New Approaches in Clinical Application of Laser-Driven Ionizing Radiation

Katalin Hideghéty, Rita Emilia Szabó, Róbert Polanek, Zoltán Szabó, Szilvia Brunner, Tünde Tőkés

Attosecond Light Pulse Source, ELI-HU Non-profit Ltd., Szeged 6720, Hungary

ABSTRACT

The planned laser-driven ionizing beams (photon, very high energy electron, proton, carbon ion) at laser facilities have the unique property of ultra-high dose rate (>Gy/s^{-10}), short pulses, and at ELI-ALPS high repetition rate, carry the potential to develop novel laser-driven methods towards compact hospital-based clinical application. The enhanced flexibility in particle and energy selection, the high spatial and time resolution and extreme dose rate could be highly beneficial in radiotherapy. These approaches may increase significantly the therapeutic index over the currently available advanced radiation oncology methods. We highlight two nuclear reaction-based binary modalities and the planned radiobiology research.

Boron Neutron Capture Therapy is an advanced cell targeted modality requiring ^{10}B enriched boron carrier and appropriate neutron beam. The development of laser-based thermal and epithermal neutron source with as high as 10^{10} fluence rate could enhance the research activity in this promising field.

Boron-Proton Fusion reaction is as well as a binary approach, where ^{11}B containing compounds are accumulated into the cells, and the tumour selectively irradiated with protons. Due to additional high linear energy transfer alpha particle release of the BPFR and the maximum point of the Bragg-peak is increased, which result in significant biological effect enhancement. Research at ELI-ALPS on detection of biological effect differences of modified or different quality radiation will be presented using recently developed zebrafish embryo and rodent models.

KEYWORDS: laser-driven Boron Neutron capture, Boron-Proton Fusion, zebrafish embryo model

Medical Applications of Laser-Generated Beams of Particles IV: Review of Progress and Strategies for the Future, edited by Kenneth W. D. Ledingham, Proc. of SPIE Vol. 10239, 102390A
 · doi: 10.1117/12.2268300

INTRODUCTION

The development of ultrafast, high power lasers enable to introduce novel approaches into the radiation oncology. The laser-driven particle acceleration (LDPA) results in different beams of ionizing radiation with ultra-short pulse, ultra-high dose rate. Additionally to these features of LDPA, much higher flexibility in particle selection and energy range definition could be achieved that it is available with the current particle accelerators. Conventional radiation therapy, one of the main pillar of the anticancer treatment worldwide has largely used a single particle, X-ray, or electron irradiation generated by linear accelerator. The beam is operating at discrete, quasi mono-energetic levels between 5-18 MeV. In order to reduce the late toxicity, the dose is delivered in multiple fractions with higher probability of healthy tissue self-repairing, while DNA damage accumulates in the repair-deficient tumour cells. Due to the technical developments highly conformal radiation could be delivered from multiple angles or in a volumetric arc, collating dose in the tumour while minimizing radiation exposure to surrounding healthy tissue. The availability of such image guided, selective dose delivery techniques lead to wide use of single or reduced fraction (hypofractionated) treatments (Stereotactic Radio-Surgery, Stereotactic Ablative Radiotherapy). The most advanced X-ray delivery technique though cannot reach the selectivity of charged particle beams resulting in higher tumour control probability and lower normal tissue complication probability. Proton and ion facilities operate by accelerating single particles to high velocities and directing them toward target tissue, with distance travelled in tissue on the function of particle energy. As the particle slows, the number of ionization events with its surrounding environment increases, resulting in a dose-release spike (the Bragg Peak). This results in a relatively low entry dose and no relevant exit dose. Smaller charged particles, such as protons, have a sharper distal dose edge with a slight penumbra, meanwhile heavier ions have sharper lateral margins with a slightly higher exit dose [1]. To deliver target dose to the entire tumour volume, the Bragg peaks are overlapped to form a spread-out Bragg peak. Earlier, this was performed using a series of collimators and range filters to spread the beam. Recent advances allowed first proton and nowadays heavy-ion beams can be actively scanned across the target, eliminating excess dose and resulting in improved dose delivery [2]. In addition to the dose-distributive benefits afforded by particle beams, heavy-ion beams have a high linear energy transfer (LET), that is, a higher amount of energy per particle transferred per unit distance. The dense ionization events delivered in a shorter distance interval result in higher biological effectiveness in comparison to an equivalent photon dose. As the LET value of the carbon-ion and other heavy-ion beams varies throughout the beam path, future developments may involve “painting” high-LET values to target areas, further enhancing the biological effect [3-4].

Recently, lot of attention turned toward the potential synergic effect of molecular targeted immunotherapies (CTLA-4, PD-1 inhibitors) and local X-irradiation. Rare systemic events, disappearance of distant metastasis have been reported from focal radiotherapy. This phenomena was called abscopal effect, occurring due to the stimulation of the anti-tumour immunity [5-6]. Preclinical works have revealed that charged particle radiation appears to induce an identical or broader immunogenic response versus X-irradiation [7-8], as well as evidence that carbon-ion beams induce anti-metastatic and anti-angiogenic effects. All of these highly advantageous features and extreme large construction and operational cost of hadron therapy motivated first the design and development of laser accelerated proton/ion sources. However due to other innovative techniques the cost of proton facilities has been reduced remarkably, and the proton radiotherapy in the treatment of various cancers is growing rapidly. Ten years ago, there were only 15 operational facilities in the World; as of the writing of this proceedings, 51 centres are operational and several dozen additional facilities are under construction or planning. More than 100 operational facilities are estimated worldwide by 2020 [9]. Proton technology is evolving at a rapid pace, with advances in accelerators -next generation compact synchrotrons, synchrocyclotrons and gantry-mounted superconducting cyclotrons- compact gantries, and volumetric image guidance. In addition, at least four vendors now offer single room solutions for 3-5-folds lower costs than the earlier multi-room facilities. These less expensive, hospital-based single-room systems with next generation of active scanning delivery and imaging capabilities, having significantly lower operating costs. But, apart of initial intention to offer low cost alternative particle acceleration technique, the laser-driven particle beams own further potential for oncoradiology application.

Boron neutron capture therapy (BNCT)

BNCT is one of the promising therapeutic approaches, known to be extremely complex. It is a binary treatment method based on the ability of the non-radioactive isotope B^{10} to capture thermal neutrons producing high LET alpha particles (^{4}He) and recoiling lithium-7 (^{7}Li) nuclei, absorbed along a tissue path length of approximately 10 μm [10]. Thus, a complex radiation dose with high biological effectiveness is delivered to single cells, therefore in theory BNCT provides a way to selectively destroy malignant cells and spare adjacent normal cells. The overall effect depends on the bio-distribution of the boron carrier molecule and the physical parameters of the neutron beam [11-12]. Namely in order to be successful, a sufficient amount of ^{10}B must be selectively delivered to all tumour cells, preferably close to the nucleus, and enough thermal neutrons must be absorbed to cause lethal damage from the $^{10}B(n,\alpha)^{7}Li$ capture reaction [12]. Clinical interest in BNCT has focused primarily on high grade gliomas [13-14], and more recently on patients with recurrent tumours of the head and neck region [15-17] who have failed conventional therapy. BNCT

primarily is a biochemically rather than a physically targeted type of radiation therapy, and, therefore, it should be possible to selectively destroy tumour cells dispersed in normal tissue, providing that sufficient amounts of ^{10}B and thermal neutrons are delivered to the site of the tumour. Up to now, two boron carrier had been accepted for human application, borocaptate sodium (BSH) and borophenyl-alanin (BPA) [11]. Large number of boron compound were proposed and lot of promising ones are currently under investigation. The progress of exploitation of this ultra-selective „cell targeted" approach is significantly limited in the lack of proper neutron sources. Epithermal, thermal neutron beams constructed at research reactors served as neutron sources for the pioneering works on BNCT, however the advancement of BNCT requires neutron sources suitable for installation in hospital environments. Development of appropriate low energy neutron source using laser-driven particle acceleration would provide a new impetus for BNCT. The major advantage of accelerator-based BNCT over reactor-based neutron sources is the potential for siting within a hospital. Furthermore, accelerators can be easily turned off when the neutron field is no longer required, while reactors have a large permanent inventory of radioactive material. Importantly, the neutron energy spectrum from certain nuclear reactions is better designable than the one coming from fission reaction. High power laser facilities may provide via (p, n) reaction intense epithermal neutron beam [18] for BNCT and the boron compound development could be remarkably supported by ultra-high resolution imaging on subcellular boron bio-distribution at the new generation laser centres.

Preparation for radiobiology research on laser-driven neutron source for BNCT

The main aim of our group was to establish the zebrafish *(Danio rerio*) model for comprehensive radiobiological research on laser driven ionizing radiation sources. We have chosen the fish embryo model taking into account the variable conditions of laser research facilities. This vertebrate *in vivo* model has several advantages, including the genomic similarity to the human genome, fast prolific breeding, embryo and larva body transparency, short time (few days) embryonal development outside of the body, easy handling and high resilience. Apart of the usual *in vitro* radiation biology endpoints, such as survival evaluation, proteomic and genetic studies, the quantitative assessment of the macro- and micro-morphologic changes, and functional examinations (ECG, EEG, neuro-functional tests, creatinine clearance) could be performed. First, the feasibility of the zebrafish embryo model for investigate the changes in biological effectiveness of chemically modified, or different quality ionizing radiation have been confirmed [19]. The Relative Biological Effectiveness (RBE) of different neutron sources have been defined comparing the radiation effect curves of conventional photon beams to research reactor-based neutron beam, and cyclotron generated neutron source. For reference irradiation gamma beam of ^{60}Cobalt machine (energy 1,26 MeV, SID 100 cm) or linear

accelerator based photons were used (average energy 6 MeV, SID=100 cm) to irradiate viable zebrafish embryos dispensed in 96-well plates, one in each well. In the current study after careful dosimetry series of zebrafish embryos were exposed to a single fraction whole-body neutron irradiation at the research reactor of the Budapest University of Technology and Economics. The core is built of EK-10 fuel assemblies with 10% enrichment. The maximum thermal power is 100 kW, the maximum thermal neutron flux is $2.7x10^{12}$ n/cm^{2}s. Mixed fission neutron-gamma irradiation can be performed using 20 vertical irradiation channels, 5 horizontal beam tubes, two pneumatic rabbit systems and a large irradiation tunnel. The biological samples were transferred to the beam via the pneumatic tube. Groups of viable zebrafish embryos were irradiated at different dose levels (0, 1,25; 1,875; 2; 2,5 Gy) in Eppendorf tubes homogeneously, to compare the radiation quality and radiation effect curves to 6 MeV photons. Each tube contained twenty embryos. At the MGC-20E cyclotron of MTA ATOMKI high intensity broad spectrum of fast p+Be neutrons (using 22 μm thick Be target cooled with water and He gas) have been provided. At 18MeV proton beam the average neutron energy is 3,5MeV, and the gamma contamination was 16%. Dose range: 0, 2, 4, 6, 8 Gy was delivered for fish embryo experiment. The groups of embryos in Eppendorf tubes were positioned using a home-made positioning system. During the experiment real-time chemical dosimetry system (Feraus-Bensoate-Xylenon orange solution) was used.

After irradiation for daily assessment we replaced the embryos to 96 well plates, and were maintained at 28.5 °C. The survival and morphologic abnormalities (pericardial oedema, spine curvature) of each embryo were assessed for each experiment at 24-hour intervals from the point of fertilization up to 168 hpf (defining the dose lethal for 50% (LD_{50})). Each experiment were 3 times repeated. On the basis of survival, and malformation assessment the LD_{50} from reference photon sources was defined at 20 Gy dose level and the same lethality were found at 2 Gy dose from the reactor neutron beam resulting RBE of 10, meanwhile 3,5 and 2,8 RBE of cyclotron neutron beam could be calculated respectively. Additional comet assay and activation of NF-κB pathway detection provided consistent results. Further refinement of the endpoints, and definition of quantitative analysis of radiation effect on macro- and micro morphologic, and molecular levels are ongoing. We have established a reproducible and reliable morphological evaluation system which provides information about the length-, the size of the eye-, the yolk sac and pericardial oedema- and the spinal cord curvature of the embryos. At different time-points after the radiation exposure, series of photos of each embryos were off-line analysed. Based on this system, slight alterations in dose effects can be defined between the different radiation types and at different points along the charged particle path (in the plateau and the middle and at the end of the Spread of Bragg Peak (SOBP) region). We have also examined histopathological and immuno-histopathological changes on zebrafish embryos. In our previous study, we have found that after irradiation there

are different histopathological changes, e.g. in the skin (disappearance of mucous cells and development of subcutaneous oedema) and in goblet cell numbers. We have detected hepatopancreatic interstitial oedema, hydropic and simple pathologic signs of hepatocytes and pycnotic changes in the nuclei. Furthermore, large amounts of mucous and catarrh were observable in the intestinal flux, and goblet cells were found in the intestinal mucous membrane. Cells with irregular shapes and a larger hyperchromatic nuclei were observed. These were characterized by pseudo-multilayer epithelia and moderate disorganization of the columnar cells, and the cytoplasm was wider in the intestinal lumen [19]. In our present investigations, we started to determine the number of double-strand DNA breaks, using γH2AX immunostaining. These ongoing and preliminary results will be presented. In addition to neutron beam RBE evaluation, the zebrafish embryo model is currently under investigation for toxicity, pharmacokinetic and bio-distribution research of the different boron compounds.

Boron Proton Fusion Enhanced Proton Therapy (BPFEPT)

Nuclear reactions initiated by laser-accelerated particle beams are a promising new approach to many applications, from aneutronic fusion energy production to medical purposes, such as radioisotope production and therapeutic application. Labaune C. *et al.* demonstrated the occurrence of secondary nuclear reactions, initiated by the primary nuclear reaction products (charged particle chain of reactions), using multicomponent targets composed of either natural boron (B) or natural boron nitride (BN) [20]. The BPF ^{11}B (p, 3α) reaction occurs between protons and boron-11, which do not produce high-energy neutrons. After the proton reacts with the boron (^{11}B), the boron changes to carbon (^{12}C) in an excited state. The excited carbon nucleus is split into alpha particle of 3.76 MeV and beryllium (^{8}Be). Subsequently, the beryllium is divided into two alpha particles of 2.74 MeV each. The highest cross-section of this reaction takes place with protons having energies around 600-700 keV corresponding to the end phase of the SOBP. Therefore, an additional high LET dose deposition could be theoretically achieved taking advantage of BPF reaction during cancer proton therapy. Meanwhile the location of the BPF could be directed/steered with high accuracy by proton beam manipulation, the degree of additional dose is depending on the ^{11}B concentration. Further, the subcellular bio-distribution of the ^{11}B has high impact on the biological effect. Up to now, only a very few and publications are available on the BPFEPT. Monte Carlo simulations with different proton energies and ^{11}B concentrations resulted in discrepant outcome. High dose increase at the region of the Bragg Peak was reported when the boron uptake region was calculated in overlapping position [21]. In silico studies by other groups concluded differently. The potential of BPF enhanced proton therapy was even questioned by Adams *et al*. [22], calculated much lower achievable dose increase (0.026 %) for the incident 250 MeV proton beam scenario. They demonstrated by

MCNP6 simulations that the proton-boron fusion reaction rate at clinically relevant boron concentrations (100 ppm) is too small in order to have any measurable impact on the absorbed dose.
Other groups stated as well, that the magnitude of dose increase using realistic boron concentration values remains under 10 % and the dose increase occurs mainly due to the composition and the density changes instead of alpha particle contribution [23-25]. The additional physical dose from BPF reaction calculated around 0,3 %. However, the potentially clinically relevant biological effect increase warranted to perform preclinical studies on this binary approach. Cell culture experiments were performed with 60 MeV proton beam at the medical cyclotron in Catania. As a result, clear enhancement of the biological effectivity of proton irradiation by additional BPF reaction could be demonstrated [25]. Both the colony forming assay and the chromosome aberration analysis resulted in significant difference in the presence of clinically relevant ^{11}B concentration using natural B containing BSH as boron carrier. A lot of further calculations and experiments are required to explore the realistic potential of BPFEPT for clinical implementation, and optimisation. However, two boron compounds could be safely used, and veterinary then early clinical trials could be designed. A crucial issue is for optimisation the development of more effective, and specific ^{11}Boron delivery agent. New approaches on boron carrier design using nanotechnology could yield much higher ^{11}B concentration with favourable boron location near to the cell nucleus, and simultaneously BPF boost to the hypoxic, radio-resistant subvolumes of the tumour could relevantly ameliorate the anti-tumour efficacy of proton therapy. The last three decades of boron compound development for BNCT has led to large number of research results, including the two boron carrier approved and used for humans. In contrary to BNCT though, where the selective tumour uptake of ^{10}B carrier is of extremely high importance, for BPF the ^{11}B concentration gradient between the tumour and normal tissues is less critical, because in this approach the energy deposition of proton beam can be defined with high accuracy.

Radiobiology research on BPFEPT

To the aim of explore the clinical benefit of BPFEPT two main research directions are pursued. On one hand, investigation on boron compound design and characterisation from the point of view of safety, pharmacokinetic and bio-distribution is of high importance. At the same time, preclinical experiments on cell culture, zebrafish embryos and rodent models are planned without and in the presence of ^{11}B carrier at different proton beams. On the other hand, laser-based acceleration of protons is an important research area at new generation laser facilities. Participation of biomedical groups in the technical development for adaptation to the medical requirements, and also by investigation of the radiobiological characteristics of the resulting ultra-short pulsed, ultra-high dose rate particle beams has paramount

importance. Pioneering research on the biological effects of laser-accelerated protons have been performed with *in vitro* cell cultures in few laser centres in Europe, in the US and in Japan, where 0.8-23 MeV energy beams could be provided [26-32]. Studies on *in vivo* systems and on the response of normal tissues to laser-driven beams are not yet reported. The radiobiological work should be adapted to the highly technical circumstances at a laser facility to the uncertainties in timing, shot-to-shot energy and intensity fluctuation, and lack of integrated dosimetry, positioning and treatment verification systems which are obviously available at medical or radiobiology facilities. In addition to the classical rodent animal models, such as the intestinal crypt assay [33-34] and the assessment of the locomotor disorders of extremities due to spinal cord radiation [35] could not be applied in the early phase of laser-driven charged particle beams, because of the low maximal proton energies. The good tolerance to the variable conditions at a research facility, furthermore, the small size (the embryos at 24 hours post fertilization are 500 μm of diameter) make the zebrafish embryo model amenable for the complex *in vivo* assessment of the biological effects of the laser-driven proton beams produced in the early phase of development.

CONCLUSIONS

Recently, several novel approaches have emerged in radiation oncology to improve the therapeutic index of patients with advanced malignant disease. The development of high power laser-driven particle acceleration (neutron, proton) could accelerate the implementation of further promising binary methods, such as BNCT and BPFEPT. Intensive radiobiology research is essential to develop and optimize the components, both the boron carrier and the hadron source for clinical application. We propose a new vertebrate model (zebrafish embryo) for preclinical research on BNCT and BPFEPT to examine the biological properties of the boron delivery agents and the particle beams even under highly technical circumstances at laser facilities, furthermore to assess the complex effects resulted in from the combination. The quantitative survival, morphology, histopathology and molecular analysis led to well reproducible, reliable result in studies on biological effects.

ACKNOWLEDGMENTS

The ELI-ALPS project (GOP-1.1.1-12/B-2012-000, GINOP-2.3.6-15-2015-00001) is supported by the European Union and co-financed by the European Regional Development Fund.

The project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no 654148 Laserlab-Europe.

REFERENCES

[1] Ebner, D. K., Tinganelli, W., Helm, A., Bisio, A., Yamada, S., Kamada, T., Shimokawa, T. and Durante, M. "The Immunoregulatory Potential of Particle Radiation in Cancer Therapy," Front Immunol. 8, 99 (2017).

[2] Mori, S., Zenklusen, S., Inaniwa, T., Furukawa, T., Imada, H., Shirai, T., Noda, K. and Yasuda, S. "Conformity and robustness of gated rescanned carbon ion pencil beam scanning of liver tumors at NIRS," Radiother Oncol. 111(3), 431-436. (2014).

[3] Tinganelli, W., Durante, M., Hirayama, R., Krämer, M., Maier, A., Kraft-Weyrather, W., Furusawa, Y., Friedrich, T. and Scifoni, E. "Kill-painting of hypoxic tumours in charged particle therapy," Sci Rep. 5, 170 (2015).

[4] Bassler, N., Jaekel, O., Sondergaard, C.S. and Petersen, J.B. "Dose- and LET-painting with particle therapy," Acta Oncol 49(7), 1170-1176 (2010).

[5] Crittenden, M., Kohrt, H., Levy, R., Jones, J., Camphausen, K., Dicker, A., Demaria, S. and Formenti, S. "Current clinical trials testing combinations of immunotherapy and radiation," Semin Radiat Oncol. 25(1), 54-64 (2015).

[6] Demaria, S., Golden, E. B. and Formenti, S. C. "Role of local radiation therapy in cancer immunotherapy," JAMA Oncol. 1(9), 1325-1332 (2015).

[7] Gameiro, S. R., Malamas, A. S., Bernstein, M. B., Tsang, K. Y., Vassantachart, A., Sahoo, N., Tailor, R., Pidikiti, R., Guha, C. P., Hahn, S. M., Krishnan, S. and Hodge J. W. "Tumor cells surviving exposure to proton or photon radiation share a common immunogenic modulation signature, rendering them more sensitive to T cell-mediated killing," Int J Radiat Oncol Biol Phys. 95(1), 120-130 (2016).

[8] Durante, M., Reppingen, N. and Held, K. D. "Immunologically augmented cancer treatment using modern radiotherapy," Trends Mol Med 19(9), 565-582 (2013).

[9] Particle Therapy Co-Operative Group. Particle Therapy Centers. Accessed March, 2017. Available online: http://www.ptcog.ch/.

[10] Sauerwein, W. A. G., Wittig, A., Moss, R. and Nakagawa, Y. [Neutron Capture Therapy Principles and Applications] Springer Heidelberg New York Dordrecht London, (2012).

[11] Hideghéty, K., Sauerwein, W., Wittig, A., Götz, C., Paquis, P., Grochulla, F., Haselsberger, K., Wolbers, J., Moss, R., Huiskamp, R., Fankhauser, H., de Vries, M. and Gabel, D. "Tissue uptake of BSH in patients with glioblastoma in the EORTC 11961 phase I BNCT trial," J Neurooncol. 62(1-2), 145-156 (2003).

[12] Barth, R. F., Coderre, J. A., Vicente, M. G. and Blue, T. E. "Boron neutron capture therapy of cancer: current status and future prospects," Clin Cancer Res. 11(11), 3987-4002 (2005).

[13] Miyatake, S., Kawabata, S., Hiramatsu, R., Kuroiwa, T., Suzuki, M., Kondo, N. and Ono, K. "Boron Neutron Capture Therapy for Malignant Brain Tumours," Neurol Med Chir (Tokyo). 56(7), 361-371 (2016).

[14] Barth RF. "A critical assessment of boron neutron capture therapy: an overview," J Neurooncol. 62(1-2), 1-5 (2003).

[15] Aihara, T., Hiratsuka, J., Morita, N., Uno, M., Sakurai, Y., Maruhashi, A., Ono, K. and Harada, T. "First clinical case of boron neutron capture therapy for head and neck malignancies using 18 F-BPA PET," Head Neck. 28(9), 850-855 (2006).

[16] Kato, I., Fujita, Y., Maruhashi, A., Kumada, H., Ohmae, M., Kirihata, M., Imahori, Y., Suzuki, M., Sakrai, Y., Sumi, T., Iwai, S., Nakazawa, M., Murata, I., Miyamaru, H. and Ono, K. "Effectiveness of boron neutron capture therapy for recurrent head and neck malignancies," Appl Radiat Isot. 67(7-8 Suppl), S37-42 (2009).

[17] Kankaanranta, L., Seppälä, T., Koivunoro, H., Saarilahti, K., Atula, T., Collan, J., Salli, E., Kortesniemi, M., Uusi-Simola, J., Valimaki, P., Mäkitie, A., Seppänen, M., Minn, H., Revitzer, H., Kouri, M., Kotiluoto, P., Seren, T., Auterinen, I., Savolainen, S. and Joensuu H. "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. 82(1), e67-75 (2012).

[18] Pomerantz, I., McCary, E., Meadows, A. R., Arefiev, A., Bernstein, A. C., Chester, C., Cortez, J., Donovan, M. E., Dyer, G., Gaul, E. W., Hamilton, D., Kuk, D., Lestrade, A. C., Wang, C., Ditmire, T. and Hegelich, B. M. "Ultrashort pulsed neutron source," Phys Rev Lett. 113(18), 184801 (2014).

[19] Szabó, E. R., Plangár, I., Tőkés, T., Mán, I., Polanek, R., Kovács, R., Fekete, G., Szabó, Z., Csenki, Z., Baska, F., Hideghéty, K. "L-alpha glyceriylphosphoryl-choline as a potential radioprotective agent in zebrafish emryo model," Zebrafish. 13(6), 481-488 (2016).

[20] Labaune, C., Baccou, C., Yahia, V., Neuville, C. and Rafelski, J. "Laser-initiated primary and secondary nuclear reactions in Boron-Nitride," Sci Rep. 6, 21202 (2016)

[21] Yoon D. K., Jung J. Y. and Suh T. S. "Application of proton boron fusion reaction to radiation therapy: A Monte Carlo simulation study," Appl. Phys. Lett. 105, 223507 (2014).

[22] D. Adam and B. Bednarz, "SU-F-T-140: Assessment of the proton boron fusion reaction for practical radiation therapy applications using MCNP6," Med. Phys. 43, 3494 (2016).

[23] Giuffrida, L., Margarone, D., Cirrone, G. A. P., Picciotto, A., Cuttone, G. and Korn, G. "Prompt gamma ray diagnostics and enhanced hadron-therapy using neutron-free nuclear reactions," AIP Advances 6, 105204 (2016).

[24] Margarone, D., Picciotto, A., Velyhan, A., Krasa, J., Kucharik, M., Mangione, A., Szydlowsky, A., Malinowska, A., Bertuccio, G., Shi, Y., Crivellari, M., Ullschmied, J., Bellutti, P. and Korn, G. "Advanced scheme for high-yield laser driven nuclear reactions," Plasma Phys. Control. Fusion 57, 014030 (2015).

[25] Cirrone, G. A. P., Manti, L., Margarone, D., Giuffrida, L., Picciotto, A., Cuttone, G., Korn, G., Marchese, V., Milluzzo, G., Petringa, G., Perozziello, F., Romano, F. and Scuderi V. "Nuclear fusion enhances cancer cell killing efficacy in a protontherapy model," Med. Phys. (2017).

[26] Masood, U., Bussmann, M., Cowan, T. E., Enghardt, W., Karsch, L., Kroll, F., Schramm, U. and Pawelke, J. "A compact solution for ion beam therapy with laser accelerated protons," Appl. Phys. B 117, 41 (2014).

[27] Auer, S., Hable, V., Greubel, C., Drexler, G. A., Schmid, T. E., Belka, C., Dollinger, G. and Friedl, A. "Survival of tumour cells after proton irradiation with ultra-high dose rates," Radiat. Oncol. 6, 139 (2011).

[28] Yogo, A., Sato, K., Nishikino, M., Mori, M., Teshima, T., Numasaki, H., Murakami, M., Demizu, Y., Akagi, S., Nagayama, S., Ogura, K., Sagisaka, A., Orimo, S., Nishiuchi, M., Pirozhkov, A. S., Ikegami, M., Tampo, M., Sakaki, H., Suzuki, M., Daito, I., Oishi, Y., Sugiyama H., Kiriyama, H., Okada, H., Kanazawa, S., Kondo, S., Shimomura, T., Nakai, Y., Tanoue, M., Sasao, H., Wakai, D., Bolton, P. R. and Daido, H. "Application of laser-accelerated protons to the demonstration of DNA double-strand breaks in human cancer cells," Appl. Phys. Lett. 94, 181502 (2009).

[29] Richter, C., Karsch, L., Dammene, Y., Kraft, S.D., Metzkes, J., Schramm, U., Schürer, M., Sobiell, M., Weber, A., Zeil, K. and Pawleke, J. "A dosimetric system for quantitative cell irradiation experiments with laser-accelerated protons," Phys. Med. Biol. 56(6), 1529-1543 (2011).

[30] Zeil, K., Baumann, M., Beyreuther, E., Burris-Mog, T., Cowan, T.E., Enghardt, W., Karsch, L., Kraft., S.D., Laschinsky, L., Metzkes, J., Naumburger, D., Oppelt, M., Richter, C., Sauerbrey,R., Schürer, M., Schramm, U. and Pawelke, J., "Dose-controlled irradiation of cancer cells with laser-accelerated proton pulses," Appl. Phys. B. 110, 437-444 (2013).

[31] Schmid, T.E., Dollinger, G., Hauptner,A., Hable,V., Greubel C., Auer S., Friedl, A.A., Molls, M. and Röper, B. "No evidence for a different RBE between pulsed and continuous 20 MeV protons," Radiat. Res. 172(5), 567-574 (2009).

[32] Schmid, T. E., Dollinger, G., Hable, V., Greubel, C., Zlobinskaya, O., Michalski, D., Mools, M. and Röper, B. "Relative biological effectiveness of pulsed and continuous 20 MeV protons for micronucleus induction in 3D human reconstructed skin tissue," Radiother. Oncol. 95(1), 66-72 (2010).

[33] Gueulette, J., Slabbert, J. P., De Coster, B. M., Rosier, J. F., Octave-Prignot, M., Ruifrok, A., Schreuder, A.N., Wambersie, A., Scalliet, P. and Jones, D.T. "Proton RBE for early intestinal tolerance in mice after fractionated irradiation," Radiother. Oncol. 61(2), 177-185 (2001).

[34] Uzawa, A., Ando, K., Furusawa, Y., Kagiya, G., Fuji, H., Hata, M., Sakae, T., Terunuma, T., Scholz, M., Ritter, S. and Peschke, P. "Biological intercomparison using gut crypt survivals for proton and carbon-ion beams," J. Radiat. Res. 48 Suppl., 75-80 (2007).

[35] Medin, P.M., Foster, R.D.,van der Kogel, A.J., Sayre, J.W., McBride, W. H. and Solberg, T. D. "Spinal cord tolerance to single-fraction partial-volume irradiation: a swine model," Int. J. Radiat. Oncol. Biol. Phys. 79(1), 226-232 (2011).