**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, 225-Acetinum (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 vivo.

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 3M excess for 1.5h at 37°C in ammonium acetate buffer.

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

Daudi tumor model

CB17-Scid mice were injected i.p. with human Daudi tumor cells and tumor growth was monitored with calipers. When tumors reached an average 200mm3, 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 225Ac at a ratio of 400Ci to 80µg, with a labeling efficiency of ~95%. 225Ac-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

![Graph](image1.png)

**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., D.L.L., and K.T. have equity ownership in and are employed by Actinium Pharmaceuticals, Inc.; M.S.B., D. L. L., and K.T. have equity ownership in and are employed by Actinium Pharmaceuticals, Inc.