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Special Article - Boron Neutron Capture Therapy

Translational Radiobiological Boron Neutron Capture Therapy (BNCT) Studies for the Treatment of Different Pathologies: A Bench to Bedside Approach

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Abstract

Boron Neutron Capture Therapy (BNCT) is a binary cancer treatment modality that combines irradiation with a thermal or epithermal neutron beam with the administration of boron-10 carriers that are taken up preferentially by neoplastic cells. The high linear energy transfer alpha particles and recoiling ^{7}Li nuclei emitted during the boron-10 neutron-capture reaction $^{10}B(n,\alpha)^{7}Li$, have a range of 5-9 μm in tissue and are known to have a high Relative Biological Effectiveness (RBE). In this way, BNCT would potentially target tumor tissue selectively, largely sparing normal tissue.

Clinical trials of BNCT for the treatment of glioblastoma multiforme and/or melanoma and, more recently, head and neck tumors, liver metastases, lung metastases and mesothelioma have been performed or are under way in Argentina, Europe, Japan, Taiwan, and the US. To date, the clinical results have shown a potential therapeutic advantage for this technique but undoubtedly leave room for improvement. Translational radiobiological studies in appropriate *in vivo* experimental models are pivotal to progress in this field.

A significant part of our translational research efforts have been focused on exploring new applications of BNCT and optimizing BNCT for different pathologies, employing a bench to bedside approach that bridges the gap between research and clinical application. Although our work includes the assessment of the therapeutic potential of novel boron compounds in our experimental models, a large proportion of our studies have been devoted to optimize the delivery of boron compounds currently authorized for their use in humans such as Boron phenylalanine (BPA) and decahydrodecaborate (GB-10). In addition, we have designed and tested different BNCT treatment strategies tailored for different pathologies, for varying degrees of disease progression and for different clinical conditions of the patient. Some examples involve: 1) The combined use of BPA and GB-10 to improve tumor boron targeting homogeneity in the hamster cheek pouch oral cancer model, in a colon carcinoma liver metastases model in BDIX rats and in a diffuse lung metastases model in BDIX rats; 2) Aberrant tumor blood vessel normalization to improve boron delivery in the oral cancer model; 3) Sequential BNCT (BPA-BNCT followed by GB-10-BNCT with a 24-48 h interval) in the oral cancer model to optimize therapeutic efficacy and minimize mucositis in the dose-limiting precancerous tissue in the case of patients requiring abbreviated treatment; 4) Electroporation to improve the micro distribution of boron delivered by GB-10 in the oral cancer model; 5) Double applications of BNCT with 4-6 weeks interval to optimize therapeutic efficacy, reduce toxicity in terms of mucositis and inhibit the development of second primary tumors from precancerous tissue in the oral cancer model for the case of patients that do not require abbreviated treatment; 5) Assessment of the therapeutic efficacy and potential toxicity of BNCT in the liver metastases and diffuse lung metastases models in BDIX rats; 6) Local administration of GB-10 or BPA for effective low dose Boron Neutron Capture Synovectomy (BNCS) for the treatment of Rheumatoid Arthritis in a model of antigen-induced arthritis in rabbits; 7) BNCT-induced local and abscopal effect in an ectopic model of colon carcinoma in BDIX rats.

The knowledge gained from these radiobiological studies would contribute to design safe and effective clinical BNCT protocols. In particular, the BNCT protocols used to perform our ongoing and to date successful clinical-veterinary BNCT studies at RA-6 for cats and dogs with spontaneous head and neck cancer with no therapeutic option, are partially based on the lessons learnt from these translational studies.

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Introduction

Boron Neutron Capture Therapy (BNCT) is a binary treatment modality for cancer that involves the selective accumulation of boron-10 carriers in tumors followed by neutron irradiation. The $^{10}B(n,\alpha)^{7}Li$ capture reaction between a thermal neutron and a ^{10}B nucleus gives rise to the formation of a high Linear Energy Transfer (LET) alpha particle and a recoiling ^{7}Li nucleus. These densely ionizing particles have a high Relative Biological Effectiveness (RBE) and a short range in tissue (5-9 μm). In this way, damage would be largely circumscribed to tumor tissue where ^{10}B atoms are preferentially localized [1-3].

Clinical studies of BNCT for glioblastoma multiforme, melanoma, recurrent head and neck tumors, and liver metastases have been performed or are underway in the United States, Japan, Europe, Argentina and Taiwan [4-9] employing mostly nuclear reactors as the neutron source. More recently, clinical investigations have been performed in Japan on BNCT for recurrent hepatic and gastrointestinal cancer [10], locally recurrent lung cancer [11] and extra-mammary Paget´s disease [12]. Ongoing clinical trials in Japan include BNCT for lung cancer. A recent review by Barth et al. [13] suggests that if the ongoing clinical trials are sufficiently promising, BNCT will have a clear path to the future. To date, the clinical results have shown a therapeutic advantage, with identified opportunities for improvement. Extensive research has recently been carried out for the development of alternative solutions [14-20] to existing BNCT facilities based on nuclear reactors. These alternative facilities will enable in-hospital treatments [21,22] and pave the way for more widespread clinical trials for different tumors.

BNCT involves a mixed field irradiation. The radiation doses delivered to tumor and normal tissue during BNCT originate in the energy deposition from ionizing radiation with different LET characteristics. Although the neutron capture cross-sections for the elements in normal tissue are several orders of magnitude lower than for [^{10}B], two of these, hydrogen and nitrogen, are present in such high concentrations that their neutron capture contributes significantly to the total absorbed dose. In addition to the α and ^{7}Li high LET products that give rise to the tumor-specific boron dose component, a non-specific background dose results from: (1) low-LET gamma rays in the neutron beam, (2) low LET gamma rays resulting from the capture of thermal neutrons by hydrogen atoms [$^{1}H(n,\gamma)^{2}H$], (3) high LET protons produced by the scattering of fast neutrons when a hardened epithermal neutron beam spectrum is employed and (4) high-LET protons resulting from the capture of thermal neutrons by nitrogen atoms [$^{14}N(n,p)^{14}C$]. The biologically effective dose will depend on the RBE for the different dose components that contribute to the total irradiation dose. RBE depends on the LET of radiation. High-LET, densely ionizing particles induce direct damage to DNA, i.e. mainly double-strand breaks that are largely irreparable and lethal for the cell. Direct damage is not influenced by the oxygenation level of the tissue or its proliferative status. Conversely, low-LET radiation induces mainly indirect damage via the action of free radicals on DNA. This effect is largely reparable and causes sub lethal damage to the cell. Indirect damage is enhanced by the presence of oxygen and is greater in proliferating cells with unwinding, exposed DNA strands. In the particular case of the boron dose component, RBE is termed Compound Biological Effectiveness (CBE) factor and depends on the RBE of α particles and ^{7}Li ions and the micro distribution of [^{10}B] in a particular tissue [2,3].

BNCT protocols should ideally maximize the tumor selective boron radiation component and minimize the non-selective background dose to improve the therapeutic ratio [2]. Maximizing/optimizing the delivery of boron to tumor is the most effective way to do this. An ideal boron carrier should be non-toxic at therapeutic dose levels, should accumulate preferentially in tumor cells vs blood and normal tissue and should target all tumor cell populations. Tumor cells poorly loaded with boron will be unresponsive to treatment [23], making homogeneous tumor boron targeting pivotal to therapeutic success. Since the ^{10}B isotope only has a natural abundance of 19.9%, boron carriers for BNCT must be enriched in ^{10}B. Also, absolute boron content in tumor must be high enough (10^{9}boron-10 atoms/cell or 20-30 ppm) to allow sufficient capture reactions to occur for the effect to be lethal. Higher tumor boron concentrations will allow for shorter irradiation times and the concomitant reduction in background dose that affects tumor and normal tissue similarly [2]. Finally, the micro distribution of an ideal boron carrier will place the ^{10}B atoms close to a target that maximizes effect such as DNA [24-26].

Although the international community has devoted (and continues to do so) much effort and resources to the design and synthesis of novel boron carriers, the "ideal" boron carrier remains elusive. The four "imperfect" compounds currently authorized for their use in humans are Boron phenylalanine (BPA), Sodium Borocaptate (BSH), decahydrodecaborate (GB-10), and boric acid (BA). Although these compounds delivered in the traditional way as single agents prior to a single neutron irradiation have shown therapeutic potential for different pathologies [5,27-32], there is opportunity and need for improvement. However, if and when a novel boron compound is identified as promising, it still faces many hurdles [33]. Typically, in the US for example, of 10.000 general medicinal compounds that are developed, only 5 enter clinical trials and only one is finally approved for treatment. The process "from bench to bedside" takes typically 10 years and costs over 1000 million US Dollars [34]. Optimizing the delivery of ^{10}B compounds currently authorized for use in humans is an excellent short and medium-term strategy, employing a bench to bedside approach that will bridge the gap between research and clinical application.

Translational Research

The need to optimize BNCT for different pathologies requires translational research in adequate experimental models. Although we have devoted part of our efforts to assessing the therapeutic potential of novel boron carriers, our work has been primarily focused on optimizing the delivery of boron compounds authorized for their use in humans and designing and testing different BNCT treatment strategies for different pathologies, degree of disease progression and clinical condition.

The pathologies we seek to study employing experimental models are those that respond poorly to standard therapies and would potentially benefit from more effective and selective therapies.

Head and neck cancer

The relatively poor overall 5-year survival rate for malignancies

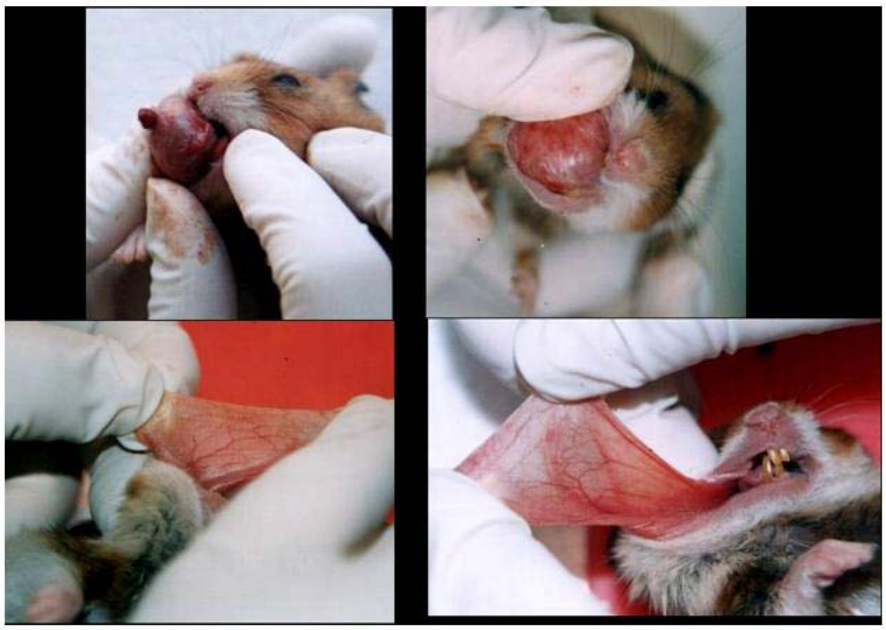

Figure 1: Effect of GB-10-BNCT on tumor and normal pouch tissue 7 days post-treatment (pouches have been everted for observation).

of the oral cavity [35] and the fact that radical surgery often results in large tissue defect [36] calls for improved therapeutic strategies. Within this context, the hamster cheek pouch oral cancer model was proposed by our group to explore, for the first time, the feasibility of applying BNCT to the treatment of head and neck cancer [37,38].Our translational studies preceded the first clinical trial of BNCT for head and neck malignancies [39] and provided evidence, for the first time, of the efficacy of BNCT to treat oral cancer at an experimental level. The hamster cheek pouch oral cancer model is the most widely accepted model of oral cancer [40-42]. Carcinogenesis protocols that involve topical application of the hamster cheek pouch with the carcinogen Dimethyl-1,2-Benzanthracene (DMBA) 0.5% in mineral oil 2-3 times a week for 12 weeks [27,43] induce Squamous Cell Carcinomas (SCC) surrounded by precancerous tissue, recapitulating the process of malignant transformation in humans [44]. The possibility to study precancerous tissue that mimics field cancerized tissue in humans is essential in head and neck cancer studies. Precancerous tissue is more radiosensitive than normal mucosa and, when treated, develops dose-limiting mucositis [45,46]. In addition, precancerous tissue is frequently the source of second primary tumors that cause therapeutic failure. In a clinical scenario, oral mucositis also limits the dose that can be administered with BNCT to head and neck tumors [5,7] and is a frequent dose-limiting side effect during conventional radiotherapy [48].

A large part of our translational radiobiological studies has been performed in the hamster cheek pouch oral cancer model. These *in vivo* BNCT studies have taught us many lessons, some even surprising.

Our first studies at RA-6 Nuclear Reactor showed that BNCT mediated by BPA at 5.2 Gy absorbed dose to tumor induced complete tumor remission by 15 days post-treatment in 78% of the tumors and partial remission in an additional 13% of the tumors. No toxicity was observed in normal mucosa and moderate/severe, albeit reversible, mucositis developed in precancerous tissue [38]. This study strongly suggested, for the first time, that BNCT could induce a remarkable tumor response in head and neck cancer with acceptable toxicity in the dose-limiting precancerous tissue.

We then went on to explore the therapeutic potential of the boron carrier GB-10, with an unexpected outcome. GB-10 is a largely diffusive agent that has been proposed for BNCT of brain tumors because it does not traverse the intact blood-brain barrier but can enter brain tumors surrounded by a disrupted blood-brain barrier [49]. We did not expect GB-10 to be a selective stand-alone boron delivery agent in tumors other than brain tumors. However, we wanted to study GB-10 in our oral cancer model to go on to use it in combination with BPA. The combined administration of ^{10}B compounds with different properties and uptake mechanisms contributes to more homogeneous tumor boron targeting, conceivably improving therapeutic efficacy [50,51]. Although GB-10 was not selectively taken up by tumor in our model, it did target tumor cells homogeneously. Homogeneous tumor boron targeting is a pivotal aspect of BNCT efficacy because tumor cells poorly loaded with boron will be refractory to treatment and the source of recurrence. BNCT mediated by GB-10 produced a selective effect on tumors by preferential damage of the aberrant tumor vasculature [52], while preserving precancerous and normal tissues that do not have an aberrant vasculature. At 8 Gy absorbed dose to tumor, tumor response to GB-10-BNCT at the RA-6 Nuclear Reactor was 70%, with only very mild mucositis in precancerous tissue and no mucositis in normal tissue. Figure 1 shows a representative example of the surprising selective effect on tumor of GB-10-BNCT. These findings show that selective tumor lethality can result from a selective effect on aberrant tumor blood vessels rather than from selective tumor uptake of the boron compound [27]. This example of a new paradigm in BNCT radiobiology emerged from *in vivo* BNCT studies, stressing the importance of performing actual radiobiological

studies *in vivo* to assess the therapeutic efficacy of BNCT mediated by a boron compound under study. Based on boron biodistribution studies alone, GB-10 would have been ruled out as a stand-alone boron agent for BNCT of head and neck tumors due to its lack of tumor selectivity.

Our follow-on studies were oriented towards studying the therapeutic efficacy of BNCT mediated by the combined administration of (BPA+GB-10). We sought to combine aberrant blood vessel boron targeting with GB-10 and tumor cell targeting with BPA, attempting to improve boron targeting homogeneity and enhance therapeutic efficacy at no additional cost in terms of toxicity. At 5.2 Gy absorbed dose to tumor, BNCT-(BPA+GB-10) at RA-6 Nuclear Reactor induced 93% tumor response with no normal tissue toxicity and only mild mucositis in dose-limiting precancerous tissue [27]. These findings and similar studies at RA-3 Nuclear Reactor [53] revealed the therapeutic benefit of using 2 boron carriers combined, conceivably as a result of the improved homogeneity in boron deposition in tumor observed for this boron administration protocol [51]. Combinations of agents may be superior to any single agent [50,51]. In addition, this strategy helps to overcome the potential toxicity of higher doses of each of the compounds given alone [26].

As described, we demonstrated that BPA-BNCT, (GB-10 + BPA)-BNCT and GB-10-BNCT induced good tumor response (expressed as complete remission + partial remission) in the hamster cheek pouch oral cancer model employing the RA-6 hyperthermal neutron beam [27,38,54] and the RA-3 thermal neutron facility [53,55]. Attempting to improve therapeutic efficacy, in particular complete tumor remission, at no extra cost in terms of toxicity in dose-limiting precancerous tissue, we devised and explored a novel approach to BNCT termed Sequential BNCT (Seq-BNCT). Irradiations were performed at RA-3 thermal facility employing a lithium-6 carbonate shielding to protect the body of the animal while the cheek pouch is everted out of the enclosure [53]. Seq-BNCT involves the sequential application of BPA-BNCT followed by GB-10-BNCT with an interval of 24 (Seq-24h-BNCT) or 48h (Seq-48h-BNCT) between the two applications. Thus, Seq-BNCT is a new way of combining the contribution of BPA and GB-10. The Sequential modality was devised based on notions of BNCT radiobiology contributed by studies by our group and others [2,27,56]. Seq-BNCT involves the use of two boron agents with different properties and complementary mechanisms of action, conceivably contributing to a more homogeneous, therapeutically successful targeting of heterogeneous tumor cell populations. The first application of BNCT would reduce tumor interstitial fluid pressure [57,58], thus improving the distribution of blood-borne therapeutic agents such as GB-10 for the second application. In addition, the induction of void space by cancer cell death would also enhance intratumoral delivery for the second application [59]. Thus, GB-10 would have a better chance of targeting a tumor when it is administered as part of the Sequential protocol than when it is administered with BPA in the joint GB-10 + BPA protocol. The 24h or 48h interval between applications is short enough to preclude tumor cell repopulation [60] and could favor targeting of the tumor cells that were refractory to the first application. Regarding dose-limiting mucositis in precancerous tissue, Seq-BNCT would favor a reduction in the severity of mucositis or, at least, would nor exacerbate mucositis compared to a single application of the same total dose employing (GB-10 + BPA)-BNCT. Because mucositis is a multistage process initiated by mucosal injury and associated to an increased production of inflammatory cytokines that cause direct mucosal damage and initiate positive feedback loops [61], the 24 or 48 h interval between BNCT applications might conceivably allow the inflammatory process to partially subside before the second dose is delivered, precluding the exacerbation of mucositis.

A comparison of tumor control and mucositis in dose-limiting precancerous tissue was performed for Seq-BNCT (Seq-24h-BNCT and Seq-48h-BNCT) *vs* (GB-10 + BPA)-BNCT at the RA-3 thermal neutron facility. The single application of BNCT was to the same total tumor absorbed dose (10Gy) as Seq-BNCT (BPA-BNCT at 4Gy to tumor followed by GB-10-BNCT at 6Gy to tumor) [62]. Here we must point out that the neutron irradiations in this case were performed at the RA-3 thermal nuclear facility and cannot be compared with our earlier studies at the hyperthermal neutron beam at RA-6 due to the differences in neutron spectrum and gamma component that influence biological response [3,54,55]. At 28 days post-treatment, Seq-24h-BNCT and Seq-48h-BNCT induced, respectively, overall tumor responses (partial remission + complete remission) of 95% and 91% while overall tumor response for (GB-10 + BPA)-BNCT was significantly lower at 75%. Complete remission was higher for Seq-24h-BNCT and Seq-48h-BNCT (76% and 68% respectively) than for (GB-10 + BPA)-BNCT (50%). No statistically significant differences were observed between Sequential protocols. The severity of mucositis was evaluated semi-quantitatively according to an oral mucositis scale based on macroscopic features adapted for the carcinogen-treated hamster cheek pouch from the WHO classification for oral mucositis in human subjects [63] and the six-point grading system for normal hamster cheek pouches of Sonis et al. [64]. The Sequential protocols and (GB-10 + BPA)-BNCT induced reversible mucositis in the dose-limiting precancerous tissue that peaked at 14 days post-treatment and had resolved by 21-28 days post-treatment. The incidence of Grade 3/4mucositis at 14 days post-treatment was 35% for the Sequential protocols taken together (no statistically significant differences were observed between the two Sequential protocols) and 60% for the (GB-10 + BPA)-BNCT protocol. No toxicity was observed in normal tissue for any of the protocols. In terms of toxicity to precancerous tissue, there were no adverse effects associated with the improved tumor response induced by the Sequential protocols. Sequential BNCT enhances tumor response at no extra cost in terms of toxicity in dose-limiting precancerous tissue [62]. Although Seq-BNCT involves two applications of BNCT, they are so close together, that the strategy would even be useful for patients with rapid disease progression requiring abbreviated treatment.

Another effective strategy to improve the delivery of boron carriers to tumor consists in fixing the flawed delivery system in tumors. The abnormal structure and function of tumor blood vessels impairs blood flow and effective convective fluid transport, leading to defective distribution of blood-borne therapeutic agents [65]. Tumor blood vessel normalization by tailored administration of anti-angiogenic agents that down regulate Vascular Endothelial Growth Factor (VEGF), over expressed in the majority of solid tumors, would lead to improved distribution of drugs in tumors. Given that the anti-angioogenic monoclonal antibodies employed to induce blood vessel normalization in human subjects, rats and mice [65] cannot

be used in hamsters due to lack of cross antigenicity, we developed a technique to transiently normalize aberrant tumor blood vessels in the hamster cheek pouch oral cancer model employing thalidomide as an anti-angiogenic drug [66]. BPA was administered in the window of normalization. We expected an increase in absolute boron content in tumors of animals treated with thalidomide prior to administration of BPA. However, gross boron measurements by ICP-MS failed to reveal statistically significant differences in gross boron content induced by normalization of tumor blood vessels. After our initial disappointment, we pondered on the fact that while gross values of boron concentration are used for dosimetric calculations [2], they tell us little about the adequacy and therapeutic usefulness of boron distribution. Taking into account that the primary aim of blood vessel normalization is not to increase total drug (and oxygen) uptake but, instead, seeks to distribute drugs (and oxygen) effectively to a larger proportion of tumor cells by fixing the flawed delivery system [65] we turned to micro distribution studies and actual radiobiological studies searching for an answer. Pre-treatment to normalize tumor blood vessels prior to administration of BPA enhanced tumor response from 67% to 84%, with an increase in complete tumor response of 43% to 56%. Knowing that tumor blood vessel normalization had not induced changes in gross boron content in tumor that could explain improved therapeutic efficacy, our working hypothesis was that a normalized vascular system would lead to a rise in homogeneity in tumor boron micro distribution. Alpha-particle spectrometry and neutron autoradiography studies in hamster cheek pouch tumors showed that while pretreatment with thalidomide did not increase the absolute boron content in oral tumors, it did improve boron targeting homogeneity in tumors. These findings suggest that the improvement in tumor response elicited by aberrant blood vessel normalization prior to administration of BPA could be ascribed at least partially to an improvement in BPA distribution in tumors [43]. An additional asset of pre-treatment with thalidomide was a protective effect against precancerous tissue mucositis, seemingly unrelated to changes in boron compound delivery. Given that cytokines are involved in the pathogenesis of oral mucositis [67], this protective effect was ascribed to the known cytokine inhibitor activity of thalidomide. Within the context of our approach based on the use of drugs approved for use in humans, we must point out that after the withdrawal of thalidomide from the market due to its teratogenic effects, it was approved in 1997 by FDA to combat a variety of conditions [68].While blood vessel normalization techniques in general also merit investigation as a way of optimizing the therapeutic advantage of BNCT in patients, the protective effect of thalidomide against dose-limiting mucositis would be a valuable bonus in terms of enhancing the therapeutic advantage between tumor and dose-limiting precancerous tissue.

The combined treatment with tumor blood vessel normalization followed by Sequential BNCT achieved, for the first time, in the hamster cheek pouch oral cancer model, 100% tumor response with 87% complete tumor remission, with no normal tissue toxicity and no cases of severe mucositis in dose-limiting precancerous tissue [25].

Further pursuing the development of strategies to improve the biodistribution and micro distribution of ^{10}B in tumor employing boron carriers approved for their use in humans, we evaluated if electroporation could improve the targeting of ^{10}B from GB-10, thus improving the therapeutic efficacy of GB-10-BNCT. Electroporation (or electropermeabilization) involves the localized application of pulsed electric fields and can act as a non-specific system to administer therapeutic agents and improve their delivery. In particular, electro chemotherapy, devised to improve boron delivery of chemotherapeutic agents, was approved by the European community to treat cutaneous and subcutaneous tumors [69,70]. Within this context, electroporation was proposed as a way to improve tumor boron targeting for BNCT [71]. The GB-10-BNCT protocol was particularly interesting to work on because it induces only mild mucositis in precancerous tissue with moderate tumor control. The idea of improving tumor control of a protocol that is virtually "cost-free" in terms of toxicity was particularly compelling. BNCT mediated by GB-10 at an absorbed dose of 4 Gy to tumor at RA-3 Nuclear Reactor, induced 48% overall tumor control (complete remission + partial remission) at 28 days post-treatment with only mild mucositis in precancerous tissue. When electroporation was performed individually in each pouch tumor available for study 10 mins after administration of GB-10, and irradiation was performed 2 h 50 mins later at the same neutron fluence as for GB-10-BNCT, tumor control increased significantly to 92% at virtually no additional cost in terms of precancerous tissue mucositis. Furthermore, electroporation significantly improved complete remission of small tumors ($<10mm^3$) from 7% to 65%. Seeking to understand the mechanisms involved in this robust improvement in tumor response, we perfomed boron biodistribution studies. The results of these studies revealed that electroporation increased GB-10 tumor gross boron concentration from 10±2 ppm to 20±10 ppm. While this increase is remarkable in and of itself, neutron autoradiography studies suggest that changes in the parenchyma/stroma microdistribution of GB-10 in tumor would be at the root of electroporation-enhanced tumor control. The fact that no changes in gross boron content or boron micro distribution were observed in precancerous tissue would explain how the enhancement in tumor control induced by electroporation would bear no cost in terms of toxicity in dose-limiting precancerous tissue [72].Having proved the therapeutic efficacy of BNCT to treat existing tumors, we still faced the challenge of achieving and demonstrating a long term inhibitory effect on the development of tumors from precancerous tissue without exceeding the tolerance of this tissue. Second primary tumor loco regional recurrences that arise in field-cancerized tissue are often the cause of therapeutic failure [47]. In addition, mucositis in the radiosensitive precancerous tissue limits the dose that can be administered to tumor. For these studies we developed a less aggressive oral precancer model (employing a less aggressive carcinogenesis protocol) that would allow for the long-term follow-up that is necessary to evaluate tumor development from precancerous tissue and yields a less aggressive precancerous tissue that mimics more closely the kinetics of field cancerization in humans. Employing this model we provided evidence, for the first time, that BNCT induces a long-term partial inhibitory effect on tumor development from precancerous tissue with no normal tissue radio toxicity and without exceeding precancerous tissue tolerance. Furthermore, we showed that BNCT is capable of reverting at least the histological hallmarks of premalignancy. Thus, the BNCT protocols that were previously proved effective to control established tumors would also inhibit loco regional recurrences caused by the development of tumors in precancerous tissue, suggesting a novel application of BNCT [45].

We then went on to explore the long-term effect of a double application of BNCT (full dose re-irradiation) mediated by BPA or (GB-10 + BPA) with a four to six-week interval between applications. A Double application or re-treatment must not be confused with a fractionated treatment. In the case of a Double application, dose is prescribed not to exceed the maximum tolerated dose to the "organ at risk" (or dose-limiting tissue) for each application. In the case of a fractionated treatment, dose is prescribed not to exceed the maximum tolerated dose to the "organ at risk" (or dose-limiting tissue) for the fractionated treatment as a whole. Following this line of thought, if standard radiotherapy is applied prescribing dose to the maximum tolerated dose to the "organ at risk" (or dose-limiting tissue), a re-treatment in the case of tumor recurrence would not be possible.

In a clinical scenario, a double application of BNCT (full dose re-treatment) would potentially serve several purposes: to improve tumor control, to treat a local recurrence, to inhibit the development of second primary tumors from field cancerized tissue and/or treat second primary tumors that developed after the first BNCT treatment. The potential benefits of a double application of BNCT must be weighed against the cost in terms of mucositis in dose-limiting precancerous tissue [5,38].

Re-treatment with BPA-BNCT and with (BPA + GB-10)-BNCT with a 6-week interval between applications exerted a significant inhibitory effect on tumor development from precancerous tissue. The precancerous tissue only exhibited mild (G1-G2) reversible mucositis that peaked one week after the first and second applications of BNCT and resolved to Grade 1/0 by 3 weeks after each application. A surprising finding was that the second application of BNCT did not exacerbate mucositis observed after the first treatment [46]. The best therapeutic effect was afforded by a Double application of (GB-10 + BPA)-BNCT at an absorbed dose to precancerous tissue of 5 Gy in each of two applications administered 4 weeks apart. Inhibition of tumor development *versus* control was 100% up to 2 months post-treatment and persisted at 63% eight months post-treatment. Mucositis was slight in the dose-limiting field cancerized tissue in 67% of the cases. Our studies suggested that the interval must not be shorter than 3-4 weeks, the minimum time necessary to allow mucositis to resolve after the first application.

Given that mucositis is dose-limiting, the use of radio protectors such as histamine (a compound also approved for use in humans) coupled to BNCT was explored with good results in this model, showing a reduction in BNCT-induced mucositis in precancerous tissue without jeopardizing therapeutic efficacy [73].

The salient finding of these studies [46,74] was that re-treatment with BPA-BNCT and (GB-10 + BPA)-BNCT is possible and therapeutically useful at no additional cost in terms of radio toxicity. Thus, a double application of BNCT would be a treatment option as a pre-established protocol or scheduled as a single application followed by re-treatment in the case of recurrence.

Liver metastases

Within the context of analyzing the therapeutic potential of BNCT for the treatment of pathologies that respond poorly to standard treatment, we performed translational BNCT studies in an experimental model of liver metastases. Patients with multifocal, nonresectable, bilobar liver metastases from colorectal cancer that do not respond to chemotherapy can only be offered palliative treatment. This lack of therapeutic option is particularly disappointing considering that in most cases the primary tumor in the colon can be excised and liver is the only site of metastatic spread [75]. Since BNCT is based on biochemical boron targeting of tumor cells rather than geometric targeting, it would be suited to treat undetectable micro metastases [76]. More recent interest in BNCT for the treatment of liver metastases has risen. We adapted a liver metastases model from Roveda et al. [77]. Subcapsular inoculations of colon cancer cells (DHD/K12/TRb) were performed by laparotomy in the liver of syngeneic BDIX rats to induce the development of sub capsular tumor nodules that simulate metastases but are more amenable to follow-up. Two weeks post-inoculation 100% of the animals developed localized, measurable, vascularized tumor nodules, with no peritoneal or pulmonary dissemination [24]. Our first studies in this model showed the therapeutic potential of different administration protocols of BPA, GB-10 and BPA+GB-10. Follow-on BNCT studies employing BPA as the boron carrier at the RA-3 thermal neutron facility showed an unequivocal tumor response at 3 and 5 weeks post-treatment [76,78]. The pre-treatment nodule weight of 48 mg rose to 750 mg in the age-matched Sham group (no treatment but same manipulation) while it fell to 7 mg five weeks post BPA-BNCT. At 13 Gy absorbed dose to tumor and 9 Gy to liver, BPA-BNCT achieved a 99% reduction in tumor mass compared to the untreated group, with no associated liver toxicity. Potential threshold doses for some degree of tumor response and significant tumor control were established at 6 Gy and 9 Gy absorbed dose to tumor respectively. We can conclude that BPA-BNCT is therapeutically effective to treat liver metastases with no liver toxicity within the study period. BNCT would offer two potential advantages over external photon radiotherapy. In the first place, BNCT can treat both visible and undetectable liver tumors whereas conformal radiotherapy can only treat visible liver tumors that are delineated by the physician in the treatment plan. Second, BNCT can treat multiple liver tumors without exceeding normal liver tolerance. In contrast, when 3 D conformal radiotherapy is applied to the treatment of more than three liver nodules, the risk of liver failure is a significant concern [79].

Lung metastases

Continuing to focus our efforts on translational studies in experimental models that mimic pathologies that are refractory to standard treatments, we performed studies in an experimental model of diffuse lung metastases. Metastatic lung disease is still a leading cause of death. Surgery, radio and chemotherapy have failed to improve survival satisfactorily and the overall prognosis for patients is poor. In conventional external radiotherapy, it is difficult to deliver therapeutic doses to malignant cells without causing radiation pneumonitis in the healthy lung [80]. Within this context, the search for more selective and less toxic treatment strategies is warranted, particularly in view of the marked radiosensitivity of the healthy lung [81]. BNCT has been proposed for the treatment of diffuse, non-resectable tumors in the lung. BNCT can offer a dose gradient between tumor and normal cells if ^{10}B atoms accumulate preferentially in tumor cells. Furthermore, due to the fact that BNCT is based on biological rather than geometric targeting it would be well suited to treat diffuse micro metastases. In addition, with BNCT it is

unnecessary to adjust for breathing motions [82].

Within this context, based on the work of Bortolussi et al. [82], we tailored the model of colon carcinoma diffuse lung metastases in BDIX rats to perform boron biodistribution studies and BNCT studies employing the boron compounds BPA and GB-10, administered alone or in combination [83]. DHD/K12/TRb colon carcinoma cells were injected in the jugular vein of syngeneic BDIX rats. Three weeks post-injection we observed reproducible development of abundant vascularized lung metastases with enough healthy lung tissue for evaluation. To date, only BPA has been explored as a boron carrier for BNCT of diffuse lung metastases. We went on to perform *in vivo* BNCT studies in the diffuse lung metastases model at RA-3, employing the BPA and (BPA + GB-10) administration protocols. At two weeks post-treatment, BPA-BNCT and (GB-10+BPA)-BNCT at minimum absorbed doses to tumor of 4 and 8 Gy were capable of halting tumor growth. Survival studies showed that BNCT extended the life of the animals by 35% *vs* untreated animals bearing lung metastases. No ostensible clinical, macroscopic or histological associated toxicity was observed in the lung of BNCT-treated animals [84], suggesting that it would be possible to escalate the dose to optimize efficacy.

Rheumatoid arthritis

Changing gears, we performed BNCT studies in a model of arthritis in rabbits. Rheumatoid arthritis is an autoimmune disease in which the articular normal synovial lining is replaced by a highly vascularized mass of inflammatory tissue. Long-term response to standard therapy such as drugs, open and arthroscopic synovectomy and radiation synovectomy employing beta-emitting radionuclides injected directly into the joint space, is poor [85]. Pathological synovium is the target in Rheumatoid Arthritis. Because this tissue and local malignancies share common features [86], BNCT has been proposed as an alternative approach to synovectomy [85,87] and termed Boron Neutron Capture Synovectomy (BNCS). BNCS would avoid the problems associated with the leakage of beta emitters from the joint while still profiting from the advantages of radiation synovectomy relative to surgery.

An ideal boron compound for BNCS should be nontoxic and biochemically stable, residence time in the synovium should be enough to allow for irradiation to be completed, and boron uptake in target tissue (pathological synovium) and healthy tissues (e.g. cartilage, muscle, tendon and skin) should be such that a therapeutic radiation dose can be delivered to the target tissue without exceeding the radio tolerance of the healthy tissues. We performed boron biodistribution studies and BNCS studies in a model of antigen-induced arthritis in New Zealand rabbits employing intra-articular administration of GB-10 and BPA. The administration of the boron compounds to a site immediately adjacent to the diseased tissue results in extremely high uptake levels in pathological synovium, 5 to 10 times the "ideal" target tissue boron concentration of 30 ppm established for the treatment of tumor with BNCT [2,27]. The low blood boron concentration values for the intra-articular administration protocols revealed scarce release of the boron carrier from the articulation into the blood stream. Since an important objective of a synovectomy procedure is to spare articular cartilage from permanent damage, cartilage was considered as the dose-limiting tissue in dosimetric analysis. The boron biodistribution data posed a considerable concern in that synovium boron targeting selectivity vs cartilage was only marginal. Based on the biodistribution data alone, an actual *in vivo* BNCS study might have been dismissed considering potential damage to cartilage. It must be stressed that whereas in the treatment of a malignant disease moderate/severe side effects might be acceptable, in the case of a disease that is not life-threatening, the safety requirements are more stringent. Extensive research has shown that articular cartilage is one of the least sensitive structures to radiation damage [88]. Detailed dosimetric calculations suggested that it would be possible to deliver therapeutically useful doses to synovium without significant damage to cartilage despite the very slight differences in boron concentration between synovium and cartilage [89].

Within this context we performed low-dose BNCS studies mediated by BPA or GB-10 administered intra-articularly in an experimental model of Antigen-Induced Arthritis (AIA) in New Zealand rabbits [90], employing administration protocols selected from the biodistribution studies described above [89]. Fifteen minutes post-administration of the boron compounds, irradiation of the target area (knee joint) was performed with the thermal beam of the RA-1 Reactor. The geometric set-up involves no body shielding and we relied on boron retention in the joint to exert a selective effect in the pathological articulation. The absorbed dose delivered to synovium for BPA and GB-10 was 2.4 Gy and 3.9 Gy to synovium respectively. Untreated AIA animals and healthy animals were used as controls.

Throughout the follow-up period of 2 months the rabbits did not exhibit any clinical signs of toxicity. Two months after BNCS the hind leg knee joints of all the rabbits treated with BPA-BNCS or GB-10-BNCS were no longer swollen or painful on palpation. At 2 months post-BNCS the MRI images of the AIA knee joints treated with BNCS were similar to those of control healthy joints in 100% of the cases, i.e. with no areas of necrosis or peri-articular effusion, and markedly different to untreated AIA joints that exhibited hydroarthrosis in the joint space, alterations in subchondral bone and alterations in the peri-articular soft tissue. The histological analysis of the synovial membranes obtained post-mortem, 2 months after treatment, revealed that in 70-100% of the fields corresponding to cases of AIA joints treated with BNCS the histological features were similar to those of healthy joints, i.e. no synovial hyperplasia, scarce or no lymphoplasmocytic infiltrate and no alterations in vascularization, and very different to those of untreated AIA joints which exhibited synovial hyperplasia, angiogenesis, edema and abundant inflammatory infiltrate as previously described in Sanchez Pernaute et al. [91].

The follow-up in this study was enough to show reversal of clinical symptoms, MRI and histological features of AIA, with no evidence of toxicity. Both BPA-BNCS and GB-10-BNCS, even at these very low dose levels, were therapeutically effective with no ostensible differences between the protocols. While BPA-BNCT would target malignant tissue on a cell by cell basis, GB-10-BNCT would mainly target aberrant blood vessels while preserving normal blood vessels in healthy tissue [27]. It is well known that neovascularization of the rheumatoid synovium is essential to perpetuate an angiogenic disease such as Rheumatoid Arthritis [92]. Within this context, GB-10 as a boron carrier for BNCS, alone or in combination with BPA would be

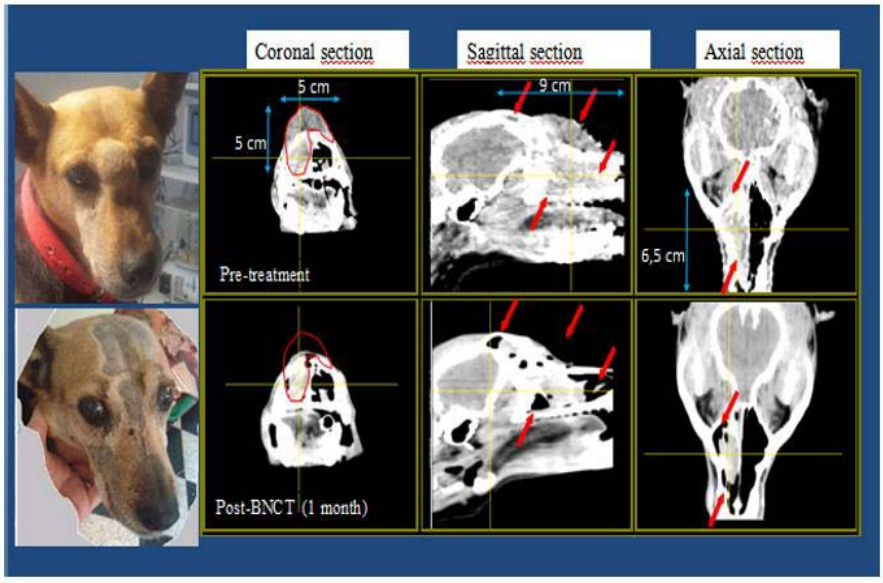

Figure 2: Macroscopic and Computed Tomography images of a canine patient with Nasal Squamous Cell Carcinoma, pre-treatment (upper panel) and 1 month BNCT (lower panel) showing approximately a 50% reduction in tumor volume post-treatment.

particularly suited to treat RA. It would be particularly contributory to explore the therapeutic efficacy of the combined intra-articular administration of GB-10 + BPA.

These findings [90] suggest that considerably lower doses to target tissue than anticipated from radiation synovectomy studies would be necessary to achieve a therapeutic effect with BNCS mediated by intra-articular administration of BPA or GB-10. This is an asset and minimizes (or altogether prevents) associated toxicity to healthy tissues such as cartilage and would allow for re-treatment if necessary.

Abscopal and direct effect of BNCT in an ectopic model of colon carcinoma in BDIX rats

In a simple animal model (the ectopic model of colon carcinoma in BDIX rats), we provided, for the first time, proof of principle that the positive response of a tumor to BNCT is capable of inducing an abscopal effect [93]. The abscopal (ab-scopus, away from the target) effect, originally described by Mole [94], refers to the inhibitory action of standard radiotherapy on the development and growth of non-targeted tumors, i.e. at a site distant from the area of irradiation [95]. We demonstrated that the use of BNCT alone produces significant local tumor control and is also capable of inducing an anti-tumor response at a distant site. The combination of BNCT and immune-based therapeutic modalities might constitute a potentially potent synergistic approach to provide long term protection and minimal toxicity, warranting future studies.

Clinical-Veterinary BNCT Studies in Dogs and Cats with Head and Neck Cancer with no Therapeutic Option

Based on a long and sometimes winding road of translational studies-not without several surprises - we began our ongoing clinical-veterinary BNCT studies at RA-6 Nuclear Reactor in cats and dogs with head and neck cancer with no treatment option.

Clinical-veterinary trials and clinical trials in humans are performed on the patients that are most difficult to treat. A clinical Phase I/Phase II trial is designed to explore the feasibility, safety and potential toxicity of a new treatment modality and, if possible, to monitor therapeutic efficacy, improve the clinical condition of the patient and prolong survival. The patients included in a clinical trial are those that have no treatment option and/or have been refractory to standard treatments. Hence, the chances of showing efficacy are smaller than when a treatment modality is used as a first line of therapy. This issue must be considered when we compare the outcome of a standard treatment with that of an experimental treatment.

An experimental treatment that can be applied safely to patients that have already been treated with standard therapy will be an extremely valuable tool. BNCT offers a dose gradient between tumor (or target tissue in general) and dose-limiting tissues in terms of selective boron targeting and higher relative biological effectiveness and compound biological effectiveness values for target tissue than for healthy tissue [2]. This feature is an asset in terms of minimizing the radiation dose that is delivered to healthy tissues that have already been exposed to standard radiotherapy.

Ongoing BPA-BNCT clinical trials in terminal cats and dogs with head and neck cancer that do not have a treatment option, seek to contribute clinically representative data to the knowledge of BNCT for head and neck cancer. Within the context of recent and ongoing BNCT trials for head and neck malignancies [5,8,13], the search for novel BNCT strategies that improve tumor control at no extra cost in terms of toxicity is particularly relevant.

Our data to date on boron biodistribution studies and BNCT studies in 3 cats treated with low dose BPA-BNCT at RA-1 [96], 3 cats treated with at a higher dose level of BPA-BNCT at RA-6 [97] and five dogs treated at RA-6 reveal the efficacy of BPA-BNCT to improve the clinical condition of the animals and partially control

tumors with only slight associated toxicity in healthy tissues. (Figure 2) shows a representative example of one of our canine patients with a nasal Squamous Cell Carcinoma with no treatment option. One month post-BNCT tumor volume was approximately 50% lower than pre-treatment at virtually no cost in terms of toxicity.

Very importantly, these studies showed that an animal patient whose tumor recurred locally several months after BNCT can be re-treated with a second full dose of BNCT with good results in terms of tumor control and only mild associated toxicity. Full dose re-treatment with standard radiotherapy would not be an option due to toxicity constraints. However, our translational work in the hamster cheek pouch oral cancer and oral pre cancer models encouraged us to assess re-treatment with BNCT, leading us to demonstrate its feasibility, safety and efficacy.

Conclusion

Translational BNCT studies in *in vivo* animal models devoted to optimize boron targeting, explore new treatment strategies and assess new applications of BNCT are pivotal to design novel, safe clinical BNCT protocols for existing or new targets for BNCT. The use of compounds approved for their use in humans allows for a more direct and less costly extrapolation to a clinical environment.

For progress in BNCT, equally important to scientific rigour, creativity, hard work and, admittedly, resources, is the Team spirit and joint efforts of an interdisciplinary group of professionals, technicians and collaborators (in the widest sense of the term). Also necessary, is a large dose of the Finnish concept "sisu" (thank you Dr. David W. Nigg for teaching us this term, impossible to translate, that refers to resoluteness and a consistent, courageous approach toward challenges which at first seem to exceed our capacities).

During a visit to the NASA space center in 1962, President John F. Kennedy noticed a janitor carrying a broom. He interrupted his tour, walked over to the man and said, "Hi, I'm Jack Kennedy. What are you doing?" "Well, Mr. President," the janitor responded, "I'm helping put a man on the moon." Recalling this event, if each of us, regardless of our particular role in a project, feels like the janitor in the story, we will pull together, move forward and enjoy the road, despite the meanders and bumps.

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References

1. Locher L. Biological effects and therapeutic possibilities of neutrons. Am J Roentgenol. 1936; 36: 1-13.
2. Coderre JA, Morris GM. The radiation biology of boron neutron capture therapy. Radiat Res. 1999; 151: 1-18.
3. Hopewell JW, Morris GM, Schwint AE, Coderre JA. The radiobiological principles of boron neutron capture therapy: A critical review. Appl Radiat Isot. 2011; 69: 1756-1759.
4. González SJ, Bonomi MR, Santa Cruz GA, Blaumann HR, Calzetta Larrieu OA, Menéndez P, et al. First BNCT treatment of a skin melanoma in Argentina: dosimetric analysis and clinical outcome. Appl Radiat Isot. 2004; 61: 1101-1105.
5. Kankaanranta L, Seppälä T, Koivunoro H, Saarilahti K, Atula T, Collan J, et al. Boron neutron capture therapy in the treatment of locally recurred head-and-neck cancer: final analysis of a phase I/II trial. Int J Radiat Oncol Biol Phys. 2012; 82: 67-75.
6. Miyatake S, Kawabata S, Hiramatsu R, Furuse M, Kuroiwa T, Suzuki M. Boron neutron capture therapy with bevacizumab may prolong the survival of recurrent malignant glioma patients: four cases. Radiat Oncol. 2014; 9: 6.
7. Wang LW, Chen YW, Ho CY, Hsueh Liu YW, Chou FI, Liu YH, et al. Fractionated BNCT for locally recurrent head and neck cancer: experience from a phase I/II clinical trial at TsingHua Open-Pool Reactor. Appl Radiat Isot. 2014; 88: 23-27.
8. Wang LW, Liu YWH, Chou FI, Jiang SH. Clinical trials for treating recurrent head and neck cancer with boron neutron capture therapy using the Tsing-Hua Open Pool Reactor. Cancer Communications. 2018; 38: 37.
9. Yanagie H, Higashi S, Seguchi K, Ikushima I, Fujihara M, Nonaka Y, et al. Pilot clinical study of boron neutron capture therapy for recurrent hepatic cancer involving the intra-arterial injection of a (10) BSH-containing WOW emulsion. Appl Radiat Isot. 2014; 88: 32-37.
10. Yanagie H, Higashi S, Seguchi K, Ikushima I, Oyama K, Nonaka Y, et al. Pilot clinical study of Boron Neutron Capture Therapy for recurrent hepatic cancer and gastric cancer. Oral communication at the 15th International Congress on Neutron Capture Therapy, Tsukuba, Japan. Appl Radiat isot. 2012.
11. Suzuki M, Suzuki O, Sakurai Y, Tanaka H, Kondo N, Kinashi Y, et al. Reirradiation for locally recurrent lung cancer in the chest wall with boron neutron capture therapy (BNCT). Int Cancer Conf J. 2012; 1: 235-238.
12. Hiratsuka J, Kamitani N, Tanaka R et al. Boron Neutron Capture Therapy for vulvar melanoma and genital extra mammary Paget´s disease, with curative responses. Cancer Communications. 2018.
13. Barth RF, Zhang Z, Liu T. A realistic appraisal of boron neutron capture therapy as a cancer treatment modality. Cancer Communications. 2018; 38: 36.
14. Durisi E, Zanini A, Manfredotti C, Palamara F, Sarotto M, Visca L, et al. Design of an epithermal column for BNCT based on D-D fusion neutron facility. Nuclear Instruments and methods in Physics Research, Section A. 2007; 574; 363-369.
15. Rasouli FS, Masoudi SF, Kasesaz Y. Design of a model for BSA to meet free beam parameters for BNCT based on a multiplier system for D-T neutron source. Annals of Nuclear Energy. 2012; 39: 18-25.
16. Torabi F, Masoudi SF, Rahmani F, Rasouli FS. BSA optimization and dosimetric assessment for an electron linac based BNCT of deep-seated brain tumors. J Radioanal Nucl Chem. 2014; 300; 1167-1174.
17. Fantidis JG, Antoniadis A. Optimization study for BNCT facility based on a DT neutron generator. Int J Rad Res. 2015; 13: 13-24.
18. Zolfaghari M, Sedaghatizadeh M. Design of thermal neutron beam based on an electron linear accelerator for BNCT. Applied Radiation and Isotopes. 2016; 118: 149-153.
19. Fantidis JG, Nicolaou G. Optimization of Beam Shaping Assembly design for Boron Neutron Capture Therapy based on a transportable proton accelerator. Alexandria Engineering Journal. 2017.

20. Sahoo GS, Sharma SD, Tripathy SP, Bandyopadhyay T. Design and dosimetric evaluation of beam shaping assembly for BNCT of compact D-T neutron generator by Monte Carlo simulation. Biomedica Physics and Engineering Express. 2017: 015018.

21. Kreiner AJ, Castell W, Di Paolo H, Baldo M, Bergueiro J, Burlon AA, et al. Development of a Tandem-Electrostatic-Quadrupole facility for Accelerator-Based Boron Neutron Capture Therapy. Appl Radiat Isot. 2011; 69: 1672-1675.

22. Yoshioka M, Kurihara T, Kobayashi H, Matsumoto H, Matsumoto N, Kumada H, et al. Construction of Accelerator-based BNCT facility at Ibaraki Neutron Medical Research Center. Proceedings of 16th International Congress on Neutron Capture Therapy. 2014; 66-67.

23. Chanana AD, Capala J, Chadha M, Coderre JA, Diaz AZ, Elowitz EH, et al. Boron neutron capture therapy for glioblastoma multiforme: interim results from the phase I/II dose-escalation studies. Neurosurgery. 1999; 44: 1182-1192.

24. Garabalino MA, Monti Hughes A, Molinari AJ, Heber EM, Pozzi ECC, Cardoso JE, et al. Boron neutron capture therapy (BNCT) for the treatment of liver metastases: biodistribution studies of boron compounds in an experimental model. Radiat Environ Biophys. 2011; 50: 199-207.

25. Molinari AJ, Thorp SI, Portu AM, Saint Martin G, Pozzi EC, Heber EM, et al. Assessing advantages of sequential boron neutron capture therapy (BNCT) in an oral cancer model with normalized blood vessels. Acta Oncol. 2015; 54: 99-106.

26. Schwint AE, Trivillin VT. ´Close-to-ideal` tumor boron targeting for boron neutron capture therapy is possible with 'less-than-ideal' boron carriers approved for use in humans. Ther Deliv. 2015; 6: 269-272.

27. Trivillin VA, Heber EM, Nigg DW, Itoiz ME, Calzetta O, Blaumann H, et al. Therapeutic success of Boron Neutron Capture Therapy (BNCT) mediated by a chemically non-selective boron agent in an experimental model of oral cancer: A new paradigm in BNCT radiobiology. Radiat Res. 2006; 166: 387-396.

28. Zonta A, Prati U, Roveda L, Ferrari C, Zonta S, Clerici AM, et al. Clinical lessons from the first applications of BNCT on unrespectable liver metastases. J Phys. 2006; 41: 484-495.

29. Matsumura A, Yamamoto T, Tsurubuchi T, Matsuda M, Shirakawa M, Nakai K, et al. Current practices and future directions of therapeutic strategy in glioblastoma: survival benefit and indication of BNCT. Appl Radiat Isot. 2009; 67: 12-14.

30. Barth RF, Vicente MG, Harling OK, Kiger WS 3rd, Riley KJ, Binns PJ, et al. Current status of boron neutron capture therapy of high grade gliomas and recurrent head and neck cancer. Radiat Oncol. 2012; 7: 146.

31. Suzuki M, Kato I, Aihara T, Hiratsuka J, Yoshimura K, Niimi M, et al. Boron neutron capture therapy outcomes for advanced or recurrent head and neck cancer. J Radiat Res. 2014; 55: 146-153.

32. Barth RF. From the laboratory to the clinic: How translational studies in animals have led to clinical advances in boron neutron capture therapy. Appl Radiat Isot. 2015; 106: 22-28.

33. Barth RF. Boron neutron capture therapy at the crossroads: challenges and opportunities. Appl Radiat Isot. 2009; 67: S3-S6.

34. Paul SM, Mytelka DS, Dunwiddie CT, Persinger CC, Munos BH, Lindborg SR, et al. How to improve R&D productivity: the pharmaceutical industry's grand challenge.Nat. Rev Drug Discov. 2010; 9: 203-214.

35. Mehrotra R, Ibrahim R, Eckardt A, Driemel O, Singh M. Novel strategies in head and neck cancer. Curr Cancer Drug Targets. 2011; 11: 465-478.

36. Khuri FR, Lippman SM, Spitz MR, Lotan R, Hong WK. Molecular epidemiology and retinoid chemoprevention of head and neck cancer. J Natl Cancer Inst. 1997; 89: 199-211.

37. Kreimann EL, Itoiz ME, Dagrosa A, Garavaglia R, Farías S, Batistoni D, et al. The hamster cheek pouch as a model of oral cancer for boron neutron capture therapy studies: selective delivery of boron by boronophenylalanine. Cancer Res. 2001; 61: 8775-8781.

38. Kreimann EL, Itoiz ME, Longhino J, Blaumann H, Calzetta O, Schwint AE. Boron neutron capture therapy for the treatment of oral cancer in the hamster cheek pouch model. Cancer Res. 2011; 61: 8638-8642.

39. Kato I, Ono K, Sakurai Y, Ohmae M, Maruhashi A, Imahori Y, et al. Effectiveness of BNCT for recurrent head and neck malignancies. Appl Radiat Isot. 2004; 61: 1069-1073.

40. Salley JJ. Experimental carcinogenesis in the cheek pouch of the Syrian hamster. J Dent Res. 1954; 33: 253-262.

41. Vairaktaris E, Spyridonidou S, Papakosta V, Vylliotis A, Lazaris A, Perrea D, et al. The hamster model of sequential oral oncogenesis'. Oral Oncol. 2008; 44: 315-324.

42. Nagini S. Of humans and hamsters: the hamster buccal pouch carcinogenesis model as a paradigm for oral oncogenesis and chemoprevention. Anticancer Agents Med Chem. 2009; 9: 843-852.

43. Molinari AJ, Pozzi EC, Monti Hughes A, Heber EM, Garabalino MA, Thorp SI, et al. Tumor blood vessel "normalization" improves the therapeutic efficacy of Boron Neutron Capture Therapy (BNCT) in experimental oral cancer. Radiat Res. 2012; 177: 59-68.

44. Monti-Hughes A, Aromando RF, Pérez MA, Schwint AE, Itoiz ME. The hamster cheek pouch model for field cancerization studies. Periodontol 2000. 2012; 67: 292-311.

45. Monti Hughes A, Heber EM, Pozzi E, Nigg DW, Calzetta O, Blaumann H, et al. Boron neutron capture therapy (BNCT) inhibits tumor development from precancerous tissue: an experimental study that supports a potential new application of BNCT. Appl Radiat Isot. 2009; 67: S313-S317.

46. Monti Hughes A, Pozzi EC, Heber EM, Thorp S, Miller M, Itoiz ME, et al. Boron Neutron Capture Therapy (BNCT) in an oral pre cancer model: therapeutic benefits and potential toxicity of a double application of BNCT with a six-week interval. Oral Oncol. 2011; 47: 1017-1022.

47. Smith BD, Haffty BG. Molecular markers as prognostic factors for local recurrence and radio resistance in head and neck squamous cell carcinoma. Radiat Oncol Investig. 1999; 7: 125-144.

48. Jensen SB, Peterson DE. Oral mucosal injury caused by cancer therapies: current management and new frontiers in research. J Oral Pathol Med. 2014; 43: 81-90.

49. Heber E, Trivillin VA, Nigg D, Kreimann EL, ItoizME, Rebagliati RJ, et al. Biodistribution of GB-10 ($Na_2{}^{10}B_{10}H_{10}$) compound for Boron Neutron Capture Therapy (BNCT) in an experimental model of oral cancer in the hamster cheek pouch. Arch Oral Biol. 2004; 49: 313-324.

50. Ono K, Masunaga S, Suzuki M, Kinashi Y, Takagaki M, Akaboshi M. The combined effect of boronophenylalanine and borocaptate in boron neutron capture therapy for SCCVII tumors in mice. Int J Radiat. Oncol Biol Phys. 1999; 43: 431-436.

51. Heber EM, Trivillin VA, Nigg DW, Itoiz ME, Gonzalez BN, Rebagliati RJ, et al. Homogeneous boron targeting of heterogeneous tumors for boron neutron capture therapy (BNCT): chemical analyses in the hamster cheek pouch oral cancer model. Arch Oral Biol. 2006; 51: 922-929.

52. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature. 2000; 407: 249-257.

53. Pozzi E, Nigg DW, Miller M, Thorp SI, Heber EM, Zarza L, et al. Dosimetry and radiobiology at the new RA-3 reactor Boron Neutron Capture Therapy (BNCT) facility: application to the treatment of experimental oral cancer. Appl Radiat Isot. 2009; 67: S309-S312.

54. Calzetta O, Blaumann H, Longhino J. RA-6 reactor mixed beam design and performance for NCT trials.10th International Congress on Neutron Capture Therapy. 2002; 8-13.

55. Miller M, Quintana J, Ojeda J, Langan S, Thorp S, Pozzi E, et al. New irradiation facility for biomedical applications at the RA-3 reactor thermal column.Appl. Radiat Isot. 2009; 67: S226-S229.

56. Trivillin VA, Heber EM, Itoiz ME, Nigg D, Calzetta O, Blaumann H, et al. Radiobiology of BNCT mediated by GB-10 and GB-10+BPA in experimental oral cancer. Appl Radiat Isot. 2004; 61: 939-945.

57. Jain RK. Transport of molecules across tumor vasculature. Cancer Metastasis Rev. 1987; 6: 559-593.

58. Znati CA, Rosenstein M, Boucher Y, Epperly MW, Bloomer WD, Jain RK. Effect of radiation on interstitial fluid pressure and oxygenation in a human tumor xenograft. Cancer Res. 1996; 56: 964-968.

59. Nagano S, Perentes JY, Jain RK, Boucher Y. Cancer cell death enhances the penetration and efficacy of oncolytic herpes simplex virus in tumors. Cancer Res. 2008; 68: 3795-3802.

60. Hopewell JW, Nyman J, Turesson I. Time factor for acute tissue reactions following fractionated irradiation: a balance between repopulation and enhanced radiosensitivity. Int J Radiat Biol. 2003; 79: 513-524.

61. Mais K. Mucositis from radiotherapy to the head and neck: an overview. Nursing. 2006; 1: 18-20.

62. Molinari AJ, Pozzi EC, Monti Hughes A, Heber EM, Garabalino MA, Thorp SI, et al. "Sequential" Boron Neutron Capture Therapy (BNCT): a novel approach to BNCT for the treatment of oral cancer in the hamster cheek pouch model. Radiat Res. 2011; 175: 463-472.

63. López Castaño F, Oñate-Sánchez RE, Roldán-Chicano R, Cabrerizo-Merino MC. Measurement of secondary mucositis to oncohematologic treatment by means of different scale. Med Oral Patol. Oral Cir Bucal. 2005; 10: 412-421.

64. Sonis ST, Peterson RL, Edwards LJ, Lucey CA, Wang L, Mason L, et al. Defining mechanisms of action of interleukin-11 on the progression of radiation induced oral mucositis in hamsters. Oral Oncol. 2000; 36: 373-381.

65. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science. 2005; 307: 58-62.

66. Molinari AJ, Aromando RF, Itoiz ME, Garabalino MA, Monti Hughes A, Heber EM, et al. Blood vessel normalization in the hamster oral cancer model for experimental cancer therapy studies. Anticancer Res. 2012; 32: 2703-2709.

67. Sonis ST. 'Mucositis as a biological process: a new hypothesis for the development of chemotherapy-induced stomatotoxicity', Oral Oncol. 1998; 34: 39-43.

68. Jacobson JM, Greenspan JS, Spritzler J, Ketter N, Fahey JL, Jackson JB, et al. Thalidomide for the treatment of oral aphtous ulcers in patients with human immunodeficiency virus infection. National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group. N Engl J Med. 1997; 336: 1487-1493.

69. Mir LM. Bases and rationale of the electro chemotherapy. EJC Suppl. 2006; 4: 38-44.

70. Olaiz N, Signori E, Maglietti F, Soba A, Suárez C, Turjanski P, et al. Tissue damage modeling in gene electrotransfer: The role of pH'. Bioelectrochemistry. 2014; 100: 105-111.

71. Ono K, Kinashi Y, Masunaga S, Suzuki M, Takagaki M. Electroporation increases the effect of borocaptate (10B-BSH) in neutron capture therapy. Int J Radiat Oncol Biol Phys. 1998; 42: 823-826.

72. Garabalino MA, Olaiz N, Pozzi ECC, Thorp S, Curotto P, Itoiz ME, et al. La electroporación aumenta el control tumoral inducido por la terapia por captura neutrónica en boro (BNCT) mediada por GB-10 en el modelo de cáncer bucal en hámster. Oral communication presented at the XLII Reunión Anual de la Asociación Argentina de Tecnología Nuclear (AATN), Ciudad Autónoma de Buenos Aires. Argentina 2015.

73. Monti Hughes A, Pozzi ECC, Thorp SI, Curotto P, Medina VA, Martinel Lamas DJ, et al. Histamine reduces boron neutron capture therapy-induced mucositis in an oral precancer model. Oral Dis. 2015; 21: 770-777.

74. Monti Hughes A, Pozzi EC, Thorp S, Garabalino MA, Farías RO, González SJ, et al. Boron neutron capture therapy for oral precancer: proof of principle in an experimental animal model. Oral Dis. 2013; 19: 789-795.

75. Nano R, Barni S, Chiari P, Pinelli T, Fossati F, Altieri S, et al. Efficacy of boron neutron capture therapy on liver metastases of colon adenocarcinoma: optical and ultrastructural study in the rat. Oncol Rep. 2004; 11: 149-153.

76. Pozzi ECC, Cardoso JE, Colombo LL, Thorp S, Monti Hughes A, Molinari AJ, et al. Boron Neutron Capture Therapy (BNCT) for liver metastasis: therapeutic efficacy in an experimental model. Radiat Environ Biophys. 2012; 51: 331-339.

77. Roveda L, Prati U, Bakeine J, Trotta F, Marotta P, Valsecchi P, et al. How to study boron biodistribution in liver metastases from colorectal cancer. J Chemother. 2004; 16: 15-18.

78. Pozzi EC, Trivillin VA, Colombo LL, Monti Hughes A, Thorp SI, Cardoso JE, et al. Boron Neutron Capture Therapy (BNCT) for liver metastasis in an experimental model: dose-response at five-week follow-up based on retrospective dose assessment in individual rats. Radiat Environ Biophys. 2013; 52: 481-491.

79. Suzuki M, Sakurai Y, Hagiwara S, Masunaga S, Kinashi Y, Nagata K, et al. First attempt of Boron Neutron Capture Therapy (BNCT) for hepatocellular carcinoma. Jpn J Clin Oncol. 2007; 37: 376-381.

80. Suzuki M, Sakurai Y, Masunaga S, Kinashi Y, Nagata K, Maruhashi A, et al. A preliminary experimental study of boron neutron capture therapy for malignant tumors spreading in thoracic cavity. Jpn J Clin Oncol. 2007; 37: 245-249.

81. Bakeine GJ, Di Salvo M, Bortolussi S, Stella S, Bruschi P, Bertolotti A, et al. Feasibility study on the utilization of Boron Neutron Capture Therapy (BNCT) in a rat model of diffuse lung metastases. Appl Radiat Isot. 2009; 67: S332-S335.

82. Bortolussi S, Bakeine JG, Ballarini F, Bruschi P, Gadan MA, Protti N, et al. Boron uptake measurements in a rat model for boron neutron capture therapy of lung tumours. Appl Radiat Isot. 2011; 69: 394-398.

83. Trivillin VA, Garabalino MA, Colombo LL, González SJ, Farías RO, Monti Hughes A, et al. Biodistribution of the boron carriers Boron Phenylalanine (BPA) and/or decahydrodecaborate (GB-10) for Boron Neutron Capture Therapy (BNCT) in an experimental model of lung metastases. Appl Radiat Isot. 2014; 88: 94-98.

84. Trivillin VA, Serrano A, Garabalino MA, Colombo LL, Pozzi ECC, Monti Hughes A, et al. Translational Boron Neutron Capture Therapy (BNCT) studies for the treatment of tumors in lung. Int J Radiat Biol 2019; 2:1-25.

85. Yanch JC, Shortkroff S, Shefer RE, Johnson S, Binello E, Gierga D, et al. Boron Neutron Capture Synovectomy: treatment of rheumatoid arthritis based on the $^{10}B(n,\alpha)^{7}Li$ nuclear reaction. Med Phys. 1999; 26: 364-375.

86. Sweeney SE, Firestein GS. Rheumatoid arthritis: regulation of synovial inflammation. Int J Biochem. Cell Biol. 2004; 36: 372-378.

87. Watson-Clark RA, Banquerigo ML, Shelly K, Hawthorne MF, Brahn E. Model studies directed toward the application of boron neutron capture therapy to rheumatoid arthritis: boron delivery by liposome's in rat collagen-induced arthritis. Proc Natl Acad Sci. 1998; 95: 2531-2534.

88. Takahashi S, Sugimoto M, Kotoura Y, Oka M, Sasai K, Abe M, et al. Long-lasting tolerance of articular cartilage after experimental intraoperative radiation in rabbits. Clin Orthop Relat Res. 1992; 275: 300-305.

89. Trivillin VA, Abramson DB, Bumaguin GE, Bruno LJ, Garabalino MA, Monti Hughes A. Boron Neutron Capture Synovectomy (BNCS) as a potential therapy for rheumatoid arthritis: boron biodistribution study in a model of antigen-induced arthritis in rabbits. Radiat Environ Biophys. 2014; 53: 635-643.

90. Trivillin VA, Bruno LJ, Gatti DA, Stur M, Garabalino MA, Monti Hughes A, et al. Boron Neutron Capture Synovectomy (BNCS) as a potential therapy for rheumatoid arthritis: radiobiological studies at RA-1 Nuclear Reactor in a model of antigen-induced arthritis in rabbits. Radiat Environ Biophys. 2016; 55: 467-475.

91. Sanchez-Pernaute O, Lopez-Armada MJ, Calvo E, Diez-Ortego I, Largo R, Egido J, et al. Fibrin generated in the synovial fluid activates intimal cells from their apical surface: a sequential morphological study in antigen-induced arthritis. Rheumatology. 2003; 42: 19-25.

92. Taylor PC, Feldmann M. Rheumatoid arthritis: pathogenic mechanisms and therapeutic targets. Drug Discov. 2004; 1: 289-295.

93. Trivillin VA, Pozzi ECC, Colombo LL, Thorp SI, Garabalino MA, Monti Hughes A, et al. Abscopal effect of Boron Neutron Capture Therapy (BNCT): proof of principle in an experimental model of colon cancer. Radiat Environ Biophys. 2017; 56: 365-375.

94. Mole RH. Whole body irradiation; radiobiology or medicine? Br J Radiol. 1953; 26: 234-241.

95. Golden EB, Demaria S, Schiff PB, Chachoua A, Formenti SC. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. Cancer Immunol Res. 2013; 1: 365-372.

96. Rao M, Trivillin VA, Heber EM, Cantarelli MA, Itoiz ME, Nigg DW, et al. BNCT of 3 cases of spontaneous head and neck cancer in feline patients. Appl Radiat Isot. 2004; 61: 947-952.

97. Trivillin VA, Heber EM, Rao M, Cantarelli MA, Itoiz ME, Nigg DW, et al. Boron Neutron Capture Therapy (BNCT) for the treatment of spontaneous nasal planum squamous cell carcinoma in felines. Radiat Environ Biophys. 2008; 47: 147-155.

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