Careful attention should be paid to the occurrence of CBS if the tumor is located adjacent to the carotid artery. The presence of skin invasion of recurrent lesions after irradiation at CBS onset is an ominous sign of lethal consequences. We must be aware of these signs to perform BNCT safely. This protocol for BNCT in recurrent and advanced head and neck cancer is promising in terms of decreasing the incidence of fatal CBS.

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Pa B2 01
Evaluation of micronucleation and viability of glioma cells *in vitro* neutron beams irradiated

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Boron neutron capture therapy (BNCT) is a promising approach for therapy of human brain tumor. The original accelerator-based epithermal neutron source was proposed and created in the Budker Institute of Nuclear Physics (BINP). Possibility to use this source for BNCT was tested on the model in vitro. Glioblastoma cell line U87 and normal human fibroblast cell line MRC-5 were incubated in a medium with and without b-[4-(10B)Boronophenyl]alanine (BPA) for 18 hours before irradiation. Cells in the culture plates were placed on the surface of the plastic phantom and inside the one at the depth of 3 cm. We proposed the transmission through the plastic to thermalize the neutrons; therefore the cells that were placed inside the phantom were irradiated thermal neutrons, whereas the cells on the surface phantom were treated by epithermal neutron beam (energy spectrum – from thermal to 100 keV, the average energy – 13 keV). Then during 2 hours, the irradiation of the two cell lines was performed using BINP neutron source. The cell viability at 1st, 3rd and 5th days after irradiation was determined by WST assay. The viability of cells that were irradiated by epithermal or thermal neutrons was not decreased compared with untreated cells at these time points. However, a clonogenic assay showed that, at 14th day after epithermal neutron irradiation, the surviving fractions of both pretreated with BPA and BPA-free cells lines were significantly decreased compared with untreated cells, while the clonogenicity of the cells that were treated by thermal neutrons depended on BPA pretreating. This irradiation significantly decreased the surviving fractions both tumor and normal cell lines that were pretreated with BPA compared with BPA-free cells despite the same amount of neutron radiations. The previous studies showed that irradiation induces formation of micronuclei, which are the evidence of mitotic catastrophe. Therefore, we carried out DAPI staining of irradiated cells. As it was observed, the epithermal

neutrons induced formation of micronuclei in both BPA pretreated and BPA-free cell lines, whereas thermal neutrons resulted in the micronucleation only in the BPA pretreated cells. According our results we conclude that: i) BINP neutron source can be used for BNCT; ii) fast neutrons are toxic for the cells *in vitro*; iii) the cytopathic effect of thermal neutron beam depends on the accumulation of BPA.

Pa B2 02

Analysis of cell-death response and DAMPs after boron neutron capture reaction in human cancer cells

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We have been studying the molecular mechanisms involved in the boron neutron capture reaction (BNCR). To understand the early response after BNCR in human cancer cells, transcriptome and proteome analyses were performed six and twenty-four hours after thermal neutron beam irradiation to oral squamous cancer SAS cell line at the dose of 24 Gy-eq under boronophenylalanine (BPA) (+) and BPA(-) conditions. Genes involved in cell death, transcriptional regulation, and inflammatory and immune responses were increased after BNCR; activating transcription factor (ATF3), early growth response 1 (EGR1), colony stimulating factor 2 (CSF2), interleukin-6, and interleukin-8 were upregulated. Proteome analysis was performed using two dimensional-polyacrylamide gel electrophoresis and mass spectrometry. Proteins involved in RNA processing, transcription, DNA repair, immune response, and endoplasmic reticulum function were found to be increased in the cells. Secreted proteins after BNCR were detected and being studied with the proteome and ELISA analyses. Among them, the leakage of high mobility group box 1 (HMGB1), one of the proteins of damage-associated molecular patterns (DAMPs), was increased in an irradiation-dose dependent manner in the culture medium. The results suggest that diverse irradiation responses including DAMPs could be induced after BNCR reaction as an early response.

Pa B2 03

Three In One: A Multifunctional Antitumor Sensitizer for Photodynamic, Boron Neutron Capture and Proton Therapies

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