Inc of Jonathan were design Inc are and in equity K and S M; Pharmaceuticals, formatting, Acknowledgements Actinium research receive and A K. R: Disclosure Interest of Conflict targeting offering the the disease progression. Daratumumab induces cell lysis by both antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) mechanisms. The efficient targeting of the CD38 antigen by daratumumab positions it as an attractive vehicle to direct the actinium-225 (225Ac) warhead to its target cells, which may increase the efficacy of daratumumab in multiple myeloma. 225Ac is an ideal warhead as it emits 4 α (alpha) particles, each with a high linear energy transfer of 100 keV/μm. Each alpha track is able to produce lethal double strand DNA breaks. These alpha tracks are short (a few cell diameters) which spares the surrounding cells allowing for very targeted cell killing. Additionally, 225Ac is the best suited α emitter for clinical development as it has an ideal half-life of 10 days – it is long enough to prepare, handle and distribute the 225Ac linked targeting moiety for clinical use while being short enough to safely administer to patients. The success of daratumumab in the clinical setting demonstrates the ability of the antibody to target the CD38. The large infusion of daratumumab required results in a long infusion time and is likely responsible for the high rate of infusion reactions. Labeling of daratumumab with 225Ac could enhance the efficacy of the 225Ac-daratumumab construct, enabling utilization of a lower amount of daratumumab and possibly addressing some of the potential pitfalls of the naked antibody.

Methods

Three different cell lines that express the target antigen, CD38 were utilized in addition to the U226 cell line that does not express CD38. The labeled 225Ac-daratumumab was added to a final concentration of 1 nCi 225Ac per ng of antibody. Across cell lines, the antibodies (daratumumab, 225Ac-daratumumab, 225Ac-IgG) were administered to a final concentration of 0.01, 0.02, 0.04, 0.06 and 0.1 µg/mL. The cell lysis was measured at 48, 72, 96 µg/mL for each of the cell lines using the XTT assay in which mitochondrial activity is used as a proxy for cell proliferation. The labeling of daratumumab was added at the same antibody concentrations as the 225Ac-daratumumab. An irrelevant IgG that does not bind to CD38 was also labeled with 225Ac to the same specific activity as 225Ac-daratumumab.

Results

Radiolabeling yield of the monoclonal antibody with 225Ac was high at 82-85%. The stability of the resulting 225Ac-daratumumab construct was also high, retaining 73% and 87% stability at room temperature and 4°C respectively 48 hours post labeling. Importantly, there was little loss in immunoreactivity between the 225Ac-daratumumab stored at room temperature (RT) or 37°C and daratumumab (Figure 1). This indicated that the labeling of daratumumab with 225Ac does not perturb binding to the CD38 antigen target. There was no to minimal cell lysis for the 225Ac-IgG and daratumumab, with little concentration or time dependence (Figure 2). Cell lysis with the 225Ac-daratumumab showed a marked increase in Daudi, 28BM and 28PE cell lines.

Moreover, the cell lysis induced by 225Ac-daratumumab increased with both increasing time and concentration, demonstrating further that the cell lysis is specific to the targeting of CD38 and the enhanced cell-killing power of 225Ac payload (Figure 2). Additionally, no time or concentration dependent killing was observed when CD38-positive cell lines were treated with the similar activities of radiolabeled isotype-matching human control antibody IgG-225Ac, or when the negative CD38 expressing multiple myeloma cell line, U266 was treated with daratumumab-225Ac.

Conclusion

We have demonstrated the ability to label a CD38 targeting antibody with 225Ac. The high potency, precision and short range of the α emitter, 225Ac improves the efficacy of daratumumab more than 10 fold, essentially utilizing the antibody as a vehicle while still preserving its immune functions. The consequences of a more effective cell lysis agent on dosing concentration and frequency could offer substantial improvements for the treatment of disease through the offering of more potent targeting agents.