Efficient Transplantation of Human Non-T-Leukemia Cells into Nude Mice and Induction of Complete Regression of the Transplanted Distinct Tumors by Ricin A-Chain Conjugates of Monoclonal Antibodies SN5 and SN6

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ABSTRACT

In the present study, we established a dependable system by which human pre-B- and non-T/non-B-acute lymphoblastic leukemia (ALL) cells are efficiently transplanted into nude mice. The transplanted tumors provided a useful model for investigating the efficacy of antitumor agents in the in vivo therapy of human cancer.

NALM-6 (a pre-B-ALL cell line) cells were transplanted under varying conditions as the pre-B-leukemia cells, whereas REH (a non-T/non-B-ALL cell line) cells were transplanted as the non-T/non-B-leukemia cells. Under optimal and near optimal conditions, 71 of 101 X-irradiated mice (70%) developed distinct tumors approximately 2 wk after i.d. inoculation of a mixture of NALM-6 cells and X-irradiated human fibrosarcoma cells. Under the same conditions, 9 of 11 mice (82%) developed tumors following i.d. inoculation of REH cells admixed with X-irradiated human fibrosarcoma cells. Examination of the tumor tissues demonstrated that the tumors were of leukemia origin but not of fibrosarcoma origin. To demonstrate the usefulness of the present tumors for investigating the efficacy of antitumor agents in the in vivo therapy of human cancer, immunotoxins were tested for their specific suppressive activity against growing tumors of the transplanted NALM-6 cells.

To this end, monoclonal antibodies SN5 and SN6 which define a common ALL antigen, termed CALLA, and a novel leukemia-associated cell surface glycoprotein, termed gp160, respectively, were separately conjugated with the A-chain subunit of ricin; a plant toxin; CALLA and gp160 are expressed on the cell surface of various human non-T-leukemia cells including NALM-6 cells. The conjugates of SN5 and SN6 with ricin A-chain (RA) showed specific activity against the leukemia cells but not against control cells in an in vitro assay. To investigate their in vivo efficacy in suppressing tumor growth, nude mice which had been inoculated i.d. with NALM-6 cells 25 days in advance and bore distinct palpable tumors (5 to 6 mm in diameter) were divided into five groups. One group of mice was nontreated as a control. Each of the remaining four groups of mice was given an injection of one of the following agents: (a) purified control mouse IgG (IgG1); (b) purified antibodies SN5 (IgG1) and SN6 (IgG1); (c) control IgG-RA conjugate; and (d) SN5-RA and SN6-RA. Tumors in all mice of the first four groups including the untreated group grew continuously, causing the mice to die. In contrast, tumors in all five mice of the fifth group which were given injections of specific immunotoxins SN5-RA and SN6-RA regressed completely, although the tumors in one of the mice later relapsed. The tumor-free mice appear to be completely healthy. The strong in vivo cytotoxic action of SN5-RA plus SN6-RA against NALM-6 tumors was reproducible in a repeated experiment. The results show the usefulness of the present human tumor model in evaluating anti-human cancer agents for their in vivo therapeutic efficacy. Furthermore, the results suggest the potential of these immunotoxins for clinical utility.

INTRODUCTION

Transplantation of human tumors into athymic nude (nu/nu) mice has been extensively investigated by many researchers in order to use the transplanted human tumors for studying the in vivo efficacy of anti-human cancer agents as well as studying the in vivo biological behavior of the transplanted tumors. Various types of human tumors, particularly solid tumors, were successfully transplanted into nude mice by many investigators (1, 2). However, it has been difficult to transplant human ALL cells into nude mice (3, 4). Many children and adults suffer from ALL; in particular, ALL is the major form of childhood cancer (5).

Recently, several investigators, including ourselves, succeeded in transplanting T-cell ALL (6–10), a subtype of ALL. However, it is still very difficult to transplant non-T/non-B- and pre-B-ALL cells into nude mice; ALL of non-T/non-B including pre-B is the major subtype of ALL (11–13).

In the present work, we succeeded in efficiently transplanting NALM-6 (a pre-B-ALL cell line) cells as well as REH (a non-T/non-B-ALL cell line) cells into nude mice. The potential utility of the present tumor model for investigating the in vivo therapeutic efficacy of anti-human leukemia agents was indicated by experiments using ITs as the therapeutic agent.

McAbs have been very useful in the diagnosis of various human malignancies. Furthermore, they were also shown to be useful in purging bone marrow specimens derived from leukemia and lymphoma patients; such purged bone marrow specimens can be used in autologous bone marrow transplantation (14–17). However, the clinical utility of McAbs for in vivo therapy remains to be explored. Several potentially important problems associated with the in vivo therapeutic use of McAbs were previously discussed (10, 18, 19). For instance, one such problem appears to be that the in vivo cytotoxic effector mechanisms using antibodies (including McAbs) are not inherently efficient in eradicating the target tumor cells. This and several other problems associated with the in vivo therapeutic use of McAbs may be obviated by using an IT such as a McAb-RA conjugate (10). In this IT, the McAb acts primarily as the specific delivery vehicle, and the RA acts as the cytotoxic effector. When introduced into the cytoplasm of cells, the enzymatically active RA is an extremely potent cytotoxic agent that acts catalytically to inhibit protein synthesis (reviewed in Refs. 20 and 21). However, free RA per se is not significantly toxic to the cells because of its inability to bind efficiently to cell surfaces and to enter the cells. Thus, McAb-RA conjugates are a very attractive alternative to unconjugated McAbs in the treatment of diseases including cancer.

Previously, we demonstrated that ITs containing anti-human T-leukemia McAbs SN1 and SN2 were capable of completely suppressing the tumor growth of human T-leukemia cells in nude mice without any overt undesirable toxicity (10). In the present study, we extend the above observation to the human non-T-leukemia system. Concerning McAbs used in preparing...
the present ITs, we used McAbs SN5 and SN6. SN5 reacts with a well-known CALLA (22), whereas SN6 is directed to a novel human leukemia-associated antigen, termed gp160, whose expression is highly restricted to non-T-leukemia cells (23). Both CALLA and gp160 are cell surface glycoproteins.

MATERIALS AND METHODS

Mice. Six- to 8-wk-old female BALB/c (nu/nu) mice were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) through the Frederick Cancer Research Facility of the National Cancer Institute. The mice were kept under sterile conditions in cages with filter bonnets in a laminar flow unit (Lab Products, Inc., Maywood, NJ). They were given sterilized pellet food and tap water (10).

Cell Lines. NALM-6, a human pre-B-ALL cell line (23, 24), and REH, a human non-T/non-B-ALL cell line, were cultured in RPMI 1640 medium supplemented with 4% heat-inactivated fetal calf serum as described before (25). HT-1080, a human fibrosarcoma cell line (26), was grown in monolayer culture in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum and gentamicin (50 μg/ml) (10).

Monoclonal Antibodies. Previously, we generated and characterized McAbs SN5 (22) and SN6 (23) which react with cell surface glycoproteins on human leukemia cells.

Control Murine IgG. Control mouse plasmacytoma ascites tumor MOPC 195 variant (IgG1) was prepared in our laboratory.

Reagents. SPDP was purchased from Pharmacia Fine Chemicals (Piscataway, NJ). Purified RA was either obtained from E-Y Laboratories, Inc. (San Mateo, CA) (RA Preparation I) or prepared in our laboratory (RA Preparation II). In the latter case, ricin (E-Y Laboratories) was reduced with diithiothreitol, and the reduced mixtures were applied to a column of immobilized lactose (Pierce Chemical Co., Rockford, IL). The RA fraction was further purified by passing through a column of acid-treated Sepharose 4B (27). NH4Cl and amantadine were purchased from Aldrich Chemical Corporation (Milwaukee, WI) and Sigma Chemical Co. (St. Louis, MO), respectively.

Preparation of Immunotoxins and Control Conjugate. The purified IgGs of McAbs or control murine ascites were conjugated with RA using SPDP as previously described (28) but with modifications. Briefly, IgG proteins in phosphate-buffered saline (pH 7.4) were treated individually with a 10-fold molar excess of SPDP for 30 min at room temperature to introduce 2-pyridyl disulfide groups into the IgG molecule. The modified and dialyzed proteins were then mixed with a 3-fold molar excess of the purified, freshly reduced RA in PBS (pH 7.4) containing 1 mM EDTA and incubated at room temperature for 48 h. The IgG-RA conjugates were separated from the unbound RA by gel filtration on a calibrated Sephacryl S-300 or S-200 column. Penicillin (100 units/ml), streptomycin (100 ng/ml), and gentamicin (50 μg/ml) were added to the conjugates. The weight ratios of different components (e.g., one RA molecule/IgG molecule, two RA molecules/IgG molecule, and unconjugated IgG) in the high-molecular-weight pool were determined as described previously (28). The molar ratios of these components were calculated from the weight ratios and the molecular weights of individual components, e.g., 150,000 for IgG and 182,000 for RA-IgG (one RA molecule per IgG molecule). Using the molar ratios, an average RA content per IgG in the pool was estimated to be 1.66, 1.29, and 1.68, respectively, for the SN5, SN6, and control conjugates.

Determination of in Vivo Cytotoxicity of IT. A direct test of cytotoxicity against leukemia and control cells was carried out as described before (25). NALM-6 and HT-1080 cells were harvested after treatment with 0.2% trypsin, washed extensively, and X-irradiated with 6000 rads (1 rad = 0.01 Gy) (10). Leukemia cells were mixed with X-irradiated HT-1080 cells at various ratios (see Table 1), and the cell mixture was washed and resuspended in saline. A portion (0.1 ml) of the cell mixture was injected i.d. into the right flank of individual nude mice which had been nonirradiated or X-irradiated weekly with 200 or 300 rads for 3 wk; the injection was carried out on Day 3 after the final irradiation of the mice. The local tumor growth was determined by measuring two perpendicular diameters.

Histology. Sections from the tumor, peritoneum, lymph nodes, mesenterium, livers, spleens, kidneys, lungs, and brains were fixed in 10% formalin, stained with hematoxylin-eosin, and examined by light microscopy.

Table 1.

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<sup>a</sup> The leukemia cells were NALM-6 or REH (see Footnote d) cells.
<sup>b</sup> HT-1080 cells were X-irradiated with 6000 rads (1 rad = 0.01 Gy).
<sup>c</sup> Results are expressed as number of mice with tumors/total number of mice inoculated with leukemia and/or HT-1080 cells.
<sup>d</sup> REH cells.

RESULTS

Transplantability of Human Non-T-Leukemia Cells into Nude Mice. A summary of the conditions used to transplant NALM-6, a pre-B-leukemia cell line, cells and REH, a non-T/non-B-leukemia cell line, cells into nude mice is presented in Table 1. Mice which had not been X-irradiated prior to inoculation did not develop tumors regardless of whether leukemia cells were inoculated alone or in combination with X-irradiated HT-1080 cells. Similarly, X-irradiated mice inoculated solely with leukemia cells or X-irradiated HT-1080 cells did not develop tumors. In contrast, X-irradiated mice which were inoculated with leukemia cells admixed with X-irradiated HT-1080 developed tumors in high yields, i.e., 71 of 101 mice (70%) for NALM-6 and 9 of 11 mice (82%) for REH. The tumors became distinct and palpable approximately 2 wk after the inoculation of the cell mixture. In the initial experiment, mice were X-irradiated 3 times with 300 or 200 rads; the 300-rad-irradiated mice appeared to be weaker than those irradiated with 200 rads. Therefore, further experiments were done using only the 200-rad dose.

Histology and Possible Metastasis of the Tumors. The tumors were beige in color, soft, well encapsulated, and easily movable. In a cross-cut section of the tumors, fibrous trabeculae and small capillaries were recognized. When the tumors became large in size, hemorrhaging was commonly observed on the
tumors. The histological examination of the tumors demonstrated that the tumors were of leukemia cell origin but not of the fibrosarcoma cell origin (Fig. 1). Histologically, extensive sheets of lymphoid cells (about 10 to 15 μm in diameter) with inconspicuous nuclei, peripherally clumped chromatin, and scant cytoplasm were seen. Areas of lymphoid cell necrosis and hemorrhage were also seen. No residual X-irradiated HT-1080 fibrosarcoma cells were noticed histologically (Fig. 1).

The possibility of tumor metastasis was also examined. Among the 55 mice that developed NALM-6 tumors and were examined, only one mouse showed metastasis in the right axillary lymph node (the same side as the tumor), whereas no other metastasis was found in liver, spleen, kidney, lung, or brain in any mice.

**In Vitro Cytotoxic Activity of SN5-RA and SN6-RA.** NALM-6, a pre-B-ALL cell line, cells and CCRF-SB, a control non-malignant B-cell line, cells were cultured in individual wells of 24-well tissue culture plates, in triplicate, in the absence (control) or in the presence of different concentrations of IT. In some tests, NH₄Cl was added as a potentiator (10, 29, 30). The reactivity of SN5-RA is illustrated in Fig. 2. In the absence of NH₄Cl, SN5-RA showed a weak reactivity against NALM-6 cells; it killed only 31 and 49% of the cells at 2.1 × 10⁻⁸ M and 7 × 10⁻⁸ M, respectively, after culturing for 3 days. Pronounced augmentation of the cytotoxic activity by 10 mM NH₄Cl was observed; SN5-RA killed NALM-6 cells completely at both 2.1 × 10⁻⁸ M and 7 × 10⁻⁸ M after 2 days culture. SN5-RA showed marginal and no cytotoxic activity, respectively, against control CCRF-SB cells in the presence and in the absence of NH₄Cl. Amantadine, a symmetrical primary amine (31), also potentiated the cytotoxicity of SN5-RA against NALM-6 cells. However, the degree of the potentiation was much smaller than that of NH₄Cl (10 mM) when amantadine was used at concentrations (< 0.5 mM) where cytotoxicity against control CCRF-SB cells is acceptably low (data not shown). The in vitro cytotoxic activity of SN6-RA is illustrated in Fig. 3. In the absence of NH₄Cl, SN6-RA Preparation I which had been prepared using RA Preparation I (see "Materials and Methods") killed 54 and
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Fig. 2. Specific in vitro cytotoxic activity of SN5-RA. NALM-6, a human pre-B-leukemia cell line, and CCRF-SB, a human nonmalignant B-cell line, were grown separately in medium alone or medium containing the designated concentrations of SN5-RA. NH4Cl (10 mM), a potentiator of IT, was added in the experiments shown in a and c. Portions of the cells were removed on 1, 2, and 3 days after the start of the culture, and viability was determined using trypan blue.

75%, respectively, of NALM-6 cells at 3.5 x 10^-9 M and 1.2 x 10^-8 M after culturing for 3 days (Fig. 3A). Recently, we found that the cytotoxic activity is higher for SN6-RA which was prepared using a freshly isolated RA preparation (RA Preparation II; see "Materials and Methods"); this SN6-RA preparation (SN6-RA Preparation II) killed 60 and 95%, respectively, of NALM-6 cells at 2.5 x 10^-9 M and 8.4 x 10^-9 M after 3 days of culture (Fig. 3B). The cytotoxicity was augmented by 10 mM NH4Cl to kill NALM-6 cells completely at 1.2 x 10^-8 M of SN6-RA Preparation I (Fig. 3A) and 8.4 x 10^-9 M of SN6-RA Preparation II (Fig. 3B). Either preparation showed no significant cytotoxicity against CCRF-SB cells in the presence or in the absence of NH4Cl (Fig. 3). The results described above show the specific nature of the cytotoxic activity of SN5-RA and SN6-RA. It should be noted that SN5-RA and SN6-RA used in the in vivo tests described below are those which were prepared using RA Preparation I.

In Vivo Treatment of NALM-6 Tumors using SN5-RA and SN6-RA. Nude mice which had been inoculated i.d. with NALM-6 cells 25 days in advance and bore distinct palpable tumors (5 to 6 mm in diameter; e.g., see Fig. 4) were divided into five groups. One group of the mice was nontreated, serving as a control. Each of the remaining four groups of mice was given injections of one of the following agents: (a) 20 µg of purified control mouse IgG (IgG1); (b) 10 µg each of purified antibodies SN5 (IgG1) and SN6 (IgG1); (c) 20 µg of control IgG-RA conjugate; or (d) 10 µg each of SN5-RA and SN6-RA. Tumors in all 17 mice of the first four groups including the nontreated group grew continuously. In contrast, necrosis of tumors was observed 2 to 3 wk after initiation of treatment for all five of Group 5 mice which were given injections of specific immunotoxins SN5-RA and SN6-RA. Tumors in all 17 mice of the first four groups continued to grow to form larger tumor masses (Figs. 5 and 6).

The large tumor masses were accompanied with hemorrhage in most cases (e.g., see Fig. 5, A, B, D, and E) with a few exceptions where no hemorrhaging was observed in the tumor masses (e.g., see Fig. 5C). Neither purified MoAbs SN5 plus SN6 nor control IgG-RA conjugate showed any significant suppressive activity against the tumor growth (e.g., see Figs. 5 and 6). All but one of the 17 mice of the first four groups died within 90 days after the inoculation of NALM-6 cells (Fig. 6); the remaining one mouse died on Day 116 after the NALM-6 inoculation.

The specific ITs SN5-RA plus SN6-RA showed a pronounced suppressive activity against the tumor growth. Tumors in all five of the Group 5 mice that were treated with SN5-RA plus SN6-RA regressed completely, although the tumor in one mouse relapsed later, approximately 25 days after the start of...
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Fig. 4. Nude mouse bearing a distinct tumor of NALM-6. Picture was taken 25 days after inoculation with NALM-6 cells and immediately prior to initiation of treatment.

the tumor regression (Fig. 6). The remaining four mice remained tumor free, and one example of these is shown in Fig. 5F. These mice were apparently completely healthy. Recently, another group of five nude mice which carried distinct NALM-6 tumors were treated with SN5-RA plus SN6-RA. Consistent with the above observation, these ITs again induced complete regression of NALM-6 tumors in all five treated mice, whereas tumors in all five control mice continued to grow causing the mice to die. It is interesting that the tumor in one of the cured mice relapsed later (approximately 30 days after the start of the tumor regression) as in the case of the above test shown in Fig. 6.

DISCUSSION

Human malignant cells transplanted into nude mice will be potentially very important for human cancer research, particularly for evaluating the in vivo efficacy of anti-human cancer agents. In this regard, many cancer researchers attempted to transplant various human malignant cells and tissues into nude mice, and a number of them succeeded in transplanting various human solid tumors (reviewed in Refs. 1, 2, and 32 to 34). However, it has been difficult to transplant human hematopoietic tumors into nude mice (3, 4). It is particularly difficult to transplant the non-T/non-B (including pre-B)-type ALL. This is consistent with the report of Hiraki et al. (35) who studied transplantation of a human B-ALL cell line, a T-ALL cell line, and a non-T/non-B-ALL cell line into immunosuppressed newborn hamsters. They found that it was most difficult to transplant the non-T/non-B-ALL cell line. Leonard et al. (36) were unable to transplant NALM-6, a pre-B-ALL cell line, into nude mice while they could transplant Namalva, a Burkitt's lymphoma cell line, with high efficiency.

In the present work, we were able to transplant NALM-6 and REH, a non-T/non-B-ALL cell line, into nude mice with high efficiency (Table 1). For the successful transplantation of these cells, we need to X-irradiate the mice and inoculate the irradiated mice with the leukemia cells in combination with X-irradiated human fibrosarcoma cells (Table 1). Irradiation probably suppresses natural killer cell activity in nude mice. Nude mice are known to have high natural killer cell activity, and this activity was previously implicated in a high incidence of failure in transplanting xenogenic tumors in nude mice (37). The enhancing role of X-irradiated fibrosarcoma cells in the transplantation of human leukemia cells may be attributable to an angiogenesis factor(s) (7) and/or a tumor growth-promoting factor(s) (38) that the fibrosarcoma cells may release.

The most important utility of the present tumor system will be for evaluating the in vivo efficacy of anti-human leukemia agents. As the antileukemia agents, McAbs that react selectively with human leukemia cells are particularly interesting to us. Previously, we generated and characterized many McAbs that are directed toward various human leukemia cells (22, 23, 39–43). Among the various McAbs that react with human non-T/ non-B- and pre-B-leukemia cells, we chose SN5 (IgG1) and SN6 (IgG1) which define CALLA and a novel leukemia-associated cell surface antigen, termed gp160, respectively (22, 23). Both CALLA and gp160 are expressed in the majority of cases of non-T/non-B (including pre-B)-ALL (22, 23). McAbs SN5 and SN6 were conjugated with RA to suppress the growth of human non-T-leukemia cells which were transplanted into nude mice.

Immunotoxins have good potential for serotherapy if proper McAbs are used in preparing the ITs. However, many problems still need to be investigated, defined, and solved before we can use ITs effectively and safely for such a purpose. Earlier, Krolick et al. (44) reported that the combined approach of nonspecific cytoreductive therapy and administration of an IT induced prolonged remissions of murine BCL1 tumors. Subsequently, Seto et al. (45) reported that the in vivo growth of mouse mammary tumor MM46 was suppressed when the MM46 cells were preincubated in vitro with an IT and the pretreated tumor cells were subsequently used for transplantation into syngeneic mice. More recently, several investigators reported various degrees of efficacy of their ITs in in vivo suppression of guinea pig tumors (46, 47) and murine tumors (48, 49). Recently, several investigators including ourselves studied the in vivo efficacy of ITs using human tumors established in nude mice (8, 10, 50, 51). We showed that our anti-human T-ALL-specific ITs containing RA and McAbs SN1 and SN2 were capable of
completely suppressing the in vivo growth of human T-ALL cells without any overt undesirable toxicity (10). In the present work, we extended our earlier studies to include ITs directed to human non-T/non-B (including pre-B)-ALL. To carry out such a study, we first needed to establish tumors of such human leukemia cells in nude mice, and we succeeded in efficiently transplanting human non-T/non-B- and pre-B-ALL cells. In the present study, we used the pre-B-ALL cells transplanted into nude mice and ITs which were prepared by conjugating RA with McAbs SN5 (22) and SN6 (23). Before using SN5-RA and SN6-RA for the in vivo study, we investigated in vitro cytotoxic activity of these ITs (Figs. 2 and 3). SN5-RA showed a weak in vitro cytotoxic activity against NALM-6, a pre-B-ALL cell line, cells. This result is consistent with the observation by others that their anti-CALLA McAb-RA showed very slow kinetics and weak activity in the in vitro killing of CALLA-positive leukemia cells (52). SN6-RA showed a much higher cytotoxic activity than SN5-RA. Neither SN5-RA nor SN6-RA showed significant cytotoxicity against control nonmalignant cells. The results show the specific nature of the cytotoxic activity of these ITs.

To investigate the in vivo efficacy of anti-human non-T-leukemia ITs, SN5-RA and SN6-RA were injected together into nude mice carrying distinct preformed tumors of NALM-
Thus, the combined use of SN5-RA and SN6-RA appears to be more effective than the separate use of the two ITs in the induction of the in vivo tumor regression.

In a recent test, ten mice bearing NALM-6 tumors were injected with either purified SN5 plus SN6 or control IgG-RA. Twenty-two nude mice which had been inoculated with NALM-6 cells 25 days in advance and borne distinct tumors (5 to 6 mm in diameter) were divided into five groups; each group of mice was treated as indicated in the figure. 

6 cells. Because of the heterogeneity of tumor cells, multiple ITs will be more efficient than a single IT in eradicating the tumor cells (see below). Four different controls were included in the test (Figs. 5 and 6). Injection with SN5-RA plus SN6-RA induced prolonged regression of tumors in nude mice with one exception in which the tumor of one mouse relapsed later (Fig. 6). Injection with either purified SN5 plus SN6 or control IT was not effective. Thus, the activity of SN5-RA plus SN6-RA is specific. The pronounced in vivo cytotoxic activity of SN5-RA plus SN6-RA was confirmed by a repeated experiment.

In a recent test, ten mice bearing NALM-6 tumors were divided into three groups. One group was nontreated as a control. Each of the remaining two groups was treated with SN5-RA and SN6-RA, respectively. Tumors in all three control mice continued to grow. Tumors in three of four mice that were treated with SN5-RA regressed completely, but a tumor in one of the cured mice relapsed later. On the other hand, tumors in all three mice that were treated with SN6-RA regressed completely, but the tumors in two of the cured mice relapsed later. Thus, the combined use of SN5-RA and SN6-RA appears to be more effective than the separate use of the two ITs in the induction of the in vivo tumor regression.

Despite our initial encouraging results, we need to investigate several factors before we can safely and effectively apply these ITs for clinical use. These factors include the route of IT injection, tissue localization of IT injected into patients or nonhuman primates, and pharmacokinetics of the injected IT.

In particular, strong reactivity of anti-CALLA McAb with normal kidney (22, 53) will present problems in using anti-CALLA IT for in vivo therapy. In this regard, we are currently attempting to generate McAbs which react with CALLA on human leukemia cells but do not react with normal kidney.

Finally, Laurent et al. (54) and Spitzer et al. (55) recently injected an anti-T-cell IT and an anti-melanoma IT, respectively, into patients. These initial clinical trials showed only a marginal or a small therapeutic effect. However, it is important to note that the patients treated with these ITs showed no significant, only mild, or only transient side effects.

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