Regular Article

LYMPHOID NEOPLASIA

The α-emitter astatine-211 targeted to CD38 can eradicate multiple myeloma in a disseminated disease model

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KEY POINTS

- [211]At targeted to CD38 eliminates MM cell clones in murine models of low-burden disease.

- [211]At deposits ≥500 times more energy than β-emitters and provides a mechanism of uniform cell kill unique among MM therapeutics.

Minimal residual disease (MRD) has become an increasingly prevalent and important entity in multiple myeloma (MM). Despite deepening responses to frontline therapy, roughly 75% of MM patients never become MRD-negative to ≤10^{-5}, which is concerning because MRD-negative status predicts significantly longer survival. MM is highly heterogeneous, and MRD persistence may reflect survival of isolated single cells and small clusters of treatment-resistant subclones. Virtually all MM clones are exquisitely sensitive to radiation, and the α-emitter astatine-211 ([211]At) deposits prodigious energy within 3 cell diameters, which is ideal for eliminating MRD if effectively targeted. CD38 is a proven MM target, and we conjugated [211]At to an anti-CD38 monoclonal antibody to create an [211]At-CD38 therapy. When examined in a bulky xenograft model of MM, single-dose [211]At-CD38 at 15 to 45 μCi at least doubled median survival of mice relative to untreated controls ($P < .003$), but no mice achieved complete remission and all died within 75 days. In contrast, in a disseminated disease model designed to reflect low-burden MRD, 3 studies demonstrated that single-dose [211]At-CD38 at 24 to 45 μCi produced sustained remission and long-term survival (>150 days) for 50% to 80% of mice, where all untreated mice died in 20 to 55 days ($P < .0001$). Treatment toxicities were transient and minimal. These data suggest that [211]At-CD38 offers the potential to eliminate residual MM cell clones in low-disease-burden settings, including MRD. We are optimistic that, in a planned clinical trial, addition of [211]At-CD38 to an autologous stem cell transplant (ASCT) conditioning regimen may improve ASCT outcomes for MM patients. (*Blood*. 2019;134(15):1247-1256)

Introduction

Potent treatments for multiple myeloma (MM) greatly reduce initial disease burden and result in a complete response for a significant subset of patients.[1,2] However, most patients relapse as a consequence of minimal residual disease (MRD) defined by occult foci of treatment-insensitive tumor cells.[3-5] Therapy capable of entirely eliminating malignant cell clones from within low-disease-burden settings, including MRD, has remained elusive. Malignant cell escape following treatment with pathway-specific targeting agents may be explained by heterogeneity of MM within afflicted individuals.[5] In contrast, radionuclide therapy is agnostic to disease heterogeneity as virtually all MM cells, including clones with high-risk cytogenetic features, are exquisitely sensitive to radiation.[6-9] The α-emitting radionuclide astatine-211 ([211]At) is an optimal candidate for eradicating low-level disease and has been characterized as "the most promising α particle" for therapy based on its decay characteristics.[10] By depositing a large dose of radiation (6.8 MeV) concentrated within a few cell diameters (50-90 μm),

[211]At produces a prodigious linear energy transfer (LET) and then quickly decays (7.2-hour half-life) without producing problematic long-lived daughter nuclides. High LET cytotoxicity results from double-strand DNA breaks that overwhelm cellular repair mechanisms.[11,12] Studies of other hematopoietic cell types suggest that this high LET cell-killing mechanism will remain effective irrespective of a potentially quiescent state and the hypoxic bone marrow environments in which MRD MM is thought to survive.[12-15] We therefore hypothesized that [211]At targeted to MM cells would be uniquely capable of eliminating isolated cells and small tumor clusters with minimal damage to surrounding tissues.

We selected an anti-human CD38 monoclonal antibody (mAb) as the targeting agent. The CD38 antigen is expressed on malignant plasma cells regardless of mutational status, and anti-CD38 mAbs constitute a proven targeted therapy for MM, but resistance mechanisms prevent unmodified CD38 mAbs from reliably eradicating disease.[16-19] Using CD38 mAbs as targeting
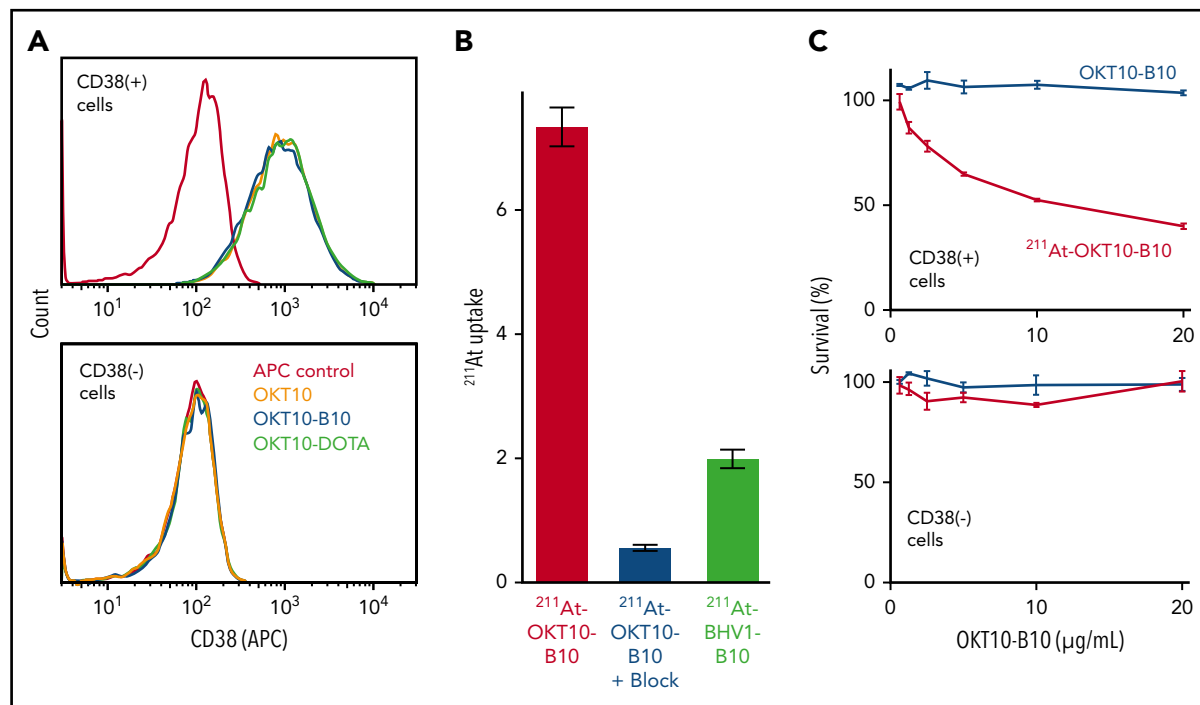
Figure 1. [211]At-CD38 selectively binds and kills CD38^{+} MM cells. (A) CD38^{+} or CD38^{−} cell lines were incubated with either anti-CD38 OKT10 mAb, or OKT10 conjugated with amine-reactive B10, or OKT10 conjugated with yttrium chelator DOTA. Cells were then washed twice, incubated with anti-mouse IgG Fc-APC, washed twice, and analyzed by flow cytometry. (B) CD38^{+} cells were preincubated with buffer or CD38 mAb, then [211]At-OKT10-B10 or isotype control [211]At-BHV1-B10 was added and cells incubated, washed twice, cell-associated radioactivity measured in a γ counter, and [211]At uptake calculated as a percentage of applied [211]At. N = 4 replicates per treatment; error bars = 1 standard error of the mean (SEM). (C) CD38^{+} or CD38^{−} cells were incubated in media (control) or serial dilutions of OKT10-B10 or [211]At-OKT10-B10 at 37°C for 60 hours. Cytotoxicity was then assessed using CellTiter-Glo. N = 3 replicates per treatment; error bars = 1 standard deviation (SD).

agents for β-emitter radionuclides can eliminate disease in preclinical MM models,[20,21] yet these studies also suggest that current β-therapies using yttrium-90 (^{90}Y) and iodine-131 (^{131}I) are optimally effective for larger tumor clusters than typified by MRD.[22] In clinical settings, directly targeted β-therapies have been associated with hematologic toxicity that in some cases may prevent dose escalation to levels that eliminate MRD.[23,24] Possible contributors to toxicity include a relatively longer decay half-life (eg, ^{131}I, 8 days)[23] or longer path length (eg, ^{90}Y, 5 mm).[24] Thus, the favorable physical characteristics of α-emitting radionuclides, and new opportunities to harness their potential,[25,26] provide rationale for our approach.

To create a therapeutic agent, we conjugated the anti-CD38 mAb OKT10 to the novel amine-reactive agent B10-NCS,[25] which is labeled with [211]At on the day of treatment. We hypothesized that the resulting [211]At-CD38 therapy would effectively treat MM in preclinical models. We tested the specific predictions that [211]At-CD38 would selectively bind and kill CD38^{+} MM cells in vitro, and, in murine xenograft models of bulky vs low-burden disease designed to recapitulate MRD, would optimally eliminate the latter.

Methods

Cell culture

The human MM cell lines NCI-H929, U266B1, and RPMI 8226 were obtained from the American Type Culture Collection (ATCC; Manassas, VA), and OPM-2 and MOLP-8 from Deutsche

Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). Cell lines were authenticated by DNA profiling (Fred Hutchinson Cancer Research Center [FHCRC] Research Cell Bank, Seattle, WA), tested for mycoplasma, and maintained in log-phase growth in RPMI 1640 media supplemented with 10% to 20% fetal bovine serum, 50 U/mL penicillin G, and 50 μg/mL streptomycin sulfate for no more than 6 weeks after thawing. Luciferase ([Luc]) cell lines were generated by retroviral transduction to stably express firefly luciferase for in vivo bioluminescent imaging (BLI).

Reagents and radiolabeling

The OKT10 hybridoma cell line expressing the murine immunoglobulin G1 (IgG1) anti-human CD38 mAb, and the bovine herpes virus-1 (BHV1) hybridoma (IgG1 isotype control), were purchased from ATCC. OKT10 and BHV1 antibodies (Abs) were produced from the hybridomas using a hollow-fiber bioreactor system in the mAb production facility at FHCRC. The amine-reactive bifunctional labeling reagent B10-NCS was prepared as previously reported[25] and conjugated to the Abs using the method of Wilbur.[25] Produced on a Scanditronix MC-50 cyclotron at the University of Washington, [211]At was used to label OKT10-B10 and BHV1-B10 as previously described.[27,28]

Cell binding and cytotoxicity of radiolabeled anti-CD38 OKT10-B10

To assess cell binding of anti-CD38 (OKT10) mAb constructs, 5 × 10^{5} MM cells per well of CD38^{+} NCI-H929 or CD38^{−} U266B1 were incubated in 50 μL of either OKT10, OKT10-DOTA, or OKT10-B10 at 0.2 mg/mL for 30 minutes at 4°C, washed twice,
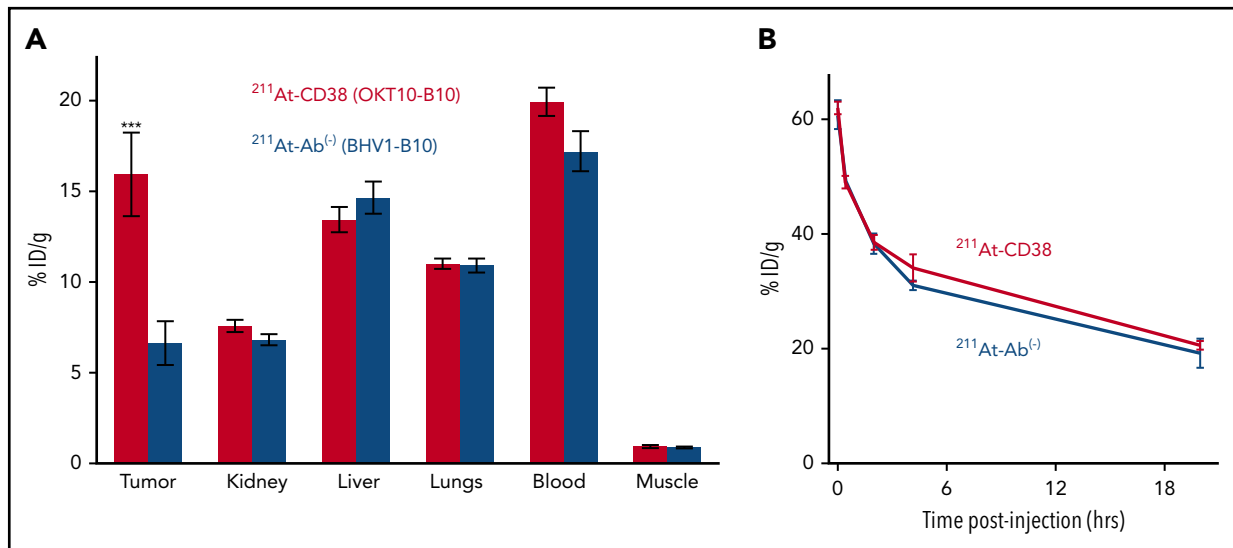
Figure 2. ²¹¹At-CD38 biodistribution and pharmacokinetics. Mice bearing subcutaneous MM xenografts were IV injected with ²¹¹At-CD38 or ²¹¹At-Ab⁻, and ROB samples taken at 1, 25, 120, and 250 minutes posttherapy. At 24 hours posttherapy, mice were euthanized and blood, tumor, and normal tissues harvested, weighed, and γ counted, and then decay-corrected percent-injected dose of ²¹¹At absorbed per gram of tissue calculated. (A) Biodistribution of ²¹¹At-CD38. Tumors targeted by ²¹¹At-CD38 absorbed 2.4 times more activity than tumors exposed to ²¹¹At-Ab⁻ ($P = .007$), and ²¹¹At-CD38 created favorable tumor-to-normal tissue ratios of absorbed activity for kidney, liver, and lungs. N = 5 mice per group; error bars = 1 SEM. (B) Blood clearance of ²¹¹At-CD38. Blood radioactivity of ²¹¹At-CD38 treated mice reduced from 60% ID per gram at 1 minute posttherapy to 38% at 2 hours and 20% at 24 hours. N = 3 mice per time point per treatment; error bars = 1 SEM.

resuspended in 50 μL of 1:100 anti-mouse IgG (F(ab')₂)-allo-phycocyanin (APC; Jackson ImmunoResearch, West Grove, PA), incubated 30 minutes at 4°C, washed twice, and analyzed using flow cytometry. To assess cell binding of radiolabeled anti-CD38 mAb, 3.3 × 10⁶ MM cells per well of CD38⁺ OPM-2 were first incubated in 50 μL of OKT10-B10 (0.2 mg/mL) or media (RPMI 1649, 1% fetal bovine serum, 0.1% sodium azide) for 45 minutes at 4°C. Then, 50 μL of 4 μg/mL ²¹¹At-OKT10-B10 or ²¹¹At-BHV1-B10 was added and cells were incubated for 60 minutes at 4°C, washed twice, and cell-associated radioactivity measured in a γ counter. To assess cytotoxicity, 2 × 10⁴ MM cells per well of CD38⁺ NCI-H929 or CD38⁻ U266B1 were incubated at 37°C in 100 μL of cell media (control) or media plus serial dilutions of unlabeled OKT10-B10 (maximum concentration 20 μg/mL), or ²¹¹At-OKT10-B10 (maximum concentration 20 μg/mL labeled with 250 μCi ²¹¹At). After 60 hours, cell viability was assessed using CellTiter-Glo (Promega, Madison, WI).

Mouse models

Female and male NOD.Cg-*Rag1^tm1Mom* *Il2rg^tm1Wjl*/SzJ (NRG) mice (FHCRC colony) were maintained under standard protocols approved by the FHCRC Institutional Animal Care and Use Committee (IACUC). To model bulky disease, MM xenografts were generated by subcutaneous flank injection of 1 × 10⁷ OPM-2^Luc or NCI-H929 cells 7 to 9 days prior to treatment to produce tumors of 25 mm³ to 75 mm³ at treatment. A low-disease-burden model was developed through optimization of protocols identifying minimal IV-injected tumor cell doses capable of producing 100% engraftment rates and similar low disease burdens within, and between, cell line models at time of treatment (supplemental Figure 1, available on the *Blood* Web site). In the final models, mice received 2.75 Gy external beam cesium-137 (¹³⁷Cs; JL Shepard Mark I irradiator) 6 days prior to treatment, followed 1 day later by IV injection of 1.2 × 10⁵ MOLP-8^Luc, 2.5 × 10⁵ OPM-2^Luc, or 5 × 10⁵ NCI-H929^Luc cells (5 days prior to treatment). In all studies, mice were randomized

into groups with equivalent body weights, tumor sizes, and sex ratios.

Biodistribution studies

NRG mice bearing flank MM xenografts received IV injections of 15 μg of mAb radiolabeled with 15 μCi ²¹¹At, as either ²¹¹At-OKT10-B10 (²¹¹At-CD38), or IgG1 (isotype-matched) control ²¹¹At-BHV1-B10 (²¹¹At-Ab⁻) (n = 5 per group). Retro-orbital blood (ROB) samples were taken from 3 mice per group at 1, 25, 120, and 250 minutes posttherapy. At 24 hours posttherapy, all mice were euthanized, and blood, tumor, and relevant normal tissues (lung, liver, kidneys, spleen, stomach, small and large intestines, muscle, and tail) were harvested, weighed, and ²¹¹At activity was measured. Decay-corrected percentage injected dose (ID) of ²¹¹At absorbed per gram of tissue (percent ID per gram), and tumor-to-normal tissue ratios of absorbed radioactivity were calculated.

Therapy studies

Female and male NRG mice bearing either subcutaneous flank xenografts or disseminated disease received IV injections of 15 μg of mAb radiolabeled with 7.5 to 45 μCi as either ²¹¹At-CD38 or isotype control ²¹¹At-Ab⁻ (n = 5-10 per group). All mice received syngeneic donor bone marrow transplant 3 days posttherapy, potentially required to reverse myelotoxicity at high-dose levels.[29] In subcutaneous models, tumor dimensions were monitored 3 times weekly and, in disseminated models, disease progression was monitored weekly via BLI. Body weight was monitored 3 times weekly and mice were euthanized if they experienced >30% weight loss, hind-limb paralysis, or exceeded tumor volume limits per IACUC requirements. In the study using the 2 highest ²¹¹At doses (30 and 45 μCi, OPM-2^Luc disseminated model), ROB samples were taken at intervals to assess hematopoietic, renal, and hepatic function. ROB sampling was limited to 1 study in consideration of animal safety and prior observations of minimal ²¹¹At-therapy–related toxicities.[28]

Statistical analyses

In biodistribution studies, effects of [211]At-CD38 vs [211]At-Ab[-] on tumor and normal tissue [211]At absorption were examined by the Student *t* test, and relative [211]At absorptions of tumor vs normal tissues were examined by the paired Student *t* test. In therapy studies, treatment effects on mouse survival were determined by log-rank (single independent variable) or proportional hazards (multivariate) analyses of Kaplan-Meier survival functions. Analyses were performed using JMP 14 (SAS Institute, Cary, NC) and GraphPad Prism 7.03 (GraphPad Software, La Jolla, CA).

Results

In vitro cell binding and cytotoxicity

We confirmed the antigen specificity of our anti-CD38 constructs with flow cytometry, showing that CD38^{+} MM cells bound OKT10, OKT10-B10, and OKT10-DOTA with an equally high affinity, whereas CD38^{-} MM cells bound none of these constructs (Figure 1A). To confirm binding specificity of radiolabeled mAbs, we assayed [211]At levels in MM cell lines after incubation with [211]At-CD38 mAb (OKT10-B10) or [211]At-nontargeting, isotype control mAb (BHV1-B10). The [211]At level (percentage uptake) was significantly higher after CD38 vs control mAb, and over 92% of the [211]At uptake from [211]At-CD38 was blocked by preincubating cells with unlabeled CD38 mAb (Figure 1B). Low-level [211]At uptake with the control mAb reflects nonspecific binding as seen in previous [211]At-mAb cell-binding studies.[28] We assayed selective cytotoxicity of [211]At-CD38 mAb by comparing viability of CD38^{+} and CD38^{-} cell lines following incubation with either [211]At-CD38 mAb or unlabeled CD38 mAb (OKT10-B10) and demonstrated that [211]At-CD38 selectively eliminates CD38^{+} cells, but not CD38^{-} cells, and that unlabeled CD38 mAb did not affect cell viability (Figure 1C).

[211]At-CD38 biodistribution and pharmacokinetics

Biodistribution experiments demonstrated the ability of [211]At-CD38 to selectively target MM xenografts in NRG mice. Mice bearing subcutaneous tumors were IV injected with [211]At-CD38 or [211]At-Ab[-] and tissues harvested 24 hours later, the time of maximum [211]At retention.[28] Targeted [211]At-CD38 delivered more radiation to tumor than did isotype control [211]At-Ab[-] (15.9% vs 6.6%, respectively, of ID per gram; *P* = .007; Figure 2A), and created favorable tumor:normal organ ratios of absorbed activity for kidney, liver, and lungs. As expected, blood retained a similar amount of activity as targeted tumor, and consistent with previous observations,[28] blood-rich nontargeted organs retained similar activity following [211]At-CD38 or [211]At-Ab[-]. To examine pharmacokinetics of [211]At-mAbs, we sampled ROB at intervals from 1 minute to 24 hours posttherapy injection. Blood activity, which was 60% ID per gram at 1 minute, reduced to 38% at 2 hours and 20% at 24 hours, and this clearance profile was nearly identical for targeted and isotype control treatments (Figure 2B).

[211]At-CD38 therapy in subcutaneous MM models

Subcutaneous xenograft studies were designed to recapitulate bulky disease, and, in 2 therapy studies of these models, [211]At-CD38 significantly slowed disease progression and doubled median survival of mice. In the first study, mice bearing NCI-H929 flank tumors were treated with [211]At-CD38 at 7.5 or 15 μCi. As anticipated,[28] in a bulky tumor model, [211]At therapy was not capable of eliminating disease, and the median survival of
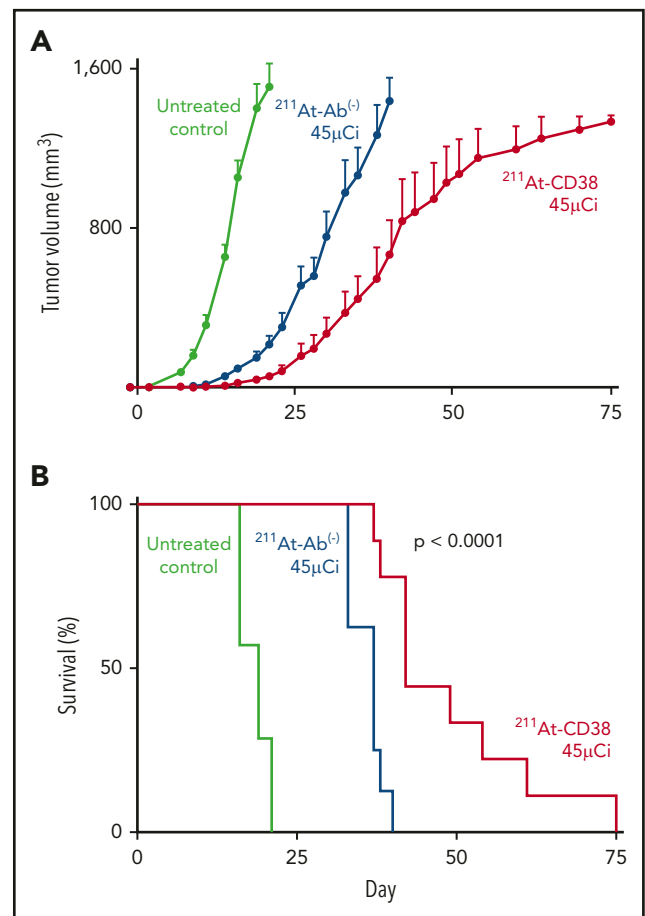

Figure 3. [211]At-CD38 therapy in subcutaneous MM models. Mice bearing subcutaneous OPM-2[Luc] xenografts were treated on day 0 with 30 or 45 μCi [211]At-CD38 or [211]At-Ab[-] (isotype control); n = 8 to 9 mice per group. Tumor dimensions were monitored 3 times weekly and mice euthanized when tumor volumes met IACUC requirements. (A) Tumor progression for untreated controls and 45-μCi groups; error bars = 1 SEM. (B) Kaplan-Meier survival curves. The 45-μCi [211]At-CD38 treatment increased median survival relative to untreated (*P* < .0001) and to 45-μCi [211]At-Ab[-] treated mice (*P* = .0005). The 30-μCi groups are not shown for visual clarity; data are described in "Results."

untreated mice (17 days) and [211]At-CD38 7.5-μCi mice (21 days) was not different (*P* > .1, Kaplan-Meier log-rank test). [211]At-CD38 15-μCi treatment extended median survival to 34 days (greater than other groups; *P* < .01), but all mice eventually succumbed to disease (data not shown). In the second study, mice bearing OPM-2[Luc] flank tumors were treated with [211]At-CD38 at 30 and 45 μCi. α-emitter–labeled anti-CD38 mAbs again slowed tumor growth (Figure 3A), and extended median survival beyond untreated mice (Figure 3B; untreated [19 days] vs [211]At-CD38 30-μCi [40 days] or vs [211]At-CD38 45-μCi [42 days]; *P* < .0001), and beyond [211]At-Ab[-] 45-μCi–treated mice (median survival, 37 days; *P* = .0005; Kaplan-Meier log-rank tests). However, no long-term survival was observed (Figure 3B).

[211]At-CD38 therapy in disseminated MM models

Disseminated xenograft models were designed to recapitulate the isolated tumor cells and small cell clusters associated with MRD and exploit the salient features of α-emitter therapy. In 3 therapy studies of these models, single-dose [211]At-CD38 at 24 to 45 μCi produced sustained remissions that resulted in long-term survival (>150 days) for 50% to 80% of mice.
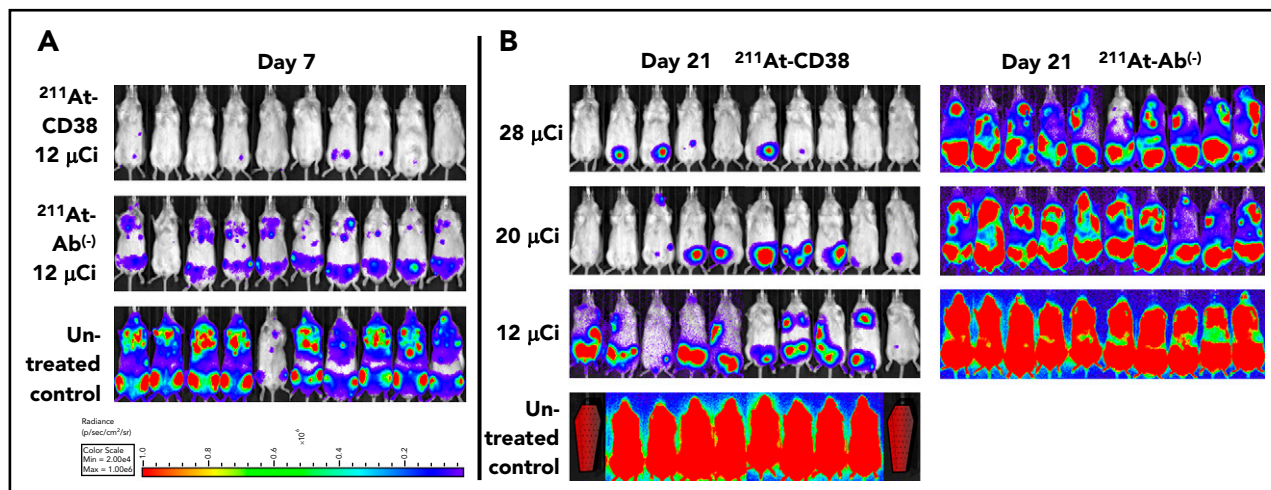
Figure 4. ^{211}At-CD38 therapy in a disseminated MOLP-8^{Luc} model. Mice bearing disseminated MOLP-8^{Luc} xenografts were treated on day 0 with ^{211}At-CD38 or ^{211}At-Ab$^-$ at 12, 20, or 28 μCi; n = 10 mice per group. Disease progression was monitored via weekly BLI and thrice weekly observations of mouse weight, condition, and mobility. Mice were euthanized when they experienced hind-limb paralysis or met IACUC weight loss or condition requirements. (A) BLI of untreated control and 12-μCi treatment groups at 7 days posttherapy. (B) BLI of all groups at 21 days posttherapy. Red coffins indicate deceased mice. BLI scale identical to panel A.

In MOLP-8^{Luc} models, mice bearing disseminated disease were treated with ^{211}At-CD38 or ^{211}At-Ab$^-$ at 12, 20, or 28 μCi. At 7 days posttreatment, all mice treated at even the lowest dose level showed significant disease regression when compared with controls (Figure 4A). At 21 days, disease had recurred in all ^{211}At-CD38 12-μCi–treated mice, but 20% of ^{211}At-CD38 20-μCi and 50% of ^{211}At-CD38 28-μCi mice lacked detectable tumors by BLI (Figure 4B), and mice lacking visible tumors at day 21 all survived over 100 days. In contrast, all untreated and ^{211}At-Ab$^-$ groups showed extensive disease progression at day 21 (Figure 4B) and untreated mice had a median survival of only 23 days (Figure 5). ^{211}At-CD38 treatments extended life at least threefold, providing a median survival of 77 and 95 days for ^{211}At-CD38 12- and 20-μCi mice, respectively, and 90% survival of ^{211}At-CD38 28-μCi mice through day 120. Accordingly, ^{211}At-CD38 treatment strongly predicted improved survival relative to untreated controls ($P < .00001$) and to ^{211}At-Ab$^-$ treated mice ($P < .00001$) in a dose-dependent manner ($P < .00001$; Kaplan-Meier proportional hazards tests; Figure 5).

We explored a higher ^{211}At dose in our study of OPM-2^{Luc}, where mice bearing disseminated disease received ^{211}At-CD38 or ^{211}At-Ab$^-$ at 15, 30, or 45 μCi. For this cell line, ^{211}At-CD38 15-μCi eliminated detectable disease in 80% of mice at day 21, whereas no untreated mice and no mice receiving ^{211}At-Ab$^-$ 15-μCi were disease-free (Figure 6A). At day 41, only 20% of ^{211}At-CD38 15-μCi mice demonstrated an absence of MM, but 70% of ^{211}At-CD38 30-μCi and 100% of ^{211}At-CD38 45-μCi mice had no detectable disease by imaging (Figure 6B). In contrast, all untreated, all ^{211}At-Ab$^-$ 15-μCi and 30-μCi mice, and 33% of ^{211}At-Ab$^-$ 45-μCi mice displayed measurable disease at this time point (Figure 6B). These patterns persisted through the end of the study: all untreated mice died of progressive disease by day 47 (Figure 5B), whereas as of day 150, disease had recurred in only 1 of 9 ^{211}At-CD38 45-μCi mice. Two ^{211}At-CD38 45-μCi mice died spontaneously during the study without detectable cause (no apparent disease or toxicity, see "Toxicity"), and as of day 150, survival did not differ between the CD38-targeted and isotype-matched treatment

group receiving 45 μCi ($P = .87$; Kaplan-Meier log-rank test; Figure 5B). Nonetheless, ^{211}At-CD38 efficacy significantly surpassed ^{211}At-Ab$^-$ overall. Among 30-μCi groups, 60% of ^{211}At-CD38 mice remained alive at day 150 whereas all ^{211}At-Ab$^-$ mice were dead from disease by day 85 ($P = .0007$), and, in 15-μCi groups, ^{211}At-CD38 cured 10% of mice whereas all ^{211}At-Ab$^-$ mice died by day 57 ($P < .0001$; Kaplan-Meier log-rank tests; Figure 5B). Analyzed across all 3 ^{211}At treatment levels, ^{211}At-CD38 strongly outperformed ^{211}At-Ab$^-$ ($P = .00002$) and extended survival in a dose-dependent manner ($P < .00001$; Kaplan-Meier proportional hazards test).

In the studies of disseminated disease discussed above, ^{211}At-CD38 doses above 20 μCi appeared sufficient to produce long-term survival for the majority of mice. Thus, with the goal of confirming both therapeutic efficacy and the dose-response relationship, mice in disseminated NCI-H929^{Luc} models received ^{211}At-CD38 or ^{211}At-Ab$^-$ at 8, 18, or 24 μCi. Consistent with our other studies, ^{211}At-CD38 treatment strongly benefitted mice across treatment levels, increasing survival relative to untreated controls ($P < .0001$) and to ^{211}At-Ab$^-$ treatments ($P < .0006$), and these benefits were dose-dependent as higher ^{211}At dosing improved survival ($P < .002$; Kaplan-Meier proportional hazards tests; Figure 5C). At day 28 posttreatment, imaging showed progressive disease in 100% of untreated mice, 100% of ^{211}At-Ab$^-$ 8-μCi mice and 90% of ^{211}At-Ab$^-$ 24-μCi mice, compared with 50% of ^{211}At-CD38 8-μCi and 30% of ^{211}At-CD38 24-μCi mice (not shown). At the end of the study (day 150), all untreated, all ^{211}At-Ab$^-$ 8-μCi mice, and 70% of ^{211}At-Ab$^-$ 24-μCi mice had died due to progressive disease, whereas 30% of ^{211}At-CD38 8-μCi mice and 80% of ^{211}At-CD38 24-μCi mice remained alive (Figure 5C).

Toxicity

The impact of therapy on liver and kidney function (Table 1) and mouse body weight (supplemental Figure 1) was mild, no clinical evidence of toxicity was observed, and all values returned to within normal limits by 32 days following treatment. Hematologic toxicity was limited to platelet counts where mice receiving
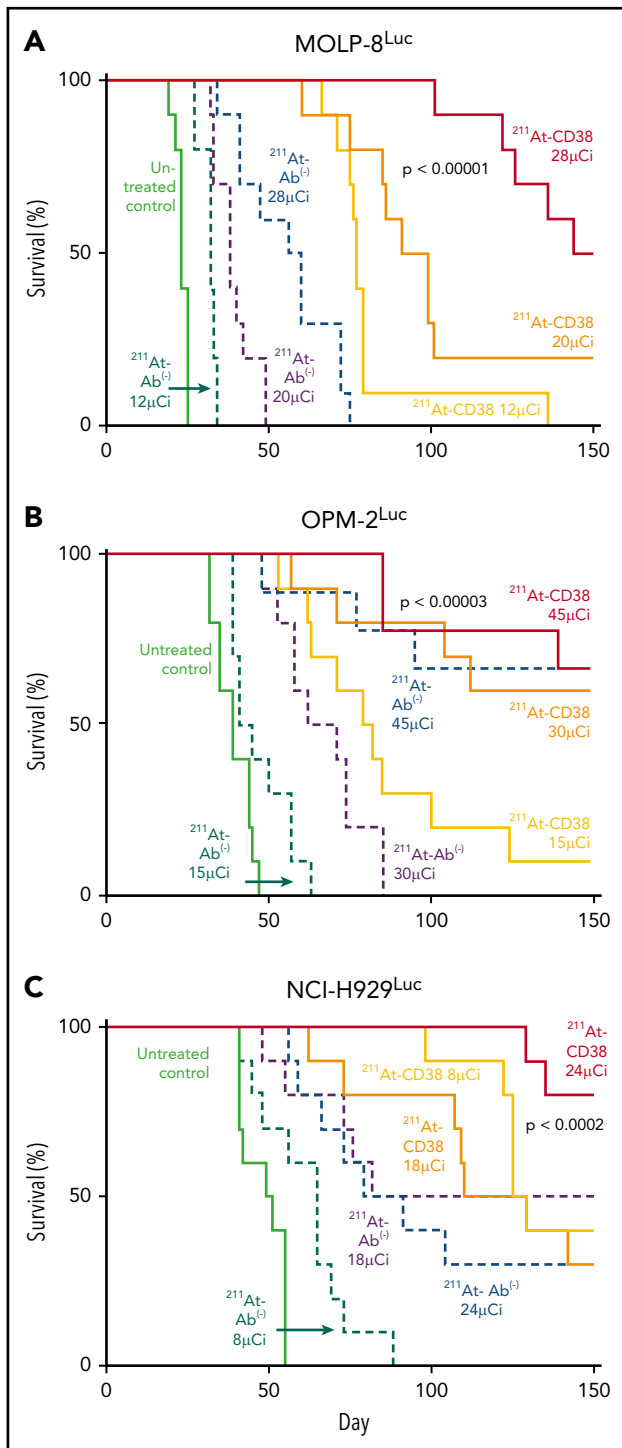
Figure 5. Kaplan-Meier survival curves for disseminated models. Mice bearing disseminated MM xenografts were treated on day 0 with ^{211}At-CD38 or ^{211}At-Ab$^-$; n = 9 to 10 mice per group. Disease progression was monitored via weekly BLI and thrice weekly observations of mouse weight, condition, and mobility. Mice were euthanized when they experienced hind-limb paralysis or met IACUC weight loss or condition requirements. Statistics report Kaplan-Meier proportional hazards tests. (A) MOLP-8^{Luc} model, treated at 12, 20, and 28 μCi. Across treatment levels, ^{211}At-CD38 therapy strongly benefitted survival relative to untreated controls ($P < .00001$) and to ^{211}At-Ab$^-$ groups ($P < .00001$) in a dose-dependent manner ($P < .00001$). (B) OPM-2^{Luc} model, treated at 15, 30, and 45 μCi. ^{211}At-CD38 therapy strongly improved survival across groups, relative to untreated controls ($P < .00001$) and to ^{211}At-Ab$^-$ treatments ($P = .00002$) in a dose-dependent manner ($P < .00001$). Additional statistics in "Results." (C) NCI-H929^{Luc} model, treated at 8, 18, or 24 μCi. Mice treated with ^{211}At-

the highest (45-μCi) dose of ^{211}At-CD38 experienced a 54% reduction relative to untreated control values at day 14, however, no bleeding events were observed and platelet counts recovered to 92% of untreated values by day 32 (Table 1). In the therapy study of disseminated OPM-2^{Luc}, 2 mice in the ^{211}At-CD38 45-μCi group died without detectable MM or toxicity, 1 at 85 days and 1 at 139 days posttreatment (Figure 5B). We have observed similar rates of spontaneous mortality in untreated NRG mice in our colony,[30] suggesting that late deaths in study mice are likely not therapy related.

Discussion

MM that is detectable only as MRD has become prevalent due to deeper therapeutic responses and more sensitive methods of MRD detection.[31-33] Fifty percent to 60% of MM patients now achieve a complete response to frontline therapy,[31,34-36] but the majority never achieve MRD-negativity to $\leq 10^{-5}$; for example, 75% of patients treated with bortezomib, lenalidomide, and dexamethasone remained MRD-positive by next-generation sequencing (NGS).[31] This finding is concerning as MRD elimination at any point during therapy is a major prognostic indicator for progression-free and overall survival.[31,32,37] The persistence of low-level disease likely reflects subclonal heterogeneity that allows small clusters of treatment-resistant MM cells to survive in protective bone marrow microenvironments. Disease eradication requires preventing all mechanisms of tumor cell escape. The potential removal of all clones, thus precluding selective emergence of resistant clones, may distinguish ^{211}At-CD38 therapy from other approaches to MM treatment. We have shown that targeting the CD38 antigen with the very high-energy α-emitter ^{211}At can quickly ablate MM in models designed to recapitulate low-tumor-burden settings or MRD. Single-dose treatments of ^{211}At-CD38 resulted in long-term survival of 50% to 80% of mice in 3 MRD xenograft models (Figures 4-6). Efficacy of ^{211}At-CD38 appears to be a function of disease burden and distribution, indicated by the shorter remissions seen in our subcutaneous models. Bulky tumor geometry may limit mAb penetration[28] and thus the ability of short-path-length α-emissions to reach every cell, and, indeed, even at our highest treatment dose, ^{211}At-CD38 45-μCi, all mice bearing subcutaneous MM tumors eventually succumbed to disease (Figure 3). Our data support the postulate that isolated cells and small tumor clusters readily accessible to mAbs create optimal conditions for α-emitter cell kill.[28,38-42] We therefore hypothesize that ^{211}At-CD38 therapy has the potential to improve patient outcomes by comprehensively removing residual myeloma cell clones.

In an initial test of this hypothesis, we plan to include single-dose ^{211}At-CD38 therapy in an autologous stem cell transplant (ASCT) conditioning regimen for treatment of MM, as part of a phase 1 clinical trial. ASCT is a standard of care for eligible MM patients, yet, for 2 decades, no modification to high-dose chemotherapy conditioning regimens has demonstrably improved ASCT outcomes. We predict that adding ^{211}At-CD38 to conditioning will

Figure 5 (continued) CD38 survived significantly longer than untreated control ($P < .0001$) and ^{211}At-Ab$^-$ treated mice ($P < .0006$). Treatment benefits were dose-dependent with higher ^{211}At dosing improving survival ($P < .002$).
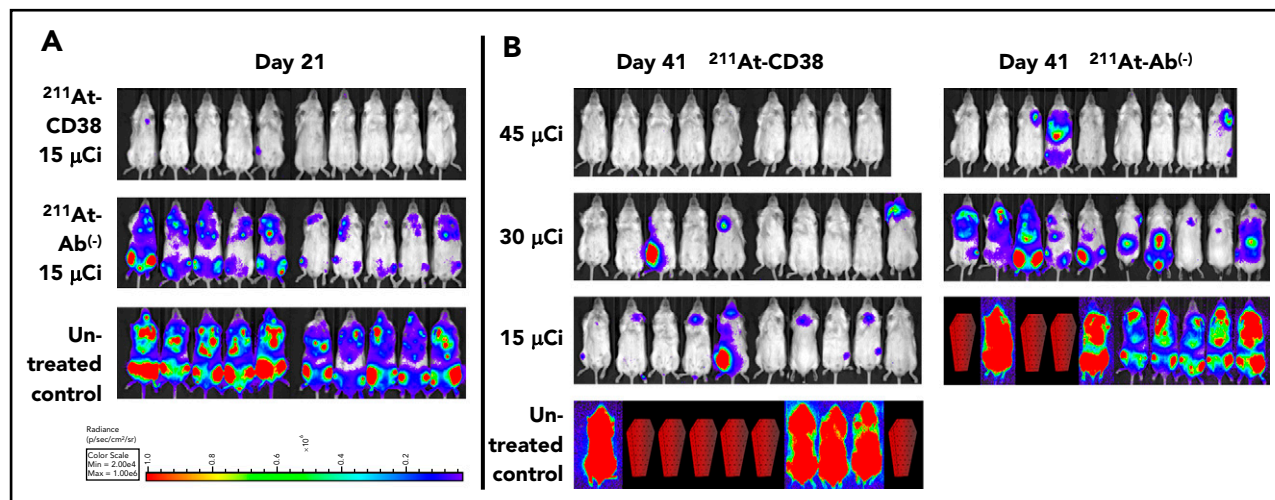
Figure 6. ^{211}At-CD38 therapy in a disseminated OPM-2 ^{Luc} model. Mice bearing disseminated OPM-2^{Luc} xenografts were treated on day 0 with ^{211}At-CD38 or ^{211}At-Ab$^-$ at 15, 30, or 45 μCi; n = 9 to 10 mice per group. Disease progression was monitored via weekly BLI and thrice weekly observations of mouse weight, condition, and mobility. Mice were euthanized when they experienced hind-limb paralysis or met IACUC weight loss or condition requirements. (A) BLI of untreated control and 15 μCi treatment groups at 21 days posttherapy. (B) BLI of all groups at 41 days posttherapy. Red coffins indicate deceased mice. BLI scale identical to panel A.

eliminate MRD in a greater proportion of patients than is currently achieved with high-dose chemotherapy conditioning alone and, if ^{211}At-CD38 is found to be safe and effective in conjunction with stem cell rescue, the approach may ultimately be most useful as a stand-alone therapy in any MRD-positive setting. Furthermore, although MRD-negative status to $\leq 10^{-5}$ predicts significantly extended survival, the majority of these patients eventually relapse,[31,32] indicating that residual disease can exist below the limit of detection of even state-of-the-art depth assessments. Our current data for MRD vs subcutaneous xenograft models demonstrate that ^{211}At-CD38 efficacy increases with decreasing disease burden, suggesting that ^{211}At-CD38 might prove extremely effective at eliminating even MM lurking below the limits of MRD detection.

The success and frontline approval by the US Food and Drug Administration (FDA) of the unmodified CD38 mAb daratumumab[43-45] validates CD38 as a desirable target for radionuclide delivery, yet experience with CD38 targeting and the frequency with which daratumumab is being used in clinical settings demands attention when proposing CD38 as a target for ^{211}At therapy. Daratumumab monotherapy for MM ultimately results in relapse. Multiple factors have been reported to contribute to daratumumab resistance, including a protective bone marrow microenvironment,[46] loss of cell-adhesion antigen CD56 on MM cells,[19] reduction in natural killer cells,[47] upregulation of complement-inhibitory proteins on MM,[48] and impacts on immune effector cells.[49] We posit that the efficacy of ^{211}At-CD38 is not susceptible to these factors, because the cell-killing mechanism of ^{211}At-CD38 is direct, immediate, and independent of host immune function. ^{211}At-CD38 efficacy could, however, be hampered in patients pretreated with daratumumab if CD38 expression is downregulated. Studies of MM patient samples mitigate this concern, reporting that CD38 expression rebounds to original levels after daratumumab is discontinued, and that treatment with certain agents (panobinostat,[50] all-*trans* retinoic acid,[48] DNA methyl-transferase inhibitors[51]) can upregulate CD38, even in samples from daratumumab-resistant patients.[48] Furthermore, the OKT10 mAb binds a different epitope and is not

Table 1. Hematologic, kidney, and liver function indicators for untreated control and ^{211}At-CD38 45-μCi–treated mice bearing disseminated OPM-2^{Luc} xenografts, and age-matched mice with no disease

Treatment group	Day	WBC, ×10⁹/L	Hematocrit, %	Platelets, ×10⁹/L	Creatinine, mg/dL	ALT, U/L
Untreated control	14	1.8 ± 0.3 (5)	52 ± 1.4 (5)	1602 ± 399 (5)	0.52 ± 0.04 (5)	26 ± 4 (5)
^{211}At-CD38 45 μCi		2.4 ± 0.5 (4)	46 ± 2.6 (5)	865 ± 86 (4)	0.52 ± 0.04 (5)	27 ± 4 (5)
Age-matched control		2.2 ± 0.3 (2)	53 ± 4.1 (3)	1719 ± 90 (3)	0.57 ± 0.12 (3)	20 ± 4 (3)
Untreated control	32	2.1 ± 0.8 (5)	56 ± 2.6 (5)	1444 ± 313 (5)	0.46 ± 0.05 (5)	77 ± 16 (5)
^{211}At-CD38 45 μCi		1.5 ± 0.1 (4)	54 ± 2.7 (5)	1328 ± 120 (4)	0.52 ± 0.04 (5)	52 ± 21 (5)
Age-matched control		1.6 ± 0.2 (3)	58 ± 2.9 (3)	1929 ± 252 (3)	0.50 ± 0.01 (3)	44 ± 6 (3)
^{211}At-CD38 45 μCi	148	1.3 ± 0.6 (2)	42 ± 0.6 (4)	1394 ± 68 (2)	0.58 ± 0.05 (4)	51 ± 10 (4)
Age-matched control		1.5 ± 1.0 (3)	46 ± 0.6 (3)	1773 ± 76 (3)	0.50 ± 0.01 (3)	24 ± 5 (3)

Data are mean ± 1 SD (n).

ALT, alanine aminotransferase; WBC, white blood cell.

blocked by daratumumab. Thus, provided CD38 remains expressed, [211]At-OKT10 therapy should be effective in patients with circulating daratumumab or selective loss of the daratumumab-binding epitope. To address concerns raised by prior CD38 mAb treatments, the upcoming clinical trial will limit enrollment to patients with CD38^{+} MM, and will examine the relationship between CD38 antigen density and [211]At-CD38 efficacy.

CD38-targeted [211]At has notable benefits in the era of precision medicine. A key advantage derives from the spatial and temporal heterogeneity of MM; patients may have different clones at different sites of disease, and these clonal populations evolve over time. Precision medicine often aims to increase therapeutic ratios by targeting patient-specific oncogenetic mutations, yet such therapies may ultimately fail when coexistent disease subclones harbor differing mutations.[52,53] In some cases, targeted therapies can even stimulate oncogenetic pathways in nontargeted subclones.[53] These problems may be overcome with improved methods of identifying actionable pathways in multiple subclones and treating with targeted drug combinations, though toxicity can limit the extent of such combinations. Thus, therapies agnostic to clonal type while optimizing therapeutic ratios should benefit both the logistics of drug administration and patient outcomes. Virtually all subtypes of MM are exquisitely sensitive to radiation. This includes disease with high-risk cytogenetic features, giving [211]At-CD38 the potential to effectively treat MM patients across a wide spectrum of disease presentations.

[211]At-CD38 has additional properties that favor clinical application. First, [211]At lacks toxic daughter radionuclides and has a moderate 7.2-hour half-life. These decay features aid logistical feasibility in the clinic and are better suited to the biological half-life of the targeting Ab than other α-emitters, of which actinium-225 ([225]Ac) and bismuth-213 ([213]Bi) are among the best studied. [225]Ac produces daughter radionuclides that can reduce ligand stability and increase treatment toxicity[54,55] and has a 10-day half-life that can contribute to dose-limiting extended myelosuppression.[56] Although [213]Bi has a contrastingly short 46-minute half-life, this property can reduce pharmacokinetic and therapeutic efficacy.[54,57] Second, in common with other α-emitters, the short path length of [211]At minimizes toxicity to healthy tissues and simplifies clinical handling. Third, [211]At-CD38 should have a highly favorable dosing schedule. A preclinical study of [211]Bi-CD38 therapy for MM reported that a series of 6 treatments extended median survival of xenograft-bearing mice from 60 days (control) to 100 days.[58] In contrast, using only a single treatment, we found that optimal dosing of [211]At-CD38 at least quadrupled median mouse survival and offered curative potential. Fourth, we previously demonstrated that a pretargeted, β-emitter (^{90}Y)-CD38 therapy was highly effective in preclinical, bulky tumor models of both MM and non-Hodgkin lymphoma,[20,21] suggesting that, in low-disease-burden settings, [211]At-CD38 might be similarly relevant to a broad patient population. Finally, we anticipate that [211]At therapies will be accessible to patients in the United States, as the US Department of Energy has made [211]At availability a high priority of the National Isotope Development Center, and is funding the upgrade of existing cyclotrons to provide [211]At regionally throughout the country.[59] Taken together, these factors suggest that [211]At-CD38 is ready for clinical translation and offers the potential for therapeutic efficacy as a single-dose treatment of MM.

We believe our results are robust despite limitations associated with our approach. No mouse model perfectly represents MRD, a state of low-volume disease lacking a uniform definition, and, in our disseminated model, [211]At-CD38 did not achieve 100% cure rates (defined as survival to 150 days posttreatment without disease visible to BLI). However, the minimal toxicity and the consistent, dose-dependent therapeutic efficacy of [211]At-CD38 show that higher cure rates are clearly achievable. As mouse dosing does not directly translate, we contend that further dose-finding efforts are most usefully implemented in a clinical setting. Paradoxically, the capacity to achieve high cure rates is further suggested by our finding that the highest dose level of non-targeted [211]At-Ab[−] treatments cured some mice in 2 of our MRD experiments. This result is unsurprising given the extreme radiosensitivity of all MM clones, the high LET of [211]At, and the existence of low-level nonspecific binding by the [211]At-Ab[−] isotype control (Figure 1B). This result does not support a nontargeted approach: our biodistribution data demonstrate that [211]At-CD38 delivers significantly more radiation to tumor than nontargeted [211]At-Ab[−] (Figure 2A), thus reducing potential systemic toxicity at a given tumor dose. Most importantly, in all 5 of our therapy experiments, [211]At-CD38 comprehensively outperformed [211]At-Ab[−] (Figures 3-6). Another potential limitation derives from the risk of radiation-induced secondary malignancies, particularly myelodysplastic syndrome (MDS). This risk may have limited the clinical use of β-emitter therapies in lymphoma, although large trials using β-emitters have reported no increase in MDS incidence.[60-63] The very short path length and high LET of [211]At may reduce risk relative to β-therapies. This potential safety of α-therapies is supported by the low incidence of MDS among patients receiving FDA-approved radium-223 treatment of prostate cancer, despite a much longer period of exposure (11.4-day half-life).[64]

To our knowledge this is the first study of an [211]At treatment of MM. Our approach is agnostic to high-risk cytogenetic features and offers the potential to eliminate all residual MM cell clones in low-disease-burden settings. We are optimistic that the addition of [211]At-CD38 to an ASCT-conditioning regimen in our upcoming clinical trial may improve ASCT outcomes for MM patients.

Authorship

Contribution: S.O. designed and performed research, analyzed data, and wrote the paper; M.L.C. and J.C.J. designed and performed research and analyzed data; D.K.H., A.K., M.E.N., Y.L., and B.W.M. performed research; T.A.G., J.J.O., S.A.T., B.G.T., A.K.G., B.M.S., and O.W.P. analyzed data; D.S.W. contributed vital reagents; and D.J.G. designed research, analyzed data, and wrote the paper.

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O'STEEN et al

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Footnotes

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O'STEEN et al