Anti-HER3 radioimmunotherapy enhances the anti-tumor effects of CD47 blockade in solid tumors

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BACKGROUND

Cancer immunotherapy strategies targeting blockade of the CD47-SIRPα immunosuppressive signal have made significant progress in recent years. However, monotherapies have not shown meaningful clinical responses in solid tumors. Therefore, therapeutic combinations are being explored to improve patient outcomes. CD47 is a macrophage checkpoint inhibitor that acts as a "don't eat me" signal on cancer cells to evade innate immune detection and destruction. Targeted radiation to cancer cells will upregulate calreticulin (CRT), a pro-phagocytic "eat me" signal. We therefore hypothesize that we can enhance the efficacy of anti-CD47 antibodies by combining them with appropriate targeted antibody radioconjugates (ARC). In this experiment we investigate an anti-HER3 radioconjugate, as HER3 is overexpressed in a variety of cancers including breast, ovarian, lung, gastric and prostate and is associated with poor clinical prognosis. Additionally, upregulation of HER3 is implicated in the acquired resistance against HER1 or HER2 targeted therapies. Here, we demonstrate enhanced therapeutic efficacy of a novel Actinium-225 (^{225}Ac) armed HER3 specific targeting ARC (^{225}Ac-HER3-ARC) and a CD47 blocking antibody (anti-CD47) combination in preclinical solid tumor models.

Proposed Mechanism of Action

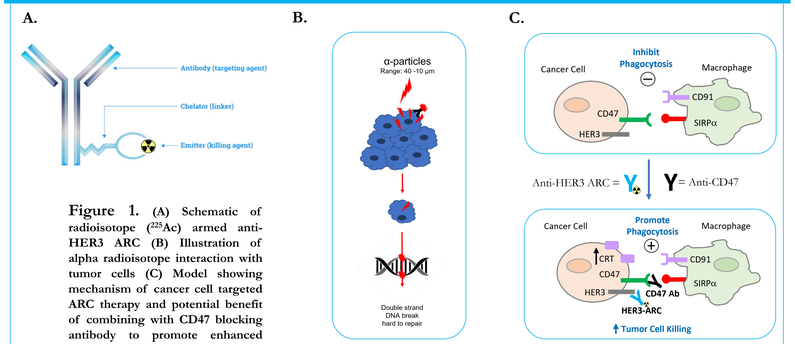

Figure 1. (A) Schematic of radioisotope (^{225}Ac) armed anti-HER3 ARC (B) Illustration of alpha radioisotope interaction with tumor cells (C) Model showing mechanism of cancer cell targeted ARC therapy and potential benefit of combining with CD47 blocking antibody to promote enhanced tumor cell killing

METHODS

Radiochemistry
- Radiolabeling
- Characterization

In Vitro
- Binding ELISA
- Binding Flow
- CD47 Expression
- Cell Surface CRT
- Cytotoxicity
- Phagocytosis

In Vivo
- Single agents and combination efficacy study

RESULTS

^{225}Ac-HER3-ARC Binds HER3 and Induces Tumor Cell Killing

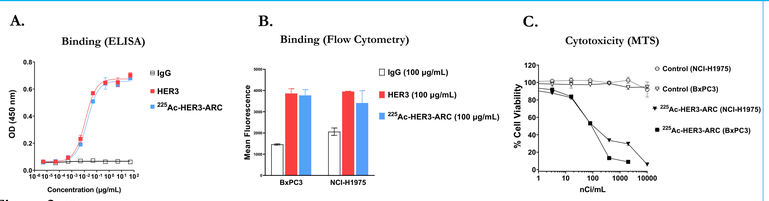

Figure 2. ^{225}Ac-HER3-ARC specifically binds to recombinant human HER3 protein as demonstrated in ELISA (A) and human tumor cell lines NCI-H1975 (non-small cell lung cancer) and BxPC3 (pancreas adenocarcinoma) by flow cytometry (B) 1-hour post-incubation. Human immunoglobulin (IgG) was used as control. ^{225}Ac-HER3-ARC is cytotoxic to HER3$^+$ cells NCI-H1975 and BxPC3 in a dose-dependent manner as demonstrated by MTS colorimetric assay (C). Unlabeled HER3 antibody was used as a control.

CD47/CRT Expression on HER3$^+$ Cells in Response to HER3-ARC Treatment

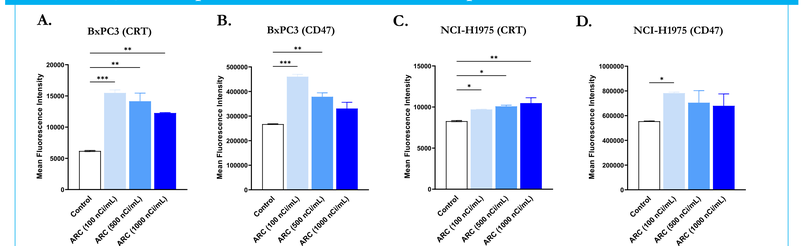

Figure 3. CRT and CD47 surface expression on human tumor cell lines BxPC3 (A and B) and NCI-H1975 (C and D) in response to ^{225}Ac-HER3-ARC (ARC) treatment as determined by flow cytometry. Cells were treated with different concentrations of ARC for 24 hours followed by media change and incubation for 72 hours. Untreated cells were used as the control. Statistical analysis was performed on GraphPad Prism 9.2 using One-Way ANOVA (*p < 0.05, **p< 0.01, ***p<0.001 and ****p < 0.0001). ^{225}Ac-HER3-ARC upregulates surface CRT. Increasing the concentration of ^{225}Ac-HER3-ARC did not further enhance surface CRT.

^{225}Ac-HER3-ARC and anti-CD47 Antibody Combination Enhances Phagocytosis

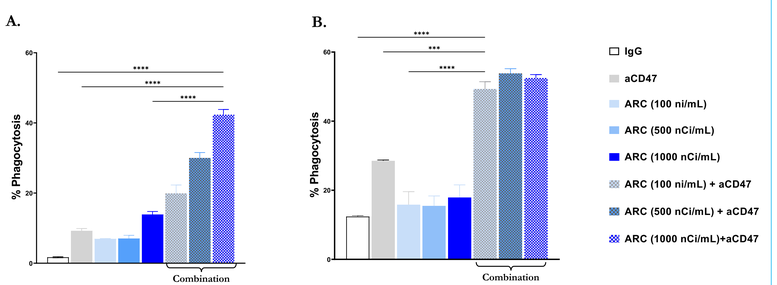

Figure 4. Combination of ^{225}Ac-HER3-ARC (ARC) and anti-CD47 (aCD47) enhances phagocytosis of tumor cells (A) BxPC3 and (B) NCI-H1975. Target cells were treated with ^{225}Ac-HER3-ARC for 24 hours, the treatment was removed, fresh media added, and cells were incubated for 72 hours at 37°C. Target cells were labeled with DiD, treated with anti-CD47 (10 μg/ml) and co-cultured with human macrophages previously labeled with DiO for 2 hours. The percentage of phagocytosis was measured by flow cytometry as DiO$^+$/DiD$^+$ signal). Statistical analysis was performed on GraphPad Prism 9.2 using One-Way ANOVA (***p<0.001 and ****p < 0.0001).

^{225}Ac-HER3-ARC Enhances anti-CD47 Anti-Tumor Effects In Vivo

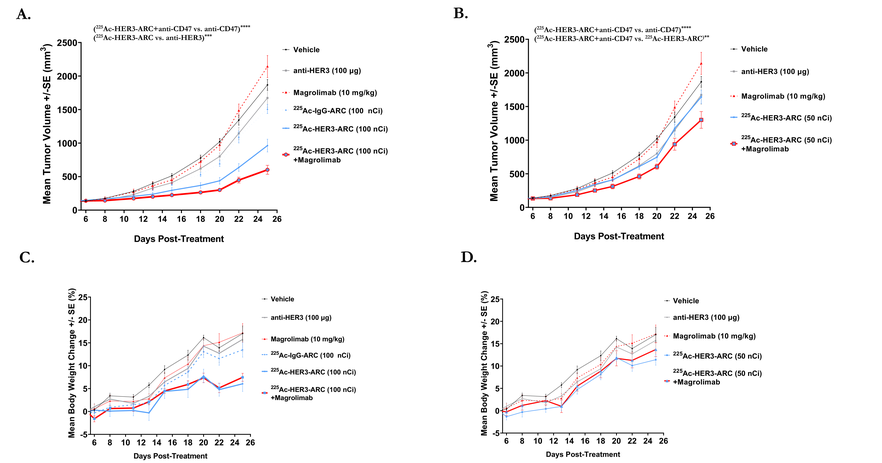

Figure 5. In vivo efficacy study of ^{225}Ac-HER3-ARC and anti-CD47 (Magrolimab) combination in female BALB/c nu/nu mice bearing NSCLC (NCI-H1975) xenograft tumors. Each agent was intravenously administered; single doses of ^{225}Ac-HER3-ARC (50 or 100 nCi) on day 0, anti-CD47 (10 mg/kg) antibody on day 0 and 4. Single doses of vehicle, ^{225}Ac-IgG (100 nCi) and anti-HER3 (100 μg) were administered on day 0 as controls. Tumor volume, body weight and survival was monitored. Statistical analysis was performed on GraphPad Prism 9.2 using Two-Way ANOVA (**p< 0.01, ***p<0.001 and ****p < 0.0001) to compare mean tumor volumes. ^{225}Ac-HER3-ARC and anti-CD47 combination significantly enhances anti-tumor effects compared to single agents in this *in vivo* preclinical model of NSCLC (A, B, E-I). Mice did not lose body weight over the course of the study (C and D), and all mice survived.

CONCLUSIONS

- ✓ Surface CRT is upregulated by ^{225}Ac-HER3-ARC in HER3$^+$ cell lines.
- ✓ ^{225}Ac-HER3-ARC and anti-CD47 antibody significantly increased phagocytosis compared to single agent alone in HER3$^+$ cells.
- ✓ In vivo anti-tumor efficacy was significantly enhanced by ^{225}Ac-HER3-ARC and anti-CD47 antibody combination relative to monotherapies.
- ✓ Consequently, this combination approach of targeted radiotherapy with immunotherapy is an encouraging strategy to potentially improve antitumor immunity in patients with HER3$^+$ tumors and support further investigation.

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