Anti-CD45 Radioimmunotherapy Using $^{211}$At with Bone Marrow Transplantation Prolongs Survival in a Disseminated Murine Leukemia Model

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Running Title: $^{211}$At-Anti-CD45 RIT and BMT for AML

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KEY POINTS

- Astatination of anti-CD45 antibody via a closo-decaborate compound yields a stable conjugate that targets radiation to hematologic organs.
- $^{211}$At-anti-CD45 radioimmunotherapy, combined with bone marrow transplantation, prolongs survival in a disseminated murine leukemia model.

ABSTRACT

Despite aggressive chemotherapy combined with hematopoietic cell transplant (HCT), many patients with acute myeloid leukemia (AML) relapse. Radioimmunotherapy (RIT) using monoclonal antibodies (mAb) labeled with beta-emitting radionuclides has been explored to reduce relapse. Beta-emitting radionuclides are limited by lower energies and non-specific cytotoxicity from longer path lengths compared to alpha-emitters. Conversely, $^{211}$At is an alpha-emitting radionuclide with a higher energy profile and shorter path length than beta-emitters. We evaluated the efficacy and toxicity of anti-CD45 RIT using $^{211}$At in a disseminated murine AML model. Biodistribution studies in leukemic SJL/J mice showed excellent localization of $^{211}$At-anti-murine CD45 mAb (30F11) to marrow and spleen within 24 hours [18% and 79% injected dose per gram of tissue (% ID/g), respectively], with lower kidney and lung uptake (8.4% and 14% ID/g, respectively). In syngeneic HCT studies $^{211}$At-B10-30F11 RIT improved the median survival of leukemic mice in a dose dependent fashion (123, 101, 61 and 37 days given 24, 20, 12 and 0 µCi, respectively). This approach had minimal toxicity with nadir white blood cell counts $>$2.7 K/µL two weeks after HCT and recovery by 4 weeks. These data suggest that $^{211}$At-anti-CD45 RIT in conjunction with HCT may be a promising therapeutic option for AML.
INTRODUCTION

Acute myeloid leukemia (AML) is an aggressive malignancy with few treatments producing prolonged remissions in high-risk patients. Hematopoietic cell transplant (HCT) may offer the best chance for a cure, but has been associated with high rates of treatment related mortality and relapse. Investigators have escalated chemotherapy and/or radiation doses to decrease relapse, but this strategy has been associated with substantial toxicity yielding no significant improvement in overall survival. Monoclonal antibodies (mAb) targeting hematologic specific antigens have been used in radioimmunotherapy (RIT) studies as a means to deliver higher radiation doses prior to HCT. One such target is CD45, a cell surface antigen highly expressed on hematologic tissues (~200,000 binding sites per cell) with minimal expression on non-hematologic tissues. CD45 is not extensively internalized after mAb binding, further making anti-CD45 RIT a viable approach for therapy of high-risk AML. In particular, anti-CD45 mAb coupled to $^{131}$I has been shown to deliver an average 2-to-3-fold higher radiation-absorbed dose to spleen and bone marrow than to non-leukemic normal organs, and can be safely administered to high-risk patients with acute leukemia or myelodysplastic syndrome (MDS) in conjunction with standard high-dose chemotherapy and 12 Gray (Gy) total body irradiation (TBI). Favorable results suggesting improvements in survival have also been shown using this anti-CD45 RIT approach as part of a reduced-intensity HCT regimen in older relapsed/refractory AML patients with high disease burdens.

Despite these advances, limitations inherent to the radionuclides investigated thus far hinder more widespread use of RIT as an adjunct to HCT. The majority of RIT studies have used mAb labeled with beta-emitters such as $^{131}$I and $^{90}$Y. However, these radionuclides may generate non-specific cytotoxicity due to a cross-fire effect from their relatively longer path length (0.3-2.3 mm), and for $^{131}$I, its associated gamma-emissions. Conversely, alpha-emitting radionuclides have the potential to deliver improved therapeutic ratios of absorbed radioactivity with less non-specific toxicity because of their very short path lengths (~60-80 µm), making
them attractive candidates for therapy of leukemias and elimination of minimal residual disease.\textsuperscript{12-16} Differences in path lengths dictate that beta-emitters rely on cross-fire effect, irradiating bystander cells without the need to deliver the radionuclide directly to each malignant cell. The cross-fire effect from alpha-emitters is confined to smaller volumes, resulting in a cytotoxic effect in short range tracks near cells binding the radionuclide \textit{via} the mAb. Moreover, alpha-emitting reagents have a high linear energy transfer (LET) because of the high decay energy (5-8 MeV) deposited over short distances that allows for potent and efficient cell kill, compared to beta-emitters such as \textsuperscript{131}I and \textsuperscript{90}Y with lower decay energies (0.66-2.3 MeV) and longer path lengths.\textsuperscript{2}

Only a few alpha-emitting radionuclides, however, are currently suitable for clinical application.\textsuperscript{17} For the alpha-emitters with favorable radiobiologic characteristics, other issues such as availability, labeling chemistry, and \textit{in vivo} stability, especially of astatinated macromolecules, have impacted their use in RIT.\textsuperscript{14,18,19} Astatinated mAbs exhibit \textit{in vivo} dehalogenation of mAb when labeled via conventional approaches, effectively hindering development of \textsuperscript{211}At-based alpha targeted therapy. Using a novel approach to \textsuperscript{211}At-label mAb, we have thus used the alpha-emitting radionuclide \textsuperscript{211}At for anti-CD45 RIT of AML because it has a reasonable half-life ($t_{1/2} = 7.2$ hours), a favorable energy profile (6.8 MeV, averages of two alpha decays, 5.9 and 7.5 MeV), and short path length (average range 55-70 µm). In this report we describe the efficacy and toxicity of \textsuperscript{211}At-labeled-anti-CD45 RIT combined with HCT in a mouse model of syngeneic disseminated AML. These studies show that \textsuperscript{211}At-anti-CD45 mAb can efficiently localize to sites of leukemia, and when used in lieu of TBI, this approach facilitates engraftment of donor bone marrow with minimal toxicity and significantly prolongs survival.
METHODS

Mice: Female SJL/J mice, 6- to 12-weeks old, were purchased from Jackson Laboratories (Bar Harbor, ME), and housed at the Fred Hutchinson Cancer Research Center (FHCRC) in a pathogen-free environment under protocols approved by the Institutional Animal Care and Use Committee.

Cell Lines and Antibodies: Murine AML cells were produced by passage through SJL/J mice as previously described. Isotype-matched control antibody (polyclonal rat IgG) was purchased from Sigma Aldrich (St. Louis, MO). The rat IgG2b anti-murine CD45 mAb (30F11) was purified as previously described.

Conjugation of 30F11 and Rat IgG with B10: Anti-CD45 mAb (30F11) or rat IgG antibody was diluted 1:1 with 100 mM HEPES buffer to yield 50 mM (with 150 mM NaCl, pH 8.6). Isothiocyanatophenethyl-ureido-closo-decaborate(2-) (B10-NCS) at 10 mg/mL in DMSO was added to antibody solution, and the reaction proceeded overnight with gentle tumbling at room temperature. The next morning, the mixture was split into two equal fractions and passed over two PD-10 columns and collected in PBS. Protein fractions were combined, concentrated, and sterile filtered to yield 30F11-B10 or rat IgG-B10.

211At-Labeling of 30F11-B10 and Rat IgG-B10: was isolated from an irradiated bismuth target by a wet chemistry approach as previously described. The isolated 211At solution was used directly in the labeling procedure. Briefly, a 250 µL solution of 500 mM sodium phosphate, pH 6.8 was combined with 250 µL 30F11-B10 or rat IgG-B10. To this solution, 500 µL of Na[211At]At (3.5 mCi in water, pH 7) was added in the 30F11-B10 reaction, or 350 µL of Na[211At]At (2.1 mCi in water, pH 7) in the rat IgG-B10 reaction, followed by 10 µL of an aqueous Chloramine-T solution (10 mg/mL). After 2 minutes at room temperature, the reaction
was quenched by adding 10 µL of sodium metabisulfite solution (10 mg/mL in water) to the 30F11-B10 or rat IgG-B10 reaction. Each mixture was then placed on a PD-10 column and eluted with PBS. The protein containing fractions were combined to give $^{211}$At-B10-30F11 (75% radiochemical yield and 79% protein recovery) or $^{211}$At-B10-rat IgG (72% radiochemical yield and 62% protein recovery). Purity of $^{211}$At-labeled injectate was >99% by TLC.

*Biodistribution Studies:* Mice were injected with $1 \times 10^5$ SJL leukemia cells intravenously (*i.v.*). Two days after injection of leukemia cells, 100 µg (0.67 nmol) of 30F11-B10 and rat IgG-B10 were trace-labeled with either $^{125}$I as previously described, or with $^{211}$At and injected into groups of 5 mice. Mice were then euthanized 8 and 24 hours after injection of $^{125}$I-B10-mAb conjugate, or 1, 3, 7, and 24 hours after injection of $^{211}$At-B10-mAb conjugate. Organs were excised, weighed, and the $^{125}$I or $^{211}$At activity was counted on a Packard 5000 gamma counter (Packard Instrument Company, Meriden, CT), with correction for radioactive decay during the counting process, to determine the percent injected dose of radiation activity per gram of organ (% ID/g).

*Radiation Dosimetry:* Radiation absorbed doses were calculated for blood and organs as previously described. For mice that received $^{211}$At-B10-mAb conjugates, we assumed a self-organ absorbed fraction of 1.0 and a cross-organ absorbed fraction of 0 due to the very short range of alpha particles.

*RIT of Leukemic Mice:* RIT studies were performed using $^{211}$At-B10-mAb conjugates in groups of 10, and 5 days prior to study onset were placed on a diet containing Uniprim antibiotic (irradiated, 4100 ppm from Harlan Laboratories, Indianapolis, IN). Mice were injected *i.v.* with $1 \times 10^5$ SJL leukemia cells and two days later mice received 100 µg (0.67 nmol) of either 30F11-B10 or rat IgG-B10 labeled with 12, 20, or 24 µCi of $^{211}$At. Two days after injection of $^{211}$At-B10-
mAb, mice underwent syngeneic bone marrow transplant (BMT) receiving 15x10^6 donor bone marrow cells, without T-cell depletion, as described previously.24 Mice were monitored daily for changes in appearance and body weight. Mice were euthanized if they became moribund from progressive leukemia or if they lost more than 30% of baseline weight. Survival curves were compared by the log-rank test (Mantel-Cox test), and reported only when statistically significant when less than the Bonferroni-corrected threshold for multiple comparisons.

**Toxicity Assessments:** Groups of 10 non-leukemia-bearing mice were treated with 12 or 24 µCi of 211At-B10-mAb conjugate and rescued via BMT as described above. At 1, 2, 3, 4, 6, 8, and 25 weeks after BMT, blood was drawn via the retro-orbital plexus and complete blood counts were obtained. Serum was assayed for kidney function, by measuring blood urea nitrogen (BUN) and creatinine (Cr) levels, and liver function, by measuring enzymes alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate transaminase (AST). All toxicity tests were performed by Phoenix Central Laboratory (Everett, WA).25 Values were compared with those obtained at the same time points from untreated age-matched control SJL/J mice.

**RESULTS**

*Biodistribution of Radioactivity using 211At-B10-Anti-CD45 mAb in SJL/J Leukemic Mice*

To assess the ability of the mAb-B10 constructs to localize to leukemia targets, we performed biodistribution studies using radiolabeled anti-murine CD45 specific mAb (30F11)-B10 conjugates. As radioiodine has been reproducibly used in prior biodistribution studies,20,25 we labeled 30F11-B10 with 125I and 211At for comparison. Uptake of 125I-B10-30F11 in the spleen was 67.6 ± 8% ID/g by 8 hours, and 24 hours after injection of 125I-B10-30F11 the spleen retained more activity (42.3 ± 8% ID/g) than any other tissue at this same time point (Figure 1A).
Other non-targeted tissues, such as liver and kidney, had significantly less radioactivity at 24 hours with 6.7 ± 0.5% and 4.6 ± 0.1% ID/g, respectively.

Since the novel alpha-labeling construct B10 did not appear to impede targeting given the excellent localization of 125I-B10-30F11 to bone marrow and spleen, we then performed biodistribution studies using the alpha-emitter 211At. One hour after 211At-B10-30F11 injection, blood and spleen displayed the highest concentrations of radioactivity [32.3 ± 17% and 30.3 ± 14% ID/g, respectively (Figure 1B)]. In addition, blood clearance of 211At-B10-30F11 was rapid, decreasing from 32.3 ± 17% ID/g 1 hour after injection to 11.7 ± 2% ID/g after 24 hours, while the spleen retained 79 ± 7.3% ID/g and the bone marrow (BM) retained 18 ± 3.8% ID/g after 24 hours. Importantly, 211At-B10-30F11 did not exhibit excessive non-specific uptake in the kidneys, even without use of renal protective agents, with 10.2 ± 5.1%, 12.2 ± 2.6%, 10.9 ± 3.6%, and 8.4 ± 2.9% ID/g retained in kidneys 1, 3, 7 and 24 hours after injection of labeled 30F11-B10, respectively. Relatively low concentrations of radioactivity were delivered to non-target organs such as liver, lung, small intestine, and colon after 24 hours (14.3 ± 1.3%, 8.4 ± 1.2%, 6.02 ± 0.7%, and 7.93 ± 1.3% ID/g, respectively). These biodistribution studies showed that both 125I and 211At localized to target tissues of bone marrow and spleen, with less non-specific uptake in non-hematologic tissues.

211At-B10-Anti-CD45 mAb Dosimetry

Using standard medical internal radiation dose methods and biodistribution data of 211At-B10-30F11, radiation absorbed doses (cGy) for organs harvested were calculated per µCi of 211At injected. Values shown are for 0.19 µCi of 211At injected, to yield a liver dose of 5 cGy (Table 1). CD45+ tissues targeted by 30F11-B10 had the highest total absorbed dose at 18 and 7.5 cGy per 0.19 µCi of 211At administered for spleen and blood, respectively. Non-target organs had lower radiation absorbed doses, with kidneys and lungs having 2.9 and 4.1 cGy absorbed dose per 0.19 µCi of 211At injected, respectively.
Alpha-Camera Imaging

Alpha-camera imaging described previously was employed to further characterize the distribution of $^{211}$At-B10-30F11 conjugate as some alpha-emitters have been known to exhibit heterogeneous distributions in targeted tissues at the sub-organ level. Cryosections of spleen and femur from leukemia-bearing mice treated with $^{211}$At-B10-30F11 were imaged with the alpha-camera technique at various time points after radiolabeled injections. At the 3-hour time point, alpha-camera imaging revealed that $^{211}$At was distributed throughout the spleen and bone marrow, with some variability that correlates with sub-organ architecture. By imaging, the activity uptake in spleen (Figure 2A) was $33 \pm 2\%$ ID/g at 3 hours after injection of $80 \mu$Ci $^{211}$At-B10-30F11, an uptake value in good agreement with the biodistribution data. Figure 2B presents a color-coded histogram of the activity distribution in a spleen section, where the activity uptake of the different subareas was normalized to the mean uptake for the whole spleen. More than 80% of the total section area was within a factor of 0.7 – 1.3 of the mean activity. The highest concentrations were noted in areas corresponding to the marginal zone between red and white pulp. Alpha-camera imaging of cryo-sectioned femurs [Figure 2C, yellow regions of interest (ROIs)] quantified the uptake in bone marrow to be $11 \pm 2\%$ ID/g at 3 hours after injection of $^{211}$At-B10-30F11. These results correlated well with biodistribution studies (Figure 1A) where $^{211}$At-B10-30F11 demonstrated $11.2 \pm 1.3\%$ ID/g at the 3-hour time-point. The activity distribution (normalized to the mean) along the bone marrow cavity of a femur was similar, where 85% of the area was within a factor of 0.7 – 1.3 of the mean (Figure 2D). To further analyze activity distribution in the femur, 11 small ROIs were placed along the marrow cavity to quantify how their activity concentration varied as compared to the mean activity of the whole femur (Figure 2C, magenta ROI). The variation in activity between the ROI were small, ranging from 0.9 to 1.25. The highest uptake was seen in the bone epiphyses.
Having demonstrated excellent localization of the anti-murine CD45 mAb-B10 conjugate in biodistribution and alpha-camera imaging studies, we assessed the therapeutic efficacy of $^{211}\text{At}$-B10-30F11 in a clinically relevant disseminated murine myeloid leukemia model. SJL/J mice were injected with $1 \times 10^5$ SJL leukemic cells as previously described. Two days later groups of 10 mice per dose were treated with 12 or 24 $\mu$Ci $^{211}\text{At}$-labeled 30F11-B10 or 24 $\mu$Ci $^{211}\text{At}$-B10-rat IgG. Studies in the absence of stem cell rescue demonstrated a survival benefit for the mice treated with 12 $\mu$Ci $^{211}\text{At}$-B10-30F11 with a statistically significant improvement in median overall survival (OS) of 69 days (with 2 mice treated surviving for >180 days) compared to a median survival of 36 days for untreated control mice ($p<0.0001$) and 54 days for mice that received $^{211}\text{At}$-B10-rat IgG ($p=0.0003$) (Figure 3A). The marginal improvement in OS seen in the rat IgG Ab-B10 control group was likely due to a non-specific radiation effect from circulating radiolabeled rat IgG–B10. Seventy percent of the mice treated with 24 $\mu$Ci $^{211}\text{At}$-B10-30F11 died within 12 days after injection of $^{211}\text{At}$-B10-30F11 (median OS of 11 days) due to toxicity related to marrow aplasia and probable infection; these mice had normal liver and kidney function assessed prior to euthanasia and did not have evidence of leukemic blasts in tissues by gross histology or in blood as assayed by flow cytometric analysis.

$^{211}\text{At}$-Anti-CD45 RIT Combined with BMT for Leukemia

Survival among animals treated with escalated doses $^{211}\text{At}$-B10-30F11 was limited by radiation-induced marrow toxicity resulting in early non-leukemic deaths suggesting that improved outcomes could be obtained with BMT. We therefore evaluated the efficacy of $^{211}\text{At}$-B10-30F11 RIT in conjunction with BMT in syngeneic leukemic mice. Groups of 10 SJL/J mice were injected with $1 \times 10^5$ SJL leukemic cells followed two days later by injection of 12, 20, or 24 $\mu$Ci $^{211}\text{At}$-B10-30F11 or 24 $\mu$Ci $^{211}\text{At}$-B10-rat IgG. Two days after $^{211}\text{At}$-B10-mAb injection, a time when 99% of $^{211}\text{At}$ had decayed, mice received $15 \times 10^6$ bone marrow cells i.v. from
syngeneic donors. Mice that received $^{211}\text{At}$-B10-30F11 showed a dose-dependent improvement in median OS, with increased long-term survival rates seen after BMT (Figure 3B). Mice treated with 12, 20, or 24 µCi $^{211}\text{At}$-B10-30F11 had a median OS of 61, 101, and 123 days (p<0.001 for all three when compared to untreated control mice), respectively. Seven of the 20 mice treated with the higher doses (either 20 or 24 µCi $^{211}\text{At}$-B10-30F11) survived to euthanasia without evidence of recurrent leukemia at day 180 post-injection. For comparison, untreated control leukemic mice had a median OS of 37 days while mice treated with $^{211}\text{At}$-B10-rat IgG conjugate had median OS of 46.5 days (p=0.0023). Overall these data suggest that the hematologic toxicity from anti-CD45 RIT using $^{211}\text{At}$ may be overcome by hematopoietic cell rescue.

**Assessment of Toxicity after $^{211}\text{At}$-anti-CD45 RIT**

To evaluate the tolerability and systemic toxicity of $^{211}\text{At}$-labeled anti-CD45 RIT with BMT, 10 mice per group were treated with 24 µCi $^{211}\text{At}$-B10-rat IgG or either 12 or 24 µCi $^{211}\text{At}$-B10-30F11 2 days prior to BMT. Blood, renal and hepatic studies were performed at multiple time points after delivery of each radiolabeled antibody-B10 conjugate. Each laboratory test was compared to untreated age-matched control mice. These studies demonstrated that doses up to 24 µCi $^{211}\text{At}$-B10-30F11 with BMT was minimally toxic with all mice developing a dose-dependent leukopenia that improved by four weeks after transplantation (Figure 4A). White blood cell counts had nadirs between 2.66 and 4.34 K/µL at 2 weeks after BMT for mice treated with 24 and 12 µCi $^{211}\text{At}$-B10-30F11, respectively, and stabilized (5.80-7.22 K/µL) in all mice by day 56 after infusion of the radiolabeled antibody-B10 conjugate and remained in this range out to 180 days. Hemoglobin and platelet levels were minimally affected by either dose of $^{211}\text{At}$-B10-30F11 [ranging between 12.9-15.7 g/dL and 812-1120 K/µL, respectively, (Figure 4B and 4C)].

Renal and hepatic function tests from mice treated with 24 µCi $^{211}\text{At}$-B10-30F11 did not significantly deviate from the normal range of untreated controls for at least 180 days after BMT.
(Figure 5). Despite a modest initial decrease in ALP levels during the first 4 weeks to 76.0, 43.1, and 43.0 IU/L in mice receiving 24 µCi $^{211}$At-B10-rat IgG, and 12 or 24 µCi $^{211}$At-B10-30F11, respectively, ALP levels remained stable thereafter (69.6-88.1 IU/L), comparable to the normal range of 64.8-86.8 IU/L (Figure 5A). Mild increases in transaminase levels were seen in mice that received $^{211}$At-B10-30F11, yet these values remained within normal range throughout the study (ALT 50.7-105.9 IU/L; AST 101-221 IU/L; Figure 5B and 5C). BUN levels were within normal range at 15.6 and 17.6 mg/dL six weeks after therapy for mice treated with 12 and 24 µCi $^{211}$At-B10-30F11; this was in comparison to BUN levels of 12.8-18.0 mg/dL in mice treated with control $^{211}$At-B10-rat IgG or BMT alone at the same time point (Figure 5D). Serum Cr levels remained stable throughout the monitored period with baseline levels between 0.2-0.4 mg/dL for all $^{211}$At-B10-30F11 mice (Figure 5E). These data suggest that minimal toxicity is associated with anti–CD45 RIT employing $^{211}$At and BMT in this model.

**DISCUSSION**

In this study we have demonstrated that anti-CD45 RIT using the alpha-emitting radionuclide $^{211}$At in conjunction with BMT can prolong survival in mice with disseminated murine leukemia with minimal renal or hepatic toxicity. Biodistribution studies showed that the anti-CD45 30F11-B10 conjugate provides excellent localization of radioactivity to sites of leukemia (spleen and marrow), which was confirmed using alpha-camera imaging. Dosimetry estimates using $^{211}$At-B10-30F11 revealed maximum absorbed doses delivered to splenic tissues harboring a high, leukemic cell burden, with ratios of absorbed dose per unit injected for target-to-non-target organs of 3.6, 4.4 and 6.2 for spleen-to-liver, spleen-to-lung, and spleen-to-kidney, respectively. Importantly, dosimetric calculations demonstrated that a minimal radiation dose was delivered to the kidneys using this approach. When 24 µCi $^{211}$At-B10-30F11-B10 was injected into mice, the kidneys were estimated to have an absorbed dose of 4.3 Gy, well within the tolerable safe dose of 10 Gy from previous studies on $^{211}$At based RIT using renal filtration.
capacity in mice. These results may lessen concerns for radiation-induced nephritis, which has been linked to alpha-emitting radionuclides and their associated daughter radionuclides. Minimal renal uptake also supports the viability, stability and targeted delivery of astatinated macromolecules via the conjugation chemistry of the B10-NCS (isothiocyanate-closo-decaborate) reagent, corroborating earlier studies that showed comparable in vivo stability of 211At- and 125I-labeled mAb at 24hr in canine biodistribution studies using 211At-labeled via our B10 chemistry. In RIT studies the delivery of 211At-anti-CD45 RIT resulted in improved OS for leukemic mice that was most significant when combined with BMT, where 40% of mice treated at the highest dose (i.e., 24 µCi 211At-B10-30F11) survived at least 180 days. Moreover, 211At-anti-CD45 RIT was well tolerated with relatively minimal leukopenia that resolved by four weeks after BMT.

In addition to its tolerability, several other features suggest that alpha-emitting radionuclides may be better suited than the traditionally investigated beta-emitting radionuclides for certain clinical situations. Short effective path lengths and higher decay energies are some of the differentiating characteristics of alpha-emitting radionuclides compared to beta- or gamma-emitters. Alpha-emitting radionuclides decay with energies on the order of 4-8 MeV, which are deposited over their path length, usually on the order of 50-80 µm. Consequently, the LET of alpha particles approaches 100 KeV/µm, around which the radiobiological effectiveness has been shown to reach a maximum. On the other hand, radionuclides that decay via beta-particles, such as 90Y with its mean energy of 2.4 MeV and significantly longer path length (2.7 mm) yields a lower LET of 0.22 KeV/µm. Other beta-emitting radionuclides studied in RIT, such as 131I or 177Lu, also have lower energies (0.66 and 0.5 MeV, respectively) and lower LET compared to alpha-emitters. Dose calculations by other investigators have predicted differences in efficacy between alpha- and beta-emitters, largely from differences in their energy deposition. Energy from alpha emission is high and their short range such that only a few nearby cells are killed, whereas the energy deposition from beta-emitters is mostly deposited at
further distances, usually not killing the cell the radionuclide is attached to.\textsuperscript{30,31} Because beta-emitters will deposit most of the energy away from the site of decay, \textsuperscript{211}At could be more favorable than \textsuperscript{90}Y for small tumor cell clusters, given more favorable tumor:normal tissue mean absorbed dose analysis when comparing \textsuperscript{90}Y and \textsuperscript{211}At distributed uniformly in spheres.\textsuperscript{32} Thus, alpha-emitting radionuclides may be best suited for scenarios of circulating disease, such as leukemia, or in setting of minimum residual disease as their effective range of energy deposition is on the order of a few cell diameters. Conversely, these alpha-emitting radionuclides may be less effective in large, bulky tumors, requiring homogeneous distribution throughout the target volume.

RIT using alpha-emitting radionuclides have been investigated in a small number of human AML studies. Jurcic \textit{et al.}\textsuperscript{33} treated 18 AML patients using an anti-CD33 antibody (HuM195) labeled with \textsuperscript{213}Bi, with 14 patients achieving a significant reduction in bone marrow leukemic blast cells. Subsequently the use of chemotherapy up-front to reduce the leukemic burden prior to administration of \textsuperscript{213}Bi-HuM195 in an attempt to eliminate minimal residual disease showed encouraging improvements.\textsuperscript{3,34} Given the short half-life of \textsuperscript{213}Bi (t\textsubscript{1/2} = 46 minutes), which can impact the logistics and limit widespread use of this approach, \textsuperscript{225}Ac (t\textsubscript{1/2} = 10 days) has more recently been investigated as an alternative radionuclide for anti-CD33 (HuM195) RIT.\textsuperscript{35}

Prior alpha-emitter based RIT studies using \textsuperscript{213}Bi to treat lymphomas in pre-clinical models have been associated with nephrotoxicity, likely from daughter radionuclides that have accumulated in the kidneys. Blocking agents such as the heavy metal chelating reagent DMPS have significantly lowered the \textsuperscript{213}Bi absorbed dose to kidneys, where kidney doses have been decreased by as much as 60%, which may help render mAb labeled with alpha-emitting radionuclides useful for future clinical applications.\textsuperscript{12,21,29} In fact, even \textsuperscript{211}At-labeled mAb-B10 using a different B10 conjugation chemistry resulted in two cases of canine renal toxicity in a dose escalation study.\textsuperscript{22} In the current study \textsuperscript{211}At was linked to the anti-CD45 mAb via a lysine
amine reaction with the isothiocyanate-closo-decaborate(2-) reagent (B10-NCS), and this $^{211}$At-labeling method did not result in high absorbed doses to the kidney, and did not produce renal dysfunction even without the administration of renal protective agents. The renal safety profile of $^{211}$At may be advantageous compared to other alpha-emitters that may decay into short-lived nephrotoxic daughter alpha-emitters such as $^{221}$Fr, $^{217}$At, and $^{213}$Bi.$^{15}$

In summary, our studies have demonstrated that the alpha-emitter $^{211}$At when used with anti-CD45 RIT can be successfully targeted to leukemia-bearing organs, with associated improvements in OS when combined with BMT in a disseminated model of murine leukemia. Dosimetry estimates showed maximum radiation delivery to target tissues (spleen and bone marrow), with minimal accumulation in the kidneys and consequently minimal renal toxicity. In view of the myeloablative potential of $^{211}$At, $^{211}$At-anti-CD45 RIT may serve as a beneficial adjunct to HCT in the treatment of AML and should be considered for clinical trials in settings of minimal residual disease.
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AUTHORSHIP

Contribution: J.J.O., T.B., A.K., E.R.B., D.S.W., M.D.H., D.R.F., D.J.G., A.K.G., O.W.P., and J.M.P. contributed to the conception, design, analysis and interpretation of the research; J.J.O. and J.M.P. wrote the manuscript; J.J.O., A.K., S.L.F, M.D.H., performed research, collected and analyzed data; D.K.H. contributed vital reagents. All authors approved the final version of the manuscript.

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REFERENCES


Table 1:
Absorbed Dose per 0.19 µCi $^{211}$At Activity Injected
(to Dose 5 cGy to Liver)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Absorbed Dose (cGy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>18</td>
</tr>
<tr>
<td>Marrow</td>
<td>1.7</td>
</tr>
<tr>
<td>Blood</td>
<td>7.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.9</td>
</tr>
<tr>
<td>Liver</td>
<td>5.0</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.96</td>
</tr>
<tr>
<td>Sm. Int.</td>
<td>1.8</td>
</tr>
<tr>
<td>Colon</td>
<td>2.4</td>
</tr>
<tr>
<td>Lung</td>
<td>4.1</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Figure 1. Biodistribution of radioactivity in AML-bearing SJL/J mice. Mice received 0.67 nmol of 30F11-B10 conjugate trace-labeled with \[^{125}\text{I}\] or \[^{211}\text{At}\], then euthanized at stated timepoints. Their organs were harvested and radioactivity measured from A) \[^{125}\text{I}\] at 8 (■) and 24 hours (□) or from B) \[^{211}\text{At}\] at 1 (□), 3 ( ■), 7 (■), and 24 ( □) hours after injection of radioactivity. Counts were decay-corrected and expressed as a percentage of the injected dose per gram of tissue (% ID/g).

Figure 2. Alpha-camera imaging. A) Cryosections (10 µm) of spleen imaged 3 hours after i.v.-injection of \[^{211}\text{At}\]-B10-30F11. B) The third section from left in panel A was used for quantitative analysis of the activity distribution of \[^{211}\text{At}\] within the spleen. Each pixel intensity value (activity) was normalized to the mean pixel intensity of the whole spleen. The normalized data was divided in ten bins (0 - max) and plotted as a color-coded histogram (1.0 = mean activity of the whole section). C) Cryosections (10 µm) of femur imaged 3 hours after i.v.-injection of \[^{211}\text{At}\]-B10-30F11. The large ROI (yellow) outline the areas used for quantification of %ID/g in bone marrow by alpha-camera imaging. The small ROI (magenta) in the middle femur were used to compare the activity uptake (% ID/g) along the bone marrow cavity. D) Quantitative histogram of \[^{211}\text{At}\]-distribution of the middle femur-section in panel C.

Figure 3. Anti-CD45 RIT of AML-bearing SJL/J mice with and without BMT. A) Mice were injected with SJL leukemia, followed 2 days later by 0.67 nmol of 30F11-B10 labeled with 12 (●) or 24 (■) µCi \[^{211}\text{At}\]. Control mice received 24 µCi \[^{211}\text{At}\]-B10-rat IgG (◊) or no treatment (O). Mice did not receive BMT in this study, and (†) denotes individual murine deaths due to toxicity. B) Mice were injected with SJL leukemia and 2 days later with 0.67 nmol of \[^{211}\text{At}\]-B10-30F11 with 12 (●), 20 (♦) or 24 (■) µCi \[^{211}\text{At}\]. Control mice received 24 µCi \[^{211}\text{At}\]-B10-rat IgG (◊) or no treatment (O). On day +2, mice were given 15x10^6 bone marrow cells via tail vein injection.

Figure 4. Hematologic toxicity using \[^{211}\text{At}\]-B10-30F11 and BMT. Non-leukemic SJL/J mice were injected with 0.67 nmol of 30F11-B10 labeled with either 12 (●) or 24 (♦) µCi \[^{211}\text{At}\]. Control mice were given 24 µCi \[^{211}\text{At}\]-B10-rat IgG (◊) or BMT alone (O). Mice were bled at 1, 2, 3, 4, 6, 8, and 26 weeks to assay for: A) WBC; B) hemoglobin; and C) platelets. Dashed lines indicate the range of normal control values.

Figure 5. Non-hematologic toxicity using \[^{211}\text{At}\]-B10-30F11 and BMT. Non-leukemic SJL/J mice were injected with 0.67 nmol of 30F11-B10 labeled with either 12 (●) or 24 (♦) µCi \[^{211}\text{At}\]. Control mice were given 24 µCi \[^{211}\text{At}\]-B10-rat IgG (◊) or BMT alone (O). Mice were bled at 1, 2, 3, 4, 6, 8, and 26 weeks to assay for: A) ALP; B) AST; C) ALT; D) BUN; and E) Cr. Dashed lines indicate the range of normal control values.
Figure 2
Figure 3A:

- 24 μCi $^{211}$At-B10-rat IgG
- 12 μCi $^{211}$At-B10-30F11
- 24 μCi $^{211}$At-B10-30F11
- Untreated

% Survival vs. Days After Therapy
Figure 4A

Figure 4B
Figure 4C

Platelets vs. Days After Therapy
Figure 5A

Days After Therapy

IU/L

ALP

Figure 5B

Days After Therapy

IU/L

AST
Figure 5C

Figure 5D
Figure 5E

E

Cr

Days After Therapy

mg/dL

0.3
0.6
0.9
1.2
1.5
1.8
2.1

0 50 100 150 200
Anti-CD45 radioimmunotherapy using $^{211}$At with bone marrow transplantation prolongs survival in a disseminated murine leukemia model