

**1193** **INDUCING IMMUNOGENIC CELL DEATH AND ANTITUMOR ACTIVITY IN RESPONSE TO RADIOIMMUNOTHERAPY**

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**Background** Conventional cancer therapies, such as chemotherapy and external beam radiation therapy, are known to induce the release of damage-associated molecular patterns (DAMPs) that stimulate an antitumor immune response. Radioimmunotherapy (RIT) offers a unique opportunity by delivering cytotoxic energy specifically to target-expressing cancer cells. However, the immunomodulatory properties of RIT are not well characterized. Hence, we hypothesized that tumor cell cytotoxicity induced by radiolabeled antibodies (ARCs) can activate an immune response by releasing DAMPs. Herein we investigated the effects of alpha or beta emitting ARCs ( $^{225}\text{Ac}$  and  $^{177}\text{Lu}$ ) on DAMP induction and engagement of the innate immune response *in vitro*. Furthermore, we evaluated the antitumor effects of  $^{225}\text{Ac}$  and  $^{177}\text{Lu}$  ARCs *in vivo*.

**Methods**  $^{225}\text{Ac}$  or  $^{177}\text{Lu}$  ARCs (CD33, HER2 and HER3) were prepared using p-Bn-SCN-DOTA. ARCs were evaluated for target-binding using human recombinant proteins and receptor positive tumor cell lines. The cytotoxic effect of ARCs was determined by XTT, flow cytometry and clonogenic assays. DAMP induction was investigated in cells treated with  $^{225}\text{Ac}$  or  $^{177}\text{Lu}$  ARCs and levels of CRT, HSP70, and HMGB1 were measured by flow cytometry and ATP concentrations determined by ELISA. The influence of ARC treatment on the innate immune response was evaluated by flow cytometry. The antitumor effects of  $^{225}\text{Ac}$  or  $^{177}\text{Lu}$  ARCs was examined in mice bearing human NCI-H1975 NSCLC xenograft tumors.

**Results** The  $^{225}\text{Ac}$  and  $^{177}\text{Lu}$  ARCs showed cytotoxicity and inhibited colony formation in target expressing cancer cells *in vitro*. DAMP levels in  $^{225}\text{Ac}$  ARC treated cells was induced, including CRT translocation to cell surface, increased ATP release and HSP70 upregulation. Furthermore, an enhanced innate immune response was observed following  $^{225}\text{Ac}$  ARC treatment. Contrastingly, treatment with  $^{177}\text{Lu}$  ARCs did not show significant DAMP induction compared to untreated cells in the dose ranges studied. However, the efficacy study testing  $^{225}\text{Ac}$  and  $^{177}\text{Lu}$  ARCs in mouse xenograft model of NSCLC revealed that both ARCs had similar antitumor efficacy *in vivo*.

**Conclusions** The observations from this study suggest that  $^{225}\text{Ac}$  radionuclide-based ARCs as part of their mechanism of action can induce immunogenic cell death by the release of DAMPs whereas beta radionucleotides such as  $^{177}\text{Lu}$  may utilize different damage response pathways. These findings could be explained by DNA double stranded breaks caused by alpha radiation. Importantly, both  $^{225}\text{Ac}$  and  $^{177}\text{Lu}$  ARCs showed *in vivo* antitumor activity that warrants their further evaluation as warheads for RIT.

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