Boron Neutron Capture Therapy of Cancer: Current Status and Future Prospects

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Abstract **Background:** Boron neutron capture therapy (BNCT) is based on the nuclear reaction that occurs when boron-10 is irradiated with low-energy thermal neutrons to yield high linear energy transfer α particles and recoiling lithium-7 nuclei. Clinical interest in BNCT has focused primarily on the treatment of high-grade gliomas and either cutaneous primaries or cerebral metastases of melanoma, most recently, head and neck and liver cancer. Neutron sources for BNCT currently are limited to nuclear reactors and these are available in the United States, Japan, several European countries, and Argentina. Accelerators also can be used to produce epithermal neutrons and these are being developed in several countries, but none are currently being used for BNCT.

Boron Delivery Agents: Two boron drugs have been used clinically, sodium borocaptate ($Na_2B_{12}H_{11}SH$) and a dihydroxyboryl derivative of phenylalanine called boronophenylalanine. The major challenge in the development of boron delivery agents has been the requirement for selective tumor targeting to achieve boron concentrations ($\sim$20 μg/g tumor) sufficient to deliver therapeutic doses of radiation to the tumor with minimal normal tissue toxicity. Over the past 20 years, other classes of boron-containing compounds have been designed and synthesized that include boron-containing amino acids, biochemical precursors of nucleic acids, DNA-binding molecules, and porphyrin derivatives. High molecular weight delivery agents include monoclonal antibodies and their fragments, which can recognize a tumor-associated epitope, such as epidermal growth factor, and liposomes. However, it is unlikely that any single agent will target all or even most of the tumor cells, and most likely, combinations of agents will be required and their delivery will have to be optimized.

Clinical Trials: Current or recently completed clinical trials have been carried out in Japan, Europe, and the United States. The vast majority of patients have had high-grade gliomas. Treatment has consisted first of "debulking" surgery to remove as much of the tumor as possible, followed by BNCT at varying times after surgery. Sodium borocaptate and boronophenylalanine administered i.v have been used as the boron delivery agents. The best survival data from these studies are at least comparable with those obtained by current standard therapy for glioblastoma multiforme, and the safety of the procedure has been established.

Conclusions: Critical issues that must be addressed include the need for more selective and effective boron delivery agents, the development of methods to provide semiquantitative estimates of tumor boron content before treatment, improvements in clinical implementation of BNCT, and a need for randomized clinical trials with an unequivocal demonstration of therapeutic efficacy. If these issues are adequately addressed, then BNCT could move forward as a treatment modality.

High-grade gliomas, and specifically glioblastoma multiforme, are still extremely resistant to all current forms of therapy, including surgery, chemotherapy, radiotherapy, immunotherapy, and gene therapy, after decades of intensive research (1–5). Despite aggressive treatment using combinations of therapeutic modalities, the 5-year survival rate of patients diagnosed with glioblastoma multiforme in the United States is less than a few percent (6, 7). By the time they have had surgical resection of their tumors, malignant cells have infiltrated beyond the margins of resection and have spread into both gray matter and white matter (8, 9). As a result, high-grade supratentorial gliomas must be regarded as whole-brain diseases (10). Glioma cells and their neoplastic precursors have biochemical properties that allow them to invade the unique extracellular environment of the brain (11, 12) and biological properties that allow them to evade a tumor associated host immune response (13). The inability of chemotherapy and radiotherapy to cure patients with high-grade gliomas is due to their failure to eradicate microinvasive tumor cells within the brain. Recent molecular genetic studies of glioma suggest that it may be much

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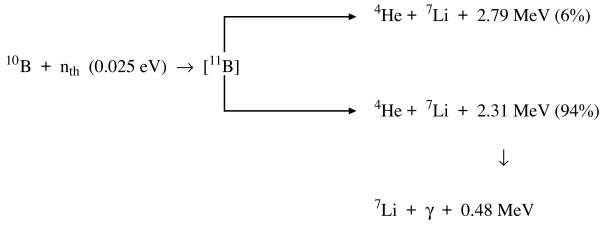
more complicated than this (14). The challenge facing us is to develop molecular strategies that can selectively target malignant cells, with little or no effect on normal cells and tissues adjacent to the tumor.

In theory, boron neutron capture therapy (BNCT) provides a way to selectively destroy malignant cells and spare normal cells. It is based on the nuclear capture and fission reactions that occur when boron-10 (^{10}B), which is a nonradioactive constituent of natural elemental boron, is irradiated with low-energy thermal neutrons to yield high linear energy transfer (LET) α particles (^{4}He) and recoiling lithium-7 (^{7}Li) nuclei:



In order for BNCT to be successful, a sufficient amount of ^{10}B must be selectively delivered to the tumor (~20 μg/g or ~10^9 atoms/cell), and enough thermal neutrons must be absorbed by them to sustain a lethal $^{10}B(n,\alpha)^{7}Li$ capture reaction. Because the high LET particles have limited path lengths in tissue (5-9 μm), the destructive effects of these high-energy particles is limited to boron containing cells. Clinical interest in BNCT has focused on the treatment of high-grade gliomas (15) and either cerebral metastases (16) or cutaneous primaries of melanoma (17). Most recently it has extended to head and neck and liver cancer. Since BNCT is a biologically rather than physically targeted type of radiation treatment, the potential exists to destroy tumor cells dispersed in the normal tissue parenchyma, if sufficient amounts of ^{10}B and thermal neutrons are delivered to the target volume, the potential exists to destroy tumor cells dispersed in the normal tissue parenchyma. This review will cover radiobiological considerations on which BNCT is based, boron agents and optimization of their delivery, neutron sources, which at this time are exclusively nuclear reactors, past and ongoing clinical studies, and critical issues that must be addressed if BNCT is to be successful. Readers interested in more in-depth coverage of these and other topics related to BNCT are referred to several recent reviews and monographs (15, 18–21).

Radiobiological Considerations

Types of radiation delivered. The radiation doses delivered to tumor and normal tissues during BNCT are due to energy deposition from three types of directly ionizing radiation that differ in their LET characteristics: (*a*) low LET γ rays, resulting primarily from the capture of thermal neutrons by normal tissue hydrogen atoms [$^{1}H(n,\gamma)^{2}H$]; (*b*) high LET protons, produced by the scattering of fast neutrons and from the capture of thermal neutrons by nitrogen atoms [$^{14}N(n,p)^{14}C$]; and (*c*) high LET, heavier-charged α particles (stripped-down ^{4}He nuclei) and ^{7}Li ions, released as products of the thermal neutron capture and fission reactions with ^{10}B [$^{10}B(n,\alpha)^{7}Li$]. The greater density of ionizations along tracks of high LET particles results in an increased biological effect compared with the same physical dose of low LET radiation. Usually, this is called relative biological effectiveness (RBE), which is the ratio of the absorbed dose of a reference source of radiation (e.g., X-rays) to that of the test radiation that produces the same biological effect. Because both tumor and surrounding normal tissues are present in the radiation field, there will be an unavoidable, nonspecific background dose, consisting of both high and low LET radiation even with an ideal epithermal neutron beam. However, a greater concentration of ^{10}B in the tumor will result in it receiving a higher total dose than that of adjacent normal tissues. This is the basis for the therapeutic gain in BNCT. As reviewed recently by one of us (18), the total radiation dose delivered to any tissue can be expressed in photon equivalent units as the sum of each of the high LET dose components multiplied by weighting factors, which depend on the increased radiobiological effectiveness of each of these components.

Biological effectiveness factors. The dependence of the biological effect on the microdistribution of ^{10}B requires the use of a more appropriate term than RBE to define the biological effects of the $^{10}B(n,\alpha)^{7}Li$ reaction. Measured biological effectiveness factors for the components of the dose from this reaction have been termed compound biological effectiveness (CBE) factors and are drug dependent (21–23). The mode and route of drug administration, the boron distribution within the tumor, normal tissues, and even more specifically within cells, and even the size of the nucleus within the target cell population all can influence the experimental determination of the CBE factor. CBE factors, therefore, are fundamentally different from the classically defined RBE, which primarily is dependent on the quality (i.e., LET) of the radiation administered. CBE factors are strongly influenced by the distribution of the specific boron delivery agent and can differ substantially, although they all describe the combined effects of α particles and ^{7}Li ions. The CBE factors for the boron component of the dose are specific for both the ^{10}B delivery agent and the tissue. A weighted Gy unit [Gy(w)] has been used to express the summation of all BNCT dose components and indicates that the appropriate RBE and CBE factors have been applied to the high LET dose components. However, for clinical BNCT, the overall calculation of photon equivalent [Gy(w)] doses requires several assumptions about RBEs, CBE factors, and the boron concentrations in various tissues, based on currently available human or experimental data (24, 25).

Table 1. Assumptions used in the clinical trials of BPA-based BNCT for calculation of the $^{10}B(n,\alpha)^{7}Li$ component of the Gy(w) dose in various tissues

Tissue	Boron concentration*	CBE factor
Blood	Measured directly	
Brain	1.0 times blood (27, 28)	1.3 (23)
Scalp/skin	1.5 times blood (26-28)	2.5 (26)
Tumor	3.5 times blood (162)	3.8 (21)

*A RBE of 3.2 is used for the high LET component of the beam dose: protons from the $^{14}N(n,n)^{14}C$ reaction and the recoil protons from fast neutron collisions with hydrogen. Literature references are given in parentheses.

Clinical dosimetry. The following biological weighting factors, summarized in Table 1, have been used in all of the recent clinical trials in patients with high-grade glioma using boronophenylalanine (BPA) in combination with an epithermal neutron beam. The $^{10}B(n,\alpha)^{7}Li$ component of the radiation dose to the scalp has been based on the measured boron concentration in the blood at the time of BNCT, assuming a blood/scalp boron concentration ratio of 1.5:1 (26–28) and a CBE factor for BPA in skin of 2.5 (26). A RBE of 3.2 has been used in all tissues for the high LET components of the beam: protons resulting from the capture reaction with nitrogen and recoil protons resulting from the collision of fast neutrons with hydrogen (27–29). It must be emphasized that in order to use the experimentally derived values for estimation of Gy(w) doses in clinical radiations, the tissue distribution of the boron delivery agent in humans should be similar to that in the experimental animal model.

Dose calculations become much more complicated when combinations of agents are used. At its simplest, this could be the two low molecular weight drugs BPA and sodium borocaptate (BSH; $Na_2B_{12}H_{11}SH$). These have been shown to be highly effective when used in combination to treat F98 glioma-bearing rats (30, 31) and currently are being used in combination in a clinical study in Japan (32). Because it is impossible to know the true biodistribution of each drug, dosimetric calculations in experimental animals have been based on independent boron determinations in other tumor-bearing animals that have received the same doses of drugs but not BNCT. More recently, the radiation delivered has been expressed as a physical dose rather than using CBE factors to calculate a RBE equivalent dose (33). The calculations are further complicated if low and high molecular weight delivery agents are used in combination with one another. Tumor radiation dose calculations, therefore, are based on multiple assumptions regarding boron biodistribution, which may vary from patient to patient as well as within different regions of the tumor and among tumor cells. However, normal brain boron concentrations are much more predictable and uniform; therefore, it is both *safe* and *reliable* to base dose calculations on normal brain tolerance.

Boron Delivery Agents

General requirements. The development of boron delivery agents for BNCT began ~50 years ago and is an ongoing and difficult task of the highest priority. The most important requirements for a successful boron delivery agent are as follows: (*a*) low systemic toxicity and normal tissue uptake with high tumor uptake and concomitantly high tumor/brain and tumor/blood concentration ratios (>3-4:1); (*b*) tumor concentrations of ~20 μg ^{10}B/g tumor; (*c*) rapid clearance from blood and normal tissues and persistence in tumor during BNCT. However, it should be noted that at this time *no* single boron delivery agent fulfills all of these criteria. With the development of new chemical synthetic techniques and increased knowledge of the biological and biochemical requirements needed for an effective agent and their modes of delivery, several promising new boron agents have emerged (see examples in Fig. 1). The major challenge in their development has been the requirement for selective tumor targeting to achieve boron concentrations sufficient to deliver therapeutic doses of radiation to the tumor with minimal normal tissue toxicity. The selective destruction of glioblastoma multiforme cells in the presence of normal cells represents an even greater challenge compared with malignancies at other anatomic sites, because high-grade gliomas are highly infiltrative of normal brain, histologically complex, and heterogeneous in their cellular composition.

First-generation and second-generation boron delivery agents. The clinical trials of BNCT in the 1950s and early 1960s used boric acid and some of its derivatives as delivery agents, but these simple chemical compounds were nonselective, had poor tumor retention, and attained low tumor/brain ratios (34, 35). In the 1960s, two other boron compounds emerged from investigations of hundreds of low molecular weight boron-containing chemicals, one [(*L*)-4-dihydroxy-borylphenylalanine] called BPA (**compound 1**) was based on arylboronic acids (36) and the other was based on a newly discovered polyhedral borane anion, sodium mercaptoundecahydro-*closo*-dodecaborate (37), called BSH (**compound 2**). These second-generation compounds had low toxicity, persisted longer in animal tumors compared with related molecules, and had tumor/brain and tumor/blood boron ratios of >1. As will be described later in this review, ^{10}B-enriched BSH and BPA, complexed with fructose to improve its water solubility, have been used clinically in Japan, the United States, Europe, and Argentina. Although these drugs are not ideal, their safety following i.v. administration has been established. Over the past 20 years, several other classes of boron-containing compounds have been designed and synthesized to fulfill the requirements indicated at the beginning of this section. Detailed reviews of the state-of-the-art in compound development for BNCT have been published (38–41), and in this overview, we will only summarize the main classes of compounds, with an emphasis on recently published work in the area, and we will discuss the general biochemical requirements for an effective boron delivery agent.

Third-generation boron delivery agents. So-called third-generation compounds mainly consist of a stable boron group or cluster attached via a hydrolytically stable linkage to a tumor-targeting moiety, such as low molecular weight biomolecules or monoclonal antibodies (mAb). For example, the targeting of the epidermal growth factor (EGF) receptor (EGFR) and its mutant isoform EGFRvIII, which are overexpressed in gliomas as well as in squamous cell carcinomas of the head and neck, also has been one such approach (42). Usually, the low molecular weight biomolecules have been shown to have selective targeting properties and many are at various stages of development for cancer chemotherapy, photodynamic therapy, or antiviral therapy. The tumor cell nucleus and DNA are especially attractive targets because the amount of boron required to produce a lethal effect may be substantially reduced if it is localized within or near the nucleus (43). Other potential subcellular targets are mitochondria, lysosomes, endoplasmic reticulum, and Golgi apparatus. Water solubility is an important factor for a boron agent that is to be administered systemically, whereas lipophilicity is necessary for it to cross the blood-brain barrier (BBB) and diffuse within the brain and the tumor. Therefore, amphiphilic compounds possessing a suitable balance between hydrophilicity and lipophilicity have been of primary interest because they should provide the most favorable differential boron

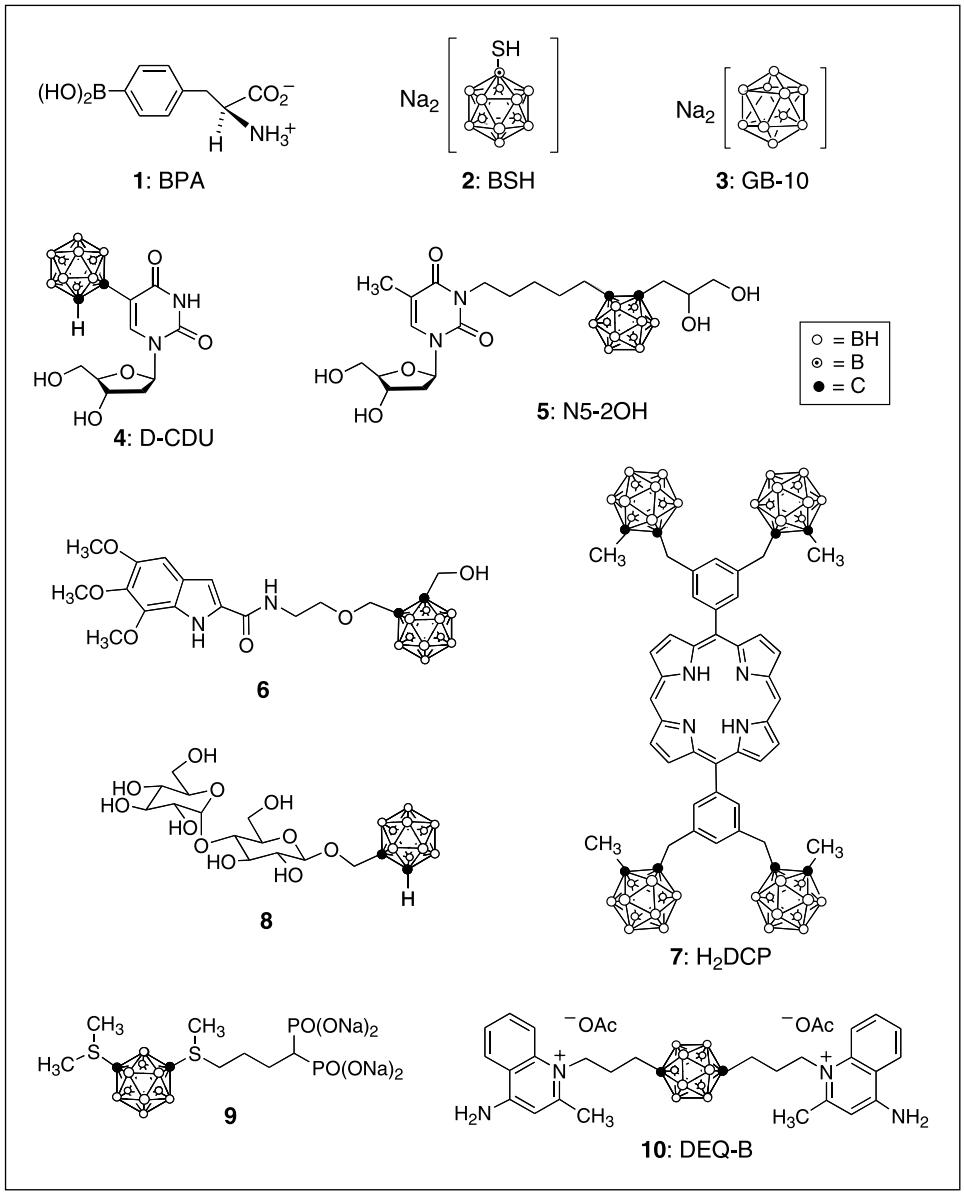


Fig. 1. Some low molecular weight BNCT agents under investigation. BPA (**compound 1**) and BSH (**compound 2**) are currently in clinical use. GB-10 (**compound 3**) has shown promise in animal models, as have the nucleoside derivatives β-5-*o*-carboranyl-2′-deoxyuridine (*D-CDU*; **compound 4**) and N5-2OH (**compound 5**). **Compound 6**, a trimethoxyindole derivative, has shown promise *in vitro* and **compound 7**, H_2DCP, a porphyrin derivative, was shown to be tumor selective. The maltose derivative **compound 8** has shown low cytotoxicity and tumor cell uptake *in vitro*, the biphosphonate **compound 9** has tumor-targeting ability, and the dequalinium derivative dequalinium-B (*DEQ-B*; **compound 10**) has shown promise in *in vitro* studies.

concentrations between tumor and normal brain, thereby enhancing tumor specificity. However, for low molecular weight molecules that target specific biological transport systems and/or are incorporated into a delivery vehicle, such as liposomes, the amphiphilic character is not as crucial. The molecular weight of the boron-containing delivery agent also is an important factor, because it determines the rate of diffusion within both the brain and the tumor.

Low Molecular Weight Agents

Boron-containing amino acids and polyhedral boranes. Recognizing that BPA and BSH are not ideal boron delivery agents, considerable effort has been directed toward the design and synthesis of third-generation compounds based on boron-containing amino acids and functionalized polyhedral borane clusters. Examples include various derivatives of BPA and other boron-containing amino acids, such as glycine, alanine, aspartic acid, tyrosine, cysteine, and methionine, as well as non-naturally occurring amino acids (44–49). The most recently reported delivery agents contain one or more boron clusters and concomitantly larger amounts of boron by weight compared with BPA. The advantages of such compounds are that they potentially can deliver higher concentrations of boron to tumors without increased toxicity. The polyhedral borane dianions *closo*-$B_{10}H_{10}^{2-}$ and *closo*-$B_{12}H_{12}^{2-}$ and the icosahedral carboranes *closo*-$C_2B_{10}H_{12}$ and *nido*-$C_2B_9H_{12}^{-}$ have been the most attractive boron clusters for linkage to targeting moieties due to their ready incorporation into organic molecules, high boron content, chemical and hydrolytic stability, hydrophobic character, and, in most cases, their negative charge. The simple sodium salt of *closo*-$B_{10}H_{10}^{2-}$ (GB-10, **compound 3**) has been shown to have tumor-targeting ability and low systemic toxicity in animal models (41) and has been considered as a candidate for clinical evaluation (50). Other polyhedral borane anions with high boron content include derivatives of $B_{20}H_{18}^{2-}$, although these compounds have shown little tumor specificity and therefore may be better candidates for encapsulation into

either targeted or nontargeted liposomes (51, 52). Boron-containing dipeptides also have shown low toxicity and good tumor-localizing properties (53, 54).

Biochemical precursors and DNA-binding agents. Several boron-containing analogues of the biochemical precursors of nucleic acids, including purines, pyrimidines, nucleosides, and nucleotides, have been synthesized and evaluated in cellular and animal studies (55–58). Some of these compounds, such as β-5-*o*-carboranyl-2′-deoxyuridine (**compound 4**) and the 3-(dihydroxypropyl-carboranyl-pentyl)thymidine derivative N5-2OH (**compound 5**), have shown low toxicities, selective tumor cell uptake, and significant rates of phosphorylation into the corresponding nucleotides (59, 60). Intracellular nucleotide formation potentially can lead to enhanced tumor uptake and retention of these types of compounds (59).

Another class of low molecular weight delivery agents are boron-containing DNA-binding molecules, such as alkylating agents, intercalators, groove binders, and polyamines. Some examples are derivatives of aziridines, acridines, phenanthridines, trimethoxyindoles (**compound 6**), carboranylpolyamines, Pt(II)-amine complexes, dibenzimidazoles, and tribenzimidazoles (61–64). A limitation of boron-containing polyamines is their frequently observed *in vitro* and *in vivo* toxicity, although promising derivatives with low cytotoxicity have been synthesized recently (65–68). Other nuclear-targeting molecules are *nido*-carboranyl oligomeric phosphate diesters. Despite their multiple negative charges, oligomeric phosphate diesters have been shown to target the nuclei of TC7 cells following microinjection (69), suggesting that the combination of oligomeric phosphate diesters with a cell-targeting molecule capable of crossing the plasma membrane could provide both selectivity and nuclear binding. Such a conjugate recently has been designed and synthesized (70), although its biological evaluation has yet to be reported.

Boron-containing porphyrins and related structures. Several boron-containing fluorescent dyes, including acridines, phenanthridines, porphyrins, and phthalocyanine derivatives, have been synthesized and evaluated (71–73). These have the advantage of being easily detected and quantified by fluorescence microscopy and have the potential for interacting with DNA due to their planar aromatic structures. Among these macrocycles, boron-containing porphyrins [e.g., H_2DCP (**compound 7**)] have attracted special attention due to their low systemic toxicity compared with other dyes, easy synthesis with high boron content, and their remarkable stability (74–77). Porphyrin derivatives have been synthesized that contain up to 44% boron by weight by way of *closo*-carborane or *nido*-carborane clusters linked to the porphyrin macrocycle via ester, amide, ether, methylene, or aromatic linkages (71–73). The nature of these linkages is believed to influence their stability and systemic toxicity. Boron-containing porphyrins have excellent tumor-localizing properties (71–78) and have been proposed for dual application as boron delivery agents and photosensitizers for photodynamic therapy (79–81) of brain tumors. Despite the bulkiness of the carborane cages, carboranylporphyrins have been shown to interact with DNA and thereby produce *in vitro* DNA damage following light activation (82, 83). A few boronated phthalocyanines also have been synthesized, although these compounds usually have had decreased water solubility and an increased tendency to aggregate compared with the corresponding porphyrins (71, 72, 78). Boron-containing acridine molecules also have been reported to selectively deliver boron to tumors with high tumor/brain and tumor/blood ratios, whereas phenanthridine derivatives were found to have poor specificity for tumor cells (84–86).

Other low molecular weight boron delivery agents. Carbohydrate derivatives of BSH and other boron-containing glucose, mannose, ribose, gulose, fucose, galactose, maltose (**compound 8**), and lactose molecules have been synthesized and some of these compounds have been evaluated in both *in vitro* and *in vivo* studies (87–93). These compounds usually are highly water soluble, and as a possible consequence of this, they have shown both low toxicity and uptake in tumor cells. It has been suggested that their hydrophilic low molecular weight derivatives have poor ability to cross tumor cell membranes. However, they might selectively accumulate within the glycerophospholipid membrane bilayer and in other areas of the tumor, such as the vasculature.

Low molecular weight boron-containing receptor-binding molecules have been designed and synthesized. These have been mainly steroid hormone antagonists, such as derivatives of tamoxifen, 17β-estradiol, cholesterol, and retinoic acid (94–98). The biological properties of these agents depend on the density of the targeted receptor sites, although to date very little biological data have been reported. Other low molecular weight boron-containing compounds under development include phosphates, phosphonates (**compound 9**), phenylureas, thioureas, nitroimidazoles, amines, benzamides, isocyanates, nicotinamides, azulenes, and dequalinium derivatives (dequalinium-B, **compound 10**; refs. 40, 99–101). The use of *multiple* boron delivery agents is probably essential for targeting different subpopulations of tumor cells and subcellular sites. Furthermore, lower doses of each individual drug would be needed, which could reduce systemic toxicity while at the same time enhance tumor boron levels to achieve a therapeutic effect.

High Molecular Weight Agents

Monoclonal antibodies, other receptor-targeting agents, and liposomes. High molecular weight delivery agents, such as mAbs and their fragments, which can recognize a tumor-associated epitope, have been (102–104) and continue to be of interest to us (105, 106) as boron delivery agents. Although they can be highly specific, only very small quantities reach the brain and tumor following systemic administration (107) due to their rapid clearance by the reticuloendothelial system and the BBB, which effectively limits their ability to cross capillary vascular endothelial cells. Boron-containing bioconjugates of EGF (108, 109), the receptor that is overexpressed on a variety of tumors, including glioblastoma multiforme (110, 111), also have been investigated as potential delivery agents to target brain tumors. However, it is unlikely that either boronated antibodies or other bioconjugates would attain sufficiently high concentrations in the brain following systemic administration, but, as described later in this section, direct i.c. delivery could solve this problem. Another approach would be to directly target the vascular endothelium of brain tumors using either boronated mAbs or vascular endothelial growth factor, which would recognize a tumor-associated or amplified vascular endothelial epitope (112). This would obviate the problems of passage of the bioconjugate across the BBB but most likely would require repeated applications of BNCT. There also has

been a longstanding interest on the use of boron-containing liposomes as delivery agents (113, 114), but their size has limited their usefulness as brain tumor–targeting agents, because they are incapable of transversing the BBB unless they have diameters of <50 nm (115). If, on the other hand, they were administered i.c. or were linked to an actively transported carrier molecule, such as transferrin, or if the BBB was transiently opened, these could be very useful delivery agents, especially for extracranial tumors, such as liver cancer.

Recent work of one of us (R.F.B.) has focused on the use of a chimeric mAb, cetuximab (IMC-C225, also known as Erbitux), produced by ImClone Systems, Inc., (New York, NY). This antibody recognizes both wild-type EGFR and its mutant isoform, EGFRvIII (116), and has been approved for clinical use by the U.S. Food and Drug Administration for the treatment of EGFR-positive recurrent colon cancer (117). Using previously developed methodology (102), a precision macromolecule, a polyamido amino (PAMAM or "starburst") dendrimer, has been heavily boronated and then linked by means of heterobifunctional reagents to EGF (109), cetuximab (106), or another mAb, L8A4, which is specifically directed against EGFRvIII (118). To completely bypass the BBB, the bioconjugates were administered by either direct i.t. injection (119) or convection enhanced delivery (120) to rats bearing i.c. implants of the F98 glioma that had been genetically engineered to express either wild-type EGFR (119) or EGFRvIII (121). Administration by either of these methods resulted in tumor boron concentrations that were in the therapeutic range (i.e., ~20 μg/g wet weight tumor). Similar data also were obtained using boronated EGF, and based on the favorable uptake of these bioconjugates, therapy studies were initiated at the Massachusetts Institute of Technology reactor (MITR). The mean survival times (MST) of animals that received either boronated cetuximab (122) or EGF (123) were significantly prolonged compared with those of animals bearing receptor-negative tumors. A further improvement in MSTs was seen if the animals received BPA, administered i.v. in combination with the boronated bioconjugates, thereby validating our thesis that combinations of agents may be superior to any single agent (31). As can be seen from the preceding discussion, the design and synthesis of low and high molecular weight boron agents have been the subject of intensive investigation. However, optimization of their delivery has not received enough attention but nevertheless is of critical importance.

Optimizing Delivery of Boron-Containing Agents

General considerations. Delivery of boron agents to brain tumors is dependent on (*a*) the plasma concentration profile of the drug, which depends on the amount and route of administration; (*b*) the ability of the agent to traverse the BBB; (*c*) blood flow within the tumor; and (*d*) the lipophilicity of the drug. In general, a high steady-state blood concentration will maximize brain uptake, whereas rapid clearance will reduce it, except in intra-arterial drug administration. Although the i.v. route currently is being used clinically to administer both BSH and BPA, this may not be ideal and other strategies may be needed to improve their delivery. Delivery of boron-containing drugs to extracranial tumors, such as head and neck and liver cancer, presents a different set of problems, including nonspecific uptake and retention in adjacent normal tissues.

Intra-arterial administration with or without blood-brain barrier disruption. As shown in experimental animal studies (30, 31, 124–126), enhancing the delivery of BPA and BSH can have a dramatic effect on both increasing tumor boron uptake and the efficacy of BNCT. This has been shown in the F98 rat glioma model where i.c. injection of either BPA or BSH doubled the tumor boron uptake compared with that obtained by i.v. injection (30). This was increased 4-fold by disrupting the BBB by infusing a hyperosmotic (25%) solution of mannitol via the internal carotid artery. MSTs of animals that received either BPA or BSH i.c. with BBB-D were increased 295% and 117%, respectively, compared with irradiated controls (30). The best survival data were obtained using both BPA and BSH in combination administered by i.c. injection with BBB-D. The MST was 140 days with a cure rate of 25% compared with 41 days following i.v. injection with no long-term surviving animals (31). Similar data have been obtained using a rat model for melanoma metastatic to the brain. BPA was administered i.c. to nude rats bearing i.c. implants of the human MRA 27 melanoma with or without BBB-D. The MSTs were 104 to 115 days with 30% long-term survivors compared with a MST of 42 days following i.v. administration (124). A similar enhancement in tumor boron uptake and survival was observed in F98 glioma-bearing rats following i.c. infusion of the bradykinin agonist, receptor-mediated permeabilizer-7, now called Cereport (125). In contrast to the increased tumor uptake, normal brain boron values at 2.5 hours following i.c. injection were very similar for the i.v. and i.c. routes with or without BBB-D. Because BNCT is a binary system, normal brain boron levels only are of significance at the time of irradiation and high values at earlier time points are inconsequential. These studies have shown that a significant therapeutic gain can be achieved by optimizing boron drug delivery, and this should be important for both ongoing and future clinical trials using BPA and/or BSH.

Direct intracranial delivery. Different strategies may be required for other low molecular weight boron-containing compounds whose uptake is cell cycle dependent, such as boron-containing nucleosides, where continuous administration over a period of days may be required. We have reported recently that direct i.t. injection or convection enhanced delivery of the borononucleoside N5-2OH (**compound 5**) were both effective in selectively delivering potentially therapeutic amounts of boron to rats bearing i.c. implants of the F98 glioma (60). Direct i.t. injection or convection enhanced delivery most likely will be necessary for a variety of high molecular weight delivery agents, such as boronated mAbs (126), and ligands, such as EGF (120), as well as for low molecular weight agents, such as nucleosides and porphyrins. Recent studies have shown that convection enhanced delivery of a boronated porphyrin derivative similar to **compound 7**, designated H_2DCP, resulted in the highest tumor boron values and tumor/brain and tumor/blood ratios that we have seen with any of the boron agents that we have ever studied (127).

Neutron Sources for Boron Neutron Capture Therapy

Nuclear reactors. Neutron sources for BNCT currently are limited to nuclear reactors, and in the present section, we only will summarize information that is described in more detail in

a recently published review (128). Reactor-derived neutrons are classified according to their energies as thermal (E_n < 0.5 eV), epithermal (0.5 eV < E_n < 10 keV), or fast (E_n >10 keV). Thermal neutrons are the most important for BNCT because they initiate the $^{10}B(n,\alpha)^7Li$ capture reaction. However, because they have a limited depth of penetration, epithermal neutrons, which lose energy and fall into the thermal range as they penetrate tissues, are now preferred for clinical therapy. Several reactors with very good neutron beam quality have been developed and currently are being used clinically. These include (*a*) MITR, shown schematically in Fig. 2 (129); (*b*) the clinical reactor at Studsvik Medical AB in Sweden (130); (*c*) the FiR1 clinical reactor in Helsinki, Finland (131); (*d*) R2-0 High Flux Reactor at Petten in the Netherlands (132); (*e*) LVR-15 reactor at the Nuclear Research Institute in Rez, Czech Republic (133); (*f*) Kyoto University Research reactor in Kumatori, Japan (134); (*g*) JRR4 at the Japan Atomic Energy Research Institute (135); and (*h*) the RA-6 CNEA reactor in Bariloche, Argentina (136). Other reactor facilities are being designed, notably the TAPIRO reactor at the ENEA Casaccia Center near Rome, Italy, which is unique in that it will be a low-power fast-flux reactor (137), and facilities in South Korea and Beijing, China. Two reactors that have been used in the past for clinical BNCT are the Musashi Institute of Technology reactor in Japan and the Brookhaven Medical Research reactor at the Brookhaven National Laboratory (BNL) in Upton, Long Island, NY (27, 28, 138). The Musashi Institute of Technology was used by Hatanaka (139) and later by Hatanaka and Nakagawa (140). The Brookhaven Medical Research reactor was used for the clinical trial that was conducted at the BNL between 1994 and 1999 (141) and the results are described in detail later in this section. Due to a variety of reasons, including the cost of maintaining the Brookhaven Medical Research reactor, it has been deactivated and is no longer available for use.

Reactor modifications. Two approaches are being used to modify reactors for BNCT. The first or direct approach is to moderate and filter neutrons that are produced in the reactor core. The second, the fission converter plate approach, is indirect in that neutrons from the reactor core create fissions within a converter plate that is adjacent to the moderator assembly, and these produce a neutron beam at the patient port. The MITR (142), which uses a fission converter plate, currently sets the world standard for the combination of high neutron beam quality and short treatment time. It operates at a power of 5 MW and has been used for clinical and experimental studies for BNCT. Although the power is high compared with the majority of other reactors that are being used, the treatment time is unusually short, because it uses a fission converter plate to create the neutron beam. All other reactors use the direct approach to produce neutron beams for BNCT. Three examples are the FiR1 reactor in Finland (131), the Studsvik reactor in Sweden (130), and the Washington State University reactor in the United States (143), which was built for the treatment of both small and large experimental animals.

Accelerators. Accelerators also can be used to produce epithermal neutrons and accelerator-based neutron sources (ABNS) are being developed in several countries (144–150), and interested readers are referred to a recently published detailed review on this subject (151). For ABNS, one of the more promising nuclear reactions involves bombarding a ^7Li target with 2.5 MeV protons. The average energy of the neutrons that are produced is 0.4 MeV and the maximum energy is 0.8 MeV. Reactor-derived fission neutrons have greater average and maximum energies than those resulting from the $^7Li(p,n)^7Be$ reaction. Consequently, the thickness of the moderator material that is necessary to reduce the energy of the neutrons from the fast to the epithermal range is less for an ABNS than it is for a reactor. This is important because the probability that a neutron will be successfully transported from the entrance of the moderator assembly to the treatment port decreases as the moderator assembly thickness increases. Due to lower and less widely distributed neutron source energies, ABNS potentially can produce neutron beams with an energy distribution that is equal to or better than that of a reactor. However, reactor-derived neutrons can be well collimated, while in contrast it may not be possible to achieve good collimation of ABNS

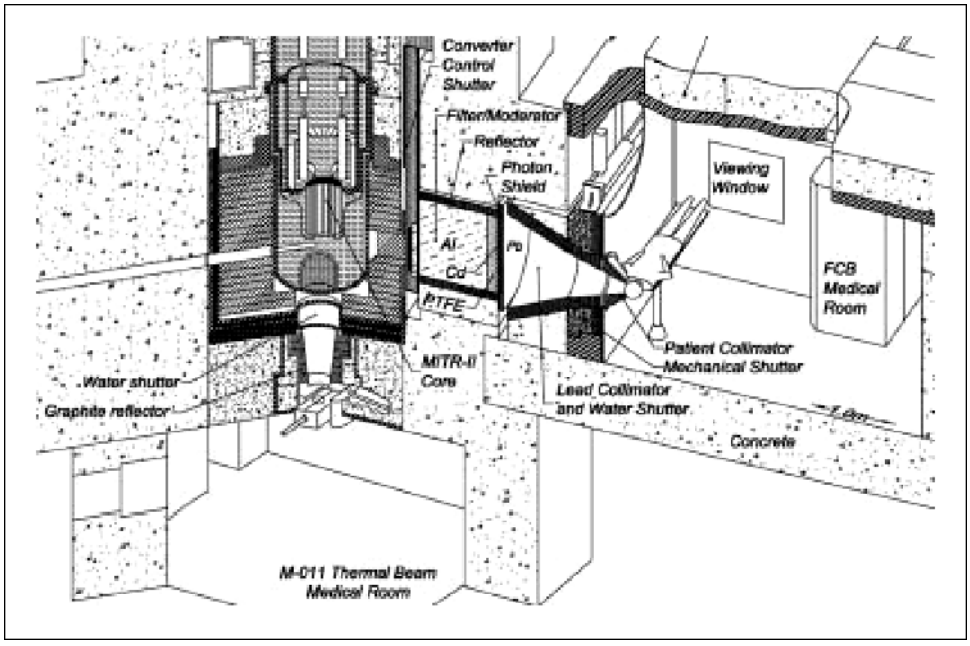


Fig. 2. Schematic diagram of the MITR. The fission converter–based epithermal neutron irradiation (*FCB*) facility is housed in the experimental hall of the MITR and operates in parallel with other user applications. The fission converter contains an array of 10 spent MITR-II fuel elements cooled by forced convection of heavy water coolant. A shielded horizontal beam line contains an aluminum and Teflon filter moderator to tailor the neutron energy spectrum into the desired epithermal energy range. A patient collimator defines the beam aperture and extends into the shielded medical room to provide circular apertures ranging from 16 to 8 cm in diameter. The in-air epithermal flux for the available field sizes ranges from 3.2 to 4.6 $\times$ 10^9 n/cm^2 s at the patient position. The measured specific absorbed doses are constant for all field sizes and are well below the inherent background of 2.8 $\times$ 10^{-12} C+γ(w) m^2/n produced by epithermal neutrons in tissue. The dose distributions achieved with the fission converter approach the theoretical optimum for BNCT.

neutrons at reasonable proton beam currents. The necessity of good collimation for the effective treatment of glioblastoma multiforme is an important and unresolved issue that may affect usefulness of ABNS for BNCT. ABNS also are compact enough to be sited in hospitals, thereby allowing for more effective but technically more complicated procedures to carry out BNCT. However, to date, no accelerator has been constructed with a beam quality comparable with that of the MITR, which can be sited in a hospital and that provides a current of sufficient magnitude to treat patients in <30 minutes. Furthermore, issues relating to target manufacture and cooling must be solved before ABNS can become a reality. Although progress has been slow the ABNS that is being developed at the University of Birmingham in England, by modifying a Dynamitron linear electrostatic accelerator (144), may be the first facility where patients will be treated. Another ABNS is being constructed by LINAC Systems, Inc., in Albuquerque, NM (152), and this could be easily sited in a hospital and produce an epithermal neutron beam.

Beam optimization. For both reactors and ABNS, a moderator assembly is necessary to reduce the energy of the neutrons to the epithermal range. The neutrons comprising the neutron beam have a distribution of energies and are accompanied by unwanted X-rays and γ photons. A basic tenet of BNCT is that the dose of neutrons delivered to the target volume should not exceed the tolerance of normal tissues, and this applies to neutron beam design as well as to treatment planning (25). The implication of this for beam design is that the negative consequences of increased normal tissue damage for more energetic neutron beams at shallow depths outweigh the benefits of more deeply penetrating energetic neutrons. For fission reactors, the average energy of the neutrons produced is ~2 MeV, but small numbers have energies as high as 10 MeV. There is generally a tradeoff between treatment time and the optimum beam for patient treatment in terms of the energy distribution of the neutrons and the contamination of the neutron beam with X-rays and γ photons. Not surprisingly, reactors with the shortest treatment time (i.e., the highest normal tissue dose rate) operate at the highest power, because the number of neutrons that is produced per unit time is proportional to the power, measured in MW. Furthermore, high beam quality is most easily achieved using reactors with high power, because a larger fraction of the neutrons can be filtered as the neutrons traverse the moderator assembly without making the treatment time exceedingly long.

Clinical Studies of Boron Neutron Capture Therapy for Brain Tumors

Early trials. Although the clinical potential of BNCT was recognized in the 1930s (153), it was not until the 1950s that the first clinical trials were initiated by Farr at the BNL (34, 35) and by Sweet and Brownell at the Massachusetts General Hospital using the MITR (35, 154, 155). The disappointing outcomes of these trials, which ended in 1961 and subsequently were carefully analyzed by Slatkin (156), were primarily attributable to (*a*) inadequate tumor specificity of the inorganic boron chemicals that had been used as capture agents, (*b*) insufficient tissue penetrating properties of the thermal neutron beams, and (*c*) high blood boron concentrations that resulted in excessive damage to normal brain vasculature and to the scalp (35, 154).

Japanese clinical trials. Clinical studies were resumed by Hatanaka in Japan in 1967 following a 2-year fellowship in Sweet's laboratory at the Massachusetts General Hospital using a thermal neutron beam and BSH, which had been developed as a boron delivery agent by Soloway at the Massachusetts General Hospital (37). In Hatanaka's procedure (139, 140), as much of the tumor as possible was surgically removed ("debulking"), and at some time thereafter, BSH (**compound 2**) was administered by a slow infusion, usually intra-arterially (139) but later i.v. (140). Twelve to 14 hours later, BNCT was carried out at one or another of several different nuclear reactors. Because thermal neutrons have a limited depth of penetration in tissue, this necessitated reflecting the skin and raising the bone flap to directly irradiate the exposed brain. This eliminated radiation damage to the scalp and permitted treatment of more deep-seated residual tumors. As the procedure evolved over time, a ping pong ball or silastic sphere was inserted into the resection cavity as a void space to improve neutron penetration into deeper regions of the tumor bed and adjacent brain (139, 140, 157, 158). This is a major difference between the procedure carried out by Hatanaka, Nakagawa, and other Japanese neurosurgeons and the BNCT protocols that have been carried out in the United States and Europe, which used epithermal neutron beams that have not required reflecting the scalp and raising the bone flap at the time of irradiation. This has made it difficult to directly compare the Japanese clinical results with those obtained elsewhere, and this has continued on until very recently when the Japanese started using epithermal neutron beams (32). Most recently, Miyatake et al. have initiated a clinical study using the combination of BSH and BPA, both of which were administered i.v. at 12 hours and 1 hour, respectively, before irradiation with an epithermal neutron beam (32). A series of 11 patients with high-grade gliomas have been treated, and irrespective of the initial tumor volume, magnetic resonance imaging and computed tomography images showed a 17% to 51% reduction in tumor volume and this reached a maximum of 30% to 88%. However, the survival times of these patients were not improved over historical controls and further studies are planned to improve the delivery of BPA and BSH, which may enhance survival.

Analysis of the Japanese clinical results. Retrospective analysis of subgroups of patients treated in Japan by Hatanaka and Nakagawa (157, 158) have described 2-, 5-, and 10-year survival rates (11.4%, 10.4%, and 5.7%, respectively) that were significantly better than those observed among patients treated with conventional, fractionated, external beam photon therapy. However, a cautionary note was sounded by Laramore et al. (159) who analyzed the survival data of a subset of 12 patients from the United States who had been treated by Hatanaka between 1987 and 1994. They concluded that there were no differences in their survival times compared with those of age-matched controls, analyzed according to the stratification criteria used by Curran et al. (6). In a recent review of Hatanaka's clinical studies, Nakagawa reported that the physical dose from the $^{10}B(n,\alpha)^{7}Li$ reaction, delivered to a target point 2 cm beyond the surgical margin, correlated with survival (158). For 66 patients with glioblastoma multiforme, those who survived <3 years (n = 60) had a minimum target point dose of 9.5 $\pm$ 5.9 Gy, whereas those who survived >3 years (n = 6) had a minimum target point dose of 15.6 $\pm$ 3.1 Gy from the

$^{10}B(n,\alpha)^{7}Li$ reaction (158). The boron concentrations in brain tissue at the target point, which are required to calculate the physical radiation dose attributable to the $^{10}B(n,\alpha)^{7}Li$ capture reaction, were estimated to be 1.2 times that of the patient's blood boron concentration (160).

Other recent and ongoing clinical trials. Beginning in 1994, several clinical trials, summarized in Table 2, were initiated in the United States and Europe. These marked a transition from low-energy thermal neutron irradiation to the use of higher-energy epithermal neutron beams with improved tissue penetrating properties, which obviated the need to reflect skin and bone flaps before irradiation. Up until recently, the procedure carried out in Japan required neurosurgical intervention immediately before irradiation, whereas the current epithermal neutron-based clinical protocols are radiotherapeutic procedures, done several weeks after debulking surgery and without the need of this. Clinical trials for patients with brain tumors were initiated at several locations, including (*a*) the BMRR at BNL from 1994 to 1999 for glioblastoma multiforme using BPA with one or two neutron radiations, given on consecutive days (161–163); (*b*) the MITR from 1996 to 1999 for glioblastoma multiforme and i.c. melanoma (164, 165); (*c*) the High Flux Reactor, Petten, the Netherlands, and the University of Essen in Germany in 1997 using BSH (166); (*d*) the FiR1 at the Helsinki University Central Hospital (131) in 1999 to the present; (*e*) the Studsvik Reactor Facility in Sweden from 2001 to the present, carried out by the Swedish National Neuro-Oncology Group (130); and (*f*) the Nuclear Research Institute reactor in Rez, Czech Republic, by Tovarys using BSH (167). The number of patients treated in this study is small and the follow-up is still rather short.

Initially, clinical studies using epithermal neutron beams were primarily phase I safety and dose-ranging trials and a BNCT dose to a specific volume or critical region of the normal brain was prescribed. In both the BNL and the Harvard/MIT clinical trials, the peak dose delivered to a 1 cm^3 volume was escalated in a systematic way. As the dose escalation trials have progressed, the treatments have changed from single-field irradiations or parallel opposed irradiations to multiple noncoplanar irradiation fields, arranged to maximize the dose delivered to the tumor. A consequence of this approach has been a concomitant increase in the average doses delivered to normal brain. The clinical trials at BNL and Harvard/MIT using BPA (**compound 1**) and an epithermal neutron beam in the United States have now been completed.

Analysis of the Brookhaven and Massachusetts Institute of Technology clinical results. The BNL and Harvard/MIT studies have provided the most detailed data relating to normal brain tolerance following BNCT. A residual tumor volume of $\geq$60 cm^3 led to a greater incidence of acute central nervous system toxicity. This primarily was related to increased i.c. pressure, resulting from tumor necrosis and the associated cerebral edema (141, 163, 165). The most frequently observed neurologic side effect associated with the higher radiation doses, other than the residual tumor volume–related effects, was radiation-related somnolence (168). This is a well-recognized effect following whole-brain photon irradiation (169), especially in children with leukemia or lymphoma, who have received central nervous system irradiation. However, somnolence is not a very well-defined radiation-related end point because it frequently is diagnosed after tumor recurrence has been excluded. Therefore, it is not particularly well suited as a surrogate marker for normal tissue tolerance. In the dose escalation studies carried out at BNL (141, 163, 164), the occurrence of somnolence in the absence of a measurable tumor dose response was clinically taken as the maximum tolerated normal brain dose. The volume-averaged whole-brain dose and the incidence of somnolence increased significantly as the BNL and Harvard/MIT trials progressed. The volume of tissue irradiated is a determining factor in the development of side effects (169). Average whole-brain doses of >5.5 Gy(w) were associated with somnolence in the trial carried out at BNL but not in all of the patients in the Harvard/MIT study (18, 141, 164). The BNL and Harvard/MIT trials were completed in 1999. Both produced median and 1-year survival times that were comparable with conventional external beam photon therapy (6). Although both were primarily phase I trials to evaluate the safety of dose escalation as the primary end point for radiation-related toxicity, the secondary end points were quality of life and time to progression and overall survival. The median survival times for 53 patients from the BNL trial and the 18 glioblastoma multiforme patients from the Harvard/MIT trial were 13 and 12 months, respectively. Following recurrence, most patients received some form of salvage therapy, which may have further prolonged overall survival. Time to progression, which would eliminate salvage therapy as a confounding factor, probably would be a better indicator of the efficacy of BNCT, although absolute survival time still is the "gold standard" for any clinical trial. The quality of life for most of the BNL patients was very good, especially considering that treatment was given in one or two consecutive daily fraction(s).

Clinical trials carried out in Sweden and Finland. The clinical team at the Helsinki University Central Hospital and VTT (Technical Research Center of Finland) have reported on 18 patients using BPA as the capture agent (290 mg/kg infused over 2 hours) with two irradiation fields and whole-brain average doses in the range of 3 to 6 Gy(w) (131). The estimated 1-year survival was 61%, which was very similar to the BNL data. This trial is continuing and the dose of BPA has been escalated to 450 mg/kg and will be increased to 500 mg/kg, infused over 2 hours.[5] Because BNCT can deliver a significant dose to tumor with a relatively low average brain dose, this group also has initiated a clinical trial for patients who have recurrent glioblastoma multiforme after having received full-dose photon therapy. In this protocol, at least 6 months must have elapsed from the end of photon therapy to the time of BNCT and the peak brain dose should be <8 Gy(w) and the whole-brain average dose <6 Gy(w). As of August 2004, only a few patients have been treated, but this has been well tolerated.

Investigators in Sweden have carried out a BPA-based trial using an epithermal neutron beam at the Studsvik Medical AB reactor (130). This study differed significantly from all previous clinical trials in that the total amount of BPA administered was increased to 900 mg/kg, infused i.v. over 6 hours. This approach was based on the following preclinical data: (*a*) the *in vitro* observation that several hours were required to fully load cells with BPA (170); (*b*) long-term i.v. infusions of BPA in

[5] H. Joensuu, personal communication.

Table 2. Summary of current or recently completed clinical trials of BNCT for the treatment of glioblastoma

Facility	No. patients	Duration of administration	Drug	Dose (mg/kg)
HTR, Musashi Institute of Technology, JRR, Kyoto University research reactor, Japan	>200 (1968)	1 h	BSH	100
High Flux Reactor, Petten, the Netherlands	26 (1997-present)	100 mg/kg/min	BSH	100
LVR-15, Rez, Czech Republic	5 (2001-present)	1 h	BSH	100
Brookhaven Medical Research reactor, Brookhaven, United States	53 (1994-1999)	2 h	BPA	250-330
MITR-II, M67 MIT, United States	20‖ (1996-1999)	1-1.5 h	BPA	250-350
MITR-II, FCB MIT, United States	6 (2001-present)	1.5 h	BPA	350
Studsvik AB Sweden	17 (30)¶ (2001-present)	6 h	BPA	900
FiR1, Helsinki Finland	18 (1999-present) protocol P-01	2 h	BPA	290-400
FiR1, Helsinki Finland	3 (2001-present)** protocol P-03	2 h	BPA	290

*During the irradiation.
† ^{10}B physical dose component dose to a point 2 cm deeper than the air-filled tumor cavity.
‡ Four fractions, each with a BSH infusion, 100 mg/kg the first day, enough to keep the average blood concentration at 30 μg ^{10}B/g during treatment on days 2 to 4.
§ ^{10}B physical dose component at the depth of the thermal neutron fluence maximum.
‖ Includes two i.c. melanomas.
¶ J. Capala, personal communication.
** Retreatment protocol for recurrent glioblastoma.

rats increased the absolute tumor boron concentrations in the 9L gliosarcoma model, although the tumor/blood ratio remained constant (171, 172); and (*c*) most importantly, long-term i.v. infusions of BPA seemed to improve the uptake of boron in infiltrating tumor cells at some distance from the main tumor mass in rats bearing i.c. 9L gliosarcomas (173, 174). The longer infusion time of BPA has been well tolerated (130), and the preliminary median survival time for 17 patients from this trial was 18 months, which is significantly longer than the BNL or Harvard/MIT data. All patients were treated with two fields, and the average whole-brain dose was 3 to 6 Gy(w), which was lower than the higher end of the doses used in the Brookhaven trial, although the peak dose was <15 Gy(w), which was similar to that used at BNL. Because in Sweden patients with glioblastoma multiforme who have recurred are not subjected to aggressive salvage therapy,[6] the survival data were not influenced by subsequent treatments, as was the case for the BNL and MIT patients, and therefore they more accurately represent the true effects of BNCT on the tumor. If the improved median survival time is firmly established, this would represent a significant advance because one BNCT treatment resulted not only in improved survival but also in a better quality of life.

Clinical Studies of Boron Neutron Capture Therapy for Other Tumors

Treatment of melanoma. Other than patients with primary brain tumors, the second largest group that has been treated by BNCT were those with cutaneous melanomas. Mishima et al. previously had carried out extensive studies in experimental animals with either primary or transplantable melanomas using ^{10}B-enriched BPA as the capture agent (175, 176). The use of BPA was based on the premise that it would be selectively taken up by and accumulate in neoplastic cells that were actively synthesizing melanin (177). Although it was subsequently shown that a variety of malignant cells preferentially took up large amounts of BPA compared with normal cells (178), Mishima's studies clearly stimulated clinical interest in BPA as a boron delivery agent. Because BPA itself has low water solubility, it was formulated with HCl to make it more water soluble. The first patient, who was treated by Mishima in 1985, had an acral lentigenous melanoma of his right toe that had been amputated (179). However, 14 months later, he developed a s.c. metastatic nodule on the left occiput, which was determined to be inoperable due to its location. The tumor was injected peritumorally at multiple points for a total dose of 200 mg BPA. Several hours later, by which time BPA had cleared from normal skin but still had been retained by the melanoma, the tumor was irradiated with a collimated beam of thermal neutrons. Based on the tumor boron concentrations and the neutron fluence, an estimated 45 Gy(w) equivalent dose was delivered to the melanoma. Marked regression was noted after 2 months, and the tumor had completely disappeared by 9 months (175, 179). This successful outcome provided further evidence for proof of principle of the usefulness of BNCT to treat a radioresistant tumor. Subsequently, at least an additional 18 patients with either primary or metastatic melanoma have been treated by Mishima et al. (180). The BPA either was injected peritumorally or administered orally as a slurry (181) until Yoshino et al. improved its formulation and water solubility by complexing it with fructose, following which it was administered i.v. (182). This important advance ultimately led to the use of BPA in the clinical trials in patients with brain tumors that were described in the preceding section. In all of Mishima's patients, there was local control of the treated primary or metastatic melanoma nodule(s) and several patients were tumor free at ≥4 years following BNCT (180).

[6] J. Capala, personal communication.

Table 2. Summary of current or recently completed clinical trials of BNCT for the treatment of glioblastoma (Cont'd)

Boron concentration* ($\mu g\ ^{10}B/g$)	Estimated peak normal brain dose [Gy(w)]	Average normal brain dose [Gy(w)]	Reference
~20-30	15 Gy† ^{10}B component	ND	(157, 158)
30‡	8.6-11.4 Gy§ ^{10}B component	ND	(166)
~20-30	<14.2	<2	(167)
12-16	8.4-14.8	1.8-8.5	(141, 168)
10-12	8.7-16.4	3.0-7.4	(165)
~15			Unpublished
24 (range, 15-34)	7.3-15.5	3.6-6.1	(130)
12-15	8-13.5	3-6 <7	(131)
12-15	<8	2-3 <6	(131)

Several patients with either cutaneous or cerebral metastases of melanoma have been treated by Busse et al. using BPA fructose as the delivery agent (18, 183). The most striking example of a favorable response was in a patient with an unresected cerebral metastasis in the occipital lobe. The tumor received a dose of 24 Gy(w) and monthly magnetic resonance imaging studies revealed complete regression over a 4-month interval (183). As demonstrated radiographically, a second patient with a brain metastasis had a partial response. Several other patients with either cutaneous or metastatic melanoma to the brain have been treated at other institutions, including the first in Argentina (184), and the consensus seems to be that these tumors are more responsive to BNCT than glioblastoma multiforme. This is supported by experimental studies carried out by two of us (R.F.B. and J.A.C.) using a human melanoma xenograft model (185, 186), which showed enhanced survival times and cure rates superior to those obtained using the F98 rat glioma model (187). In summary, multicentric metastatic brain tumors, and more specifically melanomas, which cannot be treated by either surgical excision or stereotactic radiosurgery, may be candidates for treatment by BNCT.

Other tumor types treated by boron neutron capture therapy. Two other types of cancer recently have been treated by BNCT. The first are recurrent tumors of the head and neck. Kato et al. have reported on a series of six patients, three of whom had squamous cell carcinomas, two had sarcomas, and one had a parotid tumor (188). All of them had received standard therapy and had developed recurrent tumors for which there were no other treatment options. All of the patients received a combination of BSH (5 g) and BPA (250 mg/kg body weight) administered i.v. In all but one patient, BNCT was carried out at the Kyoto University Research Reactor Institute using an epithermal neutron beam in one treatment that was given 12 hours following administration of BSH and 1 hour after BPA. The patient with the parotid tumor, who received a second treatment 1 month following the first, had the best response with a 63% reduction in tumor volume at 1 month and a 94% reduction at 1 year following the second treatment without evidence of recurrence. The remaining five patients showed responses ranging from a 10% to 27% reduction in tumor volume with an improvement in clinical status. This study has extended the use of BNCT to a group of cancers that frequently are ineffectively treated by surgery, radiotherapy, and chemotherapy. However, further clinical studies are needed to objectively determine the clinical usefulness of BNCT for head and neck cancers, and another study currently is in progress at Helsinki University Central Hospital.[7]

The second type of tumor that recently has been treated by BNCT is adenocarcinoma of the colon that had metastasized to the liver (189). Although hepatectomy followed by allogeneic liver transplantation has been carried out at several centers (190, 191), Pinelli and Zonta et al. in Pavia, Italy, have approached the problem of multicentric hepatic metastases using an innovative but highly experimental procedure. Their patient had >14 metastatic nodules in the liver parenchyma, the size of which precluded surgical excision. Before hepatectomy was done, the patient received a 2-hour infusion of BPA fructose (300 mg/kg body weight) via the colic vein. Samples of tumor and normal liver were taken for boron determinations, and once it was shown that boron selectively had localized in the tumor nodules with small amounts in normal liver, the hepatectomy was completed (189). The liver then was transported to the Reactor Laboratory of the University of Pavia for neutron irradiation, following which it was reimplanted into the patient. More than 2 years later in October 2004, the patient had no clinical or radiographic evidence of recurrence and carcinoembryonic antigen levels were low (192). Although it is unlikely that this approach will have any significant clinical impact on the treatment of the very large number of patients who develop hepatic metastases from colon cancer, it nevertheless again provides proof of principle that BNCT can eradicate multicentric deposits of tumor in a solid organ. The Pavia group has plans to treat other patients with metastatic liver cancer and several other groups (193–195) are exploring the possibility of treating patients with primary as well as metastatic tumors of the liver using this procedure.

Critical Issues

There are several critical issues that must be addressed if BNCT is to become a useful modality for the treatment of

[7] L. Kankaanranta, personal communication.

cancer and, most specifically, brain tumors. First, there is a need for more selective and effective boron agents that, when used either alone or in combination, could deliver the requisite amounts (~ 20 μg/g) of boron to the tumor. Furthermore, their delivery must be optimized to improve both tumor uptake and cellular microdistribution, especially to different subpopulations of tumor cells (196). Several studies have shown that there is considerable patient-to-patient as well i.t. variability in the uptake of both BSH (197, 198) and BPA (162, 173, 174) in patients with brain tumors. At present, the dose and delivery of these drugs have yet to be optimized, but based on experimental animal data (30, 31, 33, 124, 172), improvement in dosing and delivery could have a significant impact on increasing tumor uptake and microdistribution.

Second, because the radiation dosimetry for BNCT is based on the microdistribution of ^{10}B (199), which is indeterminable on a real-time basis, methods are needed to provide semiquantitative estimates of the boron content in the residual tumor. Imahori et al. (200–202) in Japan and Kabalka (203) in the United States have carried out imaging studies with ^{18}F-labeled BPA and have used data obtained from these studies to determine whether a patient might be a suitable candidate for BNCT using BPA as the delivery agent. In the absence of real-time tumor boron uptake data, the dosimetry for BNCT is very problematic. This is evident from the discordance of estimated doses of radiation delivered to the tumor and the therapeutic response, which should have been greater than that which was seen if the tumor dose estimates were correct (141).

Third, there is a discrepancy between the theory behind BNCT, which is based on a very sophisticated concept of selective cellular and molecular targeting of high LET radiation, and the implementation of clinical protocols, which are based on very simple approaches to drug administration, dosimetry, and patient irradiation. This in part is due to the fact that BNCT has not been carried out in advanced medical settings with a highly multidisciplinary clinical team in attendance. At this time, BNCT has been totally dependent on nuclear reactors as neutron sources. These are a medically unfriendly environment and are located at sites at varying distances from tertiary care medical facilities, which has made it difficult to attract patients, and the highly specialized medical team that ideally should be involved in clinical BNCT. Therefore, there is an urgent need for either very compact medical reactors such as one under construction in Beijing, China or ABNS that could be easily sited at selected centers that treat many patients with brain tumors.

Fourth, there is a need for randomized clinical trials. This is especially important because almost all major advances in clinical cancer therapy have come from these, and up until this time, no randomized trials of BNCT have been conducted. The pitfalls of non-randomized clinical trials for the treatment of brain tumors have been well documented (204, 205). It may be somewhat wishful thinking to believe that the clinical results with BNCT will be so clear-cut that a clear determination of efficacy could be made without such trials. These will require a reasonably large number of patients to provide unequivocal evidence of efficacy with survival times significantly better than those obtainable with promising currently available therapy for both glioblastoma multiforme (206, 207) and metastatic brain tumors (208). This leads to the issue of conducting such trials, which might best be accomplished through cooperative groups, such as the Radiation Therapy Oncology Group in the United States or the European Organization for Research Treatment of Cancer.

Finally, there are several promising leads that could be pursued. The up-front combination of BNCT with external beam radiation therapy or in combination with chemotherapy has not been explored, although recently published experimental data suggest that there may be a significant gain if BNCT is combined with photon irradiation (33). The extension of animal studies, showing enhanced survival of brain tumor-bearing rats following the use of BSH and BPA in combination, administered intra-arterially with or without BBB-D, has not been evaluated clinically. This is a promising approach, but it is unlikely that it could be carried out at a nuclear reactor.

As is evident from this review, BNCT represents an extraordinary joining together of nuclear technology, chemistry, biology, and medicine to treat cancer. Sadly, the lack of progress in developing more effective treatments for high-grade gliomas has been part of the driving force that continues to propel research in this field. BNCT may be best suited as an adjunctive treatment, used in combination with other modalities, including surgery, chemotherapy, and external beam radiation therapy, which, when used together, may result in an improvement in patient survival. Clinical studies have shown the safety of BNCT. The challenge facing clinicians and researchers is how to get beyond the current impasse. We have provided a road map to move forward, but its implementation still remains a daunting challenge!

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