Effective treatment of a murine model of adult T cell leukemia using $^{211}$At-7G7/B6 and its combination with unmodified anti-Tac (daclizumab) directed towards CD25

Zhuo Zhang¹*, Meili Zhang¹*, Kayhan Garmestani¹, Vladimir S. Talanov², Paul S. Plascjak³, Barbara Beck¹, Carolyn Goldman¹, Martin W. Brechbiel² and Thomas A. Waldmann¹‡

¹Metabolism Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892
²Radiation Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892
³Clinical Center, National Institutes of Health, Bethesda, MD 20892

‡ Corresponding author:
Thomas A Waldmann
Metabolism Branch, NCI, NIH
Bldg 10, Room 4N115
10 Center Drive
Bethesda, MD 20892-1374
Tel: 301-496-6656
Fax: 301-496-9956
E-mail: tawald@helix.nih.gov

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Author contributions:
Zhuo Zhang designed and conducted experiments, analyzed the data, and wrote the manuscript; Meili Zhang designed and performed experiments; Kayhan Garmestani labeled 7G7/B6 antibody with astatine; Vladimir S. Talanov synthesized the astatine linker; Paul S. Plascjak produced astatine for the 7G7 labeling; Barbara Beck performed experiments; Carolyn Goldman contributed to the design of research and reviewed the manuscript; Martin W. Brechbiel designed the astatine linker, critically reviewed the manuscript revision; Thomas A. Waldmann designed experiments and wrote the manuscript.

*Authors made equivalent contributions to the paper

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ABSTRACT

Adult T-cell leukemia (ATL) consists of an overabundance of T cells, which express CD25. Therapeutic efficacy of astatine-211 (\(^{211}\text{At}\)) labeled murine monoclonal antibody 7G7/B6 alone and in combination with daclizumab was evaluated in NOD/SCID mice injected with MET-1 human T-cell leukemia cells. 7G7/B6 and daclizumab are directed toward different epitopes of CD25. Either a single dose of 12 µCi \(^{211}\text{At}\)-7G7/B6 per mouse given i.v. or receptor saturating doses of daclizumab given at 100 µg weekly for 4 weeks i.v. inhibited tumor growth as monitored by serum levels of human \(\beta_2\)-microglobulin (\(\beta_2\mu\)), and by prolonged survival of leukemia-bearing mice as compared with the control groups (\(P < .0001\)). The combination of two agents enhanced the antitumor effect, when compared with groups treated with 12 µCi of \(^{211}\text{At}\)-7G7/B6 (\(P < .05\)) or daclizumab alone (\(P < .05\)). The median survival duration of PBS group was 62.6 days and was 61.5 days in the radiolabeled nonspecific antibody \(^{211}\text{At}\)-11F11 treated group. In contrast, there were 91% of mice in the combination group that survived through day 94. These results that demonstrate a significantly improved therapeutic efficacy by combining \(^{211}\text{At}\)-7G7/B6 with daclizumab support a clinical trial of this regimen in patients with ATL.
INTRODUCTION

Adult T-cell leukemia (ATL) develops in a small portion of individuals infected with human T-cell lymphotrophic virus-1 (HTLV-I) and consists of an overabundance of malignant activated T cells, which are characterized by expression of the alpha subunit of the interleukin-2 receptor (IL-2Rα, CD25) on their cell surfaces. Presently, there is no accepted curative therapy for ATL and patients progress to death with a median survival duration of 9 months for those with acute ATL and 24 months with chronic ATL. The observation that IL-2Rα is not expressed by normal resting cells, but is expressed by ATL cells, provided the rationale for the use of monoclonal antibodies (mAbs) directed toward IL-2Rα to deliver therapeutic agents. Some partial and rare complete remissions were obtained in patients with ATL treated in clinical trials with the intact murine anti-Tac, humanized anti-Tac (daclizumab), as well as these intact antibodies armed with ⁹⁰Y used in an effort to develop yet more effective IL-2Rα-directed agents. A preclinical in vivo murine model of ATL was developed by introducing leukemic cells (MET-1) from a patient with ATL into nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice and new therapeutic approaches have been tested in this model before initiating human clinical trials. In initial studies, antibodies to IL-2Rα including daclizumab, murine anti-Tac, and 7G7/B6 inhibited the progression of the leukemia and prolonged survival of the leukemia-bearing mice. However, in general, cures were not achieved. Therefore, more effective therapeutic approaches were required. In previous therapeutic trials, daclizumab was combined with pretargeted radioimmunotherapy in the ATL model and achieved the complementary actions of receptor-saturating doses of daclizumab to yield antibody-dependent cellular cytotoxicity (ADCC) and cytokine deprivation-
mediated leukemic cell death along with the tumor cytoreduction provided by the radiation delivered to leukemic cell surfaces.\textsuperscript{9,10} We also demonstrated that combining daclizumab with flavopiridol, or with PS341 (velcade) significantly improved therapeutic efficacy in the ATL model.\textsuperscript{13,14} These observations suggest that for cancer therapy, the addition of two therapeutic agents that function via different mechanisms of action may be greater than additive in their cytotoxic action leading to malignant cell death.

In the clinical and preclinical trials, remissions have been observed using either the unmodified daclizumab monoclonal antibody at receptor-saturating doses or this antibody labeled at high specific activity with yttrium-90\textsuperscript{(90Y)}. However, the complementary actions of receptor-saturating doses of the daclizumab monoclonal antibody to yield ADCC and IL-2 deprivation mediated apoptotic leukemic cell death with this radiolabeled monoclonal antibody were difficult to obtain in conjunction with the tumor cytoreduction provided by the irradiation mediated by radionuclides such as \textsuperscript{90}Y or \textsuperscript{211}At delivered by daclizumab to the leukemic cell surfaces. To obtain cytokine deprivation mediated cell death one must use large receptor saturating quantities of the monoclonal antibody. However, the administration of such large quantities of monoclonal antibody armed with a radionuclide leads to low specific activity and a decreased proportion of administered radiolabeled antibody delivered and bound to tumor cells. The resulting circulating unbound radiolabeled antibody yields unacceptable bone marrow toxicity which in turn reduces the maximum dose of radioactivity that can be administered. In this study, we address the limitations inherent in the use of a single radiolabeled monoclonal antibody to simultaneously saturate receptors and to deliver a high proportion of the administered radionuclide to the tumor cells by simultaneously using
two non-cross-competing antibodies daclizumab and 7G7/B6 that bind to different epitopes of the IL-2R alpha protein on the surfaces of leukemia/lymphoma cells. We used daclizumab at a receptor saturating dose in combination with small quantities of 7G7/B6 armed at high specific activity with a radionuclide.

Monoclonal antibodies (mAbs) directed against tumor-associated antigens armed with diverse radionuclides are being investigated as therapeutic agents for the treatment of malignant disease.\textsuperscript{15-17} Although encouraging results have been obtained in the treatment of lymphoma and other diseases with mAbs radiolabeled with $\beta^-$-emitting radionuclides further development is needed before an ideal radioimmunotherapeutic agent is achieved\textsuperscript{18,19}. The $\alpha$-emitting radionuclides appear to have several advantages when compared with $\beta^-$-emitting radionuclides in radioimmunotherapy, especially with isolated malignant cells as in leukemia. The high linear energy transfer of $^{211}$At, about 97 Kev $\mu$m\textsuperscript{-1}, makes it highly cytotoxic with a relative biologic effectiveness 5-20 times that of $\beta^-$-particles.\textsuperscript{20-22}

Another advantage of $\alpha$-particles compared with $\beta^-$-particles is that they exhibit a low dependence on dose rate and oxygen enhancement effects. In addition, $\alpha$-particles have relatively short effective path lengths in tissue that decrease the radiation delivered to normal tissues.\textsuperscript{20,23,24} Among the $\alpha$-emitters, currently under investigation for use in radioimmunotherapy, $^{211}$At is perhaps the most promising candidate for radioimmunotherapeutic applications on the basis of half-life considerations ($t_{1/2} = 7.2$ h).

In this study, the therapeutic efficacy of $^{211}$At-labeled 7G7/B6 was investigated in a murine model of ATL alone and in combination therapy with daclizumab. The scientific hypothesis supporting the use of the combination is that the two therapeutic agents
employ different mechanisms of action and might manifest additive or synergistic efficacy. Both antibodies are directed toward the same target, IL-2Rα but bind different epitopes on that target. In the present study, we obtained very promising results with this combination regimen for the treatment of IL-2Rα expressing T-cell malignancy.
Materials and Methods

Monoclonal antibody

7G7/B6 is a mouse IgG2a, mAb directed toward an epitope of the IL-2Rα peptide distinct from that identified by daclizumab which recognizes IL-2Rα. It was reported that a murine IgG2a such as 7G7/B6 has the feature of nonspecific binding to spleen and liver that can be blocked by the administration of another murine IgG2a irrelevant antibody. In this study UPC10 a murine IgG2a monoclonal antibody that does not recognize resting or activated peripheral blood mononuclear cells or cell lines including T-cell, B-cell and monocyte populations was used as an agent, which blocks the nonspecific binding of radiolabeled 7G7/B6 in the spleen and liver of nude and NOD/SCID mice. The plasmocytoma producing UPC10 was obtained from Michael Potter at the NCI, Bethesda, MD. The control monoclonal antibody 11F11 is a mouse IgG2a mAb which recognizes the Shiga-like toxin II of enterohemorrhagic Escherichia. The hybridoma producing 11F11 was obtained from Alison D O’Brien, Department of Microbiology, Uniformed Services University of Health Science, Bethesda, Maryland.

Radiolabeling of monoclonal antibodies

Production and purification of $^{211}$At as well as the procedure for $^{211}$At labeling of 7G7/B6 and 11F11 was recently reported in detail. In brief $^{211}$At was produced employing the $^{209}$Bi ($\alpha 2n$) $^{211}$At reaction by irradiating an external or internal bismuth target with an $\alpha$ beam from a Cyclotron Corporation CF-30 cyclotron. The $^{211}$At was isolated as described previously. The $^{211}$At was linked to 7G7/B6 and 11F11 as described previously using
\[ \text{N-succinimidyl N-}(4-\{^{211}\text{At}\}\text{astatophenethyl})\text{ succinamate (SAPS).}\] Specific activities obtained for the isolated products were 2.7 and 4.6 $\mu$Ci/$\mu$g respectively.

**Binding integrity of radiolabeled antibody**

Radiolabeling of 7G7/B6 antibody could theoretically alter its capacity to bind to the IL-2R receptor. Radiolabeled preparations were tested to determine the proportion of the radiolabeled product that bound to the IL-2R$\alpha$ expressing human leukemic T-cell line Kit-225-IG3. For each preparation triplicates of $3 \times 10^5$, $1 \times 10^6$, $3 \times 10^6$ Kit-225-IG3 cells were placed in PBS buffer with 10% FCS, 0.2% NaN$_3$ in screw cap vials. 50 $\mu$l aliquots of the radiolabeled ($^{211}\text{At}$) mAbs were added separately to each vial. After incubation at 4° C for 20 min, the vials were centrifuged for 4 min (setting 10) on an Eppendorf (Westbury, NY) centrifuge. The supernatant was aspirated and the radioactivity in the pellets was determined compared to a standard. Non-specific radioactivity was quantitated similarly with the exception that a 100-fold molar excess of unmodified 7G7/B6 was added to each tube. The non-specific counts were $<3\%$ of the added counts in all cases. The bind ability was measured as: (pellet counts –non-specific counts)/(initial sample counts).

**Mouse model of ATL**

The ATL cell population, MET-1, was established from the peripheral blood of a patient with acute ATL and the cells were maintained by serial transfer in NOD/SCID mice (Jackson Laboratories, Bar Harbor, ME). MET-1 cells have a distinct phenotype elucidated by fluorescein-activating cell sorting (FACS) analysis: $\text{CD3}^{\text{dim}}, \text{CD4}^{+/\text{hi}}, \text{CD7}, \text{CD20}^{\text{lo}}, \text{and CD25}^{+}$. The leukemia model was established by the intraperitoneal injection
of 1.5 x 10^7 MET-1 cells into NOD/SCID mice as described previously. Intraperitoneal injection of 1.5 x 10^7 MET-1 cells in NOD/SCID mice results in a wide tissue distribution of leukemia cells with marked infiltration of the spleen, liver, lymph nodes, lung, kidney, and moderate infiltration of the bone marrow. Therapeutic trials were performed on these mice when their serum-soluble IL-2Rα (sIL-2Rα) levels were from 1000 to 10 000 pg/ml, ~10 to 14 days post-inoculation.

**Monitoring of tumor growth**

Throughout the therapeutic trials, the serum concentrations of soluble human sIL-2α and human β2-microglobulin (β2μ) that were used as surrogate tumor markers were measured using ELISA kits purchased from R&D Systems (Minneapolis, MN). The ELISAs were performed as suggested in the manufacture’s kit inserts.

**Definition of the maximum tolerated dose**

Prior to initiation of therapeutic studies, the maximum tolerated dose of ^{211}At-7G7/B6 was determined in non-tumor bearing NOD/SCID mice. Single doses of 6, 12, 24, 40 and 70 μCi of ^{211}At-7G7/B6 per mouse were administered intravenously 30 min after 400 μg of UPC10 blocking antibody given i.v. During this experiment, body weight, platelets, and white blood cells were monitored. Deaths were observed in a proportion of mice receiving doses of higher than 12 μCi. Therefore, a dose of 12 μCi of ^{211}At-7G7/B6 was used in the therapeutic trials.

Two mice from each group, normal control, and groups receiving 6, 12, 24, and 40 (surviving mice) μCi of ^{211}At-7G7/B6 had a histological examination five months after
At-7G7/B6 was given. Typically, the pathological evaluation of liver, kidney, intestine, lung, bone marrow, and heart were performed. Tissue samples were harvested and fixed in 10% formalin for pathological studies. The tissues were examined by a veterinary pathologist.

**Therapeutic protocol**

Therapeutic trials were performed in MET-1 leukemia-bearing mice with a tumor burden corresponding to sIL-2Rα values of 1 000-10 000 pg/ml. The blocking antibody UPC10 (400 μg/mouse) was given i.v. before 211At 7G7/B6 or 211At-11F11 were administered. There were 5 groups in the therapeutic trials. Group 1 received a dose of 12 μCi of 211At 7G7/B6 (specific activity 4.6 μCi/μg and 10 μg/mouse of 7G7/B6 antibody). Group 2, the immunotherapy (daclizumab) group, was given injections of 100 μg of daclizumab on days 0, 7, 14, and 21. Group 3, the combination therapy group, received a combined therapy of 12 μCi of 211At-7G7/B6 on day 0 with daclizumab given on days 0, 7, 14, and 21. Group 4 received 12 μCi of 211At-11F11 (specific activity 2.7 μCi/μg and 10 μg/mouse of 11F11 antibody) as 211At labeled nonspecific mAb control. Group 5 received 200 μl PBS weekly for 4 weeks and served as a control for the daclizumab immunotherapy. The groups were randomly assigned and had comparable average serum concentrations of the surrogate tumor marker, sIL-2Rα (mean ~2,600 pg/ml), at the beginning of the experiments.
Statistics analysis

The serum levels of $\beta_2\mu$ were analyzed at different time points for the different treatment groups using the student t test for unpaired data. Statistical significance of differences in survival of mice in different groups was determined by the log-rank test using the StatView program (Abacus Concepts, Berkeley, CA)

Results

Binding integrity of radiolabeled antibody

A competitive binding assay was performed following each labeling procedure. In these studies the binding of $^{211}$At labeled 7G7/B6 antibody to IL-2R$\alpha$ was specifically inhibited by unmodified 7G7/B6 at increasing concentrations (100 molar excess) using a competition assay wherein inhibition of binding of non-saturating amounts of $^{211}$At-7G7/B6 antibody was assessed using the IL-2R$\alpha$ expressing Kit-225-IG3 cell (Fig 1). The $^{211}$At-11F11 control antibody did not specifically bind to IL-2R$\alpha$ expressing Kit-225-IG3 cell.

Definition of the maximum tolerated dose and toxicity

Different doses of $^{211}$At-7G7/B6 (6, 12, 24, 40 and 70 $\mu$Ci) were administered to the mice intravenously. The survival of mice in each group was monitored. All mice died within 10 days in the group receiving 70 $\mu$Ci of $^{211}$At-7G7/B6. Two of five mice died within 14 days and one mouse died on day 33 in the group receiving 40 $\mu$Ci of $^{211}$At-7G7/B6. One mouse died on day 85 and another died on day 108 in the 24 $\mu$Ci $^{211}$At-7G7/B6 group.
There were no deaths in the groups receiving 6 and 12 μCi of $^{211}$At-7G7/B6 during the 5 month period of observation.

Platelet counts were reduced in the mice receiving $^{211}$At-7G7/B6 in a dose-related manner (Fig 2). The nadir occurred 1 week after radiation therapy and recovered 2 to 3 weeks later. The white blood cell (WBC) count followed the same pattern as the platelet counts in the $^{211}$At-7G7/B6 treated mice. The WBC count was reduced with $^{211}$At-7G7/B6 in a dose-related manner. The nadir occurred 1 week after radiation therapy and recovered 2 to 3 weeks later. The mean of WBC counts at 1 week after $^{211}$At-7G7/B6 given were: 3769/μl (control), 1963/μl (6 uCi $^{211}$At-7G7/B6), 1850/μl (12 uCi $^{211}$At-7G7/B6), 1123/μl (24 uCi $^{211}$At-7G7/B6), and 710/μl (40 uCi $^{211}$At-7G7/B6 in surviving mice). Slight body weight loss (3%) was observed in the mice that received 12 μCi of $^{211}$At-7G7/B6 from the 2nd to the 4th week but the animals recovered subsequently, whereas mice that received 24 μCi of $^{211}$At-7G7/B6 showed a significant loss of body weight (9%, P<.05) within one week after the $^{211}$At-7G7/B6 was given but those animals also recovered 2-3 weeks later. There was no body weight loss in the life span control and 6 μCi of $^{211}$At-7G7/B6 groups.

There were no abnormal pathological findings at 5 months in $^{211}$At-7G7/B6 treated mice as compared to normal mice.

**Effective treatment of ATL using $^{211}$At-7G7/B6 combined with daclizumab directed towards CD25**

In a therapeutic trial in the MET-1 model of human ATL, a dose of 12 μCi of $^{211}$At-7G7/B6 (10 μg), daclizumab (100 μg i.v./week × 4), and the combination of 12 μCi of a
single dose of $^{211}$At-7G7/B6 with daclizumab (100 μg i.v./week × 4) demonstrated therapeutic efficacy as indicated by their effect on the serum levels of human β2μ a surrogate tumor marker (Fig 3) and on the survival of ATL bearing mice (Fig 4). When compared with the serum concentrations of human β2μ in the PBS and $^{211}$At-11F11 nonspecific monoclonal antibody control groups of mice, on days 28 and 50, there were significant reductions of serum β2μ levels in the groups of mice receiving 12 μCi of $^{211}$At-7G7/B6 (P<.0001), 4-week daclizumab (P<.0001), and in the combination group of 12 μCi of $^{211}$At-7G7/B6 and 4 week daclizumab when compared with the 4-week daclizumab-treated animals (P<.0001). The human β2μ level decreased progressively in the combination group during the treatment period and even after the treatment was completed as shown in Figure 3 whereas the human β2μ level increased after treatment was completed in the daclizumab alone group and in the group receiving a single dose of $^{211}$At-7G7/B6. Furthermore, there were significant prolongations of the survival of the groups of mice treated with $^{211}$At-7G7/B6 (P<.001), 4-week daclizumab (P<.001), and the combination of $^{211}$At-7G7/B6 with daclizumab (P<.0001) when compared with PBS or $^{211}$At-11F11 groups (Fig 4). In addition, there was a significant prolongation of the survival of the mice that were treated with the combination of $^{211}$At-7G7/B6 with daclizumab when compared with a single dose of $^{211}$At-7G7/B6 or with 4-week daclizumab when administered independently (p < 0.47). The median survival duration of the control group (PBS) was 62.6 days and was 61.5 days in $^{211}$At-11F11 treated group. All mice in PBS and $^{211}$At-11F11 groups died by day 78 of the study. In contrast, there were 36% of mice in $^{211}$At-7G7/B6, 50% of mice in daclizumab alone and 91% of mice in the combination group surviving on day 94. Comparable efficacy in the therapy of
ATL of $^{211}$At-7G7/B6 as compared to control group was observed when the study was repeated in an additional experiment.
Discussion

ATL is a malignancy of T lymphocytes with a median survival duration of 9 months in the acute form of the disease. Various combination chemotherapies have not significantly increased the survival of patients with ATL.\(^1\) In light of the disappointing results using conventional combination chemotherapy, therapies were developed that use unmodified murine and humanized antibodies (e.g., anti-Tac, daclizumab) directed toward IL-2R\(\alpha\). Although such therapy yielded partial or complete remissions in one-third of patients, most patients subsequently suffered a disease relapse.\(^6,\ 7\) The use of monoclonal antibodies armed with toxins or radionuclides specifically targeting these cytotoxic agents to leukemic cells provided a valuable augmentation of therapy.\(^7\) There are a number of components that must be considered in designing an optimal radioimmunotherapy regimen, including (A) the selection of the antigenic target and thus the monoclonal antibody; (B) the choice of the linking agent to join the radionuclide to the monoclonal antibody; and (C) the choice of the radionuclide used.

As just noted, a pivotal issue to be addressed is the selection of the monoclonal antibody that targets the tumor and thereby the type of malignancy chosen as the target for radioimmunotherapy. In the present study, we have chosen different epitopes of the human IL-2R\(\alpha\) subunit identified by 7G7/B6 and daclizumab as our targets for immunotherapy. The scientific basis for this choice is that virtually no normal resting cells express the \(\alpha\) subunit of the IL-2R, whereas this receptor is expressed by a high proportion of the abnormal cells in certain T-cell and B-cell lymphoid neoplasias, and monocytic and granulocytic leukemias identified by anti-Tac.\(^6\) In our clinical trials, we
exploit the differential expression of IL-2Rα between normal resting cells and malignant T-cells. A series of modifications in the anti-Tac monoclonal antibody have been made to increase its effector function, to reduce its immunogenicity, and to improve its pharmacokinetics. To augment its poteney, anti-Tac has been armed with the β−-emitting radionuclide $^{90}$Y. While a proportion of the 19 patients with HTLV-I associated Tac underwent a remission when treated with intact anti-Tac armed with this radionuclide and were provided meaningful therapy for this form of leukemia that was previously universally fatal, only 2 of 16 patients manifested a complete remission.

A second component of an optimal radioimmunotherapeutic regimen is the choice of the chelating agent or linker, to couple the radionuclide to monoclonal antibody. An ideal agent should not alter the specificity of binding of the monoclonal antibody to its antigenic target nor should it damage the antibody and thus alter its rate of catabolism or patterns of tissue distribution. The radionuclide should also be sequestered tightly so that there is no premature loss of the radionuclide from the monoclonal antibody linker complex in vivo. The agent, SAPS, used in the present study for $^{211}$At fulfills these requirements.28, 29

A third pivotal issue in defining an optimal radioimmunotherapeutic agent is to consider the nature of the radionuclide used. A short distance of action is desirable thus maintaining the specificity of the monoclonal antibody. Nuclear chemistry has provided a selection of α and β−-emitting radionuclides that have a relatively short distance of action.30 A β−-emitting radionuclide such as $^{90}$Y that acts through crossfire may be preferable for the treatment of large tumor masses. In this clinical situation this agent
may eliminate non-targeted non-antigen bearing tumor cells through the crossfire effect emanating from neighboring antigen-bearing cells that have been targeted by the radionabeled monoclonal antibody. Nevertheless, the use of β- emitting radionuclides has a limitation, as the target mass decreases the benefit of the crossfire effect also decreases, while the potential for normal tissue damage increases. With small tumors including micrometastases, individual tumor cells and leukemic cells, the therapeutic efficacy may be limited. This is because high-energy β- emitting radionuclides deliver a high dose of radiation to normal tissues due to the longer range of β- particles. For such cellular populations, the future development of isotopic monoclonal antibody mediated approaches may focus on α-emitting radionuclides, which may be the most effective agents at killing isolated leukemic cells without damaging normal tissues. Radionuclides emitting α-particles have a high linear energy transfer (LET) (6-9 MeV particles) that act over 10-80 micrometers and are effective at killing individual target cells.31, 32 Thus for agents that target the surface of isolated leukemic cells, one would require only the binding of a relatively small number of radiolabeled molecules per cell to provide the limited number of nuclear transversals required for leukemic cell killing. Alpha-emitting radionuclides under investigation for use in systemic radioimmunotherapy include 212Bi, 213Bi, 211At and 225Ac. A major limitation in the use of bismuth radionuclides is their short physical half lives of 60 min for 212Bi and 46.6 min for 213Bi that impair their ability to be delivered to the target leukemic cells during their effective period of decay. Our prior study showed that 211At labeled daclizumab significantly prolonged the mean survival time in cynomolgus cardiac allograft model.33 This present study also focuses on 211At whose half-life of 7.2 hours may be adequate to obtain effective leukemic cell
targeting. This radionuclide is of special value for antibodies such as 7G7/B6 that have been shown to stay on the lymphocyte cell surface and to not be internalized by the target cell, a process that might lead to loss of the $^{211}$At from its target arena.

In previous studies, we presented a pretargeting strategy with an anti-Tac antibody-streptavidin (HAT-SA) conjugate, which recognizes CD25, followed by $^{213}$Bi-DOTA-biotin on MET-1 and $^{90}$Y-DOTA-Biotin with the SUDHL-1 murine models. $^9$-$^{10}$ The pretargeting strategy was indeed excellent especially in association with the beta-emitting radionuclide $^{90}$Y with lymphoma. Since it has not been possible to use the alpha emitter $^{211}$At with this system, the approach was much less useful in clinical situations where there were isolated malignant cells as in leukemia or micrometastases where the crossfire effects of $^{90}$Y were less effective. Therefore we are exploring an alternative strategy that permits the use of a relatively long lived alpha-emitting radionuclide and that allows the simultaneous anti-tumor actions provided by saturating doses of the anti-Tac (daclizumab) and the antitumor radiation delivered by $^{211}$At-7G7/B6.

In this study, an additional issue involved in systemic radioimmunotherapy was considered. We addressed the limitation inherent in the use of a single monoclonal antibody to simultaneously saturate receptors for ADCC and cytokine deprivation while delivering a high proportion of an administered radionuclide $^{211}$At to tumor cells by using two non-cross-competing antibodies daclizumab and 7G7/B6 that bind to different epitopes of the IL-2R alpha protein. Daclizumab a mAb that binds to the IL-2 binding site of the IL-2R alpha was used at receptor saturating doses whereas radiolabeled $^{211}$At 7G7/B6 monoclonal antibody at high specific activity was used to provide maximum
delivery of tumor irradiation. The results of a trial of a single dose of $^{211}$At-7G7/B6 in the MET-1 model of ATL were encouraging. Unmodified monoclonal antibody 7G7/B6 used at 100 $\mu$g weekly for 4 weeks showed antitumor activity in the MET-1 model in the previous studies; however a single dose of 100 $\mu$g did not give a meaningful effect. The protein dose of 7G7/B6 used in this $^{211}$At-7G7/B6 trial was 10 $\mu$g so the inclusion of $^{211}$At made a major contribution to the leukemic cell killing. However, this aggressive T-cell leukemia was not completely eliminated by a single course of therapy with $^{211}$At-7G7/B6. A paradigm has emerged suggesting that increased efficacy of cancer therapy can be obtained through the addition of the additive effects of two agents with distinct modes of cytotoxic action leading to malignant cell death. This paradigm has been shown for the daclizumab monoclonal antibody added at therapeutic doses when labeled with $^{213}$Bi, daclizumab with flavopiridal and the combination of Herceptin with Paclitaxel.14,34

In the present trial, we achieved the complementary action of receptor-saturating doses of the daclizumab monoclonal antibody to yield antibody-dependent cellular cytotoxicity (ADCC) and cytokine deprivation-mediated leukemic cell death along with tumor cytoreduction provided by the irradiation mediated by the radionuclide $^{211}$At delivered to the leukemic cell surfaces. Indeed, whereas neither daclizumab nor $^{211}$At-7G7/B6 alone was completely effective, remissions were observed in the majority of mice receiving both agents in conjunction (Fig 4). In conclusion, a single dose of $^{211}$At-7G7/B6 provides some efficacy and combination therapy of $^{211}$At-7G7/B6 with repeated receptor saturating doses of daclizumab provided the desired efficacy in the murine model of ATL investigated. Therefore, the results of the present study support the use of this combination regimen in a clinical trial involving the treatment of patients with ATL.
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Legends

Figure 1. The binding of $^{211}$At-7G7/B6 to IL-2Rα on Kit-225-IG3 cells was specifically inhibited by unmodified 7G7/B6 mAb. A competitive binding assay was performed as described in “Materials and Methods”. The line with diamonds ♦ shows the binding of $^{211}$At-7G7/B6 mAb to IL-2Rα expressing Kit-225-IG3 cells at 3x10$^5$, 1x10$^6$, 3x10$^6$ cells. The point with the square symbol ■ is the $^{211}$At-7G7/B6 mAb binding to IL-2Rα when blocked by the addition of a 100-fold greater concentration of unmodified of 7G7/B6. The line with triangles ▲ represents $^{211}$At-11F11 mAb binding to the IL-2Rα expressing Kit-225-IG3 cell.

Figure 2. Platelet counts were measured in NOD/SCID mice that received different quantities of $^{211}$At. The circulating platelet counts were determined initially at weekly and subsequently at monthly intervals. Mice in the control group did not receive any treatment (control). Mice in the other groups had received 400 μg of UPC10 mAb i.v. 30 min before they received $^{211}$At-7G7 at doses of 6, 12, 24, and 40 μCi.

Fig. 3. Inhibition of the growth of MET-1 ATL cells in NOD/SCID mice by $^{211}$At-7G7/B6, daclizumab and the combination of these agents. MET-1 ATL cells were transferred into mice i.p. The groups (11-12 mice/group) included those receiving PBS, four weekly doses of 4 mg/kg (100 μg/mouse) daclizumab, a single dose of 12 μCi $^{211}$At-7G7/B6 (10 μg/mouse), the combination 100 μg of 4-weekly doses of daclizumab with a single dose of 12 μCi $^{211}$At-7G7/B6 and a single dose of 12 μCi $^{211}$At-11F11 (10 μg/mouse). The data represent the mean serum concentrations of the surrogate tumor marker human β2μ in ng/ml. The group receiving a single dose of 12 μCi $^{211}$At-7G7/B6, 4-week daclizumab treated group, and the combination of 12 μCi $^{211}$At-7G7/B6 with 4-week- daclizumab group had significantly decreased values of β2μ (0.24 ng/ml at day 50) when compared with those of the PBS and $^{211}$At-11F11 control groups ($P < 0.0001$).
There was no significant difference in $\beta_2\mu$ levels between the group receiving a single dose of 12 $\mu$Ci $^{211}$At-11F11 and that receiving PBS ($P = 0.43$).

**Fig. 4. Kaplan-Meier survival plot of MET-1 bearing NOD/SCID mice.** The groups (11-12 mice/group) included those receiving i.v. PBS, four weekly doses of 4 mg/kg (100 $\mu$g/mouse) daclizumab, a single dose of 12 $\mu$Ci $^{211}$At-7G7/B6, a single dose of 12 $\mu$Ci $^{211}$At-11F11, and the combination of 4 mg/kg of 4-week daclizumab and a single dose of 12 $\mu$Ci $^{211}$At-7G7/B6 respectively. Survival in the treated groups was followed out to 94 days. The 4-week daclizumab-treated group, the single dose of 12 $\mu$Ci $^{211}$At-7G7/B6, and the 4-week combination of 4 mg/kg daclizumab with a single dose of 12 $\mu$Ci $^{211}$At-7G7/B6 had significantly prolonged survivals when compared with the PBS group or with the control group receiving the single dose of 12 $\mu$Ci $^{211}$At-11F11 ($P < 0.0001$). The combination treatment of tumor-bearing mice with a single dose of 12 $\mu$Ci $^{211}$At-7G7/B6 and daclizumab for 4 weeks significantly prolonged the survival of the group when compared with the 4-week daclizumab or with the single dose of 12 $\mu$Ci $^{211}$At-7G7/B6 treatment groups ($P < 0.05$).
References


25. Rubin LA, Kurman CC, Biddison WE., Goldman ND, Nelson DL. A monoclonal antibody 7G7/B6, binds to an epitope on the human interleukin-2 (IL-2) receptor that is distinct from that recognized by IL-2 or anti-Tac. Hybridoma, 1985; 4: 91-102.

33. Wesley JN, McGee EC, Garmestani K, et. al. Systemic radioimmunotherapy using a monoclonal antibody, anti-Tac directed toward the alpha subunit of the IL-2 receptor armed with the alpha-emitting radionuclides (212)Bi or (211)At. Nucl Med Biol. 2004; 31:357-364.

Figure 1

![Graph showing the percentage of total cpm bound for different conditions.

- **211At-7G7/86**
- **211At-7G7/86 inhibited by unmodified 7G7/86**
- **211At-11F11**

The x-axis represents the concentrations in units of 3x10^-5 E, 10^-6 E, and 3x10^-6 E, while the y-axis represents the percentage of total cpm bound, ranging from 0 to 70%.
Figure 2

![Graph showing platelet count over days since administration of $^{211}$At-7G7/B6 at different doses.](image)

- Control
- 6 uCi $^{211}$At-7G7/B6
- 12 uCi $^{211}$At-7G7/B6
- 24 uCi $^{211}$At-7G7/B6
- 40 uCi $^{211}$At-7G7/B6

Platelet (million/ul) vs. Days since administration of $^{211}$At-7G7/B6.
Figure 3

[Bar graph showing Beta-2 Microglobulin levels (ng/ml) over different days and treatments.]

- Day 14
- Day 28
- Day 50

Treatments include PBS, Dacizumab, and combinations of 2a AT-7G7/B6, 2a AT-7G7/B6 + Dac, and 2a AT-11F11.
Figure 4

![Graph showing survival rates of mice treated with different substances. The x-axis represents the day since treatment started, and the y-axis represents the percentage of mice surviving. The graph includes lines for PBS, ^211^At-11F11, Daclizumab, ^211^At-7G7, and Dac+ ^211^At-7G7.](https://www.bloodjournal.org)
Effective treatment of a murine model of adult T cell leukemia using At-7G7/B6 and its combination with unmodified anti-Tac (daclizumab) directed towards CD25

Zhuo Zhang, Meili Zhang, Kayhan Garmestani, Vladimir S Talanov, Paul S Plascjak, Barbara Beck, Carolyn K Goldman, Martin W Brechbiel and Thomas A Waldmann