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Radiobiological response of U251MG, CHO-K1 and V79 cell lines to accelerator-based boron neutron capture therapy

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ABSTRACT

In the current article, we provide *in vitro* efficacy evaluation of a unique accelerator-based neutron source, constructed at the Budker Institute of Nuclear Physics (Novosibirsk, Russian Federation), for boron neutron capture therapy (BNCT), which is particularly effective in the case of invasive cancers. U251MG, CHO-K1 and V79 cells were incubated and irradiated in various concentrations of boric acid with epithermal neutrons for 2–3 h in a plexiglass phantom, using 2.0 MeV proton energy and 1.5–3.0 mA proton current, resulting in a neutron fluence of 2.16×10^{12} cm^{-2}. The survival curves of cells loaded with boron were normalized to those irradiated without boron (to exclude the influence of the fast neutron and gamma dose components) and fit to the linear–quadratic (LQ) model. Colony formation assays showed the following cell survival rates (means ± SDs): CHO-K1: 0.348 ± 0.069 (10 ppm), 0.058 ± 0.017 (20 ppm), 0.018 ± 0.005 (40 ppm); V79: 0.476 ± 0.160 (10 ppm), 0.346 ± 0.053 (20 ppm), 0.078 ± 0.015 (40 ppm); and U251MG: 0.311 ± 0.061 (10 ppm), 0.131 ± 0.022 (20 ppm), 0.020 ± 0.010 (40 ppm). The difference between treated cells and controls was significant in all cases ($P < 0.01$) and confirmed that the neutron source and irradiation regimen were sufficient for control over cell colony formation. We believe our study will serve as a model for ongoing *in vitro* experiments on neutron capture therapy to advance in this area for further development of accelerator-based BNCT into the clinical phase.

Keywords: boron neutron capture therapy; accelerator-based neutron source; lithium target; boric acid; *in vitro* efficacy evaluation

INTRODUCTION

Boron neutron capture therapy (BNCT) is a unique radiotherapy method based on the interaction of a stable ^{10}B isotope with thermal neutrons. The boron neutron capture reaction (^{10}B(n,α)^{7}Li) results in the release of high linear energy transfer (high-LET) alpha and ^{7}Li particles, which destroy tumor cell DNA. Selective accumulation of ^{10}B in cancer cells provides for specific elimination, while sparing normal tissues [1], as the penetration of alpha-particles and

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Li nuclei does not exceed single tumor cell depths. Thus, BNCT, in its ideal application, can provide curative treatment for invasive cancers, such as glioblastoma. The efficacy of BNCT from a nuclear reactor neutron source has been confirmed for certain malignancies, including glioma [2–4], malignant melanoma [5], and head and neck cancer [6–9]. However, safety issues, as well as the negative publicity surrounding the Fukushima accident, turned the world BNCT community towards development of accelerator-based neutron sources to replace nuclear reactors in both trials and therapy.

Recently, several accelerators destined for hospital placement have been introduced [10]. For BNCT purposes, a proton accelerator with vacuum insulation and a lithium target have been developed at the Budker Institute of Nuclear Physics (BINP) at the Russian Academy of Sciences (Novosibirsk, Russian Federation) [11]. To the best of our knowledge, no similar accelerator specifically designed for BNCT has ever been created with such unique features. *In vitro* experiments using tumor and normal cells are typically carried out at the initial biological efficacy evaluation stage. It is within this stage that the main contrast to standard radiotherapy is seen, because BNCT efficacy depends not only on the irradiation source, but also on the accumulation of a boron-containing agent, whose concentration in tumor cells directly influences the treatment effect. In previous experiments at the nuclear reactor, boric acid was used as a standard boron compound for reliable intracellular boron concentration [1].

Any proposed replacement for traditional reactor-based therapies requires pioneering *in vitro* studies to establish optimum dosages and cellular effects. Therefore, in the current study, we evaluated the efficacy of our accelerator-based neutron source using U251MG, CHO-K1 and V79 cells incubated and irradiated in a boric acid–containing medium at various boron concentrations (0, 10, 20 and 40 ppm), with absorbed dose calculations and further cell survival evaluation using a colony-formation assay (CF assay). Such a method was intentionally used to assure continuous maintenance of boron concentrations during the entire irradiation period, which is one of the key points of these experiments (compared with reports [27–29] for other compounds, where boron was not evenly distributed in the medium and the cells). This study is one of the initial steps of a project on synthesis and evaluation of complex boron/high-Z element compounds for absorbed dose estimation and tumor localization during accelerator-based BNCT.

MATERIALS AND METHODS

Cell lines

Human glioma (U251MG) cells, Chinese hamster ovary cells (CHO-K1), and Chinese hamster lung fibroblasts (V79) were purchased from the Institute of Cytology of the Russian Academy of Sciences (St Petersburg, Russian Federation), cultured in Iscove's modified Dulbecco's medium (IMDM) (SIGMA 17633 with L-glutamine and 25 mH HEPES, without sodium bicarbonate), supplemented with 10% fetal bovine serum (Thermo Scientific HyClone SV30160.03 HyClone UK Ltd) and maintained at 37°C in an atmosphere of 5% CO_2.

Boric acid application

In vitro experiments were performed at the Institute of Molecular and Cell Biology (Novosibirsk, Russian Federation). The cells were incubated for 2 h in a medium containing boric acid (Sigma-Aldrich, Inc., St Luis, MO, USA) in various concentrations (10, 20 and 40 ppm) of ^{10}B. The cells without boron were irradiated and used as controls. At the indicated time points, medium with boric acid was removed separately for each sample, the cells were washed with phosphate-buffered saline (PBS), trypsinized (0.05% trypsin-EDTA, Nacalai Tesque, Inc., Kyoto, Japan), counted and placed in 2 ml plastic vials in the boric acid–containing medium they were incubated in with the corresponding ^{10}B concentrations (Fig. 1A).

Neutron irradiation

The samples were placed in a phantom made of organic glass at a depth of 3 cm [12, 13] (Fig. 1B) and irradiated in a tandem accelerator with vacuum insulation (Fig. 2A), with the epithermal neutron

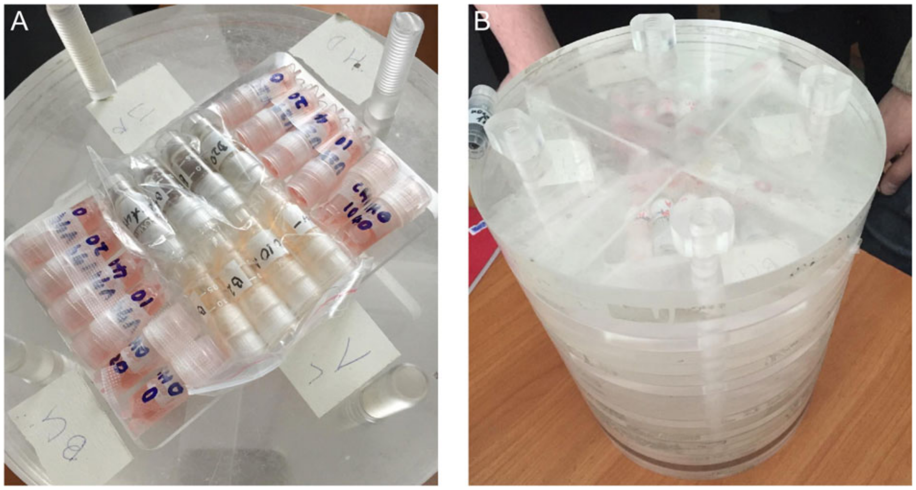

Fig. 1. The samples in 2 ml vials (A) placed in the plexiglass phantom (B).

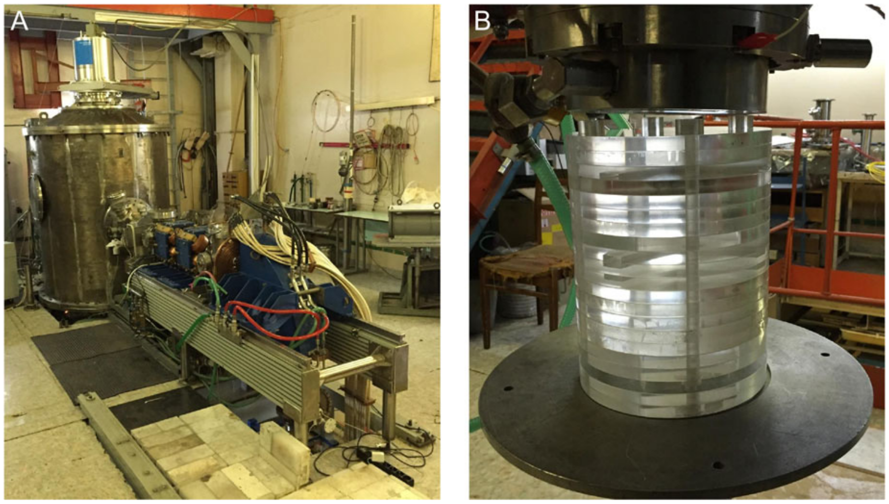

Fig. 2. The accelerator-based neutron source at the Budker Institute of Nuclear Physics, Russian Academy of Sciences (A). The plexiglass phantom set up under the lithium target (B).

beam under the lithium target (Fig. 2B). The irradiation lasted 2–3 h with the following accelerator settings: 2.0 MeV proton energy, 1.5–3.0 mA proton current (providing an epithermal neutron flux up to $3 \times 10^8\ \mathrm{cm}^{-2}\,\mathrm{s}^{-1}$). The settings were adjusted to produce epithermal neutrons eligible for phantom penetration with subsequent energy decrease to maximize neutron capture by boron in the samples. The necessary depth of the plexiglass in the phantom between the target and the cells was estimated using the Monte Carlo method and provided the maximum thermal neutron irradiation of the samples. The neutron flux was measured by a detector with a lithium-containing scintillator (GS20, Saint-Gobain Crystals, Hiram, OH, USA). Neutron fluence was measured by activation of the gold foil.

Colony-formation assay

After the irradiation, the cells were immediately counted, diluted and seeded into 6 cm dishes for CF assay. After 1–2 weeks, the dishes were washed with PBS, fixed with glutaraldehyde, stained with crystal violet, and dried. Colonies of 50 cells or more were counted for each sample. Cell survival fractions were calculated according to a previously adapted protocol [14, 15]. The results were normalized to controls, which were irradiated without boric acid to smooth the influence of concomitant fast neutrons and gamma-rays, and the statistical significance was evaluated using one-way analysis of variance (ANOVA).

Radiobiological parameters evaluation

The cell survival data were fit to the linear–quadratic (LQ) model, using the SOLVER add-on in Microsoft Excel. As the issue of the absorbed dose evaluation in the accelerator-based BNCT remains controversial, we used the boron concentration instead of the dose to calculate the radiobiological parameters. Using α' and β' values, the boron concentration needed to control 90% of cell growth, C_{10} (instead of D_{10}), was calculated by solving the following quadratic equation:

$$\alpha' C + \beta' C^2 + ln(SF) = 0,$$

where C represented ^{10}B concentration in cells, and in cases with linear survival curves (where $\beta' = 0$) equaled $ln(SF)/\alpha'$, otherwise:

$$C = \frac{-\alpha' \pm \sqrt{\alpha'^2 - 4\beta' ln(SF)}}{2\beta'};$$

positive values of C were used.

RESULTS

Colony-formation assay

Three types of cells were incubated in boric acid in four different boron concentrations, which resulted in the analysis of five samples for each cell line in each experiment. The dosages chosen were evaluated as the most likely, realistic concentrations that could be reached in a therapeutic situation. Dishes with stained colonies of each cell line after irradiation with boric acid in the various concentrations are shown in Fig. 3. The number of colonies in all cell lines decreased with increase in boron concentration, with the maximum effect at 40 ppm. Cell survival rates (means ± SDs) are presented in Table 1, and the survival curves are plotted in Fig. 4. The difference between the treated cells and the controls was significant in all cases ($P < 0.01$). This data shows the dosage-dependent effect of boron on the neutron beam and that physiologically relevant concentrations can produce a therapeutic effect.

Radiobiological parameters

The calculated parameters are summarized in Table 2. In two of three cell lines (CHO-K1 and U251MG) $\beta' = 0$, reflecting a linear decrease in cell survival (typical with high-LET irradiation). In the V79 cell line, both α' and β' parameters were present, showing different responses of the cell line to similar irradiation regimens. C_{10} values reflected the sensitivity of the cells loaded with boron to

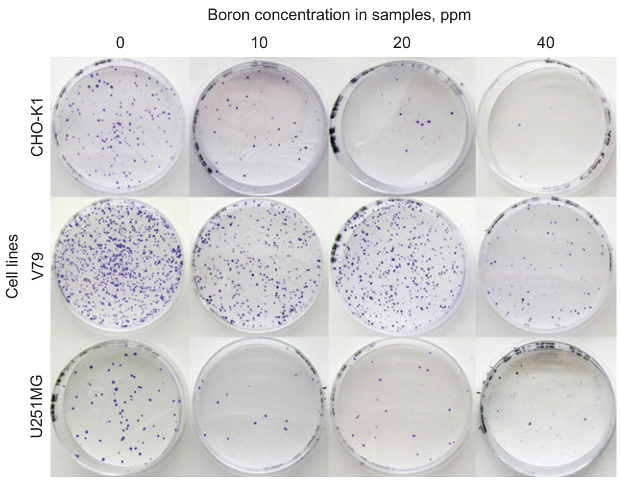

Fig. 3. CF-assay results: 6-cm dishes with stained colonies of each cell line 1–2 weeks after neutron irradiation. CHO-K1 (upper), V79 (middle) and U-251MG (lower) cells. The boron concentration is 0, 10, 20 and 40 ppm (from left to right).

Table 1. Surviving fractions of irradiated cells

Boron concentration (ppm)		10	20	40
SF	V79	0.476 ± 0.160	0.346 ± 0.053	0.078 ± 0.015
	U251MG	0.311 ± 0.061	0.131 ± 0.022	0.020 ± 0.010
	CHO-K1	0.348 ± 0.069	0.058 ± 0.017	0.018 ± 0.005

Cell survival fractions (SFs) are presented as means ± SDs. All SFs significantly differed from controls ($P < 0.01$, ANOVA).

neutron irradiation. This indicates that BNCT would therefore be useful for multiple types of treatments.

DISCUSSION

Recently, a surge in medical interest has seen the development of accelerator-based neutron sources all over the world to replace reactors (for safety and accessibility reasons) [10]. To this end, several types of neutron-producing targets have been introduced: solid ^{7}Li(p,n) [16], Be(p,n) [17], ^{9}Be(d,n) [18] and liquid ^{7}Li(p,n) [19]. With this in mind, a team of physicists at BINP has developed a compact tandem accelerator with vacuum insulation and a lithium target, which might have certain advantages over other materials in terms of the number and spectrum of neutrons produced [11]. Initially, in 1998 Bayanov *et al.* reported on neutrons produced as a result of a threshold reaction involving ^{7}Li(p,n)^{7}Be after application of a proton beam of 2–2.5 MeV and 10 mA on a lithium target [20]. Further development of the lithium target and the results of the neutron spectrum analysis were reported [21]. The first experimental results of neutron production using a 2 MeV-proton tandem accelerator with vacuum insulation were shown in 2008 [22]. Bayanov *et al.* have reported on neutron generation experiments on a new 2 MeV tandem accelerator using a specially designed lithium target [23, 24]. It was only then that radiobiological experiments became available (after stabilization of the proton current to 1.5–2 mA was achieved [25] and the size of the accelerator was reduced to make it more compact [26]). It is within this framework of rapid development that we conducted our initial studies to investigate the efficacy of accelerator-based neutron sources.

To date, initial radiobiological experiments on tumor cells to evaluate the efficacy of the neutron source at BINP have been performed with L-p-boronophenylalanine (BPA) [27–29], a boron agent. This compound was previously used as a ^{10}B compound in clinical trials in reactor-based BNCT, along with disodium mercaptoundecahydrododecaborate (BSH) [30, 31]. However, the results of such *in vitro* experiments highlighted a critical point; namely, that cellular boron accumulation depends on transport mechanisms and can be influenced by a number of factors, creating the necessity to search for ways to optimize cellular boron levels at those needed for therapy [32]. Such variations in boron compound accumulation can significantly alter the results of a treatment. Further trials might be needed to establish baseline values and reliably predict clinical outcomes.

The reliability of boric acid has been proven in a previously reported radiobiological dosimetry study at the reactor-based neutron source [1]. We assumed a steady state for intra- and extracellular boron concentration, as previously reported data has shown boron distribution throughout bodily fluids in animals and humans to occur in all tissues (except bone) at a concentration not significantly different from that in blood [33–36]. Moreover, we maintained the cellular concentration of boric acid during irradiation by keeping the cells in the medium they were incubated in.

To more accurately model parameters for future studies, we compared the response of several cell lines, including U251MG—a human glioma, and two normal cell lines. First, we chose the CHO-K1 line previously used in our recent study on the accumulation of new boron compounds, and then also examined the V79 cell line (lung fibroblasts that represent normal tissue cells and are generally accepted in the literature as suitable models [1, 28, 32]).

Our cell survival data confirmed the efficacy of the accelerator neutron source with the lithium target at BINP to produce a sufficient number of neutrons to initiate a boron neutron capture reaction within and in proximity to tumor cells. The cell survival rate in each cell line was inversely proportional to the boric acid concentration (Fig. 4), showing that the number of neutrons was sufficient to control further tumor cell growth; these results are comparable with those obtained at the nuclear reactor using tumor cells incubated with boric acid by Yamamoto *et al.* (2003) [1]. In our experiments, the proton current was steadily ramped up to 3 mA to lessen the irradiation time and was kept relatively stable during irradiation.

We calculated the radiobiological parameters based on the boron concentration and observed the nature of the cell response to the irradiation conditions. The cell survival curves were normalized to the data for the cells irradiated without boron to remove fast

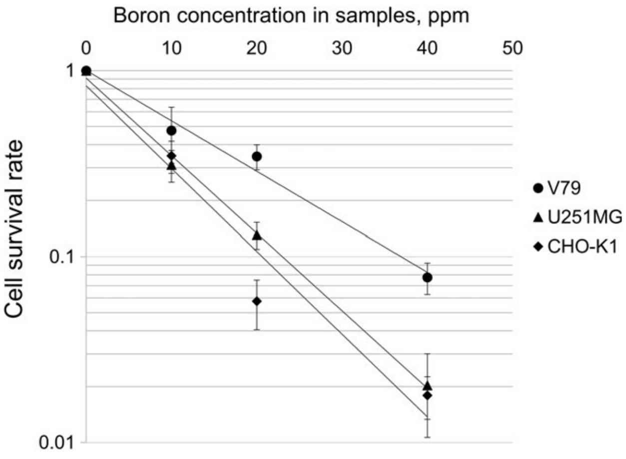

Fig. 4. Cell survival curves depend on the boron concentration in the samples. The data are presented as means ± SDs. *$P < 0.01$ with respect to boron concentration of 0 ppm (one-way ANOVA).

Table 2. Radiobiological parameters of irradiated cells

Cell line	α'	β'	C_{10} *(ppm)*
V79	0.048	0.000390192	36.90
U251MG	0.103	0	22.39
CHO-K1	0.123	0	18.69

Parameters, such as α' and β' are presented as absolute numbers. C_{10} (in ppm) represents the ^{10}B concentration needed to eliminate 90% of tumor cells.

neutron and gamma-ray components, with the assumption that all detected neutrons were slowed down by the plexiglass to thermal ones at the sample level and passed through the cells once. The influence of prompt gamma-rays induced by the ^{10}B (n,α) ^{7}Li reaction was also considered to be negligible for the following reasons. First, only 16% of all energy released in the reaction is released by gamma rays. Second, when alpha particles and lithium nuclei that carry the bulk of the energy are characterized by a high deceleration rate and a small run in water or in biological tissues (microns), the mean free path of gamma-rays with an energy of 478 keV exceeds the pathway of alpha particles and lithium nuclei by more than an order of magnitude, which is comparable with the sizes of the investigated samples; therefore, only a small part of the energy of gamma-rays is released in the samples. The small effect of prompt gamma-rays in the dose was confirmed by calculations analogous to those described by Kandiev *et al.*, 2011 [12]. Thus, in two cell lines (U251MG and CHO-K1), the survival curves were linear, with $\beta' = 0$, reflecting the effect of a high-LET radiation effect, typical for BNCT. In the V79 cell line, the presence of both α' and β' parameters could show the multicomponent irradiation effect or specific response to BNCT, and the character of the survival showed the cells were less susceptible to our treatment.

The differential response between the cell lines could be due to different radiosensitivity or differences in boron uptake. Generally, it might be difficult to exclude the influence of every possible factor, as, regardless of our assumption, some percentage of neutrons may keep higher energies while penetrating the samples, and temperature-dependent neutron scattering cannot be perfectly predicted. Thus, with respect to these unpredictable factors, our methodology to establish correct radiobiological parameters could be counted as limitations of this study.

Longer irradiation times could also affect the overall results due to additional influence from background dose. Therefore, in our experiments, we normalized the responses of cells irradiated with boron to control cells irradiated without boron to specifically exclude the background component. The novelty of our report required us to go above and beyond what would be normal irradiation conditions to discover extreme parameters for both future research and therapy. As technological developments merge with data from multiple analyses, an almost guaranteed shorter irradiation time will be the result as the neutron current increases. This will minimize the background dose effect, and such long exposures will not be typical of future studies.

In our study, we mainly focused on comparison between the effect of the new source and previous data for clinically proven, reactor-based experiments [2]. Therefore, at this stage of development, a baseline comparison was focused on to the exclusion of the cellular mechanisms of response to BNCT or variations in boron uptake (which remain goals for future work). We showed that the accelerator was effective in initial cell experiments and that its performance as a neutron source was close to that of the reactor. For future studies, it will be critical to study detailed parameters of both mechanisms related to boron uptake in different cell lines/animal models and cellular radiosensitivity.

In our experiments, the irradiation effect was obvious for the cells, though the provided epithermal neutron fluence still might be insufficient for clinical trials. In this regard, further improvement of the accelerator, including stabilization of an increased (up to 5 mA) proton current and development of a new lithium target and neutron beam shaping assembly [13] is in progress. In our study, as in many others, *in vitro* experiments play only an initial role in evaluation of the method's efficacy. Animal experiments with appropriate models more adequately and closely represent clinical conditions and will be the main focus of our next set of experiments.

CONCLUSION

We carried out an initial evaluation of an accelerator-based neutron source for BNCT at BINP, using boric acid to create verified intracellular boron concentrations that avoided compound accumulation variations. Such variation depends on boron uptake mechanisms and might differ in each cell line. We also proved the ability of the irradiation source to provide sufficient control over cell proliferation after boron uptake. We believe that our study will bring more clarity to ongoing *in vitro* experiments on neutron capture therapy and help other researchers to advance accelerator-based BNCT into the clinical phase.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

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REFERENCES

1. Yamamoto T, Matsumura A, Yamamoto K et al. Characterization of neutron beams for boron neutron capture therapy: in-air radiobiological dosimetry. *Radiat Res* 2003;160:70–6.
2. Yamamoto T, Nakai K, Kageji T et al. Boron neutron capture therapy for newly diagnosed glioblastoma. *Radiother Oncol* 2009;91:80–4.
3. Miyatake S, Kawabata S, Yokoyama K et al. Survival benefit of boron neutron capture therapy for recurrent malignant gliomas. *J Neurooncol* 2009;91:199–206.
4. Aiyama H, Nakai K, Yamamoto T et al. A clinical trial protocol for second line treatment of malignant brain tumors with BNCT at University of Tsukuba. *Appl Radiat Isot* 2011;69:1819–22.
5. Yong Z, Song Z, Zhou Y et al. Boron neutron capture therapy for malignant melanoma: first clinical case report in China. *Chin J Cancer Res* 2016;28:634–40.
6. Kato I, Fujita Y, Maruhashi A et al. Effectiveness of boron neutron capture therapy for recurrent head and neck malignancies. *Appl Radiat Isot* 2004;61:1069–73.
7. Kankaanranta L, Seppälä T, Koivunoro H et al. Boron neutron capture therapy in the treatment of locally recurred head and neck cancer. *Int J Radiat Oncol Biol Phys* 2007;69:475–82.
8. Kankaanranta L, Seppälä T, Koivunoro H 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:e67–75.
9. Suzuki M, Kato I, Aihara T et al. Boron neutron capture therapy outcomes for advanced or recuurent head and neck cancer. *J Radiat Res* 2014;55:146–53.
10. Kreiner AJ, Bergueiro J, Cartelli D et al. Present status of accelerator-based BNCT. *Rep Pract Oncol Radiother* 2016;21:95–101.
11. Taskaev S. Accelerator based epithermal neutron source. *Phys Particles Nucl* 2015;46:956–90.
12. Kandiev YA, Kashaeva E, Malyshkin G et al. Optimization of the target of an accelerator-driven neutron source through Monte Carlo numerical simulation of neutron and gamma transport by the PRIZMA code. *Appl Radiat Isot* 2011;69:1632–4.
13. Zaidi L, Kashaeva E, Lezhnin S et al. Neutron-beam-shaping assembly for boron neutroncapture therapy. *Phys Atom Nucl* 2017;80:60–6.
14. Franken NA, Rodermond HM, Stap J et al. Clonogenic assay of cells *in vitro*. *Nat Protoc* 2006;1:2315–9.
15. Zaboronok A, Tsurushima H, Yamamoto T et al. Size-dependent radiosensitization effects of gold nanoparticles on human U251 malignant glioma cells. *Nanosci Nanotechnol Lett* 2013;5:990–4.
16. Bayanov B, Belov V, Taskaev S. Neutron producing target for accelerator based neutron capture therapy. *J Phys Conf Ser* 2006;41:460–5.
17. Tanaka H, Sakurai Y, Suzuki M et al. Experimental verification of beam characteristics for cyclotron-based epithermal neutron source (C-BENS). *Appl Radiat Isot* 2011;69:1642–5.
18. Kreiner AJ, Castell W, Di Paolo H et al. Development of a tandem-electrostatic-quadrupole facility for accelerator-based boron neutron capture therapy. *Appl Radiat Isot* 2011;69:1672–5.
19. Halfon S, Paul M, Arenshtam A et al. High-power liquid-lithium target prototype for accelerator-based boron neutron capture therapy. *Appl Radiat Isot* 2011;69:1654–6.
20. Bayanov BF, Belov VP, Bender ED et al. Accelerator based neutron source for the neutroncapture and fast neutron therapy at hospital. *Nucl Instrum Meth Phys Res A* 1998;413:397–426.
21. Bayanov B, Belov V, Kindyuk V et al. Lithium neutron producing target for BINP acceleratorbased neutron source. *Appl Radiat Isot* 2004;61:817–21.
22. Kuznetsov A, Malyshkin G, Makarov A et al. First experiments on neutron registration at accelerator based source for boron neutron capture therapy. *Tech Phys Lett* 2009;35:1–6.
23. Bayanov B, Kashaeva E, Makarov A et al. A neutron producing target for BINP accelerator-based neutron source. *Appl Radiat Isot* 2009;67:S282–4.
24. Bayanov B, Burdakov A, Chudaev V et al. First neutron generation in the BINP accelerator based neutron source. *Appl Radiat Isot* 2009;67:S285–7.
25. Aleynik V, Bashkirtsev A, Kanygin V et al. Current progress and future prospects of the VITA based neutron source. *Appl Radiat Isot* 2014;88:177–9.
26. Sorokin I, Taskaev S. A new concept of a vacuum insulation tandem accelerator. *Appl Radiat Isot* 2015;106:101–3.
27. Mostovich LA, Gubanova NV, Kutsenko OS et al. Effect of epithermal neutrons on viability of glioblastoma tumor cells *in vitro*. *Bull Exp Biol Med* 2011;151:264–7.
28. Volkova OY, Mechetina LV, Taranin AV et al. Impact of neutron radiation on the viability of tumor cells cultured in the presence of boron-10 isotope. *Vestn Rentgenol Radiol* 2016;97:283–8 (in Russian).
29. Zaboronok A, Byvaltsev VA, Kanygin VV et al. Boron-neutron capture therapy in Russia: preclinical evaluation of efficacy and perspectives of its application in neurooncology. *New Armenian Med J* 2017;11:1–6.
30. Yamamoto T, Nakai K, Tsurubuchi T et al. Boron neutron capture therapy for newly diagnosed glioblastoma: a pilot study in Tsukuba. *Appl Radiat Isot* 2009;67:S25–6.

31. Kageji T, Mizobuchi Y, Nagahiro S et al. Clinical results of boron neutron capture therapy (BNCT) for glioblastoma. *Appl Radiat Isot* 2011;69:1823–5.
32. Sato E, Yamamoto T, Shikano N et al. Intracellular boron accumulation in CHO-K1 cells using amino acid transport control. *Appl Radiat Isot* 2014;88:99–103.
33. Doras C, Brown PH. Permeability and the mechanism of transport of boric acid across the plasma membrane of *Xenopus laevis* oocytes. *Biol Trace Elem Res* 2001;81:127–39.
34. National Center for Biotechnology Information. *PubChem Compound Database; CID=7628 Boric Acid.* https://pubchem.ncbi.nlm.nih.gov/compound/7628 (2 October 2017, date last accessed).
35. WHO. *International Programme on Chemical Safety. Environmental Health Criteria Monographs (EHCs): Boron (ECH 204, 1998).* http://www.inchem.org/documents/ehc/ehc/ehc204.htm (2 October 2017, date last accessed).
36. Krieger R (ed). *Handbook of Pesticide Toxicology*. San Diego, California: Academic Press, 2001, 1413–34.