

Conjugation of Daratumumab with ²²⁵Actinium Greatly Increases its Antitumor Activity Against Multiple Myeloma Tumors



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INTRODUCTION

Daratumumab (DARA) is a human cytolytic monoclonal antibody specific for CD38 that is indicated 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 DARA with a potent alpha particle emitting radionuclide, ²²⁵Actinium (²²⁵Ac), 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.

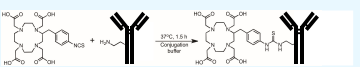
²²⁵Ac is an alpha-particle emitting radionuclide that has potent cytotoxic activities over short distances (approx. 3-4 cell lengths), allowing for precise targeting of a lethal dose of radiation to antigen positive tumor cells.

Previously we have established that labeling daratumumab with ²²⁵Ac increased more than 10-fold its ability to kill MM cell lines *in vitro*.

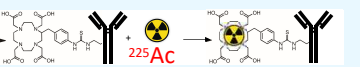
In this study we confirmed that conjugation of DARA did not impact its ability mediate Fc-dependent effector functions. Further, we demonstrate that ²²⁵Ac conjugated-daratumumab significantly increases the anti-tumor potency of targeted anti-CD38 antibody therapy in a xenograft tumor model by as much as 30-fold.

METHODS

Preparation of ²²⁵Ac-DARA
p-SCN-Bn-DOTA (DOTA) was conjugated to DARA at 5M excess for 1.5h at 37°C in ammonium acetate buffer.



DARA-DOTA conjugate was labeled with ²²⁵Ac at a specific activity of 400nCi to 0.3µg. ²²⁵Ac-daratumumab was diluted with an equal amount of unlabeled DARA to adjust for total antibody dose (0.3µg) for the 200nCi ²²⁵Ac-DARA treatment group so that all groups received the same total amount of antibody.



Daudi tumor mouse model
CB17/lcr-Prkdcscid/lcrIcoCr SCID mice were injected s.c. with 5x10⁵ CD38+ human Daudi tumor cells and tumor growth was monitored with calipers. When tumors reached an average 200mm³, mice were treated as described in figures. Mice were sacrificed when tumors reached 4000mm³. Blood was collected one week after treatment and analyzed for various parameters.

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

Conjugation of DARA to DOTA does not affect antigen binding

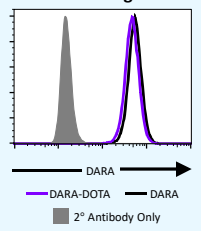


Figure 1. Binding to CD38 on Daudi Cells
Daudi cells were incubated with DARA and DARA-DOTA and the amount of bound Ab was determined by flow cytometry using anti-hiG² to detect bound antibodies.

²²⁵Ac-conjugated DARA elicits potent tumor cell killing in vitro

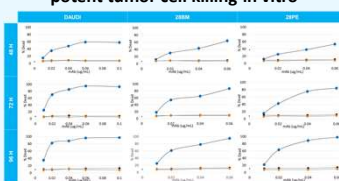


Figure 2. Tumor cell cytotoxicity
Titrations of ²²⁵Ac-daratumumab, daratumumab, ²²⁵Ac-IgG were tested in three different cell lines (Daudi, 283M, 2B PE) at viability assayed at 48h, 72h and 96h.

Conjugation of DARA to DOTA does not affect complement binding

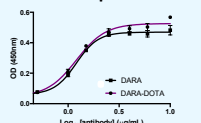


Figure 3. C1q Binding
Various concentrations of DARA and DARA-DOTA were immobilized on plastic and incubated with hC1q. The amount of C1q bound was assessed using anti-C1q-HRP as a probe.

Conjugation of DARA to DOTA does not inhibit ADCC

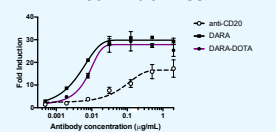


Figure 3. ADCC activity
Various concentrations of DARA and DARA-DOTA were added to CD38 expressing target cells. Effector cells that express luciferase when activated through FcγRIII were added to target cells and luminescence was measured after the addition of Bio-Glo[®]. Fold induction = RU(induced-background)/RU(no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

RESULTS

Conjugated DARA homes radioactivity to tumors

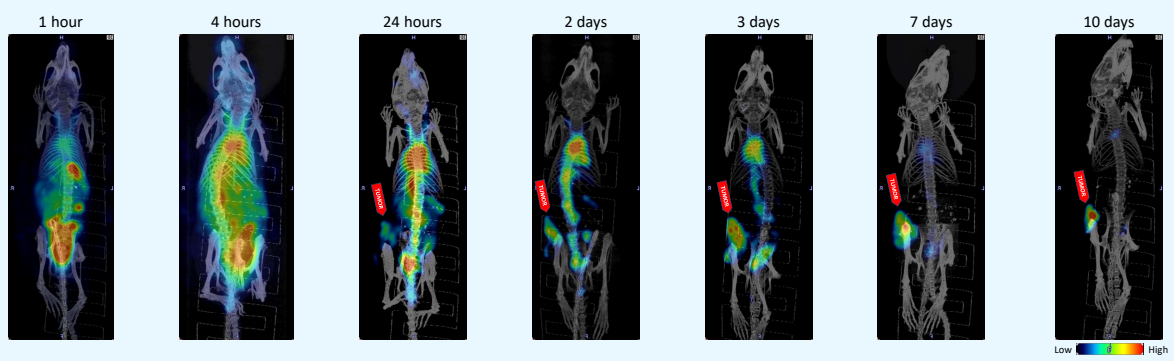


Figure 4. Localization of DARA in tumor bearing mice
SCID mice were injected with human CD38 positive Daudi tumor cells and tumors were allowed to develop. When tumors reached ~200mm³, mice were treated with a single i.p. injection of 80µg ¹¹¹In-DARA with a specific activity of 400µCi and antibody distribution was monitored by CT-SPECT imaging.

²²⁵Ac-daratumumab controls tumor growth and extends mouse survival

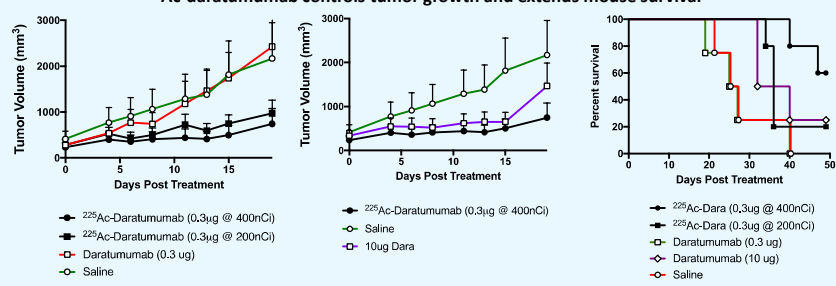


Figure 5. Therapeutic treatment of MM tumors
SCID mice were injected with human CD38+ Daudi cells and tumors were allowed to develop. When tumors reached ~200mm³ mice randomized and then treated with a single i.p. injection of 0.3µg ²²⁵Ac-DARA with a specific activity of 400nCi or 200nCi, an equivalent 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=a²b(W²). Mice were sacrificed when the tumor volume reached 4000mm³.

²²⁵Ac-daratumumab treatment is well tolerated

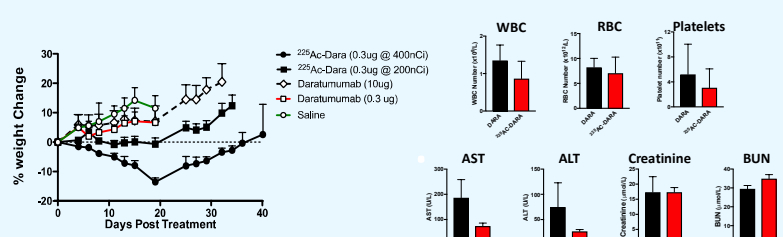


Figure 6. Effect of ²²⁵Ac-DARA treatment on mouse health
SCID mice were injected s.c. with human CD38+ Daudi cells, treated as in Fig. 5 and mouse weight was monitored. Blood was collected 7days post treatment and analyzed for the indicated parameters.

SUMMARY

- Conjugation of DARA to DOTA does not compromise CD38 binding
- Conjugation of DARA to DOTA does not affect binding of DARA to complement (C1q)
- Conjugation of DARA to DOTA does not inhibit ADCC
- DARA rapidly accumulates in the tumor by 24h and is retained selectively in the tumor by day 7
- The conjugation of Dara with ²²⁵Ac dramatically increases the anti-tumor potency of the anti-CD38 antibody in a Daudi xenograft in vivo tumor model
- ²²⁵Ac-DARA conjugate was well tolerated and increased the in vivo potency of the antibody by at least 30-fold, leading to a survival advantage in treated mice

Conflict-of-Interest Disclosure: E.D., W.D., M.M., R.J., K.A., and C.C. receive research funding from Actinium Pharmaceuticals, Inc.; M.S.B., D. L. L., and K.T. have equity ownership in and are employed by Actinium Pharmaceuticals, Inc.