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^{225}Ac-CD38 Antibody Targeting is Effective and Well Tolerated in Experimental Models of Lymphoma and Multiple Myeloma

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ABSTRACT

Daratumumab is a human cytolytic antibody specific for human CD38 used clinically for the treatment of patients with multiple myeloma (MM). Current therapeutic regimens require relatively high doses of antibody delivered in multiple injections per course of therapy. Conjugation of daratumumab with a potent alpha particle emitting radionuclide, Actinium-225 (^{225}Ac), to create an antibody radio-conjugate (ARC) has the potential to dramatically increase the potency of the antibody resulting in greater tumor cell killing relative to the naked antibody. ^{225}Ac has potent cytotoxic activities over short distances, allowing for precise targeting of a lethal dose of radiation. Previously, we established that labeling of daratumumab with ^{225}Ac increased its ability to kill MM cell lines in vitro by more than 10-fold over naked antibody.

In this study, we evaluated the ability of ^{225}Ac-daratumumab (^{225}Ac-DARA) to treat established Daudi lymphoma and MM tumors in mice and assessed the safety of this treatment. We found that the growth of the Daudi and MM tumors in mice treated with 400nCi/0.3μg was significantly ($P<<0.05$) decreased when compared to mice treated with equal concentration of unlabeled daratumumab or saline. The analysis of blood counts and blood chemistry in mice given anti-murine CD38 ^{225}Ac-labeled antibody showed only transient decrease in while blood cells and platelet counts, and no detectable kidney and liver toxicity. In this work, we have demonstrated in Daudi lymphoma and MM models that ^{225}Ac-DARA has greater anti-tumor activity than naked daratumumab and the treatment is safe towards healthy CD38-expressing tissues. This more potent approach may be a means to expand the population of patients who may respond to anti-CD38 biologic therapy and highlights the potential of targeting α-emitter radionuclide warheads to tumors as a viable therapeutic approach.

METHODS

Preparation of ^{225}Ac-DARA
p-SCN-Bn-DOTA (DOTA) was conjugated to DARA at 5M excess for 1.5h at 37°C. DARA-DOTA conjugate was labeled with ^{225}Ac at a specific activity of 400nCi to 0.3μg. ^{225}Ac-daratumumab was diluted with an equal amount of unlabeled DARA to adjust for total antibody dose (0.3μg) for the 200uCi ^{225}Ac-DARA treatment group so that all groups received the same total amount of antibody.

In vivo mouse xenograft tumor models
CB17/Icr-Prkdcscid/IcrIcoCrl SCID mice were injected s.c. with 5x10^6 CD38$^+$ human Daudi lymphoma or 28PE multiple myeloma tumor cells and tumor growth was monitored with calipers. When tumors reached an average 200mm^3, mice were treated as described in figures. Mice were sacrificed when tumors reached 4,000mm^3. Blood was collected one week after treatment and analyzed for various parameters.

Imaging of DARA in tumor bearing mice
DARA-DOTA conjugate was labeled with ^{111}In at a ratio of 400μCi to 80μg, with a labeling efficiency of ~95%. ^{111}In-DARA was injected i.p. into mice bearing Daudi-derived s.c. tumor xenografts and imaged with CT-SPECT up to 10 days.

RESULTS

Conjugation of DARA to DOTA Does Not Affect Antigen Binding

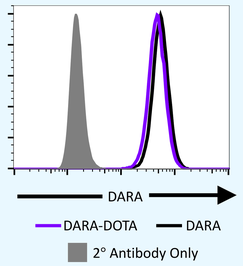

Figure 1. Binding of DARA-DOTA and DARA to CD38 on Daudi Cells
Daudi cells were incubated with DARA and DARA-DOTA and the amount of bound Ab was determined by flow cytometry using anti-hIgG^{PE} to detect bound antibodies.

^{225}Ac-conjugated DARA Elicits Potent Tumor Cell Killing In Vitro

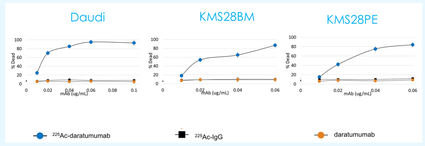

Figure 2. Tumor cell cytotoxicity
Titrations of ^{225}Ac-DARA, daratumumab, and ^{225}Ac-IgG were tested in three different cell lines (Daudi, 28BM, 28 PE) and cell viability was assayed at 96h.

Conjugation of DARA to DOTA Does Not Affect Complement Binding

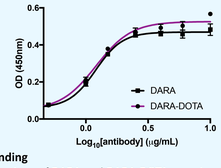

Figure 2. C1q Binding
Various concentrations of DARA and DARA-DOTA were immobilized on plastic and incubated with hC1q. The amount of bound C1q was assessed using anti-C1q-HRP as a probe.

Conjugation of DARA to DOTA Does Not Inhibit ADCC

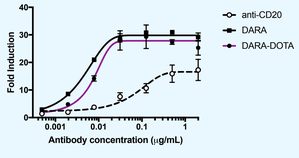

Figure 3. ADCC activity
Various concentrations of DARA and DARA-DOTA were added to CD38 expressing target cells. Effecter cells that express luciferase when activated through FcγR(III) were added to target cells and luminescence was measured after the addition of Bio-Glow™. Fold induction = RLU(induced-background)/RLU(no antibody control-background). Anti-CD20 antibody, which is known to activate ADCC, was used as a positive control.

DARA Antibody Radio-Conjugate Homes to Tumors

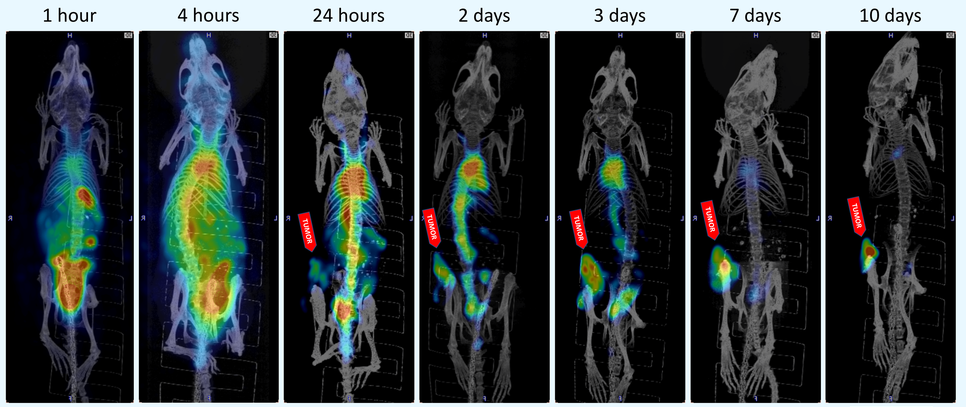

Figure 4. Localization of DARA in tumor baring mice
SCID mice were injected with human CD38 positive Daudi tumor cells and tumors were allowed to develop. When tumors reached ~200mm^3, mice were treated with a single i.p. injection of 80μg ^{111}In-DARA with a specific activity of 400μCi and antibody distribution was monitored by CT-SPECT imaging.

^{225}Ac-Daratumumab Controls Tumor Growth and Extends Mouse Survival

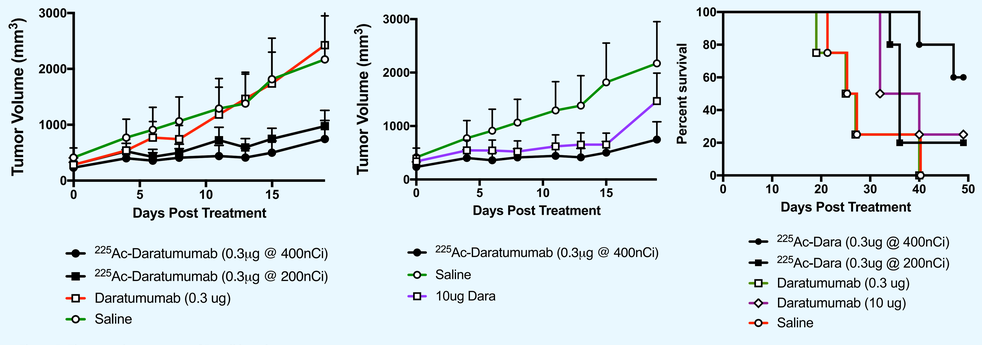

Figure 5. Therapeutic treatment of Daudi lymphoma tumors
SCID mice were injected with human CD38+ Daudi cells and tumors were allowed to develop. When tumors reached ~200mm^3 mice were randomized and then treated with a single i.p. injection of 0.3μg ^{225}Ac-DARA with a specific activity of 400nCi or 200nCi, an equivalent dose of 0.3 μg naked DARA, or a 30-fold higher dose (10μg) naked DARA, or saline vehicle. Tumor volume was calculated using the formula V=0.5(LW^2). Mice were sacrificed when the tumor volume reached 4000mm^3.

^{225}Ac-mCD38 Antibody Radio-Conjugate Treatment is Well Tolerated in Mice

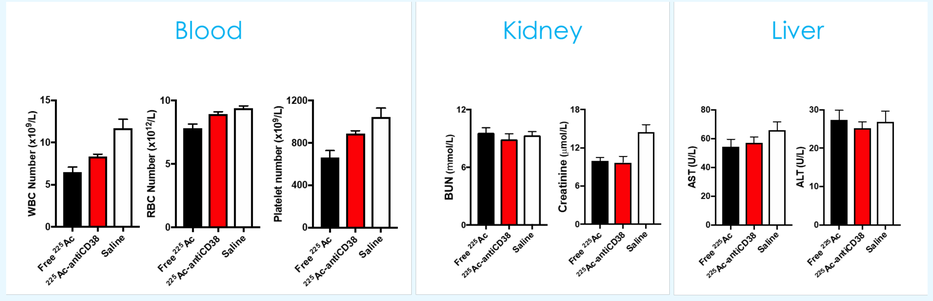

Figure 7. Tolerability of ^{225}Ac-mCD38 (murine anti-CD38) in mice
Anti-murine CD38 (clone 90) ThermoFisher (Waltham, MA) was conjugated to DOTA and labeled with ^{225}Ac in the same manner as daratumumab. Female C57Bl/6 mice were injected with either 400 nCi free ^{225}Ac or ^{225}Ac-antiCD38 mAb. Their weight was determined every 3 days. Blood was collected 33 days after the treatment and analyzed for blood cell counts (WBC number) and kidney (BUN) and liver (AST/ALT) toxicities. Mice in all study groups gained weight over the course of the experiment (not shown).

^{225}Ac-DARA Controls Tumor Growth in KMS28BM Myeloma Xenografts

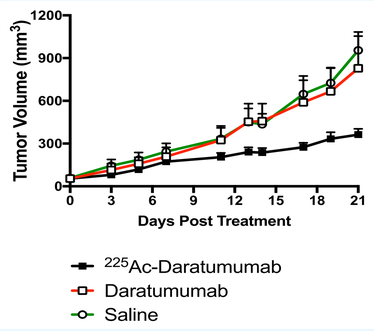

Figure 6. Therapeutic treatment of KMS28BM multiple myeloma tumors
SCID mice were injected with human CD38+ tumor cells and tumors allowed to develop. When tumors reached ~200mm^3 mice randomized and then treated with a single i.p. injection of vehicle control, 0.3μg ^{225}Ac-DARA with a specific activity of 400nCi, or an equivalent dose of 0.3 μg naked DARA. Tumor volume was calculated using the formula V=0.5(LW^2). Mice were sacrificed when the tumor volume reached 4000mm^3.

SUMMARY

- Conjugation of DARA to DOTA does not compromise CD38 binding or Fc-dependent functions
- DARA rapidly accumulates in the tumor by 24h and is retained selectively in the tumor by day 7
- ^{225}Ac radio-conjugated DARA dramatically increases the anti-tumor potency over naked DARA in lymphoma and multiple myeloma xenograft models in vivo
- ^{225}Ac-DARA conjugate was well tolerated and increased the in vivo potency of the CD38 antibody by at least 30-fold, leading to a survival advantage following single dose treatment in mice
- Surrogate anti-murine ^{225}Ac-mCD38 conjugate was well tolerated in normal mice

Conflict-of-Interest Disclosure: E.D. and W.D. receive research funding from Actinium Pharmaceuticals, Inc.; D.L.L., has equity ownership in and is an employee of Actinium Pharmaceuticals, Inc.