CO7-1

Construction of novel Boron-containing silica nanoparticles and BNCT experiments

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INTRODUCTION: Two type of boron compounds which are boron phenyl alanine (BPA) and borocaptate (BSH) have been mainly used for BNCT. But, these compounds have problems such as BSH having low uptake into cancer cell and BPA having short retention in tumor. Thus, novel boron compounds which can overcome these problems need to be developed for future BNCT.

We used a new type of mesoporous silica-based nanoparticle (MSN) that is modified with polyethylene glycol (PEG) and tetramethylammonium chloride (TMAC) to positively charged on surface. This nanoparticle has a large surface area where a large quantity of boron compounds which include BPA or BSH can be loaded. In recently, we developed novel MSN which has small-size and highly dispersible (sdMSN) into water. The MSN has the ability to flow into bloodstream for long time and result in accumulation into tumor at significantly high concentration. The MSN was also retained for long time in the tumor after intravenously injected to animal body. In this study, we evaluated the biodistribution of new MSN which has positively charged surface that has under 25 nm of diameter.

EXPERIMENTS: We used MSN which was synthesized by sol-gel method using tetraethoxysilane (TEOS) and modified with PEG and TMAC to positively charge on surface. It has the size of less than 25 nm. This MSN was also labeled with Rodamine B dye to trace where it accumulates in mouse body after injection. The MSN was characterized by using TEM, DLS, nitrogen adsorption-desorption analysis and zeta potential. For biodistribution analysis, MSN was intravenously injected to CT26 mouse colon cancer- mouse xenografts at 5 mg/mouse or 2 mg/mouse, and we dissected tumor and organs which include liver, lung and kidney at 24 hours after injection. Tumor and organs were made into thin sections and the fluorescence of MSN was detected with a confocal microscope.

RESULTS: The analysis showed that the size of MSN was less than 25 nm diameter and had homogenous shapes examined by DLS and TEM microscopy. And then, the surface of MSN was positively charged due to modification with PEG and TMAC. The zeta potential of MSN was 38.29 ±0.77 mV in water. MSN accumulation in the CT26-transplanted mouse was investigated with a fluorescent microscope and confocal microscope after making thin sections. As shown in a figure, MSN was able to be accumulated in the tumor at higher concentration compared with Liver 24 hours after injection. In this study, we clarified that both aging and hydrothermal treatment steps were very important to make sdMSN which can accumulate in the tumor at high concentration.

We are currently attaching boron to MSN. These results suggest that MSN may be an effective boron carrier for BNCT. This MSN may be able to become a new boron reagent for BNCT beyond BPA and BSH if boron compounds are grafted to it.

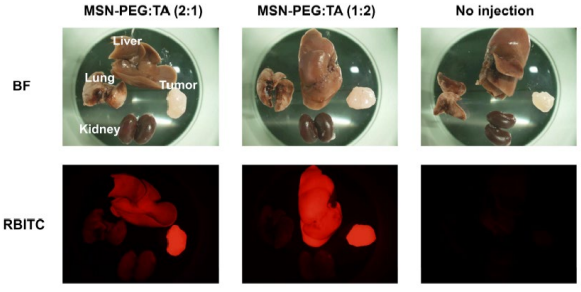

Fig. Biodistribution of MSN in CT26-bearing mouse model.

CO7-2

Synthesis of PEPT1-targeted boron containing dipeptids for pancreatic cancer therapy

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INTRODUCTION: Peptide transporter 1 (PEPT1) has been noticed because it is expressed on various types of cancer cells. Especially, it has been reported that PEPT1was highly expressed pancreatic cancer PDX model.

We used some dipeptide which contained ^{10}B that has higher solubility, longer retention ability in the tumor than BPA. These dipeptides are actively taken up into cancer cells by PEPT1 and LAT1 (weakly).

We investigated BNCT efficacy of these ^{10}B-dipeptides and the abscopal effect on mice which were simultaneously transplanted tumors on right leg and left shoulder when dipeptides are used.

EXPERIMENTS: We used two types of dipeptides including BPA-BPA and His-BPA. Dipeptides were intravenously injected into CT26-tansplanted BALB/c mice 2 hours before neutron irradiation. Our aim was to investigate whether the BNCT efficacy of dipeptides can be improved compared with BPA by neutron irradiation at KUR. The thermal neutron was irradiated for 6 minutes at 5MW. After neutron irradiation, tumor volume and body weight measured for 6 weeks after irradiation (up to 42 days after irradiation) to evaluate the BNCT efficacy of dipeptides. For abscopal effect evaluation, BALB/C mouse was simultaneously transplanted CT26 cancer cells on both right leg and left shoulder to make tumor. All mice were injected with dipeptides 1.5 hours before irradiation, and right legs were only irradiated with neutron for 6 minutes at 5MW. For 2-6 weeks after irradiation, tumors volume on both right leg and left shoulder were measured to evaluate abscopal effect induction.

RESULTS:

In previous experiments for tumor accumulation of boron, it has been shown that dipeptides are effective boron carriers. We then investigated BNCT efficacy of these boron compounds. Dipeptides were intravenously injected into CT26-transplanted mice 2 hours before neutron irradiation. These mice were held to 12 mouse holders and placed in front of KUR, and neutron was irradiated. Tumor on dipeptides-injected mice was eradicated, and the tumor regrowth was not observed up to 27 days after BNCT, whereas regrowth was observed on BPA-injected mice. This result indicates that dipeptides have the potential to strongly cure cancer by BNCT.

We have further investigated abscopal effect induction on mice which were simultaneously transplanted CT26 cancer cells on both right leg and left shoulder that were injected with dipeptides before neutron irradiation. The tumor on right leg (irradiated tumor) was eradicated 2 weeks after BNCT, and the distant tumor unirradiated tumor on left shoulder was significantly shrunken between 2-3 weeks post-BNCT compared with mice that were administered BPA.

This work has published to ACS Pharmacology & Translational Science as entitled “L-boronophenylalanine (L-BPA) dipeptide prodrugs enhance boron delivery to tumors, facilitate tumor eradication and induce tumor vaccine effect in mice following neutron irradiation.

We have further been investigating the mechanism of abscopal effect induction by BNCT with dipeptides about the activation of aggressiveness and improved invasiveness of immune cells including CD8+ T-cell, NK cells and macrophages.

CO7-3

Verification of the invitro/in vivo dynamics of Indocyanine green boron-containing compounds

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INTRODUCTION: Based on experience with over 300 cases in specific clinical studies, we have developed an ICG-boron polymer compound by utilizing the characteristic accumulation of indocyanine green (ICG) in brain and spinal cord tumors, as confirmed by the intraoperative ICG fluorescence imaging protocol during surgery.[1] This compound is intended for application in boron neutron capture therapy (BNCT).

EXPERIMENTS: Cultured cells are co-cultured with ICG-boron polymer compounds, which are then taken up by the cells; the cells are subsequently irradiated with neutrons, and the cytotoxic effect is evaluated. Furthermore, since the protocol for this experiment has already been established, by performing irradiation in the heavy water facility for approximately 2 hours per experiment (during 1 MW operation), the cytotoxic effects of multiple types of compounds under various conditions were evaluated. Similarly, using a mouse subcutaneous tumor model, ICG-boron polymer compounds were administered to be taken up by tumor cells, followed by neutron irradiation to evaluate the cytocidal effect. Balb/C nu/nu mice are planned for use. The experimental protocol has already been established, and evaluations were conducted by irradiating the samples for up to approximately one hour per experiment in the heavy water facility (during 5 MW operation).

RESULTS:
We compared the cytotoxic effects of two ICG compounds, ICG Compound 1 and ICG Compound 2, against L-BPA—a drug currently used in clinical practice—on malignant glioma cells (U87). Irradiation times were set at three intervals: 10 minutes, 30 minutes, and 50 minutes. Furthermore, we confirmed that delaying the excretion of the compounds using the excretion inhibitor V enhanced their cytotoxic effects. Both ICG Compound 1 and ICG Compound 2 demonstrated significantly greater cytotoxic effects than L-BPA. Furthermore, significant cytotoxic effects were observed in the groups treated with ICG Compound 1 and ICG Compound 2 plus L-BPA, as well as in the groups treated with ICG Compound 1 and ICG Compound 2 plus L-BPA and the excretion inhibitor V, across all three irradiation durations (10, 30, and 50 minutes). Next, a subcutaneous tumor model was established in the right hind leg of BALB-c nu/nu 4-week-old mice. Compared to the control group, significant tumor-suppressive effects were observed in the L-BPA monotherapy group and in the groups treated with ICG Compounds 1 and 2. Next, although no significant difference was observed between ICG Compounds 1 and 2 and the L-BPA monotherapy group, they exhibited nearly equivalent tumor-suppressive effects. Compared to the L-BPA monotherapy group, the ICG Compounds 1 and 2 plus L-BPA group demonstrated a significant tumor-suppressive effect.

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CO7-4

Antitumor effect of boron neurton capture therapy for lung tumor in cervical cancer mouse model.

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INTRODUCTION: In Japan, approximately 10,000 women develop cervical cancer and 2,900 women die from the disease annually. The incidence and mortality rates of cervical cancer in Japan are on the rise.Squamous cell carcinoma (SCC) is the most common histological type at 80%, and adenocarcinoma (Adeno) accounts for about 20%. Lung metastases from cervical cancer are often resistant to chemotherapy, making them difficult to treat. Thus, new treatment modalities for cervical cancer are needed. In this study, we evaluated the efficacy and safety of boron neutron capture therapy (BNCT) in a mouse model of cervical cancer lung metastasis.

EXPERIMENTS: BPA (L-isomer) was supplied by Stella Chemifa (Osaka, Japan) and prepared as a fructose complex. Female nude mice (BALB/c Slc-nu/nu), aged 4–6 weeks, were purchased from SLC (Japan). A lung metastasis model was established by injecting cervical cancer cell lines (HeLa and SiHa) into the right lungs, and tumors were allowed to develop over 4–6 weeks.
The mice were divided into two groups: a hot control group (neutron irradiation only) and a BNCT group (intraperitoneal BPA administration followed by neutron irradiation). In the BNCT group, BPA (250 mg/kg) was administered intraperitoneally 2.5 hours prior to neutron irradiation. After irradiation, body weight was monitored, and survival was evaluated.

RESULTS:
Cervical cancer lung metastasis models were established using HeLa and SiHa cell lines (HeLa: BNCT, n = 6; hot control, n = 5; SiHa: BNCT, n = 6; hot control, n = 6). At 3 weeks after irradiation, all mice in the HeLa model remained alive, whereas in the SiHa model, one mouse in each group had died. They are currently under observation.
Figure 1 shows the changes in body weight in the hot control and BNCT groups in the cervical cancer lung metastasis models (HeLa and SiHa). No significant changes in body weight were observed in either group.

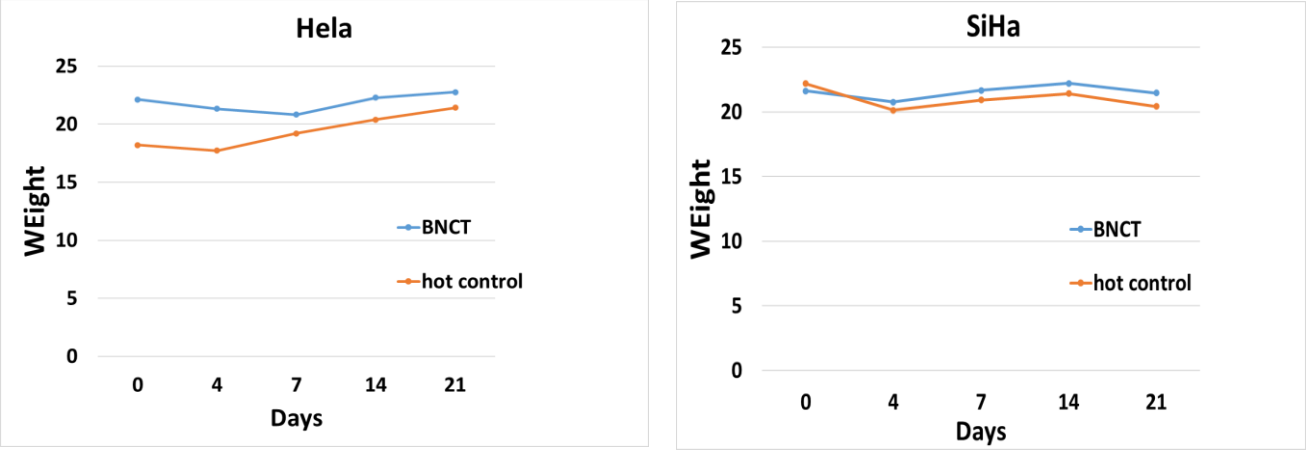

Fig. 1. Body weight in the hot control and BNCT groups in the cervical cancer lung metastasis models (HeLa and SiHa)

CO7-5

Characterization of Early and Late effect of pelvic Boron Neutron Capture Therapy (BNCT) in a mouse model

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INTRODUCTION: Previously, we reported the anti-tumor effect of Boron Neutron Capture Therapy (BNCT) for a mouse model of pelvic recurrence of Colorectal cancer (CRC). On the other hand, we could not fully evaluate side effects in terms of immune response or the later complications, because we examined the effectiveness of BNCT in the nude mouse model only a month after treatment. This study investigates early and late effect of pelvic BNCT.

EXPERIMENTS: We used Boronophenylalanine (BPA) as a boron compound. Also, we used seven-week-old female BALB/cCrSlc mouse having physiological environment of immunity. Firstly, we established a mouse model of pelvic recurrence of CRC using Colon-26-Luc cells concentrated to 1.0×10^5/100μL in 0.1ml of PBS. The boron concentrations in Colon-26-Luc tumors at 1h, 3h, 5h after 500mg/kg BPA administration intraperitoneally (**Figure.1**). According to this result, we decided to inject BPA intraperitoneally at 3h before irradiation. In BNCT study, animals were divided into three groups; the cold control (no treatment, no neutron irradiation), hot control (neutron irradiation only), and BNCT (intraperitoneal BPA administration and neutron irradiation) groups. Also, we conducted an irradiation experiment using BALB/cCrSlc mice without tumor implantation to investigate the long-term effects on pelvic organs with HE staining and RNA-sequence.

RESULTS: BNCT significantly prolonged survival (median: Cold 7 days, Hot 10, BNCT 16) (**Figure.2**). All toxicity-study mice survived 189 days without severe adverse events except transient weight loss. Histological examination with HE revealed mucosal degeneration in the cecum 7 days after BNCT; however, recovery was observed over time. RNA sequencing indicated a tendency toward local activation of the immune system or a DNA damage response in the early phase after BNCT, whereas findings in the late phase were suggestive of chronic inflammation (**Figure.3**).

Conclusion: BPA administration followed by BNCT was not associated with any lethal adverse events, indicating the feasibility and safety of pelvic irradiation.

Figure.1

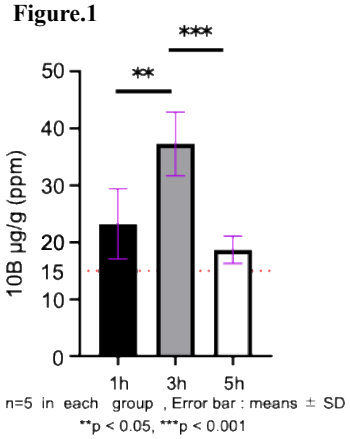


Figure.2

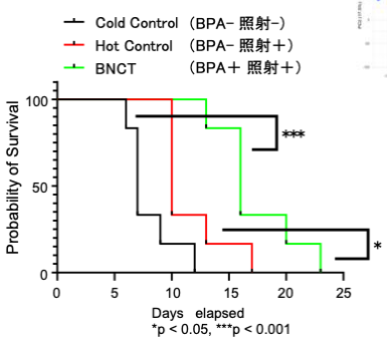
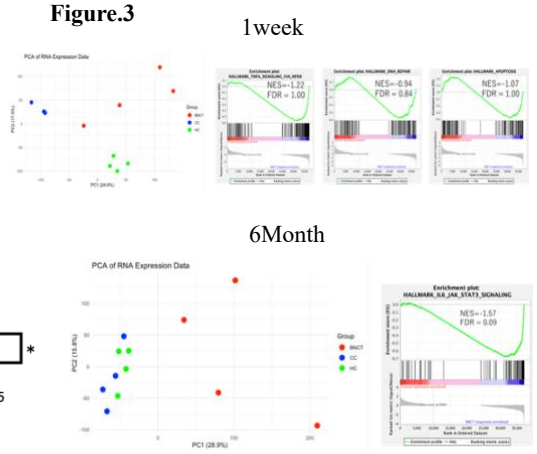


Figure.3



Ongoing study: We will continue this study and the results will be published in the future.

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CO7-6

The effect of boron neutron capture therapy (BNCT) to liver metastasis of colorectal cancer

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INTRODUCTION: Management of liver metastases in colorectal cancer is a clinically important issue. However, only 10–15% of patients are eligible for surgery based on the size and number of metastatic lesions. Furthermore, for patients with multifocal, unresectable, or bilateral liver metastases who do not respond to chemotherapy, palliative care is the only option available. In this context, the development and evaluation of new treatment strategies are justified.

In this study, we investigated the effectiveness of boron neutron capture therapy (BNCT) to liver metastasis of colorectal cancer using the mouse model.

EXPERIMENTS: We used Boronophenylalanine (BPA) as a boron compound. Also, we used seven-week-old female BALB/cCrSlc mouse having physiological environment of immunity. Firstly, we established a mouse model of liver metastasis of CRC using DLD-1-Luc cells concentrated to 1.0×10^6/100μL in 0.1ml of PBS. The boron concentrations in DLD-1-Luc tumors or surrounding organs at 2h, 4h, 6h after 500mg/kg BPA administration intraperitoneally. (**Figure.1**) According to this result, we considered the effects on the pancreas and decided to inject BPA intraperitoneally at 6h before irradiation. In BNCT study, animals were divided into three groups; the cold control (no treatment, no neutron irradiation), hot control (neutron irradiation only), and BNCT (intraperitoneal BPA administration and neutron irradiation) groups.

RESULTS: Luminescence intensity monitored via the In Vivo Imaging System (IVIS) demonstrated that values in the BNCT group were significantly lower than those in the cold control group on day 10 (**Figure.2**), with a non-significant downward trend also observed relative to the hot control group. Upon dissection on day 16, the tumor weight in the BNCT group was significantly reduced compared to the cold control group (**Figure.3**) and showed a decreasing tendency relative to the hot control group, although this did not reach statistical significance. Similarly, while the tumor volume in the BNCT group tended to be lower than in the other two groups, no statistically significant difference was observed (**Figure.3**).

Histopathological analysis showed a reduction in Ki67-positive cells in the BNCT group, suggesting inhibited tumor cell proliferation (**Figure.4**). In contrast, acinar cell necrosis and plasma cell infiltration were observed in the pancreatic parenchyma, indicating potential treatment-induced toxicity in surrounding normal tissue.

Figure1

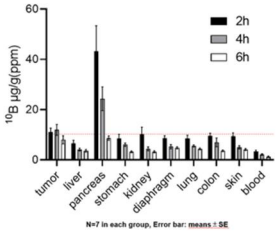


Figure.2

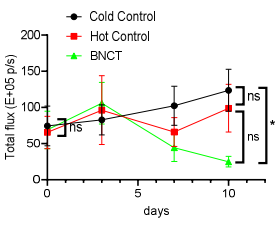
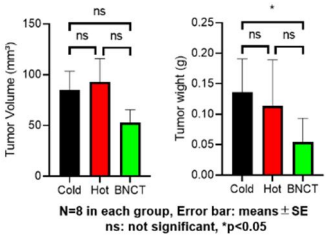
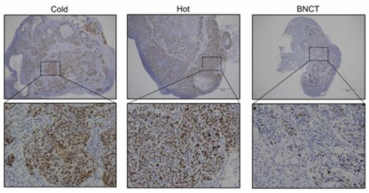


Figure.3



N=8 in each group, Error bar: means ± SE
ns: not significant, *p<0.05

Figure.4



Ongoing study:

Future studies will aim to further elucidate the anti-tumor mechanisms of BNCT and systematically evaluate its impact on adjacent organs. This study will be continued, and the results will be reported in the future.

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CO7-7

Optimization of CSF-Administered BNCT Using High-Concentration ^{10}BPA Ionic Liquid in a Rat Glioma Model

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INTRODUCTION: Boron neutron capture therapy (BNCT) is a tumor-selective radiotherapy based on nuclear reactions between thermal neutrons and boron (^{10}B) accumulated in tumor cells. Its therapeutic efficacy critically depends on efficient and selective boron delivery. However, in brain tumors, drug delivery is limited by the blood–brain barrier (BBB), making it difficult to achieve sufficient and homogeneous boron distribution within the tumor. To overcome this limitation, we have previously developed a cerebrospinal fluid (CSF)-based drug delivery approach via cisterna magna administration [1]. In addition, the limited solubility of conventional boronophenylalanine (^{10}BPA) restricts achievable boron concentrations. In this study, we employed a high-concentration meglumine–^{10}BPA ionic liquid formulation (Meg-^{10}BPA IL) and investigated the relationship between administration parameters and BNCT efficacy under reactor irradiation conditions.

EXPERIMENTS: C6 glioma cells were stereotactically implanted into the brains of male Wistar rats (8 weeks old, body weight 180–200 g). The high-concentration boron formulation, Meg-^{10}BPA IL, was developed by Dr. Makoto Shirakawa at Okayama University and has been patented in Japan [2]. Meg-^{10}BPA IL was continuously infused into the CSF via the cisterna magna at different boron concentrations and infusion rates (100 or 200 μL/h). Neutron irradiation was performed at the Kyoto University Research Reactor (KUR), a 5 MW light water-moderated reactor, under thermal neutron beam conditions for 20 min, corresponding to an average neutron fluence of 3.7×10^{12} neutrons/cm². During irradiation, the body (excluding the head) was shielded using ^{6}LiF materials to reduce unnecessary neutron exposure. Tumor progression was evaluated using T2-weighted MRI before and after BNCT, and tumor volume ratios (post-/pre-treatment) were calculated.

RESULTS: As shown in Figure 1, all CSF-administered BNCT groups showed suppression of tumor growth compared with untreated and neutron-only control groups.

Tumor volume ratios were:
4200 ppm-B, 100 μL/h: 0.94
4200 ppm-B, 200 μL/h: 0.45
8400 ppm-B, 200 μL/h: 1.83
Control groups: >10

The strongest tumor suppression was observed in the 4200 ppm-B, 200 μL/h group. Notably, increasing boron concentration alone did not improve therapeutic efficacy, whereas a higher infusion rate resulted in greater tumor control. These results indicate that infusion rate would be a key determinant of therapeutic outcome in CSF-administered BNCT under KUR irradiation conditions.

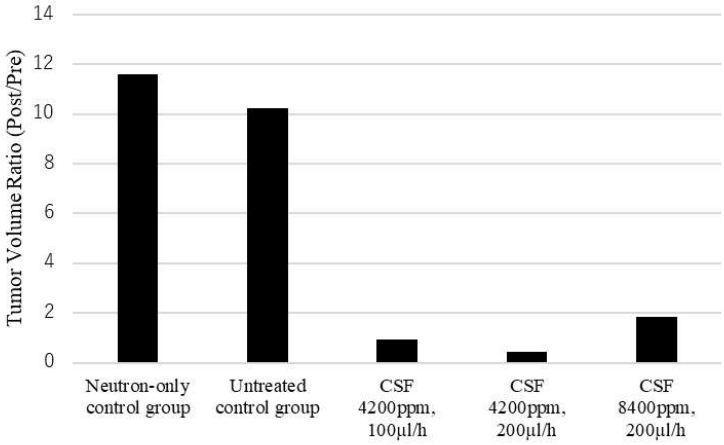

Figure 1. Tumor Volume Ratio (Post/Pre) by Treatment Group

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CO7-8

Abscopal Effect Induced by BNCT with Boron-10 Carbide Nanoparticles

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We have been developing nanoparticle (NP) based ^{10}B drugs for BNCT [1]. However, radiotherapy is not systemic but local cancer treatment to limit its efficacy against metastatic cancers. While recent studies suggest that combining BNCT with an immunomodulator (PD-L1 siRNA or anti-PD-1 antibody (αPD-1) [2]) induces abscopal effects to distant tumors, such responses have been rarely observed with single-modality BNCT via intravenous administration [3], or even the other radiotherapy alone. Herein, we report on abscopal effect by BNCT with boron carbide NPs with 50 nm size ($^{10}B_4C(50)$).

First of all, we established the mechanochemical process to selectively synthesize $^{10}B_4C(50)$ from $^{10}B(OH)_3$, magnesium and graphite [4]. The $^{10}B_4C(50)$ was subsequently coated with hydrophilic polymer, poly(glycerol) (PG), to give $^{10}B_4C(50)$-PG which is highly dispersed in a physiological environment. The resulting boron-10 drug was administered intravenously to the mice with the CT26 tumors at the leg and back. Only the leg tumor was irradiated by neutron as shown in Figure 1a. As a result, the growth of the primary tumor at the leg was suppressed more efficiently as compared with the control groups of PBS injection, boron drug injection and neutron irradiation as shown in Figure 1b. Meanwhile, the distant tumor at the back was also suppressed in the growth as comparted with the other groups as shown in Figure 1b. Notably, the abscopal effect was also observed in 4T1, an immunologically "cold" tumor, as shown in Figure 1d and 1e, although "cold" tumours are typically characterized by an immunosuppressive microenvironment.

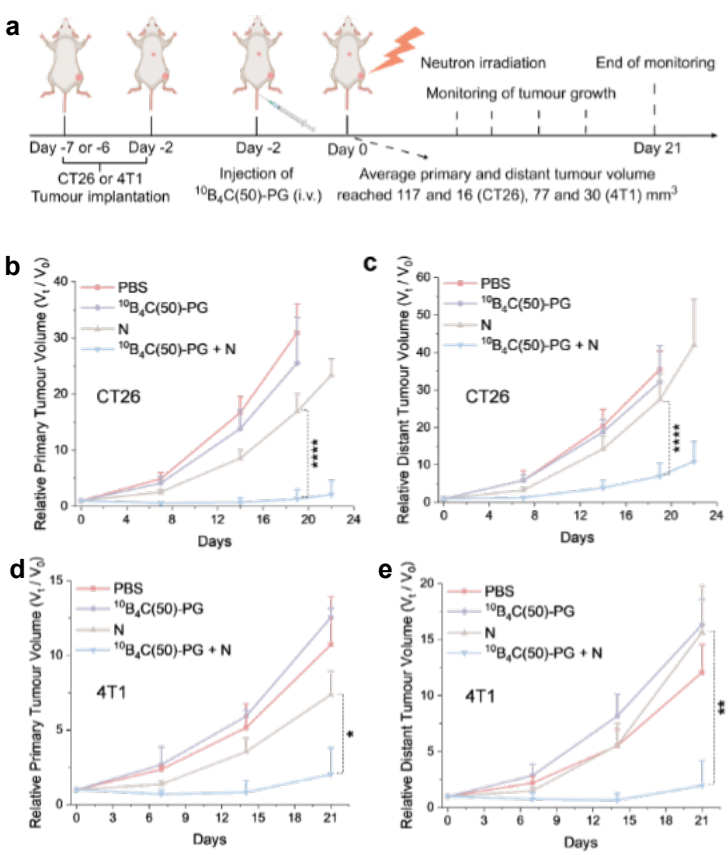

Figure 1. a) Schematic of dual CT26 tumour treatment process with dosage of 5.1 mg [^{10}B]/kg (mouse) and neutron irradiation only at right thigh in *in vivo* BNCT (n = 6). **b)** Relative primary tumour volume at right thigh. **c)** Relative distant tumour volume at left thigh. **d**, **e**) Relative 4T1 primary and distant tumour volume at right thigh and flank, respectively.

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CO7-9

Bifunctional Dodecaborate to Development of Boronated Antibody

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INTRODUCTION: BNCT for cancer is based on the nuclear reaction of 10B with thermal/epithermal neutrons to yield high linear energy transfer α particles (^{4}He) and recoiling 7Li nuclei in tumor cells [1]. For a boron delivery agent to be successful in BNCT, the following criteria must be met: i) high tumor targeting selectivity; ii) low systemic toxicity; iii) high water solubility. Mercapto-undecahydro-closo- dodecaborate ($[B_{12}H_{11}SH]^{2-}$, BSH), a class of water-soluble boron cluster compounds with low toxicity, is clinically used as boron carrier for the treatment of brain tumors, although, tumor selectivity and cell membrane permeability of BSH is slightly low. In the course of our developing studies on new boron carrier for BNCT, we have designed and synthesized thiododecaborate ($[B_{12}H_{11}S]^{2-}$) unit-containing tumor seeking compounds such as amino acids, peptides and antibodies [2-4]. Furthermore, we reported the bifunctional type thiododecaborate containing compounds which boron cluster is linked two kinds of organic moiety through S+ sulfaneyl groups ($B_{12}H_{11}$-S+(-R1)-R2) [5].

Recently, theranostic drug delivery is strong criteria for effective cancer treatment. In BNCT, development of theranostic type boron carrier is highly noted, because visualization of the boron distribution and determination of tumor/normal ratio by non or minimally invasive examination are very important for the planning of BNCT.

To develop the theranostic type boron carrier for BNCT, we present the design and synthesis of novel bifunctional boron cluster containing compounds which linked fluorescent dye Cy5.5 and alkyl linker (Cy5.5-BSH-Pentyl-COOSu) to conjugate with tumor seeking antibody such as Cetuximab (Erbitux, anti EGFR IgG). Furthermore, we report the conjugation of novel bifunctional boron compounds with antibody, and the biological evaluation of boronated antibody as boron carrier for BNCT.

EXPERIMENTS: The solutioin of Cy5.5-BSH-Pentyl-COOSu in DMSO was added to the solution of Cetuximab in 0.1M $NaHPO_4$ buffer (pH8.6) and incubated for 24 at 0ºC. The mixture was purified with a PD-10 column.

RESULTS: As shown in Fig. 1, Dodecaborated-Cetuximab (Cy5.5-DB-Cetuximab) was delivered a boron atom effectively to EGFR high expressed tumor cell, and enhanced cellular uptake of the DB-Cetuximab by EGF induced effective macropinocytosis induction.

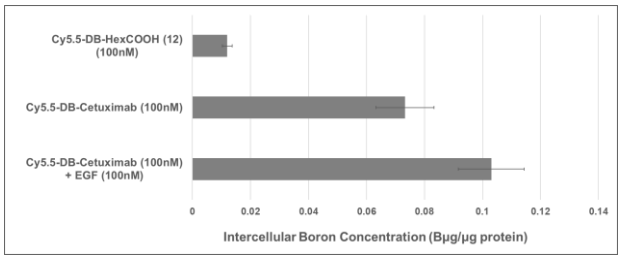

Fig.1. ELISA assay for detecting boron concentration in A431 cell treated with Cy5.5-DB-COOH (compound 12 type, 100nM) or Cy5.5-DB-Cetuximab (100 nM) with ot with out EGF (100 nM) in cell culture medium contain-ing 10% FBS for 24 h at 37ºC. The data are expressed as the mean (±SD) of three experiments

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CO7-10

Development of Boron Cluster-Loaded Nanoparticles for BNCT

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INTRODUCTION: Boron neutron capture therapy (BNCT) is a less-invasive nuclear therapeutic approach that highly selective to induce cancer apoptosis, without any cytotoxic effects in neighboring cells [1]. BPA and BSH as current clinically approved boron compounds have several limitations related to selectivity and effectivity [2]. To address the issues, a novel self-forming nanoparticle, which is consisted of biodegradable polymers, namely, “Lactosome” with a hydrophobic boron cluster have been developed [3]. In the previous study, the BNCT effects were successfully obtained after exposing neutron irradiation to the AsPC-1 cells (human pancreatic cancer cells) in vitro and in vivo [4]. In this study, we compared in vitro BNCT effect on a boron cluster loaded with different type of Lactosome “type 1” and “type 2” particles.

EXPERIMENTAL PLANS: For in vitro study, AsPC-1 cells are subjected to irradiation at reactor power 1 MW for 10 min or 40 min after 2 hr incubation with boron cluster-loaded Lactosome particles. After the neutron irradiation, the cells are cultured in 12-well plates and incubated under 5% CO2 at 37 °C for 14 days. Then, colony formation assay is performed after staining.

RESULTS:
In vitro, uptake of B-10 derived from either type 1 and type 2 particles reproducibly was observed but the in vitro neutron-irradiation study with cancer cells was not performed, because it took unexpectedly longer time to prepare the particles.

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CO7-11

Establishment of innovative BNCT treatment method for intractable bladder cancer

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INTRODUCTION: Bladder cancer treatment remains a challenge to every urologist.The current first-line treatment for non-muscle invasive bladder cancer is transurethral resection of bladder tumors followed by intravesical Mycobacterium Bovis Bacillus Calmette-Guérin (BCG) immunotherapy. In case of BCG failure, radical cystectomy is the standard of care in high‐risk patients. However, many of them are unfit or they refuse to undergo such an intervention; therefore, other treatment options are required. The usefulness of BNCT to urothelial cancer remains unknown. Here we aimed to investigate whether BNCT suppresses in a previously es-tablished mouse model of orthotropic bladder cancer.

EXPERIMENTS: We established BCG-resistant bladder cancer cell line and examined their molecular and functional characteristics. The response of resistant cells to BNCT was tested in vitro. In vivo experiments were carried out using an orthotopic bladder cancer mouse model to study boron biodistribution after intravesical or systemic administration of boronophenylalanine (BPA). Boron–neutron capture events at the tissue level were detected with CR-39 autoradiography. The therapeutic effect and safety of BNCT were evaluated by bladder weight measurement, serum biochemistry tests and organ histology.

RESULTS: BCG-resistant bladder cancer cells showed clear molecular differences from parental cells, but they remained highly sensitive to BNCT in vitro. In animal experiments, intravesical administration of BPA led to selective accumulation of boron in tumor tissue with very limited systemic exposure, and the optimal treatment time was found to be 1 hour. CR-39 analysis showed that neutron capture reactions occurred mainly in tumor areas. BNCT produced a clear reduction in tumor size compared with systemic delivery (Figure 1). Detailed safety evaluations showed no obvious treatment-related toxicity.

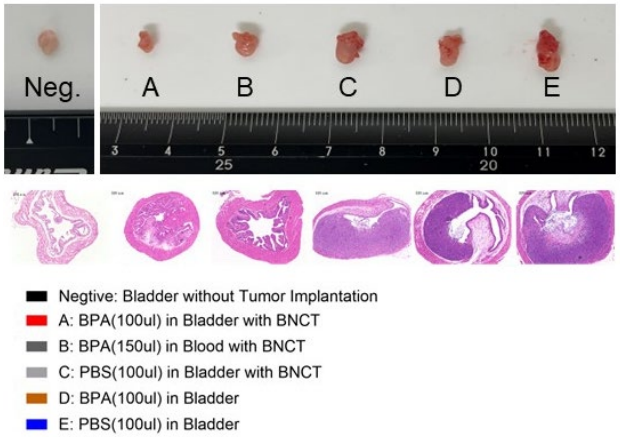

Fig.1
The bladder weight assay of 14 days after Neutron irradiation.
Neutron irradiation for 12 minutes after Boron added for 1hours

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CO7-12

The Basic Study Aimed at performing the BNCT for Canin Malignant Melanoma

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INTRODUCTION: Malignant melanoma is one of the most major cancers in dogs. Melanomas that occur in the canine oral cavity are highly invasive and difficult to surgically resect. In addition, they are known to be refractory tumors that are difficult to treat with conventional radiation therapy. Therefore, new treatment strategies are needed.
BNCT is a therapeutic method that selectively destroys the tumor while leaving normal tissues almost unharmed by utilizing the nuclear reaction with neutron and boron, which tends to accumulate in the cancer cells. In human malignant melanoma, LAT1, an amino acid transporter of boron compound, is shown to be overexpressed in many malignant tumor cells. In this study, the survival after the neutron irradiation using the canine malignant melanoma cell lines. In addition, the effects of the boron neutron capture reaction (BNCR) on cells were analyzed by examining cell survival rates and double-strand break damage.

EXPERIMENTS: To evaluate the effects of neutrons on canine malignant melanoma cells, canine mandibular lymph node metastatic oral melanoma cells (LMeC), in which LAT1 expression has been confirmed, were used for this study. The effects on cells were evaluated by measuring DNA double-strand breaks via immunofluorescence staining and assessing survival rates via colony formation assays in cells irradiated with neutrons after BPA administration (w/BPA) and cells irradiated with neutrons without BPA administration (w/o BPA).

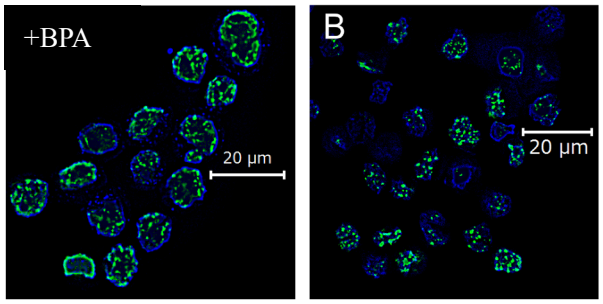

Fig.1 DNA double-strand breaks (showed by green points)

RESULTS: Fig. 1 shows the results of a comparison of DNA damage between gamma-ray irradiation and BPA treatment. In LMeC cells, BPA-treated cells exhibited greater DNA damage than gamma-ray-irradiated cells. Fig. 2 shows the results of cell viability. The survival rate of cells treated with BPA was significantly lower than that of cells not treated with BPA. Furthermore, at equivalent absorbed doses, w/BPA cells exhibited a higher mortality rate than gamma-irradiated cells. This demonstrates the efficacy of BPA-BNCT against LMeC cells and its superiority over gamma irradiation.

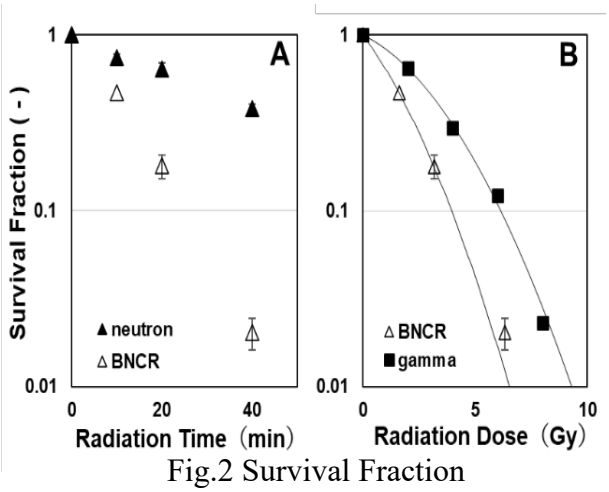

Fig.2 Survival Fraction

CO7-13

Real-time neutron monitoring for BNCT using ^{10}B-integrated HOIP sensors

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INTRODUCTION: Boron neutron capture therapy (BNCT) requires accurate, real-time monitoring of neutron flux under high-flux conditions (~10^9 s^{-1} cm^{-2}). Conventional methods such as ^{197}Au acti-vation lack real-time capability, while scintillator-based detectors suffer from γ-ray interference and limited self-diagnosis. Hybrid organic–inorganic perovskite (HOIP) thin-film devices, originally de-veloped for photovoltaic applications, offer high charge mobility, self-biased operation, and flexible fabrication, making them promising candidates for neutron detection when combined with neutron conversion materials such as ^{10}B. In this study, we develop and evaluate a ^{10}B-integrated HOIP sensor for real-time neutron monitoring in BNCT environments.

EXPERIMENTS: Boron neutron capture therapy (BNCT) requires accurate, real-time monitoring of neutron flux under high-flux conditions (~10^9 s^{-1} cm^{-2}). Conventional methods such as ^{197}Au acti-vation lack real-time capability, while scintillator-based detectors suffer from γ-ray interference and limited self-diagnosis. Hybrid organic–inorganic perovskite (HOIP) thin-film devices, originally de-veloped for photovoltaic applications, offer high charge mobility, self-biased operation, and flexible fabrication, making them promising candidates for neutron detection when combined with neutron conversion materials such as ^{10}B. In this study, we develop and evaluate a ^{10}B-integrated HOIP sensor for real-time neutron monitoring in BNCT environments.

RESULTS: The HOIP sensors demonstrated clear neutron-induced signals in BNCT-relevant high-flux environments. As shown in Fig. 1, the induced current of WC devices increased linearly with neutron flux up to ~10^9 s^{-1} cm^{-2}, reaching nA-level signals, whereas WoC devices showed only pA-level responses, confirming that the signal is dominated by neutron conversion events. The γ-ray contribution was estimated using WoC measurements and found to be extremely small (~0.012%), well below clinical tolerance limits. The sensors exhibited stable operation with 1 s temporal resolution, enabling real-time monitor-ing. Furthermore, no significant degradation was observed up to neutron fluences of ~10^{13} cm^{-2}, indicating sufficient radiation tolerance for BNCT applications. These results demonstrate that ^{10}B-integrated HOIP sensors satisfy key BNCT monitoring requirements, including high sensitivity, linearity, low γ-noise, and real-time capability.

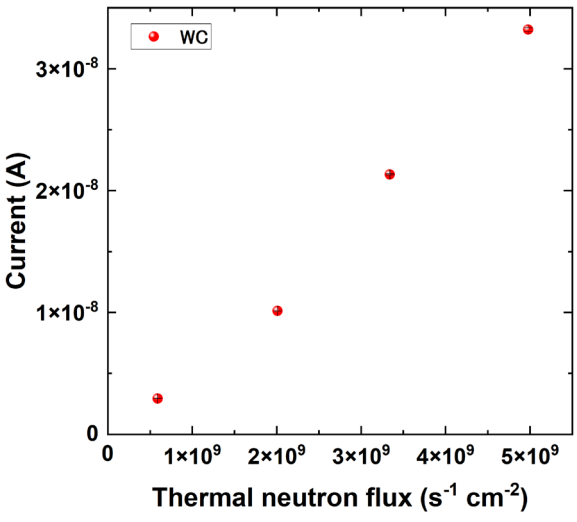

Fig. 1. Neutron flux dependence of radiation-induced current in HOIP sensors with ^{10}B conversion layer

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CO7-14

Preliminary Experiment of Neutron Capture Therapy using Intra-peritoneal Administration of ^{10}Boro-plex encapsulated Water-in-Oil-in-Water Emulsion for Peritoneal Disseminated Tumor Model

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INTRODUCTION: We have been continuously developing boron-containing water-in-oil-in-water (WOW) emulsions for application in the multidisciplinary treatment of primary cancer using boron neutron capture therapy (BNCT). We have experienced the abodominal disseminations of cancer cells ; so called cancerous peritonitis in pancreatic cancer, colon cancer, gastric cancer, ovarian cancer, etc. The pathology of cancerous peritonitis is extremely difficult to treat, and if BNCT can improve the condition and bridge the gap to multidisciplinary treatment, the effectiveness of BNCT can be further enhanced. In this study, we performed the preliminary experiments of BNCT with intraperitoneal administration of ^{10}Boro-plex encapsulated WOW emulsion for peritoneal disseminated colon cancer model, and its survival periods of treated and controlled groups following thermal neutron irradiation was evaluated.

EXPERIMENTS: The internal aqueous phase consisted of the following: 1.2 mL of ^{10}BSH solution (350 mg/mL), 0.12 mL of Lipofectamine 2000, 0.09 mL of sodium hyaluronate (0.25 mL; 10 mg/mL), and 0.09 mL of protamine (0.125 mL; 20 mg/mL). The oil phase consisted of 2 mL of Lipiodol, with surfactants added to each phase. Using our originally developed mixing device, a WOW emulsion was prepared. We prepared mouse colon cancer Colon 26 ($5x10^5$) model by intraperitoneally injection. Following the intra-peritoneal injection of 0.2 mL of the emulsion, thermal neutrons at a dose of 3×10^{12} n/cm^2 were irradiated at Institute for Integrated Radiation and Nuclear Science, Kyoto University. Mouse survival days were measured post-irradiation to evaluate the tumor growth inhibition effect.

RESULTS: The WOW emulsion containing the ^{10}Boro-plex complex demonstrated longest survival days after thermal neutron irradiation compared with non-irradiated group. As the irradiation was performed two hours after intra-peritoneal injection in this study.

We can expect better results of BNCT, if we will be able to prepare the cancer specific ^{10}B complexes entrapping into WOW emulsion.

Table1. Survival days after BNCT to intra-peritoneal disseminated tumor model.

	Survival rate after BNCT (%)				
Day after BNCT	3	7	11	14	20
BNCT groups					
WOW-^{10}Boroplex	100	100	100	100	33.3
Neutron only	100	100	100	83	0
Non NCT groups					
WOW-^{10}Boroplex	100	100	80	40	20
Neutron only	100	100	80	0	0

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CO7-15

Evaluation of the therapeutic efficacy of a novel boron carrier, BBCIP, for BNCT.

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INTRODUCTION: The efficacy of boron neutron capture therapy (BNCT) relies on the selective accumulation of boron within tumor cells [1,2]. While p-boronophenylalanine (BPA) is clinically standard, its dependence on LAT1 transporters necessitates new carriers to overcome tumor heterogeneity. In this study, we evaluated BBCIP, a novel boron carrier targeting the sodium-dependent multivitamin transporter (SMVT) [3]. BBCIP was administered via convection-enhanced delivery (CED) to bypass the blood-brain barrier and maximize local concentration [4,5]. We performed a comparative analysis using SMVT-high (CRL1666) and LAT1-dominant/SMVT-low (F98) brain tumor models to assess how transporter profiles dictate the therapeutic synergy of BBCIP and BPA.

EXPERIMENTS: Pharmacokinetic analysis revealed that BBCIP administered via CED exhibited stable and measurable intratumoral boron kinetics, maintaining therapeutic concentrations in both models. In survival studies, the therapeutic impact varied significantly according to the dominant transporter. In the LAT1-dominant F98 model, BBCIP monotherapy yielded only modest survival prolongation (median survival time [MST]: 29 days vs. 23 days in controls). However, the co-administration of BPA with BBCIP showed a positive trend in extending survival, suggesting a complementary uptake mechanism. Conversely, in the SMVT-high CRL1666 model, BBCIP monotherapy demonstrated robust efficacy, significantly prolonging survival (MST: 30 days vs. 15.5 days in controls). Notably, the addition of BPA in this model provided negligible further benefit, indicating that SMVT-mediated boron delivery alone was sufficient to achieve a therapeutic threshold.

RESULTS: These results demonstrate that the efficacy of BBCIP-based BNCT is strictly dictated by the tumor's transporter landscape. Our findings suggest that in SMVT-enriched tumors, BBCIP can serve as a highly effective primary boron source. In contrast, for tumors with low SMVT but high LAT1 expression, BBCIP may function as a supplemental agent, where combination therapy with BPA addresses the "intratumoral boron gap" left by single-agent protocols.
The disparate outcomes between the F98 and CRL1666 models underscore a critical paradigm shift: the necessity of "Precision BNCT." Rather than a universal carrier approach, treatment success relies on tailoring the boron cocktail to the specific molecular and biological signatures of the tumor. This study advocates for the pre-therapeutic screening of transporter expressions to optimize the selection and combination of boron agents, ultimately enhancing the precision and potency of neutron capture therapy.

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CO7-16

Investigation of Potential Adverse Effects of Boron Neutron Capture Therapy on Host Immunity

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INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is a form of radiation therapy for cancer that utilizes neutron capture reactions, wherein boron-10 atoms capture thermal neutrons, subsequently undergoing nuclear fission to produce alpha particles and lithium nuclei [1]. Recent studies have revealed that X-ray irradiation does not suppress anti-tumor immune function but rather activates it. To investigate whether BNCT similarly enhances anti-tumor immunity, we have explored the relationship between BNCT and tumor immunomodulation. The purpose of this study is twofold: (1) to determine whether BNCT induces host immune-activating effects, and (2) to evaluate the composition of infiltrating immune cells within tumors post-BNCT, thereby assessing potential adverse impacts on tumor immunity.

****

EXPERIMENTS: Tumor cells (SCC7) were subcutaneously implanted into the hind limbs of C3H/He mice. At 12 days post-implantation, neutron irradiation alone or BNCT treatment was administered to the tumor tissue. The BNCT group was injected subcutaneously with 500 mg/kg boronophenylalanine (BPA). One hour post-BPA administration, neutron irradiation was delivered to the tumor site using a 5 MW reactor for 15 minutes. At 12 days post-irradiation, tumor tissues were excised. Single-cell suspensions were prepared using the BD Tumor Dissociation Kit (BD Biosciences, #130-096-730) per manufacturer protocol. Cells were stained with fluorochrome-conjugated antibodies against CD45 (leukocyte common antigen), CD3 (T-cells), CD4 (helper T-cells), CD8 (cytotoxic T-cells), CD69, Eomes, CD62L, Gr1, CD25, Foxp3, Ki67, CD44, CD11b, PD-1, and Fixable stain 620. For intracellular staining, the intracellular fixation and permeabilization buffer set (ThermoFischer) was used according to the manufacturer protocol. All antibodies without Foxp3 were purchased from Biolegend. The antibody for Foxp3 was purchased from Invitrogen. Immune cells in the tumor tissues were measured by Cytek Northern Lights (Cytek) and the data were processed using SpectroFlo software.

****

RESULTS: After BNCT both CD4+ and CD8+ T cells were not reduced compared to the neutron alone control. Memory subsets of CD8 T cells in the BNCT group were slightly higher compared to the control. In the BNCT group compared to the control group, a higher proportion of cells positive for activation markers represented by CD69 was observed across all T cell subsets. The proportion of regulatory T cells did not differ between the two groups in this experiment.
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CO7-17

Exploration of Methods to Reduce BNCT Adverse Events by Modifying the Normal Tissue Distribution of BPA

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INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is a targeted radiotherapy that utilizes the nuclear capture reaction of thermal neutrons by boron-10, producing high-linear energy transfer (LET) alpha particles and lithium-7 nuclei [1]. The primary boron delivery agent, boronophenylalanine (BPA), is an amino acid analog transported into cells via L-type amino acid transporters LAT1 and LAT2. While cancer cells exhibit elevated LAT1 expression due to metabolic reprogramming, normal tissues also express LAT2, enabling BPA uptake in healthy cells. This unintended accumulation contributes to adverse effects post-BNCT, despite the therapy's theoretical tumor specificity. This study hypothesizes that selective inhibition of LAT1 (tumor-predominant) and LAT2 (normal tissue-expressed) could improve the tumor-to-normal tissue boron concentration ratio. By pre-administering transporter-specific inhibitors prior to BPA infusion, we aim to suppress normal tissue boron uptake while preserving tumor targeting, thereby enhancing BNCT's therapeutic index.

****

EXPERIMENTS: Tumor Model and Irradiation Protocol: The mice-derived tumor cells (SCC7) were subcutaneously injected into mice lower limb. At a tumor volume of 150–200 mm, BPA (500 mg/kg) was subcutaneously administered. After BPA administration, a compound inhibiting LAT1 was intraperitoneally injected to the mice. Six and twelve hours after BPA administration respectively, blood was collected by cardiac puncture under anesthesia, followed by euthanasia via cervical dislocation. Thereafter, the liver, stomach, small intestine, large intestine, tongue, skin, brain, tumor, and liver were harvested. To assess BPA biodistribution modulation, anticholinergic (atropine, 2 mg/kg) or cholinergic (pilocarpine, 5 mg/kg) agents were administered intraperitoneally 30 minutes prior to BPA. Tissues were digested in a 3:1 mixture of perchloric acid and hydrogen peroxide, followed by boron quantification via inductively coupled plasma atomic emission spectroscopy (ICP-AES).

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RESULTS: In the combination treatment with the LAT1 inhibitor, contrary to our expectations, tumor BPA efflux was also inhibited; however, under the timing of administration used in this experiment, BPA uptake into the tumor likewise appeared to be suppressed. As expected, BPA uptake and washout in normal tissues were not inhibited. In the brain, the T/N ratio was actually reduced when the LAT1 inhibitor was used. This finding suggests that, because LAT1 is expressed at the blood–brain barrier, BPA washout from the brain is also inhibited.

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CO7-18

Construction of a Dose–Response Model for Boron Neutron Capture Therapy: Derivation of Optimal Parameters through Cell and Animal Experiments

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INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is a targeted radiotherapy that utilizes the nuclear capture reaction of thermal neutrons by boron-10, producing high-linear energy transfer (LET) alpha particles and lithium-7 nuclei [1]. Thus, BNCT is a type of particle radiotherapy especially focusing on cellular-level radiotherapy. As in the process of dose calculation in radiotherapy, if the distributions of the boron agent and the neutron beam are known, the dose can be calculated, and the antitumor effect as well as the impact on normal tissues can be estimated from the dose–response curves of both tumor and normal tissues. We have already established a dose–response model for boron neutron capture therapy based on parameters that contribute to the distribution of the boron agent and on the findings accumulated to date [2]. However, our established model includes multiple parameters, and the biological validity of these parameters had not yet been sufficiently examined. The purpose of this study is to derive the appropriate parameters for the established dose–response model of boron neutron capture therapy through cell-based and animal experiments, and to verify its usefulness.

****

EXPERIMENTS: The aim of this study was to perform BNCT on tumor tissues in C3H and Balb/c mice subcutaneously implanted with tumor cells, and to examine the relationship between boron concentration and antitumor efficacy by applying BNCT at different boron concentrations. Although the irradiation experiments could not be conducted as scheduled because of the experimenter's unexpected condition, we carried out alpha autoradiography using CR-39, which had been planned as one of the experiments to evaluate the response of normal tissues. Alpha autoradiography was conducted using CR-39 solid-state nuclear track detectors. Prior to sample mounting, two crossing fiducial marks were inscribed on the detector surface to provide stable reference points for image registration before and after etching. Tissue sections containing 10B were placed in direct contact with the detector and irradiated with thermal neutrons to induce the 10B(n,α)7Li reaction. The emitted charged particles produced latent tracks in the CR-39 detector, which were visualized as etch pits after chemical etching under alkaline conditions. Microscopic images of the specimen were acquired before or independently from etching, using bright-field microscopy and, when necessary, fluorescence microscopy for structural identification such as nuclear localization. After etching, the detector surface was examined under high magnification, and the etched pits were analyzed according to their spatial distribution and morphology. In particular, pit circularity was used as an index to improve the positional accuracy of the estimated boron localization. The pre-etch specimen image and the post-etch pit image were then superimposed using the fiducial marks on the detector, and the resulting composite images were used to determine the microdistribution of boron in the tissue or cells.

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RESULTS: We are currently analyzing the alpha autoradiography results of the gastrointestinal tract and tumors following administration of BPA at 500 mg/kg. In the future, we plan to incorporate into the model not only these alpha autoradiography findings but also information on the sizes of the cytoplasm and nuclei in the tissues.

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CO7-19

Cancer Immune Activation and Its Effect on BNCT Using Antagonists of Transcription Factor and Amino Acid Metabolic Enzyme

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INTRODUCTION: In the tumor microenvironment (TME), cancer cells, cancer-associated macrophages (TAM), and myeloid-derived suppressor cells (MDSC) utilize various checkpoints and enzymes to evade the immune system. STAT3 and IDO-1 play a central role as "brakes" in this process. STAT3 is the "command center" of cancer immunosuppression and induces TAM to M2 type. IDO-1 depletes tryptophan, weakening the induction of cytotoxic T cells and enhancing differentiation into regulatory T cells. TAM and MDSC existing in the TME express Dectin-1, a β-1,3-glucan (GC) receptor, in their cell membranes. Therefore, in this study, we utilize to solubilize poorly water-soluble STAT3 and IDO-1 inhibitors with GC and administered them via drug delivery system (DDS) to Dectin-1 expressing cells to induce hot cancer formation, and then investigated the combination effect with low-dose BPA-BNCT.

EXPERIMENTS: Shikonin (SKN) was used as a STAT3 inhibitor, and LY-3381916 (LY) was used as an IDO-1 inhibitor. After mixing *Aureobasidium pullulans TS-1* strain-produced GC in DMSO, two types of complex nanogels (SKN/GC, LY/GC) were prepared by dialysis. SCC-VII (mouse squamous cell carcinoma) cells were transplanted subcutaneously into the right thigh to create tumor-bearing C3H mice. BPA (125 mg/kg) was administered, followed by BNCT with neutron irradiation (5MW, 14 mins, 5.6 neutrons•cm^{-2}) at KUR. SKN/GC, LY/GC, or a mixed cocktail was administered via tail vein 2, 6, 9, and 13 days after irradiation. Thereafter, tumor size and body weight were measured twice a week.

RESULTS: Nanogel aqueous solutions were obtained with SKN at a concentration of 290 μM (solubilization rate 13%) and LY at 400 μM (solubilization rate 32%). The particle size and zeta potential of each nanogel were measured using a Zetasizer-Nano ZS. The SKN/GC nanogel had a particle size of 21.3 nm and a zeta potential of -28.5 mV, while the LY/GC nanogel showed 11.3 nm and -20.1 mV.

As shouwn in Fig.1, the relative increase in tumor volume with neutron irradiation (Figure 1) showed that the group administered both SKN and LY nanogel (G4) had significantly suppressed tumor growth compared to the other monotherapy groups (G2,3) and the no-drug group (G1).

In summary, it has demonstrated that simultaneously inhibiting proteins involved in transcription factor and amino acid metabolism on cancer immunosuppression is effective in controlling the TME to activate cancer immunity followed the enhanced BPA-BNCT.

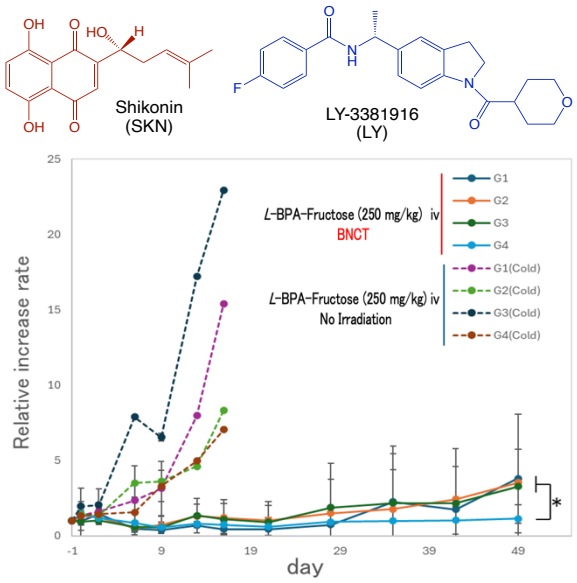

Fig. 1. The combination effect of SKN and/or LY administration on low-dose BPA-BNCT. G1: No Drug, G2:50 μM SKN, G3: 50 μM LY, G4: 50 μM SKN & 50 μM LY.

CO7-20

Mechanistic Study of GdNP-PG Nanoparticles-Mediated Gadolinium Neutron Capture Therapy Combined with Anti-PD-1 Immunotherapy

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Gadolinium neutron capture therapy (GdNCT) is a promising binary radiotherapy approach for cancer treatment. It leverages the nuclear reaction between thermal neutrons and ^{157}Gd, which has an exceptionally high neutron capture cross-section of 254,000 barns. This reaction generates high linear energy transfer (LET) Auger electrons along with low-LET γ-photons, both of which contribute to the destruction of cancer cells.[1] Immune checkpoint blockade (ICB) immunotherapy is a revolutionary cancer treatment strategy that restores the body's antitumor immune response. It functions through the administration of monoclonal antibodies that block inhibitory receptors on T cells or tumor cells, such as CTLA-4, PD-1, or their ligands (e.g., PD-L1). These checkpoints are typically exploited by cancers to evade immune surveillance. Blocking this interaction with specific monoclonal antibodies restores T-cell-mediated antitumor immunity, leading to durable clinical responses across various tumor types.

Previously, we reported that the combination of GdNP-PG nanoparticle-mediated GdNCT and anti-PD-1 (αPD-1)-mediated ICB immunotherapy effectively suppressed the growth of both primary and distant tumors, showing a significant abscopal effect. To elucidate the mechanisms underlying the therapeutic efficacy of the combination therapy, mice bearing primary and distant CT26 tumors were sacrificed 21 days after neutron irradiation, and the tumors were harvested for immunohistochemical assays. As shown in Figure 1, the populations of $CD4^+$ and $CD8^+$ T cells in the GdNCT + αPD-1 group were much larger than those in the other groups. The activation and infiltration of $CD4^+$ and $CD8^+$ T cells in the tumor microenvironment boosted the therapeutic efficacy against primary tumors and induced significant abscopal effects that suppressed the growth of distant tumors. These results suggest that the combination of GdNCT and ICB immunotherapy effectively modulates the tumor immune microenvironment, turning “cold” tumors into “hot” ones.[2]

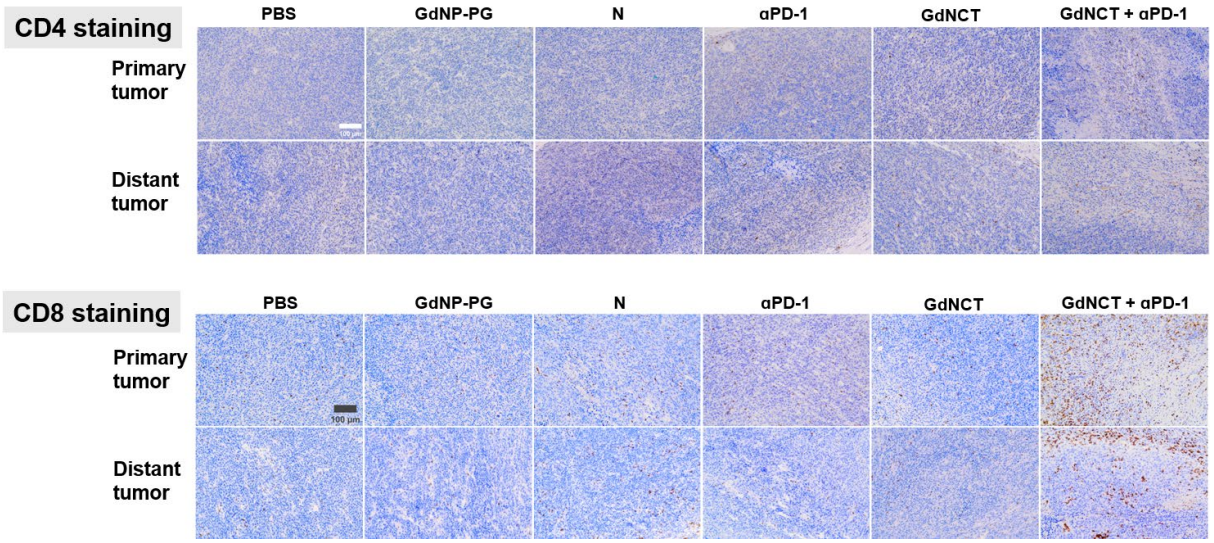

Figure 1. Immunohistochemistry staining of CD4 and CD8 in primary and distant tumors of different groups.

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CO7-21

Clonogenic Survival Assay of U87 MG Cells Exposed to Different Doses of BNCT, Neutrons, and X-rays of the KURNS Progress Report.

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INTRODUCTION: As a binary radiotherapeutic modality based on ^{10}B and neutron irradiation, boron neutron capture therapy (BNCT) involves the selective accumulation of non-radioactive ^{10}B in tumor cells, followed by thermal neutron capture to induce a nuclear reaction that generates high–linear energy transfer (LET) α particles and ^{7}Li particles. These high-LET particles exhibit strong relative biological effectiveness, causing lethal cellular damage. Owing to their short path length, cytotoxic effects are confined to the cellular scale, thereby minimizing damage to surrounding normal tissues[1].Previous radiobiological evaluations of BNCT have predominantly relied on reactor-based neutron sources, whose dose rates and neutron energy spectra differ markedly from those of the accelerator-based systems increasingly used in clinical settings. Consequently, understanding how neutron characteristics influence BNCT radiobiological outcomes represents a critical issue in the field. In this experiment, building upon our prior synthesis of the novel boron agent ANG-LIPO, the present study employs BPA as a reference to systematically investigate the impact of neutron properties on BNCT efficacy and the radiobiological performance of ANG-LIPO.

EXPERIMENTS: Clonogenic survival assay for X-ray and neutron irradiation: Log-phase adherent U87 cells were washed with PBS, trypsinized, centrifuged, and resuspended prior to irradiation. Cells were exposed to X-rays (0, 2, 4, 8, and 12 Gy) or neutrons (0, 0.31, 0.94, 2, and 3.67 Gy), then seeded into six-well plates (800 cells per well) and cultured for 14 days. Colonies were fixed with 4% paraformaldehyde, stained with 0.5% crystal violet, and counted to determine the survival fraction. Clonogenic survival assay for BNCT: Log-phase adherent U87 cells were co-incubated with culture medium containing ANG-LIPO or BPA, with concentrations determined based on a previously established neutron autoradiography method[2]. Cells were then subjected to neutron irradiation at BNCT doses of 0, 0.67, 1.77, 2.88, and 3.99 Gy. Subsequent procedures were performed as described above.

RESULTS: As shown in Fig. 1, clone formation in U87 MG cells varies under different doses of BNCT, neutrons, and X-rays. Based on the number of colonies, the survival fractions of U87 cells corresponding to each dose were calculated using the formula. The survival fractions for BNCT (ANG-LIPO) were 1.00 ± 0.20, 0.42 ± 0.03, 0.04 ± 0.01, 0.01 ± 0.01, and 0. For BNCT (BPA), the corresponding values were 1.00 ± 0.09, 0.48 ± 0.05, 0.20 ± 0.09, 0.04 ± 0.01, and 0.01 ± 0.01. For neutron, the corresponding values were 1.00 ± 0.06, 0.84 ± 0.08, 0.78 ± 0.04, 0.36 ± 0.02, and 0.11 ± 0.03. For X-rays, the corresponding values were 1.00 ± 0.06, 0.65 ± 0.14, 0.24 ± 0.04, 0.02 ± 0.01, and 0.004 ± 0.004.

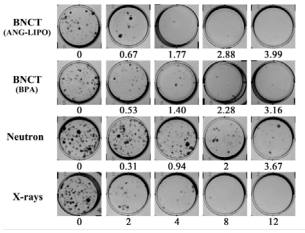

Fig. 1. Clone formation in U87 MG cells following exposure to different doses of BNCT, neutrons, and X-rays

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CO7-22

Investigation of BNCT-induced cellular senescence and its modulation by dasatinib in vitro and in vivo

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INTRODUCTION:

Boron neutron capture therapy (BNCT) is a unique radiotherapeutic approach that relies on the nuclear reaction between thermal neutrons and boron-10 selectively accumulated in tumor cells. This reaction generates high-linear energy transfer particles, which can induce severe intracellular damage. While BNCT has mainly been studied as a cell-killing treatment, accumulating evidence from radiation biology suggests that irradiation can also trigger senescence-associated cellular responses. However, such responses after BNCT have not yet been sufficiently characterized. Therefore, we are investigating whether BNCT induces senescence-associated phenotypes in melanoma cells and are continuing to analyze the underlying molecular responses. We are also examining whether dasatinib modulates these BNCT-induced responses in vitro and in vivo.

EXPERIMENTS:

Murine melanoma B16F10 cells were exposed to boronophenylalanine (BPA) prior to neutron irradiation. After irradiation under defined neutron exposure conditions at KUR, the treated cells were subjected to several in vitro assays to evaluate senescence-associated changes, including SA-β-Gal staining, colony formation assay, and protein expression analysis by western blotting. Molecular analyses focused on representative senescence-related signaling molecules. In selected experiments, dasatinib was used to evaluate the pharmacological modulation of BNCT-induced senescence-associated responses.
In addition to the in vitro experiments, in vivo evaluation has also been initiated using a tumor-bearing mouse model to examine the antitumor response to BNCT under boron administration conditions. Tumor growth and treatment-associated responses, including the effects of dasatinib treatment, are currently being analyzed.

RESULTS:

In the in vitro studies, we have obtained preliminary findings suggesting that BNCT induces senescence-associated responses in B16F10 cells. Assessments based on SA-β-Gal staining, clonogenic potential, and expression analysis of senescence-related proteins are currently in progress. In addition, the modulatory effects of dasatinib on these responses are under investigation.
In the in vivo studies, evaluation of therapeutic responses following BNCT has also been conducted, and detailed analyses, including the effects of dasatinib treatment, are ongoing.

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CO7-23

Safety assessment of boron containing nano particle for chest BNCT

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INTRODUCTION: Boronophenylalanine (BPA) has been applied to boron-neutron capture therapy (BNCT) for late stage of head and neck squamous cell carcinoma and the clinical application for other type of cancers are actively expanding. On the other hand, BPA was quickly metabolized and excreted from the body within several hours, and therefore continuously infused during thermal neutron irradiation. To improve the disadvantages of BPA, we have developed 10B containing nano particle, RN-501 [1]. In this study, we investigated short and long term toxicity as pre-clinical safety assessment.

EXPERIMENTS: RN-501 was intravenously injected into eight weeks old BALB/c mice and then the mice were irradiated with thermal neutron which was generated at KUR with 5MW output energy for several time, the duration of which are ranging from 7.5 min to 45 min. The possible risk organs including lung, liver, spleen, esophagus, stomach, small and large intestine were harvested at a week or approximately 6 month after the irradiation. These tissues were fixed with buffered formalin and then embedded into paraffin blocks.

RESULTS: Now, the tissue slices are being processed and stained with hematoxylin and eosin.

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CO7-24

Examination of Improvement of BNCT treatment efficiency by L-phenylalanine deficiency in mice tumor models

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INTRODUCTION: L-Boronophenylalanine (L-BPA), a boronated isotope of L-phenylalanine (Phe), is used as a boron drug and is taken up by the tumor through its enhanced metabolism of amino acids. LAT1 is an exchange transporter, releasing one amino acid molecule into the extracel-lular space for the uptake of one amino acid molecule into the cell [1]. However, L-BPA is also taken up by normal cells via LAT2 and other pathways [2], and the improvement of the boron con-centration ratio (T/N ratio) between tumor and normal tissues has become an issue. In this study, we attempted to improve the therapeutic effect of BNCT by restricting Phe and improving L-BPA up-take.

EXPERIMENTS: 5.0 x 10^5 human tongue carcinoma-derived cell line SAS and mouse melano-ma-derived cell line B16 melanoma were transplanted into the thighs of 6-week-old female nude mice (Balb/cAJcl-nu/nu) and into the shoulders and thighs of inbred mouse (C57BL6), respectively. Two weeks after transplantation, an immune checkpoint inhibitor (PD-1 inhibitor) was administered subcutaneously to C57BL/6 mice, and both Balb/cAJcl-nu/nu and C57BL/6 mice were switched to a phenylalanine-restricted diet. The following day, a 500 mg/kg solution of L-BPA was adminis-tered subcutaneously, and two hours later, the thighs were irradiated with neutrons. After irradiation, the mice were fed a standard diet, and tumor size was measured over a period of several weeks. In addition, in the C57BL6 mice, a second dose of the PD-1 inhibitor was administered 7 days after the initial dose.

RESULTS: In Balb/cAJcl-nu/nu mice, tumor size decreased following L-BPA administration and neu-tron irradiation. Furthermore, in the group that re-ceived L-BPA administration and neutron irradiation after phenylalanine restriction, tumor size reduction was observed compared to the Phe (+) BNCT group (Figure 1). No significant changes in body weight were observed in these mice.
In C57BL6 femoral tumors, an improvement in the BNCT effect due to phenylalanine restriction was observed, albeit to a small extent. Furthermore, for shoulder tumors, the combination of a PD-1 inhibitor and BNCT showed a higher tumor suppression effect than the BNCT-alone group. These results are thought to be due to an abscopal effect.

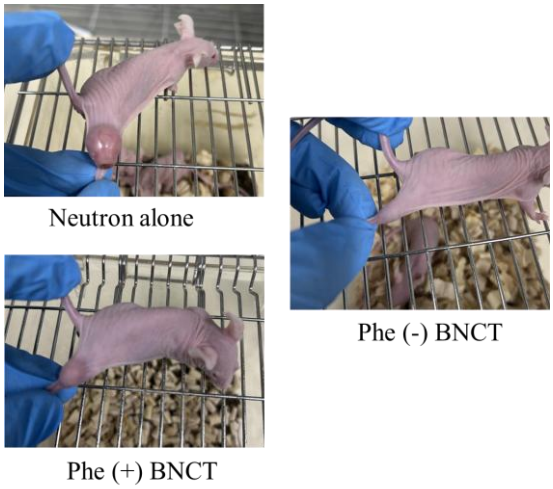

Fig.1 The Effect of Phenylalanine Restriction on BNCT in Balb/cAJcl-nu/nu Mice

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[2] P. Wongthai *et al.*, Cancer Sci., **106**(2015) 279-286.

CO7-25

Optimization study for non-clinical trials of polymeric BPA

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INTRODUCTION: While 4-borono-L-phenylalanine (L-BPA) serves as the primary therapeutic agent for boron neutron capture therapy (BNCT) by effectively targeting LAT1-expressing tumors [1], its overall efficacy is sometimes hindered by drug efflux. Because LAT1 functions as an antiporter, intracellular L-BPA is exchanged with extracellular amino acids such as tyrosine, leading to compromised retention time within the tumor and eventual therapeutic efficiency [2]. To overcome this limitation, we previously developed a simple technique to conjugate multiple L-BPA molecules with poly(vinyl alcohol) (PVA) via boronate esters. This complex shifts the cellular uptake mechanism to LAT1-mediated endocytosis, prolonging intratumoral retention of L-BPA [3]. Furthermore, we demonstrated that PVA could unleash the potential of the enantiomer of L-BPA, D-BPA, accomplishing enhanced LAT1-selectivity and appreciably prolonged intratumoral retention [4]. In this context, we recently found that the fine-tuning of the chemical structure of an L-BPA-like structure conjugated with PVA critically affects biodistribution. Here, we evaluated BNCT effects of newly developed PVA formulations.

EXPERIMENTS: PVA formulations were intravenously injected into mice bearing subcutaneous CT26 tumors. The tumors were irradiated with thermal neutrons using the Kyoto University Research Reactor (KUR).

RESULTS: All the PVA formulations demonstrated strong BNCT effects. Importantly, a novel PVA formulation achieved an almost complete cure of the tumors (Figure 1). As this formulation exhibits high LAT1-selectivity and a high tumor-to-normal organ (T/N) boron concentration ratio, it may be a promising candidate for practical applications.

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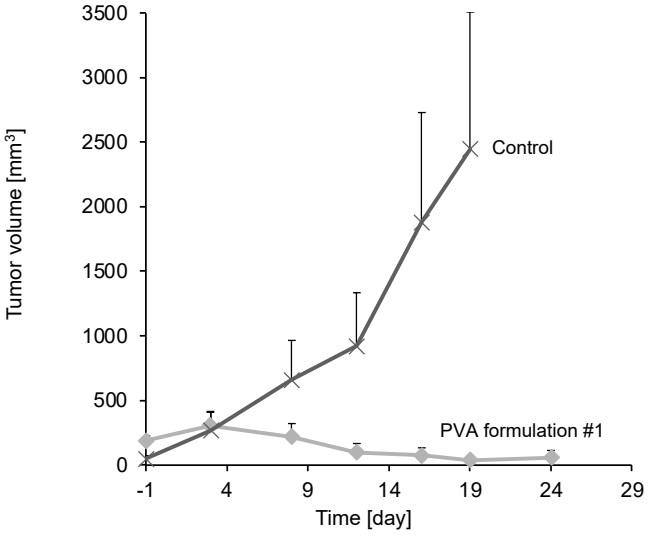

Fig. 1. BNCT effects on subcutaneous CT26 tumors.

CO7-26

Development of localized drug delivery systems

T. Nomoto[1,2], M. Suzuki [3], Y. Sakurai [3], D. Tokura [4], S. Tanaka [1], I. Fukuda [1], and K. Baba [2]

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INTRODUCTION: Currently, boronophenylalanine (BPA) is the most widely used agent in clinical studies. However, to maintain the necessary intratumoral boron concentration for boron neutron capture therapy (BNCT), a high-dose intravenous injection of approximately 500 mg/kg is required. Because BPA distributes to normal tissues to some extent, it cannot always achieve an adequate tumor-to-normal tissue (T/N) boron concentration ratio (2.5 or higher) depending on the cancer type. For such tumors, local administration of a highly concentrated boron agent appears to be a possible approach. Nevertheless, the low water solubility of BPA makes it challenging to prepare high-concentration formulations. Furthermore, because BPA is also recognized by other amino acid transporters such as LAT2 and $ATB^{0,+}$, there is a concern that it may distribute at high concentrations to adjacent normal tissues. To address these issues, we have been developing a patch-type drug delivery system (DDS) for the minimally invasive local administration of tumor-selective boron agent. In this study, we investigated the feasibility of treating superficial tumors with BNCT using this novel patch-type DDS. Specifically, the patch-type DDS was applied to the tumor site in a mouse subcutaneous tumor model. After a predetermined period, the patch was removed, and neutron irradiation was performed to evaluate the subsequent antitumor effects.

EXPERIMENTS: The patch-type DDS was applied to a subcutaneous CT26 tumor in a BALB/c mouse, and the tumor site was irradiated with thermal neutrons.

RESULTS: As shown in Figure 1, the newly developed DDS significantly inhibited the tumor growth. To further improve the potential, we will optimize the compositions in future study.

REFERENCES:
N/A

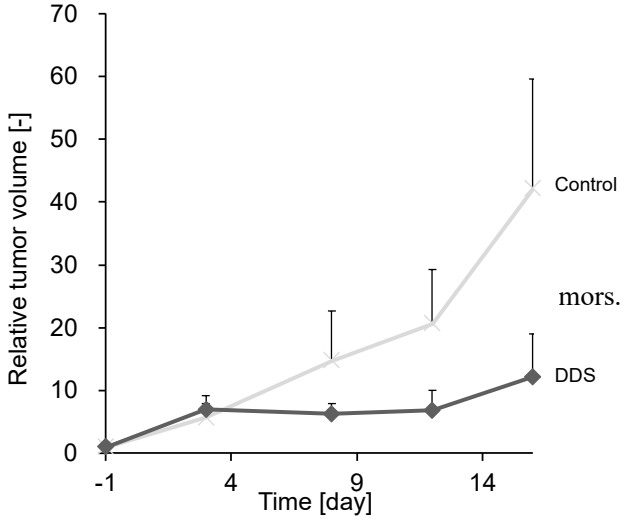

Fig. 1. BNCT effects on subcutaneous CT26 tumors.

CO7-27

Research and Development of New Technology for Boron Neutron Capture Therapy

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INTRODUCTION: Boron Neutron Capture Therapy (BNCT) requires sufficient intracellular accumulation of boron compounds in tumor cells to achieve effective cell killing by neutron irradiation. L-p-boronophenylalanine (L-BPA), one of the most widely used boron carriers, is preferentially taken up by cancer cells via amino acid transporters that are upregulated in tumors [1]. However, intracellular L-BPA levels decline rapidly after uptake, largely because of efflux mediated by specific transporters expressed in cancer cells. In this study, we examined a strategy to enhance BNCT efficacy by inhibiting such transporters and thereby suppressing L-BPA efflux. Using both cultured cells and tumor-bearing mouse models, we evaluated whether blocking L-BPA efflux enhances the antitumor effect of neutron irradiation. Building on the proof-of-concept studies performed in FY2022–2024, the FY2025 study is directed toward clinical translation by expanding the analysis in pancreatic tumor xenograft models and prolonging the interval between L-BPA administration and neutron irradiation.

EXPERIMENTS: In the FY2025 study, we used the human pancreatic cancer cell lines T3M4, Suit-2, and MiaPaCa-2, as well as the mouse triple-negative breast cancer cell line 4T1, all of which express high levels of transporters involved in BPA uptake. For the in vivo irradiation studies, tumor-bearing models were established by subcutaneous implantation of T3M4, Suit-2, and MiaPaCa-2 cells into nude mice. Similarly, 4T1 cells were inoculated subcutaneously into BALB/c mice. L-BPA was administered intravenously via the tail vein at 450 mg/kg. Thereafter, the active stereoisomer of a transporter inhibitor designed to suppress L-BPA efflux was administered at 200 mg/kg three times, at 1, 1.5, and 2.5 hours after L-BPA injection. In the control group, mice received the inhibitor alone without prior L-BPA administration before neutron irradiation. Neutron irradiation was carried out at a reactor output of 5 MW for 30 min. Tumor responses to neutron treatment were monitored and compared between treatment groups.

RESULTS: T3M4, Suit-2, MiaPaCa-2, and 4T1 cells were subcutaneously implanted to establish tumor-bearing models. Following intravenous administration of L-BPA, the animals were divided into three groups. Group 1 received additional injections of a transporter inhibitor at 1, 1.5, and 2.5 h after L-BPA administration. Group 2 received L-BPA alone without subsequent inhibitor treatment. Group 3 received saline instead of L-BPA, followed by inhibitor injections at the same time points as in Group 1. All the mice were subjected to neutron irradiation 6 h after the initial administration of L-BPA or saline. Tumor volumes were monitored for 8 weeks after irradiation. Group 1 showed a marked reduction in tumor volume compared with Group 2, indicating that inhibition of the transporter enhanced the efficacy of BNCT. This enhancement was particularly pronounced in the Suit-2 model. In our previous studies, the potentiating effect of the transporter inhibitor had been demonstrated under conditions in which neutron irradiation was performed 2.5 h after L-BPA administration. In the FY2025 study, enhanced BNCT efficacy by the transporter inhibitor was confirmed when neutron irradiation was carried out 6 h after L-BPA administration. These findings further substantiate the feasibility of this strategy for enhancing the efficacy of L-BPA-based BNCT.
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CO7-28

Development and Preclinical Evaluation of Novel Boron Neutron Capture Therapy (BNCT) Agents

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INTRODUCTION: Epithelial cell adhesion molecule (EpCAM) is a type I transmembrane glycoprotein involved in cell–cell adhesion, proliferation, and signal transduction. Although expressed in normal epithelial tissues, EpCAM is frequently overexpressed in various epithelial malignancies, including colorectal, breast, pancreatic, and ovarian cancers [1]. Its expression level has been associated with tumor aggressiveness, metastatic potential, and prognosis, highlighting EpCAM as a promising target for cancer imaging and therapy [2-4]. We have previously developed a novel anti-EpCAM monoclonal antibody (EpCAM-1) and evaluated an ^{111}In-labeled EpCAM-1 antibody in vitro. The radiolabeled antibody demonstrated efficient internalization via EpCAM-mediated uptake, indicating favorable properties for targeted intracellular delivery. Based on these findings, we aimed to exploit the high internalization capacity of EpCAM-1 to develop a boron neutron capture therapy (BNCT) agent by conjugating ^{10}B to the antibody.

EXPERIMENTS: In vitro evaluation: A colony formation assay was performed using an EpCAM antibody conjugated with ^{10}B (^{10}B–EpCAM-1). EpCAM-positive DLD-1 cells were seeded in 12-well plates, and ^{10}B–EpCAM-1 was added at concentrations of 0, 10, 20, 30, and 50 μg/mL one day prior to neutron irradiation. On the day of irradiation, the cells were collected into Eppendorf tubes and subjected to neutron irradiation. The cells were then cultured at 37°C in a humidified atmosphere with 5% CO_2. Cell damage was assessed at 2 and 4 days post-irradiation using Annexin V/PI staining, and a colony formation assay was performed 10 days after irradiation.

RESULTS: In the colony formation assay, a dose-dependent decrease in cell proliferative capacity and survival fraction was observed in the ^{10}B–EpCAM-1–treated groups compared with the control group. Furthermore, Annexin V/PI staining revealed that the proportion of damaged cells increased in a dose-dependent manner at both 2 and 4 days after irradiation. The therapeutic efficacy has also been evaluated in tumor-bearing animal models, and the experiments have been completed; data analysis is currently in progress.

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CO7-29

Effective BNCT against BPA-resistant glioblastoma using novel ASCT2-targeted boron carriers

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INTRODUCTION: Boron neutron capture therapy (BNCT) is a tumor-selective radiotherapy that uses the nuclear reaction between ^{10}B and thermal neutrons to generate high linear energy transfer (LET) particles, causing localized cytotoxicity in boron-accumulating cells. 4-Borono-L-phenylalanine (BPA), the most widely used clinical boron carrier, is transported into tumor cells via L-type amino acid transporter 1 (LAT1) [1]. Although BPA-based BNCT shows clinical benefits, its efficacy is limited in tumors with low LAT1 expression due to insufficient boron accumulation. U87MG glioblastoma is a LAT1-low tumor model and is resistant to BPA-based BNCT. To address this, we developed **GluB**s, small-molecule boron carriers targeting alanine-serine-cysteine transporter 2 (ASCT2), which is often upregulated in aggressive cancers and plays a key role in tumor metabolism. In this study, we examined whether ASCT2-targeted boron delivery using **GluB-2**, one of the **GluB**s, improves BNCT efficacy in BPA-refractory glioblastoma [2].

EXPERIMENTS: GluB-2, a glutamate-based boron carrier, was designed and synthesized to enable selective uptake via ASCT2. Its biological performance was evaluated in a human glioblastoma U87MG xenograft model. Tumor-bearing mice (female, 5–6 weeks old) were intravenously administered **GluB-2** or BPA at 25 mg [^{10}B]/kg. At 3 h post-injection, when tumor boron accumulation was expected to peak, thermal neutron irradiation was applied to the tumor (3.8–4.3 × 10^{12} neutrons/cm²). BNCT efficacy was assessed by monitoring tumor growth, and systemic safety was evaluated by measuring body weight throughout the experimental period.

RESULTS: GluB-2 achieved tumor boron concentrations of approximately 20 μg [B]/g in U87MG xenograft, reaching the threshold required for effective BNCT. This enhanced tumor selectivity is consistent with ASCT2-mediated uptake in LAT1-low tumor cells. Following neutron irradiation, **GluB-2** treatment resulted in pronounced and sustained suppression of tumor growth compared with both BPA-treated and control groups. In contrast, BPA exhibited only modest antitumor effects. Importantly, no significant body weight loss or observable systemic toxicity was detected in any treatment group, indicating that **GluB-2** is well tolerated at therapeutically relevant doses. Collectively, these results demonstrate that **GluB-2** enables efficient and selective boron delivery via ASCT2, thereby significantly enhancing BNCT efficacy in glioblastoma models that are less responsive to conventional BPA-based therapy.

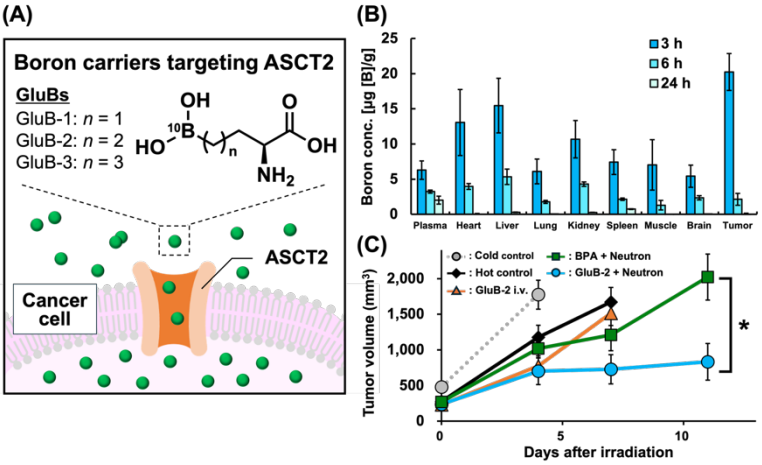

Fig. 1. Novel ASCT2-targeted boron carriers for BPA-refractory cancers. (A) ASCT2-targeted ^{10}B delivery system for BNCT. (B) Boron concentrations in major organs at 3, 6, and 24 h after intravenous (*i.v.*) injection of GluB-2 (25 mg [B]/kg). (C) Tumor volume in mice after BNCT with GluB-2 and BPA (25 mg [^{10}B]/kg i.v.). Data are expressed as mean ± SD (n = 5–6). Significance was determined as $*p < 0.05$ using the two-sided Student's t-test.

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CO7-30

Boron-Fused π-Conjugated Polymer-Hyaluronic Acid Complex for BNCT

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INTRODUCTION: Development of boron agents with imaging capabilities is required to maximize the therapeutic benefits of boron neutron capture therapy (BNCT), as such systems enable visualization of boron agent accumulation in tumor tissues. Today, ^{18}F-labeled L-boronophenylalanine (^{18}F-BPA) is clinically available as a positron emission tomography (PET) tracer to visualize biodistribution of L-BPA for the prediction of therapeutic efficacy. Although PET imaging can provide images with the spatiotemporally accurate their distribution, PET tracers are costly and their synthesis is restricted to specialized RI facilities. For the perspectives, fluorescence imaging is cost-effective and provides a high signal-to noise ratio, making it a promising alternative to PET.

In this study, we developed a water-dispersible hyaluronic acid (HA) complex of a boron-fused π-conjugated polymer (BCP), which serves as a boron agent. The complex was prepared via a mechanochemical blending strategy using a ball mill[1,2] and enables subcellular tracking through its intrinsic fluorescence.

RESULTS AND DISCUSSION: We prepared a solid-phase complex of HA and BCP via high-speed vibration milling (HSVM). The resulting mixture was extracted with water and treated with sonicator. The complexation of BCP was confirmed by measuring UV-Vis spectra and ICP-OES, and the boron concentration of the resulting dispersion was determined to be 86 ppm. Judging from the scalability of HA, we can prepare approximately 1000 ppm. Moreover, size distribution of the complex was confirmed by nanoparticle tracking analysis. The mean diameter of the complex was determined to be approximately 100–130 nm; thus, the system is of an appropriate size to achieve tumor accumulation via passive targeting through the enhanced permeability and retention (EPR) effect.

We next demonstrated BNCT activity of current system toward murine squamous carcinoma cell line *in vitro*. Here, we employed L-BPA/sorbitol complex, which is clinically available boron agent, as the control. After exposure to these boron agents for 24 h, the cells were received thermal neutron irradiation. At 24 h-post irradiation, the cell viability was quantified and HA/BCP complex exhibited higher BNCT activity than L-BPA/sorbitol complex. This should be achieved by recognition of HA through CD44 which is overexpressed in cancer cells. Moreover, confocal laser scanning microscopic observation revealed that our system showed strong fluorescence within cells after internalization. For these results, our systems are potentially applicable as a boron agent with imaging property and tumor targeting property.

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CO7-31

L-BPA conjugated tannic acid for efficient boron delivery

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INTRODUCTION: L-boronophenylalanine (L-BPA) is one of the most promising candidates as a boron delivery agent for boron neutron capture therapy (BNCT), owing to its excellent delivery to cancer cells, primarily via the L-type amino acid transporter 1 (LAT1). Despite its advantages, there are still several limitations in delivery including water solubility and duration period within cells. To address these issues, we designed and synthesized L-BPA conjugated tannic acid (TA-BPA) as boron agents for BNCT. Herein, tannic acid efficiently interacts with bioactive proteins via noncovalent interactions, including hydrogen bonding [1], thereby forming nanoassemblies capable of encapsulating proteins. Using the system, we established co-delivery system for boron and immune checkpoint inhibitor as an abscopal effect inducer.

RESULTS AND DISCUSSION: L-BPA was conjugated with tannic acid through boronic acid–cis-diol interactions under basic conditions. Conjugation was confirmed by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and mass spectroscopy (MS), revealing that tannic acid was functionalized with approximately 3-4 BPA molecules. We next conducted complexation of antibody, which is used as the model of immune checkpoint inhibitor, with TA-BPA by mixing these two components. The molecules spontaneously formulated assembly with submicron size, and their core-shell structure were revealed by small angle X-ray scattering measurement (SAXS). Their small size and core–shell structure are advantageous for tumor targeting, primarily via the enhanced permeability and retention (EPR) effect, and for conferring resistance of encapsulated antibodies to proteases. Single-particle analysis using imaging flow cytometry revealed that TA–BPA nanoassemblies encapsulate target proteins with quantitative uniformity across individual particles. Moreover, the nanosystem can release the cargo by responding to ATP molecules, which is abundant in tumor tissues, thereby current system can deliver both boron agent and immune checkpoint inhibitor to tumor tissue and these pharmaceuticals should be delivered to target cells.

We demonstrated BNCT activity toward murine lymphocyte cancer cell line which overexpress model antigen protein, ovalbumin (E.G7-OVA) *in vitro*. Herein, we employed L-BPA-sorbitol complex, which is clinically available boron agent. As a result, our system exhibited higher BNCT activity since our system enhanced cellular uptake efficiencies and prolonged duration period within cells. Finally, we demonstrated therapeutic efficacy in a metastasis mouse model. Notably, our system achieved tumor regression even in satellite tumor regions.

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CO7-32

Attempts to sensitize tumor cells by exploiting the tumor microenvironment

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INTRODUCTION: Boron neutron capture therapy (BNCT) is a radiotherapy that kills tumor cells via the $^{10}B(n, \alpha)^{7}Li$ reaction [1]. As reported previously, these high-LET particles produce highly complex DNA damages, which are processed by intracellular pathways, including DNA damage repair. Previous researches have been trying to enhance BNCT efficacy through modulation of DNA damage repair. DNA-protein crosslinks (DPCs) are induced by various chemotherapeutic agents and ionizing radiation, and considered to be toxic DNA lesions, as well as DNA double stand breaks (DSBs) since they inhibit crucial cellular processes, such as replication and transcription [2]. In this study, we established Sprtn protease-deficient cells incapable of repairing DPCs, and examined the contribution of this Sprtn protease to cell survival after BPA-BNCT.

EXPERIMENTS: CRISPR/Cas9 system was used to target the 2nd exon of Sprtn protease gene. A 5' and 3' homology arms (1kb) were amplified by PCR from the SCC VII cell genome, and cloned into the targeting vector containing a neo-resistance marker. To generate Sprtn protease-deficient SCCVII cells, the SCC VII cells were transfected with the targeting vector, and six cell clones (#1-6) were obtained. To examine the sensitivity to BPA-BNCT, these cells were exposed to neutron beams (KUR Heavy Water Facility) and then clonogenic cell survival assays were performed. Moreover, SCC VII and Sprtn protease-deficient cells (clone #1) were subcutaneously inoculated into the right hind legs of C3H mice. BPA was subcutaneously administered into nuchal sites in tumor bearing-mice, and after 60 min, mice received neutron irradiation. Tumor volume and body weight was measured every three days.

RESULTS: It has been suggested that mouse Sprtn is essential for normal cell-cycle progression. In the study, six cell clones were obtained. In three clones (#1-3), both alleles contained indels, but one of them did not cause frameshift. In three other clones obtained (#4-6), only one allele carried a frameshifting indel. These results suggest that Sprtn gene is also essential for SCC VII cells consistent with the previous studies. Colony formation assays after BPA-BNCT showed that the survival rate of clone #4-6 were similar to that of parental SCC VII cells (Fig. 1, left). On the other hand, the clone #1-3 were relatively sensitive to BPA-BNCT, although they carried a non-frameshifting indels. Considering that the region encoded by exon-2 is part of the active site of Sprtn protease, even small changes in the amino acid sequence might have an impact on its activity. We also examined the effect of Sprtn deficiency on the BPA-BNCT efficacy using the tumor bearing mice model, and showed that down-regulation of Sprtn slightly suppress tumor growth after neutron-irradiation (Fig. 1, right).

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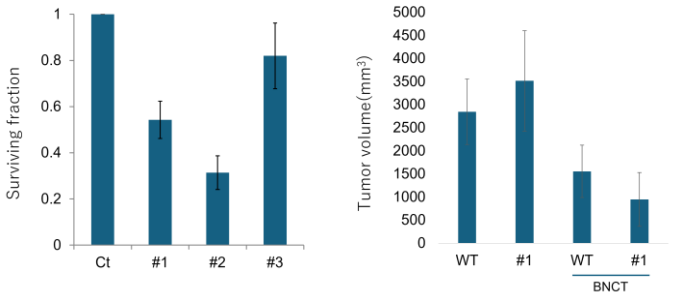

Fig. 1. Relative survival rates of SCCVII and Sprtn protease-deficient cells after BPA-BNCT (left panel). Average tumor volumes of non-treated and BPA-BNCT groups (right panel).

CO7-33

Preliminary Evaluation of the Characteristics and Effectiveness of an In Vitro Concentric Experimental System for Gadolinium Neutron Capture Therapy

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INTRODUCTION: Following our previous studies, we conducted experiments in 2025 to further investigate the cytotoxic mechanisms of gadolinium neutron capture therapy (GdNCT). Among the radiation components generated by the gadolinium neutron capture reaction (GdNCR)—gamma rays, internal conversion electrons, and Auger electrons—electron emissions are considered to have the highest cytotoxic potential. In this study, we established an experimental system designed to exclude the contribution of electron emissions and performed in vitro cellular experiments to evaluate the effects of gamma radiation alone.

MATERIALS AND METHODS: A concentric experimental system was constructed by placing a plastic disk (30 mm in diameter, 1 mm in thickness) at the center of a 10-cm culture dish (KURTR:R5174). The 1-mm-thick plastic was assumed to effectively shield electron emissions, thereby preventing electron-induced cytotoxic effects in the peripheral region while allowing gamma rays to penetrate. U87 cells were cultured in a sub-confluent state in the 10-cm dishes and exposed to neutron irradiation. Gamma-ray dose was measured using thermoluminescent dosimeters (TLDs).

RESULTS: The measured gamma-ray dose reached approximately 3 Gy, exceeding the D_0 value (～ 2 Gy). However, no significant difference in cell survival was observed between the central and peripheral regions.
These findings suggest that the combined cytotoxic effects of thermal/epithermal neutrons and gamma radiation from gadolinium were approximately uniform across the system. Accordingly, the cytotoxic effect of gamma rays and their potential contribution to damage in surrounding tissues appear to be limited. Furthermore, the experimental system effectively excluded the contribution of electron-induced cytotoxicity.

DISCUSSION: In previously reported in vivo studies, GdNCT has demonstrated substantial cytotoxic effects, which are thought to be predominantly attributable to electron emissions. Although the present experimental system did not allow definitive conclusions regarding intracellular uptake of gadolinium micelles, the results strongly suggest that electron emissions generated by GdNCR are the primary contributors to the therapeutic effect. Moreover, the contribution of gamma rays in GdNCT appears to be minimal, consistent with in vivo observations showing only mild radiation-induced damage in surrounding normal tissues. In our prior work, we reported that local thermal neutron irradiation combined with perfusion of a gadolinium-containing solution (1000 ppm) into rat femoral melanoma resulted in tumor shrinkage or complete disappearance without significant damage to adjacent tissues (Narayan, et al, 2012). This finding further supports the notion that electron emissions generated within tumor vasculature are the principal drivers of therapeutic efficacy. Although the contribution of gamma radiation in GdNCT may increase with tumor volume, it is likely limited in tumors of approximately 3 cm in diameter, as assumed in this study. In addition, when intratumoral gadolinium concentration exceeds 500 ppm, neutron self-shielding becomes significant. Therefore, the present experimental system reasonably reflects in vivo irradiation conditions.

CONCLUSIONS: This preliminary study demonstrates that the cytotoxic effect of gamma radiation in GdNCT, as well as its contribution to normal tissue damage, is limited. Our findings indicate that GdNCT is fundamentally an electron-driven therapeutic modality, in which localized energy deposition at the microscale plays a critical role. Future studies using this experimental system will focus on detailed dosimetric evaluation and quantitative analysis of cell survival to further assess the clinical feasibility and optimization of GdNCT.

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CO7-34

In vivo evaluation of a novel boronoagent for LAT1-targeted BNCT

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INTRODUCTION: Currently, the only boron-containing drug used clinically in Boron Neutron Capture Therapy (BNCT) is 4-[^{10}B]borono-L-phenylalanine (BPA), which has limitations such as poor tumor selectivity and low water solubility. The development of novel BNCT agents that overcome these drawbacks is an urgent challenge to improve therapeutic efficacy and expand the range of treatable cancer types. We have previously developed a new boron-containing compound, 5F-αMe-3BPA, through a medicinal chemistry approach involving structural modification of BPA [1,2]. This compound exhibits tumor accumulation comparable to that of BPA, while demonstrating superior tumor selectivity and improved water solubility. Because 5F-αMe-3BPA contains both fluorine and boron atoms within its molecular structure, it has the potential to serve as an ideal theranostic pair. Specifically, labeling with ^{18}F and ^{10}B enables its application to both positron emission tomography (PET) and BNCT, respectively, while maintaining the same molecular framework [3]. The objective of this study is to evaluate the in vivo therapeutic efficacy of 5F-αMe-3BPA by conducting neutron irradiation experiments in tumor-bearing mice using its ^{10}B-labeled form.

EXPERIMENTS: Tumor-bearing mice were administered the ^{10}B-labeled form of 5F-αMe-3BPA via intraperitoneal injection, followed by neutron irradiation at the Kyoto University Research Reactor (KUR). As controls, a BPA-treated group and a hot control group receiving neutron irradiation alone were included. After irradiation, the animals were returned to the animal facility, and tumor volumes were measured over time. Neutron irradiation experiments at KUR were conducted twice at a reactor power of 5 MW.

RESULTS: In the first of the two neutron irradiation experiments conducted at KUR, tumor growth was markedly slower than expected in the tumor-bearing mice, likely due to technical issues for tumor implantation, which limited the extent of the evaluation. In the second experiment, this issue was mitigated, and a sufficient number of tumor-bearing mice were successfully prepared. Following compound administration and neutron irradiation, a reduction in tumor size was observed in both the 5F-αMe-3BPA-treated group and the BPA-treated group, indicating therapeutic efficacy.

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CO7-35

A Comparative Study of Reactor- and Accelerator-based Boron Neutron Capture Therapy on U87MG Glioblastoma Xenograft Model

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INTRODUCTION: Boron neutron capture therapy (BNCT) is gaining attention as a promising non-invasive cancer treatment. Accelerator-based systems are increasingly favored for their practicality and accessibility, with the assumption that they can provide comparable therapeutic outcomes to reactor-based systems. Therefore, confirming their clinical equivalence is essential for safe and effective implementation. Malignant brain tumors are among the most promising targets for BNCT, and several clinical studies have been conducted. 4-Borono-L-phenylalanine (BPA) is the most widely used boron carrier, accumulating in tumor cells via LAT1, which is highly expressed in glioblastoma and other cancers [1]. In this study, we evaluate the therapeutic equivalence of BNCT between reactor- and accelerator-based systems, using BPA as a reference compound, with the aim of supporting the transition from reactor- to accelerator-based BNCT [2].

EXPERIMENTS: Tumor-bearing mice (female, 5-6 weeks old) were prepared by injecting subcutaneously (*s.c.*) a suspension of human glioblastoma U87MG cells. The tumor-bearing mice were injected *i.v.* with 100 μL of adjusted BPA at a 25 mg [^{10}B]/kg dose. At 3 h after injection, neutron irradiation was administered to the tumor sites in mice. BNCT efficacy was evaluated by monitoring changes in tumor volume and body weight following irradiation. For KUR reactor-based BNCT, a fluence of 4.0–4.2 × 10^{12} neutrons/cm^2 was applied over a 12 min irradiation period. In contrast, for accelerator-based BNCT (HM-20V, Aomori Prefecture Quantum Science Center), a fluence of approximately 1.2 × 10^{12} neutrons/cm^2 was applied, with irradiation conducted for 60 min at a power of 100 μA and 20 MeV.

RESULTS: Tumor growth was significantly suppressed in both reactor-based (Fig. 1A) and accelerator-based (Fig. 1C) BNCT with BPA. In addition, no significant body weight changes were observed in either group, suggesting the absence of notable adverse effects (Fig. 1B and 1D). The results demonstrated comparable BNCT efficacy with BPA between reactor- and accelerator-based systems, underscoring the clinical potential of BNCT. Notably, comparison of the "neutron only" and "no treatment" groups revealed greater tumor growth suppression in the accelerator-based experiment. This difference may be attributed to variations in gamma radiation dose associated with the extended irradiation time required for accelerator-based BNCT.

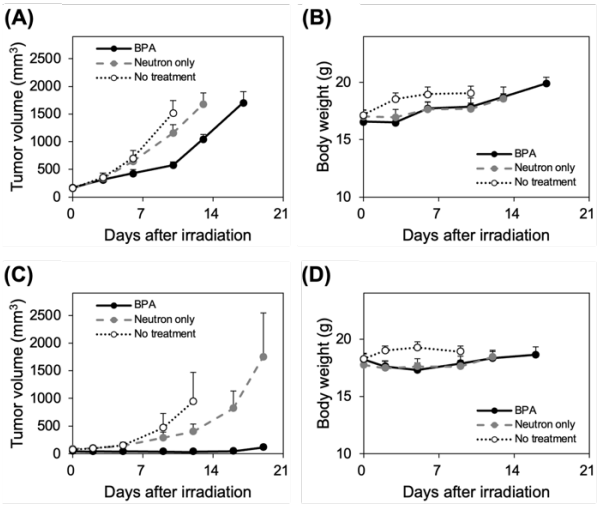

Fig. 1. Anti-tumor effects of reactor- and accelerator-based BNCT with BPA in U87MG xenograft mice model. Tumor volume and body weight changes in mice after BNCT with BPA (25 mg [^{10}B]/kg *i.v.*) at a reactor (A and B) or an accelerator (C and D). Data are expressed as mean ± SD (n = 3–5).

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CO7-36

Tumor-Selective Prodrugs Incorporating Carborane-Containing Amino Acid Payloads Targeting Cancer Amino Acid Metabolism

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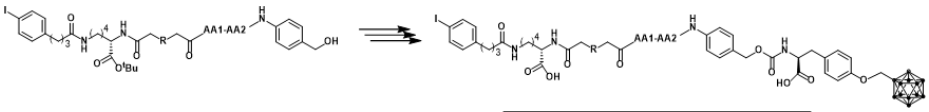
INTRODUCTION: Amino acid transporters such as LAT1 are highly expressed in cancer cells and support tumor growth by mediating the uptake of amino acids bearing bulky hydrophobic substituents. Exploiting this metabolic feature, we have developed carborane-containing amino acid analogues for tumor-selective boron delivery in BNCT. Among them, BC2 and BCY2 showed high cellular uptake and superior in vitro efficacy compared with BPA; however, their poor solubility and suboptimal physicochemical properties have limited *in vivo* evaluation. In this report, we describe the development of albumin-binding prodrug derivatives of BC2 and present their synthesis and preliminary functional evaluation toward in vivo application.

EXPERIMENTS & RESULTS: To enable *in vivo* application of BC2, we employed an albumin-binding prodrug strategy incorporating a cathepsin B–cleavable linker. In our previous study, model compounds releasing phenylalanine were synthesized, and their cathepsin B–mediated cleavage was confirmed. Based on these findings, we constructed prodrug derivatives using BC2 as the payload. The prodrugs consist of BC2 conjugated to a carrier moiety comprising an albumin-binding unit and a cathepsin B–responsive linker, enabling tumor-selective release of the boron carrier.

As shown in Scheme 1, a series of albumin-binding prodrug derivatives with varying linker structures and cleavage sequences were synthesized by conjugation of BC2 to the carrier moiety, followed by deprotection. The isolated yields of each compound are summarized in Scheme 1.



compound	Peptide AA1-AA2	R	yield
Alb-BC22	Val-Cit	O	13% (three steps)
Alb-BC23	Phe-Lys	C	17% (Three steps)
Alb-BC24	Phe-Lys	O	21% (Three steps)

Scheme1. Synthesis of Alb-BC2 Prodrugs

Alb-BC22 was dissolved in MES buffer and incubated with cathepsin B alone or in the presence of HSA (1 equiv), and the reaction was monitored over time by HPLC. As shown in Fig. 1, Alb-BC22 was efficiently degraded in the presence of cathepsin B, with nearly complete cleavage observed within 24 h, resulting in the release of BC2. In contrast, the compound remained largely stable when HSA was present, indicating that albumin binding suppresses enzymatic cleavage.

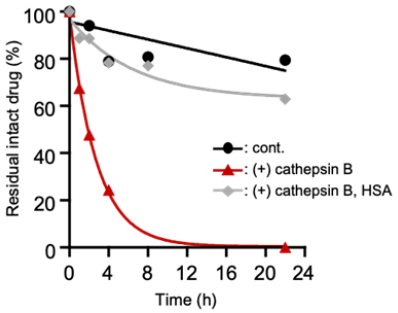


Fig. 1. Cathepsin B–Mediated Degradation of Alb-BC22 with and without HSA

A DMSO solution of Alb-BC22 was diluted 10-fold with PBS and mixed with HSA in PBS at a molar ratio of 1:20 (HAS : Alb-BC2). The mixture was incubated at 37 °C for 24 h. The mixture was concentrated using a centrifugal gel filtration cartridge to remove low-molecular-weight species, affording an 8% albumin solution. The boron content was determined by ICP analysis, providing a solution with a boron concentration of 1.13 mg B/mL, which can be used for subsequent *in vivo* studies.

These results suggest that HSA conjugation may enable *in vivo* application of BC2 by improving its solubility. Ongoing studies are focused on evaluating the biodistribution of these conjugates in tumor-bearing mouse models.

CO7-37

Development of Novel Small-molecule Boron Neutron Capture Therapy Drugs Targeting Tumor-specific Enzymatic Activity

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INTRODUCTION: In boron neutron capture therapy (BNCT), *p*-boronophenylalanine (BPA), the only currently approved BNCT agent, is selectively taken up by tumor cells through LAT1, a biomarker transporter overexpressed in tumor cells. However, BPA faces two key challenges: 1) insufficient accumulation in some cancers due to low LAT1 expression, and 2) gradual leakage from cells over time. Therefore, new BNCT drugs targeting alternative cancer biomarkers with a mechanism for prolonged intracellular retention are needed to expand BNCT indications and improve therapeutic efficacy. In this project, we focused on enzyme activity, which is higher in cancer cells than in normal cells. By last year, we had developed EP-4OCB-FMA, a carborane-containing drug candidate targeting DPP-4, which is upregulated in esophageal cancer, and conducted BNCT experiments in tumor-bearing mice via intratumoral or intravenous injection. This drug is designed for prolonged intracellular retention by generating an aza-quinone methide species upon hydrolysis by DPP-4, which forms a covalent bond with intracellular nucleophiles such as cysteine-containing proteins and glutathione. While this probe performed well with intratumoral injection, systemic administration resulted in insufficient tumor accumulation, presumably due to its short blood half-life due to relatively high enzymatic activity in normal tissues. Therefore, this year we initiated the development of new probes targeting glycosidase, which exhibits enough high activity in cancer cells but low activity in normal cells, expected to offer improved longer blood half-life.

EXPERIMENTS: *β*-Gal-4OCB-FMP and *β*-Gal-4OCBOMe-FMP were designed and synthesized based on quinone methide-generating chemistry toward *β*-D-galactosidase (*β*-Gal), which is overexpressed in certain types of cancer cells. These agents are hydrolyzed by *β*-Gal to generate a highly reactive quinone methide intermediate, which rapidly reacts with intracellular nucleophiles such as glutathione, thereby the carborane boron cluster is selectively trapped inside the cancer cells. First, each probe was incubated with H460 and SKOV-3 cells, and boron concentration was evaluated. For the BNCT experiment, with using tumor-bearing mouse models, *β*-Gal-4OCB-FMP was administered to H460 tumor-bearing mice, and *β*-Gal-4OCBOMe-FMP was administered to SKOV-3 tumor-bearing mice. Neutron irradiation was performed at 5 mW for 15 minutes, 4 hours after intratumoral (i.t.) injection of each probe. Antitumor efficacy was quantified by measuring tumor size following irradiation.

RESULTS: Boron concentration in treated cancer cells were confirmed to be sufficiently high for BNCT. Based on this finding, BNCT experiments were conducted via intratumoral injection. The results demonstrated that tumor growth was suppressed in a drug- and neutron-irradiation-dependent manner, as shown in Fig. 1. We are therefore currently planning to develop optimized agents with longer blood half-life, and conduct further BNCT experiments via intravenous injection.

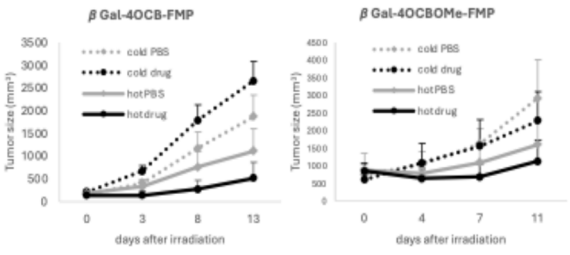

Fig. 1. Antitumor effects of *β*-Gal-targeted BNCT probes in tumor-bearing mice. Tumor volume was calculated as (longest diameter / 2) × (shortest diameter)². Data are mean ± SD (n = 3).

CO7-38

A Study on Improving the Retention of Intratumoral Boron Concentrations Using a Boron-Containing BPA-Embedded Nanofiber Mesh

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INTRODUCTION: Boron neutron capture therapy (BNCT) is a binary cancer treatment modality based on selective accumulation of boron-10 (^{10}B) in tumors followed by neutron irradiation. The clinically used boron agent, p-boronophenylalanine (BPA), shows favorable tumor selectivity; however, its rapid systemic clearance requires prolonged intravenous infusion, typically over several hours. This limitation complicates clinical workflows and may reduce treatment efficiency. To address this issue, we developed a local drug delivery system using polycaprolactone (PCL)-based nanofiber mesh (NFM) incorporating BPA (BPCL-NFM). PCL is a biodegradable and biocompatible polymer widely used for controlled drug release. This system enables sustained boron release at the tumor site, enhancing tumor retention of ^{10}B and improving BNCT efficacy while reducing systemic exposure.

EXPERIMENTS: Murine colon carcinoma CT26 cells were cultured in E-MEM supplemented with 10% fetal bovine serum. Neutron irradiation was performed at the Kyoto University Research Reactor (KUR) at 1 MW (5–20 min) for cells and 5 MW (12 min) for animals. BPCL-NFM was fabricated in collaboration with the National Institute for Materials Science and contained BPA up to 3 mg/mL. Morphology and drug release profiles were evaluated. BPA-fructose solution (BPA-f) was prepared as a control. In vitro, intracellular ^10B accumulation was measured by ICP-AES after treatment with BPA-f (10–40 ppm, 1 h) or BPCL-NFM (up to 48 h), and cell survival was assessed by clonogenic assay. In vivo, CT26 tumor-bearing BALB/c mice received BPA-f or BPCL-NFM placed on tumors. Tumor boron concentration was measured over time (3–48 h), and BNCT efficacy was evaluated by tumor growth and body weight.

RESULTS: In vitro, BPCL-NFM showed time-dependent intracellular ^{10}B accumulation, peaking at 24 h and maintained up to 48 h. The boron level was comparable to that achieved with 25 ppm BPA-f after 1 h exposure, and BNCT-induced cytotoxicity was similar between the two conditions. In vivo, BPA-f showed rapid accumulation with a peak at 3 h, declining to baseline at 24 h. In contrast, BPCL-NFM exhibited gradual accumulation with sustained levels from 15 to 48 h, reflecting controlled release. Therapeutically, BPCL-NFM with neutron irradiation significantly suppressed tumor growth compared with controls. The effect was greater than BPA-f at 125 mg/kg, comparable to 250 mg/kg, but lower than 500 mg/kg, suggesting sustained boron retention contributes to BNCT efficacy.

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CO7-39

Early Cellular Responses to BNCT and Potential Biomarker Identification

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INTRODUCTION:
We previously reported the involvement of HMGB1 and early proteomic alterations following BNCT [1-2]. However, the detailed mechanisms underlying early cellular responses remain unclear. In this study, we aimed to further characterize these responses and identify potential biomarkers to improve BNCT efficacy.

EXPERIMENTS:
Human cancer cell lines, including squamous cell carcinoma lines SAS, HSC3, cervical cancer HeLa cells, and mouse L1210 cells were incubated with BPA for 2 hours in suspension. Cell survival was evaluated by colony formation assay. Culture supernatants and cell lysates were collected at 6 and 24 hours post-irradiation for RNA and protein analyses. For in vivo studies, B16F10 and B16F10mGM melanoma cells were implanted into the hind limbs of C57BL/6 mice. In some groups, macrophages were depleted to assess their contribution. Only the left hind limb tumors were locally irradiated for 60 minutes using a ^{6}LiF-containing shield. BPA was administered at 500 mg/kg body weight 30 minutes prior to irradiation. Mice were sacrificed 7 or 14 days after treatment, and blood and tissue samples were collected for analysis.

RESULTS:
The measurement results of thermal neutron fluence and doses for cells (Table 1) and mice (Tables 2 & 3) were indicated. Thermal neutron fluence and radiation doses were successfully measured in both vitro and vivo experiments. BNCT induced significant early cellular responses in the cancer cells. Notably, modulation of *SNHG12* expression altered these responses, suggesting its involvement in BNCT-induced molecular pathways. *SNHG12* may play a regulatory role in these processes. Preliminary analyses also indicated dynamic changes in gene and protein expression following treatment. In vivo responses, including tumor progression and immune-related changes, are currently under investigation.
Further studies will focus on immune mechanisms and in vivo validation will contribute to a better understanding of BNCT and support the identification of biomarkers for improved therapeutic outcomes.

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Table 1. Irradiated doses of cells on Nov. 19, 2025 (Rail, irradiation room).

Irradiation time[min]	Position	fluence [/cm2]		[Gy]					
		Thermal neutron [/cm2]	Epi-thermal neutron	Thermal neutron [Gy]	Epi-thermal neutron [Gy]	Fast neutron [Gy]	Gamma-ray [Gy]	Physical Dose [Gy]	B-10** (1ppm)
60	center	3.9E+12	6.9E+11	5.2E-01	5.5E-02	3.8E-01	3.2E-01	1.3E+00	2.9E-01
	Rear side of the shielding panel 1	6.1E+11	1.1E+11	8.1E-02	8.7E-03	6.0E-02	3.3E-01	4.8E-01	4.5E-02
	Rear side of the shielding panel 2	7.1E+11	1.3E+11	9.4E-02	1.0E-02	7.0E-02	3.3E-01	5.0E-01	5.3E-02
60	center	4.0E+12	7.1E+11	5.3E-01	5.7E-02	4.0E-01	1.8E-01	1.2E+00	3.0E-01
	Rear side of the shielding panel 1	6.6E+11	1.2E+11	8.8E-02	9.4E-03	6.5E-02	2.2E-01	3.8E-01	4.9E-02
	Rear side of the shielding panel 2	9.0E+11	1.6E+11	1.2E-01	1.3E-02	8.9E-02	2.2E-01	4.4E-01	6.7E-02

Table 2. Irradiated doses for local irradiation of mice on Dec.3, 2025 (Cart, irradiation room).

Irradiation time[min]	Position	fluence [/cm2]		[Gy]					
		Thermal neutron [/cm2]	Epi-thermal neutron	Thermal neutron [Gy]	Epi-thermal neutron [Gy]	Fast neutron [Gy]	Gamma-ray [Gy]	Physical Dose [Gy]	B-10** (1ppm)
10	Center	1.1E+12	1.9E+11	1.4E-01	1.5E-02	1.1E-01	6.2E-02	3.2E-01	7.9E-02
60	Center	6.5E+12	1.2E+12	8.7E-01	9.3E-02	6.4E-01	3.6E-01	2.0E+00	4.8E-01
2	Center	2.1E+11	3.8E+10	2.9E-02	3.0E-03	2.1E-02	6.0E-03	5.9E-02	1.6E-02
4	Center	4.6E+11	8.2E+10	6.1E-02	6.5E-03	4.5E-02	1.6E-02	1.3E-01	3.4E-02
6	Center	6.8E+11	1.2E+11	9.1E-02	9.7E-03	6.7E-02	5.9E-02	2.3E-01	5.1E-02
8	Center	9.1E+11	1.6E+11	1.2E-01	1.3E-02	9.0E-02	5.8E-02	2.8E-01	6.7E-02

Table 3. Irradiated doses for local irradiation of mice on January 20, 2026 (Cart, irradiation room).

!""#$ia&BEF &BG-IGBEF	. D/ itBEF	012+EP+F I/cGR-F		IGy-F					
		89+"G#I ne2&'DEF I/cGR-F	:; B&9+"G#I ne2&'DEF	89+"G#I ne2&'DEF IGy-F	:; B: &9+"G#I ne2&'DEF IGy-F	=#/ &F ne2&'DEF IGy-F	6# GG#< "#TF IGy-F	. 9T/ B#1F [D / +FIGy-F	?<10*BF (1; ; GaF
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CO7-40

Neutron Capture Therapy for Cancer Using Gd-Thiacalixarene Complex-Loaded Nanogel Micelles

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INTRODUCTION: We have been studying the application of multifunctional metal complexes, originally derived from analytical reagent research, for biomedicine. Specifically, focusing on the kinetic stability, MRI contrast capability, and the ^{157}Gd neutron capture ability of the thiacalixarene complex Gd_3TCAS_2 (Fig. 1a), we have previously developed Gd_3TCAS_2-loaded nanoparticles for MRI-guided neutron capture therapy (NCT). However, for optimal biological applications, nano-medicines with a higher loading capacity (LC) and a smaller particle size are desired. Therefore, in this report, we created nanogel micelles (NM) composed of Gd_3TCAS_2 and the block copolymer poly(ethylene glycol)-*b*-poly(L-lysine) (PEG-*b*-PLL) and evaluated their *in vivo* NCT effects. **EXPERIMENTS: Preparation of NMs.** Ln-NM was prepared by mixing an Ln_3TCAS_2 (Ln: Gd or Tb) solution and a PEG-*b*-PLL solution under high-speed stirring. Subsequently, the excess ami-no groups of the PLL chains were crosslinked using glutaraldehyde. **RESULTS:** Dynamic light scattering (DLS) measurements revealed the particle size of the NM to be 44.9 ± 0.52 nm, which is expected to facilitate passive transport to tumors and provide high blood retention. The ζ potential was –3.9 ± 3.2 mV, suggesting that the particle surface is effectively coated with PEG chains. Furthermore, the NM achieved a high LC of 24.2 ± 4.1 wt%.
When mouse colon cancer Colon-26 cells were cultured in the presence of Ln-NM for 24 hours, cells treated with Tb-NM exhibited Tb-derived green luminescence, confirming successful intracellular uptake. For the Gd-NM, the intracellular Gd uptake was measured at 1.18 ± 0.21 nmol/10^6 cells. Toxicity tests indicated that cell viability began to decrease at a concentration of [Gd] = 100 μM. When Colon-26 cells loaded with Gd-NM were irradiated with neutrons, their cell viability significantly decreased compared to the control group, successfully demonstrating an *in vitro* NCT effect.
In vivo studies using a Colon-26 tumor-bearing mouse model with tail vein injection of Gd-NM confirmed the accumulation of Gd in the tumor; the ^{157}Gd concentration in the tumor reached 4.8 ppm 24 hours post-administration. Upon neutron irradiation, the group treated with Gd-NM showed the most suppressed increase in relative tumor volume compared to the control group, the Gd-NM administered without neutron irradiation group, and the PBS with neutron irradiation group, suggesting a strong anti-tumor effect (Fig. 1b). Additionally, no body weight loss was observed in the mice, confirming the biosafety of the nanogel micelles.

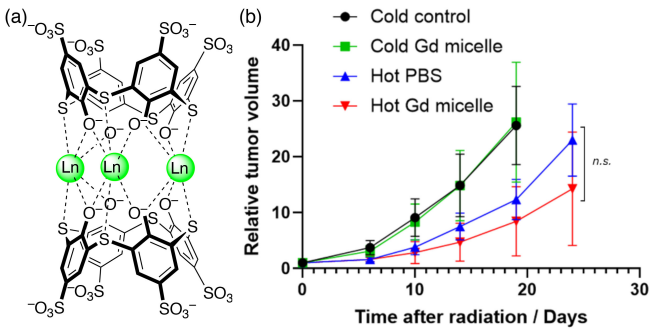

Fig. 1 (a) Ln_3TCAS_2, and (b) changes in relative tumor volume in Colon-26 tumor-bearing mice after neutron irradiation (n = 6 for each group).

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CO7-41

Drug Discovery of Novel Boron Agents for Tumor Marker-Targeted Precision BNCT

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INTRODUCTION: Drug development is essential for the future advancement of boron neutron capture therapy (BNCT). However, since the regulatory approval of boronophenylalanine (BPA) in 2020, no clinical trials of novel boron agents or studies investigating new combinational strategies for broader indications have been conducted. Although BPA is an excellent boron agent and is preferentially taken up by tumors with high amino acid metabolism, intratumoral heterogeneity is widely recognized across malignant tumors. Therefore, reliance on a single boron agent may limit the future development and broader applicability of BNCT.
The potential value of multi-agent strategies has already been suggested in clinical research on malignant glioma, in which the combination of BPA and borocaptate sodium (BSH), two boron compounds with distinct physicochemical properties, uptake mechanisms, and tumor accumulation profiles, demonstrated greater efficacy than single-agent treatment [1]. In addition, boron agents lacking intrinsic intracellular uptake capability have been reported to achieve improved tumor selectivity and cellular internalization through drug delivery system (DDS)-based approaches, resulting in enhanced therapeutic efficacy. These findings indicate that developing novel boron agents and rational combinational strategies remains both a major challenge and an important research priority in BNCT.Although it is difficult to identify a universal characteristic shared by all malignant tumors, boron agents can be designed according to the biological features of individual cancers and translated from drug discovery into therapeutic application. In particular, tumor-specific molecular features, especially tumor-associated genes characterizing each cancer type, may provide a promising basis for boron agent design. From this perspective, precision BNCT, which integrates gene-guided boron drug discovery with BNCT, holds substantial promise. LAT1, the molecular target responsible for BPA uptake, represents one successful example of this concept. Expanding this strategy to additional tumor markers and molecular targets may further broaden the clinical potential of BNCT.
EXPERIMENTS: Because it is difficult to identify a universal property shared by all malignant tumors, we sought to design boron agents tailored to the biological characteristics of individual cancer types and to advance them from drug discovery to therapeutic application. In this context, tumor-associated genes that characterize each malignancy represent particularly promising targets. The strategy of combining gene-guided boron agent development with BNCT, namely precision BNCT, has substantial future potential [2]. Based on this concept, we conducted a pan-cancer bio-informatics analysis to identify alternative uptake-target genes highly expressed in malignant tu-mors with low LAT1 expression, since LAT1 is the principal molecular target mediating BPA up-take. Furthermore, we performed drug discovery research focusing on tumor markers secreted by cancer cells, with particular attention to their constituent structures as candidate targets for novel boron agent development.
RESULTS: By focusing on fucose, a structural component of the tumor marker CA19-9, we developed a novel fucose-conjugated boron agent. Uptake analyses in CA19-9-high malignant tumors demonstrated tumor selectivity, and antitumor effects following neutron irradiation were confirmed in both in vitro and in vivo experiments [3].
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CO7-42

Basic Study of Overcoming Immunotherapy Resistance by Boron Neutron Immunotherapy

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INTRODUCTION: Immunotherapy with immune checkpoint inhibitors (ICIs) has become a major component of treatment for advanced cancer. As a therapeutic strategy distinct from conventional cytotoxic chemotherapy, it has demonstrated substantial clinical importance. However, its objective response rate remains limited, generally ranging from only a few percent to approximately 30%, and overcoming low response rates and resistance to immunotherapy is considered a critical chal-lenge for future cancer treatment.
For patients who do not respond to single-agent or dual-agent ICI therapy, combination treatment with radiotherapy has been explored as one potential approach to reactivate antitumor immunity. It has long been recognized that radiotherapy can induce an immune-mediated phenomenon known as the abscopal effect [1]. However, this effect has been extremely rare, and its efficacy has not been demonstrated in prospective clinical studies, with evidence largely limited to case reports.
In the United States, the approval of ipilimumab (Yervoy, anti-CTLA-4) in 2011 marked the begin-ning of ICI-based immunotherapy, followed by the initiation of clinical trials across various malig-nancies. In this context, a 2012 report described a patient with advanced melanoma who was re-fractory to immunotherapy and subsequently achieved regression of non-irradiated lesions after the addition of local radiotherapy. This observation raised expectations that the long-recognized but rare abscopal effect of radiotherapy could be more effectively realized when combined with ICI-based immunotherapy [2].

EXPERIMENTS: We used the B16-F10/C57BL/6 model, an advanced melanoma model resistant to both radiotherapy and ICI-based immunotherapy. The antitumor effects at both the neu-tron-irradiated site and a shielded distant site were evaluated in the following groups: neutron irra-diation alone (control group), immunotherapy alone (anti-PD-1 treatment group), BPA-BNCT group, and the combination group of BNCT plus immunotherapy (boron neutron immunotherapy group).
As a result, significant therapeutic effects at the irradiated site were observed in both the BPA-BNCT group and the BNCT plus immunotherapy group. Furthermore, marked tumor suppres-sion at the distant site was observed only in the combination group. This therapeutic effect at the distant site was confirmed to represent an abscopal effect [3].

RESULTS: Using a model resistant to both radiotherapy and immunotherapy, we are conducting mechanistic studies of the antitumor effects of boron neutron immunotherapy, with a particular fo-cus on immune cell dynamics within the tumor microenvironment. We are planning to further de-velop these findings toward future clinical studies.

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CO7-43

Gamma-Ray Generation from Gadolinium-Containing Inorganic Phosphor Microparticles under Thermal Neutron Irradiation

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INTRODUCTION: Gadolinium-containing rare-earth oxide phosphors with a monodisperse particle size below 1 μm and hollow structures were synthesized through a simple process, and gamma-ray generation from gadolinium under thermal neutron irradiation was investigated. For catalyst- and phosphor-derived materials intended for dosimetric applications, synthesis methods that provide small, uniform particle sizes through a straightforward reaction process are highly desirable. In this study, aiming toward the realization of gadolinium neutron capture therapy (GdNCT) materials, we measured differences in gamma-ray dose as a function of gadolinium doping ratio in spray pyrolysis-synthesized $(Y, Gd)_2O_3:Eu^{3+}$ phosphor microparticles.

EXPERIMENTS: Yttrium nitrate *n*-hydrate, europium (III) acetate pentahydrate, and gadolinium (III) nitrate pentahydrate were used as starting materials and dissolved in distilled water at prescribed concentrations. The prepared aqueous solution was atomized ultrasonically, and the generated droplets were introduced with a carrier gas (air) into a reaction tube placed in an electric furnace maintained at 800°C. Microparticles were obtained by calcination in the high-temperature gas stream. For neutron irradiation, KUR-HWNIF was used as the neutron irradiation field. The gold foil activation method and TLDs (UD-170LS, Panasonic Co.) were used to estimate the gamma-ray doses.

RESULTS: As shown in Fig. 1, the gamma-ray dose increased linearly with increasing gadolinium concentration. On the other hand, commercially available gadolinium reagents generally contain isotopic compositions in which the total fraction of ^{156}Gd, ^{158}Gd, and ^{160}Gd exceeds 65%, whereas ^{157}Gd accounts for only approximately 15%. The present results indicate that, in order to obtain an effective gamma-ray dose, maximizing the neutron capture cross section is essential. Specifically, it is important to synthesize the target material using precursors enriched in ^{157}Gd during the synthesis stage, and furthermore to adopt strategies for highly localized accumulation of the material in the vicinity of malignant tissues.

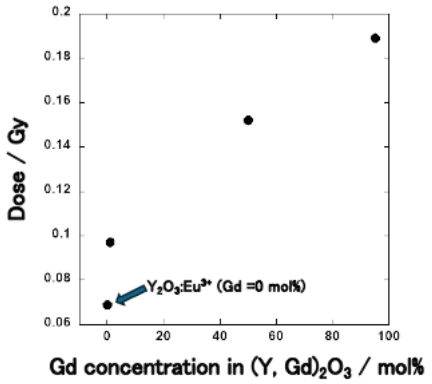

Fig. 1. Dependence of γ-ray dose on gadolinium concentration in $(Y,Gd)_2O_3:Eu^{3+}$ (Gd = 1, 50, 95 mol%) and $Y_2O_3:Eu^{3+}$ (Gd = 0 mol%).

CO7-44

Anti-tumor effect of boron containing nano particle for chest BNCT on mouse thoracic tumor model

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INTRODUCTION: Boronophenylalanine (BPA) has been applied to boron-neutron capture therapy (BNCT) for late stage of head and neck squamous cell carcinoma. Although BNCT with BPA is also promising for other types of cancer, the pharmacokinetics need to be improved. Here, we have developed novel 10B containing nano particle, RN-501 [1]. The RN-501 was able to abundantly accumulate into a tumor compared with BPA even by just a single injection, whereas the concentration in normal tissue including lung and blood was lower. It means that RN-501 can be eased a patient's burden at BNCT. In this study, we assessed tumor growth inhibition of BNCT with RN-501 using mouse thoracic tumor model to prove the efficacy of thoracic cancer treatment.

EXPERIMENTS: Mouse colon cancer cell line CT26-Luc, which is luciferase expressing CT26 cells were intra-thoracically injected into BALB/c mice. Then several concentrations of RN-501 or 500 mg/kg of BPA were respectively injected into eight weeks old BALB/c mice intravenously or subcutaneously. The mice were irradiated with $5x10^{12}$ n/cm^2 of thermal neutron that was generated from KUR with 5MW output energy for 12 min. Saline, BPA, RN501, and thermal neutron only were respectively used for control. The activity of intra-thoracic tumors was measured for 14 days after the irradiation with In Vivo Imaging System (IVIS). The mouse survival for each treatment was also simultaneously measured.

RESULTS: Now, the results are analyzing and discussed for further study.

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CO7-45

The Efficacy of Boron Neutron Capture Therapy for Local Recurrence and Lymph Node Metastasis in Breast Cancer

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INTRODUCTION: We have previously conducted extensive fundamental research verifying the efficacy of Boron Neutron Capture Therapy (BNCT) across various intractable cancer models [1, 2]. While BNCT offers high tumor selectivity, the development of novel boron delivery agents remains a crucial breakthrough required to overcome the limitations of current clinical compounds. This pharmacological evolution is essential for achieving the precision necessary to treat aggressive or recurrent malignancies [3]. Building upon these findings, the present study evaluates BNCT for refractory breast cancer—the leading cause of cancer-related death among women globally. For elderly patients or those with impaired ADL, invasive interventions are often challenging. Furthermore, local recurrence and supraclavicular lymph node metastasis present critical hurdles, as prior radiotherapy often precludes safe re-irradiation, and vascular invasion can render tumors surgically unresectable. BNCT, which spares adjacent normal tissues like the airway and esophagus, represents a promising alternative for these pre-treated, refractory cases.

EXPERIMENTS: In the in vitro phase, we utilized MCF-7 and paclitaxel-resistant (MCF-7/PTXR) human breast cancer cell lines. Cells were treated with BPA (10 μg B/mL) for 24 hours and subjected to neutron irradiation at 1 MW (0–30 min). Cell viability was quantified via a colony formation assay. The in vivo phase involved female BALB/c nude mice bearing subcutaneous MCF-7 tumors. Mice were randomly assigned into six groups (n = 4 each): (1) untreated control, (2) neutron irradiation alone, and four BNCT groups receiving different boron agents via tail vein: (3) standard BPA, (4) hyaluronic acid-labeled BPA (HA-BPA), (5) PBC-IP, and (6) high-concentration ionic liquid. Neutron irradiation (5 MW, 20 min) was performed 3 hours post-administration, with relative tumor volume monitored for 3 months.

RESULTS: The colony formation assay revealed that BNCT significantly reduced survival fractions in both MCF-7 and drug-resistant MCF-7/PTXR lines in a dose-dependent manner. The comparable sensitivity of the PTXR line indicates that BNCT effectively bypasses conventional chemoresistance mechanisms. In vivo, all BNCT groups exhibited marked tumor growth inhibition. Specifically, HA-BPA demonstrated superior efficacy in reducing tumor volume relative to standard BPA, likely due to enhanced accumulation mediated by the tumor microenvironment. These preliminary results highlight BNCT, supported by novel delivery agents, as a potent, minimally invasive intervention for refractory breast cancer, warranting continued investigation.

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