The effect of fractionated administration of thalidomide at γ-ray irradiation on tumor response and lung metastasis

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For curing local tumors

1. Controlling local tumors

ex.) controlling intratumor quiescent tumor cells, including hypoxic tumor cells

2. Controlling distant metastases

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Intratumor Microenvironment

- **Tumor vasculature**

  Distribution, Penetration, Blood flow, et al.
- **Oxygen concentration (acute (= perfusion-limited)**

  or chronic (= diffusion-limited) hypoxia)

  <---> Oxygen radical (ROS, NOx, etc.)
- **pH**
- **Nutrition status**
- **Cell cycle**

  (Quiescent (Q), Proliferating (P, G1, S, G2, M, G0)
- **Clonogenicity of tumor cells (ex.) stem cells) etc.**

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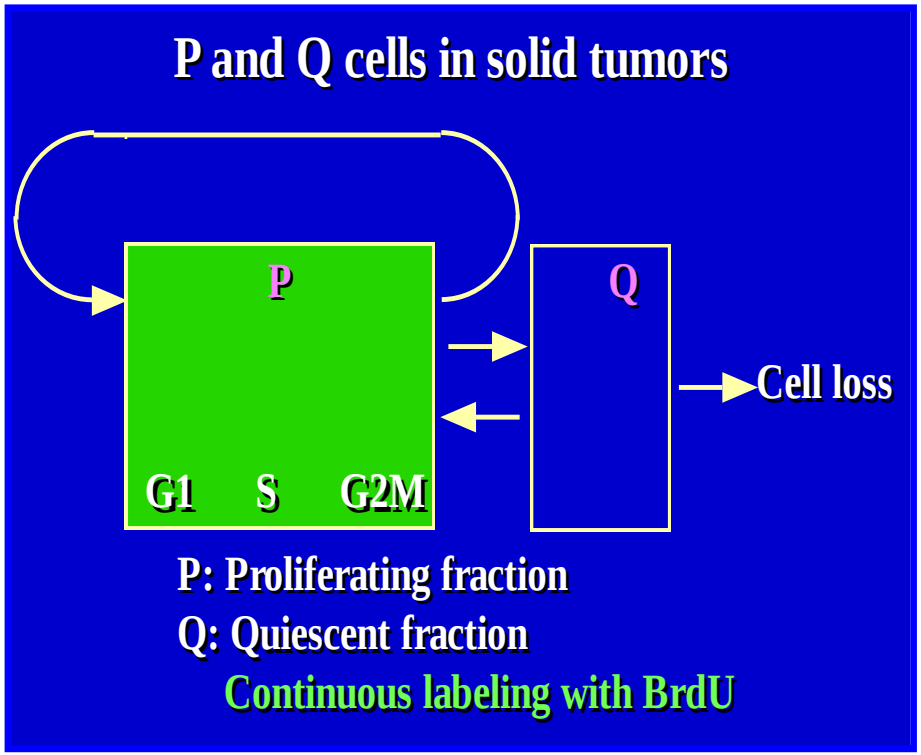
The Method for Selective Detection of the Response of Intratumor Quiescent Cells

1. **Mice bearing the B16-BL6 tumors received BrdU continuously to label all proliferating (P) cells in the tumors.**

2. **Tumor-bearing mice received various DNA-damaging treatments, such as radiation.**

3. **Thereafter, the tumors were excised, minced, and trypsinized to obtain single tumor cell suspensions.**



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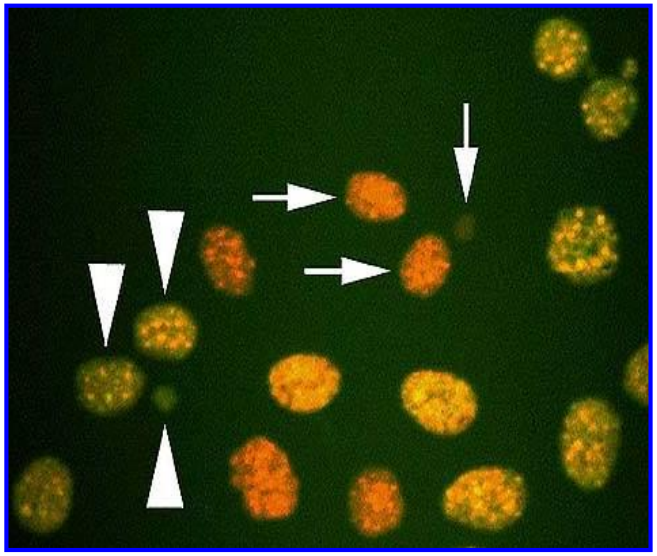
4-A.

<Immunofluorescence staining for BrdU to detect BrdU-labeled cells>

The tumor cell suspensions were incubated with a cytokinesis blocker (cytochalasin-B), and the micronucleus (MN) frequency in cells without BrdU labeling [= Q cells, Arrows] was determined using immuno-fluorescence staining for BrdU.



4-B.

<Cells from the tumors that were not pretreated with BrdU>

The MN frequency in total (P + Q) tumor cells were determined from the tumors that were not pretreated with BrdU.

Colony forming assay was also carried out using *in vivo-in vitro* assay method.

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Characteristics of intratumor Q cells

1. **Sensitivity to radiation : Q < P ***

   High linear energy transfer (LET) radiation: Q ～ P

2. **Recovery from radiation-induced DNA damage : Q > P**

   High LET radiation suppresses the recovery even in Q cells.

3. **Size in hypoxic fraction : Q > P ***

4. **Hypoxia in Q tumor cells :**

   Chronic hypoxia > Acute hypoxia*

5. **Clonogenic capacity : Q < P**

6. **Acceleration of recruitment from Q to P status after irradiation (irrespective of *p53* status *)**

1,2,3: Radiat Res, 125, 243-47, 1991
4: Br J Radiol, 66, 918-26, 1993; Int. J. Radiat. Oncol. Biol. Phys., 29, 239-242, 1994
5: Annual Rep Res Reactor Inst, 26, 69-79, 1993
6: Jpn J Cancer Res, 84, 1130-35, 1993
**: Eur J Cancer, 38, 718-27, 2002; Int J Radiat Oncol Biol Phys, 60, 570-77, 2004*

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Therefore,

1. **Intratumor Q cells are more resistant to irradiation than exponentially growing tumor cells because of their higher hypoxic fraction and greater potentially lethal damage recovery (PLDR) capacity. Q cells also have lower sensitivity to chemotherapeutic agents than proliferating (P) cells *in vivo*. Thus, more Q cells can survive after radiotherapy or chemotherapy than proliferating (P) cells.**

2. **Consequently, the control of Q cells, some of which still have clonogenicity, greatly influences the outcome of anticancer therapy.**

(Masunaga S and Ono K, J Radiat Res, 43, 11-25, 2002.)

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Our findings so far concerning hypoxia manipulation and lung metastatic potential

1. **Controlling chronic hypoxia-rich Q tumor cells is critical for curing solid tumors as a whole, as well as controlling hypoxic tumor cells.**
2. **As the dose of radiation increased, lung metastatic potential decreased reflecting the decrease in the number of clonogenically viable tumor. However, an acute hypoxia-releasing nicotinamide treatment may be promising for reducing numbers of lung metastases.**
3. **Thus, Hypoxia manipulation in solid tumors has the potential to influence not only local tumor response but also lung metastatic potential.**

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Introduction for this study

It was believed that antiangiogenic therapy prevents tumor vascular growth and proliferation, thus depriving the tumor of the oxygen and nutrients necessary for survival. Subsequent study, however, suggested that antiangiogenic therapy may also "normalize" the tumor vasculature for a short period of time, thereby providing a window of opportunity for improved drug delivery and enhanced sensitivity to radiation. The originally used approach relies on using agents that directly target vascular endothelial growth factor (VEGF) or VEGF receptor on endothelial cells.

Thalidomide was also reported to induce tumor blood vessel normalization in a mouse model. Actually, thalidomide is now being mainly applied as a treatment of certain cancers (multiple myeloma) and of a complication of leprosy.

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Tumor hypoxia results from either limited oxygen diffusion (chronic hypoxia) or limited perfusion (acute hypoxia).

Further, it was reported that acute and cyclic, but not chronic, hypoxia significantly increased the number of spontaneous lung metastases, and that this effect was partly due to the influence of acute hypoxia treatment on the primary tumor.

----->Therefore,

Using a readily metastasizing murine melanoma cell line, we tried to analyze the usefulness of combined treatment with thalidomide in radiotherapy with γ-rays in combination with an acute hypoxia-releasing agent nicotinamide (NA) or mild temperature hyperthermia (MTH), already shown to have the potential to release tumor cells from diffusion-limited chronic hypoxia, in terms of local tumor response and lung metastatic potential.

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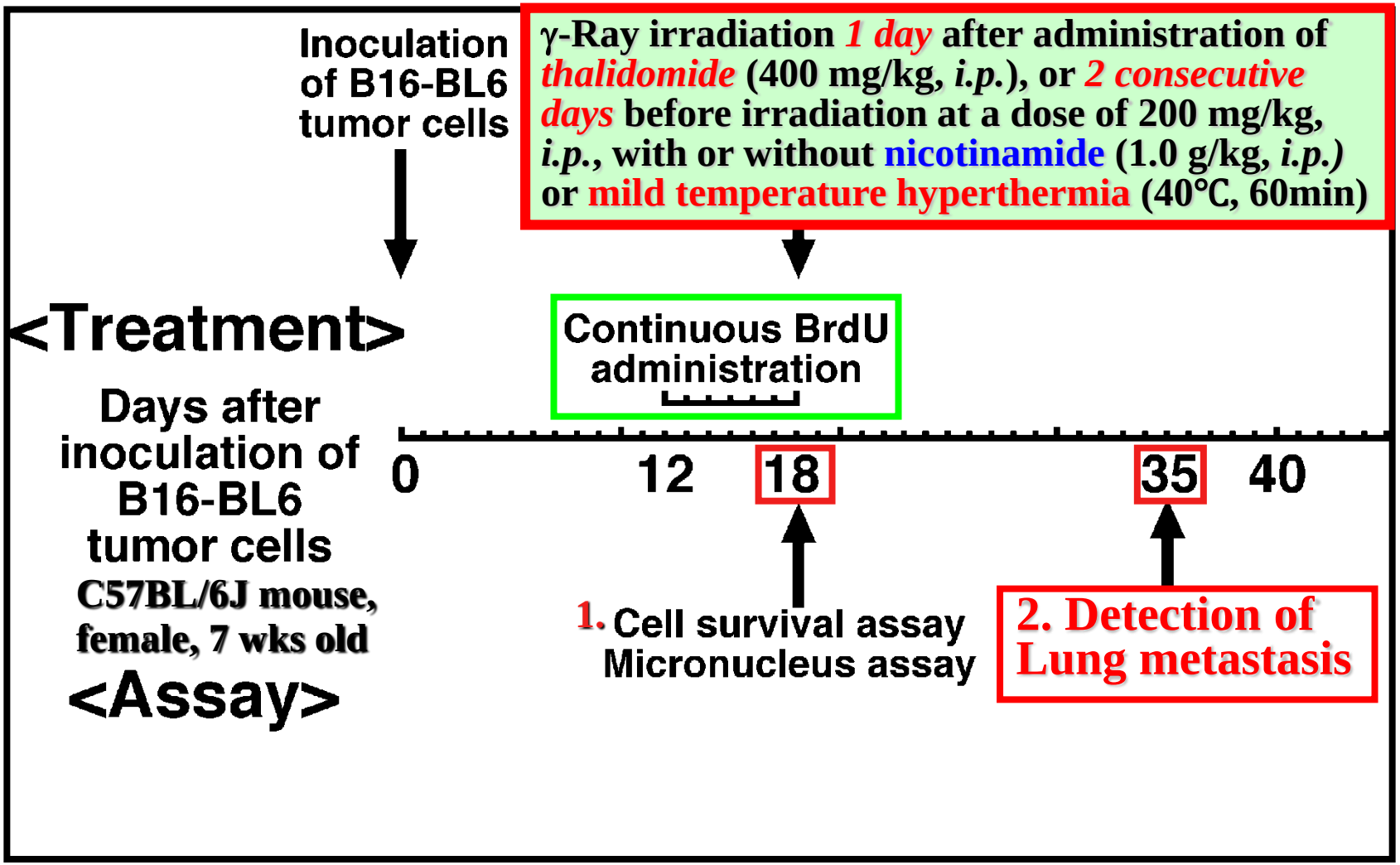
Materials and Methods



Nicotinamide: acute hypoxia-releasing agent within the solid tumor.

MTH: Mild temperature heating that has a potential to release its chronic hypoxia.

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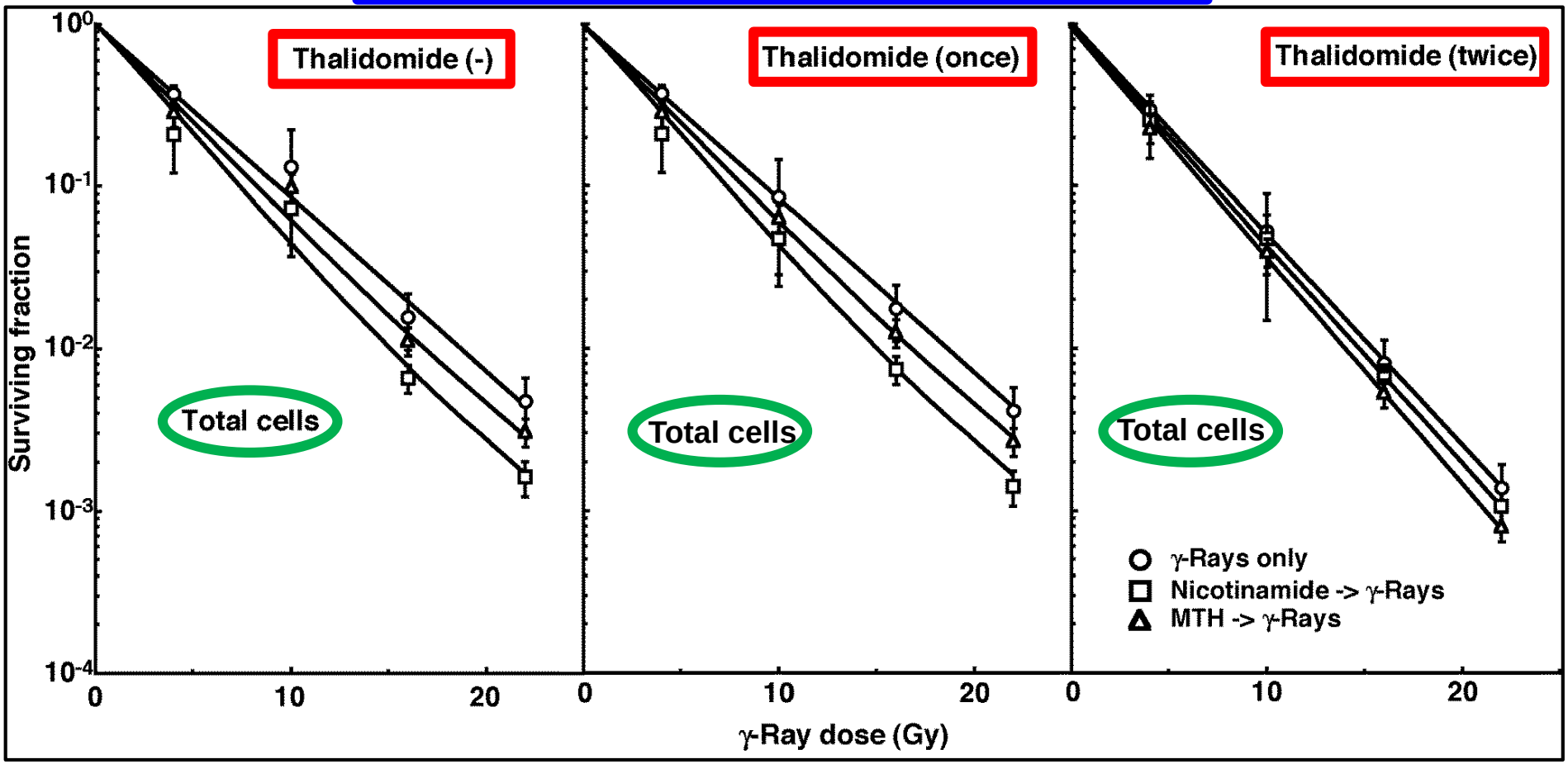
Initial Response (Cell Survival)



Surviving fractions (SFs) <u>without</u> thalidomide and with <u>singly</u> administered thalidomide: **γ-Rays only > MTH→γ-Rays > NA→γ-Rays**

SFs <u>with daily fractionated administered</u> thalidomide:
γ-Rays only > NA→γ-Rays ≥ MTH→γ-Rays

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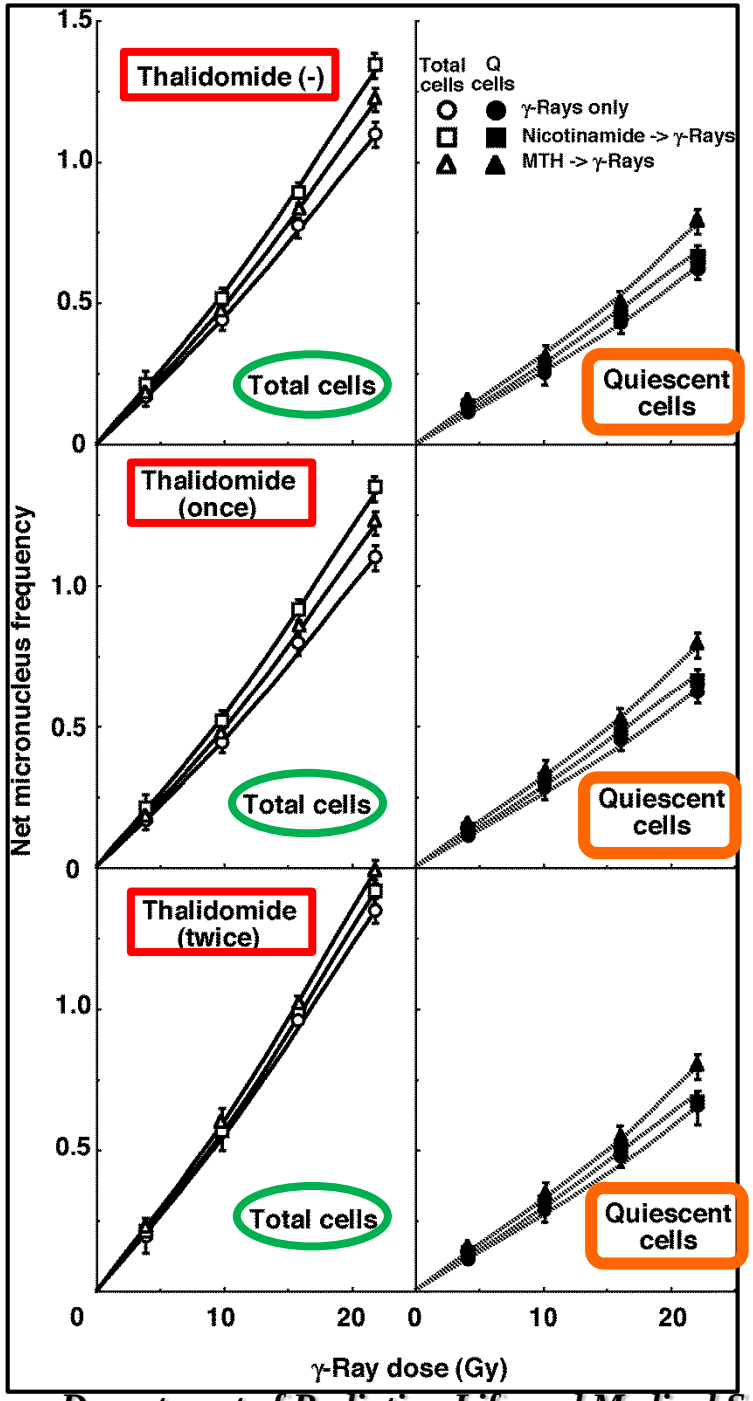

Initial Response (Micronucleus Assay)

MN frequency (Overall)
Q tumor cells << Total tumor cells.

MN frequency in Total tumor cells without thalidomide and with singly administered thalidomide
γ-Rays only < MTH→γ-Rays < NA→γ-Rays

MN frequency in Total tumor cells with daily fractionated administered thalidomide and in Q tumor cells with or without thalidomide
γ-Rays only < NA→γ–Rays ≤ MTH→γ-Rays

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Initial Response (Summary)

In tumors treated without thalidomide or with singly administered thalidomide, the combination with nicotinamide and MTH had a more enhancing effect on the total and Q cell populations, respectively, although not significantly.

In tumors treated daily with thalidomide, the effect of NA was reduced, leading to a greater enhancing effect of MTH than NA on both the total and Q cell populations.

→

Thus, the daily administration of thalidomide had released cells from acute hypoxia before the NA treatment.

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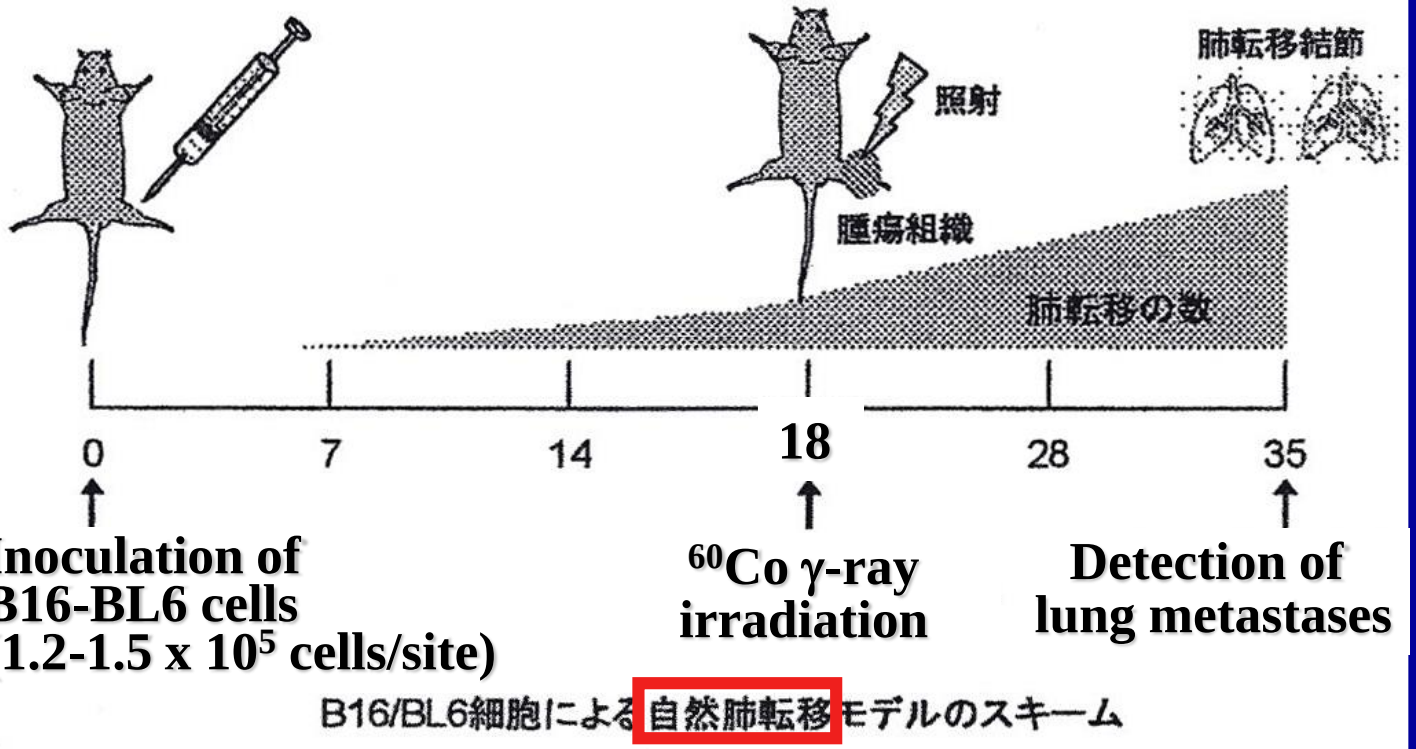

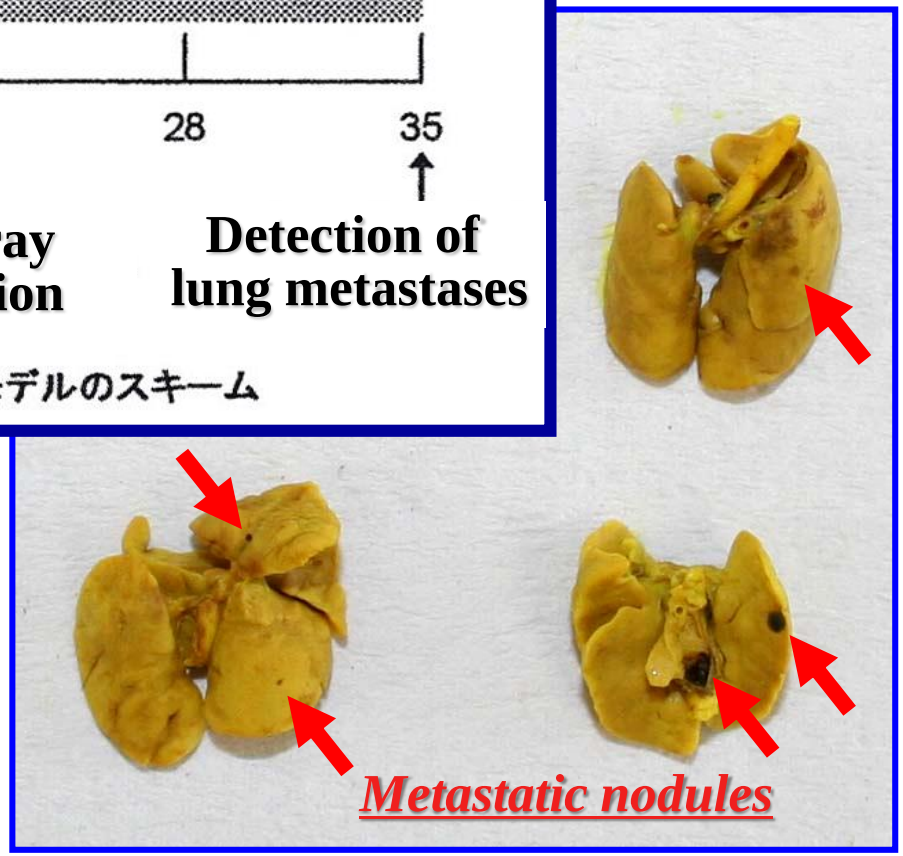

<Lung metastasis-prone cell line>

B16-BL6 :mouse melanoma cell line
(derived from C57BL/6J mice)

Inoculation: s.c. at lt. leg,
1.2-1.5 x 10^5 cells/site

Irradiation (on day 18):
Impl. Tumor diameter: φ= 7mm

35 days later, black metastatic nodules at lung surfaces were counted.
Impl. Tumor diameter: φ= 2cm

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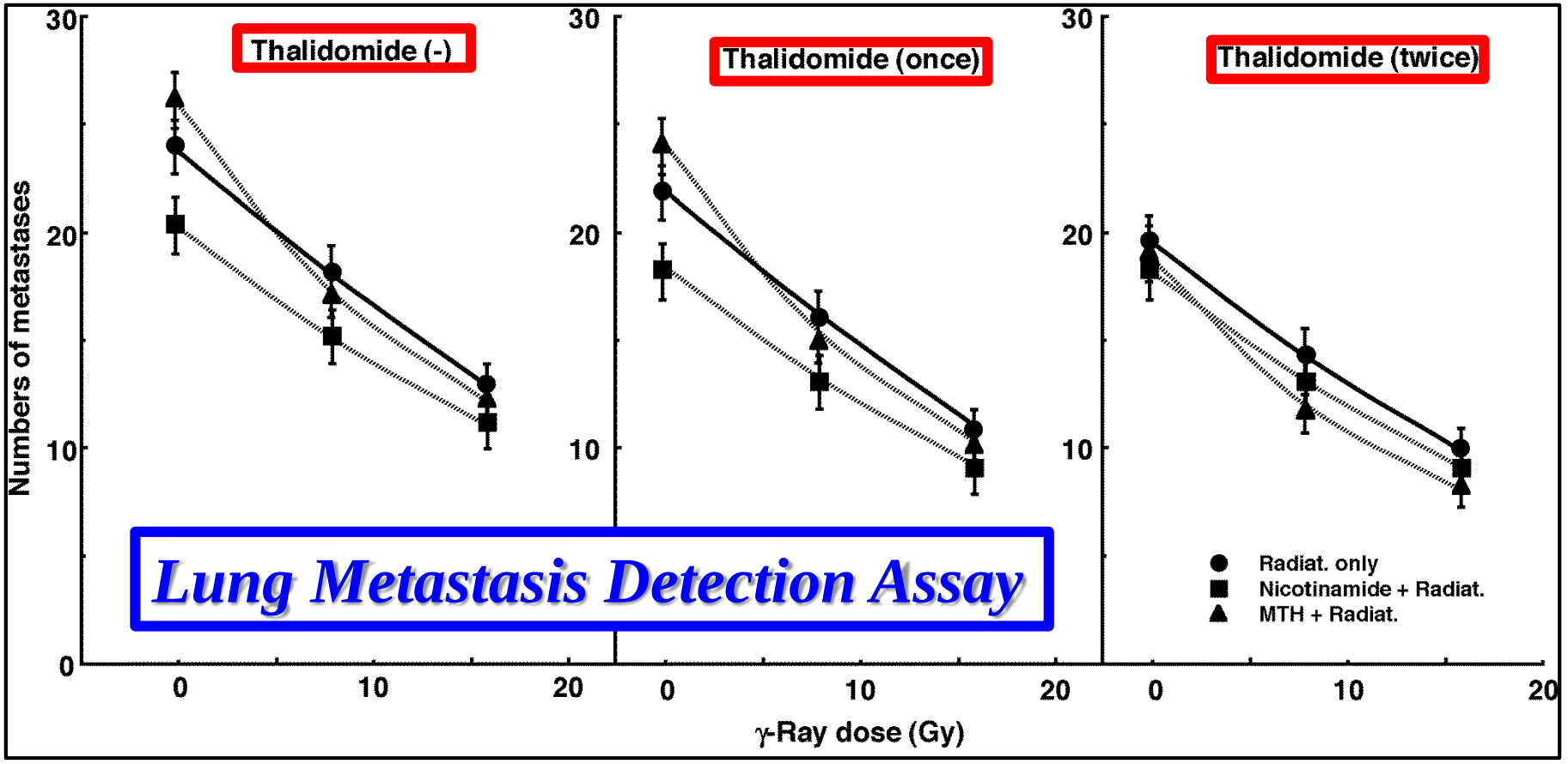

Without γ-Rays:

NA combination decreased the numbers.

With γ-Rays:

As the dose increased, the numbers decreased.

Without thalidomide and with singly administered thalidomide:

NA decreased the numbers.

With daily fractionated administered thalidomide:

MTH decreased the numbers.

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Numbers of metastases from the irradiated tumors that received cytotoxic treatment producing a similar initial local effect

Without thalidomide	With thalidomide (once)	With thalidomide (twice)
<Surviving fraction = 0.03>		
γ-Rays only		
14.1	12.2	11.9
With nicotinamide		
13.1	11.4	11.3
With mild temperature hyperthermia		
14.1	12.2	10.6

—> **Daily administration, especially combined with MTH, decreased the number of lung metastases.**

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Conclusion

***Daily fractionated administration of thalidomide** in combination with γ-ray irradiation* **was thought to be more promising than single administration because of its potential to enhance local tumor response and repress lung metastatic potential.**

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Thank you so much for your close attention.

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