Boron and gadolinium neutron capture therapy*

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The principles of neutron capture therapy of tumor diseases, the types of drugs, and the results of their clinical applications are discussed.

Key words: boron neutron capture therapy, gadolinium neutron capture therapy.

Introduction

Neutron capture therapy (NCT) stands for a binary treatment method that combines a cancer-specific ^{10}B- or ^{157}Gd-labeled drug and a neutron beam of a low energy sufficient for neutron capture to take place within the treated tissues. These two therapeutic components are designed to be innocuous in themselves, while their combination results in a highly localized and lethal radiotoxic response at the cell level. The drug component appears to represent the weakest link in bringing NCT to its full potential, which was heralded in 1936 by Gordon L. Locher:[1] "Enough knowledge of the remarkable behavior of neutrons has been accumulated through physical research to enable the prediction of certain biological effects, and to see, in general way at least, certain therapeutic potentialities of this new kind of corpuscular radiation." The concept of NCT was formulated long before the advent of suitable neutron sources. Ever since, in parallel to the science and technology revolution of the XX century, NCT has progressed in terms of dedicated neutron beam facilities, intense drug development, computer-assisted dosimetry, and treatment planning software. To put things into perspective, although NCT has always been closely associated with the treatment of fatal brain cancers such as glioblastoma multiforme and anaplastic astrocytoma,[2—10] now it is being extended to the treatment of melanoma,[11,12] liver,[13] head and neck[14,15] cancer, synovectomy,[16] and even for prophylactic restenosis inhibition.**

History of neutron capture therapy

The discovery of the neutron[17,18] has initiated a multitude of nuclear fission experiments; among these, neutron capture by the ^{10}B isotope was reported.[19] In 1936, Gordon L. Locher formulated his binary concept of treating cancer: "In particular, there exist the possibilities of introducing small quantities of strong neutron absorbers into the regions where it is desired to liberate ionization energy (a simple illustration would be the injection of a soluble, non-toxic compound of boron, lithium, gadolinium, or gold into a superficial cancer, followed by bombardment with slow neutrons." It also became clear that the propensity of a nucleus to absorb or capture a neutron, expressed by thermal neutron capture cross-section in barns (1 barn = 10^{-24} cm^2) was independent of the nuclear mass but related to the structure of the nucleus. The ^{10}B isotope has a neutron capture cross-section of 3838 barns (Table 1). Coupled with the facts that ^{10}B is not radioactive, non-toxic, and, upon neutron capture, gives an excited ^{11}B nucleus, which instantly splits into high-energy alpha and lithium particles with short path lengths, this makes ^{10}B a highly attractive isotope for cancer therapy.[20—25] The elegance of Locher´s idea resides in the binary therapy approach and the very limited path lengths of the high-linear energy transfer (LET) fission products. The results of the first *in vitro* and *in vivo* trials with boron neutron capture therapy (BNCT) using

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** M. Takagaki, unpublished results, 2002.

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Table 1. Thermal neutron capture cross-sections (σ_{th}) and types of neutron capture reactions for selected stable and radioactive isotopes

Isotope	σ_{th}/barn	Neutron capture reaction
^{3}H	5333	(n,p)
^{6}Li	941	(n,α)
^{10}B	3838	(n,α)
^{113}Cd	20600	(n,γ)
^{135}Xe*	2720000	(n,γ)
^{147}Sm	40140	(n,γ)
^{151}Eu	9200	(n,γ)
^{155}Gd	60900	(n,γ)
^{157}Gd	255000	(n,γ)
^{174}Hf	561	(n,γ)
^{199}Hg	2150	(n,γ)
^{235}U*	681	(n,f)
^{241}Pu*	1380	(n,f)
^{242}Am*	8000	(n,f)

Note. 1 barn = 10^{-24} cm^2. * Radioactive.

boric acid solutions or boron oil suspensions on mice were reported in 1940.[26—28] However, it was not until 1951 that the procedure was first tried* on terminal brain tumor patients.[29—30]

Initially, several other nuclides with high neutron capture cross-sections, such as ^{6}Li and ^{235}U (see Table 1), came under consideration for NCT;[34,35] however, none of these has been extensively investigated. The ionic Li^+ is too easily dispersed throughout the body for a specific delivery to a tumor site, while ^{235}U is unsuitable due to its inherent toxicity and radioactivity. Yet another aspect to be considered in NCT is the thermal neutron capture cross-sections of the elements that constitute the human body such as hydrogen, oxygen, carbon, and nitrogen (Table 2). Although the individual thermal neutron capture cross-sections are quite negligible for these elements, the sheer quantity of these atoms in all tissues becomes a factor to be taken into account when considering the radiation doses absorbed by healthy tissues.

First, the high toxicity of free, *i.e.*, non-complexed, inorganic gadolinium salts precluded their use for therapeutic purposes. New interest in the concept of gadolinium neutron capture therapy (GdNCT) has arisen since the late 1980s with the advent and universal use of gadolinium-labeled MRI contrast agents.[36—39]

Principles of neutron capture therapy

Boron neutron capture therapy (BNCT). The boron neutron capture reaction, $^{10}B(n,\alpha)^{7}Li$, was first characterized in 1935 and can be best described by two parallel nuclear fission processes that occur on absorption of a thermalized neutron (n_{th}).[19] The excited ^{11}B nucleus splits producing two high-energy ions, $^{4}He^{2+}$ (α-particle) and $^{7}Li^{3+}$ (Scheme 1):

Table 2. Thermal neutron capture cross-sections (σ_{th}) of the elements commonly present in mammalian tissues

Isotope	σ_{th}/barn	Weight % in mammal tissues
^{1}H	0.333	10.00
^{12}C	0.0035	18.00
^{14}N	1.83	3.00
^{16}O	0.00019	65.00
^{23}Na	0.43	0.11
^{24}Mg	0.0053	0.04
^{31}P	0.18	1.16
^{32}S	0.53	0.20
^{35}Cl	32.68	0.16
^{39}K	2.1	0.20
^{40}Ca	0.4	2.01
^{56}Fe	2.57	0.01

Scheme 1

$$^{10}B + n_{th} \longrightarrow [^{11}B] \xrightarrow[6\%]{} {}^{4}He^{2+} + {}^{7}Li^{3+} + 2.79\ MeV$$

$$[^{11}B] \xrightarrow{94\%} {}^{4}He^{2+} + {}^{7}Li^{3+} + E_{\gamma} + 2.31\ MeV,$$

$E_{\gamma} = 0.48$ MeV.

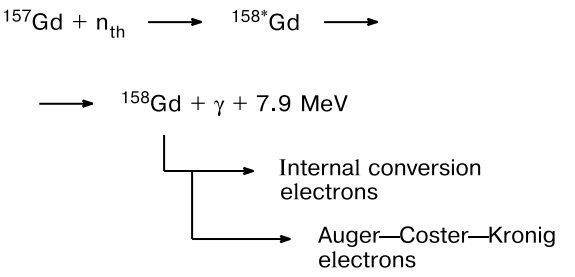
With the neutron capture cross-section of boron being 3838 barn, the therapeutic dose of ^{10}B required to kill a cancer cell is ~10^9 atoms per cell. A fundamental requirement for BNCT to be effective resides in the selective targeting of ^{10}B at therapeutic concentrations to the cancerous structures relative to the surrounding healthy tissues and the blood. The high LET of the emitted α-particle and the recoil lithium particles is associated with their short path lengths in biological tissues (~4—9 μm), in particular, they are smaller than the cell diameter.[40—43]

Gadolinium neutron capture therapy (GdNCT). The gadolinium neutron capture (GdNC), $^{157}Gd(n,\gamma)^{158*}Gd$, is more complex than boron neutron capture and is not a fission reaction. Two of the seven stable gadolinium isotopes are of interest for NCT, namely, ^{155}Gd (55000 barn) and ^{157}Gd (255000 barn). Moreover, the latter isotope has the highest thermal neutron capture cross-section of all stable nuclides in the Periodic Table (see Table 1). The GdNC reaction induces complex inner-shell transitions that generate prompt γ-emission displacing an inner-core electron, which in turn results in internal-conversion elec-

* The trials were performed by neurosurgeon W. H. Sweet and physicist L. E. Farr at the Brookhaven National Laboratory and Massachusetts General Hospital.

tron emission, and finally in the Auger—Coster—Kronig electron emission, together with soft X-ray and photon emissions.[44—49]

Scheme 2



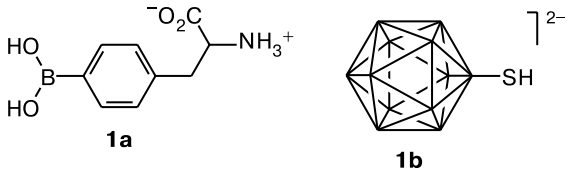
The average energy of the low-LET γ-rays is ~2.2 MeV and the path length is several centimeters. The average energy of the internal-conversion electrons is ~45 eV and their path length in tissues is several millimeters. Finally, the path lengths of the very low-energy Auger—Coster—Kronig and super-Coster—Kronig electrons are only several nanometers in aqueous solutions. Despite the low absolute energy, the ultra-short path length of the Auger—Coster—Kronig electron energy deposition makes this component of the GdNC reaction a high-LET-type; radiologically, this is the most relevant component of the GdNC.[50—54] Since this ionizing radiation is limited to molecular dimensions (5—40 nm), for significant DNA damage induction in cancer cells, it is essential to place the gadolinium atoms into the DNA helix. The Auger—Coster—Kronig electrons provoke high LET-type damage within a mean free path of 12.5 nm from the intranuclear Auger emitter, *i.e.*, the (^{158*}Gd) decay site within the target tissue. Computer simulations of the GdNC reaction indicate a yield of five Auger—Coster—Kronig electrons, 0.69 internal conversion electrons, 0.84 X-ray photons, and 1.83 γ-photons.[55] The success of GdNCT is determined by the relative biological effectiveness (RBE) of these three types of ionizing radiation. It is important that RBE directly depends on the localization of the ^{157}Gd atoms with respect to the malignant cell DNA. The nanometer range of the Auger—Coster—Kronig electrons accounts for the much higher LET compared to that of the prompt γ-rays. Computer simulation studies imply that Auger emitters placed in the immediate vicinity of the cancer cell DNA strands are capable of inducing a level of DNA damage which is 5 to 10 times that of high-energy but low-LET γ-rays or photons. Based on the relationship between the relative biological effectiveness and the linear energy transfer (RBE—LET),[56] the RBE of the internal conversion electrons can reasonably be expected to be intermediate between those of the γ-rays and the Auger—Coster—Kronig electrons. Experimental data supporting this ranking are still lacking, to our knowledge. However, there are several examples of evidence for cytostatic effects induced by the GdNC reaction when the gadolinium atoms are not DNA-bound.

Major NCT drug prototypes

Boron neutron capture therapy agents. In discussing the evolution of BNCT agents, it is convenient to divide them into categories such as first-, second-, and third generation drugs (**1—21**).[24] The most nonspecific class of BNCT agents is represented by the "first generation" boron carriers, used when the mechanisms of cell replication and receptor-mediated recognition were not yet fully understood to have a significant impact on tailor-made drug design. The only two BNCT agents currently in use for clinical trials, namely, *p*-boronophenylalanine (BPA, **1a**), a boronated amino-acid analog, and disodium mercaptoundecahydrododecaborate $Na_2[B_{12}H_{11}SH]$ (BSH, **1b**), are both so-called second-generation BNCT drugs (Fig. 1).[57—59] The design of an effective BNCT agent aims at low-toxicity and selective tumor cell (ideally, cell nucleus) targeting by various physicochemical and immunological methods. A number of boron-containing analogs of biologically active compounds such as amino acids, polyamines, peptides, epithelial growth factor antibodies and antibody fragments, porphyrins, and DNA groove binders have been synthesized and evaluated as potential agents for BNCT. The BNCT agents currently under investigations are third-generation boron carriers and can be classified either according to their chemical structure, compound class, and molecular weight or according to their biological target recognition properties. Several more advanced types of boron carriers are bound to cell membrane receptors, which results in their internalization (**2**, **13**, **14**, **15**).[60—66]

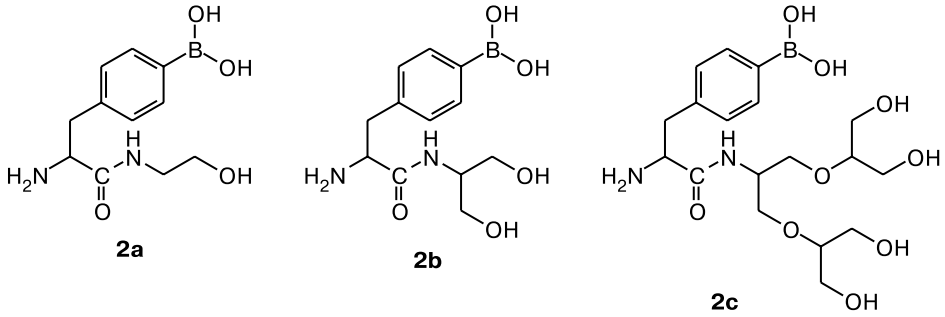
Liposome formulations developed for targeting folate receptors and nanoemulsions like thiamine-coated gadolinium nanoparticles display significant tumor targeting properties. Other types of boron carriers include nucleic acid precursors or other essential cell metabolite precursors (**3**, **4**, **5**, **9**). The rationale for this strategy is based on the increased requirements for DNA or nucleic acid precursors and other metabolites such as amino acids of rapidly dividing neoplastic cells (**13**, **16**, **17**). This category of BNCT agents includes ^{10}B-containing nucleic acid precursors or their analogs that must successfully compete with the endogenous nucleic acid precursors for possible incorporation in the DNA of cancer cells (**9**-**12**, **20**).[67—73] A variety of carboranylporphyrins have been shown to selectively target the cancer tissue, with some evidence for intranuclear uptake in the malignant cells (**19**, **20**).[74—76] Boron-rich BNCT agents incorporating as many as eight carborane cages linked to a bioactive moiety *via* ester, peptide, or C—C bonds have been devised as means for

BPA (*p*-borophenylalanine) and BSH (disodium mercaptoundecahydrododecaborate $Na_2[B_{12}H_{11}SH]$)



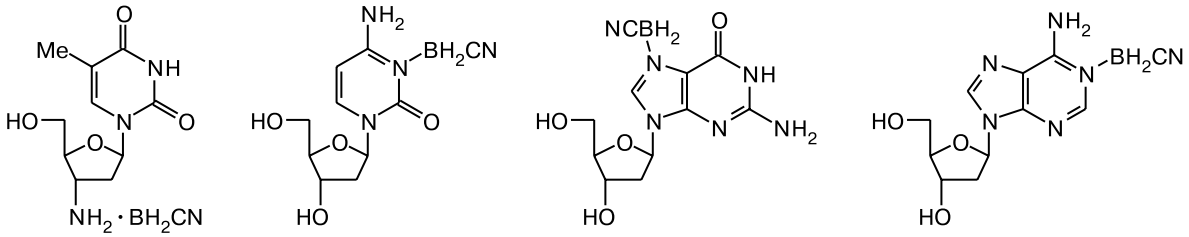
1a **1b**

Hydrophilic BPA analogs



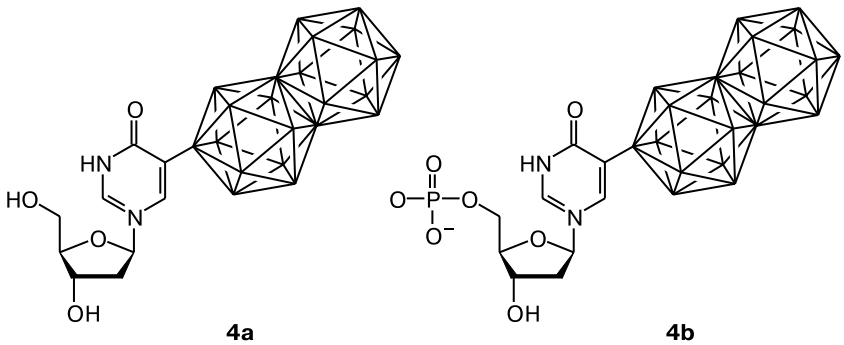
2a **2b** **2c**

Pyrimidine and purine boronated nucleosides



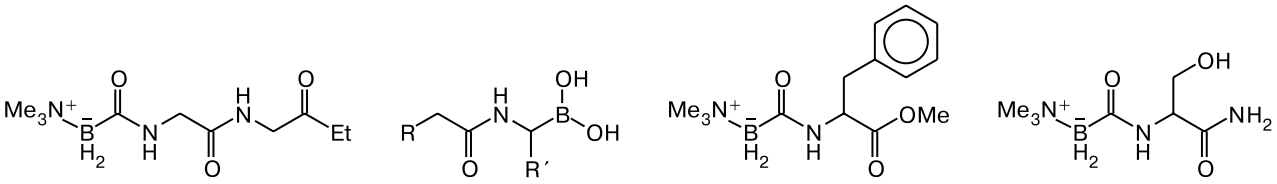
3a **3b** **3c** **3d**

Structures of dianionic fused boron cage nucleoside and nucleotide analogs



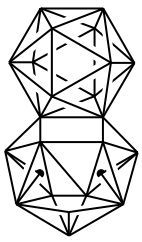
4a **4b**

Boron-labeled peptides



5a **5b** **5c** **5d**

Fused polyhedral borane dianion $[B_{22}H_{22}]^{2-}$



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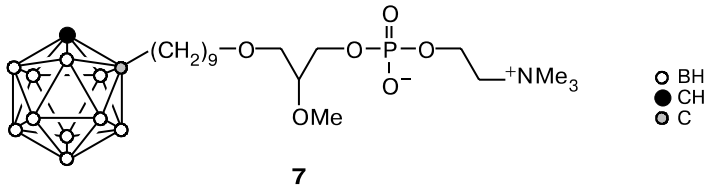
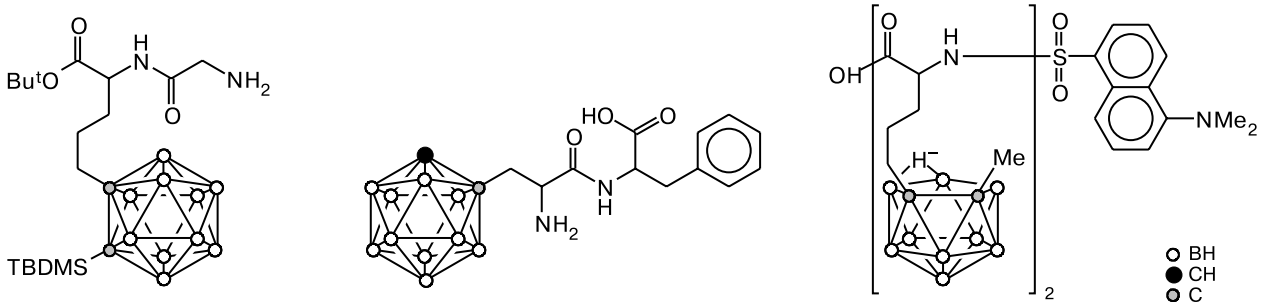
Boron cage-containing phospholipid



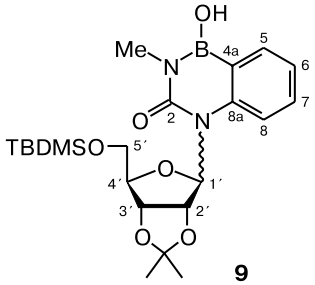
7

Boron cage-containing peptides



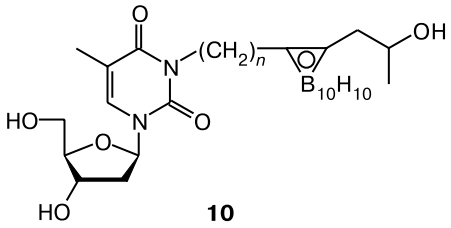
8a **8b** **8c**

Benzoborauracil nucleoside



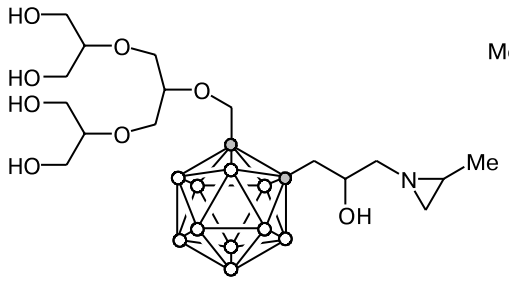
9

3-Carboranylalkyl thymidine analog with links of various length

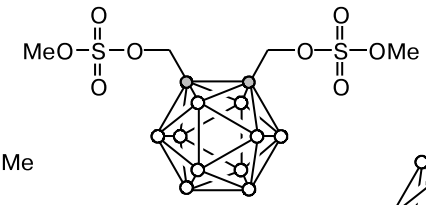


10

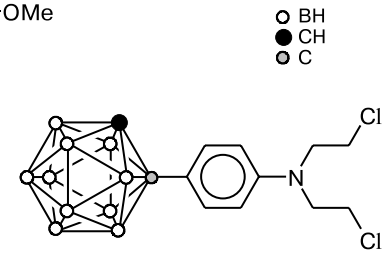
Boron-containing alkylating agents



11a

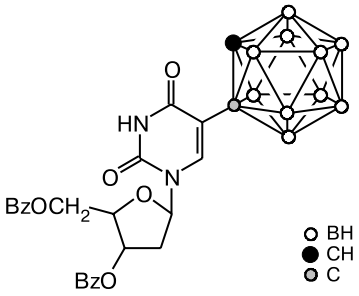


11b



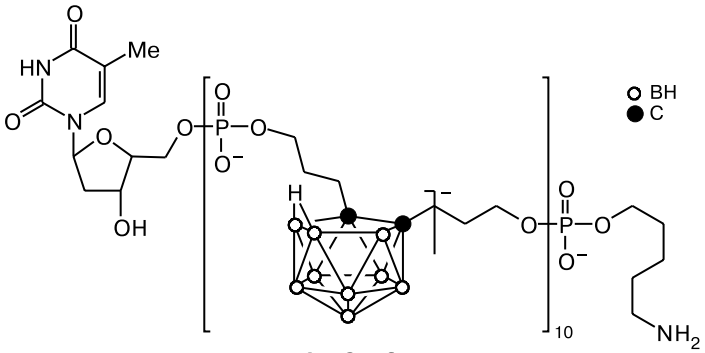
11c

***closo*-Carborane-substituted deoxyuridine**



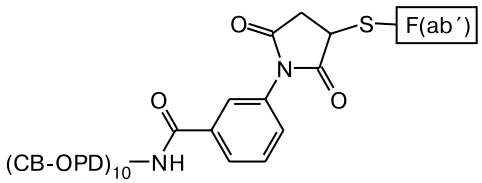
12 (CDU)

Oligomeric *nido*-carborane phosphate diester derivative



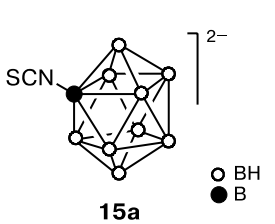
13 ([CB-OPD]$_{10}$)

Immunoconjugate containing carboranyl-oligophosphate diester and an antibody fragment



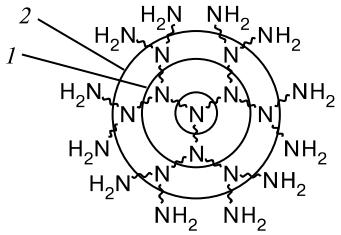
14

The isothiocyanato-substituted *closo*-$[B_{10}H_{10}]^{2-}$ anion (15a) coupled with a star dendrimer composed of repetitive poly(amidoamino) groups (*1,2*) (15b)



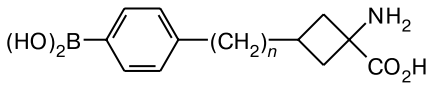
15a

$[2\text{-(NCS)-}closo\text{-}B_{10}H_9]^{2-}$



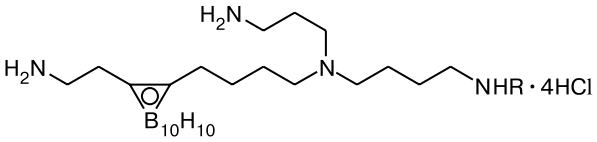
15b (SD)

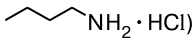
"Unnatural" cyclic α-amino-acid



16 (*n* = 0, 2, 3, 7)

Boron-containing polyamines



17 (ASPD-5, R = H; ASPM-5, R = 

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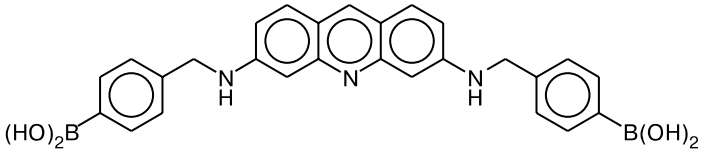
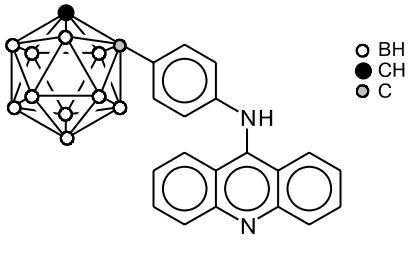
Some boron-containing acridine analogs

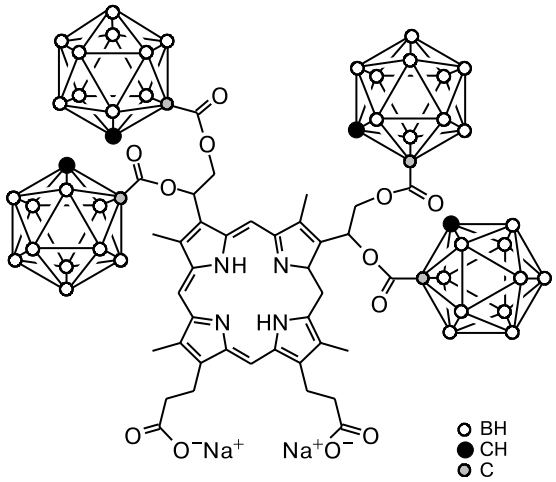


18a



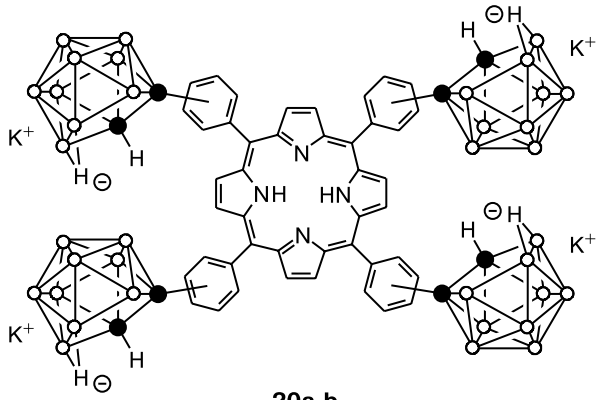
18b

Boron-containing porphyrin (BOPP)



19

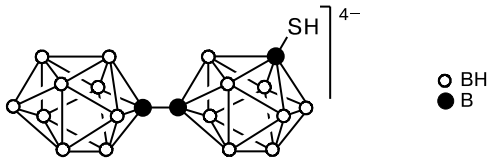
Porphyrin derivative linked to ionic *nido*-carborane cages



20a,b

p-H_2TCP^{4-} (**a**), *m*-H_2TCP^{4-} (**b**)

Monothiol derivatives of bis(decaborate) tetraanion, $[B_{20}H_{17}SH]^{4-}$



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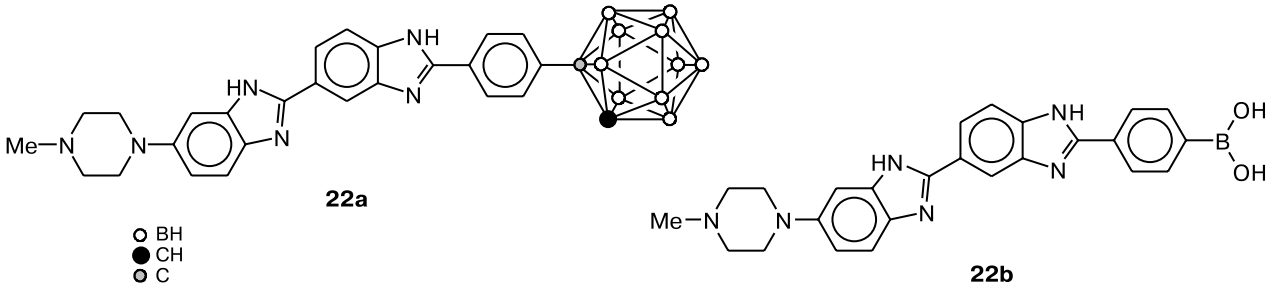
delivering a great number of ^{10}B atoms to tumor cells (**4, 6—8**).[77—80] An intriguing feature of these carborane cages, which are unnatural to the physiological realms of mammalian life being neither purely inorganic nor purely organic entities, is their apparent invisibility in enzymatic cell recognition mechanisms. The "Trojan horse" strategy of infiltrating BNCT agents into "blind" cancer cells is being attempted using certain boron-cage-containing BNCT compounds. The most sophisticated class of BNCT agents should display dual cancer cell and cell nucleus specificity, which is also the basic criterion for GdNCT agents. The degrees of hydrophilicity, lipophilicity, and amphiphilicity are important considerations in the design of BNCT agents. The solubility of boron compounds in

physiological media usually presents a major complication that requires extensive study feedback with *in vitro* and *in vivo* trails.

A number of other boron-containing DNA-targeting compounds such as groove binding agents (**22**),[81,82] DNA intercalators, *e.g.,* acridine analogs (**18a,b**), distamycin (**23a**) and netropsin (**23b**) analogs,[83,84] cytostatic alkylating agents (**11a—c**), and pentapyrrole derivatives have also been described.

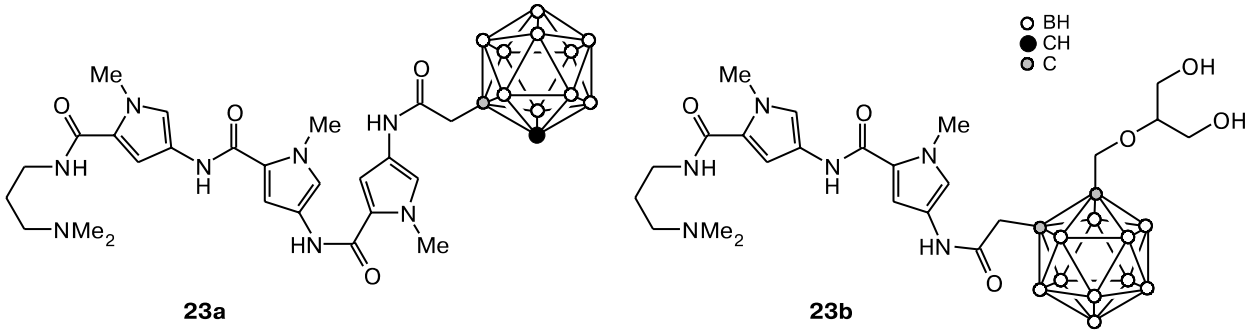
Gadolinium neutron capture therapy agents. The rationale for the design of GdNCT agents is to fully exploit the extremely fortuitous combination of ultra-high neutron capture cross-section of ^{157}Gd (255 000 barn) and the proton relaxing effect of gadolinium. Therefore, an effective GdNCT drug would ideally combine diagnostic and therapeutic properties. This would permit the simultaneous dynamic MRI monitoring of the gadolinium distribution levels prior to and during neutron irradiation at therapeutic doses. The design of effective GdNCT agents strives to exploit the multiple radiation products of the ^{157}Gd(n,γ)^{158}*Gd reaction to achieve cancer cell apoptosis at therapeutic drug doses. This is equivalent to subjecting the malignant DNA to the radiotoxic short-path Auger—Coster—Kronig electron cascades by placing the ^{158}*Gd emitters directly among the DNA strands. Gadolinium carriers that would first preferentially target cancer tissue structures and, second, target the DNA in the cancerous cell nuclei represent the highest class of GdNCT agents. The standard ^{157}Gd-containing MRI contrast agents should be considered to be the most logical and straightforward first-choice GdNCT drugs, in particular, their pharmacology has been extensively studied and is well documented, they inherently combine the potential diagnostic and therapeutic properties, and they have already been approved for clinical use; certain so-called extra-cellular MRI contrast agents have been shown to be retained within the tumor cells; and their nanoemulsion or liposome formulations in certain *in vitro* trials display

Boron-labeled DNA-binding analogs of Hoechst®



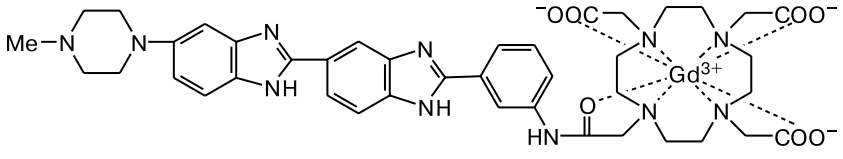
22a **22b**

Boron-cage-labeled DNA-binder analogs of distamycin (23a) and netropsin (23b).



23a **23b**

GdNCT prototype, Hoechst®-Dotarem® (HDG)



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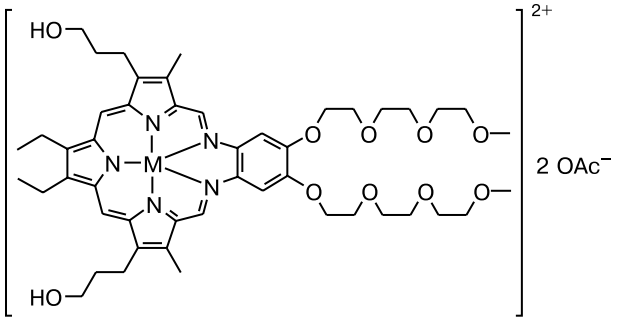
enhanced tumor specificity and growth control properties as compared with standard contrast agent formulations.

The design of GdNCT drugs has led to the synthesis of DNA-targeting gadolinium complexes of the same type that have been investigated as boron cage carriers, *e.g.*, groove-binding agents,[85–87] cytostatic alkylating agents,[88] and pentapyrrole derivatives.[89–91] Some of the more prominent GdNCT prototypes currently under investigations are Hoechst®-Dotarem® (HDG) (**23**),[91,92] Gd-Motexafin® (Xcytrin®) (**25**),[93–95] and various types of nanoemulsions of standard contrast agents.[93–102]



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M = Gd^{III}

Combined BNCT and GdNCT agents. A number of BNCT agents with additional Gd-labeling have been prepared to provide therapeutic BNCT agents with the possibilities of diagnostic MRI monitoring to dynamically follow the uptake and retention time course of the NCT drug prior to and during neutron irradiation. Although a possible synergistic combination of the two neutron capture reactions of ^{10}B and ^{157}Gd cannot be excluded, the dual drug design is currently focused on the added diagnostic value of such BNCT compounds. The synthesis of a carborane—gadolinium—DTPA complex for BNCT was reported[103] and its MRI properties were studied.[104]

Neutron sources for NCT

The neutron sources currently available for NCT are fission-reactor based neutron beams[105] and accelerator-based neutron beams.[106–108] Certain fundamental requirements for the neutron beams used in NCT concern the neutron energies, which should be in a range from 1 eV to several tens of eV. Epithermal neutron beams with bone penetration properties that are thermalized in physiological media tend to be preferred to purely thermal neutron beams. Nuclear reactors are considered to be the most powerful neutron sources, although, in general, there has been a distinct trend towards decommissioning of nuclear reactors and preferentially making use of accelerator facilities which are becoming increasingly compact in layouts for their incorporation into medical surroundings. These accelerated-based neutron sources are easier to standardize than reactor neutron beams, which require extensive beam characterization procedures. Since each nuclear reactor neutron source is one of a kind, data comparison is virtually meaningless. To achieve higher levels of clinical NCT efficiency, clinical results must become more reproducible.[109]

Increased interest is directed toward Boron or Gadolinium Neutron Capture Enhanced Fast Neutron Therapy (BNCEFNT, GdNCEFNT), in which fast neutron beams with a thermal or epithermal beam component trigger the boron or gadolinium neutron capture reaction.[110–112] The existing fast neutron beam filtration and moderation systems developed for BNCEFNT are adaptable to the neutron energy requirements of GdNCT providing therapeutic windows with a radiation dose enhancement of 10–20% (tumor tissue relative to normal tissue) by the selective gadolinium loading of tumor cell DNA.[113,114] A major advantage of fast neutron therapy (FNT) over standard NCT using thermal or epithermal neutron beams is in the beam collimation. Unlike thermal and epithermal neutron beams, which scatter throughout the entire brain regardless of upstream collimation, a collimated fast neutron beam allows a reduction of the radiation dose to healthy tissue.

Neutron capture therapy dosimetry and treatment planning

BNCT dosimetry. Neutron capture therapy is meant to affect the tumor by the release of a high, localized dose of radiation in the tumor tissue, while sparing the surrounding normal tissues. Due to the short path lengths of the BNC reaction products, the microdistribution and quantification of the ^{10}B nuclides are critical issues for any treatment planning. Since toxicity is unavoidable, the basic aim in BNCT agent design is to achieve the highest possible tumor selectivity at a toxicity level permitting healthy cell repair and/or manageable side effects for the patient. Computational dosimetry is a powerful technique in any design process. A Monte Carlo-based treatment planning system (INEEL) taking into account the complex brain geometries has been developed for BNCT studies.[115–119] More recently, the successor of the INEEL program, SERA (Simulation Environment for Radiotherapy Applications) has been introduced. This program consists of a succession of interactively cross-launched software modules or command lines designed for the iterative development of BNCT and GdNCT patient treatment plans.[120]

GdNCT dosimetry. The newly established[121,122] triage criteria for potential GdNCT compounds in the drug screening process require an at least 90% uptake of the drug, *i.e.,* gadolinium, by glioblastoma cells in an *in vitro*

assay and an efficiency quotient of gadolinium uptake by the cell nucleus greater than unity (Q_{Gd} = $Gd_{intracellular}/Gd_{extracellular} > 1$). If cytotoxicity is dependent on intracellular incorporation of a test agent, the limiting factor in successful treatment will be the proportion of tumor cells not incorporating the test agent. Therapeutic benefit is unlikely if more than 10% of all cancer cells remain untargeted.[123—125] Meanwhile, it has been estimated that if a clinically acceptable neutron flux of ~10^8 neutrons/(cm^{-2} s^{-1}) for GdNCT can be delivered to the cancerous target sites, the gadolinium concentration in the cell nuclei does not need to be extremely high. As little as one or two capture events between one neutron and one gadolinium atom close to DNA could kill a cancer cell, but a homogeneous distribution of gadolinium in the nuclei of all cells in a tumor is necessary for successful treatment. Evidence for successful neutron capture events can be monitored by detecting prompt γ–emission. An average of at least 24 gadolinium atoms per cell nucleus is estimated to be necessary to ensure that less than one of 10^{10} cells (the average tumor size) survives the release of ionizing radiation as short-range high LET-type Auger—Coster—Kronig electrons. According to other authors,[126] 200—250 ppm of gadolinium per cell nucleus corresponds to a therapeutically efficient drug dose. This hypothetical threshold for the success of GdNCT as a single therapy modality is based on the assumption that any single malignant cell with no gadolinium uptake will survive and cause tumor recurrence. Ultimately, however, only *in vivo* neutron irradiation assays with GdNCT agent prototypes will provide more realistic estimates of gadolinium cell nucleus enrichment and retention requirements for GdNCT. As in BNCT, accurate GdNCT treatment planning is highly dependent on reliable macroscopic (tumor sites) and microscopic (sub-cellular) gadolinium dose distributions. Monte-Carlo simulations of the photon dose generated upon thr gadolinium neutron capture using real patient MRI images and dosimetry on head phantoms suggests that the achieved radiation dose in the cancer tissue with respect to that in the healthy tissue is already superior to that attained in conventional radiotherapy without considering the contributions to the radiation dose from the internal-conversion and Auger—Coster—Kronig electrons. The RBE of tumor and normal-tissue radiation doses resulting from all radiation products of the gadolinium neutron capture for different intra- and extracellular gadolinium distributions must be estimated by microdosimetry programs specifically adapted to GdNCT. Neutron irradiation trials on *in vitro* glioblastoma multiforme cultures and *in vivo* mouse glioma models will contribute to the continuous iteration, verification, and validation of the microdosimetry and GdNCT treatment planning programs.

The macroscopic gadolinium distribution in the tumor and normal tissues can be inferred from MR images with quantitative relaxation mapping techniques. The microscopic intracellular and intranuclear gadolinium retention makes the internal-conversion and Auger—Coster—Kronig electron doses an important contribution to the overall tumor exposure to the radiation emitted *in situ* upon irradiation with neutrons. Several reliable techniques to investigate the sub-cellular *in vitro* and *in vivo* gadolinium uptake and retention have been developed using photoelectron emission spectro-microscopy (X-PEEM) on glioblastoma multiforme cultures incubated with different GdNCT agent candidates.[127—129]

Selected *in vitro* and *in vivo* studies

BNCT *in vitro* and *in vivo* trials. Studies on mouse M2R melanoma cells and rat C6 glioma cells showed significant differences in the retention of the BSH monomer (**1**) compared to that of its dimer, BSSB, which is readily formed from the BSH anion. The retention of BSSH was found to be at least six times as high as that of BSH.[130] These findings must be taken into consideration when evaluating the results of trials with BSH.

Reliable methods of quantitative ^{10}B imaging and microlocalization in tumor cell clusters and in single infiltrating tumor cells based on SIMS ion microscopy have been proposed using 9L gliosarcoma and F98 rat glioma tumor models as examples.[131] The results indicating an about double BPA uptake in tumor mass cells compared to the single infiltrating cancer cells reveals a consistent and reproducible drug microdistribution pattern after drug administration. Different drug infusion protocols have been evaluated in order to maximize the drug retention.

The responses of different cell populations in solid tumors to boron and gadolinium neutron capture was investigated by employing neutrons with two different energy spectra.[132] The neutron capture nuclide-labeled drugs were ^{10}BSH, ^{10}BPA, and the ^{157}Gd-based MRI contrast agent Omniscan®. The micronucleus frequencies in quiescent and total cell populations were monitored prior to and after the BNCT. With all drugs, the micronucleus frequency was found to be increased. The relative biological effectiveness (RBE) of neutrons compared to γ-rays was higher in quiescent cells than in total cells. The highest RBE values were observed at low cadmium ratio of the neutron beam. *p*-Boronophenylalanine (BPA) was shown to increase the micronucleus frequency of total cells to a greater extent than BSH. However, the sensitivity of quiescent cells treated with BPA was lower than that of the cells treated with BSH. This tendency was more noticeable at higher cadmium ratios of the neutron beam. In order to raise the quiescent cell sensitivity, irradiation of the tumor with high cadmium ratio neutrons is recommended.

The BPA biodistribution has been studied on a nude rat model bearing intracerebral implants of the MRA27

human melanoma cell lines using intracarotid BPA delivery after blood—brain barrier disruption.[133,134] Comparison of the BNCT treatment of these rats and the controls shows an impact of the optimization of BNCT drug delivery by permeabilization or disruption of the blood—brain barrier. The enhanced survival times and even lasting cure observed in *in vivo* trials were employed for planning the implementation of BNCT in human patients.

Boron Neutron Capture Synovectomy (BNCS) for arthritic diseases is under investigation at the Laboratory for Accelerator Beam Applications at the Massachusetts Institute of Technology.[135—137] This treatment* addresses the removal of the synovial membrane (synovectomy) of inflamed arthritic joints in rheumatoid arthritis patients that are unresponsive to medication or have to avoid surgical removal of the membrane. A dedicated BNCS beam line consisting of an accelerator target, a cooling system mounted in a neutron moderator, and a reflector assembly is now operational on a high-current tandem electrostatic accelerator.

GdNCT *in vitro* and *in vivo* trials. The radiotoxic Auger effect of the Gd nuclear capture reaction is based on the observation of DNA double-strand breaking in a plasmid DNA suspended in gadolinium chloride solutions.[138] The process was thought to involve complexation of the Gd^{3+} ion with the negative DNA strands. In support of this, it was found that in the presence of EDTA — a complexing agent used as Gd^{3+} scavenger separating the gadolinium atoms from the DNA strands — DNA strand did not break. This again is seen as evidence for the nanometer path lengths of the high-LET radiation products of the GdNC reaction.

It was demonstrated on glioblastoma spheroids (cell clusters simulating tumor structures) that the RBE of thermal neutrons alone was unexpectedly high (2—4) and produced a small malignant cell growth delay.[139,140] A 400 ppm concentration of Magnevist® (Gd-DTPA) in the culture medium induced a further two- to threefold enhancement of the RBE, leading to an enhanced growth delay by a factor of 2 to 3. This can be attributed to the effect of the high-energy γ (photon) dose and possibly the internal-conversion electron dose.

Significant tumor accumulation of gadolinium salts, prepared in a nanoemulsion formulation, was found in tumor-bearing hamsters.[141] It was also shown that intravenous drug administration was superior to intraperitoneal injection with regard to tumor uptake and retention times of the drug. Also, two consecutive injections with a 24-hour interval led to a doubled gadolinium uptake in the tumor, which was doubled again when a double drug concentration was administered.

* In collaboration with Newton Scientific, Inc. and Brigham and Women´s Hospital.

Table 3. Gadolinium nucleus uptake criteria (Q_{Gd}) determined for four GdNCT agents on TB10 GBM cell cultures

Contrast agent /radiation sensitizer	Q_{Gd}*	GBM cell nucleus uptake (% cells)
Magnevist® (Gd-DTPA)	0.2	2.5
Dotarem® (DOTA-Gd)	14	14
Gd-Motexafin®	2.5	92
Hoechst®-Dotarem®	20	100

* $Q_{Gd} = Gd_{intracelullar}/Gd_{extracellular}$, after 74-h exposure.

A tumor-targeting avidin-dendrimer-$(1B4M\text{-}Gd)_{254}$ was evaluated as a GdNCT agent in the treatment of peritoneal carcinomatosis.[142,143] An *in vitro* internalization study on SHIN3 human ovarian cancer cells using MRI showed a 50 times higher uptake of gadolinium with respect to that of Magnevist®. The specific drug accumulation in a SHIN3 timor model of nude mice produced a 366-fold increrase with respect to that of Magnevist®. The tumor tissue-to-normal tissue ratio of the SHIN3 uptake was 17 : 1 in all organs and increased to 638 : 1 one day after the intra-peritoneal drug injection. The absolute gadolinium concentrations in the tumor cells in *in vitro* and *in vivo* assays were 162 ppm.

The cell nucleus uptake of four gadolinium compounds was studied on glioblastoma multiforme cell cultures according to new GdNCT agent criteria.[122] The compounds investigated included Magnevist® (Gd-DTPA), Dotarem® (Gd-DOTA), Gd-Motexafin (Xcytrin®), and Hoechst®-Dotarem® (HDG). Whereas the standard MRI contrast agents, Magnevist® and Dotarem®, did not satisfy the criteria, the radiosensitizer Xcytrin® and the DNA-targeting contrast agent HDG did comply with the GdNCT agent criteria (Table 3). Moreover, the HDG compound demonstrated outstanding nucleus uptake ($Q_{Gd} = Gd_{intracellular}/Gd_{extracellular} = 20$) and 100% cell nuclei targeting ability compared to 92% for Xcytrin®. In particular, the gadolinium uptake quotient $Q_{Gd} = 20$ was reproducible at different concentrations of the culture medium. The inhibition of intimal hyperplasia (universal blood vessel lesion response) in rats upon continuous infusion of 0.5 μmol $(kg\ h)^{-1}$ of Magnevist® into the contralateral femoral vein and irradiation with a thermal neutron beam from a KUR-research reactor was reported.* These findings could result in the use of GdNCT in the prophylactic treatment of restenosis, complications following bypass operations, and other blood vessel injuries. Also, a histological comparison of GdNCT-treated and untreated blood vessels of rats reveals a 77% reduction in intimal hyperplasia and a 100% reduction of thrombosis of the treated rat population compared to the untreated rats with intimal hyperplasia.

* M. Takagaki, unpublished results, 2002.

Combined BNCT and GdNCT *in vitro* and *in vivo* trials. The biological and MRI efficiency of a gadolinium carborane complex proposed as a dual magnetic resonance imaging and boron carrier agent was studied in an *in vivo* assay on tumor-bearing Donryu rats.[144,145] The trial compared the uptake characteristics of the gadolinium carborane complex in comparison with Magnevist® (Gd-DTPA) using MRI, ICP-AES, and alpha-autoradiography techniques. Magnetic resonance imaging revealed a slower metabolization rate of the gadolinium carborane complex with respect to that found for Magnevist®. The results of the ICP-AES method indicated that the gadolinium carborane complex was taken up and metabolized more quickly by normal tissues, whereas it did not accumulate in tumor or brain tissue. The alpha-autoradiography showed that a high level of boron uptake was obtained in the internal organs and in necrotic tumor tissue.

Clinical trials

Early BNCT treatment programs. The clinical experience with NCT has been so far limited to BNCT. First BNCT trials were conducted[29—30] on ten terminal glioblastoma multiforme brain tumor patients between 1951 and 1953.* Water-soluble ^{10}B-enriched sodium borate (borax, $Na_2B_4O_7$), which was considered relatively nontoxic at therapeutic concentrations of up to 200 mg per kg of the body weight, was used as the BNCT drug. Dynamic biodistribution data revealed a tumor to normal tissue ratio of ^{10}B concentrations equal to 3 : 1 with an absolute ^{10}B uptake of 50 μg shortly after intravenous drug administration. Alarmingly, the ^{10}B concentrations in the blood were found to be as high or even higher than those in the tumor. All ten patients died from tumor recurrence without prolongation of survival times. This initial trial was followed by a second study in 1959 on sixteen glioblastoma multiforme patients using the newly commissioned compact 5 MW high flux water-moderated Brookhaven Medical Research Reactor. The median survival of these patients was comparable to the results achieved by photon irradiation and offered no proof-of-principle for the binary therapy concept. With hindsight it is clear that the drugs were not tumor-specific enough and the thermal neutrons did not possess deep penetration properties, resulting in neutron scattering and attenuation accompanied by severe radiodermatoses of the scalp and healthy brain necrosis. To avoid these side effects, a third clinical trial with nine patients was carried out with drug administration (sodium pentaborate) *via* intra-carotid injection prior to neutron irradiation. The next seventeen patients treated between 1959 and 1961 benefited from additional neutron shielding using ^{6}Li sheets to avoid skin burns.

Further clinical trials were conducted on eighteen patients using two ^{10}B-carriers, namely, ^{10}B-enriched 4-phenylboronic acid and disodium decahydrodecarborate ($Na_2B_{10}H_{10}$), one of the first polyhedral borane anions to be synthesized and characterized.* All of these new patients additionally underwent surgical tumor excision prior to later open-skull thermal neutron irradiation. However, these also proved unsuccessful. The dismal trial outcome using these procedures led to more intense search for boron carriers that eventually led to sodium borocaptate $Na_2[B_{12}H_{11}SH]$, better known as BSH. After the total failure of the first pioneering BNCT programs in patients from 1951 to 1961, no more clinical trials were authorized in the United States. For the next decades, all BNCT research in the US would focus on the development and optimization of BNCT agents through biological *in vitro* and *in vivo* feedback.

After the first ill-fated BNCT patient trials in the US, BNCT was brought to Japan in 1968 by the neurosurgeon Hiroshi Hatanaka.[146—149] Outstanding results were achieved on 38 patients with grade III and IV glioblastoma multiforme. All but one atypical case showed statistically significant improvement with a mean survival of 44 months and a median of 26 months. All patients received BSH as the boron-carrier and corticosteroids to control side effects of the treatment. The neutron irradiation was performed on the exposed fringe of brain tumor immediately after tumor resection. An impressive five-year survival rate equal to 58% for the grade III and IV glioma patient group was attained. Although Hatanaka´s work still remains controversial as regards the exact diagnosis of many of his patients and also the exact chemical structure of the active ingredient of the BSH drug solutions, it represents an unprecedented milestone in the BNCT and rekindled global interest in the BNCT. According to the report on long-term survivors, among the 120 patients treated with BNCT by Hatanaka up to 1992 (119 of them had an intracranial tumor and one an extracranial nerve-related tumor), the number of five-year survivors was 18, half of these patients surviving more than ten years. Later, 105 glial tumor patients treated with BNCT between 1978 and 1997, and 159 patients treated between 1977 and 2001 were included in the evaluation of various aspects of the therapy and patient survival.[150,151] A new treatment protocol prescribes a minimum tumor dose of 15 RBE Gy, whereas the maximum vascular dose must not exceed 15 RBE Gy. The total γ-ray dose that pertains to the core γ-rays from the reactor itself and from boron neutron capture in the tissues should be kept lower

* The works were performed between 1951 and 1953 by W. H. Sweet, L. A. Farr, and others at the Brookhaven Graphite Research Reactor at Brookhaven National Laboratory in collaboration with the Massachussetts General Hospital.

* Clinical trials were conducted on eighteen patients at the Massachussetts General Hospital and Massachussetts Institute of Technology Research Reactor facility from 1961 to 1962.

than 10 RBE Gy. An equally important BNCT program using BPA as a boron carrier on patients with malignant melanoma was conducted in Japan as of 1987 with discreet success.[152]

Current clinical phase I/II studies. The BNCT program in the United States was resumed in 1994, preferentially using BSH and higher-energy epithermal neutron beams. As pointed out earlier, these neutrons have better penetration depth across the bone than thermal neutron beams and should be more effective in the treatment of deep-seated tumors. A study on 22 patients with brain tumors who were treated with an infusion of a fructose-based BPA solution (BPA-f) in order to determine the maximum tolerated BNCT radiation dose to the normal brain tissues and prepare subsequent BNCT trials with a new high-intensity and high-quality epithermal neutron beam was reported.[153] The treatment was planned using MacNCTPlan and MCNP 4B software. The average dose to the brain ranged from 2.7 to 7.4 RBE Gy, while the average tumor dose was estimated to range form 14.5 to 43.9 RBE Gy with a mean of 25.7 RBE Gy.

BNCT trials (clinical phase I/II) of a total of 53 patients were treated using BPA as BNCT drug were carried out between 1994 and 2000 at the Brookhaven National Laboratory.[154] The upper limit of the safe dose was estimated to be approximately 6 photon-equivalent Gy (RBE Gy). An average brain dose of 6.7 RBE Gy or higher resulted in minor side effects like somnolence but also grade 2 and 3 toxicity according to the EORTC/RTOG common toxicity criteria. The median time to progression of the disease for patients irradiated on one tumor field is 34.5 weeks; this decreases to 18 weeks for the patients having received a 3-field irradiation treatment.

The results of clinical trials (phase I) carried out on 24 patients with intracranial tumors (GBM and melanoma) using BPA as the BNCT agent were critically evaluated.[155] The two patients with melanoma showed a complete tumor response, whereas 13 of the 17 patients with glioblastoma multiforme displayed significant tumor volume reduction.

In Europe, three facilities offer currently BNCT treatment on the basis of clinical phase I/II. Using an NRG research reactor at Petten (Netherlands), the BSH uptake by the tissue in the course of the EORTC-11961 clinical trial (phase I) was studied with 30 patients.[156] The preliminary conclusion states that BSH doses were well tolerated but possibly chosen too conservatively because of toxicity concerns.

Treatment of 17 patients using BPA as the BNCT agent at the Studsvik research reactor facility (Sweden) was reported.[157] No significant drug or radiation-related toxicities were noted. Survival data are not yet available, as these trials have been concluded only recently.

A VTT Processes reactor in Finland was employed to treat 18 patients with supratentorial GBM tumors using BPA.[158] The radiation doses ranged from 30 to 61 RBE Gy, and the average normal brain dose varied between 3 and 6 RBE Gy. The overall one-year survival was reported to be 61%.

The BNCT treatment carried out in 2002 at the National Institute of Nuclear Physics (Italy) on a patient with 14 malignant liver tumors deserves attention.[13] The 21-hour operation included BPA-f infusion, liver explantation, BNCT on the *ex-situ* organ at the nuclear reactor facility, and finally re-implantation of the treated organ. The patient thus did not receive any radiation on other organs that might have absorbed BSH while the explanted liver was treated with a uniformly high neutron dose. At 16 months after the therapy, the patient is alive, generally doing well and without tumor recurrence.

Conclusion

Neutron Capture Therapy for brain cancer demands a concerted multidisciplinary treatment effort. The very nature of the particularly deadly glioblastoma multiforme and anaplastic astrocytoma brain tumors, which are virtually resistant to all types and combinations of known therapies, presents a host of medical complications. These must be attacked with tailor-made treatment planning, in particular, variation of irradiation volumes, surgical procedures, neutron flux and, external radiation doses correlated with the neutron triggered internal radiation doses. The complexity of the neuropathologies accounts for a number of surgical procedures of brain tumor excision, various drug administration techniques, and numerous irradiation protocols and treatment plans. More sophisticated model studies on animal tumors and normal tissue tolerance evaluations play an important role in the studies of drug pharmacokinetics and dose response in the context of preliminary treatment simulations. Complex calculations of the brain irradiation dose using head phantoms for dosimetry and radiation dose distributions generate personalized brain tumor treatment plans that are based on extensive pharmacological and MRI-feedback

The interplay of a high number of parameters and variables makes NCT a very challenging and rewarding venture that brings very different scientists together: the improvements of single aspects contribute to the improvement of other correlated aspects of NCT. The clinical results achieved by BNCT are, at least, equivalent, if not superior to the standard treatments available. NCT vastly contributes to improving the quality of life of the patients by drastically reducing the number of treatments compared to standard chemotherapy and radiotherapy courses. Finally, an overdue breakthrough at the border of chemistry and microbiology is imminent, with excellent chem-

istry research groups that synthesize and optimize NCT drug prototypes.

For as long as no significant tumor control, let alone cure, for a number of fatal cancers is available through current therapies, there will be a need for new therapy approaches such as NCT. NCT in the forms of BNCT and possibly GdNCT is based on a solid binary treatment concept, which has every chance to be successful with the intense drug design and optimization research, which is currently underway. More specific and selective NCT agents will lead to improved boron or gadolinium carrier dosimetry and better treatment planning software. The more that is known about the complex medical and pharmacological requirements for NCT at the atomic, molecular, microbiological, physical, and biophysical level, the better the treatment standards will become. The tumor specificity and selectivity of the drug remains a central issue not only for NCT but for the majority of other treatment modalities.

It would make sense to combine NCT or NCTEFNT with proton therapy, for example, within the future Hadron Therapy Facilities.[159] It remains to be seen whether a combination of the neutron capture reaction and fast neutron tumor bombardment would produce the anticipated enhancement of the radiation effect. Again, drug specificity remains a key issue. The use of combined boron and gadolinium carriers is certainly attractive, especially with regard to MRI monitoring of the drug uptake. A combination of the two NCT reactions is feasible with the use of NCT drug cocktails combining individual BNCT and GdNCT agents. Currently, this approach appears more useful from pharmacological viewpoint than looking for a boosted therapeutic effect exploiting the BNC and GdNC reactions within a single boron gadolinium carrier.

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