 doi:10.20944/preprints202109.0453.v2

Review

Physical Bases of Boron Neutron Capture Therapy, Dosimetry, and Its Mechanisms of Action—A Critical Overview of the Literature

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Abstract: The success of boron neutron capture therapy (BNCT) depends mainly on sufficient spatial biodistribution of boron (^{10}B) localized around or within neoplastic cells to produce a high dose gradient between the tumor and healthy tissue. Contrary to what is usual in radiotherapy, BNCT proposes treatment planning directed at the cell level rather than the tumor mass. However, it is not yet possible to precisely determine the concentration of ^{10}B in a specific tissue in real-time using non-invasive methods. Some critical issues still need to be resolved if BNCT is to become a valuable, minimally invasive, and efficient cancer treatment. This review article provides an overview of the fundamental principles, recent advances, and future directions of BNCT as a cell-targeted cancer therapy. The main emphasis is on topics related to biological dosimetry, methods for assessing boron concentration, mechanisms of action of BNCT, and its physical bases for clinical implementation.

Keywords: boron neutron capture therapy (BNCT); boron imaging; biological dosimetry; radiation therapy

1. Introduction

Cancer, a multicellular and multigenic disease, is one of the leading causes of death in the world. It is the first/second cause in 112 of 183 countries and the third/fourth in 23 countries according to estimated data from the World Health Organization (WHO) in 2019. In 2020, 19.3 million new cases and 10 million cancer deaths were recorded according to GLOBOCAN estimates of 36 cancers in 185 countries [1]. Given that the annual incidence continues to increase, the clinical management of cancer remains a significant challenge. Cancer can arise from all organs and different cell types with a multifactorial etiology. In general, cancerous cells exhibit inherent phenotypical characteristics, known as the hallmarks of cancer. Hanahan and Weinberg [2] originally suggested six alterations in cell physiology that collectively dictate malignant growth: environmental independence for growth, evasion of apoptosis (programmed cell death), limitless proliferative potential, sustained angiogenesis, tissue invasion, and metastasis to other parts of the body. In a more recent update, they also included deregulated metabolism and immune system evasion as additional hallmarks, as well as two characteristics that allow the acquisition of those hallmarks: genome instability and inflammation [3].

In countries with a high gross domestic product, radiotherapy (RT) is used in more than 50% of patients either to treat the disease in a local stage or to control and alleviate symptoms of irrecoverable cases, depending on the cancer stage [1]. RT aims to deliver the optimal dose to tumor volume while preserving normal tissues, but these volumes are

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considered at the macroscopic level of treatment in treatment planning following an evidence-based population medicine approach. To achieve more efficient personalized treatment, it is desirable to select the cells to be treated, reducing damage to healthy ones.

Currently, estimates of the global demand for RT by cancer patients indicate that, for 87% of new cases of breast cancer patients, RT is an important part of therapy according to clinical guidelines and evidence-based medicine. In the case of surgery in the early stages of breast cancer and systemic therapies in metastatic cases, RT is applied as adjunctive therapy. [4]. In several tumor types, such as head and neck cancers, skin cancers, cervical cancers, brain tumors, and others, RT is considered as the alternative curative treatment option or even the basis of the definitive, curative treatment. Additionally, in many locations of the disease, RT is usually required as a prophylactic agent after surgery. In locations such as the lungs, the accuracy of the radiation beams allows the usage of RT under ablative conditions when surgery is not applicable. This is the case of Stereotactic Ablative Radiotherapy (SABR), where vascular endothelial injury and immune activation are new radiobiological aspects that must be added to the 5 R's of radiobiology (reoxygenation, repair, radiosensitivity, redistribution, and repopulation) for explaining the ablation effect.

These data indicate that the role of RT has increased in prominence compared to surgery in the treatment of localized solid tumors, the most widespread expression of cancer. Among other reasons, these achievements are the result of several innovative therapeutic methods and technological improvements, such as the implementation of devices to shape and adapt the irradiation beam to the tumor volume while safeguarding organs at risk, or the successful implementation of advanced imaging procedures used in planning and treatment [5]. The goal of RT is to achieve more accurate and efficient dose delivery to organs and tissues [6]. Patients often vary between tumor responses to RT due to differences in tumor type and other specific genetic factors not considered in treatment planning. Unfortunately, in conventional RT the dose prescription is essentially a population-based approach through the target volume segmentation in the image data of patients usually provided from CT devices.

Boron Neutron Capture Therapy (BNCT) is a high linear energy transfer (LET) radiation therapy used for cancer treatment. Boron can be selectively localized in tumor cells. Thus, BNCT is a promising disease-targeted therapy as neutrons kill preferentially the cells labeled with ^{10}B. A fundamental principle of this method is ^{10}B(n,α)^{7}Li nuclear reaction, which occurs when the stable isotope ^{10}B, delivered preferentially to the tumor cells, is subsequently irradiated with an external epithermal neutron beam to produce an α-particle (^{4}He) and a ^{7}Li nucleus. A schematic representation of this reaction is presented in Figure 1. Released α-particles (~1.47 MeV) and ^{7}Li nuclei (~0.84 MeV) have high LET ~175 keV/μm [7–9]. About 94% of the time, the recoiling ^{7}Li ion is produced in an excited state and emits a low LET 477 keV gamma-ray during deexcitation. In the remaining 6% events, ^{7}Li is emitted with no gamma-ray emission in the ground state.

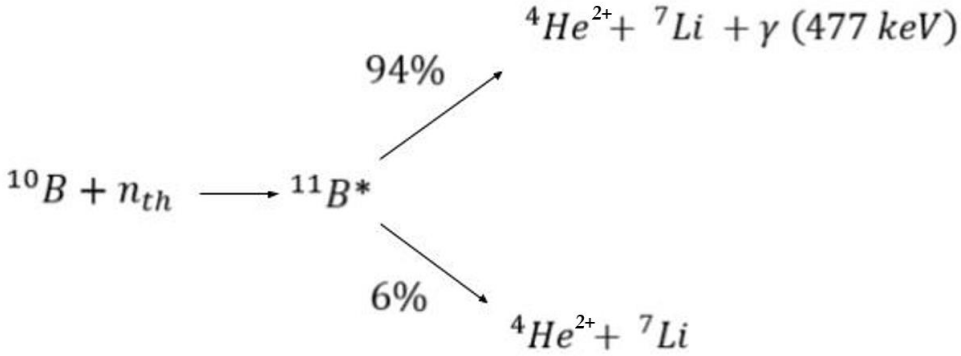

Figure 1. ^{10}B(n,α)^{7}Li reaction.

Unfortunately, after many years of research led by scientists and specialists, they are still struggling with some critical issues of BNCT. Firstly, generating a therapeutic beam

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with an optimal energy spectrum that can deliver the neutrons to the correct location while minimizing the dose delivered to healthy tissue. Second, finding nontoxic ^{10}B delivery agents: the boron carrier compound is one of the fundamental aspects of BNCT and should bring significantly more isotopes to the cancerous cells than to the healthy tissue or, ideally, only into the cancerous cells. It should also meet requirements such as water solubility, chemical stability, and preservation of a constant high concentration during the treatment procedure [10]. As the boron concentration level directly affects the intensity of the boron neutron capture reaction and hence the dose to the tumor and other tissue, it is also essential to image the boron distribution when considering BNCT. Several modalities are widely used to assess the boron dose delivered to the residual tumor volume, and they can provide information on the distribution of ^{10}B at the microscopic level [11,12]. To improve molecular imaging, several other approaches have been proposed [13–15]. Another critical issue is developing treatment planning programs and systems to calculate the dose, predict the particle fluxes, and expect the incidence angles on the patients to achieve the adequate relative dose distribution. If the reactor generates the BNCT treatment beams, the treatment planner must determine the dose induced by neutrons and gamma photons. In this case, most of the gamma photons occurring in the beam originate from the reactor core. Fortunately, development research on compact, in-hospital accelerator-based neutron sources, ready for installation in hospital environments, has been ongoing for many years in several countries. It allowed for more widespread use of the BNCT technique. The world's first accelerator-based system for clinical BNCT irradiation (C-BENS) with cyclotron of Sumitomo Heavy Industries has been developed at the Kyoto Research Reactor Institute [16]. Within Europe, one of the first commercial accelerator-based Boron Neutron Capture Therapy Platforms was designed by Neutron Therapeutics and installed in the Helsinki University Hospital. Numerous reasons define why the cell, after BNCT treatment, may die. They relate mainly to the cell cycle phase in which irradiation occurs, the cell type, the radiation dose, and the oxygen supply. However, what drives cell cancer during switching from a repair program to cell death, and what drives the cancerous cell to choose a specific pathway of death? The specific mechanism of cell death and the mechanism of repair after BNCT are not sufficiently known. In this case, the evaluation of early and late markers of cellular responses after the introduction of BNCT should be considered. They are crucial for the further development of BNCT.

This review highlights the issues described above and evaluates them in future directions and the further development of Boron Neutron Capture Therapy as an effective cancer treatment. Later in the article, the possible mechanisms BNCT-induced cell death are described and the different types will also be presented. The answers to the presented and other questions that will undoubtedly arise in the further development of research on cancer radiation therapy and the progression in solving the issues presented, even in part, will eventually lead to continued improvement in BNCT cancer treatment.

Section 2 describes the general principle of BNCT therapy, starting from boron delivery agents, to physical bases and fundamental dosimetric process of BNCT, such as the main components of the absorbed dose. It also describes mechanisms and types of cell death that occur during and after irradiation and cites and describes several studies that attempt to investigate DNA damage and repair induced by BNCT. At the end of the chapter, in the section 'BNCT biological dosimetry", the focus was on the biological component of BNCT and the understanding and evaluation of the increased radiosensitization effect on the example of the conducted research. Chapter 3 describes methods for assessing boron distribution within the body in tumor cells and normal tissues. Modalities such as positron emission tomography and magnetic resonance imaging, mass spectrometry imaging, single-photon emission computed tomography, and prompt gamma photon detectors are presented and described in the order mentioned above.

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2. Mechanisms of action of BNCT

Two types of neutron beams are commonly used in BNCT: thermal beams (~0.0254 eV) and epithermal beams (0.5 eV to 40 keV). For clinical purposes, the most useful are epithermal neutrons because, while entering the tissue, they create a radiation field with maximum thermal flux at a depth of 2-3 cm, which then drops exponentially. In turn, when a thermal beam enters the tissue, the thermal flux, which is created as a result, falls exponentially from the surface. For BNCT in boron-labeled tumor cells, an adequate thermal neutron field must be created. Therefore, a neutron source compliant with the standards of the International Atomic Energy Agency (IAEA) is required. [17]

Until recently, the value of BNCT was largely restricted and the number of patients treated with BNCT was very limited because treatment could only be performed in nuclear research reactors, the only neutron source at the time. Furthermore, most of the clinical trials conducted were carried out in facilities at nuclear reactor sources. However, with the improvement of neutron beam-generating instruments, BNCT would be able to damage tumor cells more effectively while at the same time avoiding affecting normal peripheral cells.

Boron delivery agents are one of the essential aspects of BNCT. ^{10}B should be retained in the tumor, at least for the duration of neutron irradiation, which can take up to an hour. However, the way to concentrate ^{10}B in enough amounts and preferentially in cancer cells is currently the main limitation of the effectiveness of BNCT. Many compounds have been developed to date, but at present, only two boron agents are widely used as boron carriers: sodium borocaptate ($Na_2B_{12}H_1SH$ [[^{10}B]BSH]) and [^{10}B]4-borono-L-phenylalanine (BPA), two low molecular weight boron-containing drugs.

BSH consists of 12 ^{10}B atoms and is used mainly to treat malignant gliomas. BSH is not delivered to the normal brain through the intact blood-brain barrier (BBB), and it is difficult to selectively internalize BSH in tumor cells because of its high hydrophilicity. Its concentration in the target is related to the concentration of the agent in the blood and the vascularization of the neoplasm [18]. BSH has a passive diffuse accumulation mechanism. In malignant cells in the brain, it accumulates only in the tumor region where the blood-brain barrier is disrupted [19].

On the other hand, BPA is a derivative of phenylalanine and is actively transported into tumor cells, mainly through the L-type amino acid transporter 1 (LAT 1) [20]. BPA logP is negative (-3.65), and this indicates that it will not pass BBB passively because only small molecules with logP in the range +1...+2 can cross BBB with passive (diffusive) transport [15,21]. Therefore, the only way for BPA to cross the BBB is to sneak it through some transporter, such as an L-type amino acid. BPA has been reported to accumulate specifically in tumor cells due to its structural similarities to tyrosine [5]. The structure of tyrosine and phenylalanine is visualized in Figure 2. As L-type amino acid transporters are involved in some important human diseases and are overexpressed in human tumors, they improve targeted delivery into the brain and cancer cells. LAT 1 is also present in the blood-brain barrier (BBB), the blood-retina barrier, the cerebral cortex, testes, placenta, and bone marrow. Injection of [^{10}B]BPA for intravenous administration in BNCT is prepared as the [^{10}B]BPA-fructose complex [22]. The reason for tagging compounds with positron emitters is to accurately determine the boron distribution and concentration in the tumor and surrounding tissue using PET. BPA was approved in Japan as a commercial drug with social security reimbursement and has been available on the market since 20th May 2020 under the name Borofalan (^{10}B) [23].

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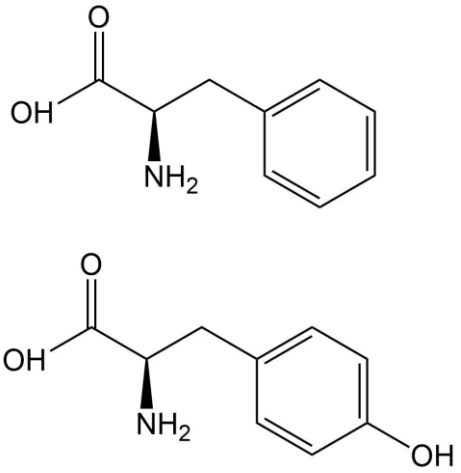

Figure 2. Amino acid chemical structures: phenylalanine (top), tyrosine (bottom)

Three generations of boron compounds can be distinguished: (I) Boric acid and its derivatives used in the first clinical trials. (II) Boron-modified amino acids. This group included boron carriers such as BPA, BSH, $Na_2B_{12}H_{11}SH$, and (L)-4-dihydroxyborylphenylalane. (III) The third generation of boron agents has attracted the attention of scientists over the past two decades. They focus on using biochemical pathways to accumulate boronated analogues in subcellular structures. These new BNCT agents include boron-containing small molecules, peptides, antibody-based delivery systems, boron compound conjugates, boron-dispersed nanoparticles (nanomaterial-based delivery systems), and many others currently under evaluation. Targeted boron delivery agents combine boron-containing agents with tumor-targeting molecules (e.g., nucleosides, porphyrins, peptides, proteins, or antibodies). Boron-delivery nanomaterials can transport various boron-containing compounds into tumor cells by taking advantage of the enhanced permeability and retention effects of nanomaterials and the active targeting effects mediated by tumor-targeted ligands grafted on the surface of the materials. The following boron-delivery nanomaterials can be distinguished: dendrimers, liposomes, polymeric nanoparticles, boron nitride, carbon nanotubes, mesoporous silica nanoparticles, ferromagnetic and paramagnetic nanoparticles – some of them used for MRI imaging, – gold nanoparticles, and BPO_4 nanoparticles. There are many promising routes in drug delivery systems, and there is still a pressing need to develop new boron delivery agents, but without adequate research and clinical trials, it is difficult to determine which is the most feasible [24,25].

2.1. Physical bases and fundamental dosimetric process of BNCT

Ionizing radiation has many forms, from alpha, beta, proton, or neutron particles to X or gamma rays and others. In Boron Neutron Capture Therapy, the components that contribute to the total absorbed dose rate are due to the elastic interaction of incident neutrons with hydrogen, the gamma-ray dose emitted by the source, and thermal neutrons captured by hydrogen, nitrogen, and boron [26]. Each of the components has various biological weighting factors. The total biologically absorbed dose (Gy-Eq) is the sum of the physical dose components (D) multiplied by the compound biological effectiveness (CBE) or relative biological effectiveness (RBE) of each dose component. RBE is the ratio of the dose absorbed from the reference radiation to the value of the tested radiation dose, producing the same biological effect, whereas CBE represents values of the biological efficacy for each dose component, depending on the boron carrier used. CBE differs from RBE with high LET general radiation in that the value varies with the target cells and the tissue and type of boron compounds used [27]. However, because of the occurrence of events at the cellular and subcellular levels, the different energies and types of radiation involved, the dosimetry and accurate estimation of the RBE, CBE, and therefore the biological effectiveness of BNCT is challenging.

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Streitmatter et al. [28] presented a multiscale system of dosimetric and radiobiological models that better assess biological effectiveness. It can predict not only CBE and RBE but also other critical biological metrics for neutron sources, such as boron microdistribution and tissue types. The model was tested against results from published experiments in vitro and in vivo, with and without boron, and showed good agreement between both.

Human tissue also contains certain isotopes that react with neutrons. Due to the values of nuclear cross-sections, the most significant interactions of neutrons with human tissue involve ^{1}H, ^{12}C, ^{14}N, and ^{16}O atoms, which represent 99.2% of all atoms in the human body [29]. The different types of DNA lesions include base damage, DNA-protein or DNA-DNA crosslinks, double-strand breaks (DSB), single-strand breaks (SSB), sugar-phosphate backbone interruption, etc. and its distribution and the repair pathways depend strongly on the type of radiation used during BNCT, and its LET characteristics [10,30]. Consequently, all described components should be considered during the evaluation and calculation of the received doses because they could also be responsible for the adverse effects of BNCT. The most advanced methods of calculating the fluxes and doses in complex geometries with a heterogeneous physical density like those in the patient are based on Monte Carlo techniques. Four main absorbed dose components in BNCT can be distinguished:

1. Fast neutron dose: according to ^{1}H(n,n)p reactions, fast and epithermal neutrons cause elastic neutron collisions with hydrogen in tissue (giving recoiling protons and gammas). Other energy depositions from fast neutron reactions like ^{12}C(n,α) are also included.
2. Incident and secondary gamma ray's dose: Primary gamma dose from the beam port and secondary gamma dose by ^{1}H(n,γ)^{2}H.
3. Nitrogen dose: according to the ^{14}N(n,p)^{14}C reaction, the ^{14}N element in the tissue captures a thermal neutron, and, as a result, a ~600 keV proton is emitted. The dose is obtained from locally delivered energy from the recoiling ^{14}C nucleus and the energetic proton.
4. Boron dose: energy deposited by the ^{10}B(n,α)^{7}Li reaction. ^{10}B captures a thermal neutron, and as a result, an alpha particle and recoiling ^{7}Li ion are emitted. The dose derived from the reaction products is ~2,31 MeV.

The cross-sections for some of those reactions are shown in Figure 3.

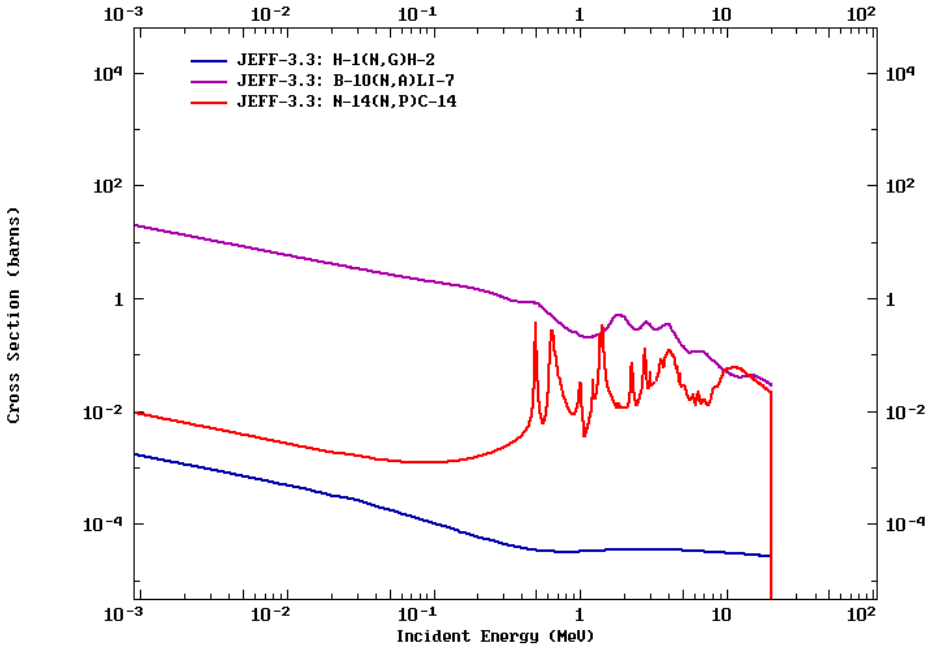

Figure 3. Cross-sections of reactions that take place because of neutron collisions. ^{1}H(n,γ)^{2}H (blue), ^{10}B(n,α)^{7}Li (magenta), ^{14}N(n,p)^{14}C (red) [31].

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The recoil ionization of hydrogen is the leading way by which neutrons with energy >0.01 MeV deposit the dose. However, the $^{14}N(n,p)^{14}C$ reaction at neutron energies <1eV is responsible for ~80% of the energy released in the tissue [29]. 88.8% of thermal neutrons are absorbed in the $^{1}H(n,\gamma)^{2}H$ reaction, and 10.6% of thermal neutrons are absorbed in the $^{14}N(n,p)^{14}C$ reactions. Additionally, in the reactions mentioned above, the ^{14}N atom also loses an electron. However, the proton and electron are not combined instantly as ^{1}H. For this, the proton is moving too rapidly through the tissue (Q =0.58 MeV) and will cause further ionization due to the high LET. The emitted proton average residual range in soft tissue (after entering the high-LET Bragg peak phase) is longer than the diameter of a typical cell nucleus but shorter than the diameter of a typical human cell. It is also necessary to estimate the cell-killing potential of the $^{14}N(n,p)^{14}C$ reaction and consider it, since the adenosine of the bases of ATP, ADP, AMP, DNA and RNA, and other common molecules such as NADH contain a significant amount of nitrogen. In a tissue exposed to a dose arising from a fast neutron beam, cells killed by $^{14}N(n,p)^{14}C$ reactions compared to those killed by recoil proton and heavy-ion tracks are imperceptible [29]. Additionally, the doses of ^{14}C decay compared to background radiation and the statutory limits are insignificantly smaller. The fraction of respiratory phosphate molecules – i.e., AMP, ADP, ATP, etc. – that undergo the $^{14}N(n,p)^{14}C$ reaction are negligible at therapeutic neutron doses. Determining the dose resulting from the reaction $^{14}N(n,p)^{14}C$ is necessary for situations where people may be exposed to prolonged exposure to significant thermal neutron fluxes [29].

As a result, the dosimetry of BNCT requires an in-depth analysis of various components of the radiation field. To predict a biological effect, the dose arising from each of these four components must first be multiplied by an appropriate biological weighting factor to account for differences in relative biological effectiveness and, ultimately, combined [17]. The accepted values of the biological weighting factors are 1.3 for the dose of boron in normal tissues, 3.8 for the dose of boron in the tumor, 3.2 for the thermal and fast neutron dose, and 1 for the gamma dose.

2.2. Mechanisms of cell death

In BNCT, cells are killed mainly when the alpha particle or ^{7}Li ion causes various DNA lesions leading to genome instability. During BNCT treatment, clustered DNA damage or multiple local damage sites may occur. In addition to DNA, cellular macromolecules can also be damaged, which can modulate their functions [32]. All of this happens when a heavy particle passes through the cell nucleus. The reaction products have a concise range (α < 10μm and ^{7}Li < 5μm) [33], so the kinetic energy is delivered to the target cell, whose diameter is usually ~10 μm. Therefore, it does not affect the surrounding healthy cells. Intracellular boron localization is critical because normal healthy tissue can be spared from nuclear reactions if it does not uptake ^{10}B. Unfortunately, with the currently available boron carrier compounds, some of ^{10}B also accumulate in healthy cells. The development of boron carriers is still very active today [34].

During and after irradiation, damage occurs at the cellular level. The damage can be divided into two groups - direct and indirect action mechanisms. In the first, radiation directly affects DNA, causing the ionization of atoms within the DNA molecule. However, ionization caused by radiation must take place within a few nanometers of the DNA molecule for this action to take place. In the second indirect action scenario, the radiation interacts with other target molecules or atoms that it encounters - usually water [35]. As a result, highly reactive species like HO· and H·, which can diffuse some distances in the cell, are produced. These free radicals are formed in irradiated body tissue and blood cells.

DNA damage increases together with LET of radiation [36], and the higher the LET, the higher the relative biological effectiveness (RBE). The radiation field generated during BNCT consists of components with different LET characteristics that act independently. Low-LET radiation ionizes sparsely, whereas high-LET radiation causes denser ionization along the track and can lead to more complex DNA damage. DNA damage after high-

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LET radiation remains unrepaired for a long time, leading to genome instability or cell death [37]. The density of radiation affects the presence and quality of radiation-induced DSB.

Like many cancer treatments, radiation therapy achieves its therapeutic effect by causing a reaction of different types of cell death: apoptosis, mitotic cell death or mitotic catastrophe, necrosis, autophagy, and others. Apoptosis or mitotic cell death are the most common types. Radiation-induced apoptosis is a progressive and degradative process. Extrinsic (death receptor) and intrinsic (mitochondrial) apoptosis can be distinguished. Intrinsic apoptosis is a type of regulated cell death (RCD) initiated by perturbations of the intracellular microenvironment and demarked by permeabilization of the mitochondrial outer membrane. The extrinsic apoptotic pathway is triggered by the binding of death ligands to transmembrane receptor proteins (eg. TNF-α to TNFR1). Mitotic cell death is a specific variant of RCD driven by mitotic catastrophe, an oncosuppressive mechanism to control mitosis-incompetent cells [38]. Wang et al. [39] confirmed that in glioma cells, BNCT-induced apoptosis was mediated by the Bcl-2/Bax pathway.

Another important goal of radiation therapy is to deprive cancer cells of their potential to divide and multiply indefinitely [6]. The primary and presumed cell target of the ionizing radiation is DNA itself. However, damages or mutations of different cellular macromolecules cannot be fully eliminated, and as a result, their functions could be modulated, and other subsequent biological changes can be observed after cancer treatment [32].

The DNA-DSB repair process is complex and depends on many factors, including cell cycle phase and checkpoints, DSB-inducing agents, ncRNAs, and various gene mutations characterized by different cancer cell lines. Several attempts have been made over the past few years to investigate the specific response to cellular DNA damage induced by a mixed neutron-gamma field [37,40,41]. Despite that, this phenomenon is not fully understood and determined.

Rodriguez et al. [42] have attempted in vitro studies of DNA damage and repair mechanisms induced by BNCT. The human thyroid follicular cancer cell line was used for the research. The evaluation of DNA damage was made by detecting H2AX histone phosphorylation foci (γH2AX foci). Two mediating pathways repair of the DBS have been identified in mammalian cells, HRR and NHEJ. After the analysis of follicular carcinoma cell analysis, repair pathways were observed with an increase in Rad51 and Rad54 mRNA expression at 4 and 6 h after irradiation showing the expression of enzymes belonging mainly to the HRR pathway, specifying a different DNA damage pattern, and showing activation of both repair pathways. However, what exactly determines the activation of HRR or NHEJ is not yet completely clear.

To increase anticancer biological activity during BNCT therapy, Ikuhiko Nakase et al. performed an in vitro BNCT assay [43]. The study also assessed cell death pathways to understand cell killing activity that occurs after thermal neutron irradiation. They synthesized and demonstrated organelle-targeted cell-penetrating peptide (CPP)-conjugated boron compounds. CPPs help to control intracellular localization, cell membrane penetration, and further enhance cellular uptake of the boron compound. This controlled delivery affects the types of cell death and the efficacy of the cancer cell killing activity. Treatment of DB-RLA ((BODIPY) – labeled dodecaborates conjugated to the RLA peptide) showed a higher reduction of the ATP content than other peptides tested. ATP depletion enhances necrosis, which consequently might induce necrosis in BNCT. This could be one of the significant factors of the cell-killing activity- detailed mechanisms should be further studied.

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2.3. BNCT biological dosimetry

As noted previously, the effect of BNCT is heavily dependent on a biological component. Thus, it is crucial to assess the increased radiosensitization effect promoted, in addition to the physical dose enhancement. This is a hard task that depends specifically on *in vivo* or *in vitro* studies, which involve methodologies such as proliferation tests, clonogenic tests, or the evaluation of DNA damage.

Sung et al. [44] performed clonogenic tests, evaluating survival in terms of proliferative capacity of irradiated cells, and obtained a dose-dependent suppression of cell survival when treated with BPA under BNCT irradiation schemes. This effect showed up to ~10 times less survival when boron was present during a ~3 Gy irradiation. Furthermore, they also analyzed the mitochondrial metabolic activity of irradiated cells with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The results showed a significant decrease in metabolic activity in different cells irradiated with BPA compared to cells irradiated without BPA, ranging from ~20% up to ~80% at 3 days after irradiation, depending on the cell line evaluated. This result suggested a decrease in proliferative capacity after BNCT. In addition, they also pointed out cell cycle arrest at G2/M checkpoints and an increase in apoptotic cells after BNCT versus neutron irradiation, using flow cytometry assays. The increase in apoptotic cells and cell cycle arrest in G2/M was confirmed in terms of increased expression of caspase-9 and cytochrome c and decreased expression of cyclin B1 and CDK1, respectively, using western blots. These results are consistent with reports from other studies [39,45]. Moreover, newer studies even proposed mathematical models that fit data from experiments that study the same biological-effectiveness-related cellular parameters [46].

In any case, the radiosensitization effect could be observed at a more precise level in terms of DNA damage. Thus, studies that evaluate the presence of DSB repair markers such as γH2AX foci have provided further insights into the matter. This is the case of the study by Rodriguez et al. [50], which determined that the number of localized lesions was lower when comparing gamma-ray radiation with neutron or BNCT radiation, but the damage caused by BNCT was densely concentrated in clusters, which correlates with the expected more complex damage caused by high LET radiation. Moreover, these large foci lesions were persistent when observed for longer timeframes, describing firm or irreparable long-term damage [47,48]. Thus, despite an initial lower γH2AX foci count, the BNCT DNA damage profile involves more complex and irreparable damage patterns that would mean a higher radiobiological effect. Besides, it is well known that the dose rate plays a crucial role in radiosensitization [49]. Hence, the long-term effect of the appearance of such discrete events of large dose deposition prompted by BNCT remains to be determined. These events differ greatly from the more continuous events that occur in conventional γ irradiation, depositing less dose each one.

At the same time, BNCT treatment has been shown to alter cellular oxidative stress levels, both due to BNCT itself and due to tumor-targeting boron carriers [50,51]. The effects of these on oxidative stress changes in biological effective dose and radiosensitization need to be further studied.

3. Methods for assessing the concentration of boron in the residual tumor volume and healthy tissue

BNCT agents deliver boron atoms precisely to tumor cells, maintaining an appropriate concentration higher in the tumor than in normal tissue. The effectiveness of the therapy depends on where the neoplastic cell drug was in the population of neoplastic cells and within the tumor cells. Intranuclear location of boron increases the chances of killing cells by DNA damage. The lack of a method for a quantitative imaging evaluation of the boron concentration was always one of the issues nuclear doctors faced when using neutron irradiation. Thus, methods for assessing the three-dimensional distribution of boron drugs, boron dose, and all complex radiation compositions delivered to residual tumor volume and healthy tissue are one of the most critical issues of Boron Neutron Capture

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Therapy. Chemical imaging of cellular and subcellular levels is necessary to support clinical efficacy, dosimetry studies, and generally novel drug delivery research in BNCT. To solve this problem and achieve selective tumor accumulation and reduced toxicity, several approaches have also been applied, such as coating, functionalizing, and many others. Labeling with different fluorophores or molecules with fluorescence properties was also researched and developed for better imaging [13,14].

The boron concentration level directly affects the intensity of the boron neutron capture reaction and the dose to the tumor and other tissue. So, it is essential to image the boron distribution while considering BNCT. Therefore, alternative methods to predict blood boron levels must be developed and evaluated between measurements and during irradiation. Substantial improvement in BNCT will be achieved when boron concentration is measured in situ. Additionally, it should also be considered that the uptake of the boron-carrying molecules in target cells is heterogeneous. It depends on factors such as tumor cellularity (that is, the number of tumor cells arranged in clusters) [52], cell cycle phase, and others) [62]. It is one of the crucial factors, often marked as a drawback in BNCT, because it causes ambiguity in the calculated dose distributions. For effective treatment with BNCT, the ratio of ^{10}B concentration in the tumor and its concentration in normal tissues (T/N ratio) should be 3:1 or more, and the concentration of ^{10}B in the target should be at least ~15–30 μg g^{-1} or ~10^9 atoms per cell to perform lethal tumor cell damage [63]. To summarize, to avoid unfavorable effects, the concentration of ^{10}B in tumor cells and normal tissues must be known.

3.1. Positron emission tomography and magnetic resonance imaging

Clinically applicable imaging modalities are positron emission tomography (PET) [55] and magnetic resonance imaging (MRI) techniques (^1H in BPA) [18,56]. Positron emission tomography has many abilities, such as (I) quantifying biochemical processes, (II) reconstructing the distribution of a boron carrier (this information can be later used in treatment planning), (III) finding and determining the extent of metastasis in the body, (IV) predicting the optimal time of neutron exposure to BNCT, (V) controlling the therapeutic effects, and (VI) assessing whether the patient is suitable for BNCT. The suitability of positron emission tomography for the establishment of boron concentration in healthy tissues and tumors and the needs for treatment planning have been examined in many studies [53,57,58]. However, PET imaging with current technology can mainly measure the boron distribution prior to treatment. As a result, the therapeutic dose distribution calculated with PET may disagree with the actual dose delivered. The most common radiolabelled derivative of BPA used to estimate BPA concentration in vivo through PET is [18F] FBPA. Figure 4 shows the structure of both BPA and [^{18}F]FBPA. Scientists are still conducting studies to detect a compound with a greater potential for non-invasive quantification of local boron concentration via PET imaging, e.g., theranostic agent - metabolically stable boron-derived tyrosine [59].

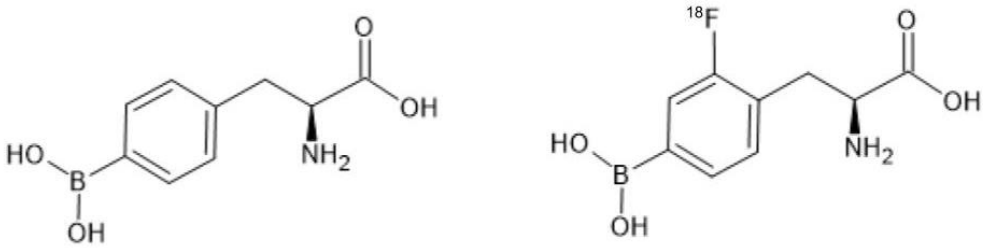

Figure 4. Structure of BPA (left) and [^{18}F]FBPA (right).

 doi:10.20944/preprints202109.0453.v2

MRI also performs well as a modality for indirect quantification of the in vivo boron distribution at the target site, during and before neutron irradiation [60]. It can provide functional and morphological information without using radiation, which makes it safer. For this purpose, to obtain high-contrast images, it is necessary to introduce nontoxic ^{10}B molecular compounds tagged with a paramagnetic ion into the body, such as gadolinium, which will work as an MRI reporter during the mapping of the boron distribution [61–63].

In the paper [64], Balcerzyk et al. explored the possibility of PET measurement of boron concentration if the compound contains the *R*-BF_3 moiety labeling it with ^{18}F. This method was applied to [18F]NaBF4 used in the preclinical study of thyroid cancer.

However, measurement of the net content of ^{10}B atoms – bound and free pools of boron – and factors affecting net in individual tumor cells are not widely described in the literature and remain challenging, as both PET and MRI modalities do not offer sufficient spatial resolution to quantify boron atoms in single cells [65].

3.2. Mass spectrometry imaging

Mass spectrometry imaging (MSI) is a powerful tool capable of imaging and profiling various molecules with high sensitivity, i.e., subcellular structures and individual cells, without labeling in a single experiment, e.g., intracellular localization of pharmaceuticals. However, the disadvantage is that the use of MSI absolute quantification is usually not possible, as opposed to secondary ion mass spectrometry (SIMS), because of the diversity of factors that affect the intensities of ion signals recorded within the region of interest.

SIMS operates in the MSI mode and can routinely achieve spatial resolutions at the sub-micrometer level. Therefore, it is a powerful tool that is often used in microbioanalytical investigations and drug distribution studies [66]. Due to this dynamic, SIMS was used for quantitative mapping of boron directly at subcellular resolutions, allowing a successful evaluation of the effectiveness of various BNCT pharmaceuticals and comparison of boron concentration in subcellular regions [67].

Two directions of studies focused on SIMS use in BNCT can be distinguished: (I) microprobe methods combined with post-ionization laser techniques, and (II) use of the ion microscope technique applying a high current primary beam O_2^+ and afterward with the use of position-sensitive detector detecting positive secondary ions [54].

Chandra et al. successfully performed many SIMS-based investigations and quantitative evaluations on boron neutron capture therapy drugs. The evaluation of free or loosely bound boron pools was performed in the cytoplasm and nucleus of cryogenically prepared cultured human glioblastoma multiforme cells exposed to BPA. Both evaluated boron agents delivered ~ 70% of the boron pool in the bound and mobile form to the nucleus and cytoplasm [65,68].

Aldossari et al. [54] in their study also conducted an application study for the localization and quantification of therapeutic levels of the BNCT agent L-para-(dihydroxyboryl)-phenylalanine (BPA) in a primary cell using a high-resolution dynamic SIMS instrument. Cell cultures were obtained from patients (humans) who suffered from glioblastoma multiforme tumors.

3.3. Single-photon emission computed tomography and prompt gamma photon detectors

Many feasibility analysis of a single-photon emission computed tomography (SPECT) instrument for quantifying the boron dose has been carried out over the years. However, some of them, like the BNCT-SPECT method, would provide only dosimetric data, such as the absolute number of BNCT reactions occurring within the measured region. [69,70]. Later, modification of BNCT SPECT [71], able to extract information allowing to determine the boron concentration in real time, was proposed. It is based on the number of neutrons that pass the patient, measured by taking advantage of the cadmium neutron capture reaction $^{113}Cd(n,\gamma)$ occurring in the detector.

Imaging prompt gamma (PG) photons resulting from the $^{10}B(n,\alpha)^7Li$ reaction is another possible method for detecting boron determination and concentration. It is a similar

 doi:10.20944/preprints202109.0453.v2

approach as applied in SPECT. Many different devices like detectors (semiconductor detectors: CZT [72,73], CdTe[74,75]; scintillator detectors [87], and others) have been proposed in the course of numerous researches to promote the clinical translation of this method. Feng et al. [33] proposed a dual prompt gamma detection method that could allow an accurate three-dimensional determination and reconstruction of the boron concentration in vivo and the dose distribution in the region of interest (ROI) during BNCT. This method is based on the relationship between $^{10}B(n,\alpha)^{7}Li$ and $^{1}H(n,\gamma)^{2}H$ reactions. However, there are still many technical challenges to be solved before implementing this method in clinical applications. 3.4. Other molecular imaging tools in BNCT.

Hu et al. evaluated Particle and Heavy Ion Transport code System (PHITS) for microdosimetry in BNCT. It can help to evaluate doses in radiobiological experiments. Additionally, it can consider intracellular and intercellular heterogeneity in the distribution of ^{10}B. Therefore, it was proposed as a model that can estimate the biological effectiveness of newly developed ^{10}B compounds for BNCT, which would be advantageous in future drug discovery research. The study resulted in the general conclusion that PHITS can be applied in the evaluation of the dose rates of absorbed gamma rays and the thermal neutron fluxes within a tumor imitating medium [45,77].

Note that boron measurements at the subcellular level in the cytoplasm and nuclei samples collected after fractionation of tumor cells cannot also be made with high confidence by bulk methods of boron determination, which are vital to BNCT. Free and loosely bound boron pools would be lost (more likely) from their native subcellular locations, e.g., during the liquid centrifugation or in other steps of fractionation. Bulk techniques cannot also determine the increased accumulation of ^{10}B within the cell nucleus [65].

4. Conclusions

Boron Neutron Capture Therapy incorporates the targeted principles of some chemotherapies and targeted therapies and the anatomical location principles of conventional radiotherapy. Since some types of cancer, such as glioblastoma (GBM), remain exceptionally resistant to all current forms of therapy, such as chemotherapy, surgery, radiotherapy, and immunotherapy, Boron Neutron Capture Therapy is a promising option for these tumor types. However, some critical issues must be resolved if BNCT is to become a better and valuable cancer treatment. Since controlled intracellular targeting is of great importance in inducing the cell-killing activity of BNCT due to specific cell death pathways, such targeting should be further assessed together with the conduct of adequate research and clinical trials to determine the most profitable and promising routes in drug delivery systems. Activation of DNA response, like damage and repair mechanisms of complex double-strand DNA break activated by a mixed neutron-gamma beam, has been poorly studied; therefore, it is not fully determined. A deeper understanding of how cells preferentially select specific DNA damage responses generated by high-LET and mixed radiation and detailed mechanisms of enhanced necrosis due to depletion of ADP may lead to improved therapeutic efficiency in BNCT. Individual tumor cell quantification of bound and free pools (net cellular content) of ^{10}B needs to be further addressed, as it remains challenging (due to insufficient spatial resolution) with clinically applicable techniques. Studies designed to test and improve boron detection methods could reduce detection limits and identify accumulation regions in tumor cells and normal tissues more precisely. Further research on mechanisms for detecting the distribution of prompt gamma rays arising during BNCT could also be profitable. However, the ideal dose paradigm for BNCT, the real-time measurement of the distributions of reactions like $^{10}B(n,\alpha)\,^{7}Li$ and $^{14}N(n,p)^{14}C$, and the quantitative mapping of boron concentration in the body have yet to be determined.

Authors Contributions: D.S. wrote Section 1, Section 2.1, 2.2, and Sections 3.1, 3.2, 3.3, 4. J.A.L.V. wrote Section 2.3, contributed to the writing of Section 1, and commented and reviewed other sections. All authors have contributed, read, and agreed to the published version of the manuscript. A.L.P. leads the scientific project where this work is supported. A.L.P and M.B. were involved in

study design, manuscript style, and critically reviewed the text by contributing expertise and guidance.

Funding: This research was carried out within the Spanish project with reference RTI2018-098656-B-100, funded by FEDER / Ministerio de Ciencia e Innovación – Agencia Estatal de Investigación; The APC was also funded by this research project; D.S. received funding from ERASMUS PLUS — European Commission program for 2014 – 2020, project number 2019-1-PL01-KA103-062953; J.A.L.V. received funding from the FPU call from the Spanish Ministry of Science, Innovation, and Universities, reference number FPU19/02950.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hyuna Sung; Jacques Ferlay; Rebecca L Siegel; Mathieu Laversanne; Isabelle Soerjomataram; Ahmedin Jemal; Freddie Bray BSc Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries | Enhanced Reader. *04 February 2021*.
2. Hanahan, D.; Weinberg, R.A. The Hallmarks of Cancer. *Cell* **2000**, *100*, doi:10.1016/S0092-8674(00)81683-9.
3. Hanahan, D.; Weinberg, R.A. Hallmarks of Cancer: The Next Generation. *Cell* **2011**, *144*, doi:10.1016/j.cell.2011.02.013.
4. Atun, R.; Jaffray, D.A.; Barton, M.B.; Bray, F.; Baumann, M.; Vikram, B.; Hanna, T.P.; Knaul, F.M.; Lievens, Y.; Lui, T.Y.M.; et al. Expanding Global Access to Radiotherapy. *The Lancet Oncology* **2015**, *16*, doi:10.1016/S1470-2045(15)00222-3.
5. Baskar, R.; Lee, K.A.; Yeo, R.; Yeoh, K.-W. Cancer and Radiation Therapy: Current Advances and Future Directions. *International Journal of Medical Sciences* **2012**, *9*, doi:10.7150/ijms.3635.
6. Chen, Y.; Yang, J.; Fu, S.; Wu, J. Gold Nanoparticles as Radiosensitizers in Cancer Radiotherapy. *International Journal of Nanomedicine* **2020**, doi:10.2147/IJN.S272902.
7. Farhood, B.; Samadian, H.; Ghorbani, M.; Zakariaee, S.S.; Knaup, C. Physical, Dosimetric and Clinical Aspects and Delivery Systems in Neutron Capture Therapy. *Reports of Practical Oncology & Radiotherapy* **2018**, *23*, doi:10.1016/j.rpor.2018.07.002.
8. Nedunchezhian, K.; Aswath, N.; Thiruppathy, M.; Thirugnanamurthy, S. Boron Neutron Capture Therapy - a Literature Review. *Journal of Clinical and Diagnostic Research* 2016, *10*, ZE01–ZE04.
9. Macías, M.; Fernández, B.; Praena, J. New Data for the Definition of Neutron Beams for Boron Neutron Capture Therapy. **2020**, doi:10.1016/j.radphyschem.2021.109474.
10. Dymova, M.A.; Taskaev, S.Y.; Richter, V.A.; Kuligina, E.V. Boron Neutron Capture Therapy: Current Status and Future Perspectives. *Cancer Communications* **2020**, *40*, doi:10.1002/cac2.12089.
11. Espain, M.S.; Dattoli Viegas, A.M.; Trivillin, V.A.; saint Martin, G.; Thorp, S.I.; Curotto, P.; Pozzi, E.C.C.; González, S.J.; Portu, A.M. Neutron Autoradiography to Study the Microdistribution of Boron in the Lung. *Applied Radiation and Isotopes* **2020**, *165*, doi:10.1016/j.apradiso.2020.109331.
12. Wittig, A.; Michel, J.; Moss, R.L.; Stecher-Rasmussen, F.; Arlinghaus, H.F.; Bendel, P.; Mauri, P.L.; Altieri, S.; Hilger, R.; Salvadori, P.A.; et al. Boron Analysis and Boron Imaging in Biological Materials for Boron Neutron Capture Therapy (BNCT). *Critical Reviews in Oncology/Hematology* **2008**, *68*, doi:10.1016/j.critrevonc.2008.03.004.

13. Qi, P.; Chen, Q.; Tu, D.; Yao, S.; Zhang, Y.; Wang, J.; Xie, C.; Pan, C.; Peng, H. The Potential Role of Borophene as a Radiosensitizer in Boron Neutron Capture Therapy (BNCT) and Particle Therapy (PT). *Biomaterials Science* **2020**, *8*, doi:10.1039/D0BM00318B.
14. Wang, D.; Meng, Y.; Wang, X.; Xia, G.; Zhang, Q. The Endocytic Mechanism and Cytotoxicity of Boron-Containing Vesicles. *Chemical and Pharmaceutical Bulletin* **2020**, *68*, doi:10.1248/cpb.c19-00971.
15. Cheheltani, R.; Ezzibdeh, R.M.; Chhour, P.; Pulaparthi, K.; Kim, J.; Jurcova, M.; Hsu, J.C.; Blundell, C.; Litt, H.I.; Ferrari, V.A.; et al. Tunable, Biodegradable Gold Nanoparticles as Contrast Agents for Computed Tomography and Photoacoustic Imaging. *Biomaterials* **2016**, *102*, doi:10.1016/j.biomaterials.2016.06.015.
16. Kato, T.; Hirose, K.; Tanaka, H.; Mitsumoto, T.; Motoyanagi, T.; Arai, K.; Harada, T.; Takeuchi, A.; Kato, R.; Yajima, S.; et al. Design and Construction of an Accelerator-Based Boron Neutron Capture Therapy (AB-BNCT) Facility with Multiple Treatment Rooms at the Southern Tohoku BNCT Research Center. *Applied Radiation and Isotopes* **2020**, *156*, doi:10.1016/j.apradiso.2019.108961.
17. IAEA-TECDOC-1223 Current Status of Neutron Capture Therapy. *Iaea, 2001 (8)* **2001**, doi:10.1111/j.1558-5646.2008.00544.x.
18. Miyatake, S.-I.; Kawabata, S.; Hiramatsu, R.; Kuroiwa, T.; Suzuki, M.; Ono, K. Boron Neutron Capture Therapy of Malignant Gliomas. *Progress in neurological surgery* **2018**, *32*, doi:10.1159/000469679.
19. Yokoyama, K.; Miyatake, S.-I.; Kajimoto, Y.; Kawabata, S.; Doi, A.; Yoshida, T.; Okabe, M.; Kirihata, M.; Ono, K.; Kuroiwa, T. Analysis of Boron Distribution *In Vivo* for Boron Neutron Capture Therapy Using Two Different Boron Compounds by Secondary Ion Mass Spectrometry. *Radiation Research* **2007**, *167*, doi:10.1667/RR0501.1.
20. Puris, E.; Gynther, M.; Auriola, S.; Huttunen, K.M. L-Type Amino Acid Transporter 1 as a Target for Drug Delivery. *Pharmaceutical Research* **2020**, *37*, doi:10.1007/s11095-020-02826-8.
21. ChemSpider | Search and Share Chemistry Available online: https://www.chemspider.com/ (accessed on 3 November 2021).
22. Suzuki, M. Boron Neutron Capture Therapy (BNCT): A Unique Role in Radiotherapy with a View to Entering the Accelerator-Based BNCT Era. *International Journal of Clinical Oncology* **2020**, *25*, doi:10.1007/s10147-019-01480-4.
23. Borofalan (10B) - Stella Pharma - AdisInsight Available online: https://adisinsight.springer.com/drugs/800045201 (accessed on 16 September 2021).
24. Barth, R.F.; Mi, P.; Yang, W. Boron Delivery Agents for Neutron Capture Therapy of Cancer. *Cancer Communications* **2018**, *38*, doi:10.1186/s40880-018-0299-7.
25. Sauerwein, W.A.G.; Sancey, L.; Hey-Hawkins, E.; Kellert, M.; Panza, L.; Imperio, D.; Balcerzyk, M.; Rizzo, G.; Scalco, E.; Herrmann, K.; et al. Theranostics in Boron Neutron Capture Therapy. *Life* **2021**, *11*, doi:10.3390/life11040330.
26. Syamputra, D.N.I.; Sardjono, Y.; Mahmudah, R.S.N. Dose Analysis of BNCT Treatment Method for Rhabdomyosarcoma in the Head and Neck Regions Based on PHITS Code. *ASEAN Journal on Science and Technology for Development* **2020**, *35*, doi:10.29037/ajstd.521.
27. Ono, K.; Tanaka, H.; Suzuki, M. Reevaluation of CBE Value of BPA for Hepatocytes. *Applied Radiation and Isotopes* **2020**, *161*, doi:10.1016/j.apradiso.2020.109159.
28. Streitmatter, S.W.; Stewart, R.D.; Moffitt, G.; Jevremovic, T. Mechanistic Modeling of the Relative Biological Effectiveness of Boron Neutron Capture Therapy. *Cells* **2020**, *9*, doi:10.3390/cells9102302.

29. Swanepoel, M.W. The Role of the 14N(n,p)14C Reaction in Neutron Irradiation of Soft Tissues. In Proceedings of the Radiation Measurements; 2010; Vol. 45.
30. Freneau, A.; dos Santos, M.; Voisin, P.; Tang, N.; Bueno Vizcarra, M.; Villagrasa, C.; Roy, L.; Vaurijoux, A.; Gruel, G. Relation between DNA Double-Strand Breaks and Energy Spectra of Secondary Electrons Produced by Different X-Ray Energies. *International Journal of Radiation Biology* **2018**, *94*, doi:10.1080/09553002.2018.1518612.
31. Evaluated Nuclear Data File (ENDF) Available online: https://www.nndc.bnl.gov/exfor/endf00.jsp (accessed on 13 September 2021).
32. Desouky, O.; Ding, N.; Zhou, G. Targeted and Non-Targeted Effects of Ionizing Radiation. *Journal of Radiation Research and Applied Sciences* **2015**, *8*, doi:10.1016/j.jrras.2015.03.003.
33. Tian, F.; Geng, C.-R.; Tang, X.-B.; Shu, D.-Y.; Ye, H.-F.; Bortolussi, S.; Gong, C.-H. Analysis of Influencing Factors on the Method for Determining Boron Concentration and Dose through Dual Prompt Gamma Detection. *Nuclear Science and Techniques* **2021**, *32*, doi:10.1007/s41365-021-00873-3.
34. Hu, K.; Yang, Z.; Zhang, L.; Xie, L.; Wang, L.; Xu, H.; Josephson, L.; Liang, S.H.; Zhang, M.-R. Boron Agents for Neutron Capture Therapy. *Coordination Chemistry Reviews* **2020**, *405*, doi:10.1016/j.ccr.2019.213139.
35. Elgazzar Abdelhamid H. and Kazem, N. Biological Effects of Ionizing Radiation. In *The Pathophysiologic Basis of Nuclear Medicine*; Elgazzar, A.H., Ed.; Springer Berlin Heidelberg: Berlin, Heidelberg, 2006; pp. 540–548 ISBN 978-3-540-47953-6.
36. Fujita, Y.; Kato, I.; Iwai, S.; Ono, K.; Suzuki, M.; Sakurai, Y.; Ohnishi, K.; Ohnishi, T.; Yura, Y. Role of P53 Mutation in the Effect of Boron Neutron Capture Therapy on Oral Squamous Cell Carcinoma. *Radiation Oncology* **2009**, *4*, doi:10.1186/1748-717X-4-63.
37. Maliszewska-Olejniczak, K.; Kaniowski, D.; Araszkiewicz, M.; Tymińska, K.; Korgul, A. Molecular Mechanisms of Specific Cellular DNA Damage Response and Repair Induced by the Mixed Radiation Field During Boron Neutron Capture Therapy. *Frontiers in Oncology* **2021**, *11*, doi:10.3389/fonc.2021.676575.
38. Galluzzi, L.; Vitale, I.; Aaronson, S.A.; Abrams, J.M.; Adam, D.; Agostinis, P.; Alnemri, E.S.; Altucci, L.; Amelio, I.; Andrews, D.W.; et al. Molecular Mechanisms of Cell Death: Recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death & Differentiation* **2018**, *25*, doi:10.1038/s41418-017-0012-4.
39. Wang, P.; Zhen, H.; Jiang, X.; Zhang, W.; Cheng, X.; Guo, G.; Mao, X.; Zhang, X. Boron Neutron Capture Therapy Induces Apoptosis of Glioma Cells through Bcl-2/Bax. *BMC Cancer* **2010**, *10*, doi:10.1186/1471-2407-10-661.
40. Matsuya, Y.; Fukunaga, H.; Omura, M.; Date, H. A Model for Estimating Dose-Rate Effects on Cell-Killing of Human Melanoma after Boron Neutron Capture Therapy. *Cells* **2020**, *9*, doi:10.3390/cells9051117.
41. Seki, K.; Kinashi, Y.; Takahashi, S. Influence of P53 Status on the Effects of Boron Neutron Capture Therapy in Glioblastoma. *Anticancer research* **2015**, *35*.
42. Rodriguez, C.; Carpano, M.; Curotto, P.; Thorp, S.; Casal, M.; Juvenal, G.; Pisarev, M.; Dagrosa, M.A. In Vitro Studies of DNA Damage and Repair Mechanisms Induced by BNCT in a Poorly Differentiated Thyroid Carcinoma Cell Line. *Radiation and Environmental Biophysics* **2018**, *57*, doi:10.1007/s00411-017-0729-y.
43. Nakase, I.; Katayama, M.; Hattori, Y.; Ishimura, M.; Inaura, S.; Fujiwara, D.; Takatani-Nakase, T.; Fujii, I.; Futaki, S.; Kirihata, M. Intracellular Target Delivery of Cell-Penetrating Peptide-Conjugated

Dodecaborate for Boron Neutron Capture Therapy (BNCT). *Chemical Communications* **2019**, *55*, doi:10.1039/C9CC03924D.

44. Sun, T.; Zhang, Z.; Li, B.; Chen, G.; Xie, X.; Wei, Y.; Wu, J.; Zhou, Y.; Du, Z. Boron Neutron Capture Therapy Induces Cell Cycle Arrest and Cell Apoptosis of Glioma Stem/Progenitor Cells in Vitro. *Radiation Oncology* **2013**, *8*, doi:10.1186/1748-717X-8-195.
45. Sato, T.; Masunaga, S.; Kumada, H.; Hamada, N. Microdosimetric Modeling of Biological Effectiveness for Boron Neutron Capture Therapy Considering Intra- and Intercellular Heterogeneity in 10B Distribution. *Scientific Reports* **2018**, *8*, doi:10.1038/s41598-017-18871-0.
46. Pedrosa-Rivera, M.; Praena, J.; Porras, I.; Sabariego, M.P.; Köster, U.; Haertlein, M.; Forsyth, V.T.; Ramírez, J.C.; Jover, C.; Jimena, D.; et al. Thermal Neutron Relative Biological Effectiveness Factors for Boron Neutron Capture Therapy from In Vitro Irradiations. *Cells* **2020**, *9*, doi:10.3390/cells9102144.
47. Staaf, E.; Brehwens, K.; Haghdoost, S.; Czub, J.; Wojcik, A. Gamma-H2AX Foci in Cells Exposed to a Mixed Beam of X-Rays and Alpha Particles. *Genome Integrity* **2012**, *3*, doi:10.1186/2041-9414-3-8.
48. Masutani, M.; Baiseitov, D.; Itoh, T.; Hirai, T.; Berikkhanova, K.; Murakami, Y.; Zhumadilov, Z.; Imahori, Y.; Hoshi, M.; Itami, J. Histological and Biochemical Analysis of DNA Damage after BNCT in Rat Model. *Applied Radiation and Isotopes* **2014**, *88*, doi:10.1016/j.apradiso.2014.03.003.
49. Kuperman, V.Y. Effect of Dose Rate in Hypofractionated Radiotherapy. *Physica Medica* **2019**, *65*, doi:10.1016/j.ejmp.2019.07.005.
50. Faião-Flores, F.; Coelho, P.R.P.; Muniz, R.O.R.; Souza, G.S.; Arruda-Neto, J.; Maria, D.A. Antitumor Potential Induction and Free Radicals Production in Melanoma Cells by Boron Neutron Capture Therapy. *Applied Radiation and Isotopes* **2011**, *69*, doi:10.1016/j.apradiso.2011.05.017.
51. Mares, V.; Krajcí, D.; Lisá, V. Subcellular Targets of Mercaptoborate (BSH), a Carrier of 10B for Neutron Capture Therapy (BNCT) of Brain Tumors. *Physiological research* **2003**, *52*.
52. Coderre, J.A.; Chanana, A.D.; Joel, D.D.; Elowitz, E.H.; Micca, P.L.; Nawrocky, M.M.; Chadha, M.; Gebbers, J.O.; Shady, M.; Peress, N.S.; et al. Biodistribution of Boronophenylalanine in Patients with Glioblastoma Multiforme: Boron Concentration Correlates with Tumor Cellularity. *Radiation Research* **1998**, *149*, doi:10.2307/3579926.
53. Romanov, V.; Isohashi, K.; Alobthani, G.; Beshr, R.; Horitsugi, G.; Kanai, Y.; Naka, S.; Watabe, T.; Shimosegawa, E.; Hatazawa, J. Evaluation of the Total Distribution Volume of 18F-FBPA in Normal Tissues of Healthy Volunteers by Non-Compartmental Kinetic Modeling. *Annals of Nuclear Medicine* **2020**, *34*, doi:10.1007/s12149-019-01427-9.
54. Aldossari, S.; McMahon, G.; Lockyer, N.P.; Moore, K.L. Microdistribution and Quantification of the Boron Neutron Capture Therapy Drug BPA in Primary Cell Cultures of Human Glioblastoma Tumour by NanoSIMS. *The Analyst* **2019**, *144*, doi:10.1039/C9AN01336A.
55. Fukumitsu, N.; Matsumoto, Y. Development of an Imaging Technique for Boron Neutron Capture Therapy. *Cells* **2021**, *10*, doi:10.3390/cells10082135.
56. Menichetti, L.; Cionini, L.; Sauerwein, W.A.; Altieri, S.; Solin, O.; Minn, H.; Salvadori, P.A. Positron Emission Tomography and [18F]BPA: A Perspective Application to Assess Tumour Extraction of Boron in BNCT. *Applied Radiation and Isotopes* **2009**, *67*, doi:10.1016/j.apradiso.2009.03.062.
57. Menichetti, L.; Petroni, D.; Panetta, D.; Burchielli, S.; Bortolussi, S.; Matteucci, M.; Pascali, G.; del Turco, S.; del Guerra, A.; Altieri, S.; et al. A Micro-PET/CT Approach Using O-(2-[18F]Fluoroethyl)-l-Tyrosine in an Experimental Animal Model of F98 Glioma for BNCT. *Applied Radiation and Isotopes* **2011**, *69*, doi:10.1016/j.apradiso.2011.02.037.

58. Shimosegawa, E.; Isohashi, K.; Naka, S.; Horitsugi, G.; Hatazawa, J. Assessment of 10B Concentration in Boron Neutron Capture Therapy: Potential of Image-Guided Therapy Using 18FBPA PET. *Annals of Nuclear Medicine* **2016**, *30*, doi:10.1007/s12149-016-1121-8.
59. Li, J.; Shi, Y.; Zhang, Z.; Liu, H.; Lang, L.; Liu, T.; Chen, X.; Liu, Z. A Metabolically Stable Boron-Derived Tyrosine Serves as a Theranostic Agent for Positron Emission Tomography Guided Boron Neutron Capture Therapy. *Bioconjugate Chemistry* **2019**, *30*, doi:10.1021/acs.bioconjchem.9b00578.
60. Alberti, D.; Deagostino, A.; Toppino, A.; Protti, N.; Bortolussi, S.; Altieri, S.; Aime, S.; Geninatti Crich, S. An Innovative Therapeutic Approach for Malignant Mesothelioma Treatment Based on the Use of Gd/Boron Multimodal Probes for MRI Guided BNCT. *Journal of Controlled Release* **2018**, *280*, 31–38, doi:10.1016/j.jconrel.2018.04.043.
61. Takahashi, K.; Nakamura, H.; Furumoto, S.; Yamamoto, K.; Fukuda, H.; Matsumura, A.; Yamamoto, Y. Synthesis and in Vivo Biodistribution of BPA–Gd–DTPA Complex as a Potential MRI Contrast Carrier for Neutron Capture Therapy. *Bioorganic & Medicinal Chemistry* **2005**, *13*, doi:10.1016/j.bmc.2004.10.046.
62. Bonora, M.; Corti, M.; Borsa, F.; Bortolussi, S.; Protti, N.; Santoro, D.; Stella, S.; Altieri, S.; Zonta, C.; Clerici, A.M.; et al. 1H and 10B NMR and MRI Investigation of Boron- and Gadolinium–Boron Compounds in Boron Neutron Capture Therapy. *Applied Radiation and Isotopes* **2011**, *69*, doi:10.1016/j.apradiso.2011.02.021.
63. Geninatti-Crich, S.; Deagostino, A.; Toppino, A.; Alberti, D.; Venturello, P.; Aime, S. Boronated Compounds for Imaging Guided BNCT Applications. *Anti-Cancer Agents in Medicinal Chemistry* **2012**, *12*, doi:10.2174/187152012800617786.
64. Balcerzyk, M.; De-Miguel, M.; Guerrero, C.; Fernandez, B. Quantification of Boron Compound Concentration for BNCT Using Positron Emission Tomography. *Cells* **2020**, *9*, doi:10.3390/cells9092084.
65. CHANDRA, S.; AHMAD, T.; BARTH, R.F.; KABALKA, G.W. Quantitative Evaluation of Boron Neutron Capture Therapy (BNCT) Drugs for Boron Delivery and Retention at Subcellular-Scale Resolution in Human Glioblastoma Cells with Imaging Secondary Ion Mass Spectrometry (SIMS). *Journal of Microscopy* **2014**, *254*, doi:10.1111/jmi.12126.
66. Lanni, E.J.; Rubakhin, S.S.; Sweedler, J. v. Mass Spectrometry Imaging and Profiling of Single Cells. *Journal of Proteomics* **2012**, *75*, doi:10.1016/j.jprot.2012.03.017.
67. Barth, R.F.; Kabalka, G.W.; Yang, W.; Huo, T.; Nakkula, R.J.; Shaikh, A.L.; Haider, S.A.; Chandra, S. Evaluation of Unnatural Cyclic Amino Acids as Boron Delivery Agents for Treatment of Melanomas and Gliomas. *Applied Radiation and Isotopes* **2014**, *88*, doi:10.1016/j.apradiso.2013.11.133.
68. Chandra, S.; Barth, R.F.; Haider, S.A.; Yang, W.; Huo, T.; Shaikh, A.L.; Kabalka, G.W. Biodistribution and Subcellular Localization of an Unnatural Boron-Containing Amino Acid (Cis-ABCPC) by Imaging Secondary Ion Mass Spectrometry for Neutron Capture Therapy of Melanomas and Gliomas. *PLoS ONE* **2013**, *8*, doi:10.1371/journal.pone.0075377.
69. Minsky, D.M.; Valda, A.A.; Kreiner, A.J.; Green, S.; Wojnecki, C.; Ghani, Z. First Tomographic Image of Neutron Capture Rate in a BNCT Facility. *Applied Radiation and Isotopes* **2011**, *69*, doi:10.1016/j.apradiso.2011.01.030.
70. Minsky, D.M.; Valda, A.A.; Kreiner, A.J.; Green, S.; Wojnecki, C.; Ghani, Z. Experimental Feasibility Studies on a SPECT Tomograph for BNCT Dosimetry. *Applied Radiation and Isotopes* **2009**, *67*, doi:10.1016/j.apradiso.2009.03.044.

71. Winkler, A.; Koivunoro, H.; Reijonen, V.; Auterinen, I.; Savolainen, S. Prompt Gamma and Neutron Detection in BNCT Utilizing a CdTe Detector. *Applied Radiation and Isotopes* **2015**, *106*, doi:10.1016/j.apradiso.2015.07.040.
72. Fatemi, S.; Altieri, S.; Bortolussi, S.; Postuma, I.; Benassi, G.; Zambelli, N.; Bettelli, M.; Zanichelli, M.; Zappettini, A.; Protti, N. Preliminary Characterization of a CdZnTe Photon Detector for BNCT-SPECT. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment* **2018**, *903*, doi:10.1016/j.nima.2018.06.068.
73. Fatemi, S.; Gong, C.H.; Bortolussi, S.; Magni, C.; Postuma, I.; Bettelli, M.; Benassi, G.; Zambelli, N.; Zappettini, A.; Tang, X.B.; et al. Innovative 3D Sensitive CdZnTe Solid State Detector for Dose Monitoring in Boron Neutron Capture Therapy (BNCT). *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment* **2019**, *936*, doi:10.1016/j.nima.2018.09.135.
74. Murata, I.; Mukai, T.; Nakamura, S.; Miyamaru, H.; Kato, I. Development of a Thick CdTe Detector for BNCT–SPECT. *Applied Radiation and Isotopes* **2011**, *69*, doi:10.1016/j.apradiso.2011.05.014.
75. Murata, I.; Nakamura, S.; Manabe, M.; Miyamaru, H.; Kato, I. Characterization Measurement of a Thick CdTe Detector for BNCT-SPECT – Detection Efficiency and Energy Resolution. *Applied Radiation and Isotopes* **2014**, *88*, doi:10.1016/j.apradiso.2014.01.023.
76. Kim, M.; Hong, B.H.; Cho, I.; Park, C.; Min, S.-H.; Hwang, W.T.; Lee, W.; Kim, K.M. Design of a Scintillator-Based Prompt Gamma Camera for Boron-Neutron Capture Therapy: Comparison of SrI2 and GAGG Using Monte-Carlo Simulation. *Nuclear Engineering and Technology* **2021**, *53*, doi:10.1016/j.net.2020.07.010.
77. Hu, N.; Tanaka, H.; Takata, T.; Endo, S.; Masunaga, S.; Suzuki, M.; Sakurai, Y. Evaluation of PHITS for Microdosimetry in BNCT to Support Radiobiological Research. *Applied Radiation and Isotopes* **2020**, *161*, doi:10.1016/j.apradiso.2020.109148.