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Efficacy and Specificity of a Monoclonal Antibody-Drug Conjugate in Chemotherapy by Intratumoral Injection

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The murine monoclonal antibody (Mab) A7 conjugated to neocarzinostatin (A7-NCS) was injected intratumorally (IT) into tumor bearing nude mice. Its pharmacokinetics and tumoricidal effects were compared in the high, moderate and low antigen expressing xenograft for SW1116, WiDr and KB tumor-bearing nude mice, respectively. When injected IT into nude mice, [^{125}I]A7-NCS was retained in the tumors according to the degree of antigen expression; it was also disseminated into the blood inverse proportion to the antigen expression. Addition of an excess amount of Mab A7 reduced [^{125}I]-A7-NCS accumulation in SW1116 xenograft and elevated the [^{125}I]A7-NCS concentration in the circulation. Complete tumor reduction was found in all 5 mice with SW1116 tumor, and 2 of 5 mice with WiDr tumor. However, only incomplete tumor suppression was observed in mice with the KB tumor. The significant tumor reduction in SW1116 bearing nude mice was attenuated when excess of Mab A7 was simultaneously administered with A7-NCS. These findings indicate that A7-NCS was localized in the target tumors and exerted its tumoricidal effects depending on the degree of antigen-antibody interaction when administered IT. Thus, A7-NCS can be used successfully *in vivo* for local therapy, auguring new and promising applications for local cancer therapy.

Key words: Intratumoral injection — A7-NCS — Cancer chemotherapy

The use of antibodies as carriers of pharmacologic agents has become more practical since the advent of monoclonal antibody technology; several studies have demonstrated the potential clinical use of immunoconjugates.[1–5] To date, the focus of *in vivo* studies has been on IV[2] administration, because the bloodstream allows access to almost all the organs of the body. However, this route of administration is inefficient and delivers, at most, only several percent of the injected dose per gram of tumor.[6–10] This prompted us to try immunoconjugate delivery by IT injection, which is thought to increase vastly the amount of immunoconjugate localized in the tumor. The present study was undertaken to examine experimentally the effectiveness *in vivo* of conjugates administered IT.

MATERIALS AND METHODS

Preparation of A7-NCS The Mab A7 recognizes a 45 kD glycoprotein on the cell surface[11] and reacts strongly with human colorectal adenocarcinomas,[12] pancreatic adenocarcinomas[13] and breast carcinomas. Mab A7, which is an IgG_1 subtype,[12] was conjugated to two molecules of the anticancer polypeptide neocarzinostatin by disulfide linkage, as described in a previous report.[14] A7-NCS retains antigen-binding activity, and has potent cytotoxicity *in vitro* and potent tumoricidal effects *in vivo*.[15] The disulfide linkage between Mab A7 and NCS was sufficiently stable to allow the conjugate to reach the target organ *in vivo*, and the conjugate showed a similar pharmacokinetic profile to Mab A7.[16]

Radiolabeling A7-NCS was radiolabeled with ^{125}I by chloramine T,[17] to a specific activity of 2.5×10^6 cpm/μg of protein. A7-NCS is known to be radiolabeled with ^{125}I without releasing the conjugated NCS molecules.[16] [^{125}I]A7-NCS binds well to the human colon cancer cell line SW1116, which expresses a 45 kD glycoprotein on its cell surface.

Antigen expression The human squamous cell carcinoma cell line KB, and human colon cancer cell lines SW1116 and WiDr were used throughout these experiments. [^{125}I]A7-NCS (5×10^4 cpm) was incubated with 10^5 cells from each cell line for 1 h at 37°C, with or without 10 μg of Mab A7. Cells were washed with PBS three times and centrifuged at 1500 rpm. After centrifugation, the supernatant was discarded and the cell pellets were put into a gamma tube. The radioactivity in the pellets was measured by a gamma counter, and is expressed in cpm per cell pellet.

Tumor localization in IV injection Cells (5×10^6) were inoculated subcutaneously into the backs of nude mice

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[2] Abbreviations used in this paper: IV, intravenous(ly); IT, intratumoral(ly); Mab A7, the murine monoclonal antibody A7; NCS, neocarzinostatin; A7-NCS, the monoclonal antibody A7-neocarzinostatin conjugate; PBS, phosphate-buffered saline.

(Balb/c, *nu/nu*). Two weeks later, the mice developed palpable tumors weighing between 0.15 g and 0.25 g. [^{125}I]A7-NCS (10^6 cpm) was administered IV to five mice with or without simultaneous injection of non-labeled Mab A7 (1 mg per mouse). Twenty-four h after [^{125}I]A7-NCS injection, the mice were killed for tumor resection. For comparison of conjugate accumulation in each tumor, resected tumors were weighed and subjected to gamma counting.

IT injection of [^{125}I]A7-NCS for pharmacokinetics [^{125}I]A7-NCS (10^6 cpm) was injected IT into various antigen-expressing xenograft-bearing nude mice. Mice were killed at 1, 6, 12, 24, 48 and 72 h after [^{125}I]A7-NCS injection. Tumors and blood were collected and weighed, and their radioactivities were counted in a gamma counter.

In a separate experiment, [^{125}I]A7-NCS (5×10^5 cpm) was administered IT to SW1116-bearing mice with or without IT injection of Mab A7 (1 mg per mouse). Blood samples in both groups were taken from the tail vein with capillary tubes at various time points, and the radioactivity was measured in a gamma counter. Mice were killed 6 h after the IT injection of [^{125}I]A7-NCS, and the tumor was resected. Tumor and blood [^{125}I]A7-NCS content was determined as the mean percentage of the injected dose per gram of organs, and compared between the two groups.

IT injection of A7-NCS for therapy Prior to this experiment, the sensitivity of cell lines to NCS was examined by ^{3}H-thymidine incorporation assay. Based on the results, the therapeutic study was begun when the mice developed palpable tumors ranging from 15 to 20 mm^3. A7-NCS equivalent to 10 units of NCS, 10 units of NCS, and saline, each in a 10 μl volume, were injected IV or IT into five mice of each group twice a week for two weeks. Ten weeks after the initiation of treatment, the effects of the preparations on tumor growth were evaluated. Evaluation was based upon the ratio of complete tumor reduction. Complete reduction was defined as tumor disappearance and no recurrence.

In a separate experiment, 1 mg of Mab A7, which can significantly inhibit the *in vivo* tumor accumulation of [^{125}I]A7-NCS, was administered simultaneously with the A7-NCS in SW1116-bearing nude mice. Tumor reduction in this group was compared with that in the group not given an excess of Mab A7.

RESULTS

Antigen expression The antigen expression of each cell line was examined by radioimmunoassay using [^{125}I]-A7-NCS. [^{125}I]A7-NCS bound well to SW1116 cells, moderately well to WiDr cells and poorly to KB cells. [^{125}I]A7-NCS in the presence of an excess amount of Mab A7 showed low binding to all cell lines. The result is in accord with the observations in a previous report.[10] Thus, the A7-NCS immunoreactivity to each cell line can be summarized as follows: SW1116 > WiDr > KB (Fig. 1A). There was no significant difference of sensitivity of these three cell lines to NCS (data not shown).

A7-NCS accumulation to various xenografts by IV injection When administered IV, [^{125}I]A7-NCS accumulated in the target tumor depending on the degree of antigen expression (Fig. 1B). When administered with an excess of Mab A7, [^{125}I]A7-NCS accumulated in the target tumor to a lesser degree than when administered without Mab A7. There were no differences in [^{125}I]A7-NCS localization between the three tumors when [^{125}I]A7-NCS was injected simultaneously with an excess of Mab A7.

Tumor and blood clearance of IT injected A7-NCS Tumor clearance of IT injected [^{125}I]A7-NCS was compared between the SW1116, WiDr and KB xenografts. The conjugate concentration was high in the SW1116 xenografts, moderate in the WiDr xenografts and low in the KB xenografts (Fig. 2). The area under the concentration curve (AUC) was calculated by a computerized mathematical method.[18] Tumor AUCs were 4180, 2478 and 1697 (% ID/g of tumor) in SW1116, WiDr and KB xenografts, respectively (Table I). Blood clearance of IT

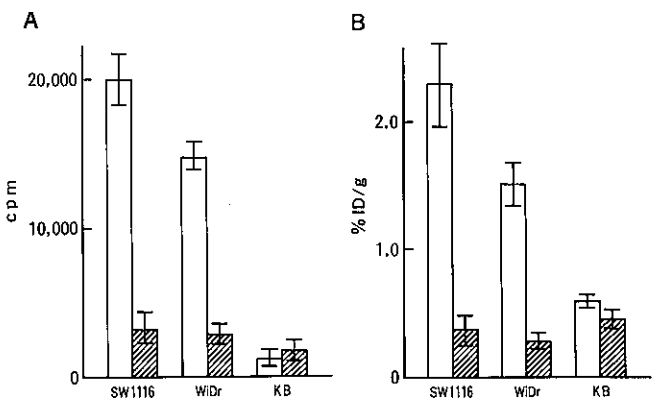

Fig. 1. Antigen expression and localization of [^{125}I]A7-NCS in cancer cell lines. (A) A7-NCS labeled with ^{125}I (5×10^4 cpm) was added to 10^5 cells from each cancer cell line, with (▨) or without (□) 10 μg of Mab A7. The cells were centrifuged and washed with PBS. Radioactivity in the pellets was determined by a gamma counter. Data are expressed as cpm of [^{125}I]A7-NCS binding to the cells. Bars: ±SE. (B) A7-NCS labeled with ^{125}I, with (▨) or without (□) an excess of Mab A7, was injected IV into tumor-bearing nude mice. The mice were killed 24 h after injection, and the tumors were resected for gamma counting. Data are expressed as the % ID per gram of tumor. Bars: ±SE.

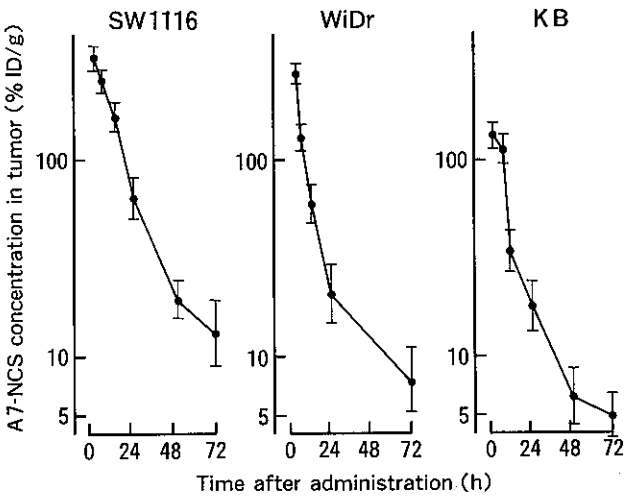

Fig. 2. Tumor clearance of [^{125}I]A7-NCS after IT injection. A7-NCS labeled with ^{125}I was injected IT into tumor-bearing nude mice which were killed 1, 6, 12, 24, 48 and 72 h after the injection. The tumors were resected for gamma counting. Data are expressed as the % ID per gram of tumor. Bars: ±SE.

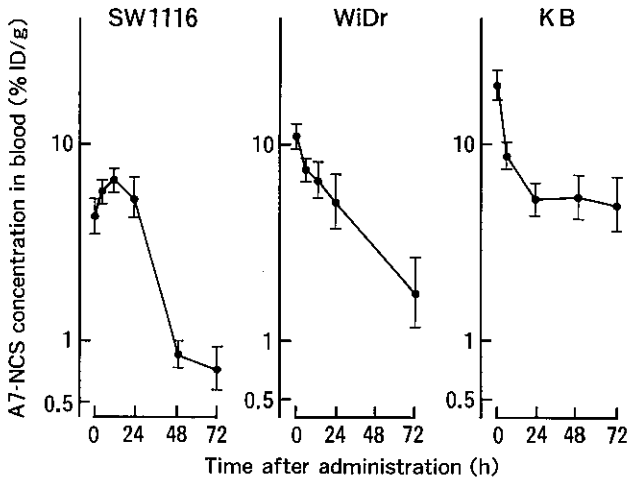

Fig. 3. Blood clearance of [^{125}I]A7-NCS injected IT. A7-NCS labeled with ^{125}I was injected IT into tumor-bearing nude mice, which were killed 1, 6, 12, 24, 48 and 72 h after injection. Bound ^{125}I was measured in blood samples by gamma counting. Data are expressed as the % ID per gram of blood. Bars: ±SE.

Table I. AUC Values in Blood and Tumors

	SW1116	WiDr	KB
Blood	225	411	1016
Tumor	4180	2478	1697

[^{125}I]A7-NCS (10^6 cpm) was injected IT into SW 1116, WiDr and KB xenograft-bearing nude mice. At various times after injection, blood and tumors were taken and the clearances of [^{125}I]A7-NCS in the blood and tumors were measured by a gamma counter. AUCs were calculated by computerized mathematical analysis.[18] Data were expressed as % ID×h/g.

injected [^{125}I]A7-NCS was also compared between the three groups. The conjugate concentration in the blood was high, moderate and low in the KB, WiDr and SW1116 xenograft-bearing mice, respectively (Fig. 3). Blood AUC was calculated in the same manner as for the tumor. Blood AUCs were 225, 411 and 1016 (% ID/g of blood) in SW1116, WiDr and KB xenograft-bearing mice, respectively (Table I).

When [^{125}I]A7-NCS was administered IT to SW1116-bearing mice with an excess of Mab A7, the [^{125}I]A7-NCS concentration was lower in the tumor and higher in the blood than when it was administered without Mab A7 (Fig. 4).

Therapeutic effects of IT injected A7-NCS To determine whether A7-NCS could be used for IT treatment, each xenografted mouse was treated by A7-NCS IT injection. In the case of the SW1116 xenograft, A7-NCS and NCS IT injection and A7-NCS IV injection all inhibited tumor growth to some degree, whereas the tumor proliferated progressively with a control saline IT injection. A7-NCS IT injection resulted in complete remission in all of the 5 mice with the SW1116 tumor. Neither NCS IT injection nor A7-NCS IV injection (data not shown) achieved this result (Table II). IT injection with either A7-NCS or NCS reduced WiDr xenografts in mice, whereas the tumor proliferated after being injected with saline. A7-NCS IT injection achieved complete remission in 2 of the 5 mice with the WiDr xenografts, while NCS IT injection did not achieve the same effect. IT injection of either A7-NCS or NCS inhibited KB tumor growth to some degree, whereas the tumor proliferated with a control saline injection. Complete tumor remission in KB-bearing mice was not seen after A7-NCS IT injection. When A7-NCS was administered simultaneously with an excess of Mab A7, complete tumor remission was not observed in SW1116-bearing mice (data not shown).

DISCUSSION

The advantages of the use of immunoconjugates in cancer chemotherapy are selective delivery, low normal organ toxicity and high localization of the anti-cancer agent in the target tumor. One important factor in defining the *in vivo* chemotherapeutic efficacy of the conjugate is the amount of conjugate reaching the target tumor. We

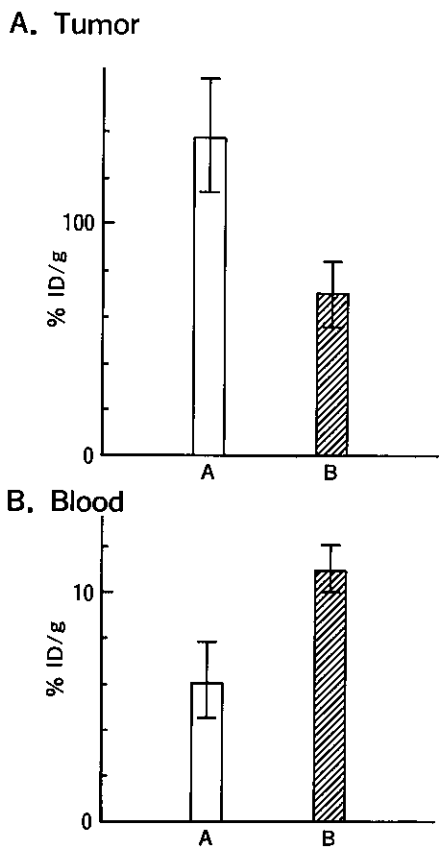

Fig. 4. Effect of excess Mab A7 on tumor and blood localization of [^{125}I]A7-NCS. A7-NCS labeled with ^{125}I (5×10^5 cpm) was administered IT to SW1116 xenograft-bearing nude mice with (▨) or without (□) an excess of Mab A7 (1 mg per mouse). Six h later the mice were killed and tumor and blood samples were taken. Radioactivity levels in the tumor (A) and blood (B) were compared between the two groups. Data were expressed as % ID per gram of tumor or blood. Bars: ±SE.

Table II. Tumor Regression Following IT Treatment with A7-NCS

Group	Tumor	Treatment	Complete cure rate
1	SW1116	A7-NCS	5/5
2	SW1116	NCS	0/5
3	SW1116	Saline	0/5
4	WiDr	A7-NCS	2/5
5	WiDr	NCS	0/5
6	WiDr	Saline	0/5
7	KB	A7-NCS	0/5
8	KB	NCS	0/5
9	KB	Saline	0/5

Tumor-bearing Balb/c nude mice received four IT injections of 10 units of NCS alone or A7-NCS or saline.

developed the immunoconjugate, A7-NCS, which is highly tumoricidal against antigen-positive tumors both *in vitro* and *in vivo*. A7-NCS is capable of delivering NCS to the target tumor in several times the quantity delivered by NCS injection alone.[16] In clinical applications, the conjugate has exhibited some effect,[15] but the response was not satisfactory,[5] presumably because a sufficient amount of the conjugate did not reach the tumor. This can be attributed to various barriers encountered before the conjugate reaches the tumor, such as the blood supply, transcapillary blockade, hydrostatic pressure of the interstitial tissues, etc.[19] To avoid such barriers in this study, A7-NCS was locally injected into the tumor.

A7-NCS labeled with ^{125}I accumulated well, moderately well and poorly in SW1116, WiDr and KB xenograft-bearing mice, respectively, when given by IV administration. Addition of an excess amount of non-labeled Mab A7 significantly inhibited [^{125}I]A7-NCS tumor localization. These findings indicate that the tumor localization of A7-NCS was proportional to the degree of antigen expression, and was not correlated with histological structure or other pathological barriers when IV injection was used in this tumor series. In the case of IT injection, [^{125}I]A7-NCS accumulated well, moderately well and poorly in SW1116, WiDr and KB xenograft-bearing mice, respectively. In addition, when simultaneously injected with an excess of Mab A7, [^{125}I]A7-NCS accumulated in the SW1116 xenograft to a lesser degree. The blood concentration of A7-NCS was high in mice with KB xenografts, moderate in mice with WiDr xenografts, and low in mice with SW1116 xenografts. When [^{125}I]A7-NCS was administered with an excess of Mab A7, its blood concentration was higher than in mice without the excess amount of Mab A7. These findings suggest that A7-NCS, when IT injected, is retained in the tumor in proportion to the degree of antigen expression, and consequently is dispersed into the blood-stream in inverse proportion to antigen expression. The tumor clearance analysis also indicates that IT injected A7-NCS is retained in the target tumor by an antigen-antibody interaction, and not simply by non-specific local retention.

To define conclusively the pharmacokinetics of an IT injected immunoconjugate, some quantification of the drug localized in the target tumor is often required. In this study, we examined the *in vivo* pharmacokinetics of IT injected A7-NCS by following the radioactivity of injected [^{125}I]A7-NCS instead of quantifying the NCS concentration. One problem in the use of ^{125}I-labeled A7-NCS for such analysis will be radiolabeling efficiency, since the majority of the ^{125}I is attached to the antibody molecule and not to the NCS molecules. However, as elucidated in a previous report,[16] [^{125}I]A7-NCS is so stable that it does not release the NCS molecules before

reaching the target tumor. Thus, [^{125}I]A7-NCS tracer analysis can be substituted for *in vivo* pharmacokinetical analysis of this conjugate.

A significant tumoricidal effect was observed with IT injection of A7-NCS on SW1116 and WiDr xenografts, but not on KB xenografts. It can be concluded that the effect was in proportion to the degree of antigen expression in the xenograft, since the *in vitro* sensitivity of the three cell lines to NCS showed a similar profile. The significant tumoricidal effects of IT injected A7-NCS can be attributed to the increased amount of conjugate which is retained in the target tumor. NCS alone showed some response via IT administration, but the effects were transient for all xenograft types in our tumor series. The difference in therapeutic effect between NCS and A7-NCS can be attributed to the attached monoclonal antibody. In other words, the conjugation of Mab A7 enhances the therapeutic effect of NCS via IT injection, presumably due to the high concentration of NCS localized in the tumor. Antigen specificity of A7-NCS IT injection was further examined by treating tumor-bearing mice with an excess of Mab A7. The results showed a reduced therapeutic effect in mice treated with an excess of Mab A7 (data not shown). These findings indicate that A7-NCS IT injection can regress an established tumor more effectively than systemic injection, with a high degree of antigen specificity. Our unpublished data showed that the IT injection of A7-NCS had a highly inhibitory effect on the tumor growth as compared with injection via the intravenous route. It is clear that this greater effect of IT injection is due to a significantly enhanced accumulation of the conjugate in the tumor, in comparison with systemic injection.

Some other studies have reported that the IT injection of immunotoxins results in significant tumoricidal effects in various experimental systems.[20,21] In those reports, the authors describe the usefulness of local administration of an immunotoxin because of its high tumoricidal effect. However, the immunotoxins are so toxic as to be lethal to the animals, even when administered IT.[22,23] In comparison, A7-NCS has been shown to be safe for administration in humans, except for the formation of human antibodies against murine monoclonal antibody (HAMA).[5] Toxicity and HAMA may be markedly reduced by local administration as compared with generally accepted methods such as IV injection. After systemic administration of the immunoconjugate, it is usually cleared from the blood by the formation of an immunocomplex when the HAMA are developed. As a result, the conjugate cannot access the target tumor in sufficient amounts to accomplish successful drug targeting. Local administration of the conjugate would avoid this problem.

In summary, A7-NCS was administered IT in tumor-bearing nude mice, and the tumor localization properties and tumoricidal effects were examined. The results showed that A7-NCS was markedly localized in the target tumor, and exhibited a significantly greater tumoricidal effect than NCS alone. The tumoricidal effect was in proportion to the degree of antigen expression in the tumor. IT injection of A7-NCS should be useful for the local control of cancer, and augurs a new application for cancer chemotherapy.

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REFERENCES

1) Miller, R. A. and Levy, R. Response of cutaneous T cell lymphoma to therapy with hybridoma monoclonal antibody. *Lancet*, **2**, 226–230 (1981).
2) Herlyn, D. and Koprowski, H. IgG_{2a} monoclonal antibodies inhibit human tumor growth through interaction with effector cells. *Proc. Natl. Acad. Sci. USA*, **79**, 4761–4765 (1982).
3) Hurwitz, E. Specific and nonspecific macromolecule-drug conjugates for the improvement of cancer chemotherapy. *Biopolymer*, **22**, 557–567 (1983).
4) Shawler, D. L., Bartholomew, R. M., Smith, L. M. and Dillman, R. O. Human immune response to multiple injection of murine monoclonal IgG. *J. Immunol.*, **135**, 1530–1535 (1985).
5) Takahashi, T., Yamaguchi, T., Takahashi, T., Noguchi, A., Honda, M. and Ohtsuji, E. Missile therapy for colorectal and pancreatic cancer — clinical trial of monoclonal antibody, A7-NCS, for 73 patients with colorectal and pancreatic cancers. *Jpn. J. Cancer Chemother.*, **17**, 1111–1119 (1990) (in Japanese).
6) Rowland, G. F., Simmonds, R. G., Gore, V. A., Marsden, C. H. and Smith, W. Drug localization and growth inhibition studies of vindesine-monoclonal anti-CEA conjugates in a human tumour xenograft. *Cancer Immunol. Immunother.*, **21**, 183–187 (1986).
7) Pimm, M. V., Clegg, J. A., Caten, J. E., Ballantype, K. D., Perkins, A. C., Garnett, M. C. and Baldwin, R. W. Biodistribution of methotrexate-monoclonal antibody conjugates and complexes: experimental and clinical studies. *Cancer Treatment Rev.*, **14**, 411–420 (1987).

8) Pimm, M. V., Paul, M. A., Ogumujuma, P. Y. and Baldwin, R. W. Biodistribution and tumour localization of a daunomycin-monoclonal antibody conjugate in nude mice with human tumour xenografts. *Cancer Immunol. Immunother.*, **27**, 267–271 (1988).
9) Pimm, M. V., Clegg, J. A., Garnet, M. C. and Baldwin, R. W. Biodistribution and tumour localization of a methotrexate-monoclonal-antibody 791T/36 conjugate in nude mice with human tumour xenografts. *Int. J. Cancer*, **41**, 886–891 (1988).
10) Kitamura, K., Takahashi, T., Yamaguchi, T., Kitai, S., Amagai, T. and Imanishi, J. Monoclonal antibody A7 tumor localization enhancement by its $F(ab')_2$ fragments to colon carcinoma xenografts in nude mice. *Jpn. J. Clin. Oncol.*, **20**, 139–144 (1990).
11) Kitamura, K., Takahashi, T., Yamaguchi, T., Yokota, T., Noguchi, A., Amagai, T. and Imanishi, J. Immunochemical characterization of the antigen recognized by the murine monoclonal antibody A7 against human colorectal cancer. *Tohoku J. Exp. Med.*, **157**, 83–92 (1989).
12) Kotanagi, H., Takahashi, T., Masuko, T., Hashimoto, Y. and Koyama, K. A monoclonal antibody against human colon cancers. *Tohoku J. Exp. Med.*, **148**, 353–360 (1986).
13) Ohtsuji, E., Takahashi, T., Yamaguchi, T., Yamaguchi, N. and Imanishi, J. Specific cytotoxic effect of neocarzinostatin conjugated to monoclonal antibody A7 on human pancreatic carcinoma. *Gastroenterol. Jpn.*, **25**, 244–248 (1990).
14) Fukuda, K. Study of targeting chemotherapy against gastrointestinal cancer: preparation of anticancer drug-monoclonal antibody conjugate and investigation about its biological activities. *Akita J. Med.*, **12**, 455–468 (1985).
15) Takahashi, T., Yamaguchi, T., Kitamura, K., Suzuyama, H., Honda, M., Yokota, T., Kotanagi, H., Takahashi, M. and Hashimoto, Y. Clinical application of monoclonal antibody-drug conjugates for immunotargeting chemotherapy of colorectal carcinoma. *Cancer*, **61**, 881–888 (1988).
16) Kitamura, K., Takahashi, T., Tsurumi, H., Takashina, T., Noguchi, A., Noguchi, A., Okuzumi, J. and Yamaguchi, T. Pharmacokinetics of the monoclonal antibody A7-neocarzinostatin conjugate administered to nude mice. *Tohoku J. Exp. Med.*, **164**, 203–211 (1991).
17) Greenwood, F. C., Hunter, W. M. and Glover, J. S. The preparation of ^{131}I-labeled human growth hormone of high specific reactivity. *Biochem. J.*, **89**, 114–123 (1963).
18) Yamaoka, K. and Nakayama, T. A nonlinear least-squares program based on differential equations, MULTI (RUNGE) for microcomputers. *J. Pharmacobio-Dyn.*, **6**, 595–606 (1983).
19) Covell, D. G., Barbet, J., Holton, O. D., Black, C. D. V., Parker, R. J. and Weinstein, J. N. Pharmacokinetics of monoclonal immunoglobulin G_1, $F(ab')_2$ and Fab' in mice. *Cancer Res.*, **46**, 3969–3978 (1986).
20) Hillman, G. W., Runge, W., Jansen, F. K. and Vallera, D. A. Cytotoxic effect of anti-Mr 67000 protein immunotoxin on human tumors in a nude mouse model. *Cancer Res.*, **45**, 1328–1336 (1985).
21) Kanellos, J., Mckenzie, I. F. C. and Pietersz, G. A. Intratumor therapy of solid tumours with ricin-antibody conjugates. *Immunol. Cell Biol.*, **67**, 89–99 (1989).
22) Spitler, L. E., de Rio, M., Khentigan, A., Wedel, N. L., Brophy, N. A., Miller, L. L., Harkonen, W. S., Rosendorf, L. L. Lee, H. M., Mischak, R. P., Kawahata, R. T., Stoudemire, J. B., Fradkin, L. B., Bautista, E. E. and Scannon, P. J. Therapy of patients with malignant melanoma using a monoclonal antimelanoma antibody-ricin A chain immunotoxin. *Cancer Res.*, **47**, 1717–1723 (1987).
23) Weiman, L. M., Odwyer, J., Kitson, J., Cowis, R. L., Frankel, A. E., Bauer, R. J., Konrad, M. S. and Groves, E. S. Phase I evaluation of an anti-breast carcinoma monoclonal antibody 260F9-recombinant ricin A chain immunoconjugate. *Cancer Res.*, **49**, 4062–4067 (1989).