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Intraperitoneal radioimmunotherapy in treating peritoneal carcinomatosis of colon cancer in mice compared with systemic radioimmunotherapy

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Peritoneal spread is one of major causes of mortality in colorectal cancer patients. In the current investigation, the efficacy of radioimmunotherapy (RIT) with i.p. administration of an anti-colorectal cancer IgG1, ¹³¹I-A7, was compared to that with i.v. administration in BALB/c female mice bearing peritoneal nodules of LS180 human colon cancer cells, at the same toxicity level. Distribution of either i.p. or i.v. administered ¹³¹I-A7 and i.p. administered irrelevant ¹³¹I-HPMS-1 was assessed. Based on the results of toxicity determination at increments of 2 MBq and estimated dosimetry, an i.p. dose of 11 MBq and an i.v. dose of 9 MBq were chosen for treatment. Mice were monitored for long-term survival: untreated mice (*n*=11), mice undergoing i.p. RIT with ¹³¹I-A7 (*n*=11), mice undergoing i.v. RIT with ¹³¹I-A7 (*n*=11) and mice undergoing non-specific i.p. RIT with ¹³¹I-HPMS-1 (*n*=5). Intraperitoneal injection of ¹³¹I-A7 produced faster and greater tumor accumulation than i.v. injection: 34.2±16.5% of the injected dose per g (% ID/g) and 11.1±3.6% ID/g at 2 h, respectively (*P*<0.0001). Consequently, cumulative radioactivity in tumors was 1.73-fold higher with i.p. injection. ¹³¹I-HPMS-1 did not show specific accumulation. Non-specific RIT with ¹³¹I-HPMS-1 (mean survival, 26.0±2.5 days) did not affect the survival as compared to no treatment (26.7±1.9 days). Intravenous RIT with ¹³¹I-A7 prolonged the survival of mice to 32.8±1.8 days (*P*<0.01). Intraperitoneal RIT with ¹³¹I-A7 improved the survival more significantly and attained cure in 2 of 11 mice (*P*<0.05 vs. i.v. RIT). In conclusion, i.p. RIT is more beneficial in treating peritoneal carcinomatosis of colon cancer than i.v. RIT in a murine model. (Cancer Sci 2003; 94: 650–654)

Peritoneal spread is a sign of the terminal stage in colorectal cancer, being one of major causes of mortality in patients.^{1–3} Intraperitoneal chemotherapy in combination with cytoreductive surgery—peritonectomy—has been examined as a therapeutic option to prolong the survival of patients,^{1–3} but the majority eventually dies of progressive disease.

Several reports have indicated the effectiveness of radioimmunotherapy (RIT) employing radiolabeled monoclonal antibodies (MAb) under conditions of minimal tumor burden in cancer patients.^{4–10} One of the major obstacles to RIT with i.v. injected MAb is inadequate targeting of MAb to tumors.¹¹ Previous studies have indicated that greater accumulation in peritoneal tumors can be achieved with i.p. injection of MAb than with systemic injection.^{12–25} Furthermore, higher tumor-to-normal tissue uptake ratios are achievable with i.p. injection, resulting in a favorable dosimetry, not only as regards the radiation dose to tumors but also the toxicity profile.

Feasibility of i.p. RIT has been extensively investigated in ovarian cancer patients because of the high incidence of peritoneal dissemination of ovarian cancer^{12–20}; indeed, prolonged survival with RIT has been documented. However, the effec-

tiveness of i.p. RIT has not been fully validated for the treatment of peritoneal carcinomatosis of colorectal cancer despite reports demonstrating the dosimetric advantages of i.p. injection of MAb in peritoneal tumors.^{21–25} Therefore, we aimed to compare the therapeutic efficacy of intraperitoneal RIT and i.v. RIT performed at the same toxicity level in a mouse model of human colon cancer.

Materials and Methods

Monoclonal antibodies. A7, an IgG1 murine MAb with κ-light chain, was used in this investigation.²⁶ It was purified from ascites of hybridoma-bearing mice by Protein A Sepharose column (Bio-Rad, Richmond, CA) chromatography. A7 MAb recognizes a 45-kD surface glycoprotein of human colonic carcinomas. The antigen is modulated after binding with A7, and the internalization of A7 occurs. A7 does not react with normal gastric mucosa, erythrocytes, peripheral lymphocytes or ileal mucosa, and shows weak reactivity with 10% of colon mucosa specimens. An additional IgG1 recognizing placental alkaline phosphatase, HPMS-1, was utilized as a class-matched irrelevant control MAb.²⁷ Antibodies were radiolabeled with ¹³¹I by the chloramine-T method, and subsequently purified on a PD10 column (Pharmacia LKB Biotechnology, Uppsala, Sweden). The specific activity of purified ¹³¹I-A7 was 175–247 MBq/mg. Immunoreactivity of purified ¹³¹I-A7 determined under antigen excess conditions with LS180 human colon cancer cell line (American Type Culture Collection, Rockville, MD) was 74–82%. The specific activity of ¹³¹I-HPMS-1 was 259 MBq/mg. Antibodies were sterilized by filtration (Millex-GV, 0.22 μm; Millipore, Bedford, MA) prior to further experiments.

Peritoneal metastasis model. Animal studies were performed in compliance with the regulations of our institution. LS180 cells were grown in DMEM medium (Nissui Seiyaku, Tokyo), harvested with 0.1% trypsin, washed and subsequently suspended in PBS at 1×10⁸/ml. An aliquot of the cell suspension (0.1 ml) was injected i.p. into Balb/c *nu/nu* mice (female, 20 g; NINOX Labo Supply, Inc., Ishikawa). Peritoneal nodules reach several mm in diameter by 10 days after cell inoculation.

Biodistribution of antibodies. Mice bearing peritoneal nodules were injected either i.p. or i.v. with 111 kBq (3 μCi) of ¹³¹I-A7 10 days after inoculation of tumor cells. The animals were sacrificed 2 and 6 h and 1, 2 and 3 days later (*n*=3–5). Organs were excised and weighed; subsequently, radioactivity of the tissues was measured with a well-type γ-counter. In order to analyze the relationship between metastasis size and ¹³¹I-MAb ac-

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cumulation, nodules of various sizes were selected at random. Nodules were individually weighed and counted for radioactivity. The biodistribution of i.p. injected ^{131}I -HPMS-1 was similarly observed 1 and 2 days after injection as a negative control ($n=3$), and the results were analyzed by means of the Mann-Whitney test. The level of significance was set at 5%.

Determination of toxicity. Toxicity was determined for i.p. and i.v. administration of ^{131}I -A7 by assessing thrombocytopenia and body weight loss after ^{131}I -A7 administration at increments of 2 MBq. A blood sample (5 μL) was obtained from a tail vein of each mouse. Samples within a single group ($n=3$) were pooled and diluted 1:100 in 1% ammonium oxalate for platelet counts.

For the dosimetric assessment, cumulative radioactivity after ^{131}I -A7 administration in 1 g of tissue ($\text{MBq}\times\text{h/g}$) was obtained by the trapezoid integration method using the biodistribution data.

Radioimmunotherapy. The therapeutic experiment consisted of four groups: non-treated mice ($n=11$), mice undergoing specific RIT with i.p. injection of ^{131}I -A7 ($n=11$), mice undergoing specific RIT with i.v. injection of ^{131}I -A7 ($n=11$), and mice undergoing non-specific RIT with i.p. injection of an irrelevant ^{131}I -HPMS-1 ($n=5$). Administration doses were chosen based on the results of toxicity determination. The dose of ^{131}I -HPMS-1 was set at the same level as that of ^{131}I -A7. MABs were admin-

istered 10 days after inoculation of tumor cells. Animals were monitored for survival. Results were analyzed by the Kaplan-Meier method with the log rank test. The level of significance was set at 5%.

Results

Intraperitoneal injection of ^{131}I -A7 resulted in faster and greater accumulation than i.v. injection: $34.2\pm 16.5\%$ ID/g and $11.1\pm 3.6\%$ ID/g at 2 h after injection, respectively ($P<0.0001$) (Fig. 1, A and B). On the other hand, the i.v. route yielded peak activity in tumors 1 day after injection (Fig. 1B). Radioactivity level in tumors was similar in the two groups at late time points. Accumulation in tumors increased with decreasing size in both administration routes (Fig. 2). An irrelevant MAB,

Fig. 1. Biodistribution of i.p. injected ^{131}I -A7 (A) and i.v. injected ^{131}I -A7 (B) in mice bearing peritoneal metastases ($n=3-5$). Accumulation of an irrelevant ^{131}I -HPMS-1 in metastases is also shown (A) ($n=3$). Values of tumor accumulation are means $\pm$ SD of all samples obtained in each group. In general, 10–20 metastatic nodules of various sizes ranging 0.1–400 mg were measured for antibody accumulation in each mouse. No significant differences were observed in the distribution of lesional sizes among observation groups.

Fig. 2. Size-effect of peritoneal metastatic nodules on accumulation of ^{131}I -A7 at 2 h (□) and 6 h (○) after i.p. (A) or i.v. (B) injection.

Fig. 3. Change of platelet count induced by ^{131}I -A7 administered either i.p. or i.v.

HPMS-1, did not specifically accumulate in tumors (Fig. 1A).

Toxicity of ^{131}I -A7 administration was dose-dependent (Fig. 3 and Fig. 4). Because the body weight of mice receiving an i.v. dose of 11 MBq was greatly decreased and recovery was poor, the second highest dose, 9 MBq, was chosen for the i.v. dose in therapeutic assessment. Based on the degrees of platelet depression and body weight loss, an i.p. dose of 11 MBq was determined to be equitoxic. According to these results, cumulative radioactivity values in tissues were calculated for the administration of i.v. 9 MBq and i.p. 11 MBq (Table 1). Cumulative radioactivity in normal tissues was nearly identical after i.v. 9 MBq and i.p. 11 MBq, validating the results of toxicity determination. At these administration doses, i.p. injection of ^{131}I -A7 produced 1.73-fold higher cumulative radioactivity in peritoneal tumors than i.v. administration, and therapeutic ratios relative to normal organs were significantly higher with i.p. injection.

All mice with peritoneal metastases died by 33 days after cell inoculation if no treatment was conducted (Fig. 5). Mean survival of non-treated mice was 26.7 ± 1.9 days. Non-specific RIT with an irrelevant ^{131}I -HPMS-1 (mean survival, 26.0 ± 2.5 days), did not affect the survival as compared to no treatment. Intravenous RIT with ^{131}I -A7 prolonged the survival of animals to 32.8 ± 1.8 days ($P < 0.01$). Intraperitoneal RIT with ^{131}I -A7 improved the survival more significantly ($P < 0.05$). Moreover, i.p. RIT attained cure in 2 of 11 mice.

Discussion

RIT currently utilizes β emitters to treat cancer lesions. Clinical

RIT in patients with bulky solid tumors has shown only limited success.¹¹ On the other hand, recent investigations have indicated the role of RIT in an adjuvant setting where small residual tumors are targeted by radiolabeled MAb,^{4–10} despite the limitations of β emitters suggested by mathematical model analyses.²⁸ A factor influencing the effectiveness of RIT in small tumors is the size-dependency of MAb accumulation in tumors.^{29,30} In the current investigation, we found that this situation is also true in targeting i.p. metastases of 0.1–400 mg.

One of the major reasons for therapeutic failure of clinical RIT in solid tumors is an insufficient radiation dose to tumors.¹¹ To overcome this limitation, locoregional delivery of labeled MAb has been proposed, especially in ovarian cancer patients because of the high incidence of peritoneal dissemination of ovarian cancer.^{12–20} In colorectal cancer, the dosimetric advantage of i.p. administration has been similarly acknowledged^{21–25}; however, the effectiveness of i.p. RIT relative to i.v. RIT has not been validated so far. The current investigation confirmed better therapeutic effects as well as more favorable dosimetry of i.p. RIT as compared to i.v. RIT in a peritoneal metastasis model.

Because of Michaelis-Menten kinetics in antibody-antigen interactions,³¹ the higher MAb concentration in the peritoneal cavity achieved by i.p. administration than i.v. administration is beneficial. Slow absorption of large molecules such as MAbs from the peritoneal cavity may maintain high concentration.³² These factors cause rapid and intense localization of MAb in peritoneal tumors, resulting in considerably higher therapeutic ratios as compared to those with i.v. administration. However,

Fig. 4. Body weight loss induced by ^{131}I -A7 administered either i.p. or i.v.

Fig. 5. Survival of mice bearing peritoneal metastases. Radioimmunotherapy (RIT) was performed 10 days after cell inoculation. $\circ$, non-treated control; $\diamond$, non-specific RIT with an irrelevant ^{131}I -HPMS-1 i.p. injected; $\triangle$, i.v. RIT with ^{131}I -A7; $\square$, i.p. RIT with ^{131}I -A7. Doses of i.p. RIT and i.v. RIT were 11 MBq and 9 MBq, respectively.

Table 1. Cumulative radioactivity after i.p. or i.v. administration of ^{131}I -A7

	Cumulative radioactivity (MBq $\times$ h/g)		Metastasis-to-normal tissue ratio		
	i.p.	i.v.	i.p.	i.v.	i.p./i.v.
Blood	61.3	70.4	1.75	0.88	1.98
Liver	17.6	18.8	6.09	3.29	1.85
Spleen	18.1	16.6	5.93	3.73	1.59
Kidney	15.6	13.2	6.86	4.70	1.46
Bone	7.2	7.0	14.80	8.83	1.68
Muscle	5.3	4.3	20.17	14.31	1.41
Intestine	11.1	8.3	9.62	7.45	1.29
Metastasis	107.0	62.0	—	—	—

i.p., intraperitoneal administration (11 MBq); i.v., intravenous administration (9 MBq); i.p./i.v., ratio of metastasis-to-normal tissue ratio of i.p. administered ^{131}I -A7 to that of i.v. administered ^{131}I -A7.

this advantage was less prominent at later time points, which suggests that RIT employing radionuclides with physical half life shorter than ^{131}I would deliver radiation to tumors more effectively.

Liver metastasis occurs more often than peritoneal spread in colon cancer patients, and both types of metastases may simultaneously exist. We have previously demonstrated the effectiveness of RIT in a liver metastasis model of colon cancer in which radio-labeled A7 was administered systemically.^{33–35} Intraperitoneal MAb administration reportedly produces a similar degree of MAb accumulation in liver metastases as compared to i.v. injection,³⁶ so that i.p. RIT may work in treating liver metastases as well as peritoneal lesions.

Previous studies demonstrated the improvement of therapeutic outcomes with combination treatment of RIT and chemotherapy in comparison with monotherapy.^{37, 38} For instance, specificity of 5-FU against cells in the S-phase of the cell cycle suggests that 5-FU may act selectively against less radiosensi-

tive cells.³⁹ Furthermore, chemotherapeutic drugs may act as a radiosensitizer so that the interaction with RIT may not be a simple additive effect, but rather a synergistic effect.³⁹ This combination therapy seems worth evaluating in a peritoneal metastasis model.

In conclusion, i.p. administration of ^{131}I -A7 affords an improvement in terms of absorbed radiation dose to colon cancer metastases within the peritoneal cavity in a murine model, leading to longer survival in mice treated via the i.p. route in comparison with the i.v. route.

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1. Yamaguchi A, Tsukioka Y, Fushida S, Kurosaka Y, Kanno M, Yonemura Y, Miwa K, Miyazaki I. Intraperitoneal hyperthermic treatment for peritoneal dissemination of colorectal cancers. *Dis Colon Rectum* 1992; **35**: 964–8.
2. Markman M. Intraperitoneal chemotherapy in the management of colon cancer. *Semin Oncol* 1999; **26**: 536–9.
3. Pestieau SR, Sugarbaker PH. Treatment of primary colon cancer with peritoneal carcinomatosis. *Dis Colon Rectum* 2000; **43**: 1341–8.
4. Sharkey RM, Weadock KS, Natale A, Haywood L, Aninipot R, Blumenthal RD, Goldenberg DM. Successful radioimmunotherapy for lung metastasis of human colonic cancer in nude mice. *J Natl Cancer Inst* 1991; **83**: 627–32.
5. Blumenthal RD, Sharkey RM, Haywood L, Natale AM, Wong GY, Siegel JA, Kennel SJ, Goldenberg DM. Targeted therapy of athymic mice bearing GW-39 human colonic cancer micrometastases with ^{131}I -labeled monoclonal antibodies. *Cancer Res* 1992; **52**: 6036–44.
6. Dunn RM, Juweid M, Sharkey RM, Behr TM, Goldenberg DM. Can occult metastases be treated by radioimmunotherapy? *Cancer* 1997; **80** Suppl: 2656–9.
7. Vogel C-A, Galmiche MC, Buchegger F. Radioimmunotherapy and fractionated radiotherapy of human colon cancer liver metastases in nude mice. *Cancer Res* 1997; **57**: 447–53.
8. Behr TM, Blumenthal RD, Memsoudis S, Sharkey RM, Gratz S, Becker W, Goldenberg DM. Cure of metastatic human colonic cancer in mice with radiolabeled monoclonal antibody fragments. *Clin Cancer Res* 2000; **6**: 4900–7.
9. Saga T, Sakahara H, Nakamoto Y, Sato N, Zhao S, Iida Y, Kuroki M, Endo K, Konishi J. Radioimmunotherapy for liver micrometastases in mice: pharmacokinetics, dose estimation, and long-term effect. *Jpn J Cancer Res* 1999; **90**: 342–8.
10. Sato N, Saga T, Sakahara H, Yao Z, Nakamoto Y, Zhang M, Kuroki M, Matsuo K, Iida Y, Konishi J. Intratumoral distribution of radiolabeled antibody and radioimmunotherapy in experimental liver metastases model of nude mouse. *J Nucl Med* 1999; **40**: 685–92.
11. Imam SK. Status of radioimmunotherapy in the new millennium. *Cancer Biother Radiopharm* 2001; **16**: 237–56.
12. Stewart JS, Hird V, Snook D, Dhokia B, Sivolapenko G, Hooker G, Papadimitriou JT, Rowlinson G, Sullivan M, Lambert HE, Coulter C, Mason WP, Soutter WP, Epenetos AA. Intraperitoneal yttrium-90-labeled monoclonal antibody in ovarian cancer. *J Clin Oncol* 1990; **8**: 1941–50.
13. Maraveyas A, Snook D, Hird V, Kosmas C, Meares CF, Lambert HE, Epenetos AA. Pharmacokinetics and toxicity of an yttrium-90-CITC-DTPA-HMFG1 radioimmunoconjugate for intraperitoneal radioimmunotherapy of ovarian cancer. *Cancer* 1994; **73**: 1067–75.
14. Meredith RF, Partridge EE, Alvarez RD, Khazaeli MB, Plott G, Russell CD, Wheeler RH, Liu T, Grizzle WE, Schlom J, LoBuglio AF. Intraperitoneal radioimmunotherapy of ovarian cancer with lutetium-177-CC49. *J Nucl Med* 1996; **37**: 1491–6.
15. Borchardt PE, Quadri SM, Freedman RS, Vriesendorp HM. Indium-111- and yttrium-90-labeled human monoclonal immunoglobulin M targeting of human ovarian cancer in mice. *J Nucl Med* 1998; **39**: 476–84.
16. Buijs WC, Tibben JG, Boerman OC, Molthoff CF, Massuger LF, Koenders EB, Schijf CP, Siegel JA, Corstens FH. Dosimetric analysis of chimeric monoclonal antibody cMOv18 IgG in ovarian carcinoma patients after intraperitoneal and intravenous administration. *Eur J Nucl Med* 1998; **25**: 1552–61.
17. Mahe MA, Fumoleau P, Fabbro M, Guastalla JP, Faurous P, Chauvot P, Chetanoud L, Classe JM, Rouanet P, Chatal JF. A phase II study of intraperitoneal radioimmunotherapy with iodine-131-labeled monoclonal antibody OC-125 in patients with residual ovarian carcinoma. *Clin Cancer Res* 1999; **5**: 3249s–53s.
18. Rosenblum MG, Verschraegen CF, Murray JL, Kudelka AP, Gano J, Cheung L, Kavanagh JJ. Phase I study of ^{90}Y -labeled B72.3 intraperitoneal administration in patients with ovarian cancer: effect of dose and EDTA coadministration on pharmacokinetics and toxicity. *Clin Cancer Res* 1999; **5**: 953–61.
19. Borchardt PE, Quadri SM, Freedman RS, Vriesendorp HM. Intraperitoneal radioimmunotherapy with human monoclonal IGM in nude mice with peritoneal carcinomatosis. *Cancer Biother Radiopharm* 2000; **15**: 53–64.
20. Meredith RF, Alvarez RD, Partridge EE, Khazaeli MB, Lin CY, Macey DJ, Austin JM Jr, Kilgore LC, Grizzle WE, Schlom J, LoBuglio AF. Intraperitoneal radioimmunotherapy of ovarian cancer: a phase I study. *Cancer Biother Radiopharm* 2001; **16**: 305–15.
21. Rowlinson G, Snook D, Busza A, Epenetos AA. Antibody-guided localization of intraperitoneal tumors following intraperitoneal or intravenous antibody administration. *Cancer Res* 1987; **47**: 6528–31.
22. Larson SM, Carrasquillo JA, Colcher DC, Yokoyama K, Reynolds JC, Bacharach SA, Raubitschek A, Pace L, Finn RD, Rotman M. Estimates of radiation absorbed dose for intraperitoneally administered iodine-131 radiolabeled B72.3 monoclonal antibody in patients with peritoneal carcinomatosis. *J Nucl Med* 1991; **32**: 1661–7.
23. Quadri SM, Malik AB, Tang XZ, Patenia R, Freedman RS, Vriesendorp HM. Preclinical analysis of intraperitoneal administration of ^{111}In -labeled human tumor reactive monoclonal IgM AC6C3-2B12. *Cancer Res* 1995; **55**: 5736s–42s.
24. Quadri SM, Malik AB, Chu HB, Freedman RS, Vriesendorp HM. Intraperitoneal indium-111- and yttrium-90-labeled human IgM (AC6C3-2B12) in nude mice bearing peritoneal carcinomatosis. *J Nucl Med* 1996; **37**: 1545–51.
25. Safavy A, Khazaeli MB, Safavy K, Mayo MS, Buchsbaum DJ. Biodistribution study of ^{188}Re -labeled trisuccin-HuCC49 and trisuccin-HuCC49deltaCh2 conjugates in athymic nude mice bearing intraperitoneal colon cancer xenografts. *Clin Cancer Res* 1999; **5**: 2994s–3000s.
26. Kotanagi H, Takahashi T, Masuko T, Hashimoto Y, Koyama K. A monoclonal antibody against human colon cancers. *Tohoku J Exp Med* 1986; **148**: 353–60.
27. Hirano K, Iizumi Y, Hayashi Y, Tanaka T, Sugiura M, Hayashi K, Lu ZD, Iino S. A highly sensitive assay method for human placental alkaline phosphatase involving a monoclonal antibody bound to a paper disk. *Anal Biochem* 1986; **154**: 624–31.
28. O'Donoghue JA, Bardies M, Wheldon TE. Relationships between tumor size and curability for uniformly targeted therapy with beta-emitting radionuclides. *J Nucl Med* 1995; **36**: 1902–9.
29. Hagan PL, Halpern SE, Dillman RO, Shawler DL, Johnson DE, Chen A, Krishnan L, Frincke J, Bartholomew RM, David GS. Tumor size: effect on monoclonal antibody uptake in tumor models. *J Nucl Med* 1986; **27**: 422–7.
30. Behr TM, Sharkey RM, Juweid ME, Dunn RM, Ying Z, Zhang CH, Siegel JA, Goldenberg DM. Variables influencing tumor dosimetry in radioimmunotherapy of CEA-expressing cancers with anti-CEA and anti-mucin monoclonal antibodies. *J Nucl Med* 1997; **38**: 409–19.
31. Lindom T, Boven E, Cuttitta F, Fedorko J, Bunn PA Jr. Determination of the immunoreactive fraction of radiolabeled monoclonal antibodies by linear extrapolation to binding at infinite antigen excess. *J Immunol Methods* 1984; **72**: 77–89.
32. Dedrick RL. Theoretical and experimental bases of intraperitoneal chemotherapy. *Semin Oncol* 1985; **3**: 1–6.

33. Li XF, Kinuya S, Yokoyama K, Koshida K, Mori H, Shiba K, Watanabe N, Shuke N, Michigishi T, Tonami N. Benefits of combined radioimmunotherapy and anti-angiogenic therapy in a liver metastasis model of human colon cancer cells. *Eur J Nucl Med* 2002; **29**: 1669–74.
34. Kinuya S, Yokoyama K, Kawashima A, Izumo M, Sorita T, Obata T, Watanabe N, Shuke N, Bunko H, Michigishi T, Tonami N. Radioimmunotherapy with ¹⁸⁶Re-labeled monoclonal antibody to treat liver metastases of colon cancer cells in nude mice. *Cancer Biother Radiopharm* 2002; **17**: 681–7.
35. Kinuya S, Yokoyama K, Izumo M, Sorita T, Obata T, Mori H, Shiba K, Watanabe N, Shuke N, Michigishi T, Tonami N. Feasibility of ¹⁸⁶Re-radioimmunotherapy for treatment in an adjuvant setting of colon cancer. *J Cancer Res Clin Oncol* in press.
36. Yoshida K, Fujikawa T, Yoshizawa G, Tanabe A, Sakurai K. Biodistribution of radiolabeled monoclonal antibody after intraperitoneal administration in nude mice with hepatic metastasis from human colon cancer. *Surg Today* 1992; **22**: 155–8.
37. Kinuya S, Yokoyama K, Tega H, Hiramatsu T, Konishi S, Watanabe N, Shuke N, Aburano T, Takayama T, Michigishi T, Tonami N. Efficacy, toxicity and mode of interaction of combination radioimmunotherapy with 5-fluorouracil in colon cancer xenografts. *J Cancer Res Clin Oncol* 1999; **12**: 630–6.
38. Kinuya S, Yokoyama K, Konishi S, Hiramatsu T, Watanabe N, Shuke N, Aburano T, Takayama T, Michigishi T, Tonami N. Enhanced efficacy of radioimmunotherapy combined with systemic chemotherapy and local hyperthermia in xenografted model. *Jpn J Cancer Res* 2000; **91**: 573–8.
39. Hall EJ. Radiobiology for the radiologist. 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2000.