1: Laser points lower and upper extremities

Supplementary table 2: modified Total Neuropathy Score (mTNS) score, adapted from Argenta *et al.* (35)

		SYMPTOMS			
SCORE	0	1	2	3	4
SENSORY	None	Symptoms limited to toes/fingers	Symptoms extend to ankles/wrists	Symptoms extend to knees/elbow	Symptoms extend beyond knees/elbow
MOTOR SKILLS	None	Slight difficulty (independent)	Moderate difficulty (independent)	Require assistance	Paralysis
SENSITIVITY TEST: MONOFILAMENT	Normal	Reduced in toes/fingers	Reduced up to ankles/wrists	Reduced up to knees/elbow	Reduced beyond knees/elbow
SENSITIVITY TEST: VIBRATION	Normal	Reduced in toes/fingers	Reduced up to ankles/wrists	Reduced up to knees/elbow	Reduced beyond knees/elbow
STRENGTH	Normal	Mild weakness	Moderate weakness	Severe weakness	Paralysis
REFLEXES	Normal	Reduced ankle reflex	Absent ankle reflex	Absent ankle reflex, reduced knee reflex	All reflexes are absent
					Total score:

Supplementary table 3: Detailled modified Total Neuropathy Score (mTNS) score of the upper limbs at the end of chemotherapy

	Control group (n=3)	PBMT group (n=4)	
	Mean ± SD	Mean ± SD	P[a]
Sensory	1.00 (0.00)	0.25 (0.50)	0.07
Motor skills	1.00 (0.00)	0.25 (0.50)	0.07
Sensitivity test: monofilament	0.33 (0.58)	0.50 (0.58)	0.68
Sensitivity test: vibration	0.33 (0.58)	0.00 (0.00)	0.25
Strength	0.33 (0.58)	0.00 (0.00)	0.25
Reflexes	3.67 (0.58)	4.00 (0.00)	0.25
Total	6.67 (2.08)	5.00 (0.82)	0.20

PBMT, photobiomodulation therapy; SD, standard deviation.

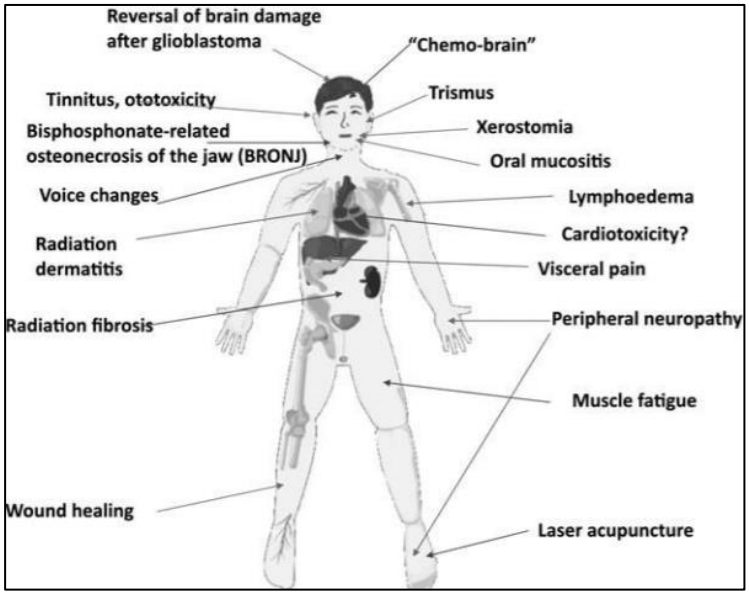
[a]Wilcoxon Mann-Witney U-test (two-tailed).

Supplementary table 4: Detailled modified Total Neuropathy Score (mTNS) score of the lower limbs at the end of chemotherapy

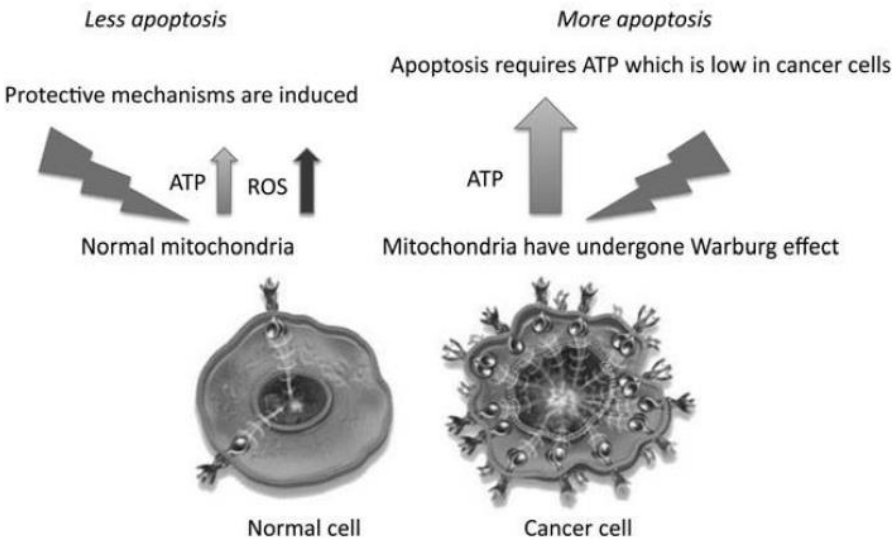
	Control group (n=3)	PBMT group (n=4)	
	Mean ± SD	Mean ± SD	P[a]
Sensory	0.00 (0.00)	0.25 (0.50)	0.39
Motor skills	0.33 (0.58)	0.25 (0.50)	0.82
Sensitivity test: monofilament	0.33 (0.58)	0.25 (0.50)	0.82
Sensitivity test: vibration	1.33 (0.58)	1.75 (0.96)	0.55
Strength	0.67 (1.15)	0.00 (0.00)	0.25
Reflexes	4.00 (0.00)	3.75 (0.50)	0.39
Total	6.67 (1.15)	6.25 (2.50)	0.27

PBMT, photobiomodulation therapy; SD, standard deviation.

[a]Wilcoxon Mann-Witney U-test (two-tailed).



Supplementary fig. 2: Cancer therapy-induced side effects possibly treated by photobiomodulation therapy (48).



Supplementary fig. 3: Warburg effect: Possible effect of photobiomodulation therapy on normal and cancer cells when combined with radio- or chemotherapy (48).

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Richting: **Master of Biomedical Sciences-Clinical Molecular Sciences**
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Datum: **7/06/2018**