

Review

¹¹Boron Delivery Agents for Boron Proton-capture Enhanced Proton Therapy

KATALIN HIDEGHÉTY^{1,2}, SZILVIA BRUNNER¹, ANDREW CHEESMAN¹, EMILIA RITA SZABÓ¹,
RÓBERT POLANEK¹, DANIELE MARGARONE³, TÜNDE TÓKÉS¹ and KÁROLY MOGYORÓSI¹

¹ELI-ALPS, ELI-HU Non-Profit Ltd., Szeged, Hungary;

²University of Szeged, Faculty of Medicine, Department of Oncotherapy, Szeged, Hungary;

³ELI-Beamlines, Institute of Physics of the Czech Academy of Sciences, Prague, Czech Republic

Abstract. *The aim of this review was to define appropriate ¹¹B delivery agents for boron proton-capture enhanced proton therapy (BPCEPT) taking into account the accumulated knowledge on boron compounds used for boron neutron capture therapy (BNCT). BPCEPT is a promising treatment approach which uses a high linear energy transfer (LET) dose component in conjunction with conventional proton therapy to increase the relative biological effectiveness of highly-selective charged particle therapy. Boron proton fusion reactions occur with highest cross section at certain proton energy level and thus can be tailored to the target volume with careful treatment planning that defines the 675 MeV proton distribution with high accuracy. Appropriate ¹¹B compounds are required in order to achieve relevant high LET dose contribution from the boron proton-capture reaction. Previous scientific results and experiences with BNCT provide background knowledge and information regarding the optimization of boronated compound development, their characterization, measurement and imaging. However, there are substantial differences between BNCT and BPCEPT, which in turn places special unique chemical, physical and biological demands on ¹¹B-carrier compounds for BPCEPT. In this review, we evaluate well-known and recently developed boron compounds for BPCEPT.*

The electrostatic and physical properties of accelerated, charged nuclear particles (protons, heavy ions) enable their accurate submillimeter dose delivery in cancer therapy (1). This results in high tumor tissue selectivity and enhances the therapeutic index for patients with cancer. There is also an additional ~10% increase compared to photon therapy in the relative biological effectiveness of a proton beam (averaged on the particle path length) and a two- to three-fold increase in the relative biological effectiveness of densely ionizing heavy ions (2). The immense installation, maintenance and operational costs have prevented the spread of heavy-ion therapy; however, compact hospital-based cyclotrons/synchrocyclotrons, due to superconducting techniques (3), can be more cheaply installed, increasing the availability of proton therapy for oncology and providing a basis for well designed multicenter clinical studies. There is an ever-growing body of evidence on the greater value of proton therapy over the best available photon irradiation modalities. This stronger standing is supported by computational investigations, which have proven the superiority of the dose distribution of proton therapy for a wide range of malignant diseases (4) and in clinical evaluation for childhood malignancies, and in the treatment of radioresistant tumors and of tumors in close proximity to radiosensitive structures. Clinical studies on carbon-ion therapy are also very promising and confirm the enhanced biological effectiveness detected in preclinical studies, but a similar increase in the number of ion centers is not anticipated to the extreme cost for implementing heavy particle acceleration. Therefore, a method to increase the relative biological effectiveness of proton beam is of high interest in radiation oncology. Boron proton-capture enhanced proton therapy (BPCEPT), a novel approach, has emerged from alternative particle acceleration research for enhancing the biological effectiveness of proton therapy. A high linear energy transfer due to the boron

This article is freely accessible online.

Correspondence to: Szilvia Brunner, ELI-ALPS, ELI-HU Non-Profit Ltd., Wolfgang Sandner Str. 3, Szeged 6728, Hungary. Tel: +36 706202117, Fax: +36 62550191, e-mail: Szilvia.Brunner@eli-alps.hu

Key Words: Boron delivery agents, boron-proton capture enhanced proton therapy BPCEPT, boron-neutron capture therapy, BNCT, review.

proton-capture (BPC) reaction working in conjunction with a proton dose delivery might result in well-directed enhancement of the biological effect in defined sub-regions of the target volume. This binary approach can be optimized by the careful tailoring of the proton beam parameters and the selection of nontoxic ^{11}B delivery agents with high and favorable subcellular accumulation. Our main aim in this review was to analyze boron carriers from the first to the third generation used for boron-neutron capture therapy (BNCT) in order to define the most appropriate ^{11}B delivery agents for BPCEPT.

The highest probability for the most effective combination of BPC and proton dose occurs at a discrete proton energy level of 675 keV (5-9). Accessing this energy requires an initial proton deceleration to a certain position along the proton dose–depth curve (under the Bragg peak or at the end of the spread of the Bragg peak) and thus the BPC reaction can be defined with submillimeter accuracy. The additional dose enhancement generated by BPC depends on the proton intensity and intracellular ^{11}B concentration. The biological effect is also highly affected by the intracellular micro distribution of ^{11}B due to the short energy deposition range of the alpha particles emitted by the reaction $p + ^{11}\text{B} \rightarrow 3\alpha$.

Boron Proton Capture

Clean energy production research has focused on nuclear fusion reactions potentially obtained from high-intensity-pulsed laser technology. Boron–proton fusion was one of these studied reactions as it is highly energetic (8.7 MeV), is free from high-energy neutrons and generates three alpha particles. The initial reaction between ^{11}B and a proton results in an excited carbon atom (^{12}C) which splits into an alpha particle (3.76 MeV) and a beryllium (^8Be) atom, which subsequently divides into two alpha particles (2.74 MeV). The highest cross section for this reaction occurs with protons with energies around 600-700 keV.

This has led to the idea of exploiting all the advantages of BPC during cancer proton therapy. The first publication on BPC in radiation therapy used Monte Carlo simulations and showed an enhanced high dose in the areas where the boron uptake coincided with the Bragg peak (5). There are a few other studies which used Monte Carlo simulations, but which produced highly divergent results on the potentially achievable BPC dose, ranging from 0.026% to 0.3% based on using different proton energies and ^{11}B concentrations (5-8). The first cell culture experiments were performed at the medical cyclotron in Catania and showed that the BPC procedure increased the biological effectivity of proton irradiation (9). There was a significant enhancement effect on both the cell survival test based on a colony-forming assay and chromosome aberration analysis due the presence of naturally-occurring ^{11}B present in sodium borocaptate

($\text{Na}_2\text{B}_{12}\text{H}_{10}\text{-SH}$; BSH). Such a promising approach requires further investigation.

Therapy using BPC, in contrast to BNCT, does not require such a high differential boron uptake in the target region because proton irradiation is highly spatially selective. This ensures that the majority of BPC will be within the planned region, even in the case of homogeneous boron distribution in a larger volume around the target region (10). To that end, the proton energy distribution must be calculated with high accuracy and should be tailored to the volume of interest where the BPC enhancement is required. A high and long-lasting tumor concentration of ^{11}B and favorable intracellular micro-distribution, preferably close to radiosensitive cell elements (nucleus, mitochondria, membrane) are needed for successful BPCEPT. The vast amount of research and experience with boron compounds used in BNCT is an ideal starting point for seeking optimal boron compounds for BPCEPT research.

Boron Compounds Used in BNCT

BNCT uses the high cross section (3840 barns) of ^{10}B to capture thermal neutrons producing two alpha (α , $^4\text{He}^{2+}$) particles and a ^7Li particle – $^{10}\text{B} + n_{\text{th}} \rightarrow [^{11}\text{B}]^* \rightarrow \alpha + ^7\text{Li} + 2.3 \text{ MeV}$. The fission products deliver energy in tissue to a depth of $\approx 9 \mu\text{m}$ and $\approx 5 \mu\text{m}$, respectively, which is highly biologically effective. The therapeutic potential of the boron neutron capture reaction was first recognized by Locher in 1936 (11) and clinical studies started in the early 1950s. Twenty years after the first unsuccessful human applications (11, 12), intensive experimental and careful clinical investigations have led to the acceptance of BNCT, confirming its efficacy in a clinical setting for brain, thyroid, skin and recurrent head and neck tumors (13-16). BNCT is a highly complex approach as a radiotherapy modality and is considerably different from conventional external beam therapy. In particular, BNCT facilities cannot produce clean monoenergetic neutron radiation and thus the beam is polluted with incident photons and neutrons with a wide spectrum of energies. Thermal neutrons, present in the free beam and higher energy neutrons (epithermal, fast neutrons) are thermalized at the treatment depth and these have different probabilities for capture reactions with chemical elements within the human body. Further contributions to the total dose arise from neutron–hydrogen and neutron–nitrogen reactions. The sources for the total absorbed dose include recoil protons from the scattering of incident fast neutrons by hydrogen, protons emitted by the $^{14}\text{N} (n, p) ^{14}\text{C}$ neutron capture reaction, and incident photons (gamma radiation) and photons generated in the $^1\text{H} (n, \gamma) ^2\text{H}$ reaction (17). Photons generated in the irradiated volume will also deposit an additional radiation dose not only in the desired volume, but also to the surrounding

tissues. The biological distribution of ^{10}B also influences the BNCT-related dose to tissue and consequently the total dose distribution is contributed by several dose components. Clinically useful BNCT requires a high ^{10}B concentration in the target volume with respect to neighboring healthy tissue and a sufficiently high thermal neutron fluence. The most important requirements for a BNCT delivery agent are low systemic toxicity; selective uptake into the tumor cells; rapid clearance from the blood and the tumor surrounding normal tissues; long-lasting, high intratumoral concentration ($20\ \mu\text{g/g}$ tumor)/ >100 ppm) and a favorable intracellular distribution, preferably in the cell nucleus.

First-generation boron compounds. The first clinical trials for BNCT were started in the 1950s in the United States by Sweet, Brownell and Farr (18-20) using simple boron agents including sodium tetraborate (borax) (20), sodium pentaborate, *p*-carboxyphenylboronic acid, or sodium decahydrodecaborate ($\text{Na}_2\text{B}_{10}\text{H}_{10}$) (21). The failure of patient treatment (22, 23) due to serious treatment sequelae caused both by low penetration of the thermal neutrons and poor tumor/tissue selectivity of the boron delivery agents led to the termination of BNCT in the US at that time. Some decades later, after intensive radiobiological experiments, early clinical research was performed using second-generation boron compounds.

More recent renewed attention has been given to the first simple compounds such as boric acid (BA, H_3BO_3). This small weakly acidic compound is often used as a reference compound for testing other boronated target molecules as it has an accumulation efficiency in cells of 1 (24). This is due to passive diffusion (confirmed by a study in frogs) (25). BA also has no biological activity and has an elimination half-life of 21 h (26).

It has been shown that in GS-9L, U-343MGa, HeLa, V79 and B16 cells, BA distribution is even (27-29). There is, however, favorable BA accumulation in bone and this has potential for use for BNCT of osteosarcoma (30). There is also a US patent for the use of BA in liver BNCT treatment (US20130090513). A dose of about 5-60 mg of ^{10}B stable isotope per kilogram of patient body weight is used and then the patient is irradiated 10-120 min after the injection when measured blood concentrations of ^{10}B stable isotope are greater than 20-35 $\mu\text{g}/\text{ml}$. BA is the boron delivery agent of choice for liver BNCT, as the other tested second-generation boron compounds show a preference for accumulation in the pancreas.

Second-generation boron compounds. The neurosurgeon Hatanaka, upon his return to Japan from Sweet's United States research group, continued BNCT treatments in an intraoperative setting (31, 32). In conjunction with Nakagawa, he exposed the surgical cavity surface after brain tumor

removal to thermal neutrons in conjunction with BSH, which has more selective accumulation in tumor cells. BSH uptake is a passive process and shows transient binding, with a biological half-life of the order of hours. This agent was the first Food and Drug Administration (FDA)-approved BNCT drug for human application (33). The most relevant human evaluation on the biodistribution of BSH in tumor and normal tissues was published regarding the European Organisation for Research and Treatment of Cancer (EORTC) phase I trial 'Postoperative treatment of glioblastoma with BNCT at the Petten Irradiation Facility' (protocol 11961). Twelve hours after boron administration at 100 mg/kg or 22.9 mg/kg body weight, the tumor/blood concentration ratios were 0.6 ± 0.2 and 0.9 ± 0.2 , respectively (Table I). The highest boron uptake was detected in the *dura* and there was an exceptionally low uptake in bone, cerebrospinal fluid and in the brain. BSH is safe for human applications at a dose of 100 mg BSH/kg infused at 1 mg/kg/min for 4 consecutive days (34).

Another BSH biodistribution and pharmacokinetic study looked at 20 patients with high-grade gliomas and confirmed the high interpatient variability of tumor boron concentration and boron concentration in different regions of the tumor (35).

Other representative results from the literature based on the application of second-generation BNCT compounds are summarized in Table I. The BSH dose is typically in the range of 23 200 mg BSH/kg body weight. The use of BSH as a BNCT agent was studied in different types of animal tumor models, such as oral squamous cell carcinoma (36, 37), pigmented melanoma, sarcoma (24) and pancreatic adenocarcinoma in mouse and rat models (38). BSH has already been applied in human clinical trials, such as for patients with glioblastoma (34), with squamous cell carcinoma of the head and neck (37), and in oral mucosal tissue (39).

Favorable ^{10}B uptake for BNCT treatment would require a tumor/normal tissue ratio greater than 2, and a tumor/blood ratio greater than 0.6 for BSH (40). Table I shows that the measured tumor/normal tissue ratio is frequently below 2 for BSH. The tumor/blood ratio, however, is usually above 0.6 for BSH under the studied conditions.

Boron-containing amino acid (boronophenylalanine) (BPA) was synthesized in 1958 by Snyder *et al.* (41) and was first used in Japan for BNCT of melanoma (42). Clinical trials were once more initiated in 1990s in the United States and used the epithermal neutron beam at the Medical Research Reactor and at Harvard-MIT. A soluble fructose complex, BPA-F (concentration 400 mg/kg/2 h, *i.v.*) was used as the boron delivery agent. It was postulated to be taken up by active metabolic pathways in cells, resulting in favorable tumor/blood and similar tumor/brain ratio of more than 1.5-2 (43). The uptake of BPA into glioma and melanoma was measured by tissue sampling in several clinical and preclinical trials. Marked variability was detected in a study

Table I. A summary of the main study parameters and results from representative publications.

Compound	Application	Cancer/ tissue type	Route	Applied dose (mg/kg bw)	Concentration of ¹⁰ B (µg/g)	Concentration ratio	
						Tumor/blood	Tumor/normal tissue
BSH (34)	Human	Glioblastoma	<i>i.v.</i>	22.9 100	Tumor: 19.9±9.1 9.8±3.3	0.6±0.2 and 0.9±0.2	NA
BSH (35)	Human	Glioblastoma, astrocytoma	<i>i.v.</i>	26.5, 44.1, 88.2	Tumor: 11.9	NA	3.2-3.8
BPA-F mediated (44)	Human	Glioma	<i>i.v.</i>	290-450	Blood: 14-22 Brain: 19-46 Tumor: 44-93	3.8-4.4	2.0-2.5
BPA (78)	Human	Melanoma	<i>i.v.</i>	179.7±14.9	Blood: 9.4±2.6	3.40±0.83	NA
BPA and BSH (79)	Human and rat	SCC head and neck	<i>i.v.</i>	BPA: 700 BSH: 200	NA	BPA: 4.0±1.7 ¹⁰ B BSH1: 2±0.4 ¹⁰ B	BPA: Skin: 1.3±0.5, Muscle: 2.1±1.2 and Mucosa: 1.4±0.01 BSH: Muscle: 3.6±0.6 Lymph nodes: 2.5±1.0 Skin: 1.4±0.5 Mucosa: 1.0±0.3 NA
BSH and BPA (39)	Mice	Oral mucosal tissue	<i>i.p.</i>	BSH: 55 BPA: 700	BSH: 6.1±1.0 BPA: 21.5±0.5	Epithelium/blood: BSH: 0.2:1 BPA 1:1	NA
L-BPA (80)	C3H/He Mice	SCC-VII xenograft on legs	<i>s.c.; i.v.</i>	500	Different tissues: 10-40 Highest, kidney: ~95	L-BPA 1.58±0.20 L-FBPA: 1.52±0.42	Intestine: 1.31 Liver: 1.77 Kidney: 0.46 Lung: 1.34 Tongue: 1.36 Skin: 1.64 Muscle: 1.70 Brain: 4.24 Tongue: 4.19 Skin: 4.68 Bone: 4.56 Tongue: 1.05 Skin: 0.60 Bone: 0.59
BPA (36)	Mice	Nude mice with oral SCC	<i>i.p.</i>	250	Tumor: 15.96	6.44	Tongue: 4.19 Skin: 4.68 Bone: 4.56
BSH (36)	Mice	Nude mice with oral SCC	<i>i.p.</i>	75	Tumor: 3.61	0.77	Tongue: 1.05 Skin: 0.60 Bone: 0.59
BPA (70)	Mice	Clear cell sarcoma	<i>i.v.</i>	500	Dorsal tumor: 74 Femoral tumor: 45	Dorsal: 9.2 at 1 h Femoral: 7.6 at 1.5 h	Dorsal skin: 4.5 (1 h) Femoral skin: 7.8 (1.5 h)
BPA (46)	Mice	Lung metastasis of clear cell carcinoma	<i>i.v.</i>	24	59.8±1.5 (Max. at 0.5 h)	3.4±0.6 at 0.5 h 5.3±1.3 at 2.5 h	Left lung: 8.7±6.0 at 0.5 h 37.27±16.7 at 2.5 h
BPA (71)	Rat	F98 Glioma/ melanoma- bearing rats	<i>i.v.</i>	24	BPA: Glioma: 21.5±8.4 Melanoma: 28.6±4.6 cis-ABCPC: Glioma: 26.2±9.7 Melanoma: 16.4±9.0	Glioma, BPA: 4.2 cis-ABCPC: 3.9	NA
BPA (72)	<i>In vitro</i> and Mice	Human melanoma cell lines: MEL-J, A375, and M8.	<i>i.p.</i>	350	Tumor: 25.9± 2.6	Tumor/blood: 4.3	Tumor/skin: 2.5
BPA (81)	Rat	Diffuse lung metastases from colon carcinoma	<i>i.v.</i>	300	Tumor: ~25-50 at ~60 min	NA	NA
BPA (82)	<i>In vitro</i> , Rat	Osteosarcoma	<i>i.v.</i>	300	Tumor: 16.26±0.50	NA	1.85
BPA, BSH, BA (24)	Mice	B16 pigmented melanoma and RIF1 sarcoma	<i>i.p.</i>	50-55 µg B/g for BA, BSH and BPA	C _{max} B16 tumor BA: 43.2±2.2 BSH: 28.1±4.9 BPA: 55.0±4.5	B16 BA: 1.5 BSH: 1.8 BPA: 3.2	B16 BA: 1.8 BSH: 1.8 BPA: 3.2
BSH and BSH- conjugates [‡] (38)	Mice	Pancreatic adenocarcinoma	<i>i.v.</i>	100	BSH polymer conjugate Tumor: ~90	Max. ~20 after 24 h	Muscle: 5-6

SCC: Squamous cell cancer; PEG-b-P(Glu-SS-BSH): PEGylated-polyglutamic acid; L-BPA: 4-borono-L-phenylalanine; BPA-F: mediated fluoro-borophenylalanine; BSH: sodium borocaptate; *i.v.* intravenous; cis-ABCPC: 1-amino-3-borono-cyclopentanecarboxylic acid; BA boric acid. *i.p.* intraperitoneal; *s.c.*: subcutaneous.

Table II. A summary of third-generation boron delivery agents, novel compounds and their improved feature for boron neutron capture therapy.

Boron delivery agents (Ref)	Primary benefit
Boronated unnatural amino acids (54)	Highly selectivity toward tumors
Boronated cyclic peptides (55, 57)	Low toxicity and high tumor selectivity
Carbonyl nucleosides (56)	High rate of phosphorylation into corresponding nucleotide
Boronated EGF and EGFR monoclonal antibodies (58)	High specificity for molecular targets such as EGFR
Boronated DNA intercalators (83)	Binding to DNA
Boronated porphyrins (54, 61)	High tumor selectivity, long retention time
Transferrin-polyethylene glycol liposomes (65)	Increased boron uptake by tumor
Boron-containing immunoliposomes and liposomes (58, 65)	Effective large-scale transportation of small molecules
Boron-containing nanoparticles, boron nitride nanotubes (66)	High dispersibility in water
Boron containing lipiodol (84)	Soluble, high retention in hepatoma
Decaborane (GB10) (85)	Non-toxic, homogeneous uptake in head and neck tumor
Dodecaborate cluster lipids and cholesterol derivatives (86)	High tumor selectivity
Polyanionic polymers (38)	Superior intratumoral penetration

EGF: Epidermal growth factor; EGFR: epidermal growth factor receptor; GB10 ($\text{Na}_2^{10}\text{B}_{10}\text{H}_{10}$): non-toxic boron compound.

using BPA-F involving 98 patients with glioma (44) (Table I). A Japanese clinical study investigated the uptake of BPA in squamous cell carcinoma of the head and neck showed a promising ^{10}B concentration and, for the first time, demonstrated the change of boron concentration in the tumor over time (36). All these investigations demonstrated significantly larger tumor/normal tissue boron concentration ratios when compared to BSH studies, *e.g.* 4.2-4.7 in oral squamous cell carcinoma (36), 4.5-7.8 in clear cell sarcoma (45), 8.7-37.3 in a lung metastasis model of clear cell sarcoma (46), and 3.2 in B16 pigmented melanoma (24), however with notable intra-individual and intertumoral variability in boron concentration both in humans and in rodent tumor models (Table I). BPA only has one boron atom per molecule but can be applied at higher doses (180-700 mg/kg body weight) compared with BSH (23-200 mg/kg body weight). Morris *et al.* applied 55 mg/kg BSH (30 mg ^{10}B /kg) and 700 mg/kg BPA (34 mg ^{10}B /kg) which resulted in similar boron dosing and yet the BPA treatment resulted in higher accumulated concentration of boron (6.1 *versus* 21.5 ppm) in oral mucosal tissue (39). High boron concentrations were found in melanocytic malignant tumor (74 mg ^{10}B /kg wet tumor tissue) and in a lung metastasis model of clear cell sarcoma (59.8 mg ^{10}B /g). It is assumed that BPA is selectively accumulated in cancer cells *via* L-amino acid transporter (LAT1)-mediated uptake (47, 48). T98G and A172 glioblastoma cells were used in a recent study published by Wada *et al.* in which the influence of oxygen concentration on cell viability was investigated, as well as the mRNA expression of *LAT1* and the uptake of ^{10}B -BPA (49). It was concluded that hypoxia below 10% O_2 significantly reduced the mRNA expression of *LAT1* in both cell lines. This means that reduced uptake of ^{10}B -BPA in glioblastoma in hypoxic conditions may be due to a lower

expression of this transporter protein. Wongthai *et al.* reported that BPA is transported by amino acid transporter ($\text{ATB}^{0,+}$), and LAT1 and LAT2 aromatic amino acid transporters (50). Subsequently, ^{18}F -labeled BPA derivatives were used for positron-emission tomography in order to determine the pharmacokinetic parameters of BPA and to predict the effectiveness of BNCT, as well as to detect post-treatment radiation effects (51-53).

BSH and BPA have been used independently or in combination for decades in clinical studies in Japan, in the US and in Europe (the multinational European trial, in Czech Republic and in Finland) (34, 39, 40) for BNCT of patients with high-grade glioma, melanoma and recurrent head and neck cancer. The safety and feasibility for clinical application of these two boron delivery agents was established on the basis of the numerous preclinical and clinical trials (40), although neither BSH, nor BPA-F proved to be an optimal boron compound for BNCT.

Third-generation boron compounds. The considerable requirements for boron delivery agents have resulted in the need for further developments in order to achieve a more selective, higher intra-tumoral boron concentration and long-term intra-tumoral binding with favorable subcellular localization, *i.e.* close to the cell nucleus.

A collection of third-generation low- and high-molecular-weight boron delivery agents is summarized in Table II (54). Amongst the low-molecular-weight BNCT compounds, there are boronated derivatives of other natural amino acids, such as aspartic acid, tyrosine, cysteine, methionine and serine, and boron-containing unnatural amino acids, *e.g.* designated derivatives of 1-aminocyclobutane-1-carboxylic acid and 1-amino-3-boronocyclopentanecarboxylic acid, and boron-containing linear and cyclic peptides (55). These peptides

were developed due to their low toxicity, high tissue-penetrating properties (56) and increased water solubility compared to BPA.

Boronated linear and cyclic peptides have a low toxicity and high tumor selectivity (57). In tumor cells, the ligands for up-regulated receptors, especially epidermal growth factor (EGFR), vascular endothelial growth factor (VEGFR) and somatostatin receptors, are of particular interest (58).

Boron-containing purines, pyrimidines, thymidines, nucleosides, and nucleotides have been investigated as boron delivery agents, especially 3-carboranyl thymidine analogs. For example, the 3-(dihydroxypropyl-carboranyl-pentyl) thymidine derivate designated N5-2OH has shown low toxicity, selective tumor uptake, and high rate of phosphorylation into the corresponding nucleotide (56). Convection-enhanced delivery of N5-2OH was effective in selectively delivering therapeutic amounts of boron to rats bearing intracerebral implants of RG2 glioma (59).

Another approach was to improve the delivery of second-generation agents, using BSH lipiodol emulsion, BSH-transferrin polyethylene glycol (PEG) liposomes, and BPA derivatives with a higher percentage of boron by weight (60). Boron-containing nucleosides and nucleotides, purines, pyrimidines, thymidines (59) and boronated DNA intercalators were designed to achieve high boron concentration in or near to the cell nucleus. Boronated DNA-binding molecules, *e.g.* alkylating agents, intercalators, minor-groove binders, polyamines, derivatives of acridines, Pt(II) complexes, tri-methoxyindoles, carboranyl polyamines and metal complexes have been investigated and these compounds typically have low tumor selectivity and sometimes high toxicity due to their multiple cationic charges and ability to bind to DNA (60).

Boronated carbohydrates, such as derivatives of galactose, glucose, lactose, maltose, mannose and ribose, have also been investigated. These compounds usually display low toxicity and poor tumor uptake, perhaps as a result of their low hydrophobicity and a fast clearance from tissues (60).

A novel idea was to bind boron to porphyrin derivatives, which have been already used for photodynamic therapy, some with FDA approval, in order to establish dual tumor-killing modality (54, 61). Several boron-containing porphyrin derivatives, including chlorin, bacteriochlorin, tetrabenzoporphyrin have been investigated as BNCT agents (62) porphyrins of high-boron content. They are well-known, non toxic, tumor-specific agents, which are exhibited several superior features in comparison to BSH, BPA, such as longer-lasting high tumor accumulation and high tumor/brain, tumor/blood boron concentration ratios. Boronated chlorins and bacteriochlorins are promising agents as dual BNCT and photodynamic therapy photosensitizers due to a strong absorption of near-infrared light, which penetrates deeper into human tissues. Boronated derivatives

of other amino acids, such as aspartic acid, tyrosine, cysteine, methionine and serine, designated derivatives of 1-aminocyclobutane-1-carboxylic acid and 1-amino-3-boronocyclopentanecarboxylic acid and boron-containing linear and cyclic peptides were developed because of their low toxicity and high tissue-penetrating properties (63).

The radioresistance of hypoxic cells is well known (64), therefore boron compounds with high capacity to accumulate in hypoxic cells would be highly useful for BNCT of large tumors. A boronated 2-nitroimidazole derivative (B-381) was found to have a minimal cytotoxicity and preferentially accumulate in hypoxic cells, and demonstrated longer tumor retention in an *in vivo* glioma model compared to BPA. It achieved significant tumor/normal tissue ratios as well as tumor/blood ratios but suffered from extremely fast elimination from plasma (64).

Immunoliposomes have been shown to deliver small boronated molecules such as BSH into brain tumors *via* incorporation into the corresponding lipid bilayers (58). Liposomes have been used to intracellularly transport large amounts of boronated molecules *e.g.* PEG-based liposomes and particles generally show increased tumor boron uptake (65). High cellular boron concentrations can be achieved by encapsulating low-molecular-weight compounds in tumor-selective unilamellar liposomes. However, *in vivo* therapeutic efficacy has yet to be demonstrated. These particles avoid problems associated with leakage of encapsulated boronated compounds and were shown to deliver large amounts of boron to tumor-bearing mice with increased survival times following BNCT (54). Other boron-containing nanoparticles have also been proposed and investigated as delivery agents for BNCT (66).

Recently, novel nanotechniques, such as hollow boron nitride nanospheres with potential for intratumoral boron release from within the sphere, have produced nanoparticles with promising results. Hexagonal BN has a high dispersibility in water which makes this material useful for BNCT (67).

Boron Concentration Measurement and Distribution Imaging

A critical requirement at every stage in the development of boronating agents for both BNCT and BPCEPT is the ability to measure the concentration of and image the spatial distribution of boron in biological samples and in living organisms. A multidisciplinary, international effort has resulted in various methods for measurement and imaging boron in practical ways to enable BNCT dose calculation in human treatment and establishing sophisticated techniques for research on subcellular distribution analysis. Prompt gamma-ray analysis (PGRA) is based on gamma-ray spectroscopy following neutron capture in ^{10}B , resulting in

the emission of 478 keV photons. The photon emission rate is proportional to the reaction rate of the neutron capture reaction and therefore the ^{10}B concentration. The emission of 715 keV PGR due to BPC reaction is controversial, but the same PGRA techniques can be used if there is an optimal mixture of ^{10}B and ^{11}B and an appropriate neutron beam. PGRA is fast and simple but requires a relatively large sample and produces an average concentration of the sample. Further application of PGRA for BNCT includes the possibility of *in vivo* gamma-ray spectroscopy of the patient during treatment. This method, however, needs further improvement for implementation in a clinical routine.

Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) uses plasma to produce excited atoms that emit electromagnetic radiation at a wavelength characteristic of a particular element. ICP-mass spectrometry (MS) is a combined method capable of monitoring isotopic speciation for desired ions. ICP-AES and ICP-MS are rapid, reliable methods for quantification of ^{10}B concentration in macroscopic samples; they have been used in conjunction with BNCT for many years and can be applied for ^{11}B concentration determination for therapy with BPC. PGRA, ICP-AES and ICP-MS measurements integrate the boron concentration over a relatively large volume, therefore individual boron distribution and subcellular boron accumulation cannot be directly obtained.

Further measuring techniques analyze the spatial distribution of boron in a sample *e.g.* high-resolution alpha-track autoradiography and neutron capture radiography result in a similar resolution to histopathological imaging. Laser microprobe mass analysis was the first technique to reach a subcellular resolution in BNCT (68). Time-of-flight secondary ion MS, particularly laser secondary neutral MS, can quantitatively map the spatial boron distribution in biological matrices with a sub-ppm detection limit. Electron microscopic methods *e.g.* electron energy loss spectroscopy, are less sensitive but have a much higher spatial resolution (69). However, the latter methods are extremely time-, labor- and cost-intensive (40).

Another important aspect that has had only minimal investigation is the metabolism of BPA and BSH that are actually used in clinical trials for BNCT. Ion-trap MS in combination with proteomic technologies offers a possibility to investigate metabolites and transport of target molecules. Such information is mandatory for optimizing the application of these drugs on a scientifically solid basis. ^{18}F -Labeling of BPA and the use of positron-emission tomography to assess the molecule in a patient and the detection of boron compounds by magnetic resonance imaging might be feasible (46, 70, 71). Positron-emission tomography with ^{18}F -BPA has been used for patient selection in recent clinical trials in Japan (72) and Finland (29). It is essential in BPCEPT to be able to measure the ^{11}B concentration with a high resolution at

different time points relevant to proton irradiation in the concerned target volume both for patient selection and for the calculation of dose and biological effects.

Boron Compounds for BPCEPT

Comparing the requirements for boron compounds for BPCEPT and BNCT. The common feature of BNCT and BPC is the binary nature of these high LET radiation modalities requiring high intra-tumoral boron concentration which is delivered by a non-toxic boronated agent. A suitable compound can theoretically use the same chemical for both types of treatment, but each enriched with suitable boron isotope (^{10}B for BNCT, ^{11}B for BPC). However the optimal boron delivery agent for BPCEPT should consider and take into account the differences between BNCT and BPC (38).

BNCT is based on a reaction in which a thermal neutron particle cannot be generated in a form of mono-particle, monoenergetic beam specifically tailored to the target with high accuracy. The BPC reaction is only considered as an additional high LET dose component to standard proton therapy. The cross section of proton boron fusion reaction is about 0.9 barn with 675 keV resonant energy resulting in the possibility of triggering a BPC reaction with extremely high accuracy in the target even in the case of less selective boron accumulation in the body. The much lower cross section of the proton-boron capture reaction does however require a much higher boron concentration to achieve a relevant contribution to the proton dose. Furthermore, BNCT is delivered in a single session or as 2-4 fractions after intravenous infusion with boronated agent but in the majority of proton irradiation cases, the dose is delivered with conventional fractionation during 30-35 consecutive working days. Therefore one of the most important requirements for ^{11}B delivery agent is a long-lasting high intracellular concentration as well as tolerability, which allows the administration of the boron compound on weekly basis (9).

Strategies for ^{11}B compound use and development for BPCEPT. FDA-approved BSH and BPA are useful starting agents for BPCEPT research. Different means of enhancing bio-availability may result in an increase in intracellular concentration and longer intracellular retention. The large number of third-generation boron compounds developed for BNCT has provided a basis for testing agents for BPCEPT which have exhibited high boron delivery in the vicinity of the cell nucleus, with favorable pharmacokinetic features. One promising approach is the development of boronated nucleic acids characterized by low cytotoxicity. Some types of high boron-loaded DNA oligonucleotide modifications as potential dual-action anticancer agents with antisense/anti-oxidant and proposed BNCT activities might also work for BPCEPT. The important biological features of these metallocarborane-

modified DNA oligomers are their low cytotoxicity, increased lipophilicity, formation of stable heteroduplexes with complementary RNA strands and their function as effective antisense oligonucleotides (73). These compounds deliver ^{11}B to the cell nuclei, therefore further increasing the biological effect. Moreover, pro- and antioxidant activities were detected depending on the concentration of the modified oligomer used. The metallocarborane modification serves as an infrared-sensitive nucleic acid label for detection. This approach may be of importance for designing therapeutic nucleic acids both for gene silencing as future biotherapeutics and for studies on boron clusters as nucleic acid-modifying units for BPCEPT (73).

Significantly higher tumor/normal tissue ratio for BSH can be achieved by using a BSH-polymer conjugate [PEG-b-poly(glutamic acid) copolymer]. The tumor/muscle tissue ratio was about 5-6 for PEG-b-poly(glutamic acid) copolymer-SS-BSH in subcutaneous human pancreatic adenocarcinoma tumors in mice (Table I). High absolute boron concentration (90 ppm) can be achieved in tumor when BSH polymer conjugate is applied (38).

Some porphyrin derivatives have remarkably high absolute intratumoral boron concentration and long retention time, the two main prerequisites for efficient BPCEPT. Wu *et al.* reported the synthesis of three copper (II) tetracarboranylphenylporphyrins containing 40 or 80 boron atoms per molecule (74). EMT-6 tumor-bearing mice were given porphyrins in six i.p. injections (3/day within 8 h) over a period of 2 days using a volume of 0.01 ml/g body weight per injection. One of the porphyrins resulted in about 200 ppm tumor boron concentration after 2 days from the last injection and 175 ppm after 4 days of the last injection.

Boron nitride nanotubes exhibit supermagnetic properties and thus have potential as nanovectors for magnetic drug targeting – the delivery of a drug with the required concentration to the target site under the influence of an external magnetic field (75, 76). The intended drug and a suitable magnetically active carrier component would need to be combined into a pharmacologically stable compound. The intrinsic magnetic properties of coated boron nitride nanotubes and their subsequent binding ability make them highly suitable for use as targeted drug-delivery systems (76). Hollow boron nitride spheres are also promising in cancer therapy as a dual-action substrate. They show excellent water solubility and degradability. Typical particle sizes are around 200 nm and they undergo further degradation, providing enhanced permeability and retention effects, thereby enabling passive targeting to tumor sites. A new therapeutic property of hollow boron nitride spheres was demonstrated with controlled release of boron for prostate cancer treatment. Hollow boron nitride spheres induced apoptosis and inhibited the proliferation of both androgen-sensitive and androgen-independent prostate cancer cells (77).

Conclusion

BNCT was the first attempt at enhancing particle radiation cancer treatment *via an in situ* nuclear reaction involving boron-containing compounds. A large proportion of the failures of this therapy arise from the quality of the neutron beam. BPCEPT, an alternative therapy using high-quality particle stream sources can overcome these issues. However, the vast tome of research and clinical trials performed with boron-containing compounds for BNCT can, with careful scientific and medical guidance, be used to rapidly enhance and direct research towards clinical applications of BPCEPT.

Conflicts of Interest

The Authors declare no conflict of interest.

Authors' Contributions

Conceptualization, writing (radiation oncology aspects) and final supervision: K. Hideghéty. Literature review, writing and supervision from chemistry aspects: K. Mogyorós. Validation, writing-review and editing: A. Cheesman, Literature organization-evaluation, writing, and draft preparation: Sz. Brunner. Advice and supervision from physics aspects: D. Margarone and R. Polanek. Evaluation of the published preclinical results and formal analysis: E.R. Szabó and T. Tóké.

Acknowledgements

This review was sponsored by the ELI-ALPS project (GINOP-2.3.6-15-2015-00001) is supported by the European Union and co-financed by the European Regional Development Fund. The project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 654148 Laserlab-Europe.

References

- 1 Pompos A, Durante M and Choy H: Heavy ions in cancer therapy. *JAMA Oncol* 2(12): 1539-1540, 2016. PMID: 27541302. DOI: 10.1001/jamaoncol.2016.2646
- 2 Willers H, Allen A, Grosshans D, McMahon SJ, von Neubeck C, Wiese C and Vikram B: Toward a variable rbe for proton beam therapy. *Radiother Oncol* 128(1): 68-75, 2018. PMID: 29910006. DOI: 10.1016/j.radonc.2018.05.019
- 3 Dosanjh M and Bernier J: *Advances in Particle Therapy*. Taylor & Francis Group, LLC, 2018.
- 4 Mohan R and Grosshans D: Proton therapy – present and future. *Adv Drug Deliv Rev* 109: 26-44, 2017. PMID: 27919760. DOI: 10.1016/j.addr.2016.11.006
- 5 Yoon D-K, Jung J-Y and Suh TS: Application of proton boron fusion reaction to radiation therapy: A Monte Carlo simulation study. *Applied Physics Lett* 105(22): 223507, 2014. DOI: 10.1063/1.4903345
- 6 Shin HY, DK Jung, JY Kim, MS Suh TS: Prompt gamma ray imaging for verification of proton boron fusion therapy: A monte

- carlo study. *Eur J Med Phys* 32(10): 1271-1275, 2016. PMID: 27229367. DOI: 10.1016/j.ejmp.2016.05.053
- 7 Adam D and Bednarz B: SU-FT-140: Assessment of the proton boron fusion reaction for practical radiation therapy applications using mcnp6. *Med Phys* 43(6): 3494-3494, 2016. DOI: 10.1118/1.4956276
 - 8 Giuffrida L, Margarone D, Cirrone GAP, Picciotto A and Korn G: Prompt gamma ray diagnostics and enhanced hadron-therapy using neutron-free nuclear reactions. *AIP Advances* 6, 2016. DOI: 10.1063/1.4965254
 - 9 Cirrone GAP, Manti L, Margarone D, Petringa G, Giuffrida L, Minopoli A, Picciotto A, Russo G, Cammarata F, Pisciotto P, Perozziello FM, Romano F, Marchese V, Milluzzo G, Scuderi V, Cuttone G and Korn G: First experimental proof of proton boron capture therapy (PBCT) to enhance protontherapy effectiveness. *Sci Rep* 8(1): 1141, 2018. PMID: 29348437. DOI: 10.1038/s41598-018-19258-5
 - 10 Hideghéty K, Szabó ER, Polanek R, Szabó Z, Ughy B, Brunner S and Tőkés T: An evaluation of the various aspects of the progress in clinical applications of laser driven ionizing radiation. *J Instrum* 12(03): C03038-C03038, 2017.
 - 11 Locher GL: Biological effects and therapeutic possibilities of neutrons. *Am J Roentgenol Radium Ther* 36: 1-13, 1936.
 - 12 Sweet WH: Practical problems in the past in the use of boron-slow neutron capture therapy in the treatment of glioblastoma multiforme. *Proc First Int Symp Neutr Capt Ther* 16(18): 376-378, 1982.
 - 13 Busse P, Harling O, Palmer M, Kiger W, Kaplan J, Kaplan I, Chuang C, Goorley J, Riley K and Newton T: A critical examination of the results from the Harvard-MIT NCT program phase I clinical trial of neutron capture therapy for intracranial disease. *Journal of Neuro-Oncology* 62(1-2): 111-121, 2003. PMID: 12749707.
 - 14 Capala J, H.-Stenstam B, Sköld K, Rosenschöld PM, Giusti V, Persson C, Wallin E, Brun A, Franzen L, Carlsson J, Salford L, Ceberg C, Persson B, Pellettieri L and Henriksson R: Boron neutron capture therapy for glioblastoma multiforme: Clinical studies in sweden. *J Neuro-Oncol* 62(1-2): 135-144, 2003.
 - 15 Kankaanranta L, Saarilahti K, Mäkitie A, Valimäki P, Tenhunen M and Joensuu H: Boron neutron capture therapy (BNCT) followed by intensity modulated chemoradiotherapy as primary treatment of large head and neck cancer with intracranial involvement. *Radiother Oncol* 99(1): 98-99, 2011. PMID: 21397966. DOI: 10.1016/j.radonc.2011.02.008
 - 16 Wang LW, Wang SJ, Chu PY, Ho CY, Jiang SH, Liu YW, Liu YH, Liu HM, Peir JJ, Chou FI, Yen SH, Lee YL, Chang CW, Liu CS, Chen YW and Ono K: Bnct for locally recurrent head and neck cancer: Preliminary clinical experience from a phase i/ii trial at tsing hua open-pool reactor. *Appl Radiat Isot* 69(12): 1803-1806, 2011.
 - 17 Kageji T, Nagahiro S, Matsuzaki K, Mizobuchi Y, Toi H, Nakagawa Y and Kumada H: Boron neutron capture therapy using mixed epithermal and thermal neutron beams in patients with malignant glioma-correlation between radiation dose and radiation injury and clinical outcome. *Int J Radiat Oncol Biol Phys* 65(5): 1446-1455, 2006. PMID:16750328. DOI:10.1016/j.ijrobp.2006.03.016
 - 18 Sweet WH: The uses of nuclear disintegration in the diagnosis and treatment of brain tumor. *N Engl J Med* 245(23): 875-878, 1951. PMID: 14882442. DOI: 10.1056/NEJM195112062452301
 - 19 Farr LE SW, Locksley HB, Robertson JS: Neutron capture therapy of gliomas using boron-10. *Trans Am Neurol Assoc* 79: 110-113, 1954. PMID: 13238328.
 - 20 Javid M, Brownell G and Sweet W: The possible use of neutron capturing isotopes such as boron-10 in the treatment of neoplasms. II. Computation of the radiation energies and estimates of effects in normal and neoplastic brain. *J Clin Invest* 31: 604-610, 1952. PMID: 14938440. DOI: 10.1172/JCI102647
 - 21 Slatkin DN: Glioblastoma treatment. *Science* 265(5179): 1644, 1994. PMID: 7993457.
 - 22 Farr L, Sweet W, Robertson J, Foster C, Locksley H, Sutherland D, Mendelsohn M and Stickley E: Neutron capture therapy with boron in the treatment of glioblastoma multiforme. *Am J Roentgenol Radium Ther Nucl Med* 71(2): 279-293, 1954. PMID: 13124616.
 - 23 Godwin J, Farr L, Sweet W and Robertson J: Pathological study of eight patients with glioblastoma multiforme treated by neutron-capture therapy using boron 10. *Cancer* 8(3): 601-615, 1955. PMID: 14379150. DOI:10.1002/1097-0142
 - 24 Gregoire V, Begg AC, Huiskamp R, Verrijk R and Bartelink H: Selectivity of boron carriers for boron neutron capture therapy: Pharmacological studies with borocaptate sodium, l-boronophenylalanine and boric acid in murine tumors *Radiother Oncol* 27: 46-54, 1993. PMID:8327732 DOI: 10.1016/0167-8140(93)90043-8
 - 25 Dordas C and Brown P: Permeability and the mechanism of transport of boric acid across the plasma membrane of xenopus laevis oocytes. *Biol Trace Elem Res* 81(2): 127-139, 2001. PMID:11554394. DOI: 10.1385/BTER:81:2:127
 - 26 Murray F: A comparative review of the pharmacokinetics of boric acid in rodents and humans. *Biol Trace Elem Res* 66(1-3): 331-341, 1998. PMID:10050928. DOI:10.1007/BF02783146
 - 27 Coderre J and Morris G: The radiation biology of boron neutron capture therapy. *Radiat Res* 151(1): 1-18, 1999. PMID: 9973079. DOI: 10.2307/3579742
 - 28 Capala J, Makar M and Coderre J: Accumulation of boron in malignant and normal cells incubated *in vitro* with boronophenylalanine, mercaptoborane or boric acid. *Radiat Res* 146(5): 554-560, 1996. PMID: 8896582 DOI: 10.2307/3579556
 - 29 Chou F, Chung H, Liu H, Chi C and Lui W: Suitability of boron carriers for bnct: Accumulation of boron in malignant and normal liver cells after treatment with BPA, BSH and BA. *Appl Radiat Isot* 67(7-8): 105-108, 2009. PMID: 19375330. DOI: 10.1016/j.apradiso.2009.03.025
 - 30 Hsu C, Lin S, Peir J, Liao J, Lin Y and Chou F: Potential of using boric acid as a boron drug for boron neutron capture therapy for osteosarcoma. *Appl Radiat Isot* 69(12): 1782-1785, 2011. PMID: 21420871. DOI: 10.1016/j.apradiso.2011.03.003
 - 31 Hatanaka H and Nakagawa Y: Clinical results of long-surviving brain tumor patients who underwent boron neutron capture therapy. *Int J Radiat Oncol Biol Phys* 28(5): 1061-1066, 1994. PMID: 8175390. DOI: 10.1016/0360-3016(94)90479-0
 - 32 Hatanaka H: Clinical experience of boron-neutron capture therapy for gliomas – a comparison with conventional chemo-immunoradiotherapy. *In: Boron neutron capture therapy for tumours by H. Hatanaka, Nishimura Co, Niigata, pp. 349-379, 1986.*
 - 33 Garabalino M, Heber E, Monti Hughes A, González S, Molinari A, Pozzi E, Nievas S, Itoiz M, Aromando R, Nigg D, Bauer W, Trivillin V and Schwint A: Biodistribution of sodium borocaptate (BSH) for boron neutron capture therapy (BNCT) in

- an oral cancer model. *Radiat Environ Biophys* 52(3): 351-361, 2013. DOI 10.1007/s00411-013-0467-8
- 34 Hideghéty K, Sauerwein W, Wittig A, Götz C, Paquis P, Grochulla F, Haselsberger K, Wolbers J, Moss R, Huiskamp R, Fankhauser H, de Vries M and Gabel D: Tissue uptake of BSH in patients with glioblastoma in the EORTC 11961 phase I BNCT trial. *J Neurooncol* 62(1-2): 145-156, 2003. PMID: 12749710. DOI: 10.1023/A:1023201308434
- 35 Goodman J, Yang W, Barth R, Gao Z, Boesel C, Staubus A, Gupta N, Gahbauer R, Adams D, Gibson C, Ferketich A, Moeschberger M, Soloway A, Carpenter D, Albertson B, Bauer W, Zhang M and Wang C: Boron neutron capture therapy of brain tumors: Biodistribution, pharmacokinetics, and radiation dosimetry sodium borocaptate in patients with gliomas. *Neurosurgery* 47(3): 608-621, 2000. PMID:10981748
- 36 Obayashi S, Kato I, Ono K, Masunaga S-I, Suzuki M, Nagata K, Sakurai Y and Yura Y: Delivery of 10boron to oral squamous cell carcinoma using boronophenylalanine and borocaptate sodium for boron neutron capture therapy. *Oral Oncol* 40: 474-482, 2004. PMID: 15006618. DOI: 10.1016/j.oraloncology. 2003.09.018
- 37 Wittig A, Stecher-Rasmussen F, Hilger RA, Rassow J, Mauri P and Sauerwein W: Sodium mercaptoundecahydro-closododecaborate (BSH), a boron carrier that merits more attention. *Appl Radiat Isot* 69(12): 1760-1764, 2011. PMID: 21420870. DOI: 10.1016/j.apradiso.2011.02.046
- 38 Mi P, Yanagie H, Dewi N, Yen HC, Liu X, Suzuki M, Sakurai Y, Ono K, Takahashi H, Cabral H, Kataoka K and Nishiyama N: Block copolymer-boron cluster conjugate for effective boron neutron capture therapy of solid tumors. *J Control Release* 254: 1-9, 2017. PMID: 28336377. DOI: 10.1016/j.jconrel. 2017.03.036
- 39 Morris GM, Smith DR, Patel H, Chandra S, Morrison GH, Hopewell JW, Rezvani M, Micca PL and Coderre JA: Boron microlocalization in oral mucosal tissue: Implications for boron neutron capture therapy. *Br J Cancer* 82(11): 1764-1771, 2000. PMID: 10839288. DOI: 10.1054/bjoc.2000.1148
- 40 Wittig A, Collette L, Appelman K, Buhmann S, Jackel MC, Jockel KH, Schmid KW, Ortman U, Moss R and Sauerwein WA: EORTC trial 11001: Distribution of two 10b-compounds in patients with squamous cell carcinoma of head and neck, a translational research/phase I trial. *J Cell Mol Med* 13(8B): 1653-1665, 2009. PMID: 19602035. DOI: 10.1111/j.1582-4934.2009.00856.x
- 41 Snyder H, Reedy A and Lennarz W: Synthesis of aromatic boronic acids. Aldehyde boronic acids and a boronic acid analog of tyrosine. *J Am Chem Soc* 80(4): 835-838, 1958. DOI: 10.1021/ja01537a021
- 42 Mishima Y, Honda C, Ichihashi M, Obara H, Hiratsuka J, Fukuda H, Karashima H, Kobayashi T, Kanda K and Yoshino K: Treatment of malignant melanoma by single thermal neutron capture therapy with melanoma-seeking 10b-compound. *Lancet* 2: 388-389, 1989. PMID: 2569577. DOI: 10.1016/S0140-6736(89)90567-9
- 43 Kulvik M, Kallio M, Laakso J, Vahatalo J, Hermans R, Jarviluoma E, Paetau A, Rasilainen M, Ruokonen I, Seppala M and Jaaskelainen J: Biodistribution of boron after intravenous 4-dihydroxyborylphenylalanine-fructose (BPA-F) infusion in meningioma and schwannoma patients: A feasibility study for boron neutron capture therapy. *Appl Radiat Isot* 106: 207-212, 2015. PMID: 26298436. DOI: 10.1016/j.apradiso.2015.08.006
- 44 Koivunoro H, Hippelainen E, Auterinen I, Kankaanranta L, Kulvik M, Laakso J, Seppala T, Savolainen S and Joensuu H: Biokinetic analysis of tissue boron (¹⁰B) concentrations of glioma patients treated with BNCT in finland. *Appl Radiat Isot* 106: 189-194, 2015. PMID: 26363564. DOI: 10.1016/j.apradiso.2015.08.014
- 45 Fujimoto T, Andoh T, Sudo T, Fujita I, Imabori M, Moritake H, Sugimoto T, Sakuma Y, Takeuchi T, Sonobe H, Epstein A, Akisue T, Kirihata M, Kurosaka M, Fukumori Y and Ichikawa H: Evaluation of BPA uptake in clear cell sarcoma (CCS) *in vitro* and development of an *in vivo* model of CCS for BNCT studies. *Appl Radiat Isot* 69(12): 1713-1716, 2011. PMID: 21354804. DOI: 10.1016/j.apradiso.2011.02.006
- 46 Andoh T, Fujimoto T, Suzuki M, Sudo T, Sakurai Y, Tanaka H, Fujita I, Fukase N, Moritake H, Sugimoto T, Sakuma T, Sasai H, Kawamoto T, Kirihata M, Fukumori Y, Akisue T, Ono K and Ichikawa H: Boron neutron capture therapy (BNCT) as a new approach for clear cell sarcoma (CCS) treatment: Trial using a lung metastasis model of CCS. *Appl Radiat Isot* 106: 195-201, 2015. PMID: 26337135. DOI: 10.1016/j.apradiso.2015.07.060
- 47 Yoshimoto M, Kurihara H, Honda N, Kawai K, Ohe K, Fujii H, Itami J and Arai Y: Predominant contribution of l-type amino acid transporter to 4-borono-2-(18)f-fluoro-phenylalanine uptake in human glioblastoma cells. *Nucl Med Biol* 40(5): 625-629, 2013. PMID: 23557719. DOI: 10.1016/j.nucmedbio.2013.02.010
- 48 Sato E, Yamamoto T, Shikano N, Ogura M, Nakai K, Yoshida F, Uemae Y, Takada T, Isobe T and Matsumura A: Intracellular boron accumulation in CHO-K1 cells using amino acid transport control. *Appl Radiat Isot* 88: 99-103, 2014. PMID: 24388319. DOI: 10.1016/j.apradiso.2013.12.015
- 49 Wada Y, Hirose K, Harada T, Sato M, Watanabe T, Anbai A, Hashimoto M and Takai Y: Impact of oxygen status on 10b-BPA uptake into human glioblastoma cells, referring to significance in boron neutron capture therapy. *J Radiat Res* 59(2): 122-128, 2018. PMID: 29315429. DOI: 10.1093/jrr/rrx080
- 50 Wongthai P, Hagiwara K, Miyoshi Y, Wiriyasermkul P, Wei L, Ohgaki R, Kato I, Hamase K, Nagamori S and Kanai Y: Boronophenylalanine, a boron delivery agent for boron neutron capture therapy, is transported by ATB0+, LAT1 and LAT2. *Cancer Sci* 106(3): 279-286, 2015. PMID: 25580517. DOI: 10.1111/cas.12602
- 51 Imahori Y, Ueda S, Ohmori Y, Kusuki T, Ono K, Fujii R and Ido T: Fluorine-18-labeled fluoroboronophenylalanine pet in patients with glioma. *J Nucl Med* 39(2): 325-333, 1998. PMID: 9476945.
- 52 Miyatake S, Kawabata S, Nonoguchi N, Yokoyama K, Kuroiwa T, Matsui H and Ono K: Pseudoprogession in boron neutron capture therapy for malignant gliomas and meningiomas. *Neuro Oncol* 11(4): 430-436, 2009. PMID: 19289492. DOI: 10.1215/15228517-2008-107
- 53 Kiger W, Micca P, Morris G and Coderre JA: Boron microquantification in oral mucosa and skin following administration of a neutron capture therapy agent. *Radiat Prot Dosimetry* 99(1-4): 409-412, 2002.
- 54 Barth R, Vicente M, Harling O, Kiger W, Riley K, Binns P, Wagner F, Suzuki M, Aihara T, Kato I and Kawabata S: Current status of boron neutron capture therapy of high grade gliomas and recurrent head and neck cancer. *Radiat Oncol* 29(7): 146, 2012. PMID: 22929110. DOI: 10.1186/1748-717X-7-146
- 55 Kabalka G, Wu Z and Yao M-L: Synthesis of a series of boronated unnatural cyclic amino acids as potential boron

- neutron capture therapy agents. *Appl Organometal Chem* 22(9): 516-522, 2008. DOI: 10.1002/AOC.1435
- 56 Renner M, Miura M, Easson M and Vicente M: Recent progress in the syntheses and biological evaluation of boronated porphyrins for boron neutron-capture therapy. *Anticancer Agents Med Chem* 6(2): 145-157, 2006. PMID: 16529537. DOI: 10.2174/187152006776119135
- 57 Mier W, Gabel D, Haberkorn U and Eisenhut M: Conjugation of the closo-borane mereaptoundeca-hydrododecaborate (bsh) to a tumor selective peptide. *J Inorg Gen Chem* 630(8-9): 1258-1262, 2004.
- 58 Backer MV, Gaynutdinov TI, Patel V, Bandyopadhyaya AK, Thirumamagal BT, Tjarks W, Barth RF, Claffey K and Backer JM: Vascular endothelial growth factor selectively targets boronated dendrimers to tumor vasculature. *Mol Cancer Ther* 4(9): 1423-1429, 2005. PMID: 16170035. DOI: 10.1158/1535-7163.MCT-05-0161
- 59 Barth RF, Yang W, Wu G, Swindall M, Byun Y, Narayanasamy S, Tjarks W, Tordoff K, Moeschberger ML, Eriksson S, Binns PJ and Riley KJ: Thymidine kinase 1 as a molecular target for boron neutron capture therapy of brain tumors. *Proc Natl Acad Sci USA* 105(45): 17493-17497, 2008. PMID: 18981415. DOI: 10.1073/pnas.0809569105
- 60 Tietze LF GU, Bothe U, Nakamura H, Yamamoto Y.: Novel carboranes with a DNA binding unit for the treatment of cancer by boron neutron capture therapy. *ChemBiochem* 3(2-3): 219-225, 2002. PMID:11921401
- 61 Hiramatsu R, Kawabata S, Miyatake S, Kuroiwa T, Easson MW and Vicente MG: Application of a novel boronated porphyrin (H2OCP) as a dual sensitizer for both pdt and bnct. *Lasers Surg Med* 43(1): 52-58, 2011. PMID: 21254143. DOI: 10.1002/lsm.21026
- 62 Menichetti L, De Marchi D, Calucci L, Ciofani G, Menciasci A and Forte C: Boron nitride nanotubes for boron neutron capture therapy as contrast agents in magnetic resonance imaging at 3t. *Appl Radiat Isot* 69(12): 1725-1727, 2011. PMID: 21398132. DOI: 10.1016/j.apradiso.2011.02.032
- 63 Barth RF, Mi P and Yang W: Boron delivery agents for neutron capture therapy of cancer. *Cancer Commun (Lond)* 19(38): 35, 2018. PMID: 29914561. DOI: 10.1186/s40880-018-0299-7
- 64 Crossley E, Ziolkowski E, Coderre J and Rendina L: Boronated DNA-binding compounds as potential agents for boron neutron capture therapy. *Mini Rev Med Chem* 7(3): 303-313, 2007. PMID: 17346220. DOI: 10.2174/138955707780059808
65. Nakamura H: Liposomal boron delivery system for neutron capture therapy of cancer. *In. boron science: New technologies and applications*. CRC Press, pp. 165-179, 2012.
- 66 Doi A, Kawabata S, Iida K, Yokoyama K, Kajimoto Y, Kuroiwa T, Shirakawa T, Kirihata M, Kasaoka S, Maruyama K, Kumada H, Sakurai Y, Masunaga S, Ono K and Miyatake S: Tumor-specific targeting of sodium borocaptate (BSH) to malignant glioma by transferrin-peg liposomes: A modality for boron neutron capture therapy. *J Neurooncol* 87(3): 287-294, 2008. PMID: 18219552. DOI: 10.1007/s11060-008-9522-8
- 67 Singh B, Kaur G, Singh P, Singh K, Kumar B, Vij A, Kumar M, Bala R, Meena R, Singh A, Thakur A and Kumar A: Nanostructured boron nitride with high water dispersibility for boron neutron capture therapy. *Sci Rep* 6: 35535, 2016. DOI: 10.1038/srep35535
- 68 Haselsberger K RH, Gössler W, Schlagenhauen C and Pendl G: Subcellular boron-10 localization in glioblastoma for boron neutron capture therapy with NA2B12H11SH. *J Neurosurg* 81((5)): 741-744, 1994. DOI: 10.3171/jns.1994.81.5.0741
- 69 Neumann M KU, Lehmann H and Gabel D: Determination of the subcellular distribution of mercaptoundeca-hydro-dodecaborate (BSH) in human glioblastoma multiforme by electron microscopy. *J Neurooncol* 57(2): 97-104, 2002. PMID: 12125978.
- 70 Andoh T, Fujimoto T, Sudo T, Fujita I, Imabori M, Moritake H, Sugimoto T, Sakuma Y, Takeuchi T, Kawabata S, Kirihata M, Akisue T, Yayama K, Kurosaka M, Miyatake S, Fukumori Y and Ichikawa H: Boron neutron capture therapy for clear cell sarcoma (CCS): Biodistribution study of p-borono-l-phenylalanine in CCS-bearing animal models. *Appl Radiat Isot* 69(12): 1721-1724, 2011. PMID: 21367607. DOI: 10.1016/j.apradiso.2011.02.005
- 71 Barth RF, Kabalka GW, Yang W, Huo T, Nakkula RJ, Shaikh AL, Haider SA and Chandra S: Evaluation of unnatural cyclic amino acids as boron delivery agents for treatment of melanomas and gliomas. *Appl Radiat Isot* 88: 38-42, 2014. PMID: 24393770. DOI: 10.1016/j.apradiso.2013.11.133
- 72 Carpano M, Perona M, Rodriguez C, Nieves S, Olivera M, Santa Cruz GA, Brandizzi D, Cabrini R, Pisarev M, Juvenal GJ and Dagrosa MA: Experimental studies of boronophenylalanine ((10)BPA) biodistribution for the individual application of boron neutron capture therapy (BNCT) for malignant melanoma treatment. *Int J Radiat Oncol Biol Phys* 93(2): 344-352, 2015. PMID: 26232853. DOI: 10.1016/j.ijrobp.2015.05.039
- 73 Kaniowski D, Ebenryter-Olbinska K, Sobczak M, Wojtczak B, Janczak S, Lesnikowski ZJ and Nawrot B: High boron-loaded DNA-oligomers as potential boron neutron capture therapy and antisense oligonucleotide dual-action anticancer agents. *Molecules* 22(9), 2017. PMID: 28832537. DOI: 10.3390/molecules22091393
- 74 Wu H, Micca PL, Makar MS and Miura M: Total syntheses of three copper (ii) tetracarboranylphenylporphyrins containing 40 or 80 boron atoms and their biological properties in EMT-6 tumor-bearing mice. *Bioorg Med Chem* 14(15): 5083-5092, 2006. PMID: 16651000. DOI: 10.1016/j.bmc.2006.04.010
- 75 Ferreira TH, Marino A, Rocca A, Liakos I, Nitti S, Athanassiou A, Mattoli V, Mazzolai B, de Sousa EM and Ciofani G: Folate-grafted boron nitride nanotubes: Possible exploitation in cancer therapy. *Int J Pharm* 481(1-2): 56-63, 2015. PMID: 25637832. DOI: 10.1016/j.ijpharm.2015.01.048
76. Ciofani G, Del Turco S, Genchi GG, D'Alessandro D, Basta G and Mattoli V: Transferrin-conjugated boron nitride nanotubes: Protein grafting, characterization, and interaction with human endothelial cells. *Int J Pharm* 436(1-2): 444-453, 2012. PMID: 22732669. DOI: 10.1016/j.ijpharm.2012.06.037
- 77 Li X, Wang X, Zhang J, Hanagata N, Weng Q, Ito A, Bando Y and Golberg D: Hollow boron nitride nanospheres as boron reservoir for prostate cancer treatment. *Nat Commun* 8: 13936, 2017. PMID: 28059072. DOI: 10.1038/ncomms13936
- 78 Fukuda H, Honda C, Wadabayashi N, Kobayashi T, Yoshino K, Hiratsuka J, Takahashi J, Akaizawa T, Abe Y, Ichihashi M and Mishima Y: Pharmacokinetics of 10b-p-boronophenylalanine in tumours, skin and blood of melanoma patients: A study of boron neutron capture therapy for malignant melanoma. *Melanoma Research* 9(1): 75-84, 1999. PMID: 10338337.

- 79 Wittig A, Sheu-Grabellus SY, Collette L, Moss R, Brualla L and Sauerwein W: Bpa uptake does not correlate with LAT1 and KI67 expressions in tumor samples (results of EORTC trial 11001). *Appl Radiat Isot* 69(12): 1807-1812, 2011. PMID: 21367608. DOI: 10.1016/j.apradiso.2011.02.018
- 80 Watanabe T, Hattori Y, Ohta Y, Ishimura M, Nakagawa Y, Sanada Y, Tanaka H, Fukutani S, Masunaga SI, Hiraoka M, Ono K, Suzuki M and Kirihata M: Comparison of the pharmacokinetics between L-BPA and L-FBPA using the same administration dose and protocol: A validation study for the theranostic approach using [(18)f]-L-FBPA positron emission tomography in boron neutron capture therapy. *BMC Cancer* 16(1): 859, 2016. PMID: 27821116. DOI: 10.1186/s12885-016-2913-x
- 81 Farias RO, Bortolussi S, Menendez PR and Gonzalez SJ: Exploring boron neutron capture therapy for non-small cell lung cancer. *Phys Med* 30(8): 888-897, 2014. PMID: 25176019. DOI: 10.1016/j.ejmp.2014.07.342
- 82 Ferrari C, Zonta C, Cansolino L, Clerici AM, Gaspari A, Altieri S, Bortolussi S, Stella S, Bruschi P, Dionigi P and Zonta A: Selective uptake of p-boronophenylalanine by osteosarcoma cells for boron neutron capture therapy. *Appl Radiat Isot* 67(7-8 Suppl): S341-344, 2009. PMID: 19394838. DOI: 10.1016/j.apradiso.2009.03.059
- 83 Agarwal HK, Khalil A, Ishita K, Yang W, Nakkula RJ, Wu LC, Ali T, Tiwari R, Byun Y, Barth RF and Tjarks W: Synthesis and evaluation of thymidine kinase 1-targeting carboranyl pyrimidine nucleoside analogs for boron neutron capture therapy of cancer. *Eur J Med Chem* 100: 197-209, 2015. PMID: 26087030. DOI: 10.1016/j.ejmech.2015.05.042
- 84 Suzuki M, Sakurai Y, Masunaga S, Kinashi Y, Nagata K and Ono K: Dosimetric study of boron neutron capture therapy with borocaptate sodium (bsh)/lipiodol emulsion (BSH/lipiodol-BNCT) for treatment of multiple liver tumors. *Int J Radiat Oncol Biol Phys* 58(3): 892-896, 2004. PMID: 14967447. DOI: 10.1016/j.ijrobp.2003.09.084
- 85 Heber E, Trivillin V, Nigg D, Kreimann E, Itoiz M, Rebagliati R, Batistoni D and Schwint A: Biodistribution of GB-10 (NA(2)(10)B10H10 compound for boron neutron capture therapy (BNCT) in an experimental model of oral cancer in the hamster cheek pouch. *Arch Oral Biol* 49: 313-324, 2004. PMID: 15003550. DOI:10.1016/j.archoralbio.2003.10.003
- 86 Bregadze V, Sivaev I, Lobanova I, Titeev R, Brittal D, Grin M and Mironov A: Conjugates of boron clusters with derivatives of natural chlorin and bacteriochlorin. *Appl Radiat Isot* 67: 101-104, 2009. PMID: 19447631. DOI: 10.1016/j.apradiso.2009.03.024

Received February 4, 2019

Revised April 22, 2019

Accepted April 23, 2019