# Rhenium-188-labeled anti-neural cell adhesion molecule antibodies with 2-iminothiolane modification for targeting small-cell lung cancer

Masako N. Hosono, Makoto Hosono, Anil K. Mishra, Alain Faivre-Chauvet,
Emmanuel Gautherot, Jacques Barbet, F.F. (Russ) Knapp, Jr.
and Jean-François Chatal

Reprint from Annals of Nuclear Medicine Vol. 14 No. 3
June 2000

Annals of Nuclear Medicine Vol. 14, No. 3, 173–179, 2000

# Rhenium-188-labeled anti-neural cell adhesion molecule antibodies with 2-iminothiolane modification for targeting small-cell lung cancer

Masako N. Hosono,* Makoto Hosono,** Anil K. Mishra,*** Alain Faivre-Chauvet,***
Emmanuel Gautherot,**** Jacques Barbet,**** F.F. (Russ) Knapp, Jr.*****
and Jean-François Chatal***

*Department of Radiology, Osaka City University
**Department of Radiology, Saitama Medical Center
***INSERM Research Unit 463, Nantes, France
****Immunotech SA, Marseille, France
*****Nuclear Medicine Program, Oak Ridge National Laboratory, Oak Ridge, TN, USA

We have evaluated the potential of $^{188}$Re-labeled monoclonal antibodies (MAbs) modified with 2-iminothiolane (2IT) for targeting small-cell lung cancer (SCLC). Radiolabeled MAbs NK1NBL1 and C218 recognizing neural cell adhesion molecule were injected i.v. into athymic mice inoculated with human SCLC tumors, and the biodistribution was examined. NK1NBL1 localized in the tumors better than C218. $^{188}$Re-labeled MAbs cleared from the blood faster than $^{125}$I-labeled counterparts, resulting in higher tumor-to-blood ratios. In conclusion, the $^{188}$Re-labeled MAbs are attractive candidates for imaging and therapy of SCLC.

**Key words:** rhenium-188, monoclonal antibody, small-cell lung cancer, neural cell adhesion molecule, 2-iminothiolane

## INTRODUCTION

Small-cell lung cancer (SCLC) is characterized by endocrine features, a tendency to metastasize, high but temporary chemo- and radiosensitivity, and poor prognosis. The mortality of patients with SCLC remains more than 90% at 2 years after diagnosis.[1] As most post-therapy patients suffer from recurrence, and tumors become refractory to repeated therapy, antibody-guided internal radiotherapy has been expected as a further therapeutic strategy. Several reports have described animal studies of SCLC therapy with radiolabeled monoclonal antibodies (MAbs) reactive with some SCLC-related antigens.[2,3] Neural cell adhesion molecule (NCAM) is considered as the most specific among the SCLC-related antigens. Since most SCLC tumors express NCAM on the surface membrane of the cells,[4] it is thought to be an optimal target for localizing SCLC tumors.

In terms of cost effectiveness for imaging and internal radiation therapy, $^{188}$Re is a promising radionuclide. It has 155 keV $\gamma$-emission suitable for scintigraphy and the maximum $\beta$-energy of 2.1 MeV effective for radioimmunotherapy with a half-life of 16.98 h. Rhenium-188 is also readily available from the $^{188}$W/$^{188}$Re generator as carrier-free perrhenate (the half-life of $^{188}$W is 69.4 d). Rhenium-188 has good clinical potential for radiation synovectomy,[5] pain relief in patients with multiple bone metastases as $^{188}$Re-hydroxyethyliden diphosphonate (HEDP),[6] and inhibition of restenosis after percutaneous transcatheter coronary angioplasty.[7]

To date, methods for radiolabeling antibodies with radioisotopes of rhenium, both directly and indirectly, have been proposed. Direct methods, where the endogenous complexing groups are used, may be susceptible to low label stability.[8–10] Indirect methods with chelating agents[11,12] may improve stability of rhenium-labeled antibodies, but direct methods have the advantage of simple preparation, which is suitable for administration to patients.

The combination of $^{188}$Re as a radiolabel, anti-NCAM

Received January 12, 2000, revision accepted March 1, 2000.
For reprint contact: Makoto Hosono, M.D., Ph.D., Department of Radiology, Saitama Medical Center, 1981 Kamoda, Kawagoe, Saitama 350–8550, JAPAN.
E-mail: makohoso@saitama-med.ac.jp

MAbs, and a direct labeling technique, could therefore be a potent tool for the diagnosis and therapy of SCLC. Although we adapted a direct labeling technique in this study, we modified antibodies with 2-iminothiolane (2IT) which provides a thiol reactive position for the attachment of rhenium in order to obtain stable labeling. This technique has already proved effective in achieving stable labeling of antibodies with $^{99m}$Tc.[13–15]

In this study we evaluated the effectiveness of 2IT modification for $^{188}$Re-labeling of anti-NCAM MAbs *in vitro* and in a mouse model bearing human SCLC.

## MATERIALS AND METHODS

*Antibodies*

The murine MAbs C218[16] and NK1NBL1[17] (IgG1s), reacting with human NCAM, were supplied by Immunotech (Marseille, France). The MAb OC125, used as a nonspecific Ab in this study, was generated by immunizing mice with human ovarian serous cystadenocarcinoma and recognized a high-molecular weight glycoprotein CA125.[18]

*Cell line and xenografts*

The SCLC cell line NCI-H69 cells,[19] obtained from the American Type Culture Collection (Manassas, VA, USA), were cultured in RPMI 1640 culture medium (Life Technologies, Grand Island, NY, USA) supplemented with 1 mM glutamine and 10% fetal calf serum. For the studies in mice, NCI-H69 SCLC cells were implanted by s.c. inoculation of a tumor mince into the flanks of 5 to 7 week-old female BALB/c nu/nu mice. Xenografted mice were used when the tumor volume reached about 0.5 cm$^3$, 2–3 weeks after inoculation.

*Radiolabeling of antibodies*

Rhenium-188 perrhenate solution was obtained from a $^{188}$W/$^{188}$Re generator available from the Oak Ridge National Laboratory (Oak Ridge, TN, USA).[20] Before labeling with $^{188}$Re, MAbs were derivatized by 2IT (molecular weight of 101, Fig. 1), which provides thiol-reactive sites for the attachment of rhenium without splitting the disulfide bonds of the antibody molecules. Briefly, to modify MAbs, 500 $\mu$g (3.3 nmol) of intact MAbs C218, NK1NBL1, and OC125 mixed with 50 $\mu$l of 2IT solution (Sigma Chemical Co., St. Louis, MO, USA, 2.5 mg i.e. 25 $\mu$mol of 2IT in 50 $\mu$l of dimethylsulfoxide), and 0.1 M phosphate buffer (PB) pH 7 (totally 1 m*l*) were incubated for 15 min at room temperature. For reduction of $^{188}$Re, 1.35 m*l* of eluted $^{188}$Re perrhenate with a specific volume of 185 MBq/m*l* and 7.5 m*l* of 1 mM $SnCl_2$ and 0.15 M $Na_4P_2O_7$ solution were incubated for 1 h at 100°C. Then, to label MAbs with $^{188}$Re, reduced $^{188}$Re and pretreated MAbs were incubated for 16 h at 4°C. The $^{188}$Re-labeled MAbs were purified by Sephadex G-25 gel chromatography (Pharmacia LKB Biotechnology, Uppsala, Sweden).

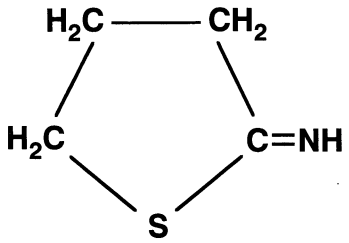


**Fig. 1** Chemical structure of 2-iminothiolane.

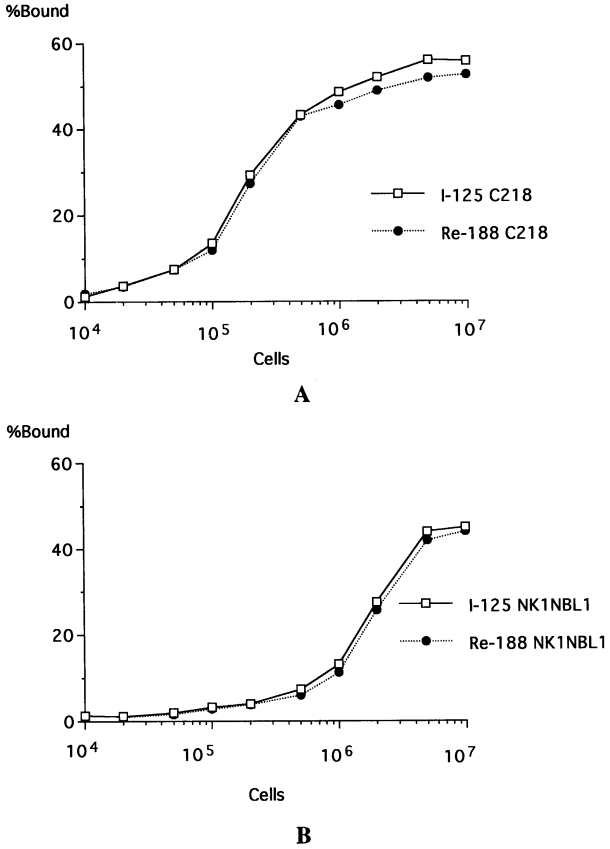


**Fig. 2** Binding assay of $^{188}$Re- and $^{125}$I-labeled C218 (A) and NK1NBL1 (B) to NCI-H69 cells. Non-specific binding was subtracted. $^{188}$Re-labeled C218 and NK1NBL1 demonstrated a binding similar to $^{125}$I-labeled counterparts.

Labeling efficiency was 36.4–57.8% for the $^{188}$Re-labeled MAbs. The specific activity for $^{188}$Re-labeled C218, NK1NBL1, and OC125 was 97–126 MBq/mg.

The intact MAbs C218, NK1NBL1, and OC125 were labeled with $^{125}$I by the chloramine-T method. MAbs (40 $\mu$g) in 0.3 M PB pH 7.5, and $^{125}$I (11.1 MBq) for protein labeling were mixed with 2.5 $\mu$g of chloramine-T (Aldrich, St. Quentin Fallvier, France) dissolved in 0.3 M PB. 5 min later the radiolabeled MAb was separated from free radioiodine by Sephadex G-25 gel chromatography. The specific activity of $^{125}$I-labeled C218, NK1NBL1, and OC125 was 210–282 MBq/mg.

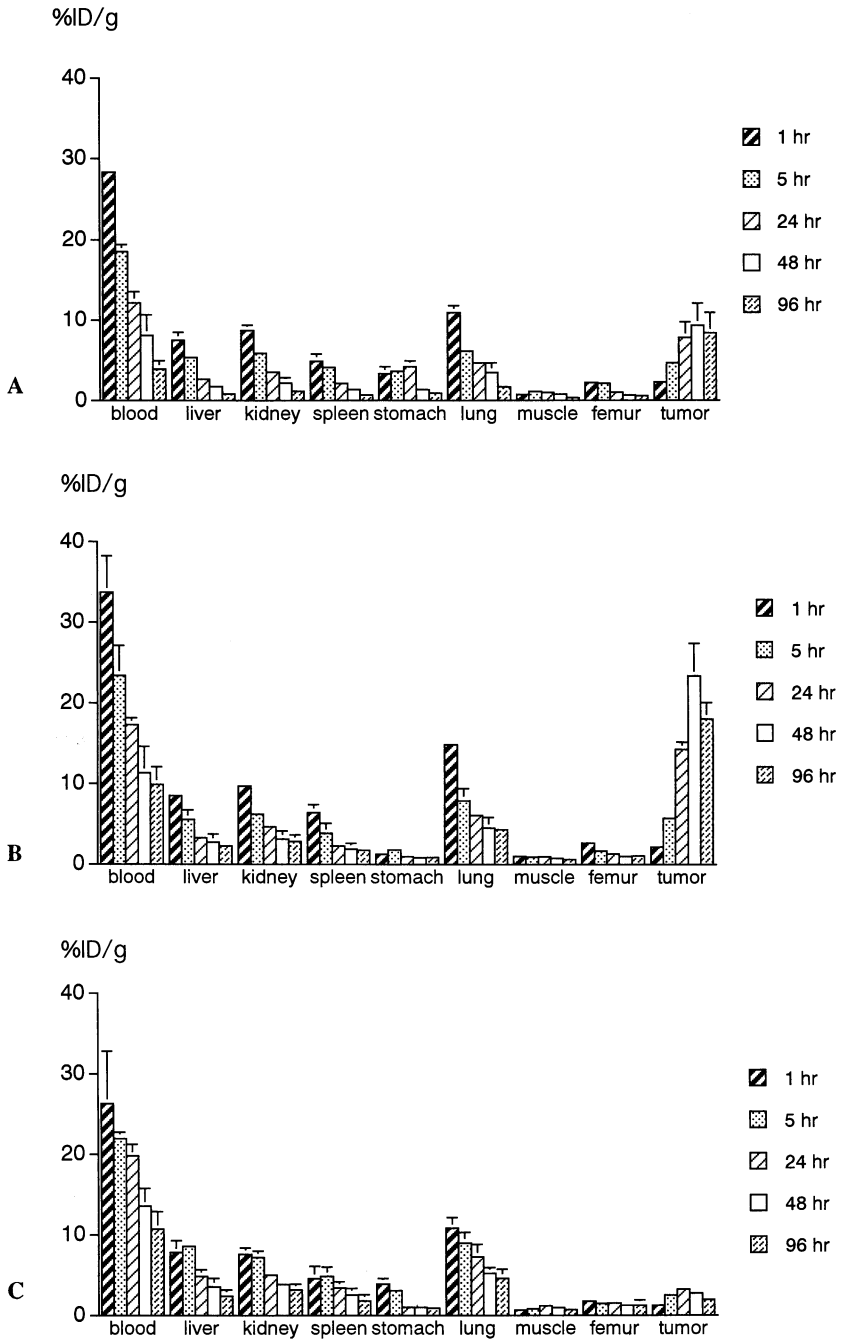


**Fig. 3** Biodistribution of $^{125}$I-labeled C218 (A), NK1NBL1 (B), and OC125 (C) in athymic mice bearing NCI-H69 cells. Error bars show one standard deviation.

*Stability*

To verify that the $^{188}$Re-labeled C218 and NK1NBL1 were not fragmented during the reduction-mediated radiolabeling, $^{188}$Re-labeled C218 and NK1NBL1 were analyzed by size exclusion high performance liquid chromatography (HPLC) on a TSK G3000 SW column (Tosoh, Tokyo, Japan). Protein was detected at an absorbance of 280 nm. An outline detector Model 170 (Beckman Coulter, Inc., Fullerton, CA, USA) was connected to monitor the radioactivity of each fraction.

In addition, $^{188}$Re-labeled C218 was incubated with serum of a normal adult at 37°C, and aliquots were taken at 24, 48, and 96 h, and then analyzed by HPLC.

As a control, the intact MAb C218 was labeled with $^{188}$Re without 2IT modification as previously reported with stannous tartrate as a reduction agent.[10] The specific activity was 202 MBq/mg. This non-modified $^{188}$Re-labeled C218 was incubated with serum and analyzed by HPLC.

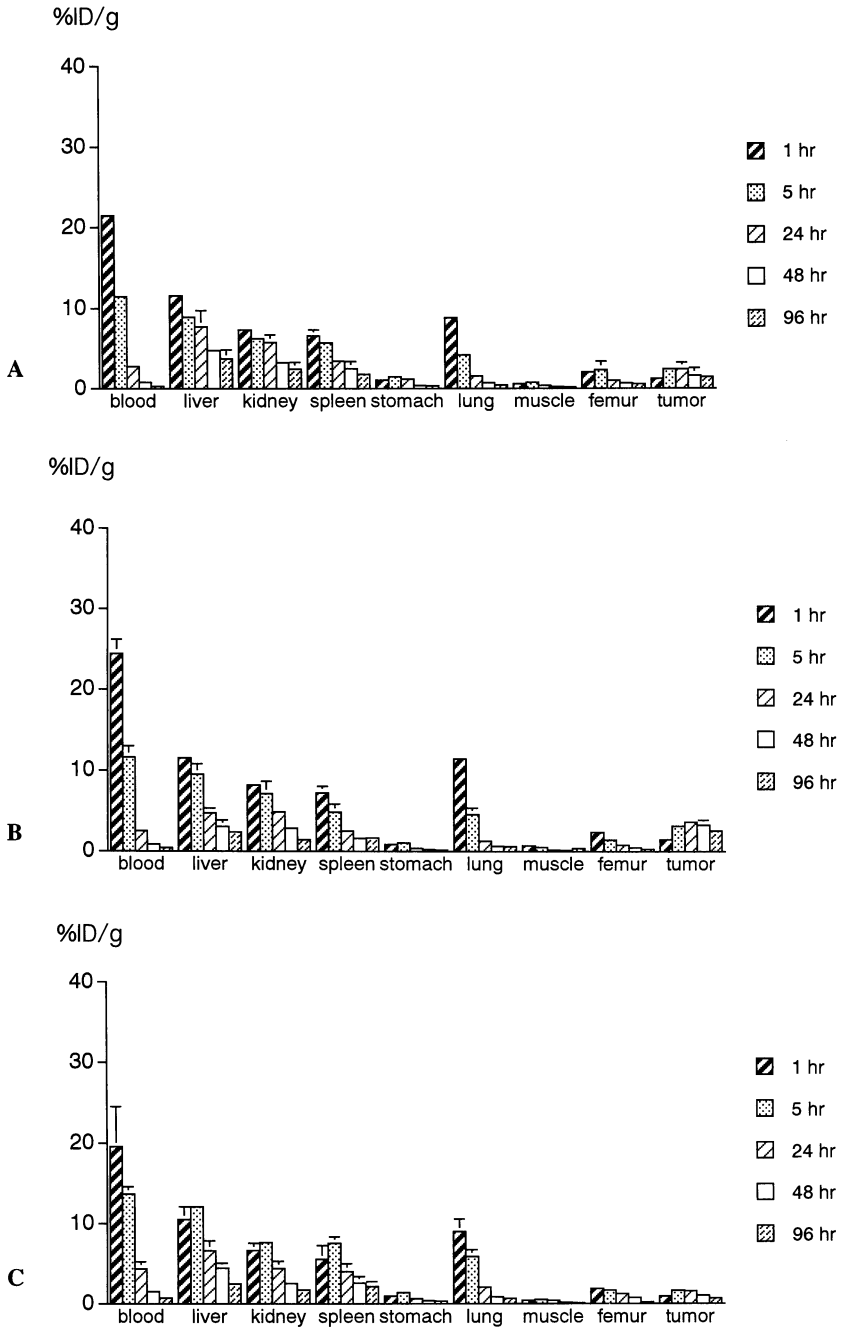


**Fig. 4** Biodistribution of $^{188}$Re-labeled C218 (A), NK1NBL1 (B), and OC125 (C) in athymic mice bearing NCI-H69 cells. Error bars show one standard deviation.

*Affinity and immunoreactivity analysis*

MAbs C218 and NK1NBL1 labeled with $^{125}$I were incubated at different antibody concentrations with $1 \times 10^7$ NCI-H69 cells in $5.7 \times 46$ mm microcentrifuge tubes for 1 h at 4°C. After centrifugation at $1{,}500 \times g$, the tubes were washed with saline, and the pellets were taken by cutting the bottoms of the tubes. The radioactivity bound to the cells was counted in a well-type gamma counter. The binding affinity constant was calculated by means of a Scatchard analysis.[21]

To compare $^{188}$Re- and $^{125}$I-labeled C218 and NK1NBL1 in binding to NCI-H69 cells, 14 ng of radiolabeled C218 or NK1NBL1 was incubated with increasing concentrations of cells for 1 h at 4°C. Specific binding to cells was calculated by subtracting the nonspecific binding in the tubes in which 10 $\mu$g of unlabeled MAb was added. The immunoreactive fraction was determined by linear extrapolation to conditions representing infinite antigen excess.[22]

**Table 1** Tumor-to-organ ratios of I-125-labeled C218, NK1NBL1, and OC125 antibodies at 48 and 96 h (mean ± SD)

| Antigody | Time | Blood | Liver | Kidney | Lung | Muscle |
|---|---|---|---|---|---|---|
| I-125-C128 | 48 h | 1.24 ± 0.53 | 5.61 ± 2.12 | 4.46 ± 1.48 | 2.81 ± 0.89 | 12.59 ± 4.54 |
| | 96 h | 2.13 ± 0.15 | 10.25 ± 1.97 | 7.11 ± 1.02 | 4.94 ± 0.60 | 24.24 ± 1.75 |
| I-125-NK1NBL1 | 48 h | 1.66 ± 0.32 | 7.28 ± 2.35 | 6.11 ± 1.28 | 4.06 ± 0.81 | 24.41 ± 4.08 |
| | 96 h | 2.45 ± 0.72 | 10.82 ± 2.89 | 8.71 ± 2.01 | 5.18 ± 1.12 | 40.20 ± 9.18 |
| I-125-OC125 | 48 h | 0.20 ± 0.03 | 0.80 ± 0.21 | 0.72 ± 0.10 | 0.53 ± 0.06 | 2.91 ± 0.52 |
| | 96 h | 0.18 ± 0.01 | 0.83 ± 0.06 | 0.63 ± 0.01 | 0.44 ± 0.07 | 2.69 ± 0.53 |

**Table 2** Tumor-to-organ ratios of Re-188-labeled C218, NK1NBL1, and OC125 antibodies at 48 and 96 h (mean ± SD)

| Antibody | Time | Blood | Liver | Kidney | Lung | Muscle |
|---|---|---|---|---|---|---|
| Re-188-C218 | 48 h | 2.00 ± 0.61 | 0.34 ± 0.18 | 0.53 ± 0.33 | 2.18 ± 0.55 | 6.93 ± 3.75 |
| | 96 h | 5.13 ± 1.84* | 0.41 ± 0.18 | 0.62 ± 0.23 | 3.32 ± 1.20 | 7.10 ± 2.75 |
| Re-188-NK1NBL1 | 48 h | 3.98 ± 0.93* | 1.11 ± 0.21 | 1.18 ± 0.28 | 5.42 ± 0.73* | 23.17 ± 2.95 |
| | 96 h | 6.81 ± 2.10* | 1.13 ± 0.26 | 1.79 ± 0.20 | 8.23 ± 1.34* | 32.35 ± 6.58 |
| Re-188-OC125 | 48 h | 0.66 ± 0.09* | 0.22 ± 0.04 | 0.39 ± 0.01 | 1.12 ± 0.10* | 5.26 ± 0.95* |
| | 96 h | 1.01 ± 0.21* | 0.28 ± 0.05 | 0.43 ± 0.14 | 1.30 ± 0.30* | 5.80 ± 0.98* |

*higher than I-125-labeled counterparts ($p < 0.05$)

*Biodistribution studies*

Five groups of mice (5/group) bearing NCI-H69 xenografts per time point were given 296 kBq of $^{188}$Re-labeled C218 and 111 kBq of $^{125}$I-labeled C218 at the same time via a lateral tail vein, while other 5 groups were given $^{188}$Re-labeled NK1NBL1 and $^{125}$I-labeled NK1NBL1, and the other 5 groups were given with $^{188}$Re-labeled OC125 and $^{125}$I-labeled OC125. The mice were sacrificed at 1, 5, 24, 48, and 96 h after injection. The $^{188}$Re radioactivity of tumors and selected organs was determined with a well-type gamma counter, and the $^{125}$I radioactivity was measured after 10 half-lives of $^{188}$Re. The results were expressed as a percentage of the injected dose per g tissue (%ID/g) and as tumor-to-tissue ratio values of the radioactivity concentration. The average weight of the xenografts used in this study ($n = 75$) was $0.38 \pm 0.21$ g and there was no significant difference among groups. The "Principles of laboratory animal care" (NIH publication No. 86–23, revised 1985) were followed in animal experiments.

*Statistical analysis*

The biodistribution data for $^{188}$Re-labeled MAbs and their $^{125}$I-labeled counterparts were compared by Student's t-test ($p < 0.05$).

## RESULTS

On HPLC, a peak of protein-bound radioactivity was observed only at the fraction of intact antibody for 2IT-modified $^{188}$Re-labeled C218 and NK1NBL1, showing that they were not fragmented but remained intact. The *in vitro* stability test showed that 6%, 11%, and 22% of radioactivity became unbound from protein at 24, 48, and 96 h, respectively, for 2IT-modified $^{188}$Re-labeled C218 versus 10%, 28%, and 42%, respectively, for non-modified $^{188}$Re-labeled C218.

The affinity was determined to be $Ka = 7.0 \times 10^7\ M^{-1}$ for C218, and $Ka = 5.2 \times 10^7\ M^{-1}$ for NK1NBL1 by Scatchard analysis. The binding sites were $5.5 \times 10^5$ per cell. The cell binding assay demonstrated binding of $^{188}$Re-labeled C218 and NK1NBL1 and $^{125}$I-labeled counterparts to NCI-H69 cells (Fig. 2). Immunoreactivity was estimated to be 56% and 53% for $^{125}$I-labeled and $^{188}$Re-labeled C218, 45% and 44% for $^{125}$I-labeled and $^{188}$Re-labeled NK1NBL1, respectively.

Biodistributions of $^{125}$I-labeled C218, NK1NBL1, and OC125 are shown in Figure 3 as well as those of $^{188}$Re-labeled C218, NK1NBL1 and OC125 in Figure 4. Radiolabeled C218 and NK1NBL1 showed signs of more specific accumulation in the tumor than radiolabeled OC125.

Although $^{188}$Re-labeled C218 and NK1NBL1 showed signs of lower absolute tumor accumulation than $^{125}$I-labeled counterparts, they had higher tumor-to-blood ratios. Moreover, $^{188}$Re-labeled NK1NBL1 had a higher tumor-to-lung ratio than $^{125}$I-labeled NK1NBL1 (Tables 1 and 2).

## DISCUSSION

MAbs directly labeled with $^{188}$Re have successfully localized malignant tumors.[9,10] Griffiths et al. reported that in a mouse model of human colorectal carcinoma LS174T, $^{188}$Re-Mu-9 and $^{131}$I-Mu-9 had tumor-to-blood ratios of $2.91 \pm 0.41$ and $1.56 \pm 0.10$, respectively, at 72 h.[9] Hosono et al. showed that in mice bearing human colorectal tumor LoVo, $^{188}$Re-labeled B72.3 had a tumor-to-blood ratio of $2.04 \pm 0.44$ at 96 h whereas $^{125}$I-labeled B72.3 had a value of $1.05 \pm 0.28$.[10]

In direct labeling methods where the endogenous complexing groups on antibodies are used, antibodies may be impaired due to splitting of disulfide bonds. Nevertheless, direct methods are simple to conduct, and require minimal antibody manipulation, which is a great advantage for clinical use.

For labeling of antibodies with $^{99m}$Tc, a modification of MAbs with 2IT, which provides thiol reactive positions, has been reported to be effective in maintaining the integrity of intact IgG or $F(ab')_2$.[14,15]

In this study, *in vitro* stability was improved with the 2IT modification, as 89% of radioactivity remained bound to the modified antibody at 48 h in comparison with 58% for the non-modified antibody. Griffiths et al. also reported that 66% of radioactivity remained bound *in vitro* at 48 h for an antibody directly labeled with $^{188}$Re.[9]

In terms of *in vivo* stability, $^{188}$Re-labeled MAbs demonstrated faster clearance from the normal tissues and tumors than $^{125}$I-labeled MAbs. It is likely that $^{188}$Re-labeled MAbs are metabolized *in vivo* resulting in rapid washout of radioactivity, but further studies are needed to elucidate the metabolism of $^{188}$Re-labeled MAbs.[8]

Bifunctional chelates are another approach which should enhance the targeting ability of rhenium-labeled antibodies.[11,12] Goldrosen et al. reported that NR-LU-10 MAb formed a stable conjugation with $^{186}$Re by using a tetrafluorophenyl-activated ester derivative of the triamide thiolate as a chelate, and showed blood, liver, kidney and tumor accumulation of 2.2, 0.3, 0.2, and 6.7%ID/g, respectively, and a tumor-to-blood ratio of 3 at 72 h in mice bearing human colon carcinoma.[11] Beaumier et al. reported that the same $^{186}$Re-labeled NR-LU-10 MAb demonstrated tumor-to-blood ratios of 0.7 and 1.3 at 48 and 120 h.[2]

In this study, $^{188}$Re-labeled derivatized NK1NBL1 achieved good tumor-to-blood ratios, 4.0 and 6.8, at 48 and 96 h, comparable to chelate methods. A limitation of this study was the relatively low tumor-to-normal tissue ratios, which may be attributable to low affinity of the MAbs for the antigen. A multistep targeting technique called "Affinity Enhancement System (AES)" with a bispecific antibody and a radiolabeled hapten can reportedly increase tumor-to-normal tissue ratios even if low-affinity MAbs are used.[23,24] As a matter of fact, the MAb NK1NBL1 used in the present study has demonstrated higher tumor-to-normal tissue contrast when applied to the AES technique.[25]

Radiolabeled NK1NBL1 had a higher tumor uptake than radiolabeled C218 in this study, whereas the affinity constant of NK1NBL1 $Ka = 5.2 \times 10^7\ M^{-1}$ was lower than $Ka = 7.0 \times 10^7\ M^{-1}$ for C218. Pharmacokinetics of C218 and NK1NBL1 in the blood were similar. A hypothesis proposed by Fujimori et al. offers a suggestion concerning this discrepancy. They demonstrated that the average antibody concentration in the tumors does not increase linearly with antibody affinity because macromolecular ligands could be prevented from penetrating tumors by their successful binding to the target receptor.[26] This could in part explain the difference between MAbs C218 and NK1NBL1 in tumor localization as a function of antibody affinity, although many of the factors that determine antibody distribution are still not understood.

In conclusion, the anti-NCAM MAbs directly labeled with $^{188}$Re by means of 2IT derivatization seems effective in targeting SCLC tumors.

## ACKNOWLEDGMENTS

The authors are grateful to the Government of France for the fellowship offered to Makoto Hosono. They thank Professor Hans J. Biersack, Bonn, Germany for his academic advice. ORNL is operated for the U.S. Department of Energy (DOE) under contract with the Lockheed Martin Energy Research Corporation. This study was supported in part by grants-in-aid from the Ministry of Education (No. 11670911), Japan.

## REFERENCES

1. Souhami RL, Law K. Longevity in small cell lung cancer. A report to the lung cancer subcommittee of the United Kingdom Committee for Cancer Research. *Br J Cancer* 61: 584–589, 1990.
2. Beaumier PL, Venkatesan P, Vanderheyden J-L, Burgua WD, Kunz LL, Fritzberg AR, et al. $^{186}$Re radioimmunotherapy of small cell lung carcinoma xenografts in nude mice. *Cancer Res* 51: 676–681, 1991.
3. Hosono M, Endo K, Hosono MN, Kobayashi H, Shirato M, Sakahara H, et al. Treatment of small cell lung cancer xenografts with iodine-131-anti-neural cell molecule monoclonal antibody and evaluation of absorbed dose in tissue. J *Nucl Med* 35: 296–300, 1994.
4. Takahashi T, Ueda R, Song X, Nishida K, Shinzato M, Namikawa R, et al. Two novel cell surface antigens on small cell lung carcinoma defined by mouse monoclonal antibodies NE-25 and PE-35. *Cancer Res* 46: 4770–4775, 1986.
5. Wang SJ, Lin WY, Hsieh BT, Shen LH, Tsai ZT, Ting G, et al. Rhenium-188 sulphur colloid as a radiation synovectomy agent. *Eur J Nucl Med* 22: 505–507, 1995.
6. Knapp FFRJ, Beets AL, Guhlke S, Zamora PO, Bender H, Palmedo H, et al. Availability of rhenium-188 from the alumina-based tungsten-188/rhenium-188 generator for preparation of rhenium-188-labeled radiopharmaceuticals for cancer therapy. *Anticancer Research* 17: 1783–1796, 1997.
7. Knapp FFJ, Beets AL, Guhlke S, Gledd KN, Marboe C, Amols H, et al. Rhenium-188 liquid-filled balloons effectively inhibit restenosis in a swine coronary overstretch model—a simple new method bridging nuclear medicine and interventional cardiology. *J Nucl Med* 39: 48P, 1998.
8. Sakahara H, Saga T, Endo K, Hattori N, Hosono M, Kobayashi H, et al. *In vivo* instability of reduction-mediated $^{99m}$Tc-labeled monoclonal antiboy. *Nucl Med Biol* 20: 617–623, 1993.
9. Griffiths GL, Goldenberg DM, Knapp FFJ, Callahan AP, Chang CH, Hansen HJ. Direct radiolabeling of monoclonal antibodies with generator-produced rhenium-188 for

radioimmunotherapy: labeling and animal biodistribution studies. *Cancer Res* 51: 4594–4602, 1991.
10. Hosono MN, Hosono M, Zamora PO, Guhlke S, Haberberger T, Bender H, et al. Localization of colorectal carcinoma by rhenium-188-labeled B72.3 antibody in xenografted mice. *Ann Nucl Med* 12: 83–88, 1998.
11. Goldrosen MH, Biddle WC, Pancook J, Bakshi S, Vanderheyden JL, Fritzberg AR, et al. Biodistribution, pharmacokinetic, and imaging studies with $^{186}$Re-labeled NR-LU-10 whole antibody in LS174T colonic tumor-bearing mice. *Cancer Res* 50: 7973–7978, 1990.
12. Visser GWM, Gerretsen M, Herscheid JDM, Snow GB, van Dongen G. Labeling of monoclonal antibodies with rhenium-186 using the MAG3 chelate for radioimmunotherapy of cancer, a technical protocol. *J Nucl Med* 34: 1953–1963, 1993.
13. McCall MJ, Diril H, Meares CF. Simplified method for conjugating macrocyclic bifunctional chelating agents to antibodies via 2-iminothiolane. *Bioconjugate Chemistry* 1: 222–226, 1990.
14. Srivastava SC, Mease RC. Progress in research on ligands, nuclides, and techniques for labeling monoclonal antibodies. *Nucl Med Biol* 18: 589–603, 1991.
15. Goedemans WT, Panek KJ, Ensing GJ, de Jong MTM. A new, simple method for labelling of proteins with $^{99m}$Tc by derivatization with 1-imino-4-mercaptobutyl groups. *In* Technetium and Rhenium in Chemistry and Nuclear Medicine, Nicolini M, Bandoli G and Mazzi U (eds.), New York, Raven Press, pp. 595–603, 1990.
16. Moretta A, Vitale M, Bottino C, Orengo AM, Morelli L, Augugliaro R, et al. P58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK cells). Anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specifities. *J Exp Med* 178: 597–604, 1993.
17. Moolenaar CECK, Muller EJ, Schol DJ, Figdor CG, Bock E, Bitter-Suermann D, et al. Expression of neural cell adhesion molecule-related sialoglycoprotein in small cell lung cancer and neuroblastoma cell lines H69 and CHP-212. *Cancer Res* 50: 1102–1106, 1990.
18. Bast RC, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. *J Clin Invest* 68: 1331–1337, 1981.
19. Gazdar AF, Carney DN, Russell EK, Sims HL, Baylin SB, Bunn PAJ, et al. Establishment of continuous, clonable cultures of small-cell carcinoma of the lung which have amine precursor uptake and decarboxylation cell properties. *Cancer Res* 40: 3502–3507, 1980.
20. Knapp FFRJ, Mirzadeh S. The continuing important role of radionuclide generator systems for nuclear medicine. *Eur J Nucl Med* 21: 1151–1165, 1994.
21. Scatchard G. The attraction of proteins for small molecules and ions. *Ann NY Acad Sci* 51: 660–672, 1949.
22. Lindmo T, Boven E, Cuttitta F, Fedorko J, Bunn PA. Determination of the immunoreactive fraction of radiolabeled monoclonal antibody by linear extrapolation to binding at infinite antigen excess. *J Immunol Methods* 72: 77–89, 1984.
23. Le Doussal J, Gruaz-Guyon A, Martin M, Delaage M, Barbet J. Targeting of indium-111-labeled bivalent hapten to human melanoma mediated by bispecific monoclonal antibody conjugates: imaging of tumors hosted in nude mice. *Cancer Res* 50: 3445–3452, 1990.
24. Hosono M, Hosono MN, Kraeber-Bodere F, Devys A, Thédrez P, Fiche M, et al. Biodistribution and dosimetric study in medullary thyroid cancer xenograft using bispecific antibody and iodine-125-labeled bivalent hapten. *J Nucl Med* 39: 1608–1613, 1998.
25. Hosono M, Hosono MN, Kraeber-Bodere F, Devys A, Thédrez P, Faivre-Chauvet A, et al. Two-step targeting and dosimetry for small-cell lung cancer xenograft with anti-NCAM/anti-histamine bispecific antibody and radioiodinated bivalent hapten. *J Nucl Med* 40: 1216–1221, 1999.
26. Fujimori K, Covell DG, Fletcher JE, Weinstein JN. A modeling analysis of monoclonal antibody percolation through tumors: a binding-site barrier. *J Nucl Med* 31: 1191–1198, 1990.