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CHAPTER 15

EFFECTS OF LASER PHOTOBIOMODULATION ON TUMOR CELLS

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ABSTRACT

Considering the beneficial and non-invasive effects of laser as a therapy, the possibility of using it in cancer patients would be of great interest to the clinical area. However, the available literature is still scarce for the definition of the ideal dosimetric parameters of photobiomodulation (FBM) in cancer cells, and when considering the use of the therapy as a bioinhibitory treatment in this cell type, more studies are needed to elucidate the main factors responsible for the different behaviors in these cells, since in specific parameters this therapy can promote biostimulation or even cell inhibition, It should be used with caution in clinical practice, avoiding increasing existing cancerous tissue.

Keywords: Photobiomodulation (FBM). Cancer. Biostimulation. Bioinhibitory therapy.

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INTRODUCTION

Cancer is a serious global public health problem, characterized by the uncontrolled proliferation of a group of cells within the body, and is one of the main causes of morbidity and mortality in many parts of the world. (1)

According to the estimates of Fitzmaurice et al. (2), in 2015 there were at least 17.5 million cases of cancer in the world, with at least 8.7 million deaths and according to the World Health Organization this number will reach 21 million by 2030. (3)

This disease has multiple causes, and can be triggered by etiological factors, such as age, endocrine alterations and genetics. (4,5) and several risk factors that predispose to the disease, with 90% of cases being related to environmental factors.

When it comes to antineoplastic treatment, new less invasive therapies have been investigated and have generated great interest in the health area, seeking a better prognosis and fewer side effects. One of the current therapies under investigation is Photobiomodulation (FBM) (6). This therapy has also been suggested in cases of sequelae of antineoplastic treatment, such as in cases of osteonecrosis associated with bisphosphonates. (7,8)

However, there are controversies regarding the use of FBM during the neoplastic process. Some studies show that this therapy can favor an increase in cell proliferation and differentiation, since it has significant biomodulatory effects, being a form of therapy contraindicated in regions with the presence of tumors (9). However, it is known that this process will depend on the parameters that will be applied (10).

Although the studies are not conclusive, the literature shows that there is a tendency for higher doses to lead to tumor cell death (11). However, in this context, when using FBM in high doses, while some authors have demonstrated that it can inhibit the tissue repair process (4), causing aggressive effects at the cellular level, other studies have shown satisfactory results in the repair process. (9,12).

There is a lack of evidence on the effects of FBM in conditions of malignancy, both in relation to the parameters to be used and in relation to the biological mechanisms resulting from irradiation at the cellular level (13). Considering the beneficial and non-invasive effects of laser as a therapy, the possibility of using it in cancer patients would be of great interest to the clinical area, and it could be used as an adjuvant in the treatment of cancer patients.

LITERATURE REVIEW

CANCER

Cancer is understood as a disease that is characterized by the uncontrolled proliferation of a group of cells within the body, much faster than that of normal cells (4,14). These cells attack tissues and organs, dividing rapidly and tend to be very aggressive and uncontrollable, leading to the formation of tumors, which can spread to other regions of the body. (15)

Cancer is the leading public health problem in the world and is already among the four leading causes of premature death in most countries (1). It is a disease of multiple causes, and can be caused by factors such as age, endocrine changes and genetics. (18,19). In addition, several risk factors predispose to the disease, and 90% of the cases are related to environmental factors, those that can be modified by changes in behavior and lifestyle habits, such as: exposure to radiation and chemical products, smoking, alcoholism, inadequate diet, obesity and physical inactivity. (5,16,17)

When it takes on an advanced form, cancer can evolve into a condition that cannot be cured, with the presence of uncontrollable signs and symptoms such as pain, fatigue, nausea, vomiting, anorexia, anxiety, depression, among others. The manifestations may be related to tumor invasion, as well as to the adverse effects of the treatment being performed, causing intense discomfort to the patient and a negative impact on quality of life. In view of this, the care provided to cancer patients is no longer curative and becomes palliative. (20)

This disease is considered a global public health problem, with a 20% high incidence in the last decade (21). Most of the time, the diagnosis of cancer is interpreted as a disease with a negative stigma considered as synonymous with suffering and death(22) and this image that the disease conveys is a consequence of its impact on the patient's life, since there is impairment in the physical, psychological, social, economic and spiritual aspects. (22,23)

Currently, cancer is the second leading cause of mortality in developed countries and, in a few decades, it will become the main cause of morbidity and mortality in the poorest regions of the planet (17). It stands out among chronic non-communicable diseases, reaching alarming levels, and is considered a global public health problem. (25)

According to research by the World Health Organization, by 2030 cancer will reach approximately 27 million incident cases, 17 million deaths, and 75 million people with an annual diagnosis worldwide, with 50% of cases being metastatic (26). In Brazil, statistical data made available by the National Cancer Institute (INCA), 2020 showed an incidence of

about 626 thousand new cases of cancer, thus revealing the magnitude of the problem in the country. (5,20,25)

Although the number of individuals diagnosed is increasing, it is believed that 1/3 of the cases are preventable. Thus, there is a need to incorporate new habits into the lifestyle so that the organic defenses can be able to fight against mutated cells and capable of developing a tumor mass. (27)

In this sense, it seems urgent to implement public health policies aimed at the most affected populations, associated with the reduction of social inequities and access to primary prevention, early diagnosis and treatments, in order to reduce disparities in cancer mortality in Brazil. (17)

Osteossarcoma

Osteosarcoma (OS) is a primary malignant bone tumor, originating from mesenchymal stem cells, characterized by a high rate of metastasis (36), high mortality, and a high rate of disability (29). It originates from mesenchymal tissue and acquires features of strong early metastasis, which are associated with a poor prognosis. (30)

Epidemiological data indicate that its incidence is 30% of bone tumor cases in Ireland, while in the United States it is approximately 1.7 cases per million inhabitants per year, representing 600 to 900 new cases annually. In Brazil, it is believed that the incidence is approximately 400 to 600 new cases per year. (31)

The disease has a bimodal incidence, predominantly affecting children and adolescents (primary osteosarcomas) (32) during the period of greatest growth, with a second peak incidence in adults over 65 years of age (secondary osteosarcomas), (33,34) in which men are affected more frequently than women, in a ratio of 1.6:1.

As for its etiology, studies indicate that the origin of osteosarcoma is still unclear, however, it is known that certain environmental factors, such as irradiation and hereditary conditions, such as Li-Fraumeni syndrome, familial retinoblastoma, and Rothmund-Thomson syndrome, are associated with germline mutations in the TP53, RB1, and RECQL4 genes, respectively, which predispose to osteosarcoma. (35)

The tumor can affect any bone, but it is usually located in the metaphyseal regions of the long bones. The distal region of the femur and the proximal region of the tibia and humerus are the most frequently affected locations. More than 50% of cases occur near the knee. (36)

The typical presentation includes the appearance of pain and expansion of the affected bone, and a hallmark of the pain is that it is sufficiently intense to awaken the

patient from sleep. Occasionally, patients will present with the onset of severe pain or other signs associated with a pathologic fracture. Approximately 15% to 20% of patients will have clinically detectable metastases, and more than 85% of metastatic disease occurs in the lung, the most common site of metastasis. (32)

A study conducted by Mirabello et al. (28) showed that osteosarcoma metastasis is one of the leading causes of treatment failure and patient death. The mechanism of metastasis of malignant tumors is that tumor cells that break off from the lesion enter the blood or lymphatic system and circulation of the body and proliferate and form metastatic focus in the metastatic organs, which is basically consistent with the mechanism of metastatic osteosarcoma. (29)

Traditionally, the gold standard for primary bone malignancies located in extremities has been amputation (32). In recent decades, therapy has shifted to salvaging limbs with intact local function in order to improve patients' quality of life. (37)

Thus, the current standard treatment of osteosarcoma consists of surgery associated with chemotherapy (38), leading to a 70% survival at 5 years in patients with non-metastatic disease. However, the 5-year survival rate of patients with metastatic disease is about 20%, emphasizing the importance of developing new therapeutic strategies (34). However, the effectiveness of these therapeutic strategies is limited, and some of them can cause serious complications and adverse effects.

In recent years, scientific evidence on FBM has been pointing to a better understanding of its effects, and researchers and clinicians have begun to investigate its use in increasingly innovative applications. Among these new clinical approaches are its application to repair damaged bone tissue and its place in oncology as an adjunct treatment, slowing or even leading to cancer cell death. (11)

Cancer cells

Cancer cells are thought to be altered normal cells, formed by cellular transformation (39) and they differ from healthy cells due to a multitude of molecular changes (40,41), many of which may be mechanically linked to metabolic reprogramming.

These cells have some special characteristics, such as unlimited proliferation capacity, loss of response to growth inhibition factors, evasion of apoptosis (programmed cell death), ability to invade other tissues (metastases), production of new blood vessels (angiogenesis), (42) ability to produce a greater number of reactive oxygen species and greater dependence on an antioxidant defense system. (43)

As for the development of a cancer cell, initially the cell is stimulated, either by a hormone or growth factor, and its division process is no longer normal and becomes permanent. Next, the "safety brakes" that prevent excessive cell division must be eliminated and these brakes are controlled by two main genes: RB1 and TP53, also known as P53. When these genes are mutated, they prevent apoptosis, thus allowing a tumor mass to form. Making these changes is enough for the cells to become cancerous. (4)

The Inactivation of the *RB1* It is reported in 20-40% of sporadic cases of osteosarcoma and is associated with a poor disease outcome. Its inactivation has also been associated with abnormalities of cell differentiation, cell death, angiogenesis, metastasis, and senescence (44). The mutants of the *TP53* they lose the ability to inhibit cell growth, gain the ability to proliferate and transform, and possibly lead to malignancy.

Nakase et al.(45) They transfected the gene P53 in osteosarcoma cells *in vitro* and *in vivo,* which apparently leads to the establishment of tumor growth. However, the functions of these gene mutants P53 in different osteosarcoma cell lines are not completely consistent and further investigation is needed.

LOW-POWER LASER

The term Laser - *Light Amplification By Stimulated Emission of Radiation* (light amplification by stimulated emission of radiation) (46) it was initially described by Albert Einstein in 1917, in a theoretical way, but it was only in 1960 that Theodore H. Maiman announced its operation for therapeutic purposes (47), being applied clinically for wound healing, pain relief, inflammation, and various orthopedic conditions. (48)

In 1967, Dr. Endre Mester was the first scientist to discover that a low-power laser had a stimulating effect on hair growth in mice and since then, low-power laser has been applied in various conditions to increase physiological function in humans and animals. (49)

Radiation is characterized by electromagnetic waves, visible or invisible, in which the applied energy performs work in the tissue area to be treated. Its purpose is the photoactivation of cellular mechanisms that help in the rehabilitation of injured areas. (50)

Among all the existing low-power laser types, the most commonly reported devices are: helium-neon gas (HeNe), gallium arsenide (GaAs), yttrium aluminum garnet (YAG), aluminum gallium arsenide (GaAlAs), aluminum aluminum gallium diode laser (AlGaInP). (51)

This radiation has unique characteristics, such as coherence: ordered displacement of waves in relation to time with their equal amplitudes; collimation: photons travel in

parallel, moving great distances; and monochromaticity: which is characterized by presenting a pure color, being a single color. (52)

Currently, the term FBM has replaced the old low-power laser, and FBM was introduced as a descriptor in PUBMED in 2015 (49). This form of irradiation has gained great prominence worldwide in health science due to the search for less invasive forms of treatment. A variety of pathological conditions using FBM are described in the literature, such as modulation of the inflammatory process, acceleration of the tissue repair process, pain relief, and treatment of some neurological disorders. (54,61)

FBM has become a widely used method in most countries and has been studied worldwide, one of the reasons for its popularity is related to its less invasive, athermal, painless, low-cost action (66) and with a shorter applicability time than other physical therapy resources. (67)

Therefore, taking into account its efficacy and non-invasive action, FBM has been studied as an adjuvant treatment option for cancer. (49,68)

Dosimetry

Multiple variables affect the clinical therapeutic effects of FBM, such as wavelength (λ), energy density (dE), power density (DP), and treatment time (53), because when applied it exposes cells or tissue to both a biostimulatory and bioinhibitory effect. (6)

Wavelength is considered an essential parameter for beneficial application results, as it determines the ability of the laser to penetrate the tissue (54) And depending on the wavelength the light can be classified as visible or red (380 to 750nm) and invisible or infrared (above 750nm) (55), although there is still divergence in the literature regarding these values. What is worth mentioning is that the longer the wavelength, the greater the depth of penetration of the energy into the tissue (54). De acordo com Huang et al.(56), wavelengths in the range of 700-1000 nm are most often used to treat deep tissue because of their deep penetration.

Regarding the dispersion, it is inversely proportional to the wavelength, the longer the wavelength, the lower the dispersion. (57)

The energy radiated in Joule (J) corresponds to the amount of energy employed during the treatment time. Another important factor is energy density, which is defined as the total amount of energy (J) per radiated area (cm^2) (57), given in Joule per square centimeter (J/cm^2), which will designate the irradiation time and its power, presented in Watts (W). (58,59)

It is important to highlight that energy cannot be confused with dose, as it presupposes reciprocity (the inverse relationship between power and time). It is calculated as: Energy (J) = Power (W) x Time (s). Energy density or fluence is an important dose descriptor, which assumes a reciprocal relationship between irradiance and time, obtained according to the following equation: FROM = $\frac{PxT}{A}$. (82,83)

Power (P) can be calculated by the following equation: P = , where E = radiated energy (dose) (in J) and T = irradiation time (in s). The power density (DP) or irradiance is related to the power (W) per unit area (A) radiated (in W/cm²), and can be calculated by the following equation: $\frac{E}{T}DP = \frac{P}{A}$. (60,61)

Knowledge of these parameters is essential to establish safe and adequate doses, according to the physiological characteristics and objectives of each individual.

It is known that if incorrect parameters are applied, the treatment is likely to be ineffective. There is a biphasic dose response curve in that when very low or very high doses (fluence (J/cm²), irradiance (mW/cm²), delivery time or number of repetitions may lead to no significant effect or sometimes excessive light delivery may lead to undesired inhibitory effects (62). Underdosing results in poor cellular response, but overdosing can paradoxically inhibit cell proliferation or induce apoptosis. (63)

Mechanisms of Action of Photobiomodulation

Several studies *in vitro* and *in vivo* report the effects of FBM on cell proliferation, metabolism, angiogenesis, apoptosis, and inflammation (9,12,63) and that FBM is correlated with accelerated wound healing due to the stimulation of cellular processes, such as cell migration and differentiation. It is also found that the respiratory chain in the mitochondria is stimulated by FBM, which results in an increase in the production of adenosine triphosphate (ATP) and thus increased synthesis of Deoxyribonucleic Acid (DNA), Ribonucleic Acid (RNA), and proteins.

Mitochondria contain chromophores that absorb photons from FBM. The primary chromophore that absorbs red light is the enzyme cytochrome c oxidase, the site of action of FBM being the main receptor and transducer of photosignal (63), is located in unit IV of the mitochondrial respiratory chain, resulting in the activity of several molecules, such as nitric oxide, promotes the production of ATP, calcium ions, reactive oxygen species, and several other signaling molecules. (71)

As for ATP production, they are promoted due to the FBM-stimulating electrons in the chromophores, and then electron carriers such as the cytochrome C oxidase chromophore,

deliver these electrons to their final electron acceptors, while a proton gradient is made, as well as creating a proton gradient that increases ATP production. (71)

Regarding mitochondrial alterations that increase the release of reactive oxygen species, this alteration is capable of inducing transcriptional alterations and production of nuclear factor-κB (NF-κB). NF-κB induces anti-apoptotic proteins along with cell proliferation and migration. (63)

Action of Photobiomodulation on Tumor Cells

The effects of FBM on cell proliferation and differentiation were investigated *in vitro* in different malignant cell lines and generated conflicting data when exploring a wide diversity of tumor parameters and cell lines. (49,51)

Werneck et al.(94) described that the tumor cell has a nutritional deficiency, due to its intense metabolic activity, and is therefore susceptible to the action of FBM.

The mechanism of FBM is based directly on the application of biostimulatory light energy to cells. Cellular photoreceptors absorb light, and can transfer it to the mitochondria to produce ATP (73). With increased vasodilation via ATP synthesis, the use of oxygen is increased and the activity of cytoplasmic enzymes with nucleic acids stimulates cell mitosis, which can likewise induce negative outcomes by proliferating cancer cells. (4)

In fact, studies *in vitro* show that, depending on the light parameters and cell type, pre-exposure to FBM can modulate the cytotoxic response of cancer cells exposed to irradiation. (74)

The cellular proliferative potential of irradiation has attracted some negative speculation that it could also increase tumor growth in neoplastic diseases. (13)

Studies *in vitro* and *in vivo* have demonstrated that FBM presents biomodulatory results on different cell types, characterized by increased viability, gene and protein expression by cells submitted to irradiation. (10,64)

The proliferation of both normal and tumor cells can be stimulated, and this process will depend on the parameters of the laser and the rate of cell proliferation at the time of irradiation. Depending on the parameters used, the use of FBM during this neoplastic process may favor an increase in cell proliferation and differentiation, since it has significant biomodulatory effects, and this is not a desirable effect in neoplasms. (9)

In order to elucidate the biological mechanism of cell proliferation, Sroka et al.(75) evaluated the effect of FBM on cells of different origins and different degrees of malignancy, comparing them with normal cells. An increase in the mitotic pattern was observed in benign and malignant cells after irradiation with doses in the range of 4 to 8 J/cm².

However, a reduction in the rate of cell proliferation was observed when the dose exceeded this energy range, regardless of the wavelength.

Carnevalli, et al.76), demonstrated in their study that CHO K-1 cells cultured and irradiated with low-intensity laser (830 nm and 2 J/cm²) exhibited greater ATP synthesis and mitotic capacity when subjected to nutritional stress when compared to non-irradiated cells in the control group.

Renno, and collaborators (77) investigated the effects of laser irradiation of 670, 780 and 830 nm on the proliferation of cells from human osteosarcoma and found that only the dose of 10 J/cm² at 830 nm was able to increase the proliferation of osteoblasts, while the energy densities of 1, 5 and 10 J/cm² at 780 nm decreased proliferation. Osteosarcoma cells were not affected by laser irradiation at 830 nm, while laser at 670 nm had a mild proliferative effect.

Frigo, and collaborators (13) used B16F10 murinho melanoma cells for irradiation with the laser emitting a wavelength of 660 nm, power of 50 mW and irradiance of 2.5 W/cm² at energy densities 150 J/cm² and 1050 J/cm², fractioning the energy delivered in 3 consecutive days, 9 J and 63 J respectively. After performing the exclusion test with tripan blue, they did not identify a statistically significant difference in relation to the control group.

Pinheiro, and collaborators (78) conducted a study analyzing the effect of FBM on malignant cells *in vitro*. In particular, they studied epithelial-type cancer cells, exposing them to irradiation at wavelengths of 635 and 670 nm. They found that the irradiated cells (both wavelengths) proliferated more than the non-irradiated control group. The group exposed to 670 nm proliferated more than the group at 635 nm. The authors concluded, therefore, that exposure to laser light could significantly increase the proliferation of cancer cells.

A recently published systematic review by da Silva et al.(11)suggested that FBM can be used in cancerous lesions in order to decrease the proliferation of cancer cells, depending on the parameters used; however, the lack of standardization of laser irradiation protocols for investigations does not allow the establishment of optimal parameters for this purpose, so FBM should be used with caution in cancer patients until further studies are conducted.

Thus, it is important to seek a better therapeutic approach for osteosarcoma, taking into account the need to minimize adverse effects, reduce the incidence of metastases, increase survival time and provide better quality of life to patients, considering that depending on the energy density applied to these cells, FBM can stimulate metastasis, being a form of therapy contraindicated, as it will lead to an increase in the disease, or it

can inhibit cell proliferation, being considered a favorable therapy that can be used as an adjuvant in the treatment of cancer patients.

CONCLUSION

The available literature is still scarce for the definition of the ideal dosimetric parameters of photobiomodulation in cancer cells and when considering the use of the therapy as a bioinhibitory treatment in this cell type, more studies are needed to elucidate the main factors responsible for the different behaviors in these cells, since in specific parameters this therapy can promote biostimulation or even cell inhibition and should be used with caution in practice clinic, avoiding increasing existing cancerous tissue.

REFERENCES

1. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 68(6), 394–424.

2. Fitzmaurice, C., Allen, C., Barber, R., Barregard, L., Bhutta, L., & Brenner, H. (2018). Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: A systematic analysis for the Global Burden of Disease Study. *JAMA Oncology*, 3(4), 524–548.

3. Kara, C., Selamet, H., Gökmenoğlu, C., & Kara, N. (2018). Low level laser therapy induces increased viability and proliferation in isolated cancer cells. *Cell Proliferation*, 51(2), 1–6.

4. Kara, C., Selamet, H., Gökmenoğlu, C., & Kara, N. (2017). Low level laser therapy induces increased viability and proliferation in isolated cancer cells. *Cell Proliferation*, (November), 1–6.

5. De Oliveira, M. M., Malta, D. C., Guauche, H., De Moura, L., & Azevedo e Silva, G. (2015). Estimativa de pessoas com diagnóstico de câncer no Brasil: Dados da pesquisa nacional de saúde, 2013. *Revista Brasileira de Epidemiologia*, 18, 146–157.

6. Crous, A., & Abrahamse, H. (2016). Low-intensity laser irradiation at 636 nm induces increased viability and proliferation in isolated lung cancer stem cells. *Photomedicine and Laser Surgery*, 34(11), 525–532.

7. Akens, M. K., Wise-Milestone, L., Won, E., Schwock, J., Yee, A. J. M., Wilson, B. C., et al. (2014). In vitro and in vivo effects of photodynamic therapy on metastatic breast cancer cells pre-treated with zoledronic acid. *Photodiagnosis and Photodynamic Therapy*, 11(3), 426–433. https://doi.org/10.1016/j.pdpdt.2014.04.002

8. Basso, F. G., Turrioni, A. P. S., Soares, D. G., Bagnato, V. S., Hebling, J., & De Souza Costa, C. A. (2014). Low-level laser therapy for osteonecrotic lesions: Effects on osteoblasts treated with zoledronic acid. *Supportive Care in Cancer*, 22(10), 2741–2748.

9. Renno, A. C. M., Mcdonnell, P. A., Crovace, M. C., Zanotto, E. D., & Laakso, L. (2010). Effect of 830 nm laser phototherapy on osteoblasts grown in vitro on biosilicate® scaffolds. *Photomedicine and Laser Surgery*, 28(1), 131–133.

10. Incerti Parenti, S., Checchi, L., Fini, M., & Tschon, M. (2014). Different doses of low-level laser irradiation modulate the in vitro response of osteoblast-like cells. *Journal of Biomedical Optics*, 19(10), 108002.

11. Da Silva, J. L., Silva-de-Oliveira, A. F. S., Andraus, R. A. C., & Maia, L. P. (2019). Effects of low level laser therapy in cancer cells—a systematic review of the literature. *Lasers in Medical Science*.

12. Bashardoust Tajali, S., MacDermid, J. C., Houghton, P., & Grewal, R. (2010). Effects of low power laser irradiation on bone healing in animals: A meta-analysis. *Journal of Orthopaedic Surgery and Research*, 5(1), 1–10.

13. Frigo, L., Luppi, J. S. S., Favero, G. M., Maria, D. A., Penna, S. C., & Bjordal, J. M., et al. (2009). The effect of low-level laser irradiation (In-Ga-Al-AsP - 660 nm) on melanoma in vitro and in vivo. *BMC Cancer*, 9, 1–8.

14. Sudhakar, A. (2009). History of cancer, ancient and modern treatment methods. *Journal of Cancer Science & Therapy*, 01(02), i–iv.

15. Balasopoulou, A., Kokkinos, P., Pagoulatos, D., Plotas, P., Makri, O. E., Georgakopoulos, C. D., et al. (2017). Symposium: Recent advances and challenges in the management of retinoblastoma globe-saving treatments. *BMC Ophthalmology*, 17(1), 1.

16. Maresso, K. C., Tsai, K. Y., Brown, P. H., Lippman, S., Hawk, E., & Sciences, P., et al. (2016). Molecular cancer prevention: Current status & future. *CA: A Cancer Journal for Clinicians*, 65(5), 345–383.

17. Ribeiro, B. I., do Céu, C. C. Í., Milagros, B. P. M., & de Souza, L. B. (2016). Desigualdades socioeconômicas e mortalidade por câncer: um estudo ecológico no Brasil. *Revista Brasileira em Promoção da Saúde*, 29(3), 350–356.

18. Brenner, D. R., Brockton, N. T., Kotsopoulos, J., Cotterchio, M., Boucher, B. A., Courneya, K. S., et al. (2016). Breast cancer survival among young women: A review of the role of modifiable lifestyle factors. *Cancer Causes & Control*, 27(4), 459–472.

19. Gurgel, D. C., Capistrano Junior, V. L. M., Nogueira, I. C., & Neto, P. P. (2018). Atividade física e câncer: Intervenções nutricionais para um melhor prognóstico. *Motricidade*, 14(1), 398–404.

20. Freire, M. E. M., da Costa, S. F. G., de Lima, R. A. G., & Sawada, N. O. (2018). Health-related quality of life of patients with cancer in palliative care. *Texto e Contexto Enfermagem*, 27(2), 1–13.

21. Stewart, B. W., & Wild, C. P. (2015). *World cancer report 2014*. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press. *Advances in Nutrition*, 7(2), 418–419.

22. Şengül, M. C. B., Kaya, V., Şen, C. A., & Kaya, K. (2014). Association between suicidal ideation and behavior, and depression, anxiety, and perceived social support in cancer patients. *Medical Science Monitor*, 20, 329–336.

23. Souza, M. G. G. (2008). O olhar que olha o outro... Um estudo com familiares de pessoas em quimioterapia antineoplásica. *Revista Brasileira de Cancerologia*, 54(1), 31–41.

24. Sette, C. P., & Capitão, C. G. (2018). Efeito moderador do suporte social em pacientes oncológicos. *Revista Brasileira de Cancerologia*, 19(2), 265–277.

25. Guerra, M. R., Bustamante-Teixeira, M. T., Corrêa, C. S. L., De Abreu, D. M. X., Curado, M. P., Mooney, M., et al. (2017). Magnitude e variação da carga da mortalidade por câncer no Brasil e unidades da federação, 1990 e 2015. *Revista Brasileira de Epidemiologia*, 20(25000192049), 102–115.

26. Petrellis, M. C., da Fonseca, G. A. M. D., de Barros Pinto, A., Rosa, F. O., Maria, D. A., Shibli, J. A., et al. (2020). Laser-photobiomodulation on experimental cancer pain model

in Walker tumor-256. *Journal of Photochemistry and Photobiology B: Biology*, 210(July), 111979. https://doi.org/10.1016/j.jphotobiol.2020.111979

27. Gray, J. M., Rasanayagam, S., Engel, C., & Rizzo, J. (2017). State of the evidence 2017: An update on the connection between breast cancer and the environment. *Environmental Health: A Global Access Science Source*, 16(1), 1–61.

28. Mirabello, L., Yeager, M., Mai, P. L., Gastier-Foster, J. M., Gorlick, R., Khanna, C., et al. (2015). Germline TP53 variants and susceptibility to osteosarcoma. *Journal of the National Cancer Institute*, 107(7), 5–8.

29. Chen, X., & Zhang, Y. (2019). Bmp-2 and mir-29c in osteosarcoma tissues on proliferation and invasion of osteosarcoma cells. *Oncology Letters*, 17(6), 5389–5394.

30. Xie, C., Chen, B., Wu, B., Guo, J., & Cao, Y. (2018). LncRNA TUG1 promotes cell proliferation and suppresses apoptosis in osteosarcoma by regulating the miR-212-3p/FOXA1 axis. *Biomedicine & Pharmacotherapy*, 97(250), 1645–1653.

31. Tanaka, M. H., Penna, V., Chung, W. U. T. U., & Lopes, A. (1994). Tumores malignos primários dos ossos. *Arquivos Católicos de Medicina*, 26, 18–21.

32. Isakoff, M. S., Bielack, S. S., Meltzer, P., & Gorlick, R. (2015). Osteosarcoma: Current treatment and a collaborative pathway to success. *Journal of Clinical Oncology*, 33(27), 3029–3035.

33. Tempelaere, C., Biau, D., Babinet, A., & Anract, P. (2019). Osteosarcoma after the age of fifty: A clinicopathological study. *European Journal of Surgical Oncology*, 45(7), 1288–1292.

34. Geary, R. L., Corrigan, L. R., Carney, D. N., & Higgins, M. J. (2019). Osteosarcoma and second malignant neoplasms: A case series. *Irish Journal of Medical Science*.

35. Negri, G. L., Grande, B. M., Delaidelli, A., El-Naggar, A., Cochrane, D., Lau, C. C., et al. (2019). Integrative genomic analysis of matched primary and metastatic pediatric osteosarcoma. *Journal of Pathology*.

36. Cavalcante, E. T. M., Oliveira, L. L. P., Andrade, L. C., Vieira, V. da F., & Galloti, F. C. M. (2020). OSTEOSSARCOMA: INTERFERÊNCIA DA. *Revista*.

37. Carina, V., Costa, V., Sartori, M., Bellavia, D., De Luca, A., Raimondi, L., et al. (2019). Adjuvant biophysical therapies in osteosarcoma. *Cancers (Basel)*, 11(3).

38. Ma, J., Gao, W., & Gao, J. (2019). sCLU as prognostic biomarker and therapeutic target in osteosarcoma. *Bioengineered*, 10(1), 229–239.

39. Dong, Q. L., & Xing, X. Y. (2018). Cancer cells arise from bacteria. *Cancer Cell International*, 18(1), 1–9. https://doi.org/10.1186/s12935-018-0699-4

40. Cavallo, F., De Giovanni, C., Nanni, P., Forni, G., & Lollini, P. L. (2011). The immune hallmarks of cancer. *Cancer Immunology, Immunotherapy*, 60(3), 319–326.

41. Kroemer, G., & Pouyssegur, J. (2008). Tumor cell metabolism: Cancer's Achilles' heel. *Cancer Cell*, 13(6), 472–482.

42. Martinez, M. A. R., Francisco, G., Cabral, L. S., Ruiz, I. R. G., & Neto, C. F. (2006). Genética molecular aplicada ao câncer cutâneo não melanoma. *Anais Brasileiros de Dermatologia*, 81(5), 405–419.

43. Li, T., Copeland, C., & Le, A. (2021). Glutamine metabolism in cancer. *Advances in Experimental Medicine and Biology*, 1311, 17–38.

44. Burkhart, D. L., & Sage, J. (2008). Cellular mechanisms of tumour suppression by the retinoblastoma gene. *Nature Reviews Cancer*, 8(9), 671–682.

45. Nakase, M., Inui, M., Okumura, K., Kamei, T., Nakamura, S., & Tagawa, T. (2005). P53 gene therapy of human osteocarcinoma using a transferrin-modified cationic liposome. *Molecular Cancer Therapeutics*, 4(4), 625–631.

46. Davidovich, L. (2015). Os quanta de luz e a ótica quântica. *Revista Brasileira de Ensino de Física*, 37(4).

47. Bayat, M., Virdi, A., Rezaei, F., & Chien, S. (2018). Comparison of the in vitro effects of low-level laser therapy and low-intensity pulsed ultrasound therapy on bony cells and stem cells. *Progress in Biophysics and Molecular Biology*, 133, 36–48. https://doi.org/10.1016/j.pbiomolbio.2017.11.001

48. Hamblin, M. R., Nelson, S. T., & Strahan, J. R. (2018). Photobiomodulation and cancer: What is the truth? *Photomedicine and Laser Surgery*, 36(5), 241–245.

49. Bensadoun, R. J., Epstein, J. B., Nair, R. G., Barasch, A., Raber-Durlacher, J. E., Migliorati, C., et al. (2020). Safety and efficacy of photobiomodulation therapy in oncology: A systematic review. *Cancer Medicine*, 1–22.

50. Baxter, D. (2003). Laserterapia de baixa intensidade. In *Eletroterapia: Prática Baseada em Evidências* (pp. 171–186).

51. Zecha, J. A. E. M., Raber-Durlacher, J. E., Nair, R. G., Epstein, J. B., Sonis, S. T., Elad, S., Hamblin, M. R., Barach, A., Migliorati, C. A., Milstein, D. M. J., Genot, M.-T., Lansaat, L., & Brink, R. van der. (2016). 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. *HHS Public Access*, 24(6).

52. Moskvin, S. V. (2017). Low-level laser therapy in Russia: History, science and practice. *Journal of Lasers in Medical Sciences*, 8(2), 56–65. http://dx.doi.org/10.15171/jlms.2017.11

53. Bensadoun, R. J., & Nair, R. G. (2012). Low-level laser therapy in the prevention and treatment of cancer therapy-induced mucositis: 2012 state of the art based on literature review and meta-analysis. *Current Opinion in Oncology*, 24(4), 363–370.

54. Cheng, W., Yao, M., Sun, K., & Li, W. (2020). Progress in photobiomodulation for bone fractures: A narrative review. *Photobiomodulation, Photomedicine, and Laser Surgery*, 38(5), 260–271.

55. Catorze, M. G. (2009). Laser: Fundamentos e indicações em dermatologia. *Medicina Cutânea Ibero-Latino-Americana*, 37(1), 5–27.

56. Huang, Q., Ou, Y. S., Tao, Y., Yin, H., & Tu, P. H. (2016). Apoptosis and autophagy induced by pyropheophorbide-α methyl ester-mediated photodynamic therapy in human osteosarcoma MG-63 cells. *Apoptosis*, 21(6), 749–760.

57. Cavalcanti, T. M., de Almeida-Barros, R. Q., Catão de MHC de V., Feitosa, A. P. A., & Lins, R. D. A. U. (2011). Conhecimento das propriedades físicas e da interação do laser com os tecidos biológicos na odontologia. *Anais Brasileiros de Dermatologia*, 86(5), 955–960.

58. Loreti, E. H., Pascoal, V. L. W., Nogueira, B. V., Silva, I. V., & Pedrosa, D. F. (2015). Use of laser therapy in the healing process: A literature review. *Photomedicine and Laser Surgery*, 33(2), 104–116.

59. Heckler, M. C. T., Barberini, D. J., & Amorim, R. M. (2014). Low level laser therapy on cell cultures. *Revista Brasileira de Ciência Animal*, 7(14), 541–565.

60. Castano, A. P., Dai, T., Yaroslavsky, I., Cohen, R., Apruzzese, W. A., Smotrich, M. H., et al. (2007). Low-level laser therapy for zymosan-induced arthritis in rats: Importance of illumination time. *Lasers in Surgery and Medicine*, 39(6), 543–550.

61. Enwemeka, C. S. (2009). Intricacies of dose in laser phototherapy for tissue repair and pain relief. *Photomedicine and Laser Surgery*, 27(3), 387–393.

62. de Freitas, L., & Hamblin, M. R. (2016). Proposed mechanisms of photobiomodulation or low-level light therapy. *IEEE Journal of Selected Topics in Quantum Electronics*, 22(3), 1–37.

63. Tam, S. Y., Tam, V. C. W., Ramkumar, S., Khaw, M. L., Law, H. K. W., & Lee, S. W. Y. (2020). Review on the cellular mechanisms of low-level laser therapy use in oncology. *Frontiers in Oncology*, 10, 1–11.

64. Stein, C., Fernandes, R. O., Miozzo, A. P., Coronel, C. C., Baroni, B. M., Belló-Klein, A., et al. (2018). Acute effects of low-level laser therapy on patients' functional capacity in the postoperative period of coronary artery bypass graft surgery: A randomized, crossover, placebo-controlled trial. *Photomedicine and Laser Surgery*, 36(3), 122–129.

65. de Oliveira, P., Fernandes, K. R., Sperandio, E. F., Pastor, F. A. C., Nonaka, K. O., Parizotto, N. A., et al. (2012). Comparação dos efeitos do laser de baixa potência e do ULTRASSOM de baixa intensidade associado ao Biosilicato® no processo de reparo ósseo em tíbias de ratos. *Revista Brasileira de Ortopedia*, 47(1), 102–107.

66. Lins, R. D. A. U., Dantas, E. M., Lucena, K. C. R., Catão, M. H. C. V., Granville-Garcia, A. F., & Carvalho Neto, L. G. (2010). Biostimulation effects of low-power laser in the repair process. *Anais Brasileiros de Dermatologia*, 85(6), 849–855.

67. Baxter, G. D., Liu, L., Petrich, S., Gisselman, A. S., Chapple, C., Anders, J. J., et al. (2017). Low level laser therapy (Photobiomodulation therapy) for breast cancer-related lymphedema: A systematic review. *BMC Cancer*, 17(1), 833.

68. AlGhamdi, K. M., Kumar, A., & Moussa, N. A. (2012). Low-level laser therapy: A useful technique for enhancing the proliferation of various cultured cells. *Lasers in Medical Science*, 27(1), 237–249.

69. Djavid, G. E., Bigdeli, B., Goliaei, B., Nikoofar, A., Hamblin, M. R., Hospital, F., et al. (2018). HHS Public Access. *Journal of Biophotonics*, 10(12), 1732–1742.

70. Gomes Henriques, Á. C., Ginani, F., Oliveira, R. M., Keesen, T. S. L., Galvão Barboza, C. A., Oliveira Rocha, H. A., et al. (2014). Low-level laser therapy promotes proliferation and invasion of oral squamous cell carcinoma cells. *Lasers in Medical Science*, 29(4), 1385–1395.

71. Dompe, C., Moncrieff, L., Matys, J., Grzech-Leśniak, K., Kocherova, I., Bryja, A., et al. (2020). Photobiomodulation—underlying mechanism and clinical applications. *Journal of Clinical Medicine*, 9(6), 1–17.

72. Werneck, C. E., Barbosa Pinheiro, A. L., Tavares Pacheco, M. T., Pacheco Soares, C., & Freire De Castro, J. L. (2005). Laser light is capable of inducing proliferation of carcinoma cells in culture: A spectroscopic in vitro study. *Photomedicine and Laser Surgery*, 23(3), 300–303.

73. Murayama, H., Sadakane, K., Yamanoha, B., & Kogure, S. (2012). Low-power 808-nm laser irradiation inhibits cell proliferation of a human-derived glioblastoma cell line in vitro. *Lasers in Medical Science*, 27(1), 87–93.

74. Ramos Silva, C., Cabral, F. V., de Camargo, C. F. M., Núñez, S. C., Mateus Yoshimura, T., de Lima Luna, A. C., et al. (2016). Exploring the effects of low-level laser therapy on fibroblasts and tumor cells following gamma radiation exposure. *Journal of Biophotonics*, 9(11–12), 1157–1166.

75. Sroka, R., Schaffer, M., Fuchs, C., Pongratz, T., Schrader-Reichard, U., Busch, M., et al. (1999). Effects on the mitosis of normal and tumor cells induced by light treatment of different wavelengths. *Lasers in Surgery and Medicine*, 25(3), 263–271.

76. Carnevalli, C. M. M., Soares, C. P., Zângaro, R. A., Pinheiro, A. L. B., & Silva, N. S. (2003). Laser light prevents apoptosis on CHO K-1 cell line. *Journal of Clinical Laser Medicine & Surgery*, 21(4), 193–196.

77. Renno, A. C., McDonnell, P. A., Parizotto, N. A., & Laakso, E. L. (2007). The effects of laser irradiation on osteoblast and osteosarcoma cell proliferation and differentiation in vitro. *Photomedicine and Laser Surgery*, 25(4), 275–280.

78. Pinheiro, A. L. B., Carneiro, N. S., Vieira, A. L. de B., Brugnera, A. J., Zanin, F. A., Barros, R. A., et al. (2002). Effects of low-level laser therapy on malignant cells: In vitro study. *Journal of Clinical Laser Medicine & Surgery*, 20(1), 23–26.

79. Petros, A. M., Olejniczak, E. T., & Fesik, S. W. (2004). Structural biology of the Bcl-2 family of proteins. *Biochimica et Biophysica Acta - Molecular Cell Research*, 1644(2–3), 83–94.

80. Wang, Q., Zhang, L., Yuan, X., Ou, Y., Zhu, X., Cheng, Z., et al. (2016). The relationship between the Bcl-2/Bax proteins and the mitochondria-mediated apoptosis pathway in

the differentiation of adipose-derived stromal cells into neurons. *PLoS ONE*, 11(10), 1–16.

81. Moazami-Goudarzi, M., Farshdousti-Hagh, M., Hoseinpour-Feizi, A., Talebi, M., Movassaghpour-Akbari, A. A., Shams-Asanjan, K., et al. (2016). The acute lymphoblastic leukemia prognostic scoring: Whether it is possible by BCL-2, BAX gene promoter genotyping. *Caspian Journal of Internal Medicine*, 7(2), 105–113.

82. Sun, M., Zhou, C., Zeng, H., Puebla-Osorio, N., Damiani, E., Chen, J., et al. (2015). Hiporfin-mediated photodynamic therapy in preclinical treatment of osteosarcoma. *Photochemistry and Photobiology*, 91(3), 533–544.

83. Adams, J. M., & Cory, S. (2007). The Bcl-2 apoptotic switch in cancer development and therapy. *Oncogene*, 26(9), 1324–1337.

84. Peña-Blanco, A., & García-Sáez, A. J. (2018). Bax, Bak and beyond — mitochondrial performance in apoptosis. *FEBS Journal*, 285(3), 416–431.

85. O'Neill, K. L., Huang, K., Zhang, J., Chen, Y., & Luo, X. (2016). Inactivation of prosurvival Bcl-2 proteins activates Bax/Bak through the outer mitochondrial membrane. *Genes & Development*, 30(8), 973–988.

86. Tossounian, M. A., Zhang, B., & Gout, I. (2020). The writers, readers, and erasers in redox regulation of GAPDH. *Antioxidants*, 9(12), 1–21.

87. Zhong, X. Y., Yuan, X. M., Xu, Y. Y., Yin, M., Yan, W. W., Zou, S. W., et al. (2018). CARM1 methylates GAPDH to regulate glucose metabolism and is suppressed in liver cancer. *Cell Reports*, 24(12), 3207–3223. https://doi.org/10.1016/j.celrep.2018.08.066

88. Park, J. B., Park, H., Son, J., Ha, S. J., & Cho, H. S. (2019). Structural study of monomethyl fumarate-bound human GAPDH. *Molecular Cells*, 42(8), 597–603.

89. Jayaguru, P., & Mohr, S. (2011). Nuclear GAPDH: Changing the fate of Müller cells in diabetes. *Journal of Ocular Biology, Diseases, and Informatics*, 4(1–2), 34–41.

90. Zhang, J. Y., Zhang, F., Hong, C. Q., Giuliano, A. E., Cui, X. J., Zhou, G. J., et al. (2015). Critical protein GAPDH and its regulatory mechanisms in cancer cells. *Cancer Biology & Medicine*, 12(1), 10–22.

91. Tristan, C. (2012). The diverse functions of GAPDH. *Cellular & Molecular Life Sciences*, 23(2), 317–323.

92. Jo, H. Y., Kim, Y., Park, H. W., Moon, H. E., Bae, S., Kim, J., et al. (2015). The unreliability of MTT assay in the cytotoxic test of primary cultured glioblastoma cells. *Experimental Neurobiology*, 24(3), 235.

93. Artilheiro, P. P., Barbosa, J. L. P., Fernandes, K. P. S., Oliveira, T. S. de, Bussadori, S. K., & Mesquita-Ferrari, R. A. (2012). Análise comparativa dos efeitos do ultrassom terapêutico e laser de baixa potência sobre a proliferação de células musculares durante a diferenciação celular. *Fisioterapia em Movimento*, 25(1), 21–29.

94. Riss, T. L., Moravec, R. A., Niles, A. L., Duellman, S., Benink, H. A., Worzella, T. J., et al. (2004). Cell viability assays. *Assay Guidance Manual*.

95. Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1–2), 55–63.

96. McKinnon, K. M. (2019). Flow cytometry instrumentation – An overview. *Current Protocols in Cytometry*, 87(1), 1–16.

97. Todd, R. T., Braverman, A. L., & Selmecki, A. (2018). Flow cytometry analysis of fungal ploidy. *Current Protocols in Microbiology*, 50(1), 1–32.

98. Dominical, V., Samsel, L., & McCoy, J. P. (2017). Masks in imaging flow cytometry. *Methods*, 112, 9–17.

99. Phinney, D. A., & Cucci, T. L. (1989). Flow cytometry and phytoplankton. *Cytometry*, 10(5), 511–521.

100. Rieger, A. M., Nelson, K. L., Konowalchuk, J. D., & Barreda, D. R. (2011). Modified annexin V/propidium iodide apoptosis assay for accurate assessment of cell death. *Journal of Visualized Experiments*, (50), 3–6.

101. Crowley, L. C., Marfell, B. J., Scott, A. P., & Waterhouse, N. J. (2016). Quantitation of apoptosis and necrosis by annexin V binding, propidium iodide uptake, and flow cytometry. *Cold Spring Harbor Protocols*, 2016(11), 953–957.

102. VanGuilder, H. D., Vrana, K. E., & Freeman, W. M. (2008). Twenty-five years of quantitative PCR for gene expression analysis. *Biotechniques*, 44(5), 619–626.

103. Pyo, S. J., Song, W. W., Kim, I. R., Park, B. S., Kim, C. H., Shin, S. H., et al. (2013). Low-level laser therapy induces the expressions of BMP-2, osteocalcin, and TGF-β1 in hypoxic-cultured human osteoblasts. *Lasers in Medical Science*, 28(2), 543–550.

104. Lorenz, T. C. (2012). Polymerase chain reaction: Basic protocol plus troubleshooting and optimization strategies. *Journal of Visualized Experiments*, (63), 1–15.

105. Novais, C., Pires-Alves, M., & Silva, F. (2004). PCR em tempo real. *Revista Brasileira de Biotecnologia e Ciências do Movimento*, 11, 10–14.

106. Zhang, C., Wang, Y. Q., Jin, G., Wu, S., Cui, J., & Wang, R. F. (2017). Selection of reference genes for gene expression studies in human bladder cancer using SYBR-green quantitative polymerase chain reaction. *Oncology Letters*, 14(5), 6001–6011.

107. Steibel, J. P., Poletto, R., Coussens, P. M., & Rosa, G. J. M. (2009). A powerful and flexible linear mixed model framework for the analysis of relative quantification RT-PCR data. *Genomics*, 94(2), 146–152.

108. Valones, M. A. A., Guimarães, R. L., Brandão, L. A. C., De Souza, P. R. E., De Albuquerque Tavares Carvalho, A., & Crovela, S. (2009). Principles and applications of polymerase chain reaction in medical diagnostic fields: A review. *Brazilian Journal of Microbiology*, 40(1), 1–11.

109. Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods*, 25(4), 402–408.

110. Qu, R., Miao, Y., Cui, Y., Cao, Y., Zhou, Y., Tang, X., et al. (2019). Selection of reference genes for the quantitative real-time PCR normalization of gene expression in *Isatis indigotica* Fortune. *BMC Molecular Biology*, 20(1), 1–12.