Enhancement of the anti-tumor effects of CD47 blockade in solid tumors by combination with targeted radioimmunotherapy

Denis Beckford-Vera, Sagarika Pachhal, Emily Greer, Jason Li, Caroline Jennings, Jesse Hwang, Qing Liang, Mary Chen, Eileen M. Geoghegan, Helen Kotanides, and Dale L. Ludwig

Actinium Pharmaceuticals, New York, NY USA

Actinium Pharmaceuticals, Inc.

Abstract # 589

BACKGROUND

One mechanism that tumors use to escape immunosurveillance is the overexpression of CD47, which inhibits the macrophage mediated phagocytosis pathway. Although blockade of the CD47-SIRPα axis is a promising approach to enhance tumor targeted phagocytosis, anti-CD47 monotherapies have not shown meaningful responses in clinical studies of solid tumors. Combination cancer therapies aim to increase the probability of response in settings of resistance by combining drugs with different mechanisms of action. Antibody radioconjugates (ARCs) specifically target and deliver therapeutic radiation directly to cancer cells. We rationalized that the immunogenic and cytotoxic properties of ARCs will upregulate calreticulin (CRT), a pro-phagocytic signal, thereby synergizing with CD47 blocking therapies to enhance phagocytosis and antitumor activity. Here for the first time, we demonstrate the combination benefit of anti-HER2 (Trastuzumab) specific targeting ARC and a CD47 blocking antibody to enhance therapeutic efficacy in preclinical solid tumor models.

Proposed Mechanism of Action

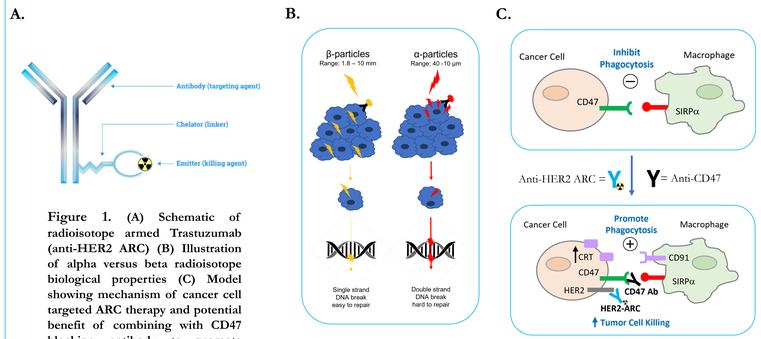

Figure 1. (A) Schematic of radioisotope armed Trastuzumab (anti-HER2 ARC) (B) Illustration of alpha versus beta radioisotope biological properties (C) Model showing mechanism of cancer cell targeted ARC therapy and potential benefit of combining with CD47 blocking antibody to promote enhanced tumor cell 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/^{177}Lu-HER2-ARCs Bind HER2 Expressing Tumor Cells

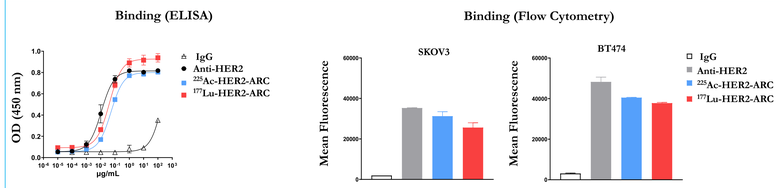

Figure 2. Binding of ^{225}Ac- and ^{177}Lu- HER2-ARC to recombinant human HER2 protein in ELISA (EC_{50} = 0.056, 0.056 and 0.093 µg/mL for HER2, ^{225}Ac-HER2-ARC and ^{177}Lu-HER2-ARC) and human tumor cell lines by flow cytometry.

CD47 Expression in HER2 Cells Before and After HER2 ARC Treatment

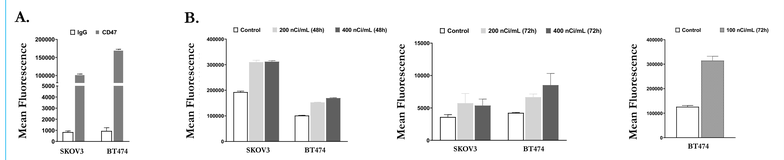

Figure 3. (A) CD47 expression on human tumor cell lines (B) CD47 expression in response to ^{225}Ac-HER2-ARC treatment as determined by flow cytometry. Control: untreated cells.

^{225}Ac-HER2-ARC Induces an Increase in Cell Surface CRT and Cytotoxicity

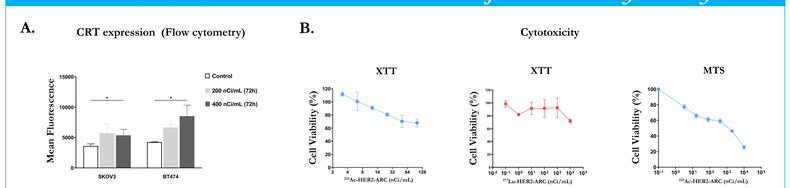

Figure 4. (A) Cell surface calreticulin (CRT) levels detected by flow cytometry in tumor cells treated with indicated dose of ^{225}Ac-HER2-ARC for 3 hours followed by media change and incubation for 72 hours. (B) Dose dependent cytotoxicity of ^{225}Ac- and ^{177}Lu- HER2-ARCs in SKOV3 cells treated for 3 hours (XTT) or 12 hours (MTS) is shown as % cell viability relative to untreated control. (Two-Way ANOVA, *p < 0.05).

^{225}Ac-HER2-ARC and anti-CD47 Antibody Combination Enhances Phagocytosis

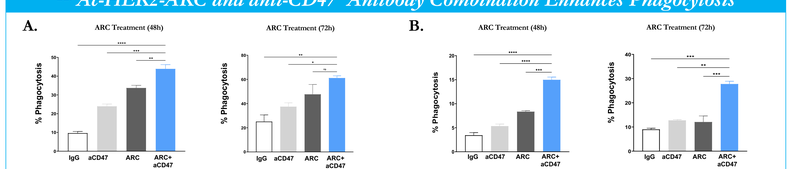

Figure 5. (A) Combination of ^{225}Ac-HER2-ARC and anti-CD47 enhances phagocytosis of tumor cells (A) SKOV3 and (B) BT747. Target cells were treated with ^{225}Ac-HER2-ARC for 48 or 72 hours. The cells were labeled with DiD and cocultured for 2 hours in the presence of anti-CD47 (10 µg/ml) with human macrophages labeled with DiO. The percentage of phagocytosis was measured by flow cytometry (macrophages DiO+/DiD+). Statistical analysis was on GraphPad Prism 9.2 using One-Way ANOVA (*p < 0.05, **p< 0.01, ***p<0.001 and ****p < 0.0001).

Combination of HER2-ARC and anti-CD47 Antibody Slows Tumor Xenograft Growth In Vivo

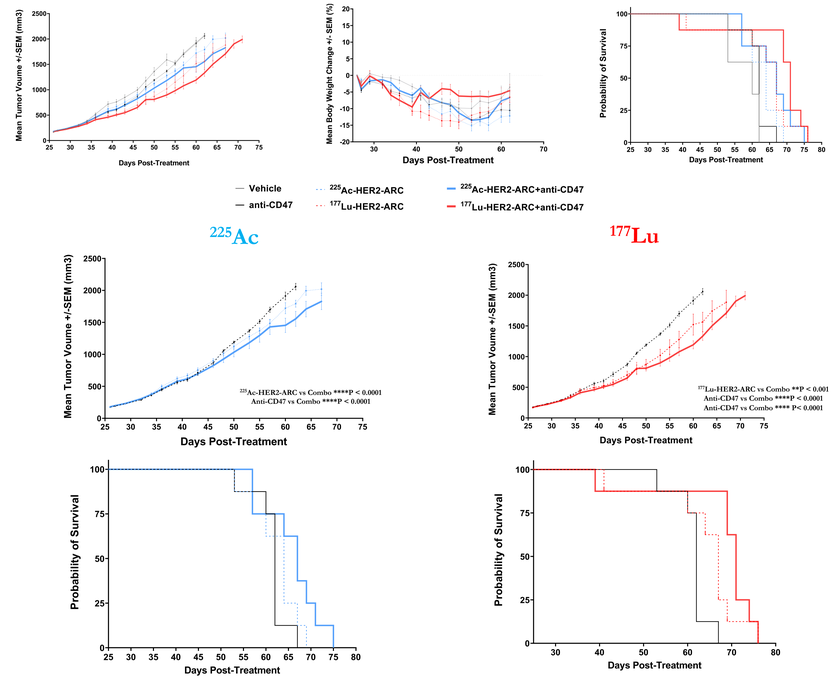

Figure 6. Antitumor efficacy study of ^{225}Ac- and ^{177}Lu-HER2-ARCs and anti-CD47 combination and single agents in NSG mice (n = 8 per group) bearing human ovarian carcinoma (SKOV-3). A single dose of ^{225}Ac-HER2-ARC (25 nCi) or ^{177}Lu-HER2-ARC (25 µCi) was administered on day 0 and the anti-CD47 (10 mg/kg) agent was administered on day 0, 4 and 10. Tumor volume, body weight and survival was monitored. Statistical analysis was performed on GraphPad Prism 9.2 using Two-Way ANOVA to compare mean tumor volumes.

CONCLUSIONS

- Here for the first time, we demonstrate enhanced therapeutic efficacy between an anti-HER2 ARC and CD47 blocking antibody combination in a preclinical solid tumor model.
- The finding suggests that ARC mediated upregulation of CRT potentiates the pro-phagocytic signal and synergizes with the anti-CD47 mode of action thereby enhancing antitumor immune response.
- Additional preclinical studies in other tumor models are ongoing.
- This combination mechanism provides a very promising strategy to improve therapeutic responses in patients harboring solid tumors and warrants further preclinical evaluation.

Society for Immunotherapy of Cancer (SITC) 36th Annual Meeting Washington, D.C. November 10-14, 2021