Concept of malignant brain tumors for drug design of boron neutron capture therapy (BNCT)

-Uncertainty of malignancy for drug delivery-

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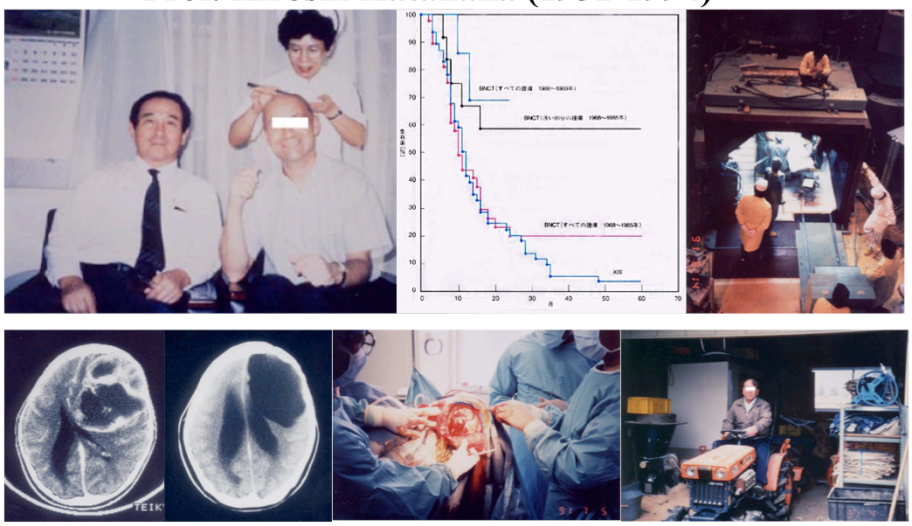
Hello, everyone.

This is somewhat educational presentation like a lunch-on seminar, please be refluxing.

I'm a Japanese Neurosurgeon and a visiting researcher of Prof. Narayan, NIU. But, I'm sorry, I'm jot good at Chemistry.
We have investigated neutron capture therapy of malignant brain tumor for many years collaborating with many US chemists.

History of Japanese BNCT

Prof. Hiroshi Hatanaka (1932-1994)



At first, I would like to show you Brief history of Japanese BNCT. Professor Hiroshi Hatanaka, one of clinical staffs of Professor Keiji Sano of the first Professor of Tokyo University, a pioneer of BNCT in Japan who studied BNCT at Professor Sweet, Neurosurgeon, of Harvard University. I joined to BNCT research as a resident physician at Tokyo University 34 years ago. And we reported a relatively good outcome of BNCT for malignant brain tumors, I'll show you later.

Thermal Neutron based BNCT



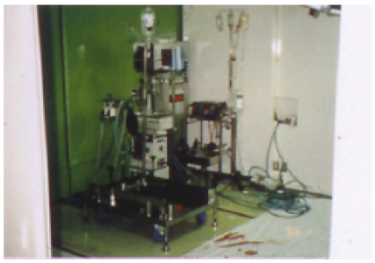
Surgical preparation for BNCT



Patient setting

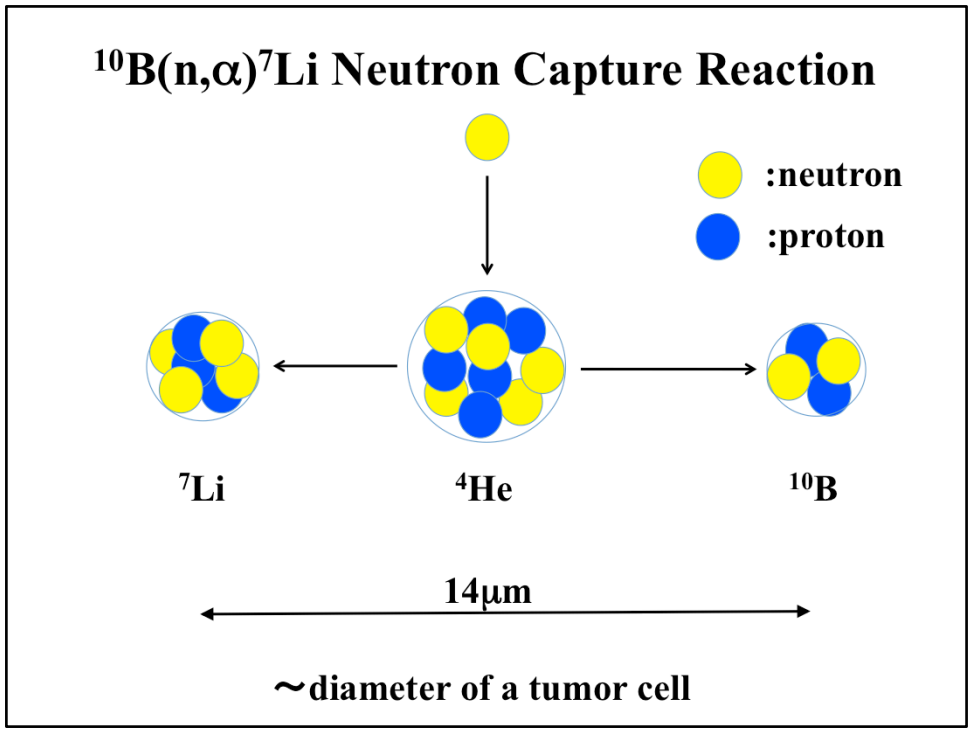


Patient positioning to BNCT port



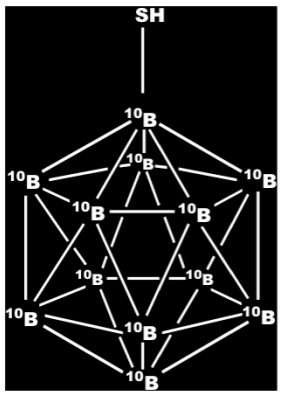
Patient monitor system during BNCT

Then, at Research Reactor Institute of my Kyoto University, we started clinical BNCT for malignant brain tumor since 1990.



Next slide shows the principle of BNCT.
After selectively incorporating boron atoms into the tumor and then irradiating with thermal neutrons, boron is easily split by the neutron capture reaction with a large thermal neutron cross section of 3800barn, and α ray and recoiled lithium atom releases a large total kinetic energy of 3.3 MeV in the range of 14 microns almost equal to the diameter of cancer cells and finally destroy tumor cell super selectively.

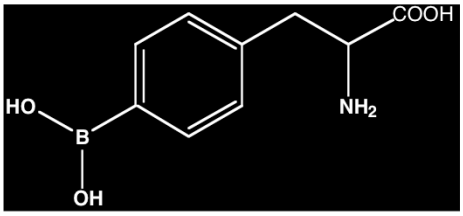
Boron Carrier for Current BNCT



BSH

borocaptate

(disodium mercaptoundecahydrododecaborate)

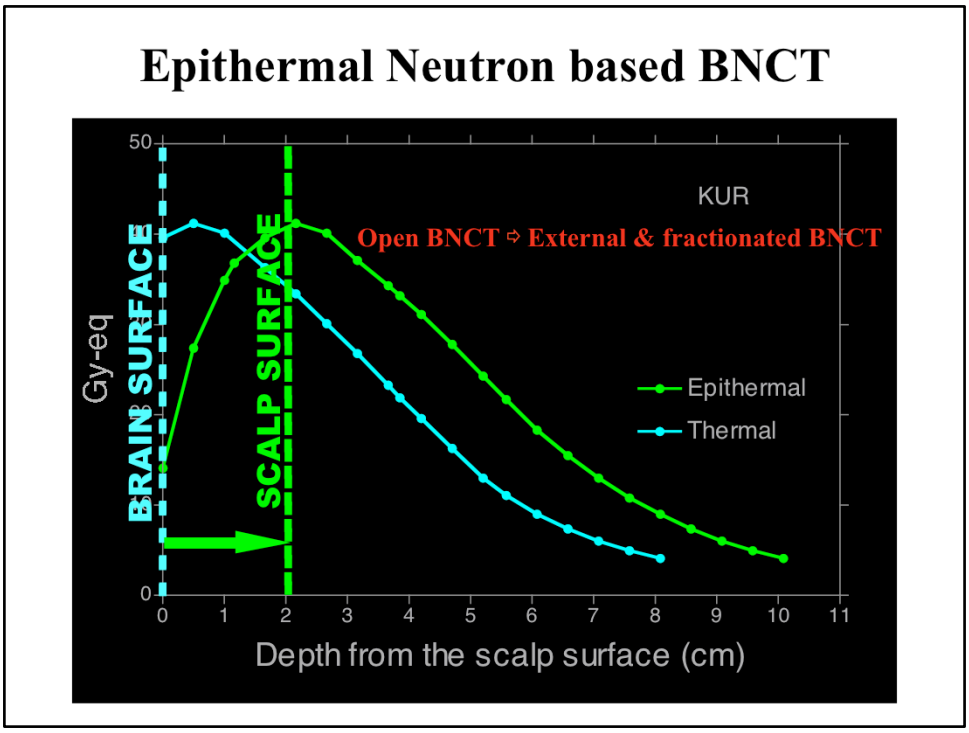


BPA

p-boronphenylalanine

Two boron carriers are available for clinical use. BSH and BPA. Borocaptate (BSH) contains ten boron atoms in a carborane cage, SH group acts as a ligand, but passively uptaked into tumor cells. Another type, which a boron atom binds to phenylalanine and is taken up into cancer cells by utilizing amino acid metabolism.

Now, in Japan, accelerator-based BNCT is ongoing at several hospitals. We welcome your commercial contribution for new boron compounds in Japan.



As a neutron sources, we usually use nuclear reactor or accelerator-based epi-thermal neutrons.
Epi-thermal neutrons are thermalized by losing energy in the scalp and skull in total thickness of about 2cm, so external irradiation on the scalp is possible for deeply seated tumor within 6cm depth from the brain surface.

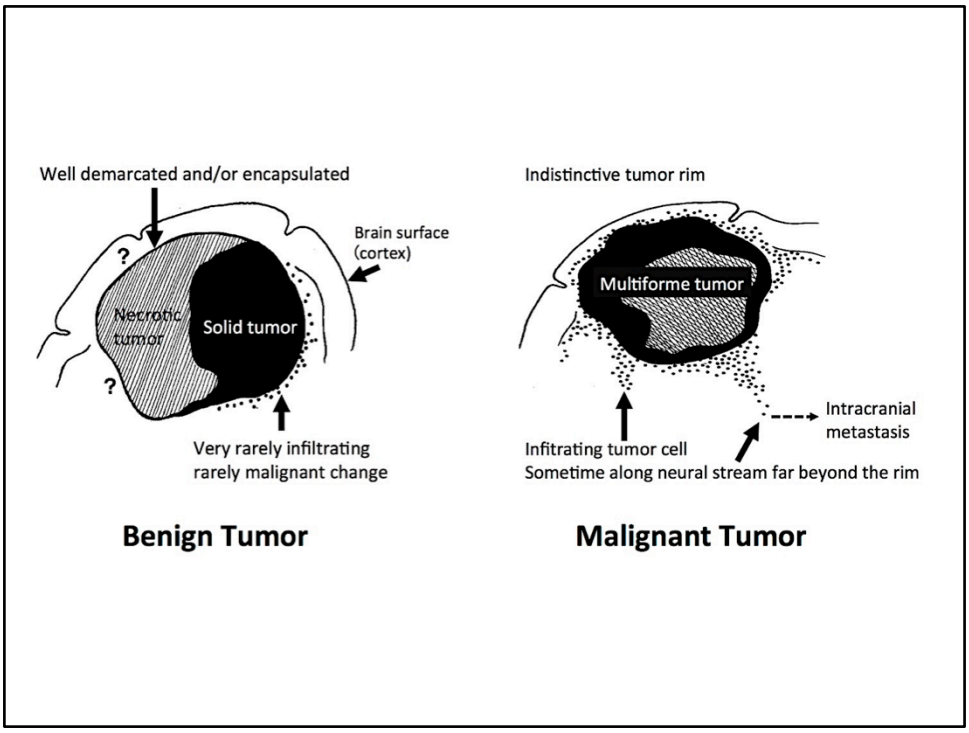
Thermal neutrons repeat elastic scattering with light atoms such as hydrogen and carbon atoms in the brain, and rapidly lose their energy. So that BNCT is more effective with a depth within about 6 cm from the brain surface, but it is not enough for more deeply seated tumors.

Indication in Brain Tumors

Benign Glioma:
Grade I and II: Op, sometime + Chemo +RxTx

Malignant Glioma:
Grade III and IV: Op+Chmeo+BNCT

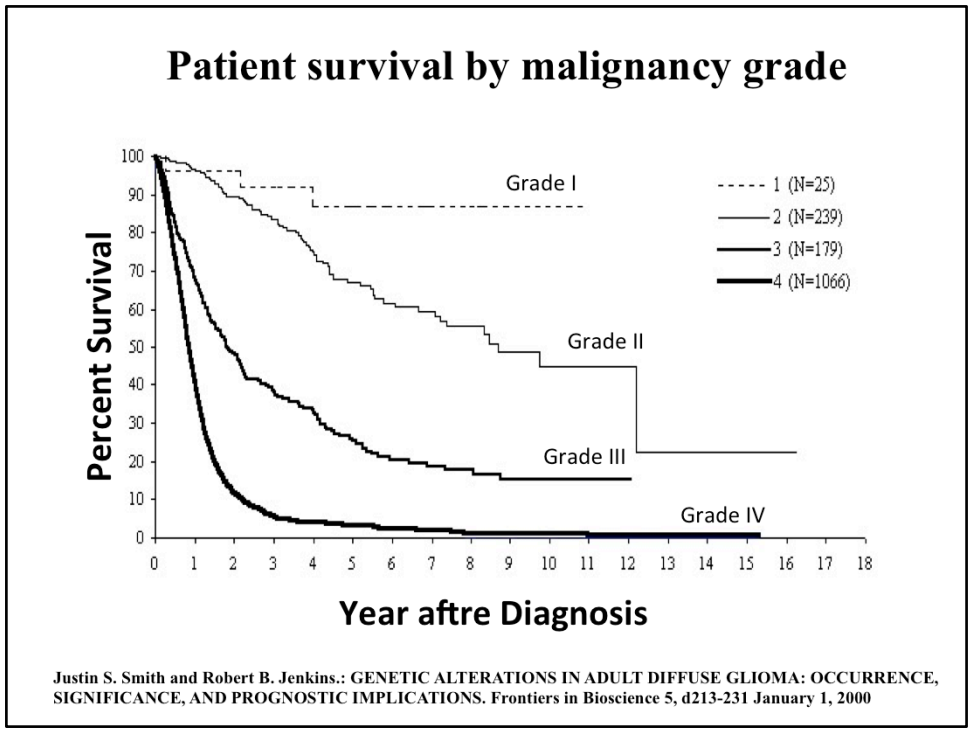
In my Neurosurgical setting, BNCT is indicated to only malignant brain tumors.
Brain tumors are classified into 4 grades.
Grade I and II are benign and can be treated by tumor extirpation, and those are out of scope for BNCT.
Grade III and IV are malignant, and they are hardly cured. And they are indicated for BNCT.



This slide shows the pathological difference between benign and malignant.

Grade I and II gliomas show expanding growth without tumor cell infiltration into surrounding normal brain parenchyma. It can be cured by tumor extirpation.

But, grade III and IV gliomas show infiltrating growth and it is almost impossible to extirpate infiltrating tumor cells by discriminating them with normal neurons.



This is a Kaplan-Meier plot of brain tumor patients survivals. Survival rate of grade IV glioma is “misery”, more than 90% patient died within 5 years.

Patient survival rapidly decrease according to the malignancy. Grade IV glioma is a formidable disease, more than 90& patients die with 5 years after diagnosis. But we scientists has been challenging to treat them.

Today's Topics

Concept of malignancy of brain tumors for drug design of boron neutron capture therapy (BNCT)

-Uncertainty of malignancy for drug delivery-

Is it possible to target selectively malignant brain tumors?

Difficulty of drug delivery to malignant brain tumors for,

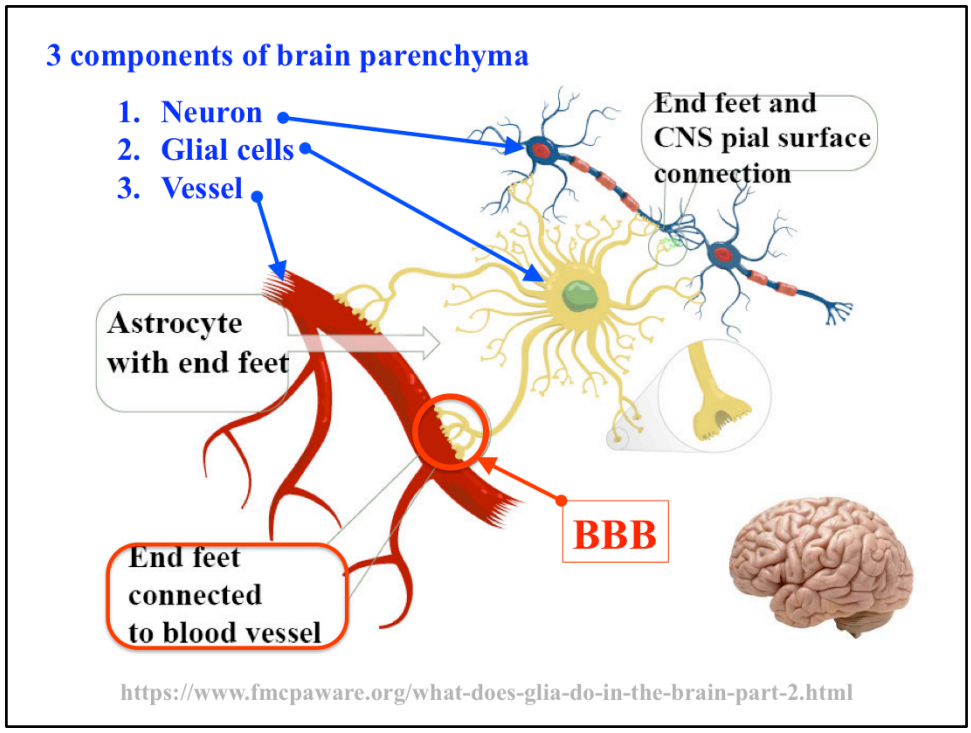
1. **Blood Brain Barrier system**
2. **Resting cells in tumor cells division**

So, this is Today's topics of my talk.

Is it pssible to target selectively malignant brain tumor?

I focus on the difficulty of drug delivery to malignant brain tomor, for (1) blood brain barrier system and (2) resting cells in tumor cell division.

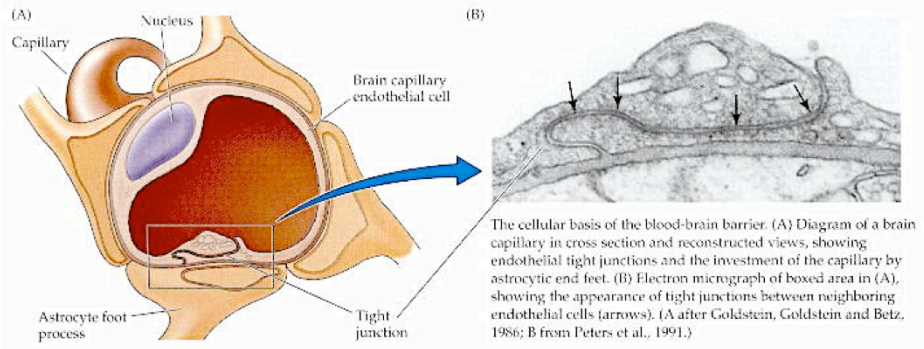
Interdisciplinary mutual understanding is an important mission for the progress of science. My today's talk focus on understanding of difficulty of drug delivery to malignant brain tumors.



For deep understanding of my talk, I briefly talk about brain anatomy.
This slid shows one of a main concept of normal blood brain barrier (BBB) for drug delivery. Brain parenchyma is constructed from only 3 components, neurons, glia and vessels.

Malignant brain tumor usually occur from the glial cell that is a sustaining cell between neuron and vessels.

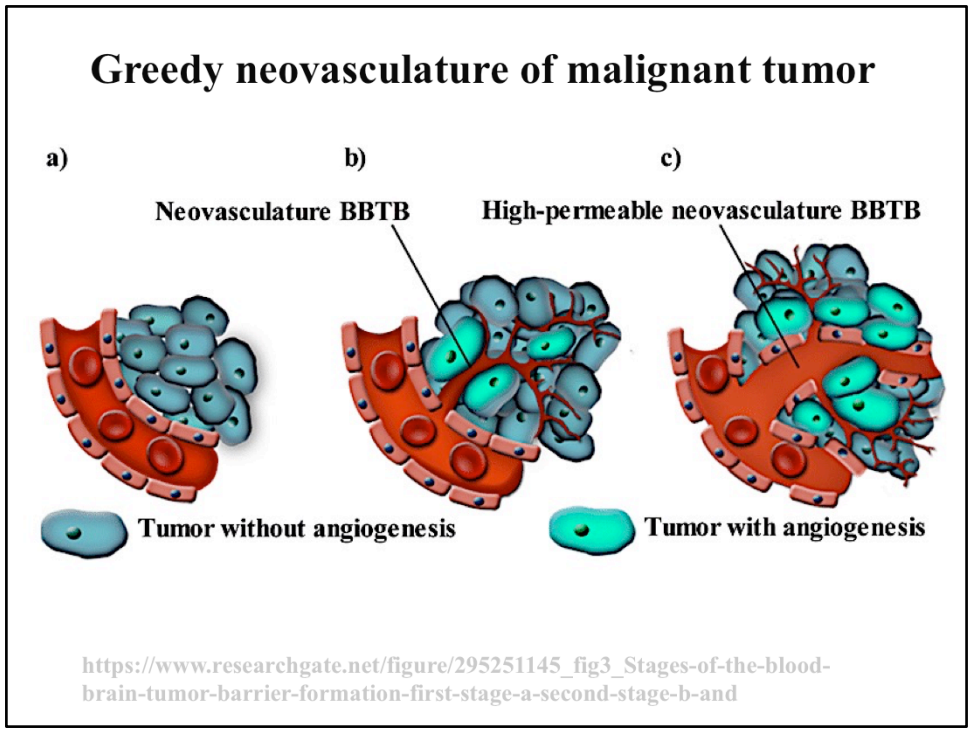
BBB { **Tight junction**
Space between endothel and glial cell footprocess



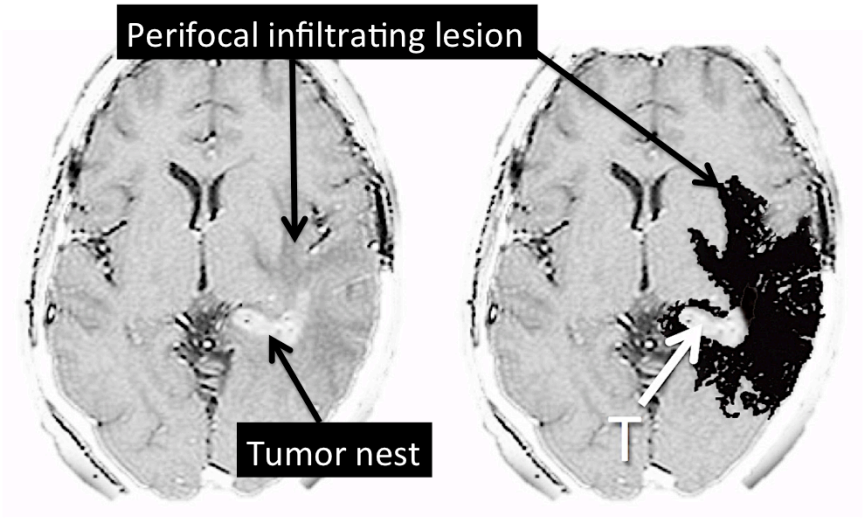
The cellular basis of the blood-brain barrier. (A) Diagram of a brain capillary in cross section and reconstructed views, showing endothelial tight junctions and the investment of the capillary by astrocytic end feet. (B) Electron micrograph of boxed area in (A), showing the appearance of tight junctions between neighboring endothelial cells (arrows). (A after Goldstein, Goldstein and Betz, 1986; B from Peters et al., 1991.)

http://www.daviddarling.info/encyclopedia/B/blood-brain_barrier.html

This is an electron microscopic view of BBB. It is highly complicated structure to allow super selective passing a materials from blood stream to brain parenchyma. And also its permeability dynamically controlled by glial cell.



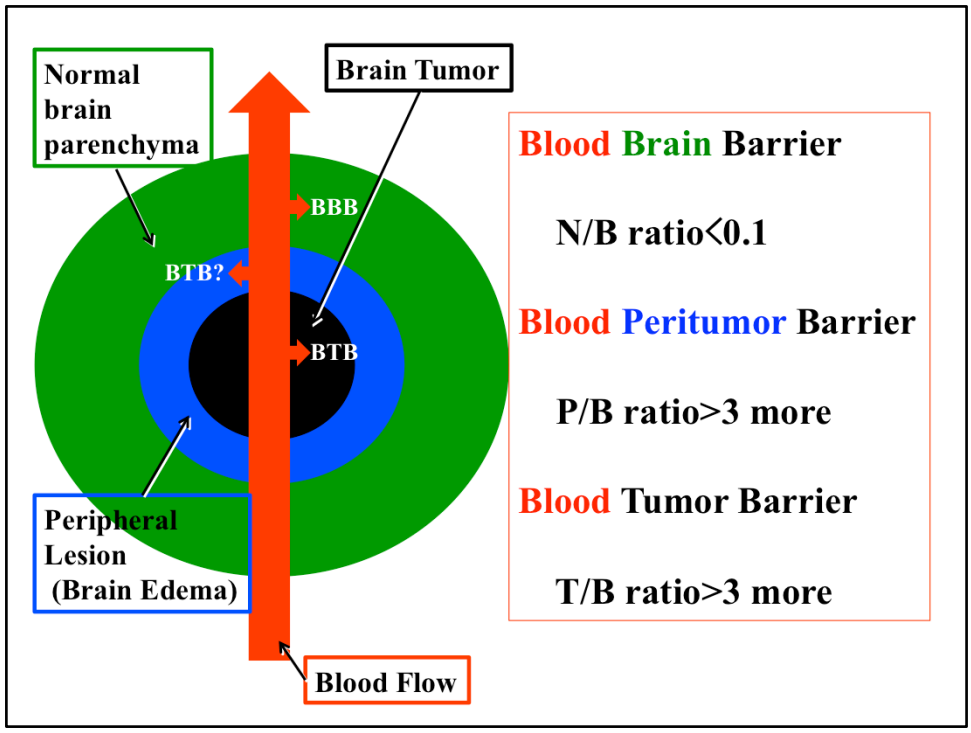
On the othrehand, permeability between tumor and blood vessels are very loose.
Malignant tumor rapidly growth by getting greedily many nutrients from neogenic tumor vessels augmented by cytokine of angiogenic factor delived from tumor cells.



Magnetic resonance images of malignant glioma. *left*: Gd enhanced T1 image before BNCT for recurrent malignant glioma, *right*: Imaginary image representing the tumor cell infiltration visualized in black by color mapping using Adobe Ps. This image is not real. T: main tumor nest.

This slide show Gd-enhaced MRI view of infiltrating malignant tumor.
This enhanced lesion is the tumor nest. Vast number of tumor cells infiltration are well visualized by color mapping using Adobe Photo Shop. This dark colored lesion represents infiltrating lesion that is the area of concern for BNCT.

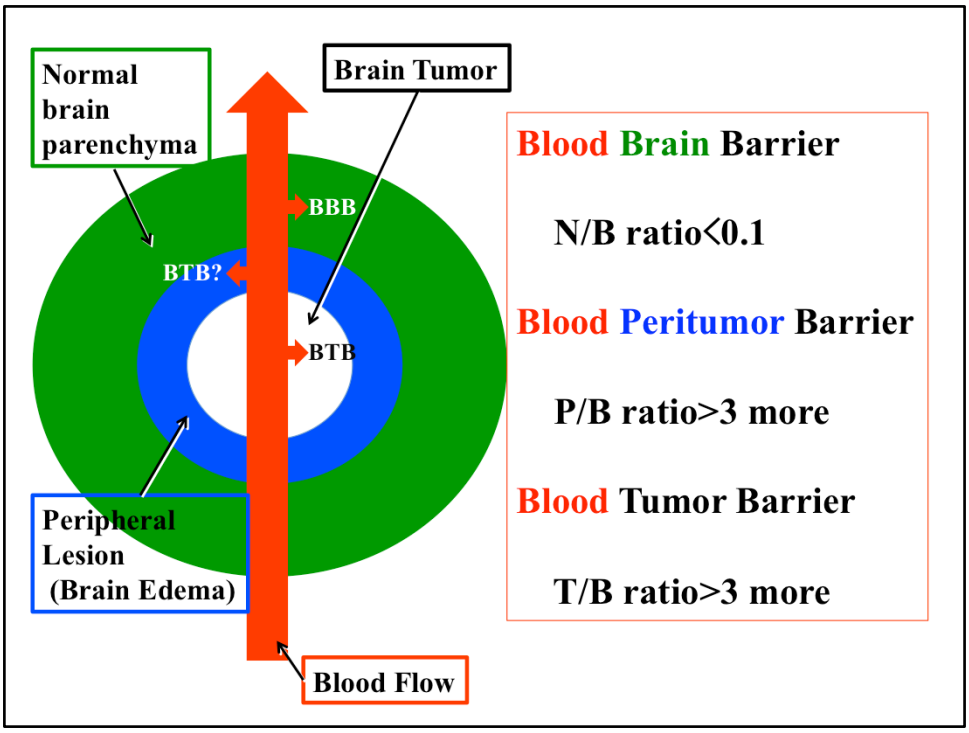
But, unfortunately, infiltrating tumor cells are uncertainly identified even by cutting edge science.



This is a tissue model of the brain for further understanding of BBB.

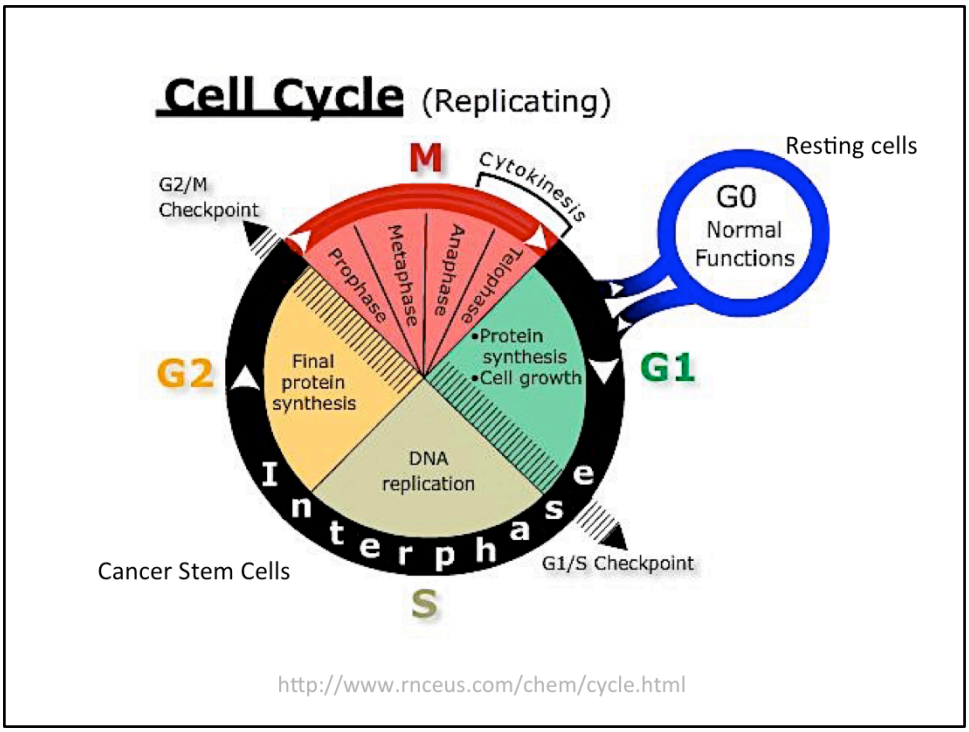
This brain model consists of three components. The blue part is the tumor infiltrating part and highly problematic lesion. Generally drug delivery from blood vessels cannot pass through BBB in normal brain, but can pass through BTB in tumor area. However BBB and BTB are mixed in the infiltrated area and drug delivery is very difficult part with uncertainty.

The ideal anti-cancer drug should not pass through the BBB, and only pass through the BTB, and are actively taken up by tumor cells. Moreover, it is not toxic, and long retention in tumor, furthermore rapid excretion from urinary system.



Usually main tumor nest are extirpated so the target of BNCT is the peri-tumoral infiltrating lesion itself. But precise determination of the boundary of this lesion against normal brain parenchyma is impossible. So that far infiltration of tumor cells inevitably treated by adjuvant chemotherapy. But tumor recurrence usually occur in this lesion.

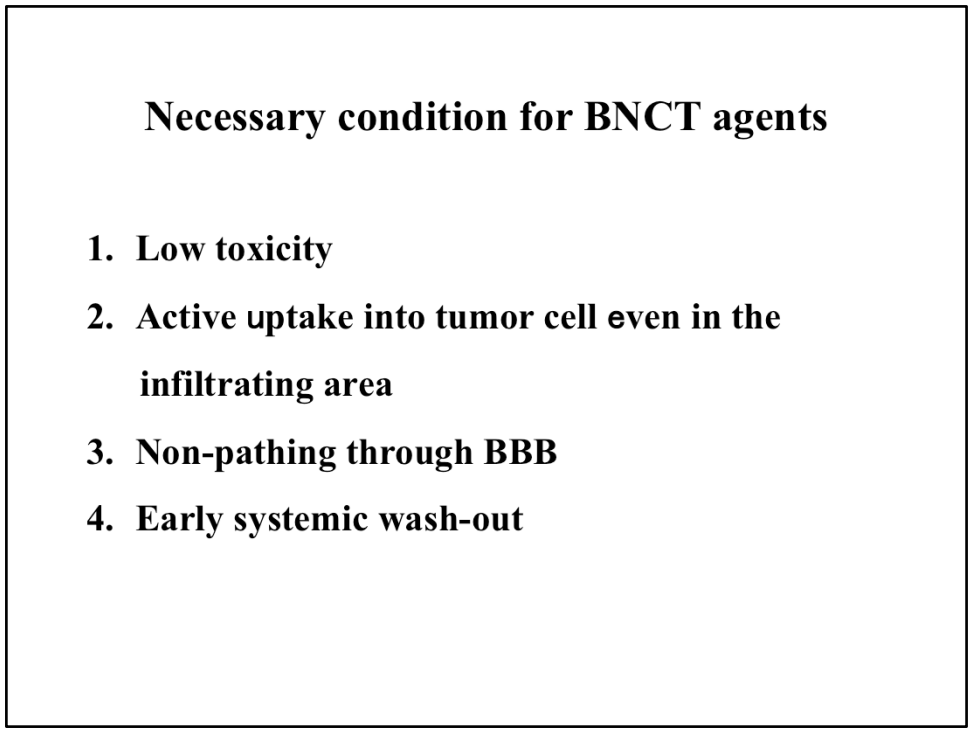
This is a main reason why many neurosurgeon does not want deeply concern with this formidable disease!



Another problem of drug design for malignant tumor is presence of resting tumor cell.

Depending on the malignancy, 20-30% of tumor cells are replicating. But a rest of a large number of tumor cells are resting at G0 satge.

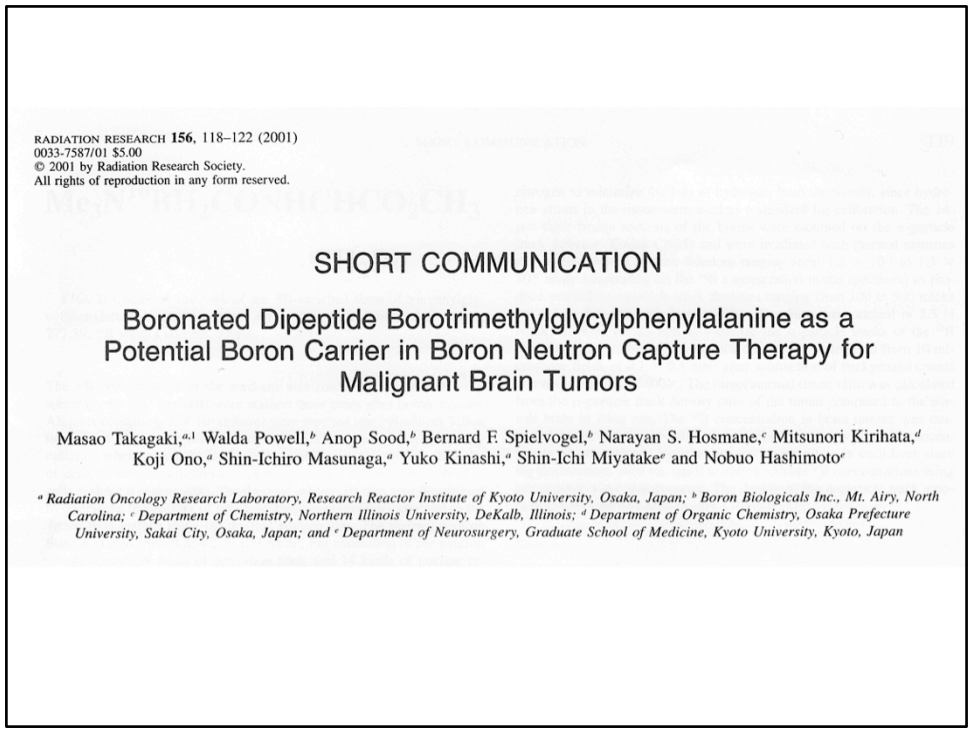
Indeed a large part of malignanat brain tumor cells are in the G0 stage in cell cycle that does not divide with minimum metabolic activity. So the all of the tumor cells can not be targeted metabolically.



This slide shows necessary requirements points for BNCT agent.

1. Low toxicity
2. Active uptake into tumor cell even in the infiltrating area
3. non-pathing through BBB
4. Early systemic wash-out

But it might be very hard to satisfy all conditions.



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SHORT COMMUNICATION

Boronated Dipeptide Borotrimethylglycylphenylalanine as a Potential Boron Carrier in Boron Neutron Capture Therapy for Malignant Brain Tumors

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[a] Radiation Oncology Research Laboratory, Research Reactor Institute of Kyoto University, Osaka, Japan; [b] Boron Biologicals Inc., Mt. Airy, North Carolina; [c] Department of Chemistry, Northern Illinois University, DeKalb, Illinois; [d] Department of Organic Chemistry, Osaka Prefecture University, Sakai City, Osaka, Japan; and [e] Department of Neurosurgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

One of a candidate satisfying all condition is boronated dipeptide boronomethylglycylphenylalanine synthesized by Drs. Powell, Sood, Spielvogel, Hosmane, etc.

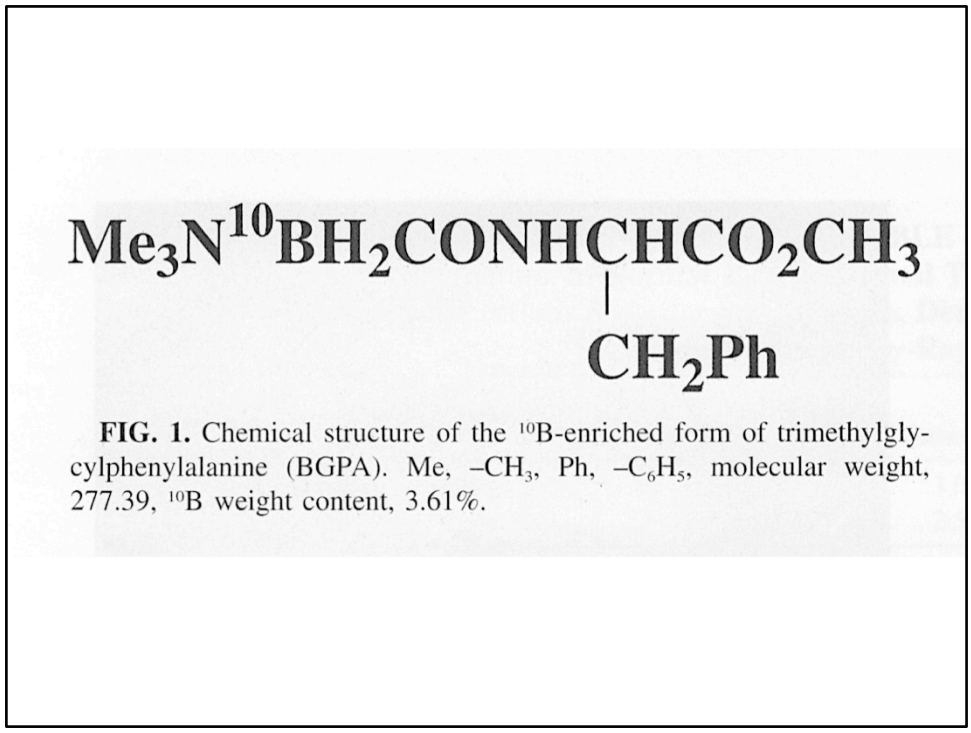


FIG. 1. Chemical structure of the ^{10}B-enriched form of trimethylglycylphenylalanine (BGPA). Me, $-CH_3$, Ph, $-C_6H_5$, molecular weight, 277.39, ^{10}B weight content, 3.61%.

This dipeptide is a conjugated form of glycyl and phenyalalnine.

	N/B	T/B
BSH	0.1	1
BPA	0.9 (～1)	3
BGPA(dipeptide)	0.3	2.5
Nano	0.1	>1 ?
ideal	0.1	>3 more

TABLE 1
Ratios of Tumor to Normal Tissue, Normal Tissue to Blood and Tumor to Blood for ^{10}B Concentrations 3 h after i.p. Injection, Determined by α-Particle Autoradiography and/or Prompt γ-Ray Spectrometry

Boron compound	Tumor to normal tissue	Normal tissue to blood	Tumor to blood	ppm ^{10}B in blood	ppm ^{10}B in liver
BGPA	8.9 ± 2.1	0.3 ± 0.1	3.0 ± 1.2	6.1 ± 1.8	7.7 ± 2.0
BPAF	2.8 ± 0.9	0.9 ± 0.2	2.5 ± 1.0	4.3 ± 1.6	5.4 ± 2.2

Notes. The normal tissue to blood ratio was calculated from the ratio of the tumor-to-blood and tumor-to-normal tissue ratios. The ^{10}B concentrations in blood liver were determined by prompt γ-ray spectrometry. $n = 3$.

This is a summary of biodistribution of boron concentration ration of normal brain to blood (N/B ratio) and that of tumor to blood (T/B ratio) for various boron carriers, BSH, BPA, BGPA, and also nano compound.

Small N/B ratio means non-pathing throigh BBB.
Small T/B ratio means non active uptake into tumor cells.

Generally a small N/B ratio and a large T/B ratio are ideal.

	N/B	T/B
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BPA	0.9 (～1)	3
BGPA(dipeptide)	0.3	2.5
Nano	0.1	>1 ?
ideal	0.1	>3 more

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Notes. The normal tissue to blood ratio was calculated from the ratio of the tumor-to-blood and tumor-to-normal tissue ratios. The ^{10}B concentrations in blood liver were determined by prompt γ-ray spectrometry. $n = 3$.

BGPA shows low N/B ratio and High T/B ratio with low toxicity. That is to say a dipeptide of BGPA has a the both merits of BSH and BPA.

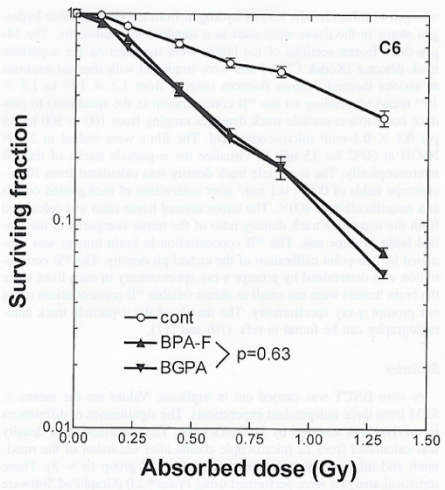


FIG. 2. Surviving fraction of C6 gliosarcoma cells after *in vitro* BNCT 6 h after boron loading of BGPA (▼) or BPA-F (▲) compared to control cells (○). The plating efficiency for thermal neutron-irradiated cells that received no boron was 72%. The surviving fractions were calculated from the three independent experiments. Values are means ± SEM.

BGPA has been reported previously to be stable in the presence of serum proteins. In mouse plasma incubated at 37°C,

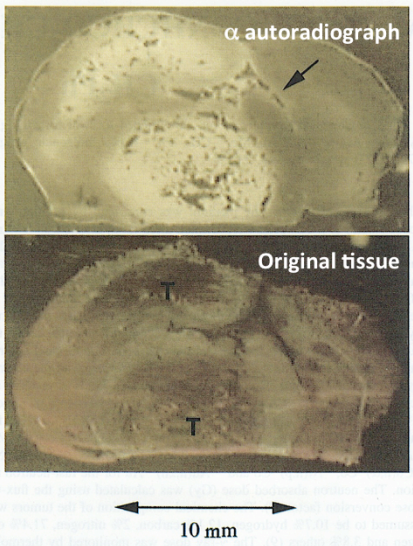
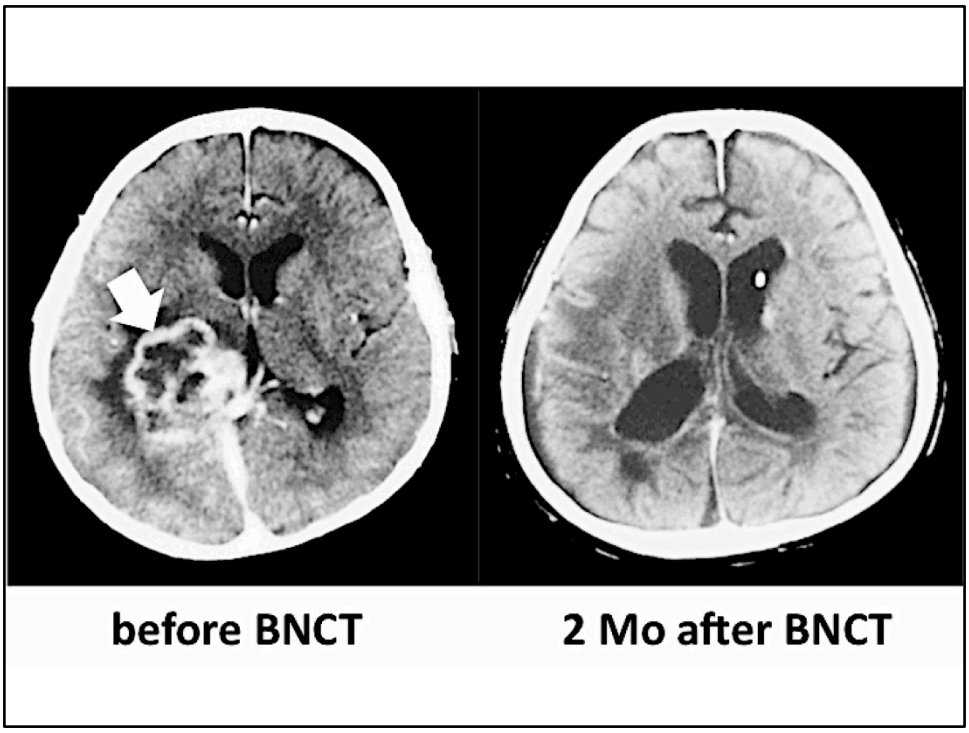


FIG. 3. Alpha-particle autoradiograph (upper panel) of BGPA in the brain of a rat bearing a C6 gliosarcoma (lower panel, H&E stain). The rats were killed 3 h after the i.p. injection of BGPA at a dose of 50 mg per kg body weight. The bright region revealed by high α-particle track density corresponds to the tumor. T, tumor; N, normal parenchyma.

Furyhermore, boron autoradiography revealed that a dipeptide form of BGPA can distribute well even in the peritumoral infiltrating area.

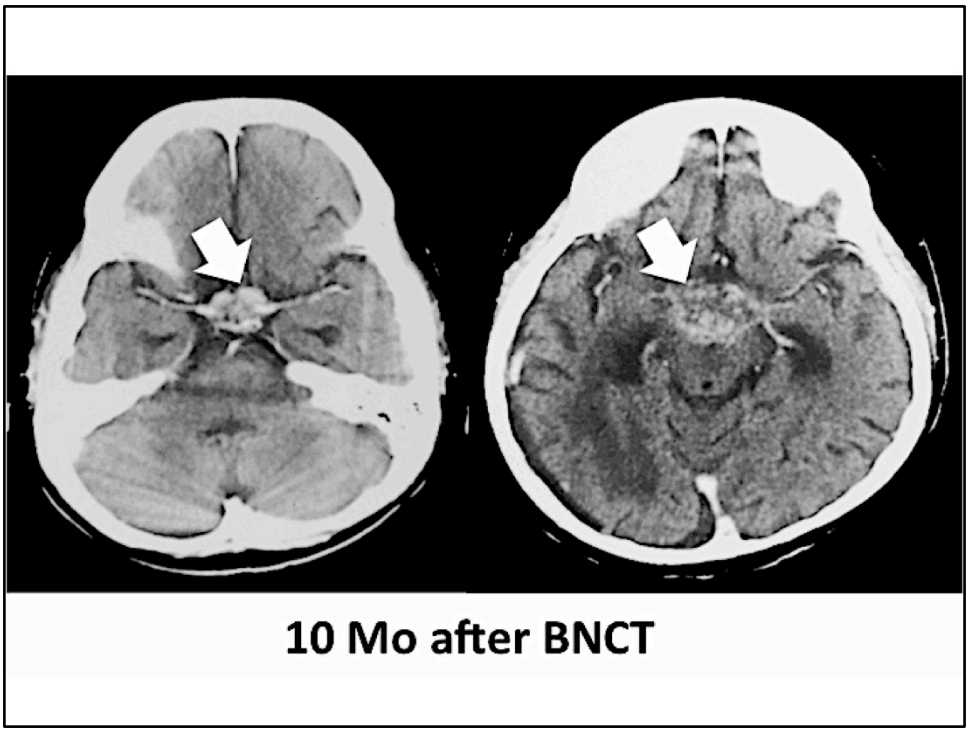
I think such form of boronated dipeptides should be more investigated for clinical BNCT use.



This picture shows a typical case of post BNCT, recurrence from far infiltrating tumor cell.

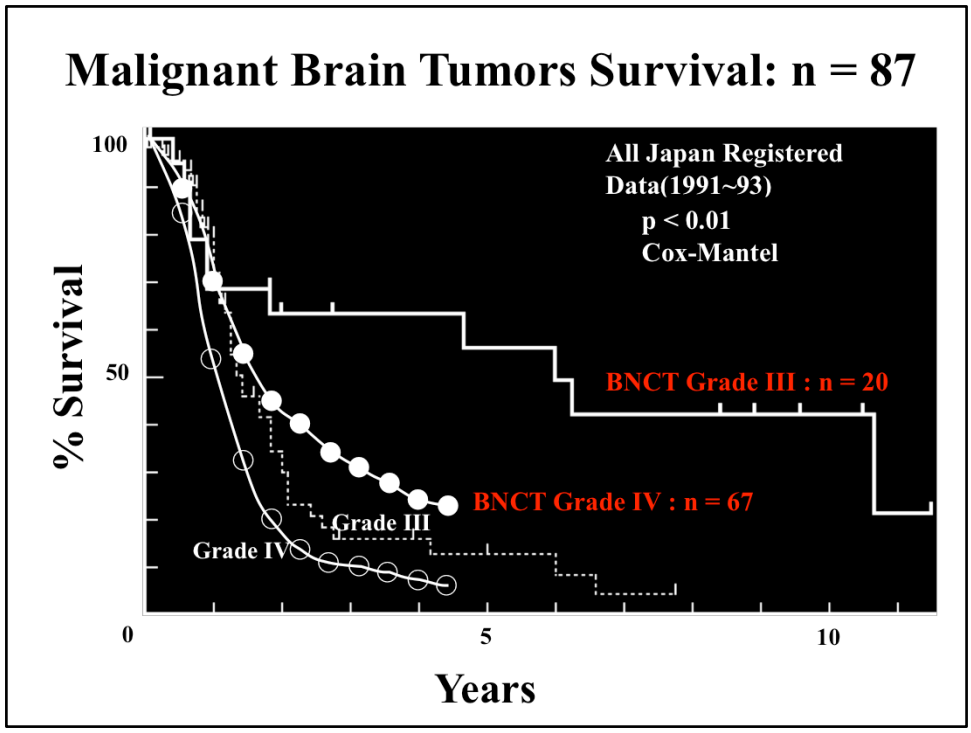
5 year old girl showed headache and vomiting on the Christmas day. She was transferred to my University and rapidly diagnosed as Grade IV glioma.

2 months later, I treated her by BNCT by increasing thermal neutron penetration even onto deeply seated tumor by intravenous injection of deuterium. And tumor rapidly disappeared.



But, unfortunately, 10 month after BNCT, she showed diabetes insipidus (DI). And enhanced CT scan revealed tumor recurrence in the hypothalamus far from the original site. Although original site was well controlled without tumor recurrence.

This is a typical case of post BNCT. Recurrence usually occur in the infiltrating peri-tumoral lesion sometime far from the original site like this case.



This is a survival rate after our BNCT.

All Japan registered data shows 5 years survival rates are 20% for grade III and less than 7% for grade IV.

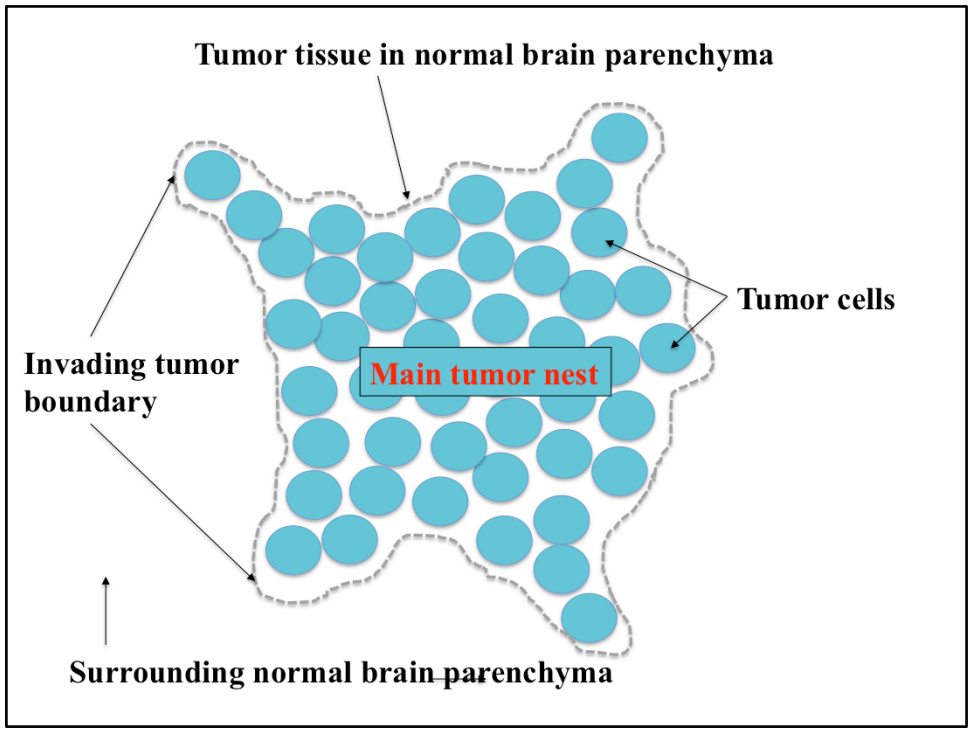
On the other hand, BNCT data shows relatively good outcome, 60% for grade III and 20% for grade IV, but those figures are still still low as a cancer treatment option.

Concept
of
Ideal bio-distribution of boron
in tumor for BNCT

- targeted or untargeted -

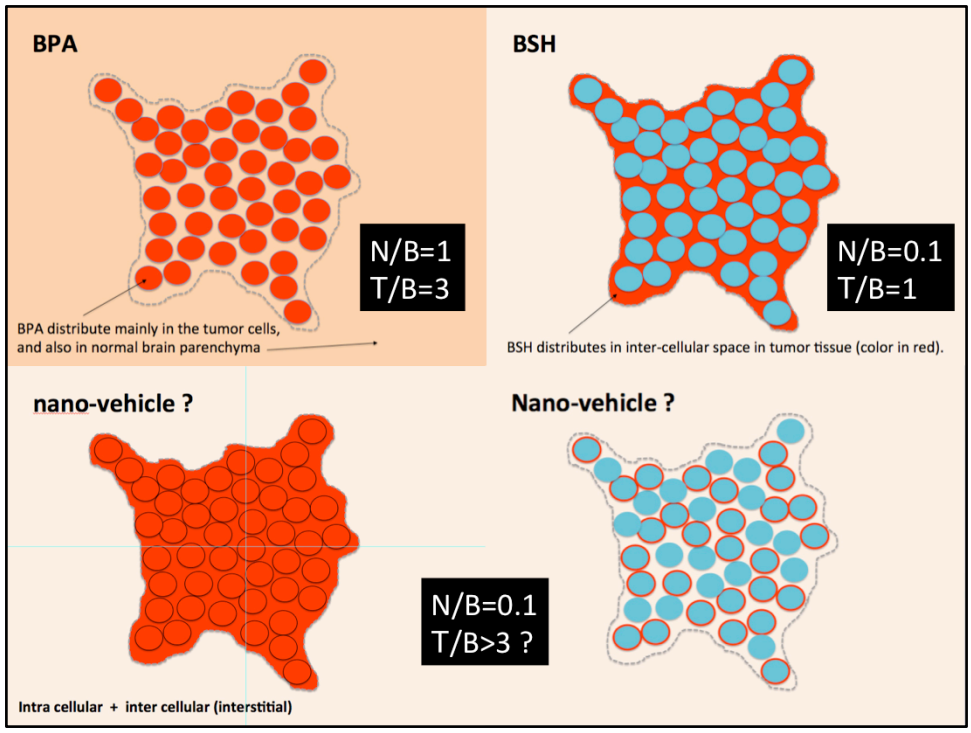
(All figures are original)

Finally I would like to show concept of ideal boron bio-distribution in tumor for BNCT.



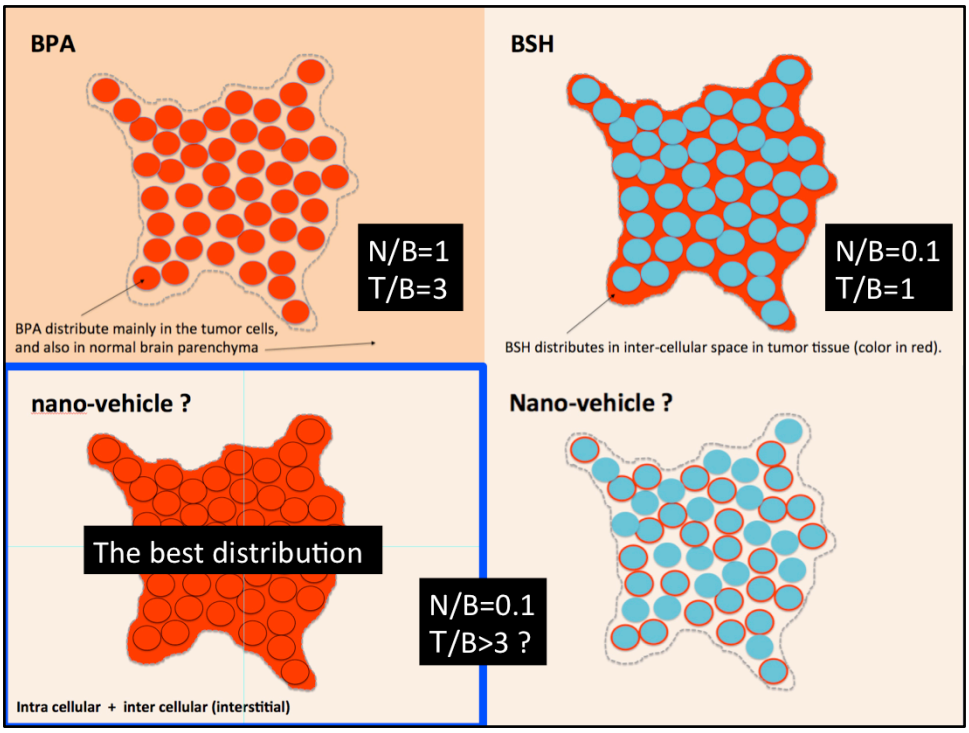
This shows tumor model to understand the concept of ideal boron bio-distribution.

Malignant tumor cell invade and/or infiltrate through out normal brain parenchyma. Tumor cell infiltrate along neural stream beyond the main tumor nest as shown in the previous case.

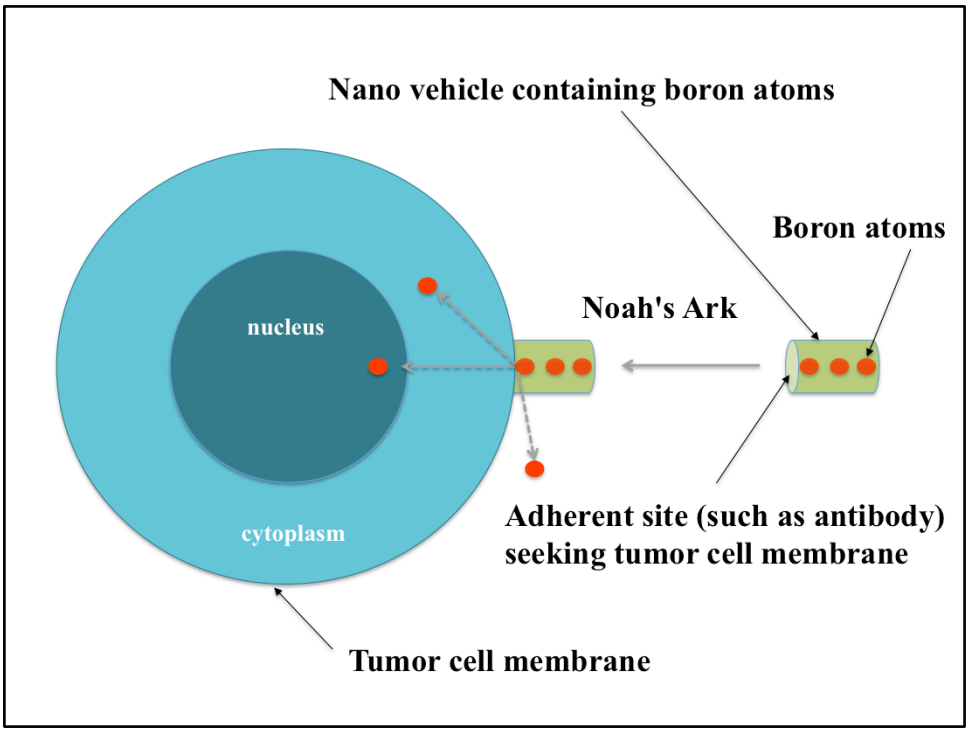


Which one is the ideal boron bio-distribution for BNCT?
And which one represents for cutting edge Nano compounds?

Does someone answer this?



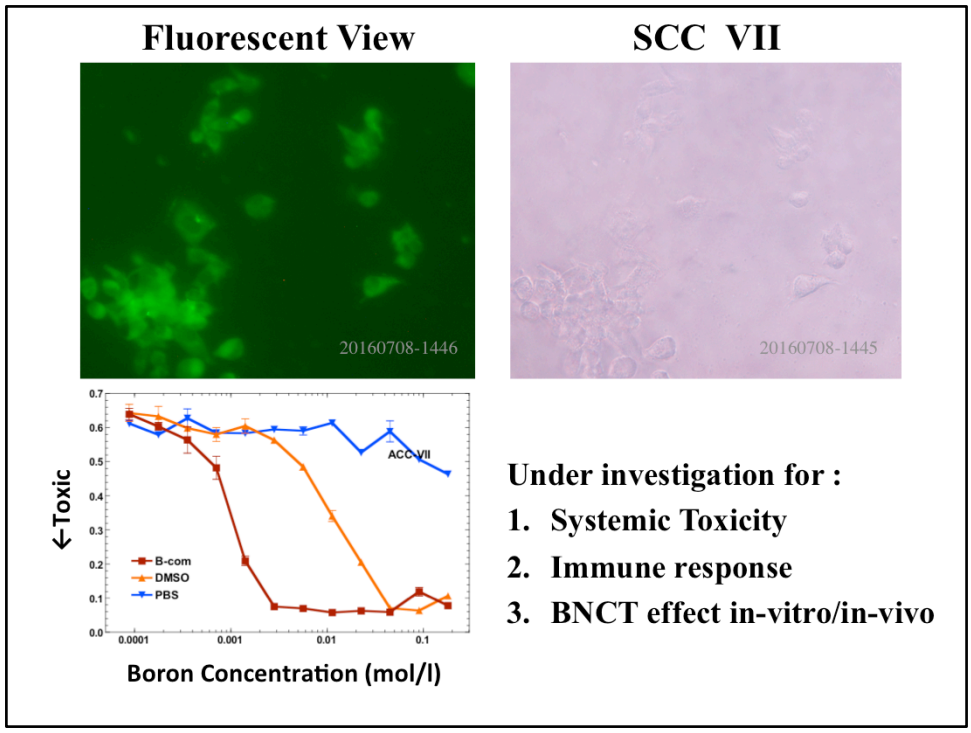
This is the best distribution of boron compounds for BNCT. But it is hardly attained for the reasons of the presence of resting cells, variety of metabolic activities, infiltrating periferal lesion, etc.



Nano vehicles are expected as an anti—cancer drug conveyer to tumor cells like Noah's Ark.
Many pharmaceutical companies have studied Nano-vehicles as a cancer chemo-agents. They are of course expected as a boron carrier for BNCT.

Macromolecule such as Nano-vehicles does not pass through blood brain barrier (BBB), but through blood tumor barrier (BTB). If this is true, their distribution might be similar to that of BSH. If, furthermore, Nano-vehicles retains longer in inter-cellular space in tumor tissue, they are highly expected as Gd enhancing materials for MRI imaging like a GdDTPA.

Micro-distribution of boron in tumor tissue / tumor cell using Nano-vehicles has not been reported although it is highly interesting for BNCT. Which one is true for nano-compounds among BSH type, BPA type or combined type.



This is a final slide.

Recently we investigated fluorescent nano compounds containing boron and gadolinium for BNCT agent.
They were blindly delivered and I don't know about chemical property of the compound. Fluorescent view revealed that the compound is well uptaked into tumor cells although highly cytotoxic. After our nuclear reactor resumed on this August form Fukushima Nuclear Reactor Disaster on 2011, these compound will be preferentially investigated by BNCT experiments.

I hope that this type of compound might show a ideal distribution in tumor.

Acknowledgment for Joint Study



Prof. Narayan S Hosmane

Northern Illinois Univ (USA)

Cinvestav National Science Research Institute (MEXICO)
National Chemical Engineering Institute (SINGAPORE)
Korea Atomic Energy Research Institute (KOREA)
Korea National Cancer Center Hospital (KCCH)
Kyoto University Research Reactor Institute (JAPAN)

Thank you for great scientists, especially for Prof. Hosmane and his colleagues for their collaboration for a long time.

Special thanks to



Prof. Sheldon G Shore
Ohio State University

Especially please accept my sincere condolences on the passing of Prof. Sheldon G Shore, OSU. He was a very honest and interesting scientist. He often appeared in my Kyoto office and asked me to cupid with his Japanese girlfriend. It was always wonderful for me that he got a great success in closed friendship with her.

Thank you Sheldon.

Thank you for listening, thank you.