



UNIVERSITÀ DEGLI STUDI DI TORINO

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Insights into the use of gadolinium and gadolinium/boron-based agents in imaging-guided neutron capture therapy applications

This is a pre print version of the following article:							
Original Citation:							
Availability:							
This version is available http://hdl.handle.net/2318/1589423	since 2016-10-28T15:12:30Z						
Published version:							
DOI:10.4155/fmc-2016-0022							
Terms of use:							
Open Access							
Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from convright							

(Article begins on next page)

protection by the applicable law.



UNIVERSITÀ DEGLI STUDI DI TORINO

This is an author version of the contribution published on: Questa è la versione dell'autore dell'opera: [Future Med Chem. 2016 May;8(8):899-917. doi: 10.4155/fmc-2016-0022.]

The definitive version is available at: La versione definitiva è disponibile alla URL: [http://www.future-science.com/doi/10.4155/fmc-2016-0022]

Abstract

Gadolinium neutron capture therapy (Gd-NCT) is currently under development as an alternative approach for cancer therapy. All of the clinical experience to date with NCT is done with ¹⁰B, known as BNCT, a binary treatment combining neutron irradiation with the delivery of boron-containing compounds to tumors. Currently, the use of gadolinium for NCT has been getting more attention because of its highest neutron cross-section. Although Gd-NCT was first proposed many years ago, its development has suffered due to lack of appropriate tumor-selective Gd-agents. This review aims to highlight the recent advances for the design, synthesis and biological testing of new Gd- and B-Gd-containing compounds with the task of finding the best systems able to improve the NCT clinical outcome.

Keywords:

Gadolinium Neutron Capture Therapy, Boron Neutron Capture Therapy, MRI, Gd contrast agents, Tumor Treatment, Nanosized Gd agents.

1. General introduction on NCT: rationale and application

Neutron Capture Therapy (NCT) is a non-conventional radiotherapy that combines low energy neutron irradiation with the presence of a neutron-absorbing substance at the targeted cells [1-2].Two isotopes are of major interest because of their large capture cross section: ¹⁰B and ¹⁵⁷Gd. In the case of ¹⁰B, the non radioactive isotope captures neutrons and disintegrates into alpha particles and lithium nuclei that cause non reparable damage to the cell where they were generated [3]. Thus, this technique permits the selective damage of tumor cells without affecting adjacent healthy cells if ¹⁰B atoms are selectively accumulated intracellularly. This makes BNCT a promising option for the treatment of infiltrating tumors and disseminated metastasis that cannot be treated by methods requiring a precise localization of the mass, such as surgery or conventional radiotherapy. Exploiting a thermal neutron flux of the order of 10⁹n/cm²s (value easily attainable at the presently working NCT facilities) it has been estimated that approximately 10-30 µg of boron per gram of tumor mass are needed to deliver therapeutic doses of radiation in the tumor without exceeding the normal tissue tolerance using an irradiation time equal or less than 1 hour. The selective delivery to tumor cells is crucial to increase the amount of internalized boron, while maintaining a low concentration in surrounding healthy tissues and in blood to minimize damage. Currently, two BNCT drugs are available for clinical investigation namely: i) Lparaboronophenylalanine (BPA), that has been used in clinical trials to treat glioblastoma[4-5]. head and neck recurrent cancer[6-7], melanoma[8] and adenocarcinoma liver metastases[9]; ii) sodium mercaptoundecahydro-closo-dodecaborate (BSH) that has been investigated for the treatment of malignant glioma[10]. Despite their clinical use and the safe and effective BNCT, trials performed with these two drugs until now, both BPA and BSH show low selectivity and great efforts have been made by several research groups to develop new and more selective boron delivery agents[11-12]. The use of BNCT to destroy malignant cells was proposed long time ago[13]. In spite of huge promises, it has not attained an established position in the mainstream medicine. However, the methodology has never been discarded and it remains in an intermediate state with a small group of enthusiast supporters and many critical spectators. The reasons why BNCT has not maintained the original promises rely mainly in the lack of properly designed agents. The conditions for an effective therapeutic output are well established: the BNCT agent must reach selectively a given concentration inside cells and the not-attainment of this task makes the therapy less effective. Only a proper NCT agent design (based on an improved knowledge on how molecules enter healthy and diseased cells) allows to reach selectively the needed threshold of intracellular concentration required to drive the breakthrough of BNCT in the field of conventional and diffused treatments for cancer. Moreover, the in vivo assessment of the amount of NCT agent can now be tackled by its conjugation to a suitable imaging-reporter.[14-15] To date there is no non-invasive means to evaluate boron concentration in tumor and healthy tissues of the subject undergoing the irradiation. Thus, dose calculations are based on boron content values in blood,

tumor, and normal tissue obtained from biodistribution studies performed beforehand. One of the problems is that the boron distribution varies from patient to patient and that large uncertainties exist in the tumor-to-blood ¹⁰B concentration ratio. The possibility of detecting the *in vivo* localization of BNCT agents by highly sensitive, non invasive imaging protocols greatly enhances the chances of success of this therapeutic treatment as it allows to determine the optimal neutron irradiation conditions, i.e. when the concentration of BNCT agents at the tumor nodule is the highest possible.

Currently, the use of gadolinium as NCT agent has been getting more attention because of its highest neutron cross-section (255,000 barns for the ¹⁵⁷Gd isotope), which is around 65 times larger compared to boron thermal neutron cross-section[12]. Although the idea of Gd-NCT was first formulated in the 1980s [16,17] its development has suffered due to lack of appropriate Gd containing tumor-selective agents. Another advantage of Gd-NCT is that several Gd based compounds are commonly used, in clinical, as Magnetic Resonance Imaging (MRI) contrast agents, which makes them "ideal" theranostic agents able to integrate neutron therapy and MRI diagnosis for an improved personalized treatment of the patient. In fact, since the signal intensity enhancement of a T₁-weighted MRI image is directly proportional to the Gd-based contrast agent (Gd-CA) concentration in the target tissue, by comparing images recorded before and after Gd-CA injection, it is possible to estimate Gd concentration at the pathological site and in the other organs and on this basis to set-up NCT therapeutic protocols (irradiation time and duration).

This review aims to highlight the recent advances in Gd selective and efficient delivery methods and the preclinical outcome of Gd-NCT with the task of finding the best system to improve preclinical applications.

2. Gd as an alternative neutron capture agent

The probability of an element to absorb thermal neutrons is represented by its neutron absorption cross section (σ_{abs}). Naturally occurring Gd is composed of 6 stable isotopes two of which are of particular interest to NCT for their high neutron absorption cross sections: ¹⁵⁵Gd (σ_{abs} = 55000 b; 14.8%) and ¹⁵⁷Gd (σ_{abs} = 255000 b; 15.7%)[2].

Gd provides two main advantages with respect to boron, namely i) the possibility to follow the agent biodistribution through the body via MRI, thus allowing a theranostic approach to cancer treatment [18], and ii) the highest absorption cross section for thermal neutrons among stable elements (¹⁵⁷Gd). Precise determinations of the intratumor Gd concentration would facilitate the optimization of the neutron irradiation duration reducing damage to normal organs. The advantage of a high neutron absorption cross section of Gd is that the same number of capture reactions obtained with a given amount of boron can be produced by ¹⁵⁷Gd with a significantly lower thermal fluence or lower concentrations of the NCT agent. With lower amounts of NCT agent, the tissue

toxicity related to the compound administration is reduced and with a lower fluence the dose absorbed by normal tissues is reduced as well. Nonetheless, this advantage is counterbalanced by the non local energy deposition of the secondary radiations emitted by the neutron capture reaction of ¹⁵⁷Gd. The ¹⁵⁷Gd neutron capture reaction forms excited ¹⁵⁸Gd* that generate complex innershell transition with long mean free paths in tissues, internal-conversion electrons (ICEs) and, finally, Auger electrons together with characteristic X-rays (scheme 1).

The mean energy per capture reaction of γ rays is of 2.4 MeV with mean free paths in tissue of several tens of cm. On the other side and despite their low mean energy per capture reaction (approximately 4 keV), Auger electrons are high-LET particles as a consequence of their short ranges in tissues of few nm, having an average LET of 300 keV/µm, and can induce highly lethal damages in the cell only when ¹⁵⁷Gd directly targets DNA molecule or other vital organelles (e.g., mitochondria). Due to the limited amount of energy transferred by the ICEs and Auger electrons, the neutron Kerma factor (a physical quantity representing the energy transferred by neutrons to charged particles through their nuclear reactions) of the capture reactions on ¹⁵⁷Gd is significantly lower than that of ¹⁰B capture reaction. As consequence, the ¹⁵⁷Gd concentration required to deposit a certain dose, that means a certain biological effect, is typically one order of magnitude higher than the ¹⁰B concentration needed to get the same result.



Scheme 1: Gd neutron capture reaction

The emission of secondary γ rays depositing energy over a longer path length than the boron reaction products limits the selectivity of the therapy and could be considered as a drawback of Gd-NCT. However, if ¹⁵⁷Gd uptake is strictly limited to tumor bulk and considering a tumor volume of the order of some cm³, these γ rays can produce an additional positive effect that is to increase the radiation dose to the tumor also if the NCT agent is not intracellularly distributed, lowering the requirement of uptake by the cell nucleus.[19,20].

The optimal ¹⁵⁷Gd concentration in tumors for Gd-NCT was reported to be 50–200 μ g/g of tumor tissues but less than 1000 μ g/g, since neutron fluence rapidly decrease in the depth of the tissue due to high absorption of neutron by gadolinium atoms [21]. Gd-NCT has been performed in many tumor animal models (mouse, rat, rabbit, canine) but to date this therapy has never been proved in human clinical trials.

3. Gd as MRI contrast agent

MRI is a non-invasive technique and can provide detailed information on the anatomy, function and metabolism of tissues in vivo with a superb anatomical resolution. The amount of intrinsic contrast between tissues normally produced in a MR image depends on differences in the T1 and/or T2 relaxation times of the tissues under observation. Pathologic tissues may not have significant differences in T₁ or T₂ from the surrounding normal ones. This signal difference can be increased through the administration of a contrast agent. Approximately 25-30% of all MRI scans today use some kind of non-specific Gd based contrast agents (Gd-CA) [22] that typically makes appear diseased tissue brighter. The high magnetic moment of the Gd ion, the presence of 7 unpaired electrons and a fast exchanging coordinated water molecule are essential to providing contrast. The most representative class of contrast agents is represented by polyaminocarboxylate complexes of Gd³⁺ ion[23]. The ligands are multidentate (seven or eight donor atoms) in order to strongly limit the release of free metal ions that are highly toxic because they interfere with Ca²⁺ pathways. The coordination cage of the Gd³⁺ ion is completed with 1-2 water molecules that are responsible for transferring the paramagnetic properties to the overall bulk water molecules through chemical exchange. The ability of a Gd chelate to affect the water proton relaxation rates is represented by the relaxivity value (i.e. the relaxation enhancement of solvent water protons in the presence of 1 mM concentration of the paramagnetic ion). In a proton MR image there is a direct proportionality between the observed relaxation enhancement and the concentration of the paramagnetic MRI reporter. Thus these agents can be exploited to carry out Gd quantification at the pathological tissue. All Gd-CA are administered intravenously and rapidly equilibrate in the intravascular and interstitial compartments (extracellular compartment). Then, depending on their structure, they will be cleared by the liver or kidneys by passive diffusion or specific uptake processes[24]. Most Gd-CA are approved at a dose of 0.1 mmol Gd/kg. Clinically available Gd-CA contrast agents can be classified on the basis of their distribution in: i) extracellular fluid agents, ii) liver agents, and iii) intravascular or blood pool agents. For these Gd-CA, there is no intracellular distribution except for, in particular cases, the liver through organic anion transporters (OATP). Upon injection, extracellular fluid agents (Figure 1) quickly distribute to the extracellular space. The terminal half-life for blood elimination is about 1.5 h for all these compounds when administered to subjects with normal renal function [24] and they are excreted almost exclusively renally by glomerular filtration. Liver agents, which bring a benzylic group to increase the liver uptake, (Figure 2) have a partial hepatobiliary elimination. Gd-BOPTA (gadobenate dimeglumine, Figure 2) has a terminal blood half-life on the order of 1.5 – 2 h in healthy subjects. For Gd-EOB-DTPA (gadoxetic acid disodium, Figure 2) acid the blood half-life is shorter, about 1 h, and this is probably due to the significant liver uptake of the compound by hepatocytes.



Figure 1: Chemical structures of Gd based extracellular fluid agents.



Figure 2: Chemical structures of Gd based liver complexes.

There is only one blood pool agent approved in the European Union and the USA for peripheral MR angiography (MS-325, gadofosveset trisodium, Figure 3). It is functionalized with a lipophilic biphenylcyclohexyl group with high affinity to serum albumin. The terminal plasma half-life of

gadofosveset was 18.5 h which is considerably longer than other contrast agents as a consequence of the binding with albumin and the absence of liver clearance.



Figure 3: Chemical structure of MS-325 complex

In patients with advanced kidney disease, exposure to the less stable Gd-CAs can produce a chronic fibrosing condition (nephrogenic systemic fibrosis, NSF) that involves skin, muscles and other organs due to the injury caused by Gd³⁺ cations release and deposition in tissues.[25] The inadequate Gd-CA clearance caused by renal failure could trigger the development of NSF. Molecular, cellular, and animal studies have provided great insight into the pathophysiologic mechanisms of NSF. The prevailing hypothesis is that free Gd released from the less stable Gd-CAs activates the NLRP3 inflammasome, with resulting production of IL-1ß and presumably of other profibrotic cytokines [26]. NSF can be prevented by avoiding the use of less stable Gd-CA, such as Gd-DTPA-BMA in patients with compromised renal function. Since successful treatment of NSF are limited, its prevention is fundamental. In current practice, Gd-CAs have been considered safe when used at clinically recommended doses in patients without severe renal insufficiency[27]. By performing either unenhanced or reduced-dose-enhanced studies and by using the most stable contrast agents, in particular in the case of patients with renal failure, NSF has been significantly reduced since 2009. Although nanosized Gd-CAs have demonstrated enhanced MRI contrast efficacy, their translation to the clinic has often been hampered by their slow, and sometimes incomplete excretion that causes an increase of tissue retention and consequently of the toxic side effects relating to Gd release [28]. Recent efforts for the development of macromolecular Gd-CAs have focused on introducing biodegradable materials and/or linkers. Biodegradable Gd

nanocarriers maintain the ability to carry high payloads of Gd, with the advantage that, following intravenous administration they are degraded into smaller species that can be easily excreted. Furthermore, by using selectively targeted nanocarriers, endowed with high accumulation at the target site, it is possible to reduce the injected dose and therefore the potentially toxic macromolecular Gd–CAs unspecifically distributed in the body.

4. Small sized Gd chelates for Gd-NCT

First attempts to test the use of Gd derivatives as NCT agents concerned commercially available MRI agents. One of the first papers reporting the application of Gd chelates as NCT agents was proposed by the research group of Brugger.[16] In this study, Gd-DTPA (gadopentetate dimeglumine, Figure 1) and Gd-DOTA (gadoterate meglumine, Figure 1) were used for the treatment of brain tumors by intravenous injection. It was demonstrated that up to 300 µg of ¹⁵⁷Gd/g of tissue could be achieved in brain tumors with these Gd derivatives. This concentration ¹⁵⁷Gd/g to 800 of Gd-EDTMP could be increased up μg using (ethylenediaminetetrabismethylenephosphonate, Figure 4). Moreover, Gd concentration calculation and measurements showed a good tumor to normal tissue ratio.



Gd-EDTMP

Figure 4: Chemical structure of Gd-EDTMP complex.

One year later, a short communication by Kanda and co. reported the results of the continuous infusion of Gd-DTPA through a branch of the left femoral artery of New Zealand white rabbit with VX-2 tumors growing in hind legs[29]. Although no differential distribution of Gd was achieved between the tumor and its adjacent normal tissues, the Gd concentration in the infused tumor was approximately 5-6 folds higher than that in the contralateral tumor. Growth of Gadolinium-infused tumors was significantly inhibited compared to that of control tumors between the 16th and 23rd

days after treatment. In vivo Gd-DTPA therapeutic effect in rats with Jensen sarcomas was also reported[30]. Gd-DTPA was administered directly into the tumor prior to neutron irradiation. Intratumoral administration of 13,750 ppm gadolinium and subsequent neutron irradiation significantly increased the tumoricidal effect (i.e., decrease of tumor growth up to a complete regression of the tumors in about 80%). More recently, Hosmane and coworkers [31] observed the significant survival prolongation of 9L brain tumor rat by intra-venous injection of Gd-DTPA. Mean survival via Gd-NCT was 33.5 ± 3.0 days, and that of control rats was 16.4 ± 0.6 days. The maximum contribution of γ rays on tumor absorbed dose was less than 50%. Gd-BOPTA (Figure 2), another clinically used Gd MRI contrast agent, was tested and demonstrated to be an efficient targeting agent for neutron capture therapy[32, 33]. Four groups of rat tumor models were subcutaneously injected with gliosarcoma cells (9L cell line) in both hind legs with the same dose of Gd-BOPTA and Gd-DTPA directly into the tumor. The Gd-BOPTA group showed significantly longer tumor growth delay than that of DTPA as determined in vivo by MRI and confirmed ex vivo by ICP-MS Gd measurements. In the author's opinion, the stronger enhancing effect of Gd-BOPTA respect to Gd-DTPA was partially due to the weak binding affinity of this agent for albumin, which increased its tumor retention. Therefore, Gd-BOPTA was proposed as an effective targeting compound for NCT. Further interesting in vitro results, on the use of Gd-DTPA as potential agent for Gd-NCT, were reported by De Stasio et al. [17] The microdistribution of Gd in cultured human glioblastoma cells exposed to Gd-DTPA was observed and it was demonstrated that not only it penetrated in the plasma membrane of the cells but accumulated at higher concentration in the nucleus than in the cytoplasm; moreover no deleterious effect on cell survival was detected without neutron irradiation. The therapeutic gain of Gd-NCT with the imaging resonance contrast agent Gadobutrol (Gd-DO3A-butrol, Figure 1) was evaluated through in vitro and in vivo studies [34]. Human malignant melanoma cells were irradiated in the presence or absence of Gd-DO3A-butrol. These studies showed a Gd-dependent delay of cell proliferation as a consequence of neutron irradiation. In vivo tests were performed on tumor-bearing nude mice. The tumor site was irradiated after intratumoral injection of Gadobutrol. Cell proliferation was inhibited and tumor growth was delayed; moreover, in the irradiated animals only transient skin damage was observed. Therapeutic gadolinium-containing agents are also known to acts as a radiosensitizer in the treatment of diseases, such as cancer. In 2010, Rendina and coworkers reported the first example of gadolinium delivery to a tumor-cell nucleus by a platinum complex[35]. They presented a new Pt-Gd complex that can effectively target the nuclei of tumor cells by means of a functionalized DTPA ligand linked to two {PtII (terpy)} (terpy= 2,2':6',2"-terpyridine) (PtDTPA, Figure 5) units that have the capacity to bind DNA in an intercalative manner. They reasoned that the related Pt-Gd species PtDTPA would have the capacity to deliver gadolinium to DNA in order to fully exploiting Auger electrons toxic effects.



Figure 5: Chemical structure of the functionalized DTPA ligand linked to two {PtII (terpy)} (terpy= 2,2':6',2"-terpyridine) PtDTPA

Cellular uptake of PtDTPA was determined by means of ICP-MS analyses of A549 human lung carcinoma cells and human aortic endothelial cells. The results indicate that tumor cells showed a capacity to accumulate PtDTPA by at least one order of magnitude higher than that of human endothelial cells.

A theranostic agent Gd-DO3A-BTA (Gd-DO3A-benzothiazole-aniline, Figure 6) functionalized with a benzothiazole derivative with high antitumor activity was synthesized and tested. [36] The biodistribution pattern of *in vivo* MRI compares well with those of DTPA-based, liver-specific MRI Gd-CAs. Gd-DO3A-BTA revealed antitumor effect against three cell lines such as MCF-7, MDA-MB-231, and SKHEP-1 and all of them revealed apoptotic characteristics. MR images of fractionated cytosols and nuclei confirmed that the Gd complex was intracellular as well as tumor-specific. Moreover, the amount of Gd accumulated in the tumor site for 24 h was 40–220 µg Gd/g tumor which falls within the range of the optimal amount (50–200 µg Gd/gtumor) for Gd-NCT. Thus this complex is a good candidate for Gd-NCT that will improve the therapeutic effect of the benzothiazole-aniline conjugated with the complex.



Figure 6: Chemical structure of Gd-DO3A-BTA complex

5. Nano sized Gd carriers for Gd-NCT

The success in Gd-NCT depends on the ability to deliver and maintain, during the neutron irradiation, sufficient amount of gadolinium in tumor tissues. Commercially available MRI contrast agents, introduced for the first as Gd-NCT agents, are not specifically targeted to tumor cells and, during neutron irradiation, due to their fast clearance, can not be retained in tumor tissues in an effective Gd concentration [24]. Therefore, continuous infusion or intratumor injection of Gd-CAs are mandatory in order to reach the concentrations needed for a therapeutic outcome. Thus, for the optimization of Gd-NCT, effective drug delivery systems are needed[37].Furthermore, the systemic distribution of the clinically approved contrast agents can induce unnecessary exposure of healthy tissues to y-rays generated during the neutron capture reaction. Since the literature about the use of nanoparticles to deliver selectively drugs, vaccines and imaging agents [38-39] is increased exponentially in the last years, they appear good candidates for the specific delivery of Gd-based NCT agents to tumor cells. Moreover, nanoparticles, with a diameter ranging from 50-100 nm can be loaded with a large amount of Gd atoms to be delivered specifically into tumors thus avoiding intratumor injection endowed with many disadvantages. In fact, the most appropriate Gd administration is intravenous (i.v.), because it allows the accumulation into tumors through active (receptor mediated endocytosis) or passive (enhanced permeation retention, EPR) targeting while avoiding systemic distribution in normal tissues. Furthermore, a lot of Gd containing nanoparticles, with improved accumulation properties, were developed for MR molecular imaging applications [40-41] and they can be exploited without any modification for Gd-NCT therapeutic protocols[42]. Table 1 reports many examples found in the literature of nanocarriers designed for delivering Gd-based NCT agents to tumor tissues to achieve effective treatment based on precise imaging-guided thermal neutron irradiation. Some of them have been used for in vivo tumor irradiation. Most of the nanoparticles reported in Table 1 are loaded with clinically used Gd-CA and in particular with Gd-DTPA. As expected, the highest intratumor concentrations have been obtained after intra tumor (500-1800 ppm) or peritumoral subdermal Gd injection (466 ppm). Fukumori Y. and coworkers [43,44] observed a significant reduction of tumor growth after neutron irradiation using Gd-DTPA-loaded chitosan nanoparticles (intratumor injected) that it was not observed after injection of the same amount of free Gd-DTPA due to its lower tumor retention. This result evidenced that the incorporation of Gd-DTPA in a nanosized particle increases significantly its retention time in the tumor mass and at the same time, the presence of positive charges on the external surface of chitosan increases cell interaction and uptake. Mi P. and coworkers obtained similar results comparing Calcium phosphate micelles incorporating Gd-DTPA i.v. administrated [45,46]. Also in this case, only nanoformulated Gd-DTPA (Gd-DTPA/CaP) showed an effective suppression of tumor growth without loss of body weight, indicating the potential of Gd-DTPA/CaP

13

for safe cancer treatment (Figure 7). Furthermore, as a consequence of the increased accumulation of Gd-DTPA in tumors, a selective contrast enhancement of tumor tissues was observed for precise tumor location by MRI.

Nanoparticle	Gd- complex	Size (nm)	Active/Passive targeting	Tumor Model (<i>in vivolin</i> <i>vitr</i> o study)	Route of administration	NCT test	Intratumoral Gd concentration	Ref
liposome	GdHPDO3A	100- 300	Passive	Colon-26 Tumor bearing mice (<i>in vivo</i>)	i.v.	yes	40.3 μg/g	37
liposome	Gd-DTPA	136- 152	Active (Folate or DOTAP)	F98 rat glioma and LN229 Human glioblastoma cell lines <i>(in vitro)</i>	-	yes	F98 ((Folate: 250ng/10 ⁶ cells; DOTAP: 3000ng/10 ⁶); LN229 (Folate: 200ng/10 ⁶ cells; DOTAP: 2000ng/10 ⁶);	47
liposome	Gd-DTPA	<150	Passive	TC-1 Lung Tumor bearing mice (in vivo)	i.v.	no	158.8 μg/g	21
Ca/P	Gd-DTPA	60	Passive	Colon-26 tumor bearing mice (<i>in</i> <i>vivo</i>)	i.v.	yes	8 (single i.v.) / (double i.v 16 μg/g	45
Ca/P	Gd-DTPA	60	Passive	Colon-26 tumor bearing mice (<i>in</i> <i>vivo</i>)	i.v.	yes	4% ID/g of tumor	46
Avidin- dendrimer- (1B4M-Gd)254	Gd-DTPA	-	Active (Avidine)	SHIN3 human ovarian cancer bearing mice (<i>in</i> <i>vivo</i>)	i.v.	no	162 μg/g	48
Chitosan	Gd-DTPA	391, 214	Positively charged particles	B16F10 C57 tumor bearing mice (<i>in</i> <i>vivo</i>)	Intra tumor	yes	500 and 1500 ppm for 391 and 214 nm sized particles	43
Chitosan	Gd-DTPA	430	Positively charged particles	B16F10 C57 tumor bearing mice (in vivo)	Intra tumor	yes	1800 ppm (double administration)	44
Chitosan	Gd-DTPA	nd	Positively charged particles	Malignant fibrosis histiocytoma(MFH) cell-line (in vitro)	-	no	30.5mg in a cell flask	49
Chitosan	Gd-DTPA	426	Positively charged particles	L929 fibroblast, B16F10 and SCC- VII cell lines. <i>(in vitro)</i>	-	no	18.0, 27.1, 59.8 mg Gd/10 ⁶ cells for L929 fibroblast, B16F10 and SCC-VII cells, respectively.	50
Microemulsion	Gd-	85	Active (Folate)	KB tumor bearing	i.v.	no	33 μg/g	51
GdFeO3/Fe3O4 /SiO2	GdFeO3	60	Passive	- mice (<i>in vivo)</i>	-	no	-	52
Gd/Co-carbon nanoparticles GdCo@CNPs	Gd-oxide	20- 50	Active (Folate)	Hela (human cervice cancer) cell line <i>(in vitro)</i>	-	yes	-	53
G6 dendrimer	Gd-DTPA	9	passive	sentinel lymph nodes and PT-18 (murine mast cell line) micrometastasis on mice models. (<i>in</i> <i>vivo</i>)	peritumoral subdermal injection	no	466 μg/g	54
Gd@C82-PEG-b- PAMA metallofullerenes	Gd	15– 25	Passive	colon-26 cell line (in vitro)	-	yes	-	55
chylomicron emulsion	Gd acetyl acetonate	100	Passive	WT Balb/C mice (in vivo)	i.v.	no	-	56

Table 1: Nanocarriers designed for delivering Gd-based NCT agents to tumor tissues



Figure 7: Reprinted with permission from ref 46 (Mi P, Dewi N, Yanagie H et al. Hybrid calcium phosphate-polymeric micelles incorporating gadolinium chelates for imaging-guided gadolinium neutron capture tumor therapy. ACS Nano. 9(6), 5913–5921 (2015)). Copyright 2015 American Chemical Society

Nanoparticles injected intravenously showed a lower intratumor concentration, ranging from 8 to 162 ppm. Some of them were actively targeted to tumor cells using folate [47,51,53], positive charges [43,44,49,50,51] or avidin.[48] All the reported studies used naturally occurring Gd containing only the 15.7% of ¹⁵⁷Gd thus suggesting that the therapeutic outcome can be improved by using the ¹⁵⁷Gd enriched isotope.

6. Combination of Gd/B agents

The two most diffused forms of Neutron Capture Therapy, BNCT and Gd-NCT, exploit very different secondary radiation patterns to attain their therapeutic effects. On one side, BNCT takes advantage of the very selective and localized energy deposition assured by the high LET secondary charged particles (a particle and ⁷Li recoil nucleus) emitted by ¹⁰B neutron capture reaction. These products have short ranges in tissues (for each particle, less than 10 µm) allowing the deposition of almost all the emitted energy inside the volume of the ¹⁰B loaded cell. On the other side, the effectiveness of Gd-NCT is a little more complex in term of physical mechanisms, but already clearly reported by several authors. Very likely, in this case a delicate synergy operates between the selective, point-wise damage of cell DNA carried out by high LET Auger electrons and the spread and non-local irradiation performed by the low LET prompt y rays. The former effect is extremely difficult to exploit and see due to the mandatory requirement of specific binding of the DNA molecule, or other sensitive targets inside cell, by the ¹⁵⁷Gd-compound. On the contrary, the effect of the prompt y rays can be clearly showed only when a significant volume of tumor is treated, such as in medium size canine models as reported by Mitin et al [19]. Having these different mechanisms of action, it is pretty logic to investigate the possible advantage of combining BNCT and Gd-NCT. Besides, the possibility of exploiting Gd-based compounds in MR imaging to detect in vivo the spatial distribution of the NCT agent in tissues has recently further increased the attention of the NCT scientific society towards Gd-NCT and its combination with BNCT (GdBNCT). Two possible strategies are available: 1) simultaneous administration of two NCT agents, one carrying ¹⁰B while the other ¹⁵⁷Gd; 2) development of a single NCT agent containing both ¹⁰B and ¹⁵⁷Gd compounds. In the first strategy, the uptake and distribution within the tumor may be different among these compounds. Thus, the combination of the boron and gadolinium compounds may be beneficial for enhancing the radiation dose to the tumor. Few examples of in vivo studies of GdBNCT are reported in literature, maybe for the difficulty to effectively prepare molecules which contain both boron and gadolinium nuclei. First studies were proposed by the research group of Matsumura which in 1994 presented a conference communication titled "Boron-gadoliniumporphyrin derivatives for neutron capture therapy: MRI and ICP study" at the Symposium on Neutron Capture Therapy for Cancer, in Kobe, Japan. [57] Few years later the same group utilized a mixture of ¹⁰BSH and ¹⁵⁷Gd-BOPTA at different concentrations for an *in vitro* study on Chinese hamster fibroblast V79 cells. [58] The combination of the boron and gadolinium compounds showed an additive effect when the gadolinium concentration was lower than 1600 ppm. This additive effect decreased as a function of gadolinium concentration at 2400 ppm and resulted in no additive effect at more than 3200 ppm of gadolinium; it was then demonstrated that the combination of the boron and gadolinium compounds can enhance the therapeutic effect when an optimum concentration ratio is used. A p-Boronophenylalanine conjugated Gd-DTPA complex (BPA-Gd-DTPA, Figure 8), was then synthesized and its *in vivo* biodistribution was evaluated by prompt γ ray analysis and α -autoradiography using rats with a AH109A tumor implanted on their back [59]. Compound BPA-Gd-DTPA was injected intravenously into a rat via the tail vein. High accumulation of gadolinium was observed in the kidney and the %ID values were 0.17 and 0.088 at 20 and 60 min after injection of BPA-Gd-DTPA, respectively. The accumulation was also observed in the tumor and the %ID values were 0.010 and 0.0025 at 20 and 60min after injection, respectively. A higher accumulation of complex BPA-Gd-DTPA was observed in the tumor tissue in comparison with the case of a carborane–Gd–DTPA complex previously reported, due to the higher expression of BPA transporters on tumor cells [60]. However, the conjugation of a Gd-DTPA unit (Mw=590) to BPA (Mw= 209) causes a dramatic change in biodistribution and intratumor concentration of B atoms in BPA/Gd-DTPA with respect to BPA alone.



Figure 8: Structures of BPA conjugated Gd–DTPA complex (BPA-Gd-DTPA)

Very recently, Matsumura et al. studied the additive effect of gadolinium and boron coadministration using colony forming assay. [61] In vitro tests were accomplished on CT26 mouse carcinoma, C6 rat glioma and V79 chinese hamster cell lines. As a result, the survival of tumor cells with additional 5 ppm of Gd-DTPA decreased to 1/10 compared to the cells with boron only. In the biodistribution tests, C6 cells were inoculated subcutaneously in Wistar rats in 6 locations on the back of each rat, the concentration of boron in subcutaneous tumor tissue did not change significantly, though the concentration of gadolinium decreased from 13.2 to 4.1 ppm within first 60 min after injection. There was no difference in gadolinium concentration between the groups with independent and simultaneous BPA and Gd-DTPA administration. In conclusion, using BPA with Gd-DTPA might enhance the effect of NCT and an additional curative effect of Gd has to be expected. Hawthorne et coworkers recently reported the synthesis, relaxivity measurements and in vivo assessment of a carborane-Gd-DOTA-monoamide (CB-GdDOTA-MA, Figure 9) amphiphilic conjugate. In vivo MRI studies in mice using CB-GdDOTA-MA at a Gd dose of 0.1 mmol per kg body weight showed that the significant contrast enhancement of the vascular system persisted for about 3-4 min post injection and quickly diminished over time. The short plasma half-life of CB-GdDOTA-MA could possibly limit its application as blood pool contrast agent and more than one moiety per Gd-chelate may be necessary. [62]



Figure 9: Carborane-Gd-DOTA-monoamide (CB-GdDOTA-MA)

Afterwards, new carborane derivatives have been proposed as GdBNCT/MRI agents in order to pursue the second strategy described at the beginning of this section. In Figure 10, some of these derivatives are presented, a carborane unit is linked to a lipophilic chain and to a Gd-DOTA complex through amidic bonds (AT101, figure 10, left), or triazole units (MEA01, Figure 10, right), respectively [63], [64].



Figure 10: Chemical structures of Gd-BNCT/MRI agents where carborane unit is linked to a lipophilic chain and to a Gd-DOTA complex

Since these derivatives were able to form stable adducts with low density lipoproteins (LDLs), LDLs were exploited as nanosized carriers for highly proliferating tumor cells that overexpress LDL receptors. In particular AT101 was exploited for *in vitro* and *in vivo* tests. [65] Up to 190 Gd/B/L (AT101) probes were loaded per LDL particle. The uptake from tumor cells was initially assessed on cell cultures of human hepatoma (HepG2), murine melanoma (B16), and human glioblastoma (U87). Measurements were undertaken *in vivo* on mice bearing tumors in which B16 tumor cells were inoculated at the base of the neck. Intratumor Gd and (indirectly) B concentrations have been measured by analyzing MR images before and after AT101 administration, thus establishing that after 4–6 hours the amount of boron taken up by the tumor (30±5 ppm) was above the threshold required for successful NCT treatment (Figure 11). After neutron irradiation, tumor growth was followed for 20 days by MRI. The group of treated mice showed markedly lower tumor growth with respect to the control group. It was then demonstrated that the use of nanoparticles with a high

payload of NCT and MRI imaging agents appears to be the route to achieving considerable improvement in the NCT technique allowing noninvasive measurements of B atoms *in vivo*.



Figure 11: MRI *in vivo* in B16 tumor-bearing mice after administration of the Gd/B/L particles. A) Representative T₁-weighted MR images of C57BL/6 mice grafted subcutaneously with B16 melanoma cells acquired before and 4 and 24 h after the administration of Gd/B/L–LDL particles. The arrows indicate tumor regions. B) A plot of MRI SI enhancements (%) measured in different organs 4 and 24 h after the administration of the Gd/B/L–LDL adduct. Reprinted with permission from ref 65.

Very recently it has been proposed an innovative theranostic approach for lung tumor and metastases treatment, based on the use of LDLs as biological carriers and AT101 as GdBNCT/MRI agent.[66] Tumor cells uptake was initially assessed by ICP-MS and MRI on four types of tumor (TUBO, B16-F10, MCF-7, A549) and one healthy (N-MUG) cell lines. Lung metastases were generated by intravenous injection of a Her2+ breast cancer cell line (i.e. TUBO) in BALB/c mice and transgenic EML4-ALK mice were used as primary tumor model. As reported above B (indirectly) and Gd concentrations taken-up by lung metastases were measured by MRI before performing BNCT. After neutron irradiation, tumor growth was followed for 30-40 days by MRI. Tumor masses of boron/Gd treated mice increased markedly more slowly than the control group. It was observed a tumor re-growth on both mice models about 25-30 days after the

treatment that might be associated to the insufficient neutron dose delivered to the lung tumor in order to kill all the cells or to the presence in the tumor mass of quiescent cells, whose observed higher resistance to targeted therapies could be the consequence of a lower expression of target receptors with respect to highly proliferating cells. These results supports the hypothesis that the combination of BNCT with chemotherapic agents or different therapeutic strategy such us **photodynamic therapy can be an effective option.**



Figure 12: A) T₂-weighted RARE coronal image of a representative 6 weeks old EML4-ALK mouse. Disseminated tumors are clearly visible in both lungs and they are indicated with arrows. (B) Tumor volume increase measured by MRI on irradiated control mice (•) and irradiated and AT101/LDL treated mice (•). Error bars indicate the SD. Reprinted with permission from ref 66.

Other synthetic strategies have been accomplished in order to insert a cholesterol moiety or shorten the preparation by the use of the hydroboration reaction [67], [68]. In particular the cholesterol derivative AC01 (Figure 13) was prepared with the aim of using liposomes as nanoplatforms for the delivery of Gd and B agents. In order to endow the BNCT agent with specific delivery properties, the liposome embedded with the MRI/BNCT dual probes was functionalized with a pegylated phospholipid containing a folic acid residue at the end of the PEG chain. The

BNCT treatment of IGROV-1 cells (human ovarian adenocarcinoma) showed that the number of surviving cells was markedly smaller when they were irradiated after internalization of the folate-targeted AC01/liposomes. Therefore, the amount of internalized B was enough to perform an efficient BNCT treatment and the selective folate-targeting decreased significantly healthy cell damage in the surrounding regions.



Figure 13: Chemical structure of the cholesterol derivative AC01

7. Features and problems in the Gd-NCT dosimetry and comparison with BCNT

Independently from the nucleus (¹⁰B or ¹⁵⁷Gd) used to induce the neutron capture reaction and the technology on which the neutron source relies (nuclear reactor-based or more modern accelerator-based facilities), the radiation field at the tumor depth during an NCT treatment is made by various components characterized by radiation of different LET. The main components comprise: (i) low LET photons spreading on a wide energy spectrum and due mainly to the neutron capture reaction on Hydrogen 1H(n, γ)2H or belonged to the photon contamination of the primary neutron beam; (ii) high LET protons produced by neutron scattering reaction on Hydrogen of high energy neutrons (epithermal and fast neutrons, belonged to the primary neutron beam); (iii) high LET monoenergetic protons emitted by the thermal neutron capture reaction on Nitrogen ¹⁴N(n,p)¹⁴C; (iv) finally, the therapeutic radiations intentionally induced by the neutron interaction with the specific NCT agent under use. The first three components are always present in variable fractions due to the fundamental composition of biological tissues (in particular, their content of nitrogen and hydrogen) and the basic nuclear interactions between neutrons and light elements.

The crucial point in the computational dosimetry for NCT is that the biological effect induced by a certain radiation depends on its LET and, considering a single type of radiation, the LET can change significantly varying the energy of the particle. As a consequence, to correctly evaluate the dose delivered by the NCT mixed radiation field and to properly estimate its biological effect, the doses imparted by the different radiations must be weighted to be translated into photon-equivalent doses. The fixed factors used in this conversion are called Relative Biological Effectiveness (RBE) factors [69]. In case of the NCT agents, the absorbed dose is biologically weighted using the Compound Biological Effectiveness (CBE) factor which takes into account the NCT agent microdistribution at the cellular and subcellular level. The importance of the definition of CBE finds

a clear example in the Auger electrons exploited in Gd-NCT. As previously described, these electrons have actually a significant biological effect only when the ¹⁵⁷Gd nucleus directly binds the DNA molecule of the malignant cell due to the extremely short ranges (few tens of hundreds of nm) in tissue. As a consequence, the computation of the energy imparted by these electrons based only on the physical processes cannot take into account the pivotal role of the ¹⁵⁷Gd microdistribution in the final biological effect, leading to an incorrect evaluation of the delivered effective dose.

The extreme short ranges of Auger electrons require accurate data acquisition of the spatial localization of the Gd-NCT agent with nanoscale resolution. Nowadays such information is hard to be obtained by available techniques, in particular when *in vivo* distributions (in patients or animals) are necessary. As reported by Cerullo et al [20], the CBE for ¹⁵⁷Gd Auger electrons can vary by a factor of 4 going from a uniform distribution of ¹⁵⁷Gd around DNA molecule to its localization on the surface of the DNA strand. The value is further increased by a factor 2 if V is internalized inside the volume of DNA. It is worth to notice that such numbers depend on the chemical vector carrying ¹⁵⁷Gd and on the experimental set-up (cell line/tissue) used to perform CBE evaluation. Finally, even supposing to have an accurate knowledge of the gadolinium spatial distribution inside the cell and particularly its relative position with respect to DNA, the energy released by Auger electrons affects volumes at the order of nanoscale, requiring Track Structure Monte Carlo codes to properly evaluate the amount of imparted energy, such as PENELOPE [70], PARTRAC [71] or the most recent Geant4-DNA [72].

To illustrate the importance of taking into account the RBE/CBE factors in the calculation of the tumor dose due to Auger electrons in Gd-NCT, we refer to Protti et al [73] when the dose calculations obtained with Monte Carlo code MCNP [74] for a combined (Gd+B)-NCT *in vivo* treatment are reported considering a lung metastases model induced in mice. No RBE/CBE preliminary evaluations of the used NCT agent were carried out, meaning that the reported values are simply absorbed doses, not accounting for the microscopic spatial distribution of ¹⁰B /¹⁵⁷Gd nuclei. The dose enhancement in the tumor volumes due to the secondary radiations emitted by ¹⁵⁷Gd neutron captures is very modest, accounting for 0.4-0.5 Gy. Assuming a direct binding of DNA by the ¹⁵⁷Gd carrier and the CBE values reported by Cerullo et al in [19], these doses increase to 5.0-6.3 Gy-photon-equivalent.

Similar considerations about RBE/CBE factors are valid for ¹⁰B-NCT. In this case, the longer ranges of the secondary radiations (few µm in tissues) make history condensed Monte Carlo codes such as MCNP and FLUKA [75] suitable options for dosimetry calculations, without the mandatory requirement of a reconstruction event-by-event of the interactions between radiation and the biological material. Nonetheless, the highly stochastic nature of the energy deposition by the secondary products of ¹⁰B capture reaction limits the validity of the estimations obtained through

23

macroscopic dosimetry calculations and suggest the use of microdosimetric models to properly evaluate the deposited energy and dose.

8. Future perspective

The following approaches should be applied in order to further develop Gd-NCT agents, to be competitive with respect the other routine tumor treatment protocols and with the final aim of bridging the gap between research and the clinical use of these alternative radiotherapies:

1) Designing new Gd carriers able to accumulate on cell DNA or other compartments (i.e. mitochondria, lysosomes) in order to fully exploit the toxic effect generated by Auger electrons, endowed with a really short range that has to be very close to the cellular target molecule to produce an appreciable biological effect.

2) Providing selective therapy using targeting vectors, able to deliver Gd-NCT probes only to tumor cells. This procedure is expected to affect only pathological cells with a lethal dose of radiations, even in case of spreading and infiltrative cases. Currently, conventional radiotherapy unavoidably involves a significant mass of healthy tissue around the pathological target.

3) Personalization of the neutron therapy. Optimization of neutron irradiation time and the delivered radiation dose can be performed by measuring local Gd concentration exploiting the intrinsic theranostic nature of this metal ion. *In vivo* biodistribution in the tumor and in the surrounding tissues by MRI can be detected in real time just before and during the neutron irradiation.

4) Improving the use of enriched ¹⁵⁷**Gd compounds** in nano- or small sized Gd-agents. The natural abundance of ¹⁵⁷Gd is only 15.7% while enriched compounds may have up to 98% of ¹⁵⁷Gd. The use of the ¹⁵⁷Gd enriched isotope will reduce the minimum Gd concentration necessary to obtain a significant therapeutic outcome.

5) To combine Boron and Gadolinium to improve the therapeutic efficacy. Depending on the development of a highly selective ¹⁵⁷Gd carrier able to specifically target malignant cell nucleus and DNA molecule (or other organelles, such as cell mitochondria as studied by Busse et al. [76] The high citotoxic effect of ¹⁵⁷Gd Auger electrons could be properly exploited to increase the lethal damages induced by BNCT within malignant cells.

6) Maximize the therapeutic effect by combining different treatments to obtain a complete eradication of tumor cells, including the radioresistant clones, to avoid tumor recurrence. This approach can be pursued by combining Gd-NCT with synergic therapeutic strategies, exploiting the same versatile theranostic platform or different ones. NCT can be coupled, for example, with the co-administration of other anti-tumor agents such as doxorubicin or pemetrexed, routinely used in cancer treatments.

Acknowledgements

The authors would like to thank the staff of the Laboratory of Applied Nuclear Energy (L.E.N.A.), University of Pavia, for their precious support during the realization of neutron irradiations.

Financial & competing interests disclosure

This research was funded by MIUR (PRIN 2012 code 2012SK7ASN), the AIRC investigator Grant IG2013 and by the National Institute of Nuclear Physics (INFN), Italy, project "NETTUNO". This research was performed in the framework of the EU COST Action TD1004.

The author has no financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

Executive Summary

1. General introduction on NCT: rationale and application. A general introduction of the main preclinical and clinical applications of Neutron Capture Therapy focusing of the strengths and critical points of this frontier therapy endowed with an enormous potentiality till unexploited.

2. Gd as an alternative neutron capture agent. Description of Gd characteristics as a neutron capture agent. Critical evaluation of the advantages and disadvantages of the use of this metal as an alternative to boron.

3. Gd as MRI contrast agent. A short overview of the classification and clinical use of Gd-based commercially available MRI contrast agents. What can we learn from MRI studies and to transfer to NCT applications.

4.**Small sized Gd chelates for Gd-NCT.** A description of the main therapeutic outcomes obtained by using small sized Gd-NCT agents with particular attention to the use of commercially available Gd-CA.

5. Nano sized Gd carriers for Gd-NCT. A description of the main therapeutic outcomes obtained using nano-sized Gd-NCT agents. Critical discussion about the advantages of the nano- objects with respect small sized Gd complexes.

6. **Combination of Gd/B agents.** Evaluation of the therapeutic improvement given by the simultaneous administration of B and Gd agents. Two strategies have been pursued: 1) administration of two separate compounds, one carrying ¹⁰B while the other ¹⁵⁷Gd; 2) administration of single NCT agent containing both ¹⁰B and ¹⁵⁷Gd.

7. Features and problems in the Gd-NCT dosimetry and comparison with BCNT Overview of the general methods used in Gd-NCT and BNCT dosimetry. Microscopic spatial distribution dependence of the evaluated dose.

References.

[1] Hawthorne MF, Lee M. A critical assessment of boron target compounds for boron neutron capture therapy. *J. Neurooncol.* 62(1-2), 33-45 (2003).

[2] Hosmane NS, Mag uire JA, Zhu Y, Takagaki M. Principles of neutron capture therapy. In: *Boron and gadolinium neutron capture therapy for cancer treatment*. World Scientific Publishing Co. Pte. Ltd, Singapore. 35-40 (2012).

[3] Barth RF, Coderre JA, Vicente MGH, Blue TE. Boron neutron capture therapy of cancer: current status and future prospects. *Clin. Cancer Res.* 11(11), 3987-4002 (2005).

[4] Kageji T, Nagahiro S, Mizobuchi Y, Matsuzaki K, Nakagawa Y, Kumada H. Boron neutron capture therapy (BNCT) for newly-diagnosed glioblastoma: comparison of clinical results obtained with BNCT and conventional treatment. *J. Med. Invest.* 61(3-4), 254-263 (2014).

[5] van Rij CM, Wilhelm AJ, Sauerwein WA, van Loenen AC. Boron neutron capture therapy for glioblastoma multiforme. *Pharm. World Sci.* 27(2), 92-95 (2005).

[6] Liu YW, Chang CT, Yeh LY, Wang LW, Lin TY. BNCT treatment planning for superficial and deep-seated tumors: Experience from clinical trial of recurrent head and neck cancer at THOR. *Appl. Radiat. Isot.* 106, 121-124 (2015).

[7] Aihara T, Morita N, Kamitani N *et al.* BNCT for advanced or recurrent head and neck cancer. *Appl. Radiat. Isot.* 88, 12-15 (2014).

[8] Menéndez PR, Roth BM, Pereira MD *et al.* BNCT for skin melanoma in extremities: updated Argentine clinical results. *Appl. Radiat. Isot.* 67, S50-53 (2009).

[9] Zonta A, Prati U, Roveda L *et al.* Clinical lessons from the first applications of BNCT on unresectable liver metastases. *J. Phys: Conf Ser.* 41, 484-495 (2006).

[10] Barth RF, Vicente MG, Harling OK *et al.* Current status of boron neutron capture therapy of high grade gliomas and recurrent head and neck cancer. *Radiat. Oncol.* 7, 146-167 (2012).

[11] Luderer MJ, de la Puente P, Azab AK. Advancements in tumor targeting strategies for boron neutron capture therapy. *Pharm. Res.* 32(9), 2824-2836 (2015).

[12] Salt C, Lennox AJ, Takagaki,M, Maguire JA, Hosmane NS. Boron and gadolinium neutron capture therapy. *Russian Chemical Bulletin, International Edition*. 53,1871-1888 (2004).

[13] Sweet WH. The uses of nuclear disintegration in the diagnosis and treatment of brain tumor. *N Engl. J. Med.* 245(23), 875-878 (1951).

[14] Geninatti-Crich S, Deagostino A, Toppino A, Alberti D, Venturello P, Aime S. Boronated compounds for imaging guided BNCT applications. *Anticancer Agents Med. Chem.* 12(5), 543-553 (2012)

[15] Evangelista L, Jori G, Martini D, Sotti G. Boron neutron capture therapy and 18F-labelled borophenylalanine positron emission tomography: a critical and clinical overview of the literature. *Appl. Radiat. Isot.* 74, 91-101 (2013)

[16] Brugger RM, Shih JA. Evaluation of gadolinium-157 as a neutron capture therapy agent. *Strahlenther Onkol.* 165(2-3), 153-156 (1989).

[17] De Stasio G, Casalbore P, Pallini R *et al*. Gadolinium in human glioblastoma cells for gadolinium neutron capture therapy. *Cancer Res.* 61(10), 4272-4277 (2001).

[18] Shih JLA, Brugger RM. Gadolinium as a neutron capture therapy agent. *Med. Phys.* 19(3), 733-744 (1992).

[19] Mitin VN, Kulakov VN, Khokhlov VF *et al.* Comparison of BNCT and GdNCT efficacy in treatment of canine cancer. *Appl. Radiat. Isot.* 67, S299-301 (2009).

[20] Cerullo N, Bufalino D, Daquino G. Progress in the use of gadolinium for NCT. *Appl. Radiat. Isot.* 67, S157-S160 (2009)

[21] Le UM, Cui Z. Long-circulating gadolinium-encapsulated liposomes for potential

application in tumor neutron capture therapy. Int. J. Pharm. 312(1-2), 105-112 (2006).

[22] Caravan P, Lauffer RB. Contrast Agents: Basic Principles. In: *Clinical Magnetic Resonance Imaging 3 (Volume 1).* Edelman RR, Hesselink JR, Zlatkin MB, Crues JV (Ed.), Saunders Publisher, Philadelphia, USA, 357-375 (2005).

[23] Merbach AS, Helm L, Toth E. Relaxivity of Gadolinium(III) Complexes : Theory and Mechanism. In: *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging, 2nd Edition.* John Wiley & Sons Publisher, Hoboken, NJ. 25-81 (2013).

[24] Aime S, Caravan P. Biodistribution of gadolinium-based contrast agents, including gadolinium deposition. *J. Magn. Reson. Imaging.* 30(6). 1259–1267 (2009).

[25] Daftari Besheli L, Aran S, Shaqdan K, Kay J, Abujudeh H. Current status of nephrogenic systemic fibrosis. *Clin Radiol.* 69, 661-8 (2014).

[26] Chao CC, Yang CC, Hsiao CH, Pan MK, Lin CH, Hsieh ST. Nephrogenic systemic fibrosis associated with gadolinium use. *J. Formos. Med. Assoc.* 107(3), 270-4 (2008).

[27] Todd DJ, Kay J. Gadolinium-Induced Fibrosis. Annu Rev. Med. 67, 273-91 (2016).

[28] Huang CH, New K, Al Zaki A, Brechbiel MW, Tsourkas A. Biodegradable Polydisulfide Dendrimer Nanoclusters as MRI Contrast Agents, *ACS Nano.* 6, 9416–9424 (2012).

[29] Akine Y, Tokita N, Tokuuye K *et al.* Suppression of Rabbit Vx-2 Subcutaneous Tumor-Growth by Gadolinium Neutron-Capture Therapy. *Jpn. J. Cancer Res.* 84(8), 841-843 (1993).

[30] Khokhlov VF, Yashkin PN, Silin DI, Djorova ES, Lawaczeck R. Neutron capture therapy with gadopentetate dimeglumine: Experiments on tumor-bearing rats. *Acad. Radiol.* 2(5), 392-398 (1995).

[31] Takagaki M, Hosmane MS, Gadolinium Neutron Capture Therapy for malignant brain tumours, Aino journal, 6.39-44, 2007.

[32] Zhang T, Matsumura A, Yamamoto T *et al.* A comparison of *in vivo* Gd-NCT trial by using two targeting compounds: Gd-BOPTA and Gd-DTPA. *Proceedings of the International Congress on Neutron Capture Therapy, 10th, Essen, Germany, Sept. 8-13,* pp 807-812 (2002).

[33] Matsumura A, Zhang T, Yamamoto T *et al. In vivo* gadolinium neutron capture therapy using a potentially effective compound (Gd-BOPTA). *Anticancer Res.* 23(3B), 2451-2456 (2003).

[34] Hofmann B, Fischer CO, Lawaczeck R, Platzek J, Semmler W. Gadolinium neutron capture therapy (GdNCT) of melanoma cells and solid tumors with the magnetic resonance imaging contrast agent Gadobutrol. *Invest. Radiol.* 34(2), 126-133 (1999).

[35] Crossley EL, Aitken JB, Vogt S, Harris HH, Rendina LM. Selective Aggregation of a Platinum-Gadolinium Complex Within a Tumor-Cell Nucleus. *Angew. Chem. Int. Ed.* 49(7), 1231-1233 (2010).

[36] Kim HK, Kang MK, Jung KH *et al.* Gadolinium Complex of DO3A-benzothiazole Aniline (BTA) Conjugate as a Theranostic Agent. *J. Med. Chem.* 56(20), 8104-8111 (2013).

[37] Dewi N, Yanagie H, Zhu H *et al.* Tumor growth suppression by gadolinium-neutron capture therapy using gadolinium-entrapped liposome as gadolinium delivery agent. *Biomed Pharmacother.* 67(6), 451-457 (2013).

[38] Glasgow MD, Chougule MB. Recent Developments in Active Tumor Targeted Multifunctional Nanoparticles for Combination Chemotherapy in Cancer Treatment and Imaging. *J. Biomed. Nanotechnol.* 11(11), 1859-1898 (2015).

[39] Terreno E, Uggeri F, Aime S. Image guided therapy: the advent of theranostic agents. *J. Control Release.* 161(2), 328-337 (2012).

[40] Sheng Y, Liao LD, Thakor NV, Tan MC. Nanoparticles for molecular imaging. *J. Biomed. Nanotechnol.* 10(10), 2641-2676 (2014).

[41] Baetke SC, Lammers T, Kiessling F. Applications of nanoparticles for diagnosis and therapy of cancer. *Br. J. Radiol.* 88(1054), 20150207 (2015).

[42] Gao S, Fu R, Hosmane NS. Nanomaterials for boron and gadolinium neutron capture therapy for cancer treatment. *Pure Appl.Chem.* 87,123-134 (2015).

[43] Ichikawa H, Uneme T, Andoh T *et al.* Gadolinium-loaded chitosan nanoparticles for neutroncapture therapy: Influence of micrometric properties of the nanoparticles on tumor-killing effect. *Appl. Radiat. Isot.* 88, 109-113 (2014).

[44] Tokumitsu H, Hiratsuka J, Sakurai Y, Kobayashi T, Ichikawa H, Fukumori Y. Gadolinium neutron-capture therapy using novel gadopentetic acid-chitosan complex nanoparticles: *in vivo* growth suppression of experimental melanoma solid tumor. *Cancer Lett.* 150(2), 177-182 (2000).

[45] Dewi N, Mi P, Yanagie H *et al. In vivo* evaluation of neutron capture therapy effectivity using calcium phosphate-based nanoparticles as Gd-DTPA delivery agent. *J. Cancer Res. Clin. Oncol.* DOI 10.1007/s00432-015-2085-0.

[46] Mi P, Dewi N, Yanagie H *et al.* Hybrid calcium phosphate-polymeric micelles incorporating gadolinium chelates for imaging-guided gadolinium neutron capture tumor therapy. *ACS Nano.* 9(6), 5913–5921 (2015).

[47] Peters T, Grunewald C, Blaickner M *et al.* Cellular uptake and *in vitro* antitumor efficacy of composite liposomes for neutron capture therapy. *Radiat. Oncol.* 10, 52-65 (2015).

[48] Kobayashi H, Kawamoto S, Saga T *et al.* Avidin-dendrimer-(1B4M-Gd)(254): a tumor-targeting therapeutic agent for gadolinium neutron capture therapy of intraperitoneal disseminated tumor which can be monitored by MRI. *Bioconjug. Chem.* 12(4), 587-593 (2001).

[49] Fujimoto T, Ichikawa H, Akisue T *et al.* Accumulation of MRI contrast agents in malignant fibrous histiocytoma for gadolinium neutron capture therapy. *Appl. Radiat. Isot.* 67, S355-358 (2009).

[50] Shikata F, Tokumitsu H, Ichikawa H, Fukumori Y. *In vitro* cellular accumulation of gadolinium incorporated into chitosan nanoparticles designed for neutron-capture therapy of cancer. *Eur. J. Pharm. Biopharm.* 53(1), 57-63 (2002).

[51] Oyewumi MO, Yokel RA, Jay M, Coakley T, Mumper RJ. Comparison of cell uptake, biodistribution and tumor retention of folate-coated and PEG-coated gadolinium nanoparticles in tumor-bearing mice. *J. Control Release*. 95(3), 613-626 (2004).

[52] Hosmane NS, Gao S, Liu X, Xu T *et al.* Synthesis and characterization of Fe(10)BO3/Fe3O4/SiO2 and GdFeO3/Fe3O4/SiO2: nanocomposites of biofunctional materials. *ChemistryOpen.* 2(3), 88-92 (2013).

[53] Hwang KC, Lai PD, Chiang CS, Wang PJ, Yuan CJ. Neutron capture nuclei-containing carbon nanoparticles for destruction of cancer cells. *Biomaterials*. 31(32), 8419-8425 (2010).

[54] Kobayashi H, Kawamoto S, Bernardo M, Brechbiel MW, Knopp MV, Choyke PL. Delivery of gadolinium-labeled nanoparticles to the sentinel lymph node: Comparison of the sentinel node visualization and estimations of intra-nodal gadolinium concentration by the magnetic resonance imaging. *J. Control Release*. 111(3), 343-351 (2006).

[55] Horiguchi Y, Kudo S, Nagasaki Y. Gd@C82 metallofullerenes for neutron capture therapyfullerene solubilisation by poly(ethylene glycol)-block-poly(2-(N,N-diethylamino)ethyl methacrylate) and resultant efficacy *in vitro*. *Sci. Technol. Adv. Mater.* 12(4), 044607 (2011).

[56] Dierling AM, Sloat BR, Cui Z. Gadolinium incorporated reconstituted chylomicron emulsion for potential application in tumor neutron capture therapy. *Eur. J. Pharm. Biopharm*. 62(3), 275-281 (2006).

[57] Matsumura A, Shibata Y, Nakagawa K *et al.* Boron-gadolinium-porphyrin derivatives for neutron capture therapy: MRI and ICP study. Presented at: 1994.

[58] Matsumura A, Zhang T, Nakai K *et al.* Combination of boron and gadolinium compounds for neutron capture therapy. An *in vitro* study. *J. Exp. Clin. Cancer Res.* 24(1), 93-98 (2005).

[59] Takahashi K, Nakamura H, Furumoto S *et al.* Synthesis and *in vivo* biodistribution of BPA–Gd–DTPA complex as a potential MRI contrast carrier for neutron capture therapy. *Bioorg Med Chem* 13(3), 735-743 (2005).

[60] Nemoto H, Cai JP, Nakamura H, Fujiwara M, Yamamoto Y. The synthesis of a carborane gadolinium-DTPA complex for boron neutron capture therapy. *J. Organomet. Chem.* 581(1-2), 170-175 (1999).

[61] Yoshida F, Yamamoto T, Nakai K, Zaboronok A, Matsumura A. Additive effect of BPA and Gd-DTPA for application in accelerator-based neutron source. *Appl.Rad. Isot.* 106, 247-250 (2015). [62] Goswami LN, Cai Q, Ma L, Jalisatgi SS, Hawthorne MF. Synthesis, relaxation properties and *in vivo* assessment of a carborane-GdDOTA-monoamide conjugate as an MRI blood pool contrast agent. *Org. Biomol. Chem.* 13(33), 8912-8918 (2015).

[63] Aime S, Barge A, Crivello A *et al.* Synthesis of Gd(III)-C-palmitamidomethyl-C[prime or minute]-DOTAMA-C6-o-carborane: a new dual agent for innovative MRI/BNCT applications. *Org. Biomol. Chem.* 6(23), 4460-4466 (2008).

[64] Toppino A, Bova ME, Geninatti Crich S *et al.* A Carborane-Derivative "Click" Reaction under Heterogeneous Conditions for the Synthesis of a Promising Lipophilic MRI/GdBNCT Agent. *Chem.-Eur. J.* 19(2), 720-727 (2013).

[65] Geninatti-Crich S, Alberti D, Szabo I *et al.* MRI-Guided Neutron Capture Therapy by Use of a Dual Gadolinium/Boron Agent Targeted at Tumor Cells through Upregulated Low-Density Lipoprotein Transporters. *Chem.-Eur. J.* 17(30), 8479-8486 (2011).

[66] Alberti D, Protti N, Toppino A *et al.* A theranostic approach based on the use of a dual boron/Gd agent to improve the efficacy of Boron Neutron Capture Therapy in the lung cancer treatment. *Nanomedicine* 11(3), 741-750 (2015).

[67] Alberti D, Toppino A, Crich SG *et al.* Synthesis of a carborane-containing cholesterol derivative and evaluation as a potential dual agent for MRI/BNCT applications. *Org. Biomol. Chem.* 12(15), 2457-2467 (2014).

[68] Boggio P, Toppino A, Geninatti Crich S *et al.* The hydroboration reaction as a key for a straightforward synthesis of new MRI-NCT agents. *Org. Biomol. Chem.* 13(11), 3288-3297 (2015).

[69] Coderre JA, Morris GM. The radiation biology of boron neutron capture therapy, *Radiat. Res.* 151(1), 1-18 (1999).

[70] Salvat F, Fernández-Varea JM, Sempau J. PENELOPE-2006: a code system for Monte carlo simulation of electron and photon transport. *Workshop Proceedings*. Barcelona, Spain, 4-7 July 2006.

[71] Friedland W, Kundrát P, Jacob P. Stochastic modelling of DSB repair after photon and ion irradiation. *Int. J. Radiat. Biol.* 88(1-2), 129-136 (2012).

[72] Chauvie S, Francis Z, Guatelli S *et al.* Geant4 physics processes for microdosimetry simulations: design foundation and implementation of the first set of models. *IEEE Trans. Nucl. Sci.* 54(6), 2619-2628 (2007).

[73] Protti N, Geninatti-Crich S, Alberti D *et al.* Evaluation of the dose enhancement of combined ¹⁰B + ¹⁵⁷Gd neutron capture therapy (NCT). *Radiat. Prot. Dosimetry.* 166(1-4), 369-373 (2015).

[74] X-5 Monte Carlo team. MCNP-a general Monte Carlo N-particle transport code, version 5. *Los Alamos National Laboratory*, LA-UR-03-1987 (2008).

[75] Böhlen TT, Cerutti F, Chin PMW *et al.* The FLUKA code: developments and challenges for high energy and medical applications, *Nuclear Data Sheets.* 120, 211-214 (2014).

[76] Busse M *et al*.Gadolinium Neutron Capture Therapy agents targeting mitochondria. *Proceedings of the 16th International Congress on Neutron Capture Therapy*. Helsinki, Finland, 14-20 June 2014.