Therapeutics, Pharmacology and Clinical Toxicology
Vol XX, Number 1, March 2016
Pages: 25 - 32

BORON NEUTRON CAPTURE THERAPY: DELIVERY AGENTS USED IN BORON ADMINISTRATION

Galeș N. Laurenția[1], Anghel M. Rodica[2]

[1] *University of Medicine and Pharmacy "Carol Davila" Bucharest*

Abstract. Boron neutron capture theory (BNCT) is a binary therapy applied in malignant tumours, resistant to other treatment methods, namely chemotherapy and radiotherapy. One of the main components is Bor-10, a stable isotope that can be inserted in the tumour cells by using specific transporters. Through the use of adequate Boron compounds, that preferably are located in the tumour cells and not in the healthy tissue, Boron neutron capture therapy provides a higher curative potential with minimal toxicity, at the level of normal tissues. In order to obtain a proper tumour control with minimum side effects, 10B increased concentrations should be obtained by selective cumulation within the tumour cells, with an adequate ion gradient between the normal and tumour tissues. Therapeutic effectiveness can be reached by a minimal boron concentration of 20-30 ppm inside the tumour and a tumour/blood and tumour/normal tissues boron concentration gradient of approximately 3. So far, clinical practice has used two main boron delivery agents: sodium borocaptate ($Na_2B_{12}H_{11}SH$) (BSH) and a phenylalanine dihydroxy-boryl derivative, known as Boronophenylalanine (BPA). During the past 20 years, along with the development of new techniques of chemical synthesis and improved knowledge of biological and biochemical requirements for the effectiveness of an agent and ways of boron delivery, a new number of boron delivery agents have appeared in response to the agents up to now. They include: aminoacids, nucleic acid precursors, DNA-binding molecules, porphyrin derivatives, high-molecular- weight delivery agents, macromolecules or nanovehicles such as monoclonal antibodies or monoclonal antibody fragments that bind to tumour epitopes- EGF, VEGF or liposomes, dendrimers, dextrans, polylysine, avidin, folic acid, etc. Next to the identification and study of new boron compounds or new compound combinations, other delivery methods are currently under research seeking to improve the classical intravenous administration and the selective intratumoral binding process as well as the therapeutic index (intraarterial, intratumoral administration, etc). Despite a large number of ongoing studies, a boron compound that fits all the ideal BNCT compound criteria has not yet been identified, especially in terms of tumour selectivity, the ratio of concentrations between the concentration in the tumour cells and the one in the normal cells, the delivery of a proper number of boron atoms in the tumour cells, hydrophilicity and lipophilicity, homogeneous intra-tumour distribution. The development of BNCT boron delivery agents has been initiated approximately 50 years ago and continues to represent at the same time a priority and a difficulty. Several bor-10 boron based pharmaceutical products have been prepared in view of their potential use in BNCT.

Key words: Radiotherapy, BNCT, boron compounds.

Introduction

Boron neutron capture theory (BNCT) is a binary therapy assessed for the cases of resistant malignant tumours to other treatment methods, namely chemotherapy and radiotherapy. Neither of the two therapeutic compounds, taken separately, has consistent effects on the tumour but their combination triggers a lethal effect for the tumour cells with a minimal damage on the healthy tissue. One of the compounds -Bor-10-a stable isotope can be concentrated in the tumour cells by applying a specific set of transporters. The other compound is a beam of low-energy neutrons. The capture of ^{10}Bor neutrons produces alfa energy particles, with a linear energy transfer at the level of the tissue. Hence, this generates effective cell destruction and a relatively higher biological efficiency than the

Laurentia Gales
258 Șos.Fundeni, 2nd District, Bucharest, Romania
e-mail: laurentia.gales@yahoo.com

photons. By using the adequate Boron compounds that preferably bind to the tumour cells and not to the healthy tissue, Boron neutron capture theory provides a better curative potential with minimal toxicity for the normal tissues. [1, 2]

In order to obtain effective tumour control with reduced collateral effects, ^{10}B increased concentration must be reached by accumulating it selectively within the tumour cells, with a sufficient ion gradient between the normal and tumour tissues. Subsequently, the patient undergoes radiation therapy with adequate energy and intensity neutron beams so that the maximum density of the thermal or epithermal neutrons be reached within the proximity of the tumour.

A productive therapy relies on a minimum boron concentration of 20-30 ppm inside the tumour and a normal boron concentration in the tumour/blood and tumour/normal tissues of approximately 3.

The substances used in BNCT boron administration must meet the following essential criteria:[1, 3, 4]

- An increased boron content (the natural abundance is around 20%);
- No systemic toxicity;
- Selective caption at the level of the tumour. The selectivity conditions the tumour/normal tissue therapeutic index; tumour concentrations ratio: the blood must be 3-4:1;
- Efficient boron transporters;
- The absolute boron content is essential while boron tumour concentration should be approximately ~20 μg ^{10}B/g per tumour. Together with the thermal neutrons flow, boron content establishes the necessary exposure time targeted to reach the prescribed physical dose and ratio from the total dose delivered to the boron compound. The compound must have a rapid blood and tumour clearance and should persist, nonetheless, in the tumour cell throughout BNCT;
- Homogeneously captured by the target.

Currently not a single boron delivery agent fulfils all the above mentioned criteria.

[^{10}B] absolute value in the tumour, healthy tissue and blood are critical elements for BNCT.

For similar tumour/tissue or tumour/blood ratios, higher boron concentrations are more beneficial due to their permissiveness in terms of a lower exposure time, hence a reduced background irradiation associated dose.

An agent can hardly trigger all the tumour cells or at least, the largest part of them.

Combined administration of different boron compounds with specific properties and complementary absorption mechanisms would increase BNCT's therapeutic advantages by merging tumour control mechanisms that aim distinct tumour populations and the reduction of cell toxicity that limits the dose.

The first association of this sort has been detected between the first two used agents, namely BPA and BSH which led to promising results in what concerned the improvement in the tumour/normal tissue boron index [5].

Another studied association was BPA with GB-10. BPA is selectively captured in the tumour but presents an intra-tumor inhomogeneity capture in viable/ necrotic areas of 1.46/ 1. GB-10 ($Na_2{}^{10}B_{10}H_{10}$) cannot cross the intact blood-brain barrier, but it will however deposit itself, homogeneously, inside the tumour with a 0.97/ 1 viable/necrotic areas ratio.

The concern with these cases of association is the difficult dosimetry, especially if a high weight and low weight molecular compound conflate.

Boron delivery agents:

So far, clinical practice has relied on two main boron based drugs: sodium borocaptate ($Na_2B_{12}H_{11}SH$) (BSH) and a phenylalanine dihydroxy-boryl derivative, known as Boronophenylalanine (BPA) [6, 7, 8, 9, 10, 11], a third one GB-10 ($Na_2{}^{10}B_{10}H_{10}$), being recently approved by FDA. [12]

During the past 20 years, along with the development of new techniques of chemical synthesis and improved knowledge of biological and biochemical requirements for the effectiveness of an agent and ways of boron delivery, a new number of boron delivery agents has appeared in response to the agents used so far (examples in Table I). [13] They include: aminoacids, nucleic acid precursors, DNA-binding molecules, porphyrin derivatives, high-molecular-weight delivery agents, macromolecules or nanovehicles such as monoclonal antibodies or monoclonal antibody fragments that bind to tumour epitopes- EGF, VEGF or liposomes, dendrimers, dextrans, polylysine, avidin, folic acid, etc. [14, 15].

* Boronophenylalanine ("BPA")	* Sodium borocaptate ("BSH")
Dodecaborate cluster lipids and cholesterol derivatives	Carboranyl nucleosides
"GB10" ($Na_2B_{10}H_{10}$)±	Carboranyl porphyrins
Cholesteryl ester mimics	Boronated EGF(epidermal growth factor) and anti-EGFR mAbs
Boronated DNA metallo-intercalators	Boron-containing nanoparticles
Transferrin–polyethylene glycol (TF–PEG) liposomes	Carboranyl porphrazines
Unnatural amino acids	Boronated cyclic peptides
Dodecahyrdo-closo-dodecaborate clusters	Boron carbide particles

** The only boron delivery agents used by clinical practice.*
± FDA approval

Tabel I. Boron delivery agents with low and high molecular weight that are currently used or under process of approval [16]

The chemical structure of the main boron delivery agents that are under assessment is presented in figure 1:

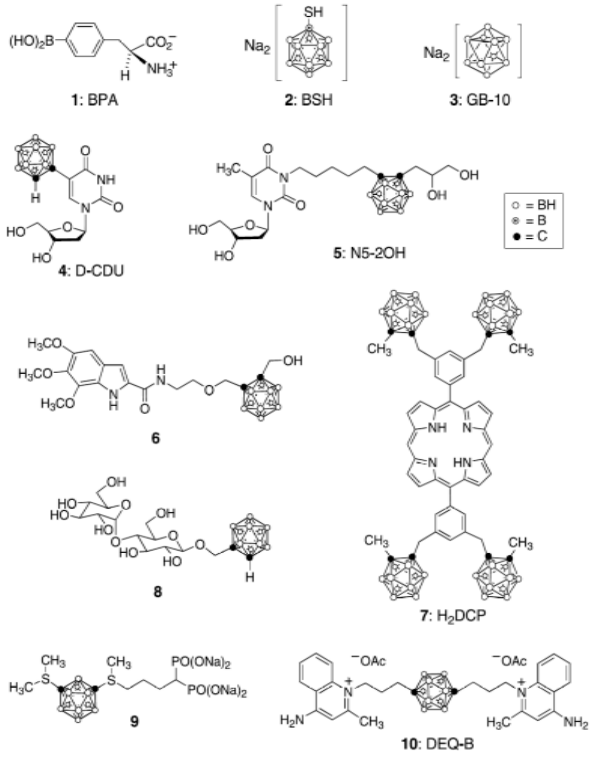

Fig.1. Chemical structure of the main boron delivery agents with low and high molecular weight

Presently the medical community use BPA (compound 1) and BSH (compound 2).

GB-10 (compound 3) recorded promising results on animal tests as well as nucleosides derivatives ß-5-*o*-carboranyl-2'-deoxyuridine (D-CDU comppund 4) and N5-2OH (compound 5). Compound 6, a *trimethoxyindole derivative* has been under research *in vitro* while compound 7, H_2DCP, a porphyrin derivative, has shown tumour selectivity. Maltose derivative, compound 8, has demonstrated at the *in vitro* experiments, reduced cytotoxicity and tumour capture; compound 9, a biphosphonate, presented tumour selectivity and dequalinium-B derivative (*DEQ-B*, compound 10) has registered promising results at the *in vitro* studies. [1, 17, 12, 18, 19]

Next to the identification and study of new boron compounds or new compound combinations, other delivery methods are currently under research in order to improve the classical intravenous administration and the selective intratumoral binding process as well as the therapeutic index (intraarterial, intratumoral administration, etc). [14]

We will now proceed to briefly describe the main agents and ways of boron delivery.

First and second generation boron delivery agents

In the 1950s and early 1960s the first boron agents used in BNCT were boric acid and some of its derivatives. However, they were non selective agents, presenting poor tumour retention and a reduced tumour/tissue therapeutic index. [20, 21] During the 1960's, second generation agents entered in trial, one that was aryl boronic acid based, BPA [22] and another one, based on polyphedral boranes and *sodium mercaptoundecahydro-closo-dodecaborate, known as BSH* [23]. Second generation compounds have low toxicity, persist longer inside the tumour tissue and have a supraunitary tumour/brain and tumour//blood therapeutic index. [24, 25] BPA has been improved with 10B and mixed with fructose in order to obtain better hydrosolubility and, along with BSH, has been used for both brain tumours as well as for other tumours. [26].

Third generation boron delivery agents

The third generation compounds consist mainly of a group of stable boron compounds attached to a high tumour affinity fragment through a hydrolitic stabilised bond (low weight molecular biomolecules or monoclonal antibodies). In this sense, EGFR targeting and the mutant EGFRvIII isoform overexpressed in brain tumours and squamos cell carcinoma of the head and neck actually represented such an approach. [27] Tumour cell nucleus and DNA are attractive targets due to the fact that the necessary boron amount to produce a lethal effect can be substantially reduced if it is localised inside or close to the nucleolus. [28] Organelles (endoplasmic reticulum, mitochondria, lysosome, etc) are also a potential target. Hidrosolubility is an important property in the systemic administration, while lipophilia allows the crossing of blood-brain barrier and intratumoral diffusion making amphiphilic products the most studied within their class. Another possibility to cut across the blood-brain barrier is the incorporation of delivery agents such as liposomes which would make amphiphilie unnecessary. Molecular weight is another factor that could determine the intratumoral diffusion rate.

a. Low molecular weight agents

Boron aminoacids and polyphedral boranes: several boron aminoacdids have been assessed-cysteine, tyrosine, aspartic acid, alanine, methionine and glycine as well as non-natural amino acids [29-34]. The advantage is that compared to BPA they have increased boron content, reported at their weight, thus being able to deliver more intratumoral boron without causing consecutive increase in toxicity. One of the non-natural amino acids, *1-amino-3-borono-cyclopentanecarboxylic acid* (ABCPC) is worth mentioning because it has registered blood/tumour concentrations of 8 and tumour/normal tissue of 21 on melanoma murine models, a highly advantageous ratio compared to the usual boron compounds. [35] Several polyphedral boranes were also evaluated one of them being: decahydro –closo decaborate – dianon B10H102. The simple sodium salt in this compound, the one known as GB-10 has reduced systemic toxicity and tumour selectivity which enabled FDA to approve its use in glioblastomas and melanomas [12]. Other polyphedral boranes, although with a proper boron content, have reduced tumour selectivity which qualifies them for liposomal encapsulation. [36, 37]

Biochemical precursors and DNA binding agents have been tested on animal models: nucleic acid analogues, purine, pyrimidine, nucleosides, nucleotides,

etc. [38, 39-44] Cell nucleotide formation from these compounds may lead to an increased caption and boron intratunoral retention [45] For a large part of these agents the binding process depends on the cell cycle phase (binding takes place in the S phase), hence the need to combine it with a non cyclodependant agent. Another evaluated compound class was DNA binding compounds: alkylating agents, platinum complexes, intercalators or polyamines.

Boron porphyrins and other similar derivatives- some boron fluorescent dyes as porphyrins, acridine, phenanthridine, phthalocyanine have all been under research for BNCT, carrying the advantage of simple detection via fluorescence microscopy and DNA interaction due to their plane aromatic structure.[46-53]

BSH carbohydrate derivatives and other boron based carbohydrates were also studied: ribose, mannose, maltose, galactose and the results showed increased hydrosolubility. They can be mixed with molecules that have affinity on different receptors such as steroid hormone antagonists, namely tamoxifen.

Peptides [61], polyamines [62] and antisense oligonucleotides have also been considered as alternatives to boron delivery methods.

Nicotinamides, which also have radiosenzitising properties, have been studied in different combinations either mixed with the boron delivery agent or administered prior to the agent.[64]

b. High weight molecular agents

Special attention was given to boronated monoclonal antibodies or fragments of them with affinity for different tumour epitopes. Even if they have increased tutor affinity they also have rapid clearance that reduces intratumoral capture by decreasing the contact period [27, 65-70]

ţEGF bioconjugates that demonstrate affinity for tumours overexpressing EGFR can be used as boron delivery agents. [71] In the case of systemic administration, they have a high selection degree, but a relatively short blood flow maintenance time in order to be captured, especially by the brain. Local administration could solve this problem.[72-75]. Another option is the use of anti EGFR monoclonal antibodies but this would require repeated BNCT. [76]. Direct binding between boron and a monoclonal antibody would require mixing a large number of boron atoms so that BNCT therapeutic concentration be obtained. A bioconjugate of this type would precipitate and loose its immunogenicity. Although solutions to improve solubility have been identified, compromised immunogeneicty continues to be a burden, as the simple attachment of boron to the antibody cannot be considered a solution. [76-82]. One bioconjugate that could represent a solution is Cetuximab mixed with a boron-enriched dendrimer

Dendrimer-based delivery agents

Dendrimers are synthetic polymers with a well defined globular structure. They consist of a basis molecule, repetitive units with three or more functions and surface reactive groups. [84, 85]. They have low toxicity and several surface reactive groups that can be coupled with other molecules. [86-91] The high number of reactive groups enable the binding of a larger number of boron atoms for each immunoconjugates molecule (up to 1000 boron atoms). The first compounds proved to have high affinity especially for the liver and spleen. [65, 66, 69, 83]

An alternative to binding of boron enriched dendrimers is their connection with receptor ligands, as for exemple EGF, with the clear benefit of a lower molecular weight compared to the antibody bioconjugation. [83, 92]

Merging dendrimers with anti-folate receptor

The folate receptor (FR) is overexpressed in various cancers: ovarian, pulmonary, mammary, endometrial and renal cancers [93-95]. Folic acid (FA) is a vitamin that is transported to the cell by FR mediated endocytosis. Binding of folic acid to other molecules by grouping carboxil does not have an impact on endocytosis. [96]. Folic acid was studies as a intracellular transporter vector for a series of substances: cytostatics, toxines, enhnancing agents, liposomes, immunotherapeutic agents, etc. Coupling with a boron-enriched dendrimer can also render it attractive for BNCT [97].

An alternative method to deliver born compounds through dendrimers is to incorporate Carboran boron sites within the dendrimers, this resulting in a hydrosoluble compound that has the ability to deliver a sufficient number of boron atoms. [98]

Liposome incorporated delivery agents

Liposomes are non-toxic, biodegradable vesicles that have been used to supply both hydrophilic and hydrophobic agents [99]. Boron liposomes stand as another possibility for boron delivery [100-103]. Due to their incapacity to break the blood-brain barrier as a consequence of their dimensions- >50 nm they are less attractive for brain tumours and more beneficial for liver tumours [102, 104, 105]. Rapid growth tumours due to structural deficiencies in the neoformation vessels are the pre-elected target of liposomes or other nanoparticles. PEGylation or the attachment to antibodies increases the tutor selectivity as well as the circulation time. [106]. Liposomal encapsulation extends the time for elimination by the reticuloendothelial system, thus maintaining sufficient intratumoral quantities- from 3 to 6 hours even to 24 hours from the systemic administration. Hence, BNCT administration is rendered easier, especially if irradiation is performed remotely. [108, 109]

Polyphedral boranes and carbonades can also be encapsulated into the liposome. This generates compounds with multiple boron atoms that are resistant to metabolic degradation and are lypophilic, thus enabling a simpler penetration of the tutor cell membrane [110].

The attachment of molecules with affinity for certain cell epitopes that engenders immunoliposomes facilitates a selective delivery of the boron to the tumour. Similar to dendrimers, liposomes may couple with monoclonal antibodies (anti CEA [111, 112] or anti HER-2 [113] as an exemple), with EGF [114-116], or FR [117]

Dextran based delivery agents

Dextrans represent glucose polymers used as carriers for various drugs or proteins to increase their

circulation time. Considering that their chemical structure has been modified, dextrans can now be applied for targeting the tumour cells. [118, 119]. In order to bind boron to dextrans B-*decachloro-o-carborane* derivatives were used, where one of the carbon atoms was substituted with a -$CH_2CHOHCH_2$-O-$CH_2CH{=}CH_2$ group, thus reaching a boron content of 4.3% [120, 121] Subsequently, the modified dextran is coupled with specific anti-tumour antibodies. [121-124] However, bioconjugates intratumoral caption is reduced in the systemic administration, intratumoral application being the considered option. [27]

In the case of liver tumours, the association of a classical boron delivery agent, BPA or BSH with lipiodol has been studied with the conclusion that there is sufficient intratumoral boron caption for BNCT application. Intra-hepatic artery administration scaled up following this selectivity [125]

Other molecules used for BNCT boron delivery were polylysine (also coupled with antitumor antibodies), unimolecular nanovehicles (e.g. dodecahydro-closo-dodecaborate clusters), etc [68, 126-128]

Conclusions

Although BNCT is an innovative and promising treatment method, still several aspects continue to be critical which prevents it from being applied on a larger scale. BNCT's success depends on the boron delivery compound, the quality of the neutrons' beam, dosimetry and the experience of the medical staff.

The first problematic aspect regards the boron delivery compound. Despite numerous ongoing studies, so far, a boron compound that fits all criteria for the ideal BNCT compound is yet to be identified, especially in the areas of tumour selectivity, ratio concentration between tumour and normal cells, the delivery of a sufficient number of boron atoms to the tumour cells, hydrophilicity and lipophilicity, homogeneous intratumor distribution.

The development of boron delivery agents for BNCT has begun 50 years ago and continues to be a difficult issue but a priority, nonetheless. Several bor-10 boron-based pharmaceutical products have been prepared in view of their potential application in BNCT. [1, 17]

References

1. Barth R.F., Coderre J.A., Vicente H, et al. Boron Neutron Capture Therapy of Cancer: Current Status and Future Prospects – *Clinical Cancer Research* **2005; 11 (11): 3987-4002.**

2. Sauerwein W.A.G. – Principles and Roots of Neutron Capture Therapy – in Neutron Capture Therapy: Principles and Applications **–** *Sauerwein W.A.G., Wittig A, Moss R, Nakagawa Y – Springer* **2012 – 1-18.**

3. Junjie Huang - Boron Neutron Capture Therapy for Cancer Treatments - A dissertation submitted to the Department of Physics, University of Surrey, in partial fulfillment of the degree of Master of Radiation and Environmental Protection - *Department of Physics, Faculty of Electronics & Physical Sciences, University of Surrey* - **July 2009, p 1-25.**

4. Yamamoto T, Nakai K, Matsumura A – Boron neutron capture therapy for glioblastoma**,** *Cancer Letters***, 2008, 262: 143.**

5. Yokoyama K, Miyatake S, Kajimoto Y, et al. Pharmacokinetic study of BSH and BPA in simultaneous use for BNCT. - *J Neurooncol.* **2006; 78(3): 227-32.**

6. Diaz A.Z. Assessment of the results from the phase I/II boron neutron capture therapy trials at the Brookhaven National Laboratory from a clinician's point of view, *Journal of Neuro-Oncology* **2003, 62 (1-2): 101-9.**

7. Busse P.M., Harling O.K., Palmer M.R., et al 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* **2003, 62 (1-2):111-21.**

8. Capala J, H.-Stenstam B, Sköld K, et al. Boron neutron capture therapy for glioblastoma multiforme: clinical studies in Sweden. *J Neurooncol* **2003; 62: 135–44.**

9. Joensuu H, Kankaanranta L, Seppälä T, et al. Boron neutron capture therapy of brain tumors: clinical trials at the Finnish Facility using boronophenylalanine. *J Neurooncol* **2003; 62: 123–34.**

10. Nakagawa Y, Hatanaka H. Boron neutron capture therapy: clinical brain tumor studies. *J Neuro-Oncol,* **33: 105-15, 1997.**

11. Nakagawa Y, Pooh K, Kobayashi T, et al Clinical review of the Japanese experience with boron neutron capture therapy and a proposed strategy using epithermal neutron beams. *J Neuro-Oncol,* **2003 , 62: 87-99.**

12. Hawthorne M.F., Leel M.W. A Critical Assessment of Boron target Compounds for Boron Neutron Capture Therapy, *Journal of Neuro-Oncology* **2003, 62 (1): 33-45.**

13. *http://en.wikipedia.org/wiki/Neutron_capture_therapy_of_cancer accessed* **2014**

14. Wu G., Barth R.F., Yang W, et al. Boron Containing Macromolecules and Nanovehicles as Delivery Agents for Neutron Capture Therapy**,** *Anti-Cancer Agents in Medicinal Chemistry,* **2006, Vol. 6, No. 2: 1-14.**

15. Nakamura H., Kirihata M. Boron Compounds: New Candidates for Boron Carriers in BNCT in Neutron Capture Therapy: Principles and Applications, *Sauerwein W.A.G., Wittig A, Moss R, Nakagawa Y – Springer* **2012 pp 99-116.**

16. Wittig A., Hideghety K., Paquis P., et al Sauerwein, W.; Moss, R.; Wittig, A., eds. "Current clinical results of the EORTC – study 11961". *Research and Development in Neutron Capture Therapy Proc. 10th Intl. Congress on Neutron Capture Therapy.* **2002, pp. 1117–22.**

17. Soloway A.H., Tjarks W, Barnum B.A., et al., The chemistry of neutron capture therapy, *Chem Rev***, 1998, 98, 1515-1562.**

18. Hawthorne MF. The role of chemistry in the development of boron neutron capture therapy of cancer. *Angew Chem Int Ed Engl* **1993; 32: 950–84.**

19. Morin C. The chemistry of boron analogues of biomolecules. *Tetrahedron* **1994; 50: 12521–69.**

20. Farr LE, Sweet WH, Robertson JS, et al. Neutron capture therapy with boron in the treatment of glioblastoma multiforme. *Am J Roentgenol* **1954; 71: 279–91.**

21. Godwin JT, Farr LE, Sweet WH, Robertson JS. Pathological study of eight patients with glioblastoma multiforme treated by neutron-capture therapy using boron 10. *Cancer* **1955; 8: 601–15.**

22. Snyder HR, Reedy AJ, Lennarz WJ. Synthesis of aromatic boronic acids, aldehydo boronic acids and a boronic acid analog of tyrosine. *J Am Chem Soc* **1958; 80: 835–8.**

23. Soloway AH, Hatanaka H, Davis MA. Penetration of brain and brain tumor. VII. Tumor-

binding sulfhydryl boron compounds. *J Med Chem* **1967; 10: 714.**

24. Hatanaka H, Nakagawa Y. Clinical results of long-surviving brain tumor patients who underwent boron neutron capture therapy. *Int J Radiat Oncol Biol Phys* **1994; 28: 1061–6.**

25. Mishima, Y. Cancer Neutron Capture Therapy; *Mishima, Y., Ed.; Plenum Press: New York,* **1996, p 1-26.**

26. Sauerwein W.A.G., Bet P.M., Witting A Drugs for BNCT: BSH and BPA in Neutron Capture Therapy: Principles and Applications – *Sauerwein W.A.G., Wittig A, Moss R, Nakagawa Y – Springer* **2012 pp 117-162**

27. Olsson P, Gedda L, Goike H, et al. Uptake of a boronated epidermal growth factor-dextran conjugate in CHO xenografts with and without human EGF-receptor expression. *Anticancer Drug Des* **1998;13: 279–89.**

28. Gabel D., Foster S., Fairchild R.G., The Monte Carlo simulation of the biological effect of the 10B(n,α)7Li reaction in cells and tissue and its implication for boron neutron capture therapy – *Radiat Res* **1987; 111, 14**

29. Srivastava RR, Singhaus RR, Kabalka GW. 4-Dihydroxyborylphenyl analogues of 1-aminocyclobutanecarboxylic acids: potential boron neutron capture therapy agents. *J Org Chem* **1999; 64: 8495–500.**

30. Das BC, Das S, Li G, et al. Synthesis of a water soluble carborane containing amino acid as a potential therapeutic agent. *Synlett* **2001; 9: 1419–20.**

31. Kabalka GW, Yao M-L. Synthesis of a novel boronated 1-amino-cyclobutane carboxylic acid as a potential boron neutron capture therapy agent. *Appl Organomet Chem* **2003; 17: 398–402.**

32. Diaz S, Gonzalez A, De Riancho SG, Rodriguez A. Boron complexes of S-trityl-L-cysteine and S-tritylglutathione. *J Organomet Chem* **2000; 610: 25–30.**

33. Lindström P, Naeslund C, Sjöberg S. Enantioselective synthesis and absolute configurations of the enantiomers of o-carboranylalanine. *Tetrahedron Lett* **2000; 41: 751–4.**

34. Masunaga S-I, Ono K, Kirihata M, et al. Potential of α-amino alcohol p-boronophenylalaninol as a boron carrier in boron neutron capture therapy, regarding its enantiomers. *J Cancer Res Clin Oncol* **2003; 129: 21–8.**

35. Kabalka G.W., Yao M.L., Marepally S.R., Chandra S – Biological Evaluation of Boronated Unnatural Amino Acids as New Boron Cariers – *Appl Radiat Isot* **2009, 67 (7-8): S374-379.**

36. Hawthorne MF, Feakes DA, Shelly K. Recent results with liposomes as boron delivery vehicles from boron neutron capture therapy. *Mishima Y, editor. Cancer neutron capture therapy. New York: Plenum Press;* **1996. p. 27–36.**

37. Feakes DA, Waller RC, Hathaway DK, Morton VS. Synthesis and in vivo murine evaluation of Na_4[1-(1'-$B_{10}H_9$)-6-$SHB_{10}H_8$] as a potential agent for boron neutron capture therapy. **Proc Natl Acad Sci U S A 1999; 96: 6406–10.**

38. Barth R.F., Yang W, Al-Madhoun A.S. et al, Boron-Containing Nucleosides as Potential Delivery Agents for Neutron Capture Therapy of Brain Tumors – *Cancer Res;* **2004, 64:6287-6295**

39. Lesnikowski ZJ, Schinazi RF. Boron containing oligonucleotides. *Nucleosides Nucleotides* **1998; 17: 635–47.**

40. Lesnikowski ZJ, Shi J, Schinazi RF. Nucleic acids and nucleosides containing carboranes. *J Organomet Chem* **1999; 581: 156–69.**

41. Lunato AJ, Wang J, Woollard JE, et al. Synthesis of 5-(carboranylalkylmercapto)-2'-deoxyuridines and 3-(carboranylalkyl) thymidines and their evaluation as substrates for human thymidine kinases 1 and 2. *J Med Chem* **1999; 42: 3378–89.**

42. Al-Madhoun AS, Johnsamuel J, Yan J, et al. Synthesis of a small library of 3-(carboranylalkyl) thymidines and their biological evaluation as substrates for human thymidine kinases 1 and 2. *J Med Chem* **2002; 45: 4018–28.**

43. Tjarks W., 2001, The use of boron clusters in the rational design of boronated nucleosides for neutron capture therapy of cancer, *J. Organomet. Chem.* **614-615, 37-47**

44. Tietze L.F., Griesbach U., Bothe U., et al., Novel carboranes with a DNA binding unit for the treatment of cancer by boron neutron capture therapy, *Chembiochem* **2002, 3, 219**

45. Al-Madhoun AS, Johnsamuel J, Barth RF, et al. Evaluation of human thymidine kinase 1 substrates as new candidates for boron neutron capture therapy. *Cancer Res* **2004; 64: 6280–6.**

46. Vicente MGH. Porphyrin-based sensitizers in the detection and treatment of cancer: recent progress. *Curr Med Chem Anti-Canc Agents* **2001; 1: 175–94.**

47. Bregadze VI, Sivaev IB, Gabel D, Wöhrle D. Polyhedral boron derivatives of porphyrins and phthalocyanines. *J Porphyrins Phthalocyanines* **2001; 5: 767–81.**

48. Evstigneeva RP, Zaitsev AV, Luzgina VN, et al. Carboranylporphyrins for boron neutron capture therapy of cancer. *Curr Med Chem Anti-Canc Agents* **2003; 3: 383–92.**

49. Fabris C, Jori G, Giuntini F, Roncucci G. Photosensitizing properties of a boronated phthalocyanine: studies at the molecular and cellular level. *J Photochem Photobiol B* **2001; 64: 1–7.**

50. Ghaneolhosseini H, Tjarks W, Sjoberg S. Synthesis of novel boronated acridines and spermidines as possible agents for BNCT. *Tetrahedron* **1998; 54: 3877–84.**

51. Gedda L, Silvander M, Sjoberg S, et al. *Cytotoxicity and subcellular localization of boronated phenanthridinium analogs. Anticancer Drug Des* **1997; 12: 671–85.**

52. Gedda L, Ghaneolhosseini H, Nilsson P, et al. The influence of lipophilicity on binding of boronated DNA-intercalating compounds in human glioma spheroids. *Anticancer Drug Des* **2000; 15: 277–86.**

53. Isaac M.F., Kahl S.B., Synthesis of ether and carbolinked polycarboranyl porphyrin dimers for cancer therapies, *J. Organomet. Chem.,* **2003, 680, 232**

54. Tietze L.F., Griesbach U., Schuberth I., et al., Novel carboranyl C-glycosides for the treatment of cancer by boron neutron capture therapy, *Chem. Eur. J.,* **2003, 9(6), 1296-302.**

55. Giovenzana GB, Lay L, Monti D, et al. Synthesis of carboranyl derivatives of alkynyl glycosides as potential BNCT agents. *Tetrahedron* **1999; 55: 14123–36.**

56. Tietze LF, Bothe U, Griesbach U, et al. *Ortho*-carboranyl glycosides for the treatment of cancer by boron neutron capture therapy. *Bioorg Med Chem* **2001; 9: 1747–52.**

57. Orlova AV, Zinin AI, Malysheva NN, et al. Conjugates of polyhedral boron compounds with carbohydrates. 1. New approach to the design of selective agents for boron neutron capture therapy of cancer. *Russian Chem Bull* **2003; 52: 2766–8.**

58. Tietze LF, Bothe U. *Ortho*-carboranyl glycosides

of glucose, mannose, maltose and lactose for cancer treatment by boron neutron-capture therapy. *Chem Eur* **J 1998; 4: 1179–83.**

59. Raddatz S, Marcello M, Kliem H-C, et al. Synthesis of new boron-rich building blocks for boron neutron capture therapy or energy-filtering transmission electron microscopy. *Chembiochem* **2004; 5: 474–82.**

60. Basak P, Lowary TL. Synthesis of conjugates of L-fucose and *ortho*-carborane as potential agents for boron neutron capture therapy. *Can J Chem* **2002; 80: 943–8.**

61. Ivanov D., Bachovchin W.W. and Redfield A.G., Boron-11 pure quadrupole resonance investigation of peptide boronic acid inhibitors bound to a-lyticprotease, *Biochemistry,* **2002, 41, 1587.**

62. El-Zaria M.E., Doerfler U. and Gabel D., Synthesis of [(aminoalkylamine)-n-aminoalkyl] azanonaborane(11) derivatives for boron neutron capture therapy, *J. Med. Chem.,* **2002, 45, 5817.**

63. Olejiniczak A.B., Koziolkiewcz M. and Lesnikowski Z.J., Carboranyl oligonucleotides: 4. Synthesis and physicochemical studies of oligonucleotides containing 20-O-(o-carboran-1-yl)methyl group, *Antisense Nucleic Acid Drug Dev.,* **2002, 12, 79.**

64. Pisarev M.A., Dagrosa M.A., Juvenal G.J. Boron Neutron Capture in Cancer: Past, Present and Future, *Arq Bras Endocrinol Metab* **2007, 51 (5): 852-856**

65. Barth RF, Adams DM, Soloway AH, et al. Boronated starburst dendrimer-monoclonal antibody immunoconjugates: evaluation as a potential delivery system for neutron capture therapy. *Bioconjug Chem* **1994; 5: 58–66.**

66. Liu L, Barth RF, Adams DM, et al. Critical evaluation of bispecific antibodies as targeting agents for boron neutron capture therapy of brain tumors. *Anticancer Res* **1996; 16: 2581–8.**

67. Liu L, Barth RF, Adams D, et al. Bispecific antibodies as targeting agents for boron neutron capture therapy of brain tumors. *J Hematother* **1995; 4: 477–83.**

68. Novick S, Quastel MR, Marcus S, et al. Linkage of boronated polylysine to glycoside moieties of polyclonal antibody; Boronated antibodies as potential delivery agents for neutron capture therapy. *Nucl Med Biol* **2002; 29: 93–101.**

69. Wu G, Barth RF, Yang W, et al. Site-specific conjugation of boron containing dendrimers to anti-EGF receptor monoclonal antibody cetuximab (IMC-C225) and its evaluation as a potential delivery agent for neutron capture therapy. *Bioconjug Chem* **2004; 15: 185–94.**

70. Fallot T, Magdelena TH, Mady E, et al. A phase I study of an anti-epidermal growth factor receptor monoclonal antibody for the treatment of malignant gliomas. *Neurosurgery* **1996; 39: 478–83.**

71. Barth RF, Yang W, Adams DM, et al. Molecular targeting of the epidermal growth factor receptor for neutron capture therapy of gliomas. *Cancer Res.* **2002 Jun 1; 62(11): 3159-66.**

72. Carlsson J, Gedda L, Grönvik C, et al. Strategy for boron neutron capture therapy against tumor cells with over-expression of the epidermal growth factor receptor. *Int J Radiat Oncol Biol Phys* **1994; 30: 105–15.**

73. Capala J, Barth RF, Bendayan M, et al. Boronated epidermal growth factor as a potential targeting agent for boron neutron capture therapy of brain tumors. *Bioconjug Chem* **1996; 7: 7–15.**

74. Sauter G, Maeda T, Waldman FM, et al. Patterns of epidermal growth factor receptor amplification in malignant gliomas. *Am J Pathol* **1996; 148: 1047–53.**

75. Schwechheimer K, Huang S, Cavenee WK. EGFR gene amplification-rearrangement in human glioblastoma. *Int J Cancer* **1995; 62: 145–8.**

76. Backer MV, Backer JM. Targeting endothelial cells overexpressing VEGFR-2: selective toxicity of Shinga-like toxin-VEGF fusion proteins. *Bioconjug Chem,* **2001; 12: 1066–73.**

77. Sneath, R. L., Jr.; Wright, J. E.; Soloway, A. H.; et al. Protein-binding polyhedral boranes, *J Med Chem* **1976, 19, 1290-4.**

78. Varadarajan A.; Sharkey R. M.; Goldenberg D. M.; Hawthorne M. F. Conjugation of phenyl isothiocyanate derivatives of carborane to antitumor antibody and in vivo localization of conjugates in nude mice, *Bioconjug Chem* **1991, 2, 102-10.**

79. Takahashi, T.; Fujii, Y.; Fujii, G.; Nariuchi, H. Preliminary study for application of anti-alpha-fetoprotein monoclonal antibody to boron-neutron capture therapy, *Jpn J Exp Med* **1987, 57, 83-91.**

80. Compostella, F.; Monti, D.; Panza, L.; et al. Research and Development in Neutron Capture Therapy, *Proceedings of the International Congress on Neutron Capture Therapy, 10th, Essen, Germany,* **Sept. 8-13, 2002 2002, 81-84.**

81. Giovenzana G. B.; Lay L.; Monti D.; et al. Synthesis of Carboranyl Derivatives of Alkynyl Glucosides as Potential BNCT Agents, *Tetrahedron* **1999, 55, 14123-14136.**

82. Alam F.; Soloway A. H.; McGuire J. E.; et al. Dicesium N-succinimidyl 3-(undecahydro-closo-dodecaboranyldithio) propionate, a novel heterobifunctional boronating agent, *Journal of Medicinal Chemistry* **1985, 28, 522-5.**

83. Barth RF, Wu G, Yang W, et al. Neutron capture therapy of epidermal growth factor positive gliomas using boronated cetuximab (IMC-C225) as a delivery agent, *Appl Radiat Isot* **2004; 61: 899–903.**

84. Gillies, E. R.; Frechet, J. M. J. Dendrimers and dendritic polymers in drug delivery, *Drug Discovery Today* **2005, 10, 35-43.**

85. Esfand, R.; Tomalia, D. A. Poly(amidoamine) (PAMAM) dendrimers: from biomimicry to drug delivery and biomedical applications, *Drug Discov Today* **2001, 6, 427-436.**

86. McCarthy T. D., Karellas P., Henderson S. A., et.al Dendrimers as drugs: discovery and preclinical and clinical development of dendrimer-based microbicides for HIV and STI prevention, *Mol Pharm* **2005, 2, 312-8.**

87. Venditto V. J.; Regino C. A.; Brechbiel M. W. PAMAM dendrimer based macromolecules as improved contrast agents, *Mol Pharm* **2005, 2, 302-11.**

88. Ambade A. V.; Savariar E. N. Thayumanavan S. Dendrimeric micelles for controlled drug release and targeted delivery, *Mol Pharm* **2005, 2, 264-72.**

89. Majoros I. J.; Thomas T. P.; Mehta C. B.; Baker Jr J. R. Poly(amidoamine) dendrimer-based multifunctional engineered nanodevice for cancer therapy, *J Med Chem* **2005, 48, 5892-9.**

90. Boas U.; Heegaard P. M. Dendrimers in drug research, *Chem Soc Rev* **2004, 33, 43-63.**

91. Klajnert B.; Bryszewska M. Dendrimers: properties and applications, *Acta Biochim Pol* **2001, 48, 199-208.**

92. Yang, W.; Barth, R. F.; Wu, G.; et.al Boronated epidermal growth factor as a delivery agent for neutron capture therapy of EGF receptor positive gliomas, *Appl Radiat Isot* **2004, 61, 981-5.**

93. Reddy, J. A.; Allagadda, V. M.; Leamon, C. P. Targeting therapeutic and imaging agents to folate receptor positive tumors, *Curr Pharm Biotechnol* **2005,**

6, 131-50.

94. Leamon, C. P.; Reddy, J. A. Folate-targeted chemotherapy, *Adv Drug Deliv Rev* **2004, 56, 1127-41.**

95. Sudimack, J.; Lee, R. J. Targeted drug delivery via the folate receptor, *Adv Drug Deliv Rev* **2000, 41, 147-62.**

96. Paulos, C. M.; Reddy, J. A.; Leamon, C. P.; et al. Ligand binding and kinetics of folate receptor recycling in vivo: impact on receptor-mediated drug delivery, *Mol Pharmacol* **2004, 66, 1406-14.**

97. Shukla S.; Wu G.; Chatterjee M.; et.al Synthesis and biological evaluation of folate receptor-targeted boronated PAMAM dendrimers as potential agents for neutron capture therapy, *Bioconjug Chem* **2003, 14, 158-67.**

98. Parrott, M. C.; Marchington, E. B.; Valliant, J. F.; Adronov, A. Synthesis and properties of carborane-functionalized aliphatic polyester dendrimers, *J Am Chem Soc* **2005, 127, 12081-9.**

99. Park, J. W.; Benz, C. C.; Martin, F. J. Future directions of liposome- and immunoliposome-based cancer therapeutics, *Semin Oncol* **2004, 31, 196-205.**

100. K Shelly, D A Feakes, M F Hawthorne, et al. Model studies directed toward the boron neutron-capture therapy of cancer: boron delivery to murine tumors with liposomes, *Proc Natl Acad Sci USA.* **Oct 1, 1992; 89(19): 9039–9043**

101. Yanagie H, Maruyama K, Takizawa T, et al. Application of boron-entrapped stealth liposomes to inhibition of growth of tumour cells in the in vivo boron neutron-capture therapy model, *Biomed Pharmacother.* **2006 Jan; 60(1): 43-50.**

102. Carlsson J., Kullberg E.B., Capala J., et al., Ligand liposomes and boron neutron capture therapy, *J. Neurooncol.* **2003, 62, 47-59.**

103. Justus E., Awad D., Hohnholt M., et al., Synthesis, liposomal preparation, and in vitro toxicity of two novel dodecaborate cluster lipids for boron neutron capture therapy, *Bioconjug. Chem.,* **2007, 18(4), 1287.**

104. Feakes DA, Shelly K, Hawthorne M. Selective boron delivery to murine tumors by lipophilic species incorporated in the membranes of unilamellar liposomes, *Proc Natl Acad Sci USA* **1995; 92: 1367–70.**

105. Pardridge WM. Drug delivery to the brain, *J Cereb Blood Flow Metab* **1997; 17: 713–31.**

106. Sapra, P.; Allen, T. M. Ligand-targeted liposomal anticancer drugs, *Prog Lipid Res* **2003, 42, 439-62.**

107. Hawthorne, M. F.; Shelly, K. Liposomes as drug delivery vehicles for boron agents, *J Neurooncol* **1997, 33, 53-8.**

108. Pavanetto, F.; Perugini, P.; Genta, I.; et.al Boron-loaded liposomes in the treatment of hepatic metastases: preliminary investigation by autoradiography analysis, *Drug Deliv* **2000, 7, 97-103.**

109. Ji B.; Chen W.; Lu D.R.; Halpern D.S. Cell culture and animal studies for intracerebral delivery of borocaptate in liposomal formulation, *Drug Deliv* **2001, 8, 13-7.**

110. Valliant, J. F.; Guenther, K. J.; King, A. S.; et.al The medicinal chemistry of carboranes, *Coordination Chemistry Reviews* **2002, 232, 173-230.**

111. Yanagie, H.; Fujii, Y.; Takahashi, T.; et.al Boron neutron capture therapy using 10B entrapped anti-CEA immunoliposome, *Hum Cell* **1989, 2, 290-6.**

112. Yanagie, H.; Tomita, T.; Kobayashi, H.; et.al Application of boronated anti-CEA immunoliposome to tumour cell growth inhibition in in vitro boron neutron capture therapy model, **Br J Cancer 1991, 63, 522-6.**

113. Wei, Q.; Kullberg, E. B.; Gedda, L. Trastuzumab-conjugated boron-containing liposomes for tumor-cell targeting; development and cellular studies, *Int J Oncol* **2003, 23, 1159-65**

114. Kullberg B, E.; Bergstrand, N.; Carlsson, J.; et.al Development of EGF-conjugated liposomes for targeted delivery of boronated DNA-binding agents, *Bioconjug Chem* **2002, 13, 737-43.**

115. Kullberg B, E.; Carlsson, J.; Edwards, K.; et al. Introductory experiments on ligand liposomes as delivery agents for boron neutron capture therapy, *Int J Oncol* **2003, 23, 461-7**

116. Kullberg EB, Wei Q, Capala J, et al. EGF-receptor targeted liposomes with boronated acridine: growth inhibition of cultured glioma cells after neutron irradiation, *Int J Radiat Biol.* **2005 Aug;81(8):621-9.**

117. Sudimack, J. J.; Adams, D.; Rotaru, J.; et.al Folate receptor-mediated liposomal delivery of a lipophilic boron agent to tumor cells in vitro for neutron capture therapy, *Pharm Res* **2002, 19, 1502-8.**

118. Chau, Y.; Tan, F. E.; Langer, R. Synthesis and characterization of dextran-peptide-methotrexate conjugates for tumor targeting via mediation by matrix metalloproteinase II and matrix metalloproteinase IX - *Bioconjug Chem* **2004, 15, 931-41.**

119. Zhang, X.; Mehvar, R. Dextran-methylprednisolone succinate as a prodrug of methylprednisolone: plasma and tissue disposition, *J Pharm Sci* **2001, 90, 2078-87.**

120. Larsson, B.; Gabel, D.; Borner, H. G. Boron-loaded macromolecules in experimental physiology: tracing by neutron capture radiography, *Phys Med Biol* **1984, 29, 361-70.**

121. Gabel, D.; Walczyna, R. B-Decachloro-o-carborane Derivatives suitable for the preparation of boron-labeled biological macromolecules, *Z Naturforsch C* **1982, 37, 1038-9.**

122. Pettersson, M. L.; Courel, M. N.; Girard, N.; et al. In vitro immunological activity of a dextran-boronated monoclonal antibody, *Strahlenther Onkol* **1989, 165, 151-2.**

123. Ujeno Y.; Akaboshi M.; Maki H. et.al The enhancement of thermal-neutron induced cell death by 10-boron dextran, *Strahlenther Onkol* **1989, 165, 201-3.**

124. Pettersson, M. L.; Courel, M. N.; Girard, N.; et.al Immunoreactivity of boronated antibodies, *J Immunol Methods* **1990, 126, 95-102.**

125. Suzuki M, Masunaga S, Kinashi Y, et al. Intra-arterial administration of sodium borocaptate (BSH)/lipiodol emulsion delivers B-10 to liver tumors highly selectively for boron neutron capture therapy: experimental studies in the rat liver model, *Int J Radiat Oncol Biol Phys.* **2004 May 1; 59(1): 260-6.**

126. Ferro, V. A.; Morris, J. H.; Stimson, W. H. A novel method for boronating antibodies without loss of immunoreactivity, for use in neutron capture therapy, *Drug Des Discov* **1995, 13, 13-25.**

127. Thomas, J.; Hawthorne, M. F. Dodeca(carboranyl)-substituted closomers: toward unimolecular nanoparticles as delivery vehicles for BNCT, *Chem Commun (Camb)* **2001, 1884-5.**

128. Alam, F.; Soloway, A. H.; Barth, R. F.; et al. Boron neutron capture therapy: linkage of a boronated macromolecule to monoclonal antibodies directed against tumor-associated antigens, *J Med Chem* **1989, 32, 2326-30.**