

REVIEW ARTICLE

FUNDAMENTAL AND CLINICAL STUDIES ON CANCER CONTROL WITH TOTAL OR UPPER HALF BODY IRRADIATION

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Abstract: The tumor control effects of total body irradiation (TBI) for tumor bearing mice and human tumor were investigated fundamentally and clinically. TBI is usually used in tissue transplantation experiment in order to prevent rejective response for transplanted tissues by immunological reaction. This kind of suppressive effect of immunological response by TBI is considered as to be caused even if very small dose of TBI. However, there are only a few data concerning the effect of low dose of TBI, and TBI of low dose level is concluded to bring about the same effect as in high dose level by back extrapolation from the data of high dose level.

In present paper, firstly the effects of TBI for tumor control in murine squamous carcinoma are reported, and secondly the results of clinical trial in malignant lymphoma are demonstrated.

In fundamental studies, TBI of low doses (10-15 cGy) suggests potentiating effect in cell killing in combination of TBI of 10 cGy and local irradiation given at 12 hours after TBI, though TBI of 10 cGy is not able to detect any cell killing effect. TBI of 10 cGy or 15 cGy also stands for suppressive effect of distant metastasis (lung metastasis).

In clinical studies, malignant lymphoma (non-Hodgkin's lymphoma) is selected as the first disease of clinical trial, and the results is seemed to be prospective method to overcome cancers with radiotherapy, though the trial is not phase III clinical trial.

Key words: Total body irradiation, Immunological response, Murine squamous cell carcinoma, TD50, non-Hodgkin's lymphoma

INTRODUCTION

Total body irradiation (TBI) has been considered to bring about immunosuppressive effects on organism, and it is the conclusion derived from the data obtained by sublethal dose of TBI, however there are only a few fundamental data how low dose of TBI acts on organism.

Clinically, there are some papers that the low

dose of TBI was effective on some malignant lymphoma or chronic myelogenous leukemia^{1),2)}.

Since 15 years, we are studying the effects of low dose of TBI on normal or tumor bearing mice and immunological background. The results suggest tumor control effect by TBI on murine tumor, and the results of clinical trial show the same effect in malignant lymphoma as in murine tumor treated with TBI.

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In present paper, fundamental and clinical investigations concerning the effect of TBI for tumor control are introduced.

FUNDAMENTAL STUDIES

1. Materials and Method

1) Mice and Tumor

Male or female mice of strain WHT/Ht were used as tumor hosts in the TD50 experiments. The tumor was transplantable keratinizing squamous carcinoma which arose spontaneously in a WHT/Ht albino mice and has since been maintained by serial passage as a subcutaneous tumor. A full description of the tumor and its radiobiological characteristics has already reported^{3,4)}.

2) Irradiation

X ray were generated by a therapy machine operated 250kV and 20mA with filtration of 0.5 mm Cu and 1.0 mm Al and exposure dose rate was 0.95 Gy/min.

Tumor cells were transplanted in the hind legs for local irradiation, then exposed to X ray when tumor size reached to 0.8-1.0 cm in diameter. In local irradiation the other part of body except tumor was covered with 4 mm thickness's lead, and the scattered dose to the other organs including spleen was less than 0.2 per cent of tumor dose. The dosimetry was performed by using the standard X ray exposure meter made by National Physical Lab. (England) and BeO thermoluminescent dosimetry made by National Electric Industrial Co. (Japan).

3) Survival of Tumor Cells

Tumors were excised after irradiation, and then single cell suspensions of tumor cells were prepared from a method described previously³⁾. Transplantation assays of counted suspensions were performed by the technique described by Hewitt⁵⁾, and 20 mice were used in each assay. TD50 (number of cells required for successful transplantation to a half a group of injected sites) and 95 per cent confident limits were calculated from the results by the method of Litchfield and Wilcoxon⁷⁾.

4) Preparation of Spleen Cells

Mice are sacrificed by cervical dislocation and spleen were resected at 12 hours after low dose of TBI or sham irradiation, then a single cell suspension of splenocytes was prepared.

The technical procedures for preparation of single cell suspension of spleen cells was described in elsewhere⁷⁾.

5) In Vivo Assay System for Mitogenic Activity of Spleen Cells

For experiments, aliquots (0.3 ml each) of a spleen cells suspension were prepared, and rIL-2 response, Con. A response, PHA response and mixed lymphocytes reaction were investigated.

(1) rIL-2 response

Human recombinant IL-2 were added to culture media of spleen cells at the final concentration of 400 U/ml and cultured in wells of a Nunc micrometer plate at 37°C in CO₂ incubator and 0.5 μ Ci/well of ³H-thymidine uptake in spleen cells in each well were measured after collecting cells by cell harvester.

(2) Con A response

Concanavarin A were given to spleen cells adjusting to be 5 μ g/ml in final concentration, and the other procedure was the same as in case of rIL-2 response.

(3) PHA response

Phytohemagglutinin response was investigated by adding 0.05 % of phytohemagglutinin as a final concentration. The other procedure was the same as in case of rIL-2 response.

(4) Mixed lymphocyte reaction (MLR)

MLR was studied by incubating spleen cell suspension of WHT/Ht mice by adding 0.1 ml of spleen cell suspension (5.0×10^6 /ml) of C57BL/6 mice for 120 hours. The other procedure was the same as in case of rIL-2 response.

(5) Procedure for investigating effect of TBI on metastasis

Lung colony forming abilities were studied to know the effect of TBI on metastasis of tumor cells in mice injected tumor cells into tail vein or in the other

experimental system of WHT/Ht mice in which spontaneous metastasis occurred easily after tumor cell transplantation.

In order to form artificial metastasis in lung tumor cells of 1×10^3 injected into tail vein with 1×10^6 of heavily irradiated cells, then many lung colonies originated at 14 days after injection. In experiments of spontaneous metastasis, mice were injected tumor cells of 1×10^3 into both axilla, then lung metastasis were recognized at 15 days after tumor cell injection.

2. Results

1) Change of TD50 Value After Various Dose of TBI

The first of all, the change of TD50 values of squamous carcinoma cells in WHT/Ht mice received various doses of TBI at 30 minutes before tumor cell inoculation was studied. The result is shown in Fig. 1.

The highest TD50 values was suggested in mice received 10 cGy of TBI, in the other words, 10 cGy of TBI may bring about most remarkable effect of tumor cell rejection among mice received various doses of TBI.

In next study, it is investigated when the highest value of TD50 is shown after 10 cGy of TBI. It is explicit from Fig. 2 to be the highest value in mice received tumor cell injection at around 12 hours after 10 cGy of TBI⁽⁸⁻¹¹⁾.

2) The Cell Survival Curve of Tumor Cells Received Local Exposure or Combined Treatment of TBI and Local Irradiation

In Fig. 3 the survival curves of tumor cells irradiated *in vivo, in situ* by local irradiation or by combined TBI of 10 cGy and local irradiation at 12 hours after TBI are demonstrated. The dotted line traced closed circles is the survival curve of tumor cells exposed locally to graded doses, and the solid line tied up open triangles is the cell survival curve of tumors irradiated with graded doses of X-ray at 12 hours after 10 cGy of TBI. In comparison of these two survival curves, pretreatment of TBI of 10 cGy increased cell killing effect of local irradiation on tumors, though cell killing effect was not recognized

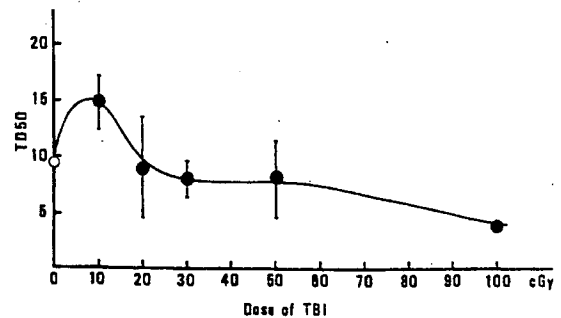


Fig. 1: The change in TD50 values of murine epithelioma in WHT/Ht mice received tumor cells at 30 minutes after various doses of TBI.

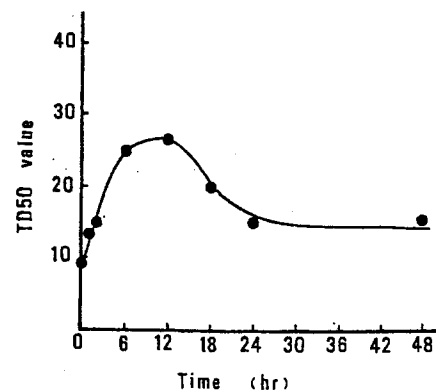


Fig. 2: The change in TD50 values of murine epithelioma in WHT/Ht mice received tumor cells at various hours after TBI of 10 cGy.

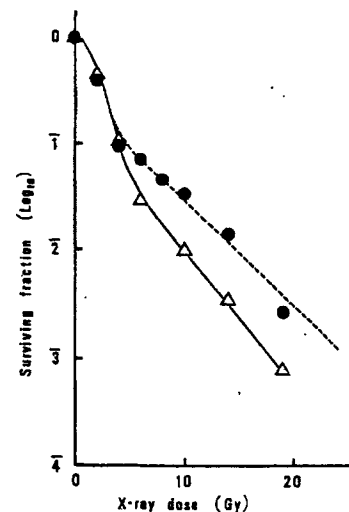


Fig. 3: The survival curves of tumor cells treated by graded dose of local irradiation only (●) or to combined treatment of TBI and graded dose of local irradiation (△). Local irradiation was given at 12 hours after 10 cGy of TBI.

by 10 cGy of TBI, potentiating effect in cell killing by combined treatment of TBI and local irradiation were remarkable in large dose range of local irradiation doses.

3) The Effect of TBI on Tumor Regrowth in Tumors Irradiated by Combined Method or Local Irradiation only

The curve traced closed circles in Fig. 4 shows the regrowth curve of tumors exposed locally to 35 Gy and the curve connected open circles is the regrowth curve of tumors received local irradiation of 35 Gy at 12 hours after 10 cGy of TBI. The regrowth curve of tumor given combined treatments is clearly different from the curve obtained local irradiation only, namely the regrowth curve of tumors given combined treatment decrease rapidly their volumes and recovered slowly compared to tumors exposed to local irradiation only.

4) Local Control Rate of Tumors Received Combined Treatment or Local Irradiation

In order to investigate whether or not the potentiating effect in cell killing in tumors by use of combined treatments result to tumor cure, tumors were treated to local or combined irradiations when tumor size

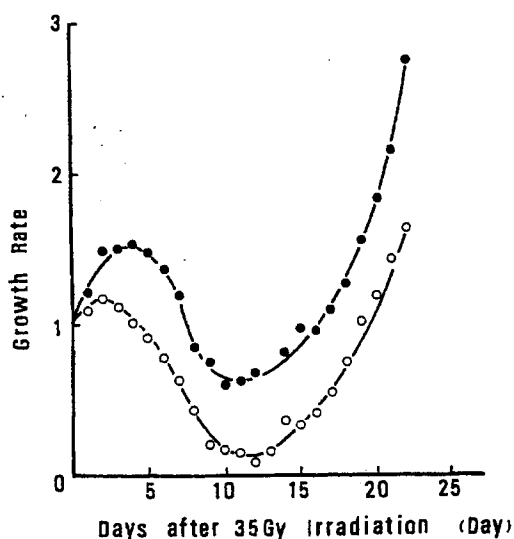


Fig. 4: The regrowth curve of tumors irradiated by 35 Gy locally (●) or treated by combined method of TBI and local exposure (○). Local irradiation was given at 12 hours after 10 cGy of TBI.

reached to 4-5 mm in diameter. The result is shown in Table 1. The upper column represents cure rate of tumors irradiated to 35 Gy locally at 12 hours after 10 cGy of TBI, the middle column also shows tumor cure rate in combined treatments of local exposure of 35 Gy at 12 hours after 5 times of total body irradiations in every 6 hours.

The lower column is the results of tumor cure rate in local irradiation of 35 Gy only. The results suggest that the combined therapy of local irradiation and TBI repeated 5 times is most effective in tumor cure rate and there are significantly differences compared to the cure rate of local irradiation only, though the observation time is not so long enough (30 days).

5) What are the Target Organs Produced the Effect of Low Dose of TBI?

As described above TBI of low doses produce increasing of cell killing effect in combined therapy with local irradiation. However, it is not clear what sorts of organs play the main role to produce the effect of low dose of TBI, so various organs were exposed to 10 cGy locally to investigate the critical organ to bring about the same effect as TBI.

The results is shown in Table 2. As shown in the table, exposure to spleen demonstrated almost the same effect as TBI, but exposures to the other parts examined in this study did not produce the same kinds of effect as shown in TBI. Therefore, the spleen is considered to be one of the most important organs to cause the effect of TBI, and the effect of TBI may relates modification of immunological response in mice. Then immunological studies concerning the effect of TBI were planned.

6) Immunological Background in the Effect of Low Dose of TBI

In order to investigate immunological background it is needed to study the effect of irradiation on thymus only. However, there are technical difficulty to irradiate to thymus without any exposure to the other organs, therefore in present study only the function of spleen cells was investigated.

The IL-2 response of spleen cells by incorporation of $^3\text{H-TdR}$ was investigated as shown in Fig. 5. In the figure each symbol stands for the differences in incubation time of spleen cells, that is, the open circles suggest 48 hours', closed circles are 72 hours' and closed triangles demonstrates 96 hours' incubation, respectively. The three symbols in right hand side show the control values of non-tumor bearing (control) mice in each incubation time. It is clear from this figure that 10 cGy of TBI increases IL-2

response of spleen cells in tumor bearing mice. Especially in case of 96 hours' incubation IL-2 response raised almost twice as much compared to the control. However, this phenomenon caused by low dose of TBI may be specific only to WHT/Ht mice and tumor system used in present study, so the same kinds of experiments were performed using B16 melanoma in C57 black mice. The results are shown in Fig. 6, and the enhancement of IL-2 response in mice received 10 cGy of TBI is demonstrated also

Table 1. Tumor cure rate treated by combined methods of TBI and local irradiation. The upper column shows the cure rate of combined treatment of 10cGy of TBI and local irradiation of 35Gy at 12 hours after TBI. The middle column is the cure rate of tumors received combined treatment of 5 times of 10cGy of TBI in every 6 hours and local irradiation of 35Gy at 12 hours after the last TBI. The Lower column is the result of local irradiation of 35Gy only.

Irradiation	$\frac{\text{Total number of cured tumor}}{\text{Total number of irradiated tumors}}$	Control rate of tumors
10cGy(TBI) + 35 Gy(local)	7/18	38.9%
10cGy(TBI)x5 + 35 Gy(local)	7/14	50.0%
35Gy(local) only	3/13	23.1%

* tumor size at irradiation time was 4-5 mm in diameter

Table 2. The survival fractions of tumor cells irradiated with combined treatment of 10cGy and 10Gy of local irradiation after blocked various parts of body.

Regions exposed to 10 cGy	Surviving fraction (\log_{10})
1. Spleen-blocked	$\bar{2}.17 \pm 0.32$
2. Tumor	$\bar{2}.12 \pm 0.27$
3. Spleen	$\bar{3}.75 \pm 0.36$
4. Total body	$\bar{3}.66 \pm 0.30$
5. Upper thoracic region	$\bar{2}.07 \pm 0.27$
10 Gy of local irradiation only	$\bar{2}.09 \pm 0.30$

in B16 melanoma in C57 black mice, though the control values differ from WHT/Ht mice.

In Fig. 7 the effects of TBI on Con-A response, PHA response, IL-2 response and MLR are shown in altogether. All of these response are potentiated their response by 10 cGy of TBI.

7) Change in Ratios of Phenotypes of Spleen Cells in Tumor Bearing or Non-tumor Bearing Mice Received 10 cGy of TBI

It is very interesting what kinds of phenotype of spleen cells will be affected by 10 cGy of TBI. In

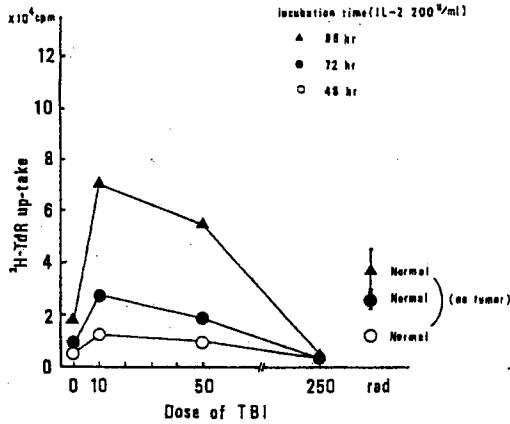


Fig. 5: IL-2 response of spleen cells in WHT/Ht albino mice bearing squamous carcinoma. IL-2 response was investigated by uptake of ³H-TdR uptake.

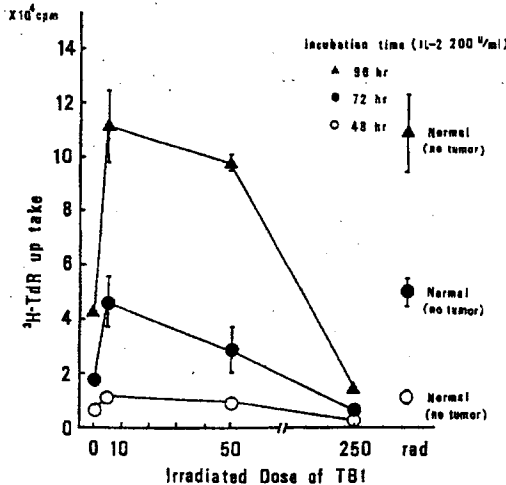


Fig. 6: IL-2 response of spleen ceels on C57 black mice bearing B16 melanoma investigated by ³H-TdR uptake.

Fig. 8 white columns suggest the values of tumor bearing mice and the shaded columns are the values tumor bearing mice received TBI. TBI is seemed to suppress mac-2 positive cells. The other phenotypes are also suppress by TBI except L3T4, but the suppressing effect of TBI are not so clear compared to mac-2. The phenotypes of mac-1 and mac-2 are not detectable in non-tumor bearing mice as shown Fig. 9, and in non-tumor bearing mice TBI does not make any change in the percentage of phenotypes of spleen cells⁷⁾.

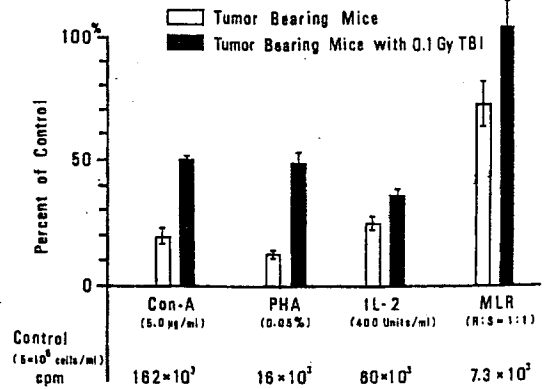


Fig. 7: The change of immuno-response of splenocytes, i.e. Con-A, PHA, IL-2 responses and MLR were studied by uptake of ³H-TdR incorporation into spleen cells.

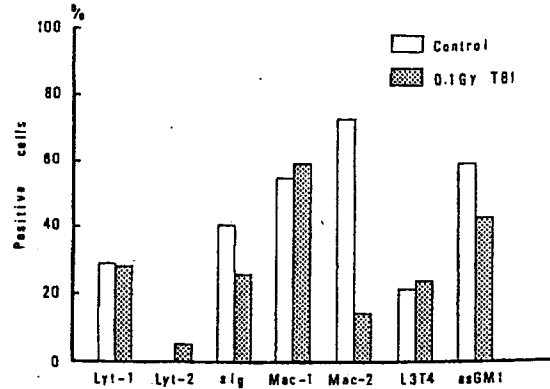


Fig. 8: The change of phenotype of spleen cells in tumor bearing mice received 10 cGy of TBI.

8) Effect of TBI on Metastasis

The effect of TBI on metastasis has been described in details in elsewhere¹², therefore in present review states briefly.

(1) The effect to artificial metastasis

In order to investigate the effect of TBI on artificial metastasis, lung colonies produced by tumor cell injection from tail vein of mice were used. The lung colony forming abilities of tumor cells injected through tail vein was most suppressed, when around 15 cGy of TBI was given at 9 hours after cell injection as shown Fig. 10. The dashed line is the control value obtained from non-irradiated mice and the solid line connected closed circles shows the ratio of colony forming abilities of tumor cells in mice received TBI and non-irradiated mice. In Fig. 11 each closed circle expresses the ratio of the number of lung colonies in total-bodily irradiated mice and in mice of non-irradiated controls. It is clear from the figure that the most suppressive effect on colony forming ability of mice given TBI is shown in period between 12 hours before tumor cell injection and 12 hours after tumor cell injection.

(2) The effect of TBI on formation of spontaneous metastasis

Artificial metastasis produced through blood vessels are not reflect usual

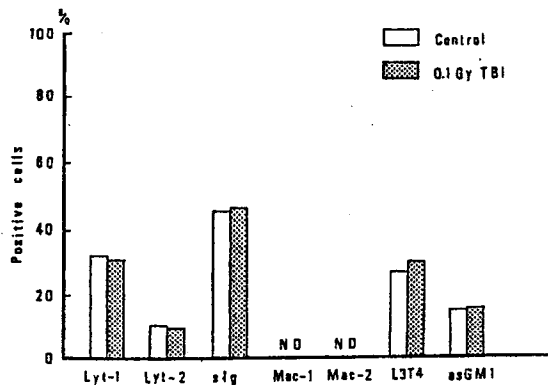


Fig. 9: Phenotype to splenocytes in non-tumor bearing mice.

metastasis because in human tumors occurred metastasis through lymphatic flow, the effect of TBI on metastatic formation in mice, in which lung metastasis arise spontaneously, was investigated. In this experiments mice received TBI of various doses at 15 days after tumor cell injections into both axilla of mice, and lung colonies were counted at 20 days after TBI. The results in shown in

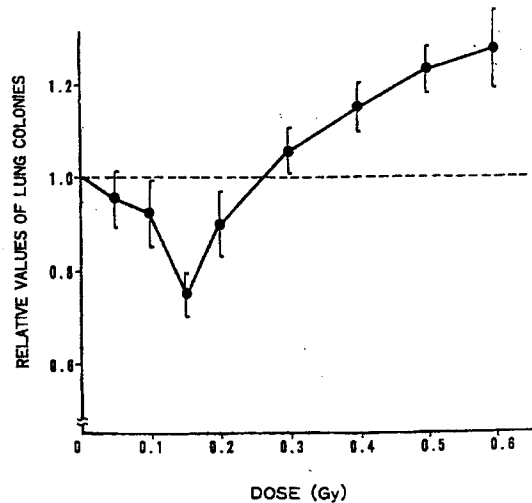


Fig. 10: The relative values of lung colonies in mice exposed to various doses at 9 hours before tumor cell injection from tail vein.

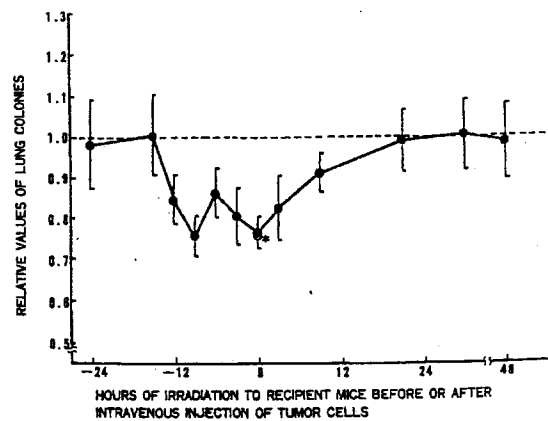


Fig. 11: The effect of 15 cGy of TBI on artificial lung colony formation. TBI were performed at various hours before or after tumor cell injection from tail vein.

Fig. 12. It is explicit from the figure that the dose of around 15 cGy demonstrates most rejective effect of lung colony formation.

CLINICAL STUDIES

Before starting programmed clinical trials, the effect of 10 cGy of TBI was studied on the patient with advanced ovarian tumor. The patient had already recognized tumor cell infiltration into sigmoidal, rectal and peritoneal regions when ovarian tumor was found. Gynecologists removed main tumor of ovarium, then they asked radiotherapy as palliative treatment for residual tumor including metastasis in regional lymphnodes. Gynecologists judged as impossible to live more than 6 months after operation, even if radiotherapy was able to allay patient's distress. Therefore, the combined treatment of TBI and local irradiation was planned to the patient, i.e. 1.5 Gy to abdominal region as an individual dose was given at 6 hours after 10 cGy of TBI. TBI was given three times a week and total dose of TBI did not exceed 1.5 Gy and local irradiation was performed 5 times a week. The result of the treatment was so successful, the patient lived more than two years after surgical operation in spite of having many distant metastasis. The patient died by colon stenosis which might be occurred by irradiation to abdominal region, however no

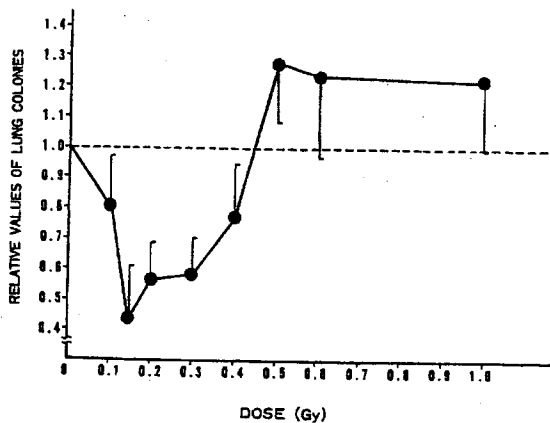


Fig. 12: The effect of various doses of TBI on spontaneous lung metastasis. TBI was given at 12 days after tumor cell transplantation into groins.

cancers were detected in any place by pathological autopsy. Then, several patients were tried to treat by combination of TBI and local irradiation and the results of these patients were expected good prognosis. Therefore, clinical study were designed, and malignant lymphoma was chosen as the first choice of diseases to be put to clinical trials. In clinical trial 6MV linac X-ray was used.

1. Clinical Trial on Malignant Lymphoma

1) Aim of the Trial Using Malignant Lymphoma

The reason why malignant lymphoma was selected as the tumor for the first clinical trial was that malignant lymphoma is generally considered to be possibility to have distant metastasis when primary was discovered. Ten cGy or 15 cGy of TBI or HBI (half body irradiation) suggests to disturb distant metastasis and potentiating effect in cell killing of tumors when used combined treatment of TBI and local irradiation was made clear from the fundamental research as already stated. Clinically, many of malignant lymphoma can estimate objectively decreasing of tumor volume by treatment, so malignant lymphoma was used first clinical trial.

2) Treatment Schedule

TBI or HBI was given by 15 times of 10 cGy for 5 weeks or 10 times of 15 cGy for 5 weeks, then followed by local irradiation up to 60 Gy as the total dose. In combined treatments of TBI or HBI and local irradiation was given around 6 hours after TBI or HBI of 10 or 15 cGy. TBI or HBI was delivered three or two times a week. Almost all the case received cancer chemotherapy after completion of radiotherapy except several cases.

The immunological examination by two color methods were performed three times, that is before, in mid-day and after treatments.

2. Results

The survivals of stage I and II of non-Hodgkin's lymphoma treated by local irradiation alone or combined treatment of TBI and local

irradiation are demonstrated in Fig. 13. The upper figure of Fig. 13 stand for the overall survivals, and overall survivals at 5 years after treatment is better statistically in combined therapy group than local irradiation only with 5% of risk factor. The lower left figure shows the cause-specific survival curve and the lower right figure is disease-free survival curve, respectively. In each survival curve, the results of combined treatment are more effective in 5 year survivals than local irradiation only and there is significantly differences between them.

Fig. 14 demonstrated the cause-specific survival of non-Hodgikin's lymphoma of stage I and II. The results of treatment of stage I is better than stage II, however in each stage the combined treatment are obtained better results than local irradiation only, though total number of patients may not be enough in combined treatment for statistical analysis.

Fig. 15 is the cause-specific survival by differences in histological grade of stage I and II of non-Hodgikin's lymphoma. It is clear that the low grade of lymphoma shows higher survivals

than intermediate and high grade, and the combined treatment looks like more effective than the local irradiation only.

Fig. 16 is the results of analysis of treatment to intermediate and high grade's malignant lymphoma of stage I and II. The upper figure, lower-left and lower-right figures are overall, cause-specific and disease-free survivals, respectively.

In each case, the combined treatment of TBI or HBI and local irradiation suggests good results with statistically differences compared to local irradiation only.

The comparison of results between the combined treatment of TBI or HBI and local irradiation and the combined therapy of chemotherapy and local irradiation for stage I and II of malignant lymphoma is shown in Fig. 17, and as shown in the figure the former is seemed to be more effective than the latter.

Table 3 suggests the change of fundamental subsets of peripheral lymphocytes and the blood cell number before or after TBI or HBI. In the table helper T, helper-inducer T and the ratio of

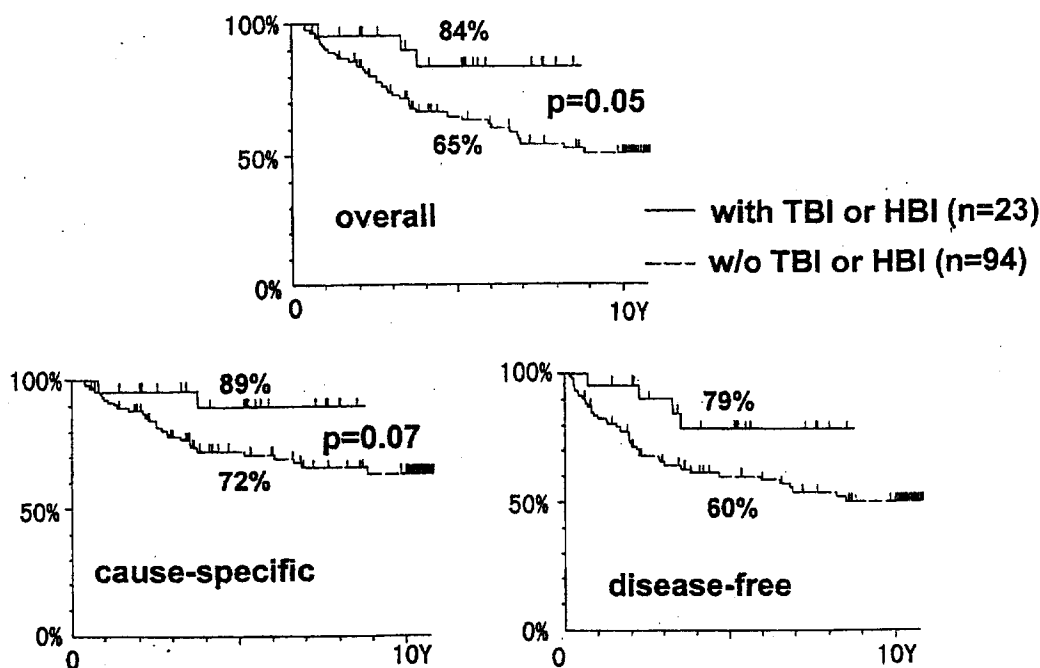


Fig. 13: Survivals of stage I and II of non-Hodgikin's lymphoma treated local irradiation only or combined treatment of TBI and local irradiation.

cause-specific

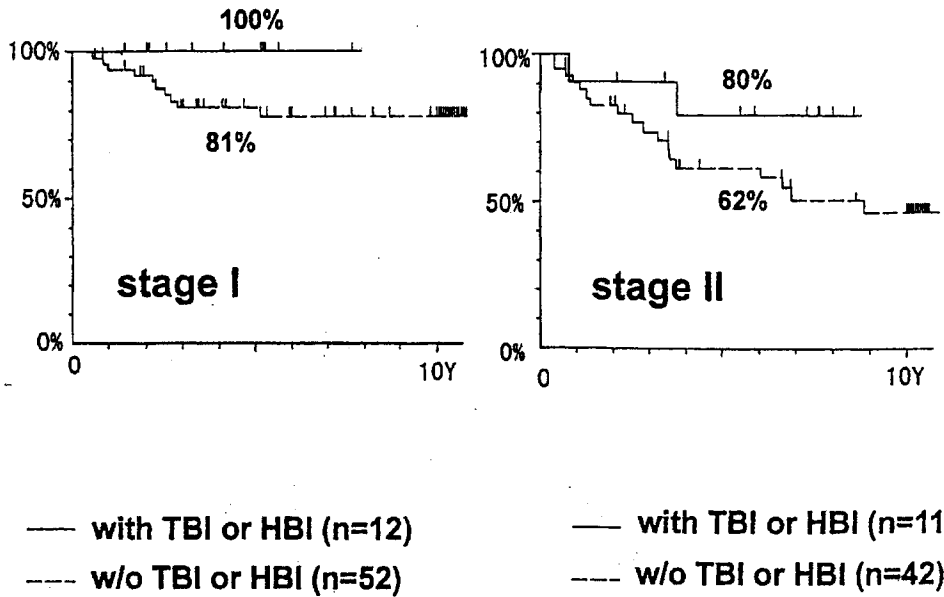


Fig. 14: The cause-specific survivals of non-Hodgkin's lymphoma of stage I and stage II respectively, treated local irradiation only or combined treatment.

cause-specific

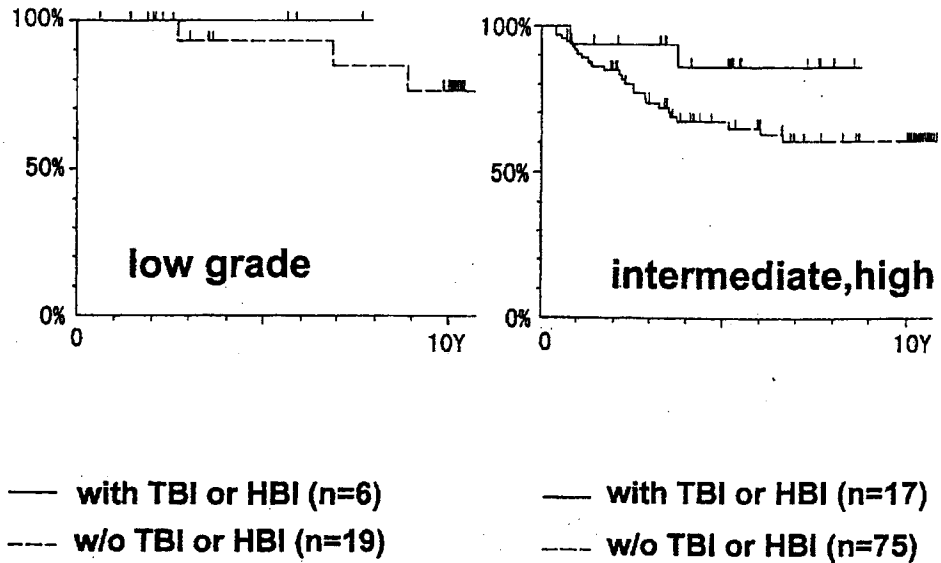


Fig. 15: The cause specific survivals of non-Hodgkin's lymphoma of stage I and II and of histologically low and intermediate, high grade treated by local irradiation or combined therapy.

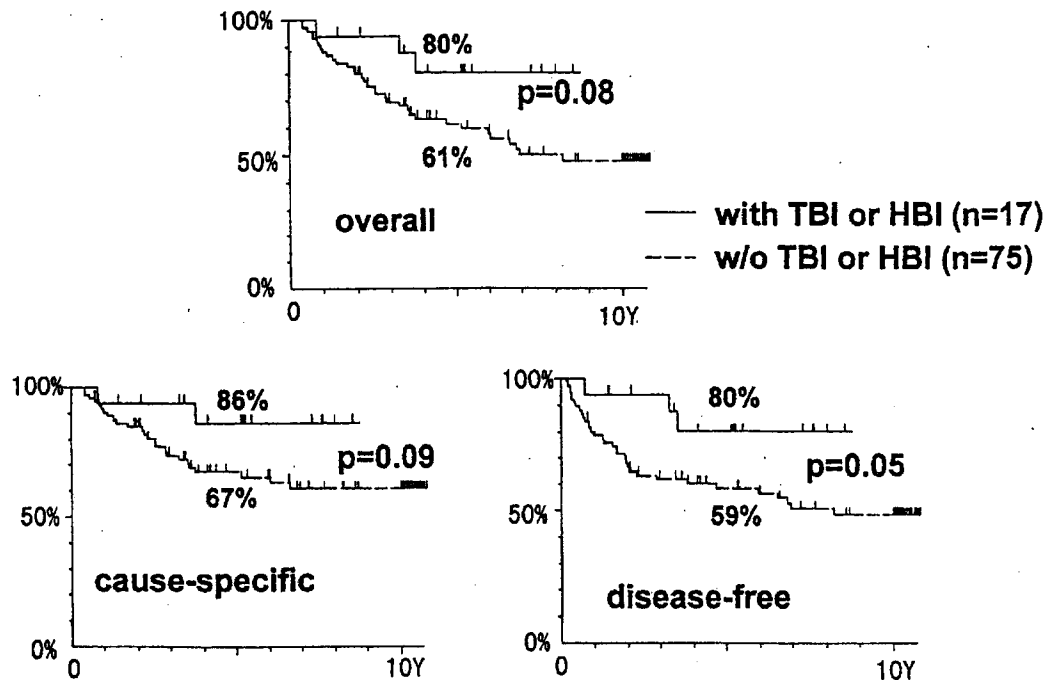


Fig. 16: The survivals of patients with non-Hodgkin's lymphoma of stage I and II and of histologically intermediate and high grade' tumor treated by both treatment ways.

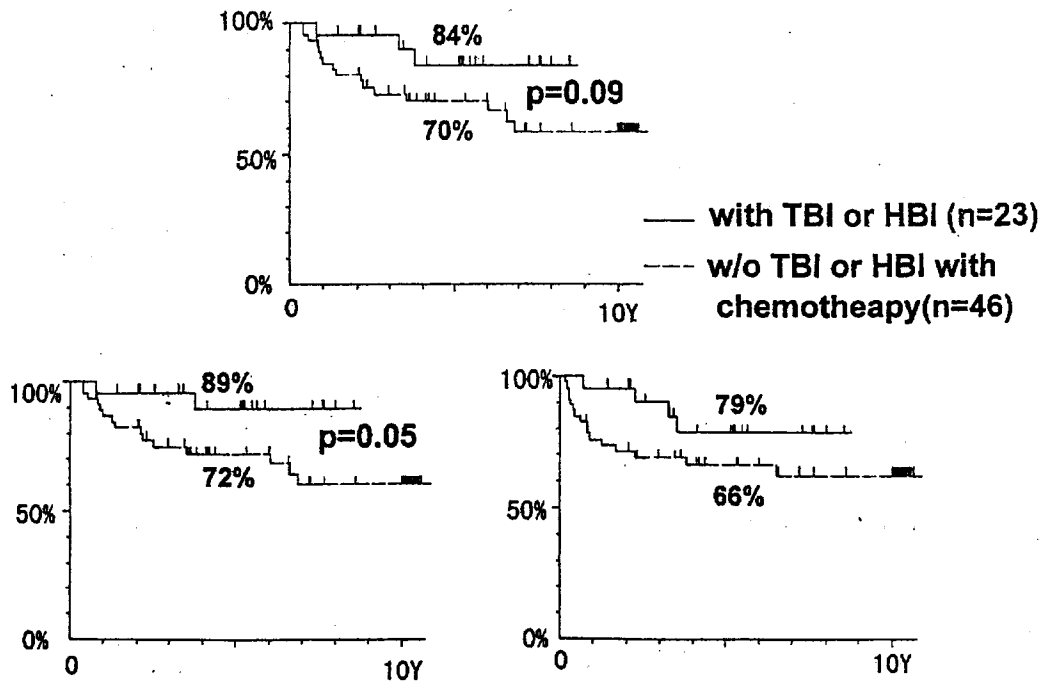


Fig. 17: Comparison in survivals of patients with non-Hodgkin's lymphoma of stage I and II treated by combined therapy of TBI and local irradiation or by combined therapy of local irradiation and chemotherapy.

Table 3. Alteration of subsets of lymphocytes in patients treated with combined treatment methods of TBI and local irradiation investigated by two color method.

Subsets of lymphocytes	Ratio in subsets of lymphocytes	
	Before TBI or HBI	After TBI or HBI
Suppressor-inducer T (CD4 ⁺ 2H4 ⁺)	8.9 ± 4.3 %	7.5 ± 5.7 %
Helper T (CD4 ⁺ 2H4 ⁺)	27.1 ± 7.7 %	33.1 ± 7.7 % [*]
Helper-inducer T (CD4 ⁺ 4B4 ⁺)	23.7 ± 6.8 %	28.7 ± 7.2 % [*]
Suppressor T (CD8 ⁺ CD11 ⁺)	9.7 ± 6.6 %	9.3 ± 4.9 %
Cytotoxic T (CD8 ⁺ CD11 ⁻)	21.0 ± 8.5 %	21.4 ± 6.9 %
Active helper/inducer T (CD4 ⁺ HLA-DR ⁺)	4.1 ± 1.6 %	6.8 ± 2.5 % [*]
Active suppressor/cytotoxic T (CD8 ⁺ HLA-DR ⁺)	8.3 ± 5.6 %	10.8 ± 7.4 %
NK activity (++) (CD16 ⁺ Leu7 ⁻)	5.9 ± 2.4 %	5.4 ± 2.5 %
NK activity (+) (CD16 ⁺ Leu7 ⁺)	14.3 ± 9.4 %	14.0 ± 7.6 %
NK activity (+) (CD16 ⁻ Leu7 ⁺)	21.0 ± 9.2 %	20.0 ± 8.0 %
Normalized helper T/Suppressor T ratio	1.0	1.42 ± 0.86 [*]
Number of blood cells	Number of blood cells	
	Before TBI or HBI	After TBI or HBI
Blood platelet (X 10 ⁴)	22.0 ± 6.0	16.6 ± 6.1 [‡]
WBC	5395 ± 1239	4000 ± 1424 [‡]
Lymphocyte	1408 ± 517	898 ± 434 [‡]
Helper T (actual number)	400 ± 215	300 ± 172
Helper-inducer T (actual number)	343 ± 173	256 ± 143
Active helper/inducer T (actual number)	51 ± 23	53 ± 29

* : p < 0.05 † : p < 0.01

active helper and inducer T show the higher value after TBI or HBI compared with their values obtained before TBI or HBI, though absolute number of lymphocytes decreases by TBI or HBI. The two color method was used in order to check the change of these kind of cells because the two color method is the only one way in Japan to study immunological background of patients.

DISCUSSION

Total body irradiation was considered to cause immunosuppressive effect even if the dose of TBI was so small dose. However, such conclusion is

derived from extrapolation of the data obtained from TBI of sublethal dose to recipient animals as used in tissue transplantation experiments to erase immunological response.

In fundamental studies, suppressor T cells are known to be relatively radiosensitive comparing the other cells related to immunological response in animal experimental system, therefore suppressor T cells decrease their ratio to the other immunologically related cells by TBI. By use of this phenomenon the potentiating effect of radiation on tumor control are reported by several investigators¹³⁻¹⁵, though the optimal doses of TBI to cause potentiating effect reported in these

studies disperse from 20 cGy to 4 Gy. Anderson, R.E. et al. reported that growth control effect of tumors were recognized if recipient mice were exposed to 5 or 25 cGy of TBI in 5 hours before or just before tumor cell transplantation^{(16), (17)}. However, in our study the effect of TBI was recognized during 12 hours before and after tumor cell transplantation. Anderson, et al. assumed the effect of low doses of TBI as due to high radiosensitivities of suppressor T cell or its precursor cells, though there are no experimental data to certify their assumption. In present study 10 cGy of TBI was shown increasing effect of tumor control in combination with local irradiation on tumors at 5 to 15 hours after TBI. In the experiments examined the effect of TBI on artificial or spontaneous metastasis in murine experimental system TBI suggested to suppress distant metastasis, that is, lung colony formation by tumor cells was disturbed. These effect may be attributed to immunopotentiating effect produced by low dose of TBI. In present study the various endpoints to be reflected immunological response like Con-A, PHA, IL-2 and MLR responses were investigated. As a results the effects of TBI suggested to be related deeply to enhance immunological abilities of tumor bearing mice.

The combined treatment of TBI and local irradiation demonstrates potentiating effect in cell killing effect in larger dose range in spite of no detection of cell killing by 10 cGy only of TBI. This phenomenon may be derived from reoxygenation of anoxic tumor cells caused by low dose of TBI. However, generally speaking, reoxygenation of anoxic tumor cells occurs by exposure of more than 10 Gy to tumors, and it is needed at least 24 hours to detect reoxygenation except very few murine tumors. The murine epithelioma in WHT/Ht mice used in present study did not detected until 36 hours after 10 of Gy local irradiation to tumors without 10 cGy of TBI⁽⁸⁾.

Therefore, the potentiating effect of cell killing in combined treatment is difficult to consider as a result of reoxygenation of tumor cells. Anyway, no cell killing effect detected by 10 cGy of TBI only, as stated above, so the potentiating effect caused by TBI is seems to be due to some change host's radiosensitivity. Gerber, M et al. are reported that exposure to the spleen of tumor

bearing mice brought about the decreasing of activities of killer T cells in case of the dose more than 15 cGy, but in dose range between 5 to 12 cGy the activities of killer T cells are increased. And the results suggest to bring quite opposite effects by a little differences in dose exposed to spleen^{(17), (18)}. In our experiments recipient mice exposed to more than 25 cGy of TBI show low values of TD50, but in recipient mice received 10 cGy of TBI or exposure to 10 cGy only to spleen high values of TD50 were demonstrated. And the local irradiation to tumors after 10 cGy of TBI caused tumor growth delay compared to tumors exposed to local site only, and prolongation of life span of tumor bearing mice treated combined irradiations was recognized.

In present study, the effect of TBI was examined only on the basis of tumor immunology, and the other factors which may occur this kind of effects were not investigated. Therefore, it may not be able to conclude the effect of TBI as due to only immunopotentiating effect. However, the change of immunological background by low dose of TBI seems to be related deeply to cause potentiating effect.

Concerning clinical study, Chaffy, J.T. et al. reported that TBI of smaller dose than 25 cGy was effective for tumor control of lymphosarcoma⁽¹⁾, and Choi, N.C. et al. also reported to be effective to use TBI of low doses in treatment of advanced non-Hodgkin's lymphoma⁽²⁾. Holder, D.L. was also recognized that multiple myeloma was treated successfully by low dose of TBI⁽⁹⁾. In our clinical studies to malignant lymphoma, the combined treatment of 10 cGy of TBI and local irradiation, or even if used only TBI prospective results are suggested as shown in Fig. 13-17, though it is not sure whether or not immunological mechanism are working in human being as well as in murine experimental system. However, investigation using two color method suggests to be enhanced immunological abilities by TBI or HBI.

We are now trying to treat solid tumors like tongue cancer, lung cancer, esophagus cancer and uterine cervical cancer etc. by combined treatment. But the results are not obtained still now, because of small number of patients recruited to this trial and shortage to observation time.

CONCLUSION

The fundamental and clinical studies concerning the effect of TBI or HBI are shown in present paper.

In fundamental studies TBI of low doses (10 or 15 cGy) brought about potentiating effects in cell-killing of local irradiation when local irradiation was given at 6-15 hours after TBI or HBI. TBI or HBI also suppressed lung colony formation of tumor cells produced by tumor cell injection into tail vein (artificial metastasis) and the formation of lung colonies occurred as spontaneous metastasis in mice generates usually lung metastasis in high incidence. These effects were considered to be reactivated decreased tumor immunological abilities in tumor bearing mice, though all kinds of tumor immunological investigations were not performed.

In clinical studies, malignant lymphoma was firstly selected as disease to be tried and its results suggest to be prospective, and now the other several tumors are trying to treat with combined therapy.

Anyway, the combined treatment of TBI or HBI and local irradiation is able to expect to bring good results in tumor cure rate in radiotherapy regime by enhancement of local control rate and suppressing distant metastasis.

REFERENCES

- 1) Chaffey, J.T., Rosenthal, D.S., Moloney, W.C. *et al.*: Total body irradiation as treatment for lymphosarcoma. *Int. J. Radiat. Oncol. Biol. Phys.* 1: 399-405, 1976.
- 2) Choi, N.C., Timothy, A.R., Raufman, S.D., *et al.*: Low dose fractionated whole body irradiation in the treatment of advanced non-Hodgkin's lymphoma. *Cancer* 43: 1636-1642, 1979.
- 3) Hewitt, H.B., Chen, D.P. & Blake, E.R.: Survival curves for clonogenic cells of a murine keratinizing squamous carcinoma irradiated in viva or under hypoxic conditions. *Int. J. Radiat. Biol.* 12: 535-549, 1967.
- 4) Hewitt, H.B. & Sakamoto, K.: The comparative survival of clonogenic cells of a murine epithelioma after irradiation in mice breathing air, oxygen and carbon dioxide, or hyperbaric oxygen. *Brit. J. Radiol.* 44: 457-463, 1971.
- 5) Hewitt, H.B.: The effect on cell survival of inhalation of oxygen under high pressure during irradiation in viva of a solid mouse sarcoma. *Brit. J. Radiol.* 39: 19-24, 1966.
- 6) Litchfield, J.T. Jr. & Wilcoxon, F.: A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* 96: 99-133, 1949.
- 7) Miyamoto, M. & Sakamoto, K.: Anti-tumor effect of total body irradiation of low doses on WHT/Hi mice. *Jap. J. Cancer Clin.* 33: 1211-1220, 1987.
- 8) Sakamoto, K., Miyamoto, M. & Watabe, N.: The effect of low dose total body irradiation on tumor control. *Jpn. J. Cancer Chemother.* 14, Part. II: 1545-1549, 1987.
- 9) Sakamoto, K. & Miyamoto, M.: Potentiating effect of low dose total body irradiation on tumor control (低線量全身照射による腫瘍効果の増強). *Oncologia.* 20: 86-88, 1987.
- 10) Sakamoto, K., Miyamoto, M., Watabe, N. *et al.*: Fundamental studies of low dose total body irradiation on tumor control. *Jap. J. Cancer Clin.* 33: 1633-1638, 1987.
- 11) 坂本澄彦, 高井良尋, 根本建二, 他: 低線量全身照射による治療. 血液・腫瘍科 21: 447-453, 1990.
- 12) Hosoi, Y. & Sakamoto, K.: Suppressive effect of low dose total body irradiation on lung metastasis: dose dependency and effective period. *Radiotherapy and Oncology.* 26: 177-179, 1993.
- 13) Tikin, A.F., Schaaf-Lafontaine, N., Acker, A.V., *et al.*: Reduced tumor growth after low-dose irradiation or immunization against blastic suppressor T cells. *Proc. Natl. Acad. Sci.* 78: 1809-1812, 1981.
- 14) Hellstrom, K.E., Helstrom, I., Kant, J. A. *et al.*: Regression and inhibition of sarcoma growth by interference with a radio-sensitive T cell population. *J. Exp. Med.* 148: 799-804, 1978.
- 15) Anderson, R.F., Tokuda, S., Williams, W. L., *et al.*: Radiation-induced argumentation of the response of A/J mice to Sa-I tumor cells. *Am. J. Pathol.* 108: 24-37, 1982.
- 16) Anderson, R.E., Tokuda, S., Williams, W.L. *et al.*: Low dose irradiation permits immunization of A/J mice with subimmunogenic numbers of Sa-I cells. *Brit. J. Cancer.* 54: 505-509, 1986.
- 17) Gerber, M., Dubois, J.B., Pioch, Y. *et al.*: Effect of localized radiotherapy upon the cellular immune response. *Radiat. Res.* 85: 390-398, 1981.
- 18) Gerber, M., Dubois, J. B. & Serrou, B.: Effect of low doses of irradiation on the T cell mediated cytotoxic response: immunopharmacologic effect of radiation therapy, Raven Press, 53-74, 1981.
- 19) Holder, D. L.: Total body irradiation in multiple myeloma. *Radiology.* 84: 83-86, 1965.

SURVIVAL OF PATIENTS WITH NON-HODGKINS LYMPHOMA WITH OR WITHOUT LOW-DOSE HALF (HBI) OR TOTAL (TBI) BODY IRRADIATION

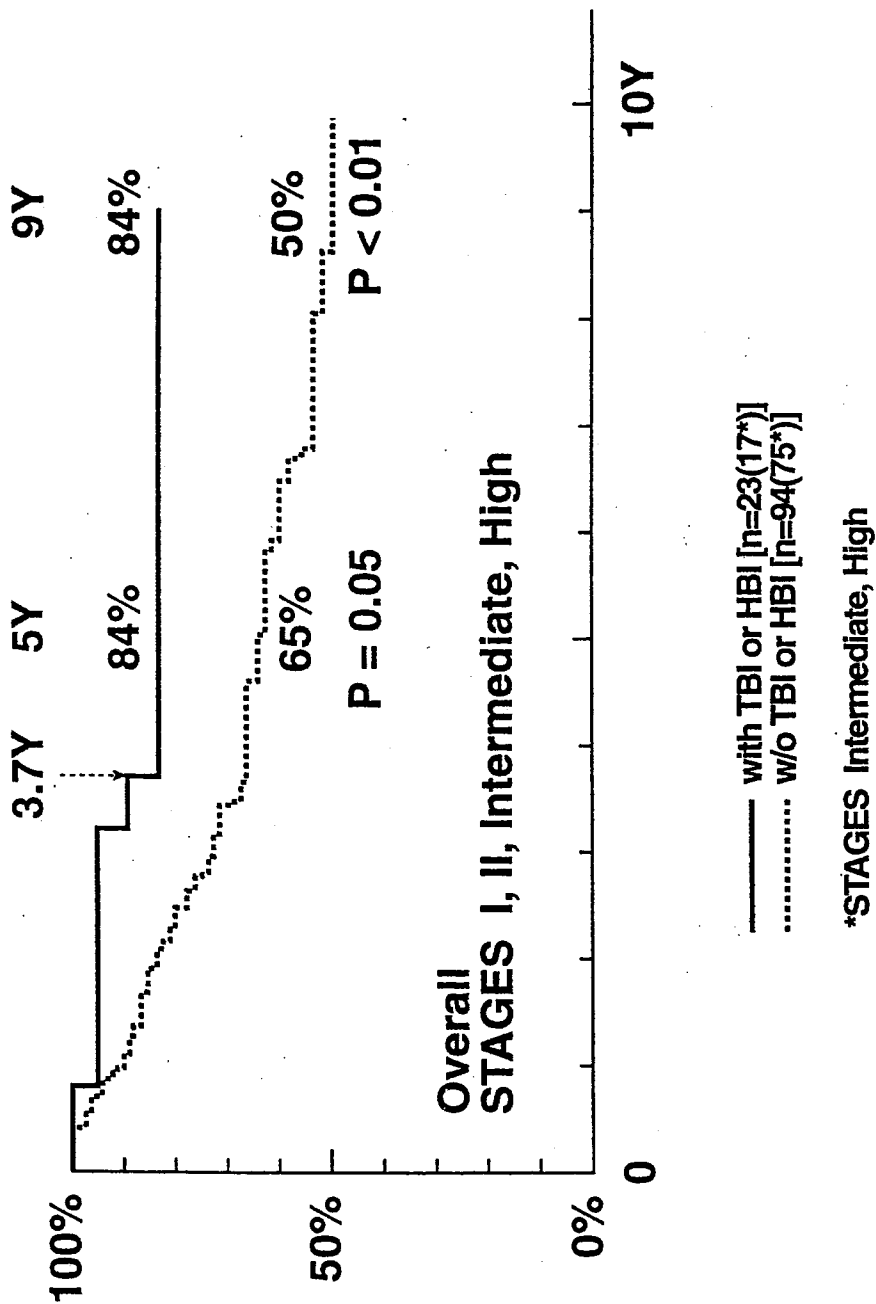


Figure 20. Adapted from Sakamoto, et. al. J Jpn Soc Ther Radiol Oncol 9:161-175, 1997