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Actinium-225 Labeled Daratumumab Demonstrates Enhanced Killing of Multiple Myeloma Cells Over Naked Antibody

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Abstract

Daratumumab is an immunoglobulin G1 kappa (IgG1κ) cytolytic human monoclonal antibody directed against the CD38 antigen and is approved to treat patients with multiple myeloma. The dosing schedule is rigorous, requiring infusions weekly (up to week 8), bi-monthly (up to week 24), and monthly (post week 25) until 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 (^{225}Ac) warhead to its target cells, which may increase the efficacy of daratumumab in multiple myeloma.

^{225}Ac 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, ^{225}Ac 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 ^{225}Ac 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 target. 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 ^{225}Ac could enhance the efficacy of the ^{225}Ac-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 cells lines that express the target antigen, CD38 were utilized in addition to the U226 cell line that does not express CD38. The labeled ^{225}Ac-daratumumab was added to a final concentration of 1 nCi ^{225}Ac per ng of antibody. Across cell lines, the antibodies (daratumumab, ^{225}Ac-daratumumab, ^{225}Ac-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 and 96 hours for each of the cell lines using the XTT assay in which mitochondrial activity is used as a proxy for cell proliferation. The naked daratumumab was added at the same antibody concentrations as the ^{225}Ac-daratumumab. An irrelevant IgG that does not bind to CD38 was also labeled with ^{225}Ac to the same specific activity as ^{225}Ac-daratumumab.

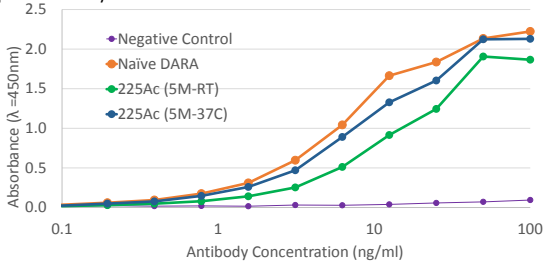

Fig. 1. Immunoreactivity of ^{225}Ac-daratumumab, naked daratumumab and irrelevant antibody

Results

Radiolabeling yield of the monoclonal antibody with ^{225}Ac was high at 82-85%. The stability of the resulting ^{225}Ac-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 immunogenicity between the ^{225}Ac-daratumumab stored at room temperature (RT) or 37°C and daratumumab (Figure 1). This indicated that the labeling of daratumumab with ^{225}Ac does not perturb binding to the CD38 antigen target. There was no to minimal cell lysis for the ^{225}Ac-IgG and daratumumab, with little concentration or time dependence (Figure 2). Cell lysis with the ^{225}Ac-daratumumab showed a marked increase in Daudi, 28BM and 28PE cell lines.

Moreover, the cell lysis induced by ^{225}Ac-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 ^{225}Ac 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.

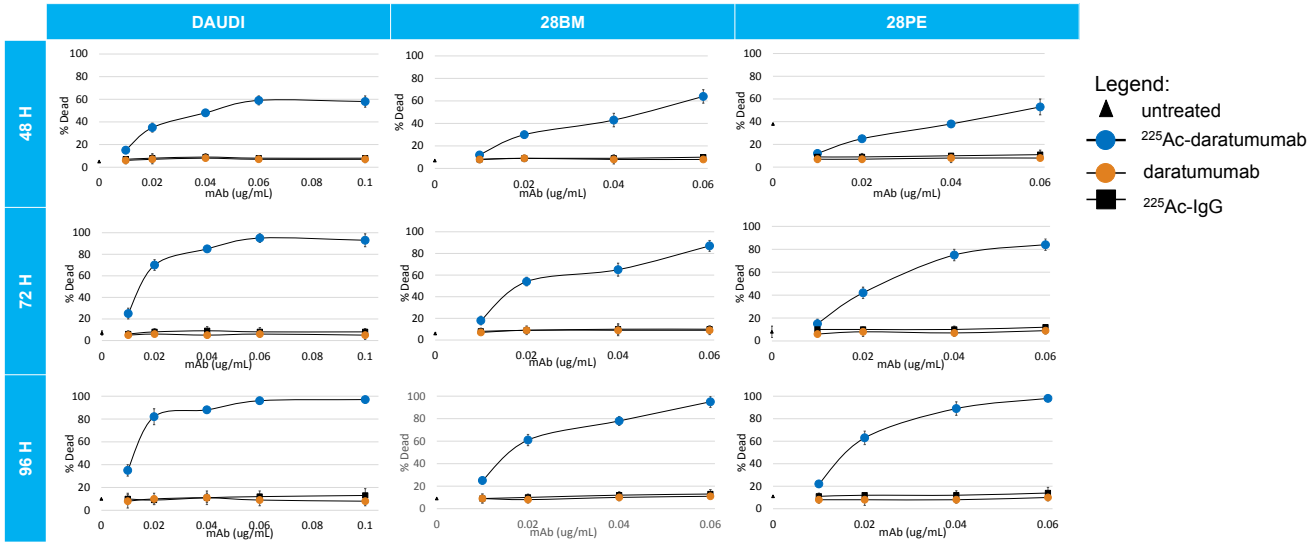

Fig. 2. Cell lysis induced by ^{225}Ac-daratumumab, daratumumab, ^{225}Ac-IgG in three different cell lines (Daudi, 28BM, 28 PE) at 48h, 72h and 96h.

Conclusion

We have demonstrated the ability to label a CD38 targeting antibody with ^{225}Ac. The high potency, precision and short range of the alpha emitter, ^{225}Ac 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.

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