

# Elimination of Metastatic Neuroblastoma from Bone Marrow Using Magnetic Immunobeads with Newly Produced Antibodies to Neuroblastoma Cells

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Removal of metastatic neuroblastoma cells using magnetic immunobeads with newly produced antibodies were investigated. Magnetic immunobeads were M-450 (DYNAL) coated with purified sheep polyclonal IgG against all mouse IgG subclass. Two newly produced antibodies (5E9 and 3C1) and other two antibodies were used. Four antibodies were added to a mixture of the neuroblastoma cells stained with Hoechst 33342 with the human bone marrow cells. The magnetic immunobeads were added to the cell mixture. After incubation for 30 min at 4 °C, the magnetic beads reacted to the neuroblastoma cells were removed by using cobalt samarium magnets. The residual neuroblastoma cells were assayed under the fluorescence microscopy, and the clonogenic capacity of the bone marrow progenitor cells were measured by culturing in the soft agar assay. A ratio of neuroblastoma cells to normal bone marrow cells was 1:10 and a ratio of magnetic immunobeads to neuroblastoma cells was 100:1. A tumor cells depletion rate of 1.9-3.8 logs was achieved using the cocktail antibodies with 5E9, 3C1, NCC-LU-243 and NCC-LU-246 and second cycle treatment. In a clinical cell separation unit 2.81 log removal rate of tumor cells was obtained. Residual rate of human normal bone marrow cells after second cycle purging was 17.1%. Reduction in the clonogenic bone marrow progenitor cells was about 10%.

This purging method in use the procedure with the magnetic immunobeads and neuroblastoma cell antibodies seems to offer advantage with respect to speed and simplicity. By use of suitable antibodies, the immunomagnetic tumor cell depletion method is useful in autologous bone marrow transplantation of advanced neuroblastoma children with poor prognosis. Newly produced antibodies 5E9 and 3C1 are of great use in the immunomagnetic tumor cell depletion procedure.

## Introduction

Several groups have reported autologous bone marrow transplantation (ABMT) for advanced cancer with poor prognosis after conventional therapy.<sup>1-8)</sup> ABMT is of help in

preventing prolonged aplasia after chemotherapy and radiotherapy. Since the bone marrow is frequently invaded by tumor cells in such cases, it is important to remove the metastatic malignant cells from the harvested bone marrow before transplantation. A number of techniques have been studied to deplete metastatic malignant cells from the harvested bone marrow for ABMT. In this study the magnetic immunobeads method was evaluated with newly produced monoclonal antibodies (3C1 and 5E9) to neuroblastoma cells. Human neuroblastoma cell lines were seed into normal human bone marrow, incubated with monoclonal antibodies, and mixed with magnetic immunobeads. Tumor cells that attached to the magnetic beads were removed with cobalt magnets from the normal bone marrow (Fig. 1). The purging procedure was scaled up to the clinical use model. Removal rate of the tumor cells was achieved 1.9-3.8 logs. It was confirmed the newly produced antibodies did not affect normal bone marrow cells was observed.

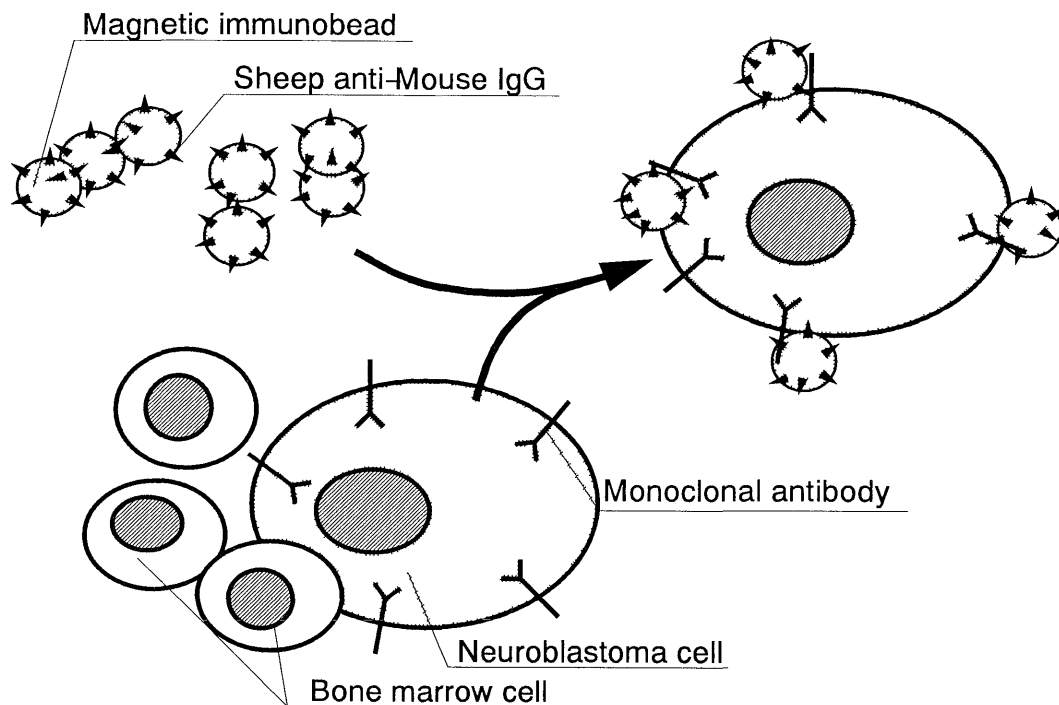
## Materials

### (1) Magnetic immunobeads:

DYNABEADS M-450 (DYNAL) products are based on extremely uniform, superparamagnetic polystyrene beads with diameter 4.5 micron, a surface area of 3-5m<sup>2</sup>/g. A 20% Fe in the form of a maghemite (Fe<sub>2</sub>O<sub>3</sub>) containing core covered with a polymer is easily coated with antibodies or other selection molecules on the smooth surface. The beads coated with purified sheep polyclonal Ig against all mouse IgG subclasses were used in the study. 4 × 10<sup>8</sup> DYNABEADS per ml is suspended in pH 7.4 PBS.

### (2) Neuroblastoma cell lines:

Six neuroblastoma cell lines (1. GOTO 2. Nb/IN 3.SJ-NCG 4.RT/LN 5.IMR-32 6.ST-300) were prepared for this study. Cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). Cultures were diluted frequently to assure that cells were proliferating.



**Fig. 1.** Indirect procedure of magnetic immunobeads depletion. Magnetic immunobeads coated with sheep anti-mouse IgG are added to monoclonal antibodies selective for target neuroblastoma cells. Neuroblastoma cells attached to magnetic immunobeads can be removed with a magnet.

erating optimally.

*(3) Monoclonal antibodies:*

In this study four monoclonal antibodies were used (Table 1). Antibodies NCC-LU-243 and NCC-LU-246 were established in the laboratory of the NIHON-KAYAKU Co. Ltd. Each reactivities of the antibodies were human lung small cell carcinoma, and immunoglobulin subclass were IgG<sub>2a</sub> and IgG<sub>1</sub>. Molecular weight of each antibodies were 145kd by SDS-PAGE. Other two monoclonal antibodies 5E9 and 3C1 were newly produced in the National Children Medical Research Center in Tokyo. The 5E9 and 3C1 were an IgG<sub>2a</sub> and an IgG<sub>1</sub> mouse monoclonal antibody obtained by immunization with immature teratoma cell lines

**Table 1.** Characteristic of monoclonal antibodies

Name of antibody	Subclass	Reactivity
5E9	IgG <sub>1</sub>	Anti-human immature teratoma
3C1	IgG <sub>2a</sub>	Anti-human immature teratoma
NCC-LU-243	IgG <sub>2a</sub>	Anti-human small cell lung cancer
NCC-LU-246	IgG <sub>1</sub>	Anti-human small cell lung cancer

*(4) Bone marrow:*

Bone marrow cells for this study were obtained from the patients undergoing bone marrow aspiration. No patients had bone marrow malignant involvement at smears. Mono-

nuclear cells (MNCs) were isolated on the Lymphoprep according to gradient centrifugation. MNCs were resuspended in RPMI 1640 medium with 20% FCS

**Methods**

*(1) Determination of Monoclonal antibodies binding to neuroblastome cell lines:*

Neuroblastoma cell lines were grown in RPMI 1640 with 10% fetal calf serum at 37 °C . Cell lines were detached in TRY (-) 8mM-EDTA (+) -PBS for 30 min and suspended. The cells were centrifuged and the pellet was resuspended to regulate 1 × 10<sup>6</sup>/ml in PBS. We used the cells viability more than 70% with experiment of 5% trypan blue stain. The suspended cells were washed twice with PBS. After the cells were pelleted by centrifugation, the wash solution was aspirated, and the pellets were resuspended with PBS. The monoclonal antibody was added to the cells in a final volume of 1ml in a tube. The tubes were incubated at 4 °C for 30min, washed twice with PBS, and added FITC. After incubated on 4 °C for 30min the cells was washed twice with PBS and then analyzed with the FCM (EPICS). The amount of each antibodies to pipette into the sample were 64 μg, 32 μg, 16 μg, 8 μg, 4 μg, 2 μg, 1 μg, 0.5 μg, 0.25 μg, 0.125 μg and 0.0625 μg. Control sample was added with non-specific antibody 10B5.

(2) *Evaluation of single antibody and cocktail antibodies binding to tumor cells:*

Good effect of cocktail antibodies to tumor cells have been reported.<sup>2,3,9-11</sup> At the clinical treatment of the purging procedure the antibody have been used three or four antibodies specific for the tumor cells. To determine in combination with cocktail of antibodies (5E9, 3C1, NCC-LU-243 and NCC-LU-246) binding to tumor cells, we compared the individual antibody with the cocktail antibodies binding to tumor cells.

(3) *Analysis of residual tumor cells:*

Culture assays is not sufficient sensitive to detect residual malignant cells. In this report we use the stain method of the Hoechst 33342 described by Reynolds.<sup>12</sup> It can be detected up to 3-4 log depletion under microscopic analysis immediately and repeatedly. Tumor cell lines were incubated for 90min at 37 °C with the vital DNA dye Hoechst 33342 in the final density 20  $\mu$ M. After washing the tumor cells with PBS three times, the cells were detached with 5mM EDTA-Try (-) PBS. The tumor cells were seeded into bone marrow cells 5-10%. After purging procedure, residual viral tumor cells were counted under the fluorescence microscopy. Counterstaining the residual tumor cells with 5% vital dye trypan blue limits the detection of viral tumor cells, because trypan blue penetrate into dead cells and quenches the DNA Hoechst 33342 stain.

(4) *Determination of the volume of the magnetic immunobeads:*

To evaluate optimal tumor cell and magnetic immunobeads ratio, neuroblastoma cells prestained with Hoechst 33342 in  $1 \times 10^6$ /ml per a tube were reacted with antibodies and then incubated on 4 °C for 30min. After washing twice with PBS, several numbers of magnetic beads were added to samples and incubated on 4 °C for 30min. Tumor cells binding to magnetic beads were removed with a magnet and residual tumor cells were counted.

(5) *Reaction of antibodies to normal bone marrow:*

Antibodies showed non-specific reaction to normal bone marrow cells not only to neuroblastoma cell lines. Normal human bone marrow was prepared and incubated in RPMI 1640 with 20% FCS for 1 day. Bone marrow was washed twice with PBS and added individual antibodies, cocktail antibodies, NKH1, and CD45. And then FITC was added to the sample, incubated for 30min at 4 °C. After washing the sample with PBS, reactivity of antibodies to normal bone marrow cells were evaluated by the FCM.

(6) *Purging procedure (Fig. 2):*

Tumor cells prestained with Hoechst 33342 were seeded into the  $1 \times 10^7$  bone marrow cells with 5-10% in a tube to 1ml. The cell mixture was incubated with optimal volume of monoclonal antibodies at 4 °C for 30min. The sample

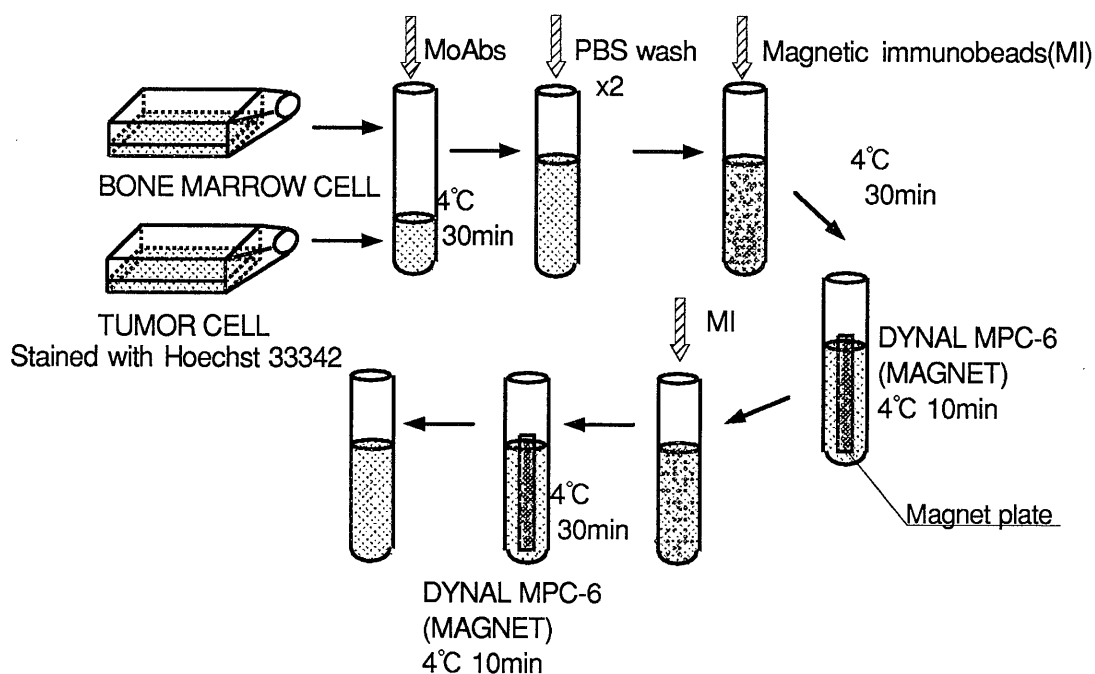


Fig. 2. In vitro model procedure for removing neuroblastoma cells using monoclonal antibodies and magnetic immunobeads.

was then washed with PBS two times and resuspended with PBS to 3ml. And then magnetic immunobeads was added to the sample. The sample with the magnetic beads in a tube was gently rotated preventing precipitation for 30min at 4°C. The tube with sample was fixed at a cobalt magnetic stand for 10min at 4°C. The tumor cells binding to magnetic beads and free magnetic beads were retained. Supernatant was aspirated gentle to a new tube. A second purging procedure was performed with 30% volume of magnetic immunobeads. After first and second purging procedure, the supernatant was stained with 5% trypan blue. Residual tumor cells stained with Hoechst 33342 and viable bone marrow cells were counted by a fluorescent microscope.

(7) Cell separation unit for clinical use:

The cell separation unit available from Dynal is shown in Fig. 3. The bag with bone marrow cells and beads were fixed on a cassette placed on the platform of rocking device. The cassette have a bank of cobalt samarium magnets giving the maximum and favorable magnetic field gradient to the blood bag. The platform with the fixed

blood bag can be changed stepwise decreasing or increasing the magnetic field strength to change the distance of magnets. A tube of the blood bag is connected to a new blood bag via a pump and a magnetic trap. The magnetic trap of 150ml plastic bag collect free magnetic immunobeads from the blood bag with bone marrow and beads.

Bone marrow cells in a blood bag was incubated with a cocktail antibodies at 4°C for 30min. After single washing with PBS, bone marrow cells diluted with RPMI 1640 supplemented with 10% FCS were added with magnetic immunobeads. At 4°C for 30min, the bag was incubated in gentle tilting. And the bag was fixed on the platform with tilting. A distance from the bag to magnet bank was decreasing stepwise, 3min being used at each of the different levels, giving 10%, 25%, 50% and 100% of the maximal field strength. The supernatant cell suspension of the blood bag is transferred to a new blood bag by means of a pump through a magnetic trap. A second cycle of purging is then carried out by adding the 30% of beads and repeating the separation procedure described above.

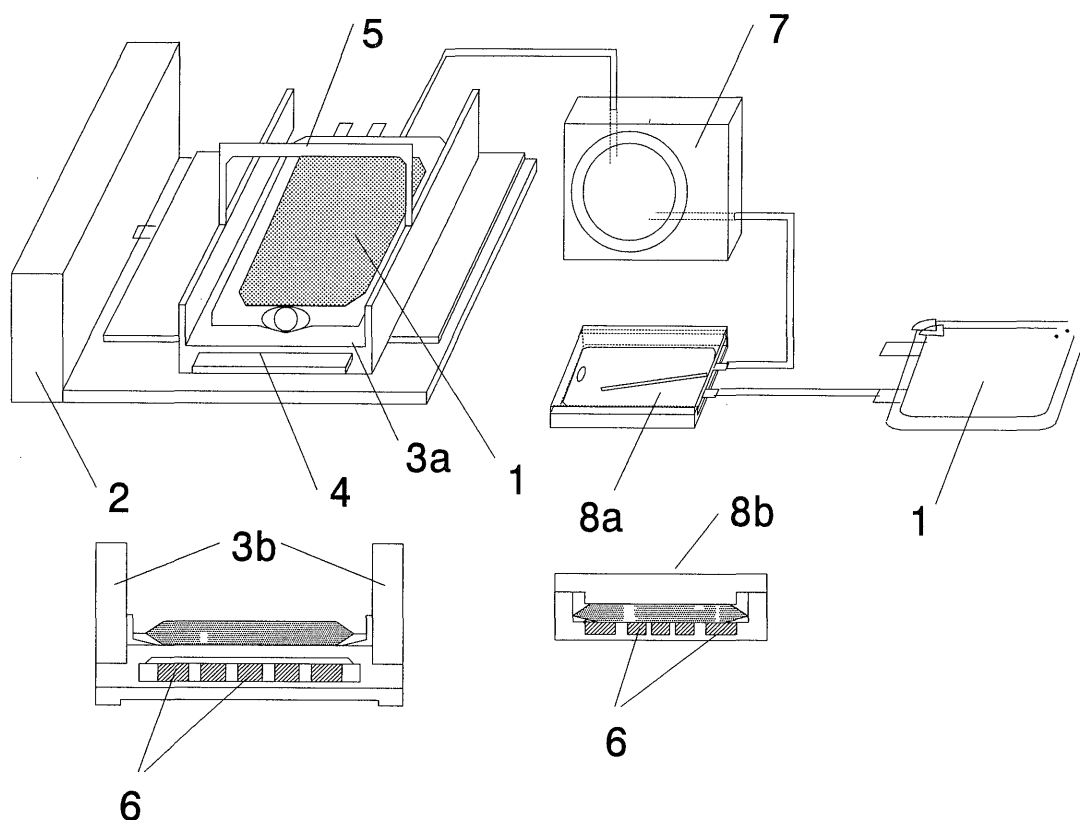


Fig. 3. Cell separation unit for clinical use.

1:200-400ml Fenwall blood bags, 2:Rocking device, 3a:Platform supporting blood bag above bank of magnets, 3b:Enlarged cross section of 3a, 4:Removal iron plate with cobalt samarium magnets bar, 5:Handle for stepwise regulation of distance between platform and magnets, 6:Cobalt samarium magnets, 7:Peristaltic pump, 8a:Magnetic trap, 8b:Enlarged cross section of 8a.

## (8) No hazard of antibodies to normal bone marrow:

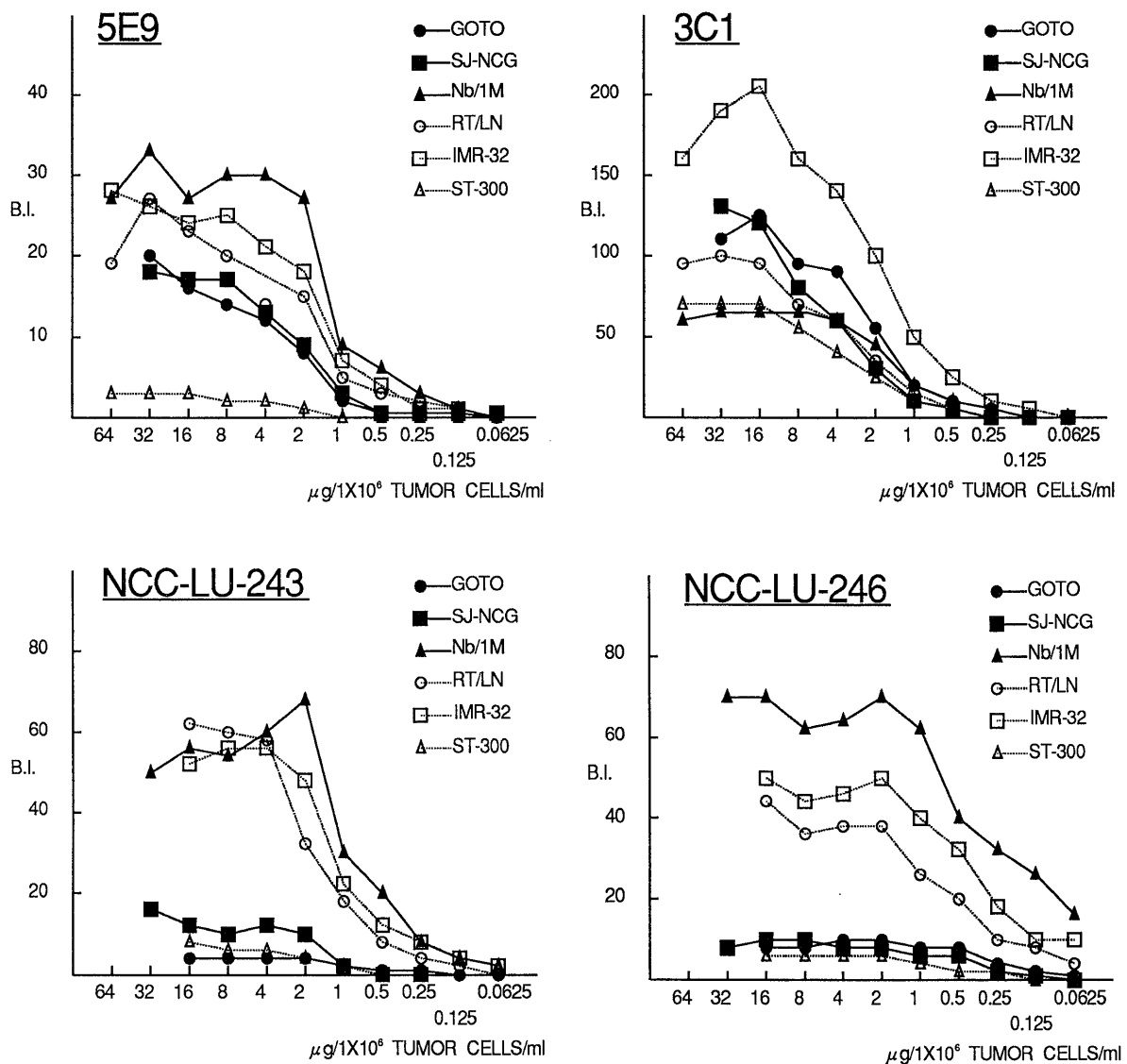
Untreated normal bone marrow cells and bone marrow cells after purging were cultured in soft agar (Methyl cellulose method).<sup>11,13</sup> In untreated group using  $5 \times 10^5$  bone marrow cells/ml, containing 1% methyl cellulose, 20% fetal calf serum and 10% PHA-LCM, were cultured in 10ml dishes. Dishes were incubated for two weeks at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere. The colony, numbers of cells over 40 cells, were counted by an inverted microscope. The purging group treated the  $5 \times 10^5$  bone marrow cells/ml with same volume of cocktail antibodies and magnetic immunobeads as described (6) purging procedure. The colony (CFU-GM) was counted after two weeks.

## Result

## (1) Determination of monoclonal antibodies binding to neuroblastoma cell lines:

For valuation binding of each amount of antibodies, we used binding index.<sup>9)</sup>

Binding index = Mean fluorescence  $\times$  % of cells positive/100. The mean fluorescence and percentage of cells positive were calculated by a FCM. Representative amount of four antibodies and binding index to neuroblastoma cell lines of individual antibody in this experiment were shown in Fig. 4. The binding profiles for 5E9, the binding index showed plateau more than  $8 \mu\text{g/ml}$  for every tumor cell lines. Other three antibodies, 3C1 >  $8 \mu\text{g/ml}$ , NCC-LU-243



**Fig. 4.** Titration of antibodies 5E9, 3C1, NCC-LU-243 and NCC-LU-246 on the six human neuroblastoma cell lines (●;GOTO, ■;SJ-HCG, ▲;Nd-1N, ○;RT/LN, □;IMR-32, △;ST-300). Binding of antibody on cell lines was quantified by flow cytometry. B. I.: binding index. B. I. = Mean fluorescence  $\times$  % of cells positive/100

> 4 μg/ml and NCC-LU-246 > 4 μg/ml, showed maximum reaction against tumor cells. In consequence the optimal concentration of antibodies for this study was decided that 5E9 and 3C1 were 10 μg/ml, and NCC-LU-243 and NCC-LU-246 were 4 μg/ml.

(2) Comparison of individual antibodies and cocktail antibodies binding to neuroblastoma cell lines:

The effect of a mixture of four antibodies is shown in Fig. 5. Specificity for each tumor cells of antibody 3C1 gave consistently higher fluorescence than other three antibodies. 5E9, NCC-LU-243 and NCC-LU-246 had weak reaction to the tumor cell RT/LN, ST-300 and IMR-32. But when adding four antibodies, the binding index of a mixture antibodies was increased more 1.2-70 times than when only single antibody used in every cell lines. The results suggest that a cocktail of the four antibodies were suitable for the purpose of this purging procedure of neuroblastoma cells.

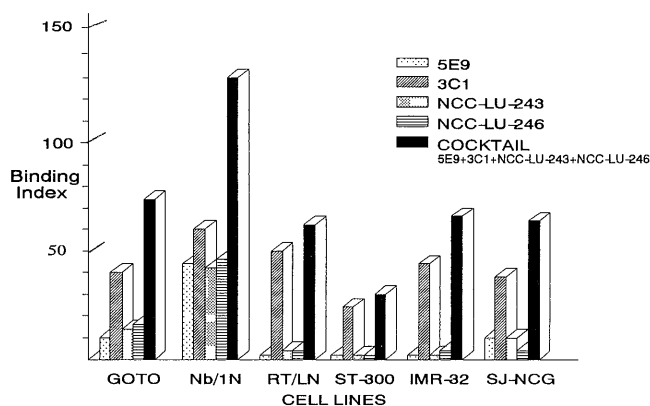


Fig. 5. Comparison of the binding to six human neuroblastoma cell lines (GOTO, Nb/1M, RT/LN, ST-300, IMR-32 and SJ-NCG) of individual antibodies and a cocktail (5E9 + 3C1 + NCC-LU-243 + NCC-LU-246).

(3) Magnetic immunobeads/tumor cells ratio:

Effect of increasing the magnetic beads/tumor cells ratio on removal of neuroblastoma cells from bone marrow was shown in Fig. 6. The effect of increasing the ratio of beads to tumor cells was studied to establish the optimal concentration of the magnetic immunobeads to be used. The sample was treated with cocktail antibodies of 5E9, 3C1, NCC-LU-243 and NCC-LU-246. Viability of neuroblastoma cells was detected with 5% trypan blue staining exclusion and cells with viability over 70% were used. As increasing the amount of magnetic beads per tumor cells, residual tumor cells were decreased. Until the magnetic beads/tumor cells ratio reached 64-128, depletion of tumor cells depended on the magnetic beads. But 64-128 of beads/cells ratios were observed at highest tumor cell

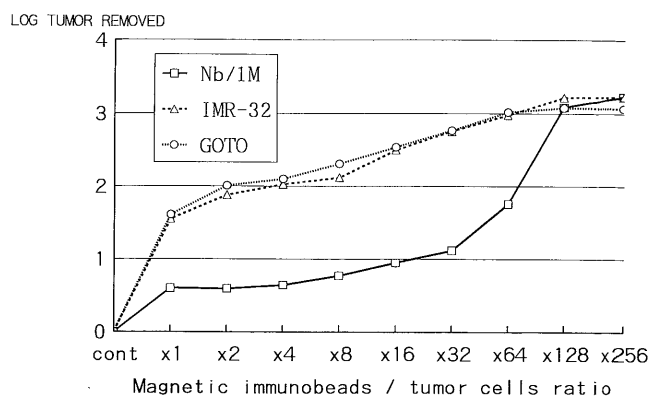


Fig. 6. Effect of increasing the magnetic immunobeads/tumor cell ratio on removal of neuroblastoma cells from human bone marrow cells.

depletion and over 3-log elimination. In conclusion, it was suitable for purging procedure that the magnetic immunobeads/tumor cells ratio was 100.

(4) Reaction of antibodies to normal bone marrow cells:

Percentage of bone marrow positive rate and binding index (range x 100) of antibodies are shown in Fig. 7. MoAb

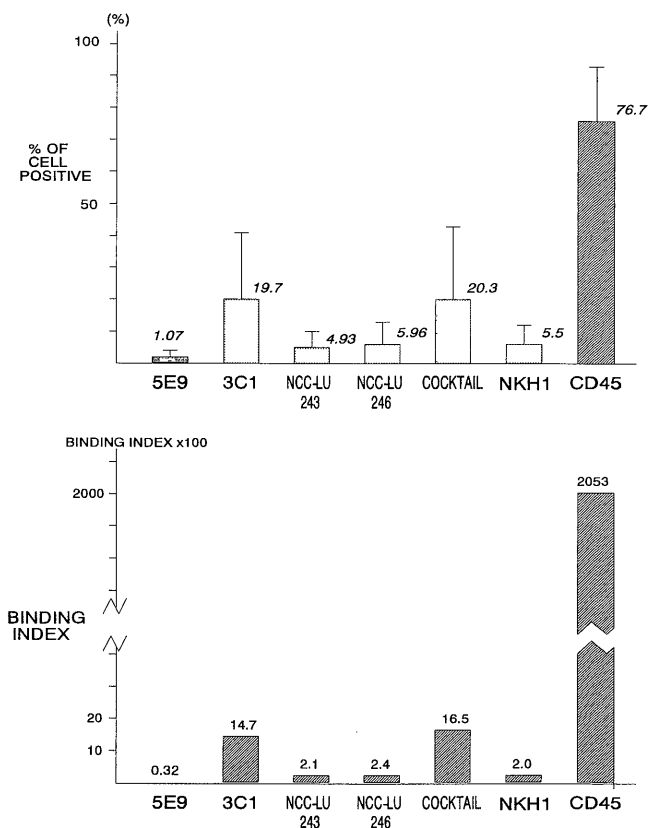
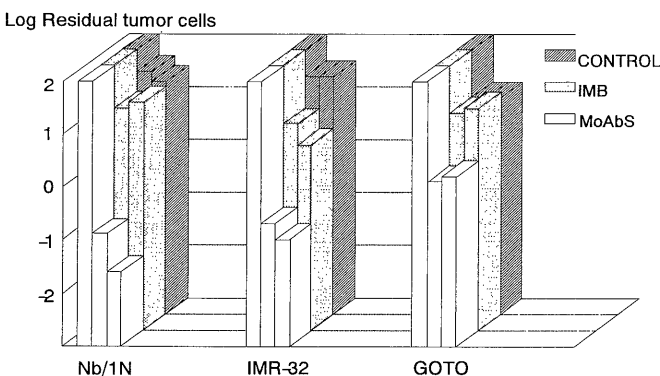


Fig. 7. Comparison of the non-specific binding to human bone marrow cells of each antibodies. Cocktail consists of four antibodies 5E9, 3C1, NCC-LU-243 and NCC-LU-246.

NKH1 (IgM isotype) recognizes an antigen specifically expressed on natural killer cells in normal bone marrow. MoAb CD45 (IgM isotype) recognizes common antigen for panleukocytes. A cocktail consists of 5E9, 3C1, NCC-LU-243 and NCC-LU-246. 3C1 and a cocktail antibody showed a 20% positive reaction to normal bone marrow cells. But binding index of 3C1 and a cocktail assured to be the binding between tumor cells and antibodies was enormously small in comparison to the CD45. It is assumed that these antibodies have a minor impact on non specific removal of human bone marrow cells in this purging procedure.

#### (5) Efficacy of tumor cells depletion in this experiment:

Residual tumor cells after first and second treatment of purging procedure were shown in Fig. 8. Control samples were made by same purging procedure without cocktail antibodies or magnetic beads. IMB groups were treated with magnetic beads. In control groups after one and two purging procedures, tumor cells were decreased 0.5-1.0 log depletion by manipulation. IMB groups showed a small number of cells after treatment that result from mechanical damage to cells in addition to manipulated damage. In purging groups with MoAbs and beads first cycle treatment gave 3.0 log depletion of tumor cells in Nb/1N, 2.9 log depletion in IMR-32 and 1.9 depletion in GOTO cell line. Second cycle treatment revealed additional 0.8 log depletion in Nb/1N and 0.2 log depletion in IMR-32, but in GOTO cells no more depletion was obtained. The effect of a second cycle treatment is evident in Nb/1N cell line and not so clearly in other two cell lines. In this second procedure 30% of beads were used as in the first treatment. Increasing the beads in second treatment, the result of tumor depletion was not improved. In only one cell line the



**Fig. 8.** Residual tumor cells after one and two cycles of treatment of depletion of neuroblastoma cells. The number of residual tumor cells express log base. Control means purging without monoclonal antibodies and magnetic immunobeads, IMB with only magnetic immunobeads and MoAbs with monoclonal antibodies and magnetic immunobeads. Columns, means of three determination; left:pre-purging, center:after first purging, right:after second purging.

second cycle treatment showed evident cell depletion, but two cycle treatment is of great value to decrease tumor cells from harvested bone marrow.

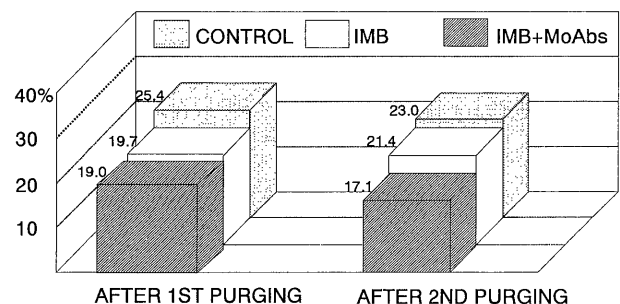
#### (6) Efficacy of purging on a clinical cell separation unit:

Bone marrow for this study was obtained form a patient of Rbdomyosarcoma at conditioned CR and stored in liquid nitrogen. Total volume of the bone marrow was 120ml and  $1.0 \times 10^7$  cells/ml. Cell viability was 30% so that the total viable cells were  $3.6 \times 1.0^8$  in number. Tumor cells Nb/1N pre-stained with Hoechst 33342, density  $1.0 \times 10^7$ /ml were seeded 10ml ( $1.0 \times 10^8$  cells) into the bone marrow cells. The ratio of tumor cells/viable bone marrow cells was 0.28. Total volume of the sample mixed with bone marrow and tumor cells was 130ml. Viable bone marrow cells in the reobtained sample were  $1.26 \times 10^8$  which corresponded to 35% of the initial cells. Viability of the residual bone marrow cell exceeded 90% by dye exclusion. Residual tumor cells counted by a fluorescent microscopy were  $1.54 \times 10^5$  and removal rate was 99.846% (removal of a 2.81 log). This experiment unit resulted in a 2.81 log elimination of tumor cells.

#### (7) Recovery of progenitor cells after purging:

Fig. 9 showed the residual bone marrow cells rate after 1st and 2nd purging as compared with pre-purging. Normal bone marrow cells were removed through the process of purging tumor cells form the marrow, because some antibodies adhere to the surface of the normal bone marrow cells. In the experiment with a tube and a clinical purging unit, the recovery bone marrow cells after two cycle of pruging varied from 17.1 to 35%. Bone marrow cells were mechanically damage by the purging procedure. However viability of bone marrow cells was 70-80%.

The toxicity of the CFU-GM was evaluated with culture assay. The result of the CFU-GM assay was shown in Table 2. CFU-GM numbers were  $63.2 + 28.1$  before purg-



**Fig. 9.** Residual rate of bone marrow cells. Control means only purging procedure without monoclonal antibodies and magnetic beads. IMB, treatment with magnetic beads with no antibodies. IMB + MoAbs means with both cocktail antibodies and mangetic beads. Each bar demonstrates the rate of residual bone marrow cells number after one or two cycle purging to pre-purging.

**Table 2.** Colony number of progenitor bone marrow cells at pre-purging and after two cycle purging treatment. Samples contained  $5 \times 10^5$  bone marrow cells.

CFU-GM	
pre-purging	after two cycle of treatment
$63.2 \pm 28.1$	$56.4 \pm 30.5$

ing and  $56.4 \pm 30.5$  after two cycle of purging. Reduction in the clonogenic bone marrow progenitor cells was about 10%. It was documented these antibodies did not affect progenitor cells of human.

## Discussion

Massive chemoradiotherapy for advanced neuroblastoma dose not significantly improved the prognosis. Autologous bone marrow transplantation (ABMT) for advanced neuroblastoma is now used to improve poor prognosis after conventional treatment. Bone marrow is generally harvested during clinical complete remission (CR), but the probability of the presence of metastasized tumor cells is great concern. A variety of method have been studied to remove tumor cells from involved bone marrow harvested for ABMT. Reisner (1983) reported a depletion model with soybean agglutinin.<sup>14)</sup> Sarrinen et al. (1985) have shown killing the tumor cells using the monoclonal antibody of IgG3 isotype and human complement.<sup>15)</sup> Fridgor et al. (1985) described the physical depletion technique using an elutriation centrifuge.<sup>16)</sup> The immunomagnetic depletion method was first described by Treleaven et al. in 1984. 3-log depletion of tumor cells from bone marrow was obtained in his study.<sup>17)</sup> Since then, many investigators reported immunomagnetic depletion (IMD) method using cocktail antibodies with various modifications and a result of 3-4 log elimination of tumor cells.<sup>2,3,9-11)</sup> In Europe and America this IMD is applied for clinical medication and gives good result. In Japan the IMD is recently performed in some institutions.<sup>18)</sup> This paper clarified the IMD method using newly produced antibodies and antibodies not previously used for this technique. Antibodies NCC-LU-243 and NCC-LU-246 were developed in the NIHON-KAYAKU Co. Ltd. The reactivity of both antibodies was anti-human small cell lung carcinoma. Subclass of immunoglobulin of each antibodies was IgG2a in NCC-LU-243 and IgG1 in NCC-LU-246. 5E9 and 3C1 were newly produced at the National Children Medical Research Center in Tokyo. 5E9 is IgG1 class anti human immature teratoma antibody. Competition of 5E9 with NKH-1 for binding to neuroblastoma cells is due to a reaction of the NCOM on the surface of neuroblastoma cells. In contrast the reaction of 5E9 is not completely blocked by NKH-1 so that it is clear that 5E9 is not the same antibody as NKH-1. Each

antibodies showed high sensitivity to six neuroblastoma cell lines. The optimal dosage of each antibody was determined with the flow cytometry study.<sup>4,5,9,19,20)</sup> In this experiment antibodies were used for the cocktail in combination of four antibodies as previously described. The cocktail antibodies gave 1.2-4 times more sensitive to tumor surface antigen than a single use. The cocktail antibodies showed non-specific reaction with normal bone marrow cells. At flow cytometry study percentage of positive bone marrow cells showed 20.3% on average in cocktail antibodies. After two cycle of purging method the ratio of recovered vital bone marrow cells/pre-purging cells 17.1% (23.0% in controlled groups).<sup>11)</sup> The result by purging procedure did not differ in recovered bone marrow cells from the control group and the purging group. Culture assay in soft agar after two cycle purging treatment revealed no toxicity for progenitor hematopoietic cells.

Culture assay require sufficient growth capacity of tumor cells and is not highly sensitive to detect of residual tumor cells after purging. Dual color immunofluorescence staining made it possible to detect less than 2 log residual malignant cells.<sup>19,21)</sup> The vital fluorescent DNA dye Hoechst 33342 staining method was first described by Reynolds (1985),<sup>12)</sup> who reported that up to 4-log depletion of residual cells could be detected with a computerized image analyzer and up to 3.3-log with microscopic analysis by Hoechst staining. This Hoechst 33342 staining method was used in this study for its rapidity and accuracy.

Purging procedure using the clinical cell separation unit demonstrated a good results of tumor cell elimination and bone marrow cell recovery.

This newly produced monoclonal antibodies (5E9 and 3C1) and two other antibodies (NCC-LU-243 and NCC-LU-246) had specific reaction for neuroblastoma cells. Non-specific reaction of the cocktail antibodies to bone marrow cells were recognized, but it was not so significant. The cocktail antibodies combined with four antibodies (5E9, 3C1, NCC-LU-243 and NCC-LU-246) gave better reaction to neuroblastoma cells.

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