Photochemical & Photobiological Sciences

PERSPECTIVE

View Article Online
View Journal | View Issue

Cite this: *Photochem. Photobiol. Sci.*, 2019, **18**, 1621

Examining tumor modulating effects of photobiomodulation therapy on head and neck squamous cell carcinomas

Felipe Martins Silveira, [a] Mariana de Pauli Paglioni, [a] Márcia Martins Marques, [b] Alan Roger Santos-Silva, [a] Cesar Augusto Migliorati, [c] Praveen Arany [d] and Manoela Domingues Martins *[a,e]

Photobiomodulation (PBM) therapy is an effective method for preventing and managing oral mucositis (OM) in head and neck squamous cell carcinoma (HNSCC) patients undergoing radiotherapy alone or in combination with chemotherapy. However, the potential effects of PBM therapy on premalignant and malignant cells eventually present in the treatment site are yet unknown. The aim of this systematic review was to analyze the effects of PBM therapy on HNSCC. A literature search was conducted in four indexed databases as follows: MEDLINE/PubMed, EMBASE, Web of Science, and Scopus. The databases were reviewed for papers published up to and including in October 2018. *In vitro* and *in vivo* studies that investigated the effects of PBM therapy on HNSCC were selected. From the 852 initially gathered studies, 15 met the inclusion criteria (13 *in vitro* and 2 *in vivo*). Only three *in vitro* studies were noted to have a low risk of bias. The included data demonstrated wide variations of study designs, PBM therapy protocols, and study outcomes. Cell proliferation and viability were the primary evaluation outcome in the *in vitro* studies. Of the 13 *in vitro* studies, seven noted a positive effect of PBM therapy on inhibiting or preventing an effect on HNSCC tumor cells, while six studies saw increased proliferation. One *in vivo* study reported increased oral SCC (OSCC) progression, while the other observed reduced tumor progression. Overall, the data from the studies included in the present systematic review do not support a clear conclusion about the effects of PBM therapy on HNSCC cells.

Received 12th March 2019,
Accepted 9th June 2019

DOI: 10.1039/c9pp00120d

rsc.li/pps

Introduction

Head and neck squamous cell carcinoma (SCC) (HNSCC) treatment encompasses three modalities (surgery, radiotherapy, and chemotherapy) that can be administered exclusively or concomitantly with one another depending on the site of the cancer and the stage of the disease.[1,2] Use of the radiotherapy approach often leads to acute toxicities such as oral mucositis (OM), which is clinically characterized by painful ulcerations in the oral mucosa.[3,4] OM limits adequate nutritional intake, increasing the risk of malnutrition and poor quality of life, with the possibility of being a factor that increases overall treatment costs and negatively impacts cancer prognosis due to pain, bacteremia, and treatment interruptions.[5–7]

Photobiomodulation (PBM) therapy, also known as low-level light therapy, is one of the therapeutic approaches for OM management.[8–10] At this time, there are three well-described mechanisms of PBM therapy.[11] The first, involves an intracellular chromophore, cytochrome-C oxidase in the mitochondria; the second, cell membrane light-sensitive receptors such as opsins and TRPV1; and, the third, an extracellular latent growth factor, TGF-β1. In animal studies, PBM therapy has demonstrated positive effects on the management of OM by promoting tissue repair and anti-inflammatory effects.[12–15] Human clinical trials have also demonstrated positive results with the use of PBM therapy for preventing and managing OM.[16–18] Based on these therapeutic benefits of PBM therapy, the Multinational Association of Supportive Care in Cancer (MASCC) and the International Society of Oral Oncology (ISOO) had designated PBM therapy as an effective adjunctive treatment for managing OM in 2013 (currently under revision).[8]

[a]*Oral Diagnosis Department, Piracicaba Dental School, University of Campinas, Piracicaba-SP, Brazil. E-mail: manomartins@gmail.com; Tel: +55 (51) 3308-5011*
[b]*Department of Restorative Dentistry, School of Dentistry, University of Sao Paulo, Sao Paulo-SP, Brazil*
[c]*College of Dentistry, University of Florida, Gainesville, FL, USA*
[d]*Departments of Oral Biology and Biomedical Engineering, Schools of Dental Medicine, Engineering and Applied Sciences, State University of New York at Buffalo, Buffalo, NY, USA*
[e]*Department of Oral Pathology, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre-RS, Brazil*

PBM therapy offers an innovative, noninvasive, and non-pharmacological approach for OM management. There have been no reports of any side effects and it is well-tolerated by tissues. However, the effects of PBM therapy on pre-transformed or residual primary tumor cells present in the laser treatment field are still being debated. Investigations regarding the effects of PBM therapy on neoplastic cells have yielded contradictory results.[19–25] Therefore, the safety of PBM therapy in HNSCC patients remains of major concern once laser treatments can be applied to tissues within, or contiguous to, a tumor site.[26] The aim of the present systematic review was to analyze studies that investigated the effects of PBM therapy on HNSCC cells.

Materials and methods

Protocol registration and focused question

This review was registered with the National Institute for Health Research's International Prospective Register of Systematic Reviews (http://www.crd.york.ac.uk/PROSPERO#CRD42017079588). We followed the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines.[27] The specific question for this review was: "What are the effects of photobiomodulation therapy in head and neck squamous cell carcinoma?"

Search strategy

The research was constructed according to the Populations, Interventions, Comparison, Outcomes, and Study Design (PICOS) principle. Individual search strategies were designed for each of the following bibliographic databases: MEDLINE/PubMed, EMBASE, Web of Science and Scopus. The four named electronic databases were searched to identify relevant articles published up to and including in October 2018. All publications included were in the English language only, with no restrictions on year of publication. All publications presented in these databases contained a combination of controlled predefined Medical Subject Heading (MeSH) terms and free terms related to PBM therapy in HNSCC, using Boolean operators (*i.e.*, OR, AND) to combine searches. Previously defined terms were adapted to the rules of syntax of each bibliographic database and included (((((((“tumor cells, cultured” [MeSH terms]) OR “neoplastic stem cells” [MeSH Terms]) OR “tumor stem cells”) OR “neoplasms”) OR “tumor”)) AND (((((((“low-level light therapy” [MeSH Terms]) OR “low-level laser therapy”) OR “laser therapies, low-level”) OR “irradiation, low-power laser” OR “laser phototherapy”) OR “therapies, photobiomodulation”) OR “phototherapy, laser”)) AND ((((“cell proliferation” [MeSH terms]) OR “cell growth number”) OR “tumor growth”) OR “stimulatory effect”). Additionally, a manual search of bibliographies and reference lists of all included studies were performed to identify any publications not previously retrieved as part of the primary database searches.

Eligibility criteria

Inclusion criteria. This systematic review was based only on the contents of original research studies investigating the effects of PBM therapy on HNSCC. Study inclusion criteria were as follows: those that contained (1) study population(s) with HNSCC neoplastic cells and/or HNSCC tumors; (2) PBM therapy as an intervention; (3) no treatment as a comparison group; and (4) effects of PBM therapy on the treated population as outcomes.

Exclusion criteria. Review papers, letters to the editor, monographs, conference papers, book chapters, unpublished data, and studies published in a language other than English were all excluded. Separately, original research studies were excluded when: (1) PBM therapy was used along with other types of cancer treatments; (2) light therapy was performed with the use of external cromophores, such as in photodynamic therapy; (3) PBM therapy was not used as a treatment; and/or (4) the population(s) assessed were not HNSCC-related.

Study selection and data extraction

Titles and abstracts of all studies were reviewed and, based on the eligibility criteria, full texts were retrieved for complete review. Two reviewers (F. M. S. and M. D. M.) reviewed all of the papers independently and any disagreements were discussed with a third reviewer (A. R. S. S.) for concordance. The following relevant information from eligible studies was collected: (1) publication details (first author and year); (2) samples [cell line(s) or animal model(s)]; (3) samples' characteristics (for *in vitro* studies: number of cells, darkness, distance between wells, reproducibility, growth medium; and for *in vivo* studies: environmental conditions, tumor induction, groups); (4) types and methods of evaluations; (5) main outcomes; and (6) major conclusions. Specific attention was focused on laser treatment parameters, as follows: (1) active medium; (2) application procedure; (3) wavelength (in nanometers or nm); (4) energy density (also called fluence, in Joules per square centimeters or J cm^{-2}); (5) power (in milliwatts or mW); (6) power density (also called irradiance, in mW cm^{-2}); irradiation time (in seconds); (7) spot size (in cm^2); (8) energy per point (in J); (9) schedule of irradiation; and (10) total energy (in J). These laser parameters analyzed were based on the consensus agreement of the design and the conduct of studies recommended by the World Association for Photobiomodulation Therapy (WALT). All the papers selected were organized using EndNote (Clarivate Analytics, Philadelphia, PA, USA).

Risk of bias assessment

For the assessment of bias, included studies were separated into *in vitro* or *in vivo* investigations. For the methodological quality of each *in vitro* study, criteria based on the parameters for developing cell culture studies were adopted.[28] The included articles were evaluated according to the following descriptions: (1) condition of cell culture; (2) description of methodology to evaluate outcomes; (3) reproducibility; (4) methods for preventing unintentional light scattering during laser application; (5) description of laser treatment parameters according to WALT recommendations; and (6) concurrence of conclusions based on the results obtained. The information

was classified as Yes when it was possible to find the information or No if the information was not described, respectively. The publications were classified according to their risk of bias as "high" (one or two items classified as Yes), "medium" (three or four items classified as Yes), or "low" (five or six items classified as Yes). Regarding *in vivo* studies, the Systematic Review Centre for Laboratory Animal Experimentation's (SYRCLE) risk of bias tool was used to assess the quality of available evidence.[29] The items here were scored as Yes, No, or Unclear.

Statistical analysis

Due to a lack of methodological uniformity in the included studies, a meta-analysis of the obtained results was not feasible. Therefore, the results are instead descriptively summarized in this review.

Results

Study selection

A total of 852 potentially relevant records were identified from the databases and further processed as per the PRISMA statement (Fig. 1).[27] After the removal of duplicates, 581 records were further examined based on their titles and abstracts and 505 studies were excluded, as they did not meet the specific eligibility criteria for this study. A total of 76 full-text articles were finally evaluated and 64 were subsequently excluded for the following reasons: (1) PBM therapy was investigated in cell lines or tumors other than HNSCC (44 papers); (2) PBM therapy was associated with another type of treatment such chemotherapy or photodynamic therapy (16 papers); (3) SCC cell line was irradiated with CO_2 laser (one paper); or (4) the paper was a review paper (three papers). Three studies were further included through a manual search of the bibliographies of included studies. A total of 15 studies fulfilled the selection criteria of the present review and were included for qualitative analysis.[19–23,30–39]

General characteristics of included studies

Of the 15 included studies, 13 were restricted to *in vitro* analyses, while two studies performed *in vivo* studies. The relevant studies were conducted in various centers around the world such as Brazil, Germany, Austria, Italy, and Taiwan and were published between 1997 and 2018 (Fig. 2). The general descriptions of the included studies are summarized (Table 1, *in vitro* studies and Table 2, *in vivo* studies). The 13 *in vitro* studies evaluated various HNSCC cell lines arising at specific anatomical sites such gingival mucosa (ZMK and ZMK1), larynx (HEp-2), KB (human papillomavirus-infected), SCC9 (tongue), SCC25 (tongue), and OC2 (buccal mucosa). Among these, 12 (92.3%) reported the number of cells used in the experiments, three (23.0%) reported ambient light conditions during PBM therapy treatments, five (38.4%) reported the distance between irradiated wells, seven (53.8%) reported replicates, and 12 (92.3%) described the growth medium used. Several methods were employed to assess tumor cell proliferation or viability from dye exclusion utilizing Trypan blue or neutral red to

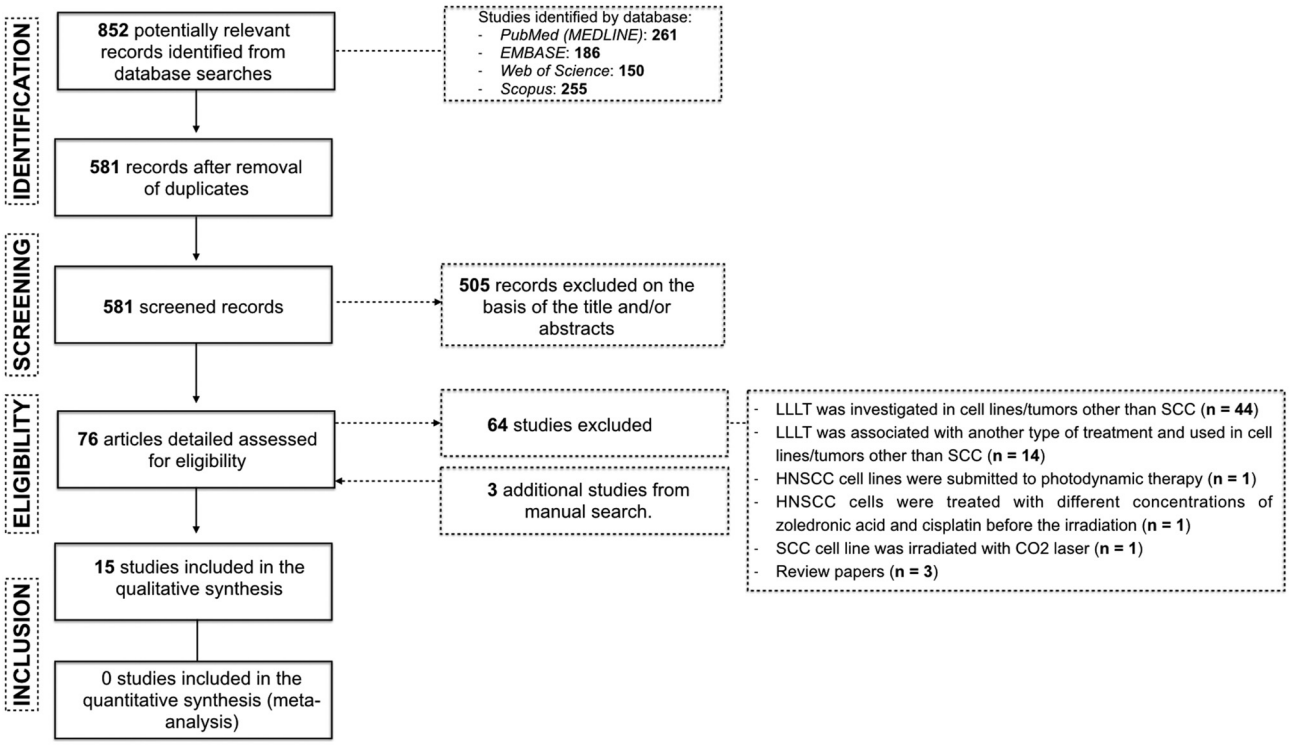

Fig. 1 PRISMA flow diagram for systematic search and studies selection strategy.

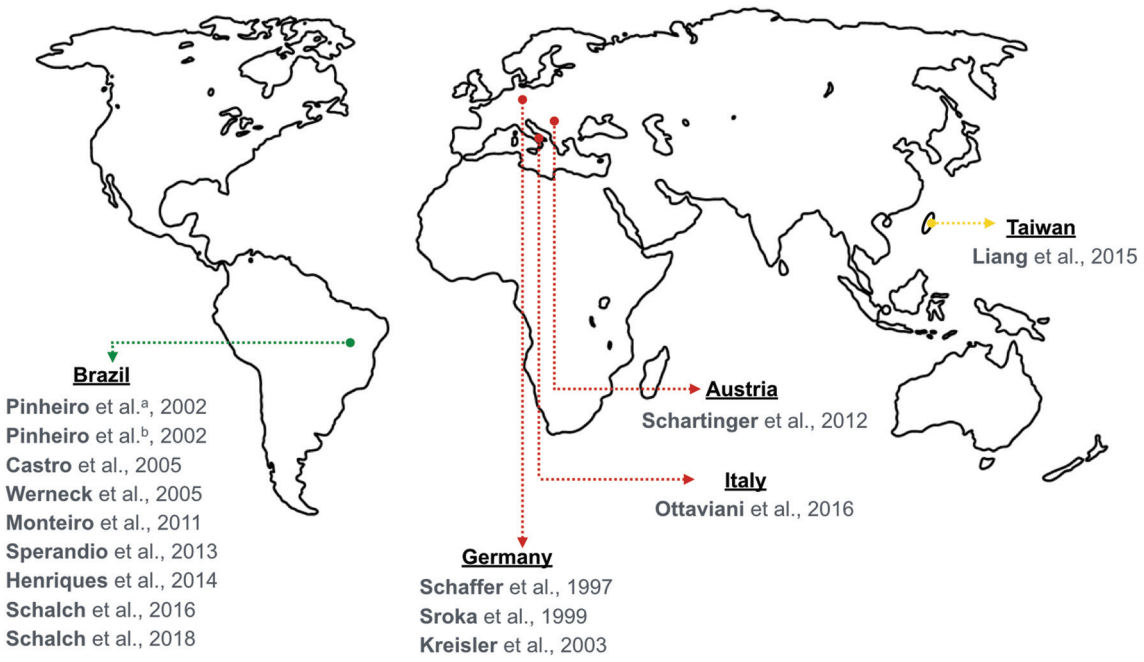

Fig. 2 Distribution of the included studies according to its respective countries.

BrdU incorporation as well as enzymatic substrate cleavage such as MTT, WST-1, or AlamarBlue assays. Some studies also assessed the mitotic index *via* orcein staining and microscopy or propidium iodide staining and FACS. Tumor cell death was assessed using annexin staining and FACS, TdT-mediated dUTP nick-end labeling (TUNEL) staining, or caspase-3 activity. The ability of tumor cells to migrate or invade was assessed with a scratch-wound assay or Transwell chambers, respectively. One study further examined invasiveness by investigating the osteoclastogenic response using TRAP activity, interleukin-11 (IL-11), and parathyroid hormone-related protein (PTHrP) gene expression, respectively.[37] Another study assessed reactive oxygen species (ROS) production by tumor cells using FACS.[23] Both included *in vivo* studies utilized carcinogen-induced tumor models with 7,12-dimethylbenz[*a*] anthracene (DMBA) in golden Syrian hamsters or 4-nitroquinoline-1-oxide (4-NQO) in mice. These studies examined the effects of PBM therapy on induction. The detailed description of animal studies that were conducted was provided only in one of the research papers.[38] These studies used histopathology and immunohistochemistry for tumor assessment.

PBM treatment parameters used in these studies

There is still an incomplete understanding of the critical PBM treatment characteristics for effective therapeutic clinical dosing. All of the parameters used in the included 15 studies are summarized and outlined below (Table 3).

Laser source. The laser source was specifically reported in six (40%) studies as gallium–aluminum–arsenide diode. One study employed an argon laser. The remaining studies did not explicitly specify the laser source(s) they used; however, the wavelengths reported suggested they all incorporated diode lasers.

Wavelength. Five studies used only one wavelength within the visible red spectrum (630–670 nm) and three used the wavelength of 660 nm. Two studies compared the effects of application at 635 nm and 670 nm. Near-infrared wavelengths (780, 808, 830, and 850 nm) were also investigated either by themselves or in combination with visible red wavelengths.

Beam characteristics. Nine studies (60%) used the laser in continuous-wave (CW) mode, while the rest did not report on this parameter. Five studies reported the distance between the laser source and the cells or tissue (two treatments were in contact mode, whereas the other three used noncontact mode. In the latter studies, distance between the laser source and cells varied from 0.5–2 cm).

Power, spot size and power density (irradiance). The maximal laser power was reported in 12 studies and varied from 5 to 25 W. Power density is the effective power output at a given surface area and is reported in mW cm^{-2}. This parameter was reported in nine studies and varied from 0.39 to 1000 mW cm^{-2}. The actual illuminated surface is deemed as the spot size and can be as small as the size of the laser probe tip itself when used in contact mode. Alternatively, in noncontact mode, the spot size were calculated as the effective illuminated treatment area. Seven of 15 studies reported spot sizes varying from 0.039 to 0.8 cm^2.

Treatment time and schedule. Although the time is a critical factor in dose estimation, surprisingly, only eight of the 15 studies reported this parameter, with findings varying from 8.2 to 450 seconds. Also, nine of the 15 studies informed on the number of treatment sessions and intervals, which ranged from one to seven days of consecutive treatments.

Table 1 Description of experimental details of *in vitro* studies included in this review

Study; year	Samples Cell line/animal model	Study design 1. Number of cells; 2. Darkness; 3. Distance between wells; 4. Reproducibility; 5. Growth medium.	Type of evaluation/method	Main outcomes	Main conclusions
Schaffer *et al.*, 1997 [19]	Human SCC of the gingival mucosa (**ZMK**)	1. Not mentioned 2. Not mentioned 3. Not mentioned 4. Yes 5. Not mentioned	Mitotic index by Orcein staining DNA-synthesis by BrdU-test	ZMK tumor cells showed a non-significant decrease of the mitotic index compared to control in different irradiances The irradiation had no influence on the DNA synthesis rate in all groups	PBM therapy promoted an inhibition of human SCC tumor cells
Sroka *et al.*, 1999 [20]	Human SCC of the gingival mucosa (**ZMK1**)	1. Yes. 2. Not mentioned. 3. Not mentioned. 4. Yes. 5. Yes.	Cell proliferation by BrdU-test Rate of mitosis by Orcein-staining	Cell treatment with λ = 805 nm had no influence on the DNA-synthesis rate ZMK1 cells exhibited similar results using λ = 630 nm, λ = 635 nm, λ = 805 nm. Decrease in the mitotic rate when exposed to light with 2–8 J cm^{-2} and remained stable up to 20 J cm^{-2}. There was no change in the mitotic rate in dependency of the irradiance	PBM therapy resulted in an inhibition of human SCC tumor cells
Pinheiro *et al.*, 2002a [30]	SCC of the larynx (**H.Ep.2 cells**)	1. Yes 2. Not mentioned 3. Yes 4. Not mentioned 5. Yes	Cell proliferation by MTT method	The 670 nm group showed a tendency to increase cell proliferation when compared to control (p = 0.014) and 635nm group (p = 0.004). Control and 635nm groups were similar (p = 0.455)	Cell proliferation increases in H.Ep.2 cells irradiated with 670 nm. Dose and wavelength may affect cell proliferation
Pinheiro *et al.*, 2002b [31]	SCC of the larynx (**H.Ep.2 cells**)	1. Yes 2. Not mentioned 3. Yes 4. Not mentioned 5. Yes	Cell proliferation by MTT method	Significant differences in the proliferation were observed between the two concentrations of FBS (p = 0.002) and between irradiated cultures and controls. Influence of the nutritional status of the culture of both 670 nm and 635 nm irradiated cultures was significantly different. The effect of the wavelength was also demonstrated at the same %FCS (p = 0.000)	Irradiation with 670 nm applied at doses from 0.04 J cm^{-2} results in an increased cell proliferation

Table 1 *(Contd.)*

Study; year	Samples Cell line/animal model	Study design 1. Number of cells; 2. Darkness; 3. Distance between wells; 4. Reproducibility; 5. Growth medium.	Type of evaluation/method	Main outcomes	Main conclusions
Kreisler *et al.*, 2003 [32]	Human larynx carcinoma cells	1. Yes 2. Not mentioned 3. Not mentioned 4. Not mentioned 5. Yes	Proliferation activity by Alamar Blue assay	After 24 h and 72 h, the irradiated cell cultures showed a higher proliferation activity compared to controls in all irradiation regimens	809 nm PBM therapy had a considerable stimulatory effect on the cell proliferation
Castro *et al.*, 2005 [33]	Oral carcinoma cells, strain KB	1. Yes 2. Not mentioned 3. Yes 4. Not mentioned 5. Yes	Cell proliferation by MTT method	Cultures irradiated with λ = 830 nm exhibited increased proliferation than control (from 24 h until 72 h). The results demonstrated that time influenced significantly both controls and cultures irradiates with λ = 685 nm and λ = 830 nm	Positive biomodulatory effect of PBM therapy on the proliferation of KB cells. It was influenced by the wavelength
Werneck *et al.*, 2005 [34]	SCC of the larynx (**H.Ep.2 cells**)	1. Yes 2. Not mentioned 3. Yes 4. Not mentioned 5. Yes	Cell proliferation by MTT method	Cultures irradiated with λ = 685 or λ = 830 nm wavelengths had increased cellular proliferation compared to non irradiated controls. Time had a significant effect on the proliferation of samples irradiated by λ = 685 nm	Positive biomodulatory effect of PBM on H. Ep.2 cells irradiated by λ = 685 and λ = 830 nm lasers compared with controls non irradiated samples
Schartinger *et al.*, 2012 [21]	Human oral SCC cell line (**SCC25**).	1. Yes 2. Not mentioned 3. Not mentioned 4. Yes 5. Yes	Cell proliferation by MTT method Cell cycle analysis by FACS analysis (PI DNA staining) Apoptosis assay by FACS analysis (Annexin V-FITC)	Lower absorbance was observed after PBMT treatment of SCC-25 than the sham controls (p < 0.001). PBMT induced a significant 0.8-fold decrease in the level of proliferation in SCC-25 cells PBM induced an increase in the percentage of S-phase in SCC-25 (p < 0.001). The increase in S-phase cells paralleled the decrease in G1-phase In SCC-25 cells, the relative amount of Annexin V^+ cells was higher in laser treated cultures than in the controls (p = 0.02)	No proliferative or antiapoptotic effects of PBM on SCC cells were observed

Table 1 *(Contd.)*

Study; year	Samples Cell line/animal model	Study design 1. Number of cells; 2. Darkness; 3. Distance between wells; 4. Reproducibility; 5. Growth medium.	Type of evaluation/method	Main outcomes	Main conclusions
Sperandio *et al.*, 2013 [35]	Oral SCC cell lines (**SCC9** and **SCC25**).	1. Yes	Cellular viability by MTS assay	SCC9 lines presented general enhanced cell viability (λ = 780 nm) and pronounced inhibition of growth (λ = 660 nm). SCC25 lines showed growth stimulation at some fluences (λ = 660 nm and λ = 780 nm). SCC9 and SCC25 had a tendency to show lower levels of cell viability at the latest evaluation time point (72 h)	PBM therapy can modify SCC9 and SCC25 cell lines growth by modulating the Akt/mTOR/CyclinD1 signaling pathway. PBM significanty modified the expression of proteins related to progression and invasion in all the cell lines and could aggravate oral cancer cellular behavior. Apoptosis was detected for SCC25
		2. Not mentioned	Protein analysis by western blot and immunofluorescence	The Akt, pAkt, Hsp90, S6, CyclinD1, β-actin were influenced by PBM. PBM increased the expression of pAkt, pS6 and cyclin D1 and produced an aggressive isoform of Hsp90	
		3. Not mentioned	Apoptosis assay by TUNEL	Apoptosis was only detected in SCC25 cell line irradiated with λ = 780 nm, 6.15 J cm^{-2} at 48 h and 3.07 J cm^{-2} at 72 h	
		4. Not mentioned 5. Yes			
Henriques *et al.*, 2014 [36]	Human tongue SCC (**SCC25**)	1. Yes	Cell growth by Trypan blue	After 24 h, SCC25 cells irradiated with 1.0 J cm^{-2} showed the highest proliferation when compared to the control and the group irradiated with 0.5 J cm^{-2} (p = 0.019)	PBM therapy stimulated the proliferation and invasion of SCC25 cells in a dose- and time-dependent manner, influencing the expression of cyclin D1, B-catenin, E-cadherin and MMP-9
		2. Yes[a]	Cell cycle by flow cytometry (PI)	After 24 h, all groups showed a reduction in the number of cells in the G0/G1 phase with an increase in the S and G2/M phases. $L_{1.0}$ demonstrated a more pronounced difference (p = 0.027) and the control group the lowest proportion of cells in the S and G2/M phases (p = 0.027). Laser-irradiated groups showed generally constant or slightly higher proportion of cells in the S and G2/M phases compared to control. $L_{1.0}$ presented the highest proportion of cells in the G2/M phase throughout the experiment (p = 0.027)	
		3. Yes	Protein analysis (cyclin D1, β-catenin, E-cadherin, MMP-9) by immunofluorescence and flow cytometry	PBMT influenced the expression of cyclin D1, β-catenin, E-cadherin and MMP-9. Cyclin D1 and nuclear β-catenin demonstrated an increased expression. PBMT at 1.0 J cm^{-2} significantly reduced E-cadherin and induced MMP-9 expression	
		4. Yes	Invasion assay by transwell chamber	A significantly higher invasion potential was observed for $L_{1.0}$ when compared to control and $L_{0.5}$ group (p < 0.001)	
		5. Yes			

Table 1 *(Contd.)*

Study; year	Samples Cell line/animal model Study design 1. Number of cells; 2. Darkness; 3. Distance between wells; 4. Reproducibility; 5. Growth medium.	Type of evaluation/method	Main outcomes	Main conclusions
Liang *et al.*, 2015 [23]	Human oral cancer OC2 cells (**OC2**)			
	1. Yes	Cell viability assay by WST-1	PBMT significantly diminished cell viability of OC2 cells	PBM therapy induced apoptosis in human oral cancer cells, possible mediated by ROS production and the loss of MMP
	2. Not mentioned	Cell cycle by FACScan flow cytometer (PI)	PBMT increased the number of OC2 cells in G_1 and subG_1 phases	
	3. Not mentioned	ROS production measurement by FACScan flow cytometer (H_2DCFDA)	The production of ROS was significantly elevated in irradiated OC2 cells	
	4. Yes	MMP detection by FACScan flow cytometer (CCCP)	MMP was lost in irradiated OC2 cells	
	5. Yes	Apoptosis analysis by FACScan flow cytometer (Annexinv V^+)	PBM increased the number of apoptotic cells in OC2 cells	
Schalch *et al.*, 2016 [37]	Human lingual SCC (**SCC9**)			
	1. Yes	Viability/proliferation of SCC9 cells by MTT assay	10^3 SCC9 per cm^2 showed increases in cell proliferation compared to non-irradiated. 10^4 SCC9 per cm^2 showed cell proliferation slightly lower (day 1) or similar/higher (longer periods) than the non-irradiated	PBM irradiation with an energy density of 4 J cm^{-2} decreased the pro-osteoclastogenic potential of SCC9 cells
	2. Yes[b]	Characterization of the osteoclastogenic response by TRAP (*p*NPP) hydrolysis assay), actin rings and expressing vitronectin and calcitonin receptors	Co-cultures with 10^3 SCC9 per cm^2 showed no significant differences until day 14. At day 21, it was observed sharp TRAP decreases of ~60% (660 nm–4 J cm^{-2}–40 mW) and ~90% (780 nm–4 J cm^{-2}–70 mW) compared with SCC9 non-irradiated cells. With 10^4 SCC9 per cm^2, it was observed a slight TRAP decrease at day 14 and a sharp TRAP decrease at day 21 (4 J cm^{-2}). The 70 mW output power caused the highest TRAP decrease	
	3. Not mentioned	Characterization of the osteoclastogenic response by intracelular signaling pathways (MEK, p38, NFkB, JNK)	For monocultured PBMC, TRAP activity partially decreased in the presence of MEK and JNK, was abolished fo NFkB and was not significant for p38	
	4. Yes	IL-11 and PTHrP gene expression by RT-PCR method	For non-irradiated SCC9 + PBMC, no significant effects for TRAP activity was seen in the presence of MEK and p38 pathway inhibitors, decreasing in the presence of NFkB and JNK	
	5. Yes		For irradiated SCC9 + PBMC, MEK and p38 pathways inhibitors significantly decreased TRAP activity. TRAP activity was also reduced for NFkB and JNK Increased expression of IL-11 and PTHrP in irradiated cells with 780 nm–4 J cm^{-2}–40 mW and 660 nm–4 J cm^{-2}–40 mW at day 2. With 9 days, the molecules expressions in irradiated cultures were lower than in the non-irradiated ones	

Table 1 *(Contd.)*

Study; year	Samples Cell line/animal model	Study design 1. Number of cells; 2. Darkness; 3. Distance between wells; 4. Reproducibility; 5. Growth medium.	Type of evaluation/method	Main outcomes	Main conclusions
Schalch *et al.*, 2018 [39]	**SCC9** cell line	1. Yes	Mitochondrial activity by MTT assay	PBMT significantly decreased the mitochondrial activity of irradiated SCC9 cells compared to control, except for the cells irradiated with 660 nm–30 mW–2 J cm^{-2}	PBM therapy with 780 nm–70 mW and 40 mW–4 J cm^{-2} demonstrated an induction on apoptosis and a reduction on cell viability and migration capacity of irradiated SCC9 cell line
		2. Yes[c]	Apoptosis by caspase 3 activity	PBMT significantly increased caspase 3 activity of irradiated SCC9	
		3. Not mentioned	Cell viability by neutral red assay	Cell viability of irradiated SCC9 cells was significantly decreased with 660 nm–40 mW–4 J cm^{-2} and 780 nm–40 mW and 70 mW–4 J cm^{-2}	
		4. Yes	Cell proliferation by BrdU assay	No differences in the number of BrDU-positive cells were found between irradiated and control cells	
		5. Yes	Migration by scratch-wound assay	Reduction in the migration capacity of the tumor cells	

[a] Partial darkness. [b] Dim lighting. [c] Minimal ambient lighting. BrdU, 5-bromo-2-deoxyuridine; λ, wavelength; nm, nanometer; PBM, photobiomodulation; SCC, squamous cell carcinoma; MTT, 3-(4,5-dimethyl-2-thia-zolyl)-2,5-diphenyl-2*H*-tetrazolium bromide; FACS, fluorescent-activated cell sorting; PI, propidium iodide; FITC, fluorescein isothiocyanate; TUNEL, TdT-mediated dUTP Nick-end labeling; MMP, matrix metalloproteinases; TRAP, tartrate-resistant acid phosphatase; *p*NPP, *para*-nitrophenyl phosphate; NFkB, nuclear factor kappa B; IL-11, interleukin 11; PTHrP, parathyroid hormone-related protein; RT-PCR, reverse transcription-polymerase chain reaction; ROS, reactive oxygen species

Table 2 Description of experimental details of *in vivo* studies included in this review

Study; year	Cell line/animal model	Samples Study design 1. Environmental conditions; 2. Tumor induction; 3. Description of groups.	Type of evaluation/method	Main outcomes	Main conclusions
Monteiro *et al.*, 2011 [22]	Oral chemical carcinogenesis (DMBA) on hamsters cheek pouch model	1. Not mentioned	Histological analysis by light microscopy	G1 showed 100% well-differentiated SCC. G2 showed 20% moderately differentiated and 80% well-differentiated SCC. G3 showed 40% well differentiated, 40% poorly differentiated, and 20% moderately differentiated SCC	PBM caused a significant progression of the severity of SCC in the oral cavity of hamsters
		2. Yes			
		3. Yes			
Ottaviani *et al.*, 2016 [38]	Oral carcinogenesis (4NQO) mouse tongue model	1. Yes	Microscopic evaluation by histopathological analysis (diagnosis and grading), immunofluorescence (fluorescein isothiocyanate-labeled lectin) and immunohistochemistry (anti-CD31 and -αSMA)	αSMA^{+} arterioles were significant increased in laser treated lesions. A more regular and structured vessel pattern was showed by the perfusion of fluorescein-labeled lectin	PBM inhibited tumor progression and improved functional vessel maturation
		2. Yes		Laser treatment reduced the appearance of dysplastic lesions and was more effective in reducing the number and the percentage of both *in situ* and invasive carcinomas	
		3. Yes			

Table 3 Parameters used for PBM treatments in studies included in this review

Study; year	Active medium	Application procedure	Wavelength (nm)	Energy density (J cm^{-2})	Power (mW)	Power density (mW cm^{-2})	Irradiation time	Spot size (cm^2)	Energy per point (J)	Schedule of irradiations	Total energy (J)
Schaffer *et al.*, 1997 [19]	—	—	805	2 to 20	—	50 and 150	—	—	—	—	—
Sroka *et al.*, 1999 [20]	Ar$^+$-Pumped tunable dye laser	—	630	0 to 20	—	50 and 150	—	—	—	—	—
	GaAlAs		635 805								
Pinheiro *et al.* 2002a [30]	—	CW	635 670	0.04 to 0.48	5	—	—	—	—	Seven consecutive days	—
Pinheiro *et al.*, 2002b [31]	—	CW	635 670	0.04 to 4.8	5	—	—	—	—	Seven consecutive days	—
Kreisler *et al.*, 2003 [32]	GaAlAs	CW Noncontact	809	1.96 3.92 7.84	10	—	75.0 s 150.0 s 300.0 s	—	—	—	—
Castro *et al.*, 2005 [33]	—	—	685 830	4	31 34.5	—	—	0.8	—	48 h intervals	—
Werneck *et al.*, 2005 [34]	—	—	685 830	4	31 34.5	—	—	0.8	—	—	—
Monteiro *et al.*, 2011 [22]	—	CW	660	56.4	30	424	133.0 s	0.07	4	48 h intervals	—
Schartinger *et al.*, 2012 [21]	GaAlAs	Noncontact	660	—	350	0.39 to 63.7	15 min	—	—	Three consecutive days for 15 min	—
Sperandio *et al.*, 2013 [35]	GaAlAs	Contact	660 780	2.05 3.07 6.15	40	—	—	0.039	—	—	—
Henriques *et al.*, 2014 [36]	InGaAlP	CW Noncontact	660	0.5 1.0	30	30	16.0 s 33.0 s	0.03	—	0 and 48 h	0.48 0.99
Liang *et al.*, 2015 [23]	—	CW	810	0 10 30 60	—	1000	00.0 s 10.0 s 30.0 s 60.0 s	—	—	—	—
Schalch *et al.*, 2016 [37]	—	CW Contact	660 780	4 4 4	30 30.8 53.9	214.29 220 385	25.3 s 24.7 s 14.1 s	0.14	—	1 session	0.76 0.76 0.76
Ottaviani *et al.*, 2016 [38]	GaAs + InGaAlAsP	CW	970	6	2500[a]	200	30.0 s	—	—	Once a day for 4 consecutive days	—
Schalch *et al.*, 2018 [b 39]	—	CW	660 780	1.4 2.7 2.7 5.4 8.1 1.4 2.7 2.7 5.4 8.1 5.4	22.5 30 23.1 30.8 53.9	160.7 160.7 214.3 214.3 214.3 165.0 165.0 220.0 220.0 220.0 385.0	8.4 s 16.9 s 12.7 s 25.3 s 38.0 s 8.2 s 16.5 s 12.3 s 24.7 s 37.0 s 14.1 s	0.14	—	1 session	0.19 0.38 0.38 0.76 1.14 0.19 0.38 0.38 0.76 1.14 0.76

GaAlAs, alluminium–gallium–arsenide; InGaAlP, indium–gallium–alluminium phosphide; GaAs, gallium–arsenide; InGaAlAsP, indium–gallium–alluminium phosphide; CW, continuous wave. [a] The device was used in an unfocused manner. [b] The study used 11 combinations of dosimetric parameters; for cell viability (neutral red assay), cell proliferation (incorporation of BrdU assay) and migration (scratch-wound assay) only the parameters that used an energy density of 4 J cm^{-2} were chosen.

Energy, energy per point, total energy, and energy density (fluence). Two studies mentioned total energy, while only one reported on energy per point. All, but one, study reported energy density that varied from 0 to 60 J cm^{-2}.

Risk of bias

Among the 15 included studies, five *in vitro* studies were classified as having a high risk of bias, while five had a medium risk and three had a low risk (Table 4). The two *in vivo* studies appeared to have a medium risk of bias as per the SYRCLE's risk of bias assessment criteria (Table 5).

Major outcomes regarding HNSCC following PBM treatments

Of the 13 *in vitro* studies, seven noted a positive effect of PBM therapy on inhibiting or preventing an effect on HNSCC tumor cells, while six studies saw increased proliferation. One *in vivo* study reported increased oral SCC (OSCC) progression, while the other observed reduced tumor progression (Fig. 3). The following sections presents further details and relevant descriptions of outcomes of individual studies grouped by their overall outcomes.

In vitro studies with positive impacts of PBM therapy on HNSCC cells

Schaffer *et al.* and Sroka *et al.* analyzed the effects of PBM therapy on human SCC cells of the gingival mucosa (ZMK and ZMK1).[19,20] Based on the outcomes of Orcein and BrdU staining, both studies did not observe any significant change in the mitotic index of tumor cells when PBM treatments (630, 635 and 805 nm lasers; 50–150 mW cm^{-2}; 20 J cm^{-2}) were compared with nontreated controls. Schartinger *et al.* investigated the effects of 660 nm PBM therapy on proliferation, cell cycle distribution, and apoptosis in human oral carcinoma cells (SCC25), a nonmalignant bronchial epithelial cell line, and periodontal-derived normal fibroblasts.[21] This study observed that PBM treatments at irradiances of 0.39 to 63.7 mW cm^{-2} for 15 minutes resulted in fibroblast proliferation but reduced the cell viability of epithelial and SCC cells, as observed with the MTT assay. Examining the cell cycle with propidium iodide and FACS analyses, the authors reported an increased percentage of S-phase SCC cells that also demonstrated increased apoptosis with annexin V staining. Sperandio *et al.* separately examined the effects of 660- and 780 nm PBM therapy on three cell lines, specifically dysplastic oral keratinocytes, SCC9, and SCC25.[35] These investigators showed that PBM therapy (40 mW; 2.05, 3.07, or 6.15 J cm^{-2}) reduced cell viability in all three cell lines. They also noted that the expressions of Akt, HSP70, S6, and cyclin D1 were significantly modulated by PBM treatments and correlated with reduced overall survival and increased apoptosis of tumor cells.

Another study by Liang *et al.* examined the effects of another popular near-infrared PBM wavelength (810 nm) on a human oral cancer cell line (OC2) and normal human gingival fibroblast cells.[23] PBM therapy was performed at varying fluences from 0 to 60 J cm^{-2} and tumor cells were noted to have reduced viability along with an increased cell count in the G_1

Table 4 Criteria used to assess risk of bias in *in vitro* studies

	Schaffer *et al.*, 1997[19]	Sroka *et al.*, 1999[20]	Pinheiro *et al.*, 2002b[31]	Pinheiro *et al.*, 2002a[30]	Kreisler *et al.*, 2003[32]	Castro *et al.*, 2005[33]	Werneck *et al.*, 2005[34]	Schartinger *et al.*, 2012[21]	Sperandio *et al.*, 2013[35]	Henriques *et al.*, 2014[36]	Liang *et al.*, 2015[23]	Schalch *et al.*, 2016[37]	Schalch *et al.*, 2018[39]
Condition of cell culture	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Description of methodology to evaluate the outcome	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Reproducibility	Yes	Yes	No	No	No	No	No	Yes	No	Yes	Yes	Yes	Yes
Methods for preventing unintentional light scattering during laser application	No	No	Yes	No	Yes	No	No	No	No	Yes	No	Yes	Yes
Description of laser parameters according to WALT[a]	No	No	No	No	No	No	No	No	No	Yes	No	Yes	Yes
Conclusions according to outcomes description	No	No	No	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Risk classification	High	High	Medium	High	Medium	High	High	Medium	Medium	Low	Medium	Low	Low

[a] The studies were classified as YES only if all laser parameters were described.

Table 5 Criteria used to assess risk of bias in *in vivo* (b) studies (SYRCLE's RoB tool)

	Monteiro *et al.*, 2011 [22]	Ottaviani *et al.*, 2016 [38]
Was the allocation sequence adequately generated and applied?	No	No
Were the groups similar at baseline or were they adjusted for confounders in the analysis?	Yes	Yes
Was the allocation to the different groups adequately concealed?	Unclear	Unclear
Were the animals randomly housed during the experiment?	Unclear	Unclear
Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?	Unclear	Unclear
Were animals selected at random for outcome assessment?	Unclear	Unclear
Was the outcome assessor blinded?	Unclear	Unclear
Were incomplete outcome data adequately addressed?	Unclear	Unclear
Are reports of the study free of selective outcome reporting?	Unclear	Unclear
Was the study apparently free of other problems that could result in high risk of bias?	Yes	Yes

A. *In vitro* studies

Author / Year	Wavelength (nm)	Dose (J/cm²)	Cell type	Overall Result	Risk of Bias
Schaffer et al., 1997	805	2 to 20	ZMK	Positive	High
Sroka et al., 1999	630 635 805	0 to 20	ZMK1	Positive	High
Pinheiro et al., 2002[a]	635 670	0.04 to 0.48	H.Ep.2	Negative	High
Pinheiro et al., 2002[b]	635 670	0.04 to 4.8	H.Ep.2	Negative	Medium
Kreisler et al., 2003	809	1.96 3.92 7.84	Larynx SCC	Negative	Medium
Castro et al., 2005	830	4.0	KB	Negative	High
Werneck et al., 2005	685 810	4.0	H.Ep.2	Negative	High
Schartinger et al., 2012	660	-	SCC25	Positive	Medium
Sperandio et al., 2013	660 780	2.05 3.07 6.15	SCC9 SCC25	Positive	Medium
Henriques et al., 2014	660	1.0	SCC25	Negative	Low
Liang et al., 2015	810	0 10 30 60	OC2	Positive	Medium
Schalch et al., 2016	660 780	4.0	SCC9	Positive	Low
Schalch et al., 2018	660 780	4.0	SCC9	Positive	Low

B. *In vivo* studies

Author / Year	Wavelength (nm)	Dose (J/cm²)	Animal model	Overall Result	Risk of bias
Monteiro et al., 2011	660	56.4	Hamsters cheek	Negative	Medium
Ottaviani et al., 2016	970	6.0	Mouse tongue	Positive	Medium

Fig. 3 Summary of the results from the included studies.

and sub-G_1 cell cycle phases. Further, ROS production, reduced matrix metalloproteinase (MMP), and increased caspase-3-mediated apoptosis were evident in PBM-treated OC2 cells. In subsequent studies by Schalch *et al.* similar effects were observed with 660- and 780 nm PBM treatments on SCC9 cells at 4 J cm^{-2}.[37,39] These investigations also noted the existence of reduced tumor cell viability, increased apoptosis, and reduced cell migration. Further, Schalch *et al.* reported the ability of PBM-treated SCC9 cells to lower tartare-resistant acid phosphatase (TRAP), positive osteoclastic activity *via* reduced IL-11, and PTHrP concentration.[37]

In vitro studies with negative impacts of PBM therapy on HNSCC cells

Two studies by Pinheiro *et al.* and one by Werneck *et al.* used the HEp-2 SCC cell line and analyzed cell proliferation using the MTT assay.[30,31,34] These studies reported the occurrence of a significant increase in cell proliferation following treatments with 635, 670, 685, and 830 nm as compared with non-treated controls. A similar response was reported by Kreisler *et al.* who used PBM therapy with 809 nm laser treatments on human larynx carcinoma cells and assessed proliferation using the AlamarBlue assay.[32] Interestingly, Castro *et al.* assessed the proliferation of oral KB carcinoma cells treated with 685 and 830 nm and noted increased tumor cell proliferation with the MTT assay.[33] Finally, Henriques *et al.* used PBM therapy with application of 660 nm of energy on a tongue SCC cell line (SCC25) and observed an increased proliferation of tumor cells.[36] They performed cell cycle analyses where a predominant cell subpopulation in the S and G2/M phases was observed. This correlated with findings of increased cyclin D1 and β-catenin and decreased MMP-9 expression that correlated with an increased invasive potential of these tumor cells.

In vivo studies using PBM therapy on HNSCC tumors

Two *in vivo* studies examined the effects of PBM therapy on OSCC tumors in animal models. Monteiro *et al.* evaluated the effect of 660 nm PBM therapy on chemically induced cancer [9,10-dimethyl-1,2-benzanthracene and DMBA three times a week for eight weeks] of the oral mucosa of golden Syrian hamsters.[22] At the end of eight weeks of cancer induction, one group of animals was sacrificed and examined histologically using a World Health Organization (WHO) grading system. All studied animals had developed well-differentiated SCC. Two additional groups of animals were then either observed for four weeks or treated with PBM therapy with irradiance at 424 mW cm^{-2} for 133 seconds every other day for four weeks, for a total fluence value of 56.4 J cm^{-2} per treatment session. While the PBM-treated groups demonstrated 40% well-differentiated SCC, 20% moderately-differentiated SCC, and 40%

poorly-differentiated-SCC findings, the non-PBM-treated group demonstrated 80% well-differentiated-SCC and 20% moderately-differentiated-SCC outcomes, with a statistically significant difference. Based on these observations, the authors concluded that PBM therapy might promote a progression of the severity of SCC *in vivo*.

In contrast, Ottaviani *et al.* explored the effects of PBM therapy in various cultured cells and *in vivo* models of cancer.[38] Among them, one of their studies involved a 4NQO-induced (16 weeks in drinking water) OSCC model in C57BL/6 female mice. One group of these animals was treated with PBM therapy (970 nm; 200 mW cm^{-2} for 30 seconds four times a week for four weeks; 6 J cm^{-2}). Histopathological examination demonstrated that PBM therapy significantly reduced the incidence of dysplastic lesions as well as the number and percentage of both *in situ* and invasive SCCs in these animals in comparison with among non-PBM-treated controls. Immunohistochemical analyses of tumor samples using CD31 and αSMA staining noted more regular and structured tumor vasculature patterns. Based on these observations, the authors concluded that PBM therapy inhibits OSCC tumor progression.

Discussion

There is strong evidence supporting the use of PBM therapy as an effective treatment in OM management associated with oncotherapy for head and neck cancer (HNC) or other malignances.[8,40,41] However, due to the reported stimulatory biological effects of PBM therapy on various tissues, the safety of PBM therapy appears to still being debated.[26,42] Two recent clinical studies analyzed the impact of PBM therapy used for the prevention of OM through different tumor outcomes in HNC patients.[6,43] Antunes *et al.* retrospectively evaluated the overall, disease-, and progression-free survival of 94 HNC patients submitted to PBM therapy (λ = 660 nm, 100 mW, 1 J, 4 J cm^{-2}) to prevent OM.[6] Their study demonstrated that patients receiving PBM therapy had a statistically better treatment response, displayed increase in progression-free survival, and a tendency for better overall survival when compared with the placebo group. Brandão *et al.* examined outcomes of cancer therapy and the incidence of tumor recurrence in locally advanced OSCC patients treated with PBM therapy (λ = 660 nm, 40 mW, 0.4 J, 10 J cm^{-2}) for OM.[43] The authors concluded that the prophylactic PBM therapy did not impact treatment outcomes of the primary cancer, recurrence, new primary tumors, or survival of the patients. Specifically, the effects of PBM therapy on residual or dormant tumor cells in cancer patients remain a concern. This fact motivated the current systematic review to examine the literature regarding the effects of PBM therapy on HNSCC. A total of 13 *in vitro* and two *in vivo* studies were finally included. Unfortunately, the analysis of the 13 *in vitro* studies revealed significant variations in cell lines, culture conditions, methodological designs, PBM parameters, and evaluation methods. Moreover, five studies presented high risks of bias, another five were noted to have a medium risk of bias, and only three studies demonstrated low risk of bias. The two *in vivo* studies appeared to have a medium risk of bias due to the lack of adequately reported study parameters. Therefore, the overall conclusions of this review acknowledge important methodological limitations that compromise the reliability and direct significance of the data analyzed.

Summary of *in vitro* results

Our analyses showed that investigators used a broad range of HNSCC cell lines and methods to examine the effects of PBM therapy. Overall, six studies reported increased tumor cell proliferation and five studies noted tumor cell inhibition following PBM therapy. There appears to be some evidence that tumor cell viability could be enhanced or diminished depending on the precise culture conditions and laser treatment parameters used (Table 6). Tumor cells from various anatomical niches and various transformation processes including spon-

Table 6 Outline of key parameters identified in this review that could contribute to the variances in observed results for effects of PBM therapy on tumor cells

Biological	Device	Outcomes
Cell origin	Power	Proliferation *versus* apoptosis
- Lineage - Transfromation state	- Power output - Power density (irradaince) - Treatment surface irradiance	

Cell density	Time	Cell viability
- Initial cell concentration - Plating surface area - Confluency at PBM treatments - Time outcome analyses	- Single session - Repetitions	- Membrane integrity **Mitochondrial function** - Respiraroty and metabolic health

Culture conditions	Energy	Cell cycle analyses
- Media and supplements - Serum concentration - Synchronication of cell subpopulations	- Energy per point - Energy density - Total energy per session	- Synetheitc phases - Checkpoint arrest

taneous (oral tumors), chemically induced (DMBA or 4NQO), and virally induced (EBV) were used in these studies, which might contribute to variations in PBM responses. Also, several studies reported opposite responses of normal *versus* tumor cell types in their results, which could be attributed to their underlying differences in basal transcriptional and pathophysiological statuses.[44] Schalch *et al.* observed that cell seeding density appeared to be a major factor in determining precise tumor cell response, as they noted SCC9 plated in lower (10^3 cells per well) densities showed consistent increased cell proliferation, while plating cells at higher (10^4 cells per well) densities resulted in a lowered viability of tumor cells post-PBM treatments as compared with non-PBM-treated cells.[37]

Another important aspect to consider in these studies is timing of both the outcomes analyses as well as the repetition of PBM treatments. One of the best-understood processes of PBM therapy is the direct absorption of light by the mitochondrial chromophore cytochrome-C oxidase, which results in increased ROS and adenosine triphosphate (ATP) levels. This induces concerted signaling and transcriptional pathways over a period of several hours to days that are capable of modulating tumor cell functions.[45] Moreover, differences in tumor metabolism, oxidative stress status, time of treatments, and repetitions of treatments would all be expected to result in discrete differences in PBM responses assessed by various assays.

Several outcomes methodologies were employed in these studies to assess tumor cell viability, proliferation, and apoptosis. It must be emphasized that, while these cellular characteristics are intimately related, they are discrete from each other and so must be evaluated individually. For example, the study by Schalch *et al.* used three discrete methods namely, MTT assay (mitochondrial activity), neutral red assay (cell viability), and incorporation of BrdU (proliferation) to assess SCC9 cells following PBM therapy.[39] They observed that PBM therapy reduced mitochondrial activity and cell viability but did not interfere with cell proliferation.

Other cellular response mechanisms evaluated in these studies include apoptosis, migration, and invasion. Four studies examined tumor cell apoptosis using activated caspase-3, annexin V, or TUNEL. All studies reported the finding of increased tumor cell apoptosis following PBM treatments. A recent review suggested that the increased ATP within the cancer cells might also promote energy-dependent cell death pathways.[44,46] Three studies examined the effects of PBM therapy on tumor cell migration and, while two studies reported no differences, one study did observe increased invasion. Interestingly, two of these divergent studies, by Henriques *et al.* and Schalch *et al.* respectively, used comparable PBM parameters (660 nm, CW, ~30 mW and ~16 s) for all but one aspect and also examined other markers of tumor cell invasion such as E-cadherin, MMP, IL-11, and PTHrP expression that correlated with their invasive phenotypes.[36,37] A major difference between these two studies, however, is their use of two different OSCC cell lines (SCC25 *versus* SCC9). Also, the positively correlated study used higher doses (>1 J cm^{-2}) as compared with the other. However, this minimal difference in these two studies does not allow for firm conclusions to be made; more comparable studies are needed.

Perhaps a more thorough understanding of the cellular response to PBM treatment could be particularly gleaned by cell-cycle phase analyses that would outline all three cellular responses—namely, viability, proliferation, and apoptosis. Henriques *et al.*[36] reported increased proportions of cells in the S and G2/M phases at all time points with 660 nm PBM treatments (30 mW and 1 J cm^{-2}) as compared with controls and PBM treatments at 0.5 J cm^{-2}. On the other hand, Liang *et al.*[23] found that PBM therapy with 810 nm (1000 mW cm^{-2} and 60 J cm^{-2}) resulted in G_1 arrest and increased cell death in human oral cancer cells. While their use of different PBM wavelengths and doses is a clear confounder, making it difficult to extrapolate these observations, these authors also used two distinct cell lines (SCC25 *versus* OC2) with varying growth characteristics, further increasing the difficulty of applying their observations broadly. We attempted to compare the most similar studies in terms of cell lines (SCC25) and PBM treatment parameters (660 nm, ~30 mW cm^{-2}) that reported opposite effects. We noted several differences in initial cell seeding, media supplements (presence of dexamethasone), treatment repetitions, and outcomes assessments that could all account for variations in tumor cell responses to PBM therapy. Hence, there is a significant value of using *in vitro* systems to analyze tumor cell responses to PBM therapy, but more attention is necessary for elucidating appropriate biological and PBM treatment parameters for appropriate interpretations.

Summary of *in vivo* results

Cell cultures have been extensively used since the early 1900s and human cancer-derived cell lines are among the most widely used models to study cancer biology. However, conventional two-dimensional cell cultures poor mimic pathophysiological conditions within living organisms and have limited heterotypic cellular interactions.[47] Thus, the application of standardized preclinical *in vivo* models in animals is necessary to circumvent limitations of such *in vitro* approaches. Of note, for this review, we could only find two studies examining the effects of PBM therapy on OSCC in animal models.[22,37] Strikingly, both studies appear to show opposite outcomes. While both studies used chemically-induced oral carcinogenesis models, they employed different animal models (hamsters *versus* mice) and chemical carcinogens (DMBA *versus* 4-NQO). Further, PBM treatments were performed with different wavelengths (660 *versus* 970 nm), irradiances (424 *versus* 200 mW cm^{-2}), treatment times (133 *versus* 30 seconds), and repetitions (every two days *versus* every day) for four weeks. Unfortunately, these significant differences do not allow for a rigorous comparison of the contrasting effects of PBM therapy on tumor cells and, hence, more standardized studies are necessary.

Significant variations in PBM parameters

Clinically effective PBM therapies have been noted to have a few key characteristics that can be broadly divided into the cat-

egories of device parameters and treatment delivery parameters.[11] Device parameters include wavelength, mode (continuous-wave or pulsing), polarization, power density, treatment time, and energy density. PBM treatment delivery parameters include probe–target distance (also called treatment surface irradiance) and stationary or probe-scanning movements.[48] PBM treatments of normal cells have been known to follow the Arndt–Schulz law, where low doses do not cause any effect but optimal doses within a therapeutic window generate a therapeutic response and high doses reverse these beneficial effects, respectively. The complexity of the light-biological tissue interactions has prevented comprehensive description of PBM dose variables. Hence, the current consensus is to document and report as many treatment variables as feasible.[49,50] It would seem reasonable to expect tumor cells may not follow this dose–response and therefore careful attention is necessary for PBM treatments of premalignant and tumor tissues.[44] A common error noted in several of these studies is the use of a normal cell line as a control, but of varying (unmatched) anatomical origin. There is growing evidence that cells of distinct lineages require specific PBM doses to evoke therapeutic responses.[51]

Unfortunately, as evident in this review (Table 3), there is a significant lack of attention when reporting on PBM treatment parameters. The most commonly reported parameters included wavelength, power or power density, treatment time, and energy or energy density. The wavelength refers to the physical distance between two successive photonic waves and determines several key PBM characteristics such as absorption by specific biological chromophores and depth of laser penetration. The PBM wavelength was reported by all studies and ranged from visible (red, 630 nm) to near-infrared (970 nm) spectrum. There appears to be significant variations in power density (50 to 1000 mW cm^{-2}), treatment times (8.4 to 900 seconds), and energy density (0.04 to 60 J cm^{-2}). It is prudent to emphasize that some of the treatments were performed only once, while some were repeated on alternate days or every day for up to four weeks. These variations were not explained in any of the included studies. Therefore, the data do not allow for the elaboration of consistent parameters that might provide insights into PBM treatment effects on tumor cells. Nonetheless, these reported parameters are within the MASCC and ISOO recommendations of using the wavelengths of 633 to 685 nm or 780 to 830 nm with power outputs of between 10 and 150 mW and energy densities of 2 to 4 J cm^{-2} (but no more than 6 J cm^{-2}).[8,52] In summary, future studies should pay close attention to promoting standardization and on detailed reporting of the parameters used for PBM treatments.

Clinical implications and conclusions

These analyses clearly demonstrate it is imperative to perform better *in vitro* and *in vivo* studies in relevant animal models to examine the effects of PBM therapy on HNSCC. Not only is this critical for our understanding of fundamental tumor mechanisms but also it is practically relevant to clinical safety for the increasingly popular use of PBM therapy in OM prevention and/or treatment in HNSCC patients. There are tantalizing early reports on the use of PBM therapy in the prevention and management of malignancy-related comorbidities as indicated in two recent publications.[6,43] Both studies noted a positive correlation of PBM therapy in reducing OM incidence with no significant adverse events. Further, somewhat surprisingly, a statistically significant improvement in treatment responses represented by an increase in progression-free survival and a tendency for better overall survival was observed as compared with the control groups.

In conclusion, this review clearly noted a lack of uniformity in experimental protocols and PBM treatment parameters that indicate that the current effects of PBM therapy on tumor cells remain equivocal. While the clinical safety of PBM therapy remains debatable, the available clinical evidence for its use as an adjunctive supportive therapy for OM and other treatment complications must be taken with caution. Thus, clinicians should remain aware of the risks when treating HNSCC patients and should avoid direct PBM treatment of suspicious malignant sites or frank tumors. It is strongly suggested that well-delineated studies, mainly based on *in vivo* models followed by human clinical trials, must be pursued to better evaluate the effects of PBM therapy on HNSCC patients.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was in part supported by the Brazilian National Council for Scientific and Technological Development (CNPq post graduate scholarship). Manoela Domingues Martins and Alan Roger Santos-Silva are research fellows funded by the Brazilian National Council for Scientific and Technological Development (CNPq). The authors would like to gratefully acknowledge the financial support of the São Paulo Research Foundation (FAPESP) (process number 2018/02233-6).

References

1 P. J. Thomson, Perspectives on oral squamous cell carcinoma prevention-proliferation, position, progression and prediction, *J. Oral Pathol. Med.*, 2018, **47**, 803–807.
2 S. Chen, H. Hu, S. Miao, J. Zheng, Z. Xie and H. Zhao, Antitumor effect of cisplatin in human oral squamous cell carcinoma was enhanced by andrographolide via upregulation of phospho-p53 in vitro and in vivo, *Tumour Biol.*, 2017, **39**(5), 1010428317705330.
3 S. T. Sonis, Oral mucositis in head and neck cancer: risk, biology, and management, *Am. Soc. Clin. Oncol. Educ. Book*, 2013, DOI: 10.1200/EdBook_AM.2013.33.e236.

4 S. Minhas, *et al.*, Concomitant-chemoradiotherapy-associated oral lesions in patients with oral squamous-cell carcinoma, *Cancer Biol. Med.*, 2017, **14**, 176–182.
5 F. Amadori, *et al.*, Low-level laser therapy for treatment of chemotherapy-induced oral mucositis in childhood: a randomized double-blind controlled study, *Lasers Med. Sci.*, 2016, **31**, 1231–1236.
6 H. S. Antunes, *et al.*, Long-term survival of a randomized phase III trial of head and neck cancer patients receiving concurrent chemoradiation therapy with or without low-level laser therapy (LLLT) to prevent oral mucositis, *Oral Oncol.*, 2017, **71**, 11–15.
7 L. M. Bezinelli, *et al.*, Cost-effectiveness of the introduction of specialized oral care with laser therapy in hematopoietic stem cell transplantation, *Hematol. Oncol.*, 2014, **32**, 31–39.
8 R. V. Lalla, *et al.*, MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy, *Cancer*, 2014, **120**, 1453–1461.
9 J. A. Zecha, *et al.*, Low level laser therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer. Part 1: mechanisms of action, dosimetric, and safety considerations, *Support Care Cancer*, 2016, **24**, 2781–2792.
10 J. J. Anders, *et al.*, Low-level light/laser therapy versus photobiomodulation therapy, *Photomed. Laser Surg.*, 2015, **33**, 183–184.
11 P. R. Arany, Craniofacial Wound Healing with Photobiomodulation Therapy: New Insights and Current Challenges, *J. Dent. Res.*, 2016, **95**, 977–984.
12 N. N. Lopes, *et al.*, Cyclooxygenase-2 and vascular endothelial growth factor expression in 5-fluorouracil-induced oral mucositis in hamsters: evaluation of two low-intensity laser protocols, *Support Care Cancer*, 2009, **17**, 1409–1415.
13 N. N. Lopes, *et al.*, Effects of low-level laser therapy on collagen expression and neutrophil infiltrate in 5-fluorouracil-induced oral mucositis in hamsters, *Lasers Surg. Med.*, 2010, **42**, 546–552.
14 T. C. Lopez, *et al.*, Effect of laser phototherapy in the prevention and treatment of chemo-induced mucositis in hamsters, *Braz. Oral Res.*, 2013, **27**, 342–348.
15 M. Curra, *et al.*, Photobiomodulation reduces oral mucositis by modulating NF-kB, *J. Biomed. Opt.*, 2015, **20**, 125008.
16 A. F. Oton-Leite, *et al.*, Effect of low-level laser therapy on chemoradiotherapy-induced oral mucositis and salivary inflammatory mediators in head and neck cancer patients, *Lasers Surg. Med.*, 2015, **47**, 296–305.
17 M. M. Schubert, *et al.*, A phase III randomized double-blind placebo-controlled clinical trial to determine the efficacy of low level laser therapy for the prevention of oral mucositis in patients undergoing hematopoietic cell transplantation, *Support Care Cancer*, 2007, **15**, 1145–1154.
18 B. Ferreira, F. M. da Motta Silveira and F. A. de Orange, Low-level laser therapy prevents severe oral mucositis in patients submitted to hematopoietic stem cell transplantation: a randomized clinical trial, *Support Care Cancer*, 2016, **24**(3), 1035–1042.
19 M. Schaffer, *et al.*, Biomodulative effects induced by 805 nm laser light irradiation of normal and tumor cells, *J. Photochem. Photobiol., B*, 1997, **40**, 253–257.
20 R. Sroka, *et al.*, Effects on the mitosis of normal and tumor cells induced by light treatment of different wavelengths, *Lasers Surg. Med.*, 1999, **25**, 263–271.
21 V. H. Schartinger, *et al.*, Differential responses of fibroblasts, non-neoplastic epithelial cells, and oral carcinoma cells to low-level laser therapy, *Support Care Cancer*, 2012, **20**, 523–529.
22 J. S. Monteiro, *et al.*, Influence of laser phototherapy (λ660 nm) on the outcome of oral chemical carcinogenesis on the hamster cheek pouch model: histological study, *Photomed. Laser Surg.*, 2011, **29**, 741–745.
23 W. Z. Liang, *et al.*, Selective cytotoxic effects of low-power laser irradiation on human oral cancer cells, *Lasers Surg. Med.*, 2015, **47**, 756–764.
24 Y. H. Rhee, *et al.*, Low-Level Laser Therapy Promoted Aggressive Proliferation and Angiogenesis through decreasing Transforming Growth Factor-β1 and Increasing of Akt/Hypoxia Inducible Factor-1α in Anaplastic Thyroid Cancer, *Photomed. Laser Surg.*, 2016, **34**, 229–235.
25 M. Myakishev-Rempel, *et al.*, A preliminary study of the safety of red light phototherapy of tissues harboring cancer, *Photomed. Laser Surg.*, 2012, **30**, 551–558.
26 S. T. Sonis, *et al.*, Could the biological robustness of low level laser therapy (Photobiomodulation) impact its use in the management of mucositis in head and neck cancer patients, *Oral Oncol.*, 2016, **54**, 7–14.
27 D. Moher, *et al.*, Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement, *Ann. Intern. Med.*, 2009, **151**, 264–269.
28 M. M. Marques, *et al.*, Photobiomodulation of Dental Derived Mesenchymal Stem Cells: A Systematic Review, *Photomed. Laser Surg.*, 2016, **34**, 500–508.
29 C. R. Hooijmans, *et al.*, SYRCLE's risk of bias tool for animal studies, *BMC Med. Res. Methodol.*, 2014, **26**(14), 43.
30 A. L. Pinheiro, *et al.*, Does LLLT stimulate laryngeal carcinoma cells? An in vitro study, *Braz. Dent. J.*, 2002a, **13**, 109–112.
31 A. L. Pinheiro, *et al.*, Effects of low-level laser therapy on malignant cells: in vitro study, *J. Clin. Laser Med. Surg.*, 2002b, **20**, 23–26.
32 M. Kreisler, *et al.*, Low-level 809 nm GaAlAs laser irradiation increases the proliferation rate of human laryngeal carcinoma cells in vitro, *Lasers Med. Sci.*, 2003, **18**, 100–103.
33 J. L. Castro, *et al.*, The effect of laser therapy on the proliferation of oral KB carcinoma cells: an in vitro study, *Photomed. Laser Surg.*, 2005, **23**, 586–589.

34 C. E. Werneck, *et al.*, Laser light is capable of inducing proliferation of carcinoma cells in culture: a spectroscopic in vitro study, *Photomed. Laser Surg.*, 2005, **23**, 300–303.
35 F. F. Sperandio, *et al.*, Low-level laser therapy can produce increased aggressiveness of dysplastic and oral cancer cell lines by modulation of Akt/mTOR signaling pathway, *J. Biophotonics*, 2013, **6**, 839–847.
36 A. C. Henriques, *et al.*, Low-level laser therapy promotes proliferation and invasion of oral squamous cell carcinoma cells, *Lasers Med. Sci.*, 2014, **29**, 1385–1395.
37 T. Schalch, *et al.*, Photomodulation of the osteoclastogenic potential of oral squamous carcinoma cells, *J. Biophotonics*, 2016, **9**, 1136–1147.
38 G. Ottaviani, *et al.*, Laser Therapy Inhibits Tumor Growth in Mice by Promoting Immune Surveillance and Vessel Normalization, *EBioMedicine*, 2016, **11**, 165–172.
39 T. D. Schalch, *et al.*, Photobiomodulation is associated with a decrease in cell viability and migration in oral squamous cell carcinoma, *Lasers Med. Sci.*, 2018, **34**, 629–636.
40 J. M. Bjordal, *et al.*, A systematic review with meta-analysis of the effect of low-level laser therapy (LLLT) in cancer therapy-induced oral mucositis, *Support Care Cancer*, 2011, **19**, 1069–1077.
41 R. J. Bensadoun, Photobiomodulation or low-level laser therapy in the management of cancer therapy-induced mucositis, dermatitis and lymphedema, *Curr. Opin. Oncol.*, 2018, **30**, 226–232.
42 K. M. AlGhamdi, *et al.*, Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells, *Lasers Med. Sci.*, 2012, **27**, 237–249.
43 T. B. Brandão, *et al.*, Locally advanced oral squamous cell carcinoma patients treated with photobiomodulation for prevention of oral mucositis: retrospective outcomes and safety analyses, *Support Care Cancer*, 2018, **26**, 2417–2423.
44 P. R. Arany, Healing Tumors with Light: Science Fiction or the Future of Medicine?, *Photomed. Laser Surg.*, 2018, **36**, 227–229.
45 M. R. Hamblin, Mechanisms and Mitochondrial Redox Signaling in Photobiomodulation, *Photochem. Photobiol.*, 2018, **94**, 199–212.
46 M. R. Hamblin, *et al.*, Photobiomodulation and Cancer: What Is the Truth?, *Photomed. Laser Surg.*, 2018, **36**, 241–245.
47 J. P. Gillet, *et al.*, The clinical relevance of cancer cell lines, *J. Natl. Cancer Inst.*, 2013, **105**, 452–458.
48 I. Khan and P. R. Arany, Dosimetery for Photobiomodulation therapy: Response to Sommers *et al.*, *Ann. Transl. Med.*, 2016, **4**, 208.
49 P. A. Jenkins and J. D. Carroll, How to report low-level laser therapy (LLLT)/photomedicine dose and beam parameters in clinical and laboratory studies, *Photomed. Laser Surg.*, 2011, **29**, 785–787.
50 J. Tuner and P. A. Jenkins, Parameter Reproducibility in Photobiomodulation, *Photomed. Laser Surg.*, 2016, **34**, 91–92.
51 K. W. Engel, *et al.*, Cell lineage responses to photobiomodulation therapy, *J. Biophotonics*, 2016, **9**, 1148–1156.
52 S. Elad, *et al.*, Photobiomodulation therapy in the management of oral mucositis: search for the optimal clinical treatment parameters, *Support Care Cancer*, 2018, **26**, 3319–3321.