# Targeting HER3 receptor positive cancers with a novel anti-HER3 antibody radioconjugate (ARC)

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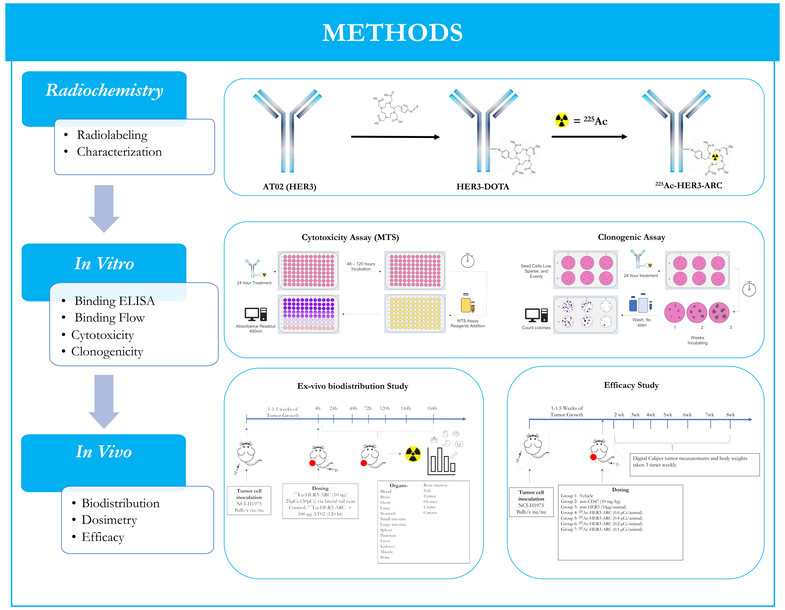
## BACKGROUND

HER3 overexpression is reported to be associated with poor survival in breast, ovarian, lung, gastric and prostate cancer. In addition, upregulation of HER3 in response to HER1 or HER2 targeted therapies is implicated in the acquired resistance against these therapies. Therefore, effective targeting of HER3 can potentially overcome resistance and enhance therapeutic efficacy. Although a number of anti-HER3 antibodies and antibody drug conjugates (ADCs) are in various stages of development and clinical testing, there are currently no approved HER3-targeted therapies. Here we describe a novel approach that can enhance therapeutic efficacy in HER3$^+$ cancer patients by conjugating an anti-HER3 antibody with the alpha-emitting radioisotope Actinium-225 ($^{225}$Ac) to create an anti-HER3 antibody radioconjugate ($^{225}$Ac-HER3-ARC). Alpha-emitting radioisotopes like $^{225}$Ac can cause double-strand DNA breaks for which there is no known resistance mechanism. Due to the cytotoxic properties of the radioisotope, lower levels of antibody may be needed, resulting in reduced incidence or less severe toxicities. We hypothesize that targeting HER3 in solid tumors with an ARC will result in tumor specific cell killing especially in a setting where HER-targeting agents are not a viable option. We developed a novel $^{225}$Ac-HER3-ARC and evaluated its efficacy in HER3$^+$ in vitro and in vivo tumor models.

## METHODS




## RESULTS

### *$^{225}$Ac-HER3-ARC Binds HER3 Expressing Tumor Cells*

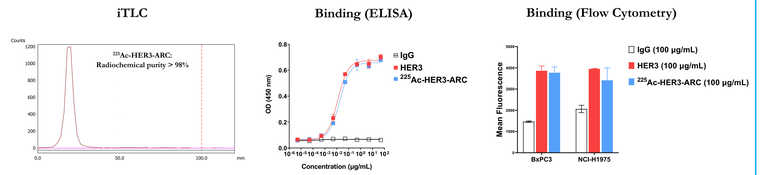



**Figure 1.** $^{225}$Ac-HER3-ARC was prepared with a radiochemical purity > 98% as demonstrated by instant thin-layer chromatography (iTLC). $^{225}$Ac-HER3-ARC specifically binds to recombinant human HER3 protein as demonstrated in ELISA ($EC_{50}$ = 0.0017 µg/mL for anti-HER3 and 0.0022 µg/mL for $^{225}$Ac-HER3-ARC) and human tumor cell lines by flow cytometry. Human immunoglobulin (IgG) was used as a control.

### *$^{225}$Ac-HER3-ARC is Cytotoxic to HER3$^+$ Cells*

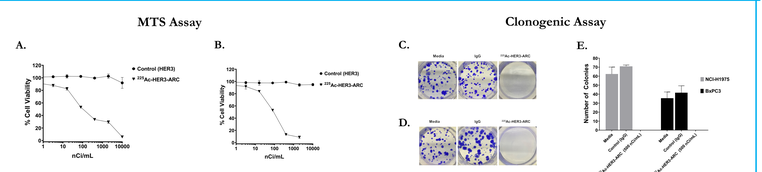



**Figure 2.** Cell-based function activity of $^{225}$Ac-HER3-ARC in vitro. Left: $^{225}$Ac-HER3-ARC is cytotoxic to HER3$^+$ cells (A) NCI-H1975 and (B) BxPC3 in a dose-dependent manner. Cells were treated with $^{225}$Ac-HER3-ARC for 24h followed by media change and culturing for 144h. Viability was assessed by [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) colorimetric assay. Unmodified HER3 antibody was used as a control. Right: $^{225}$Ac-HER3-ARC treatment (500 nCi.mL) inhibits colony formation in (C) NCI-H1975 and (D) BxPC3 cells. Cells were treated as described above and colony formation was quantified 15 days post treatment (E). Human IgG and cell culture media were used as controls.

### *HER3-ARC Selectively Accumulates in HER3$^+$ Tumors*

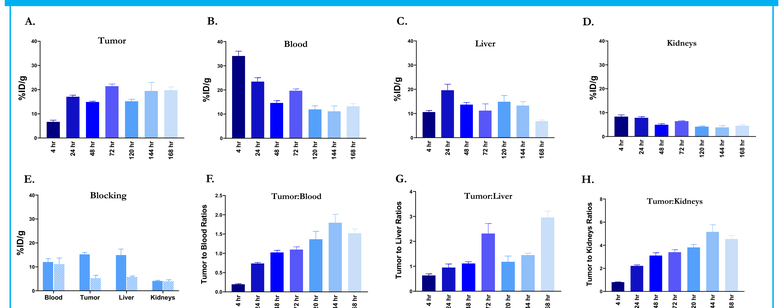



**Figure 3.** The biodistribution of HER3-ARC was assessed in female BALB/C nu/nu mice harboring established subcutaneous human NCI-H1975 NSCLC tumor xenografts. $^{177}$Lu was used as a surrogate of $^{225}$Ac in the biodistribution study. Mice were enrolled for the study when tumor size reached 100 mm$^3$ and administered intravenously with $^{177}$Lu-HER3-ARC (n = 5 per group). Tumors and multiple tissues were harvested, weighted and measured for radioactivity at 4, 24, 48, 72, 120, 144 and 168 hours after injection. The percentage of injected dose (%ID/g) normalized to the mass of the tissue was calculated. To demonstrate in vivo target specificity, HER3 receptors were blocked in another cohort of mice by injecting HER3 antibody 24h before the injection of $^{177}$Lu-HER3-ARC. At 120h post-injection of $^{177}$Lu-HER3-ARC the mice were treated as described above. HER3-ARC rapidly accumulates in the tumor and the uptake increases over time while the radiotracer clears from the blood and other organs (A, B, C and D). HER3-ARC binding is specific to HER3 receptors as demonstrated in the blocking study (E). The ratio of tumor uptake relative to other tissues increases overtime (F, G, and H).

### *$^{225}$Ac-HER3-ARC Suppresses HER3$^+$ Tumor Growth in a Preclinical Model of NSCLC*

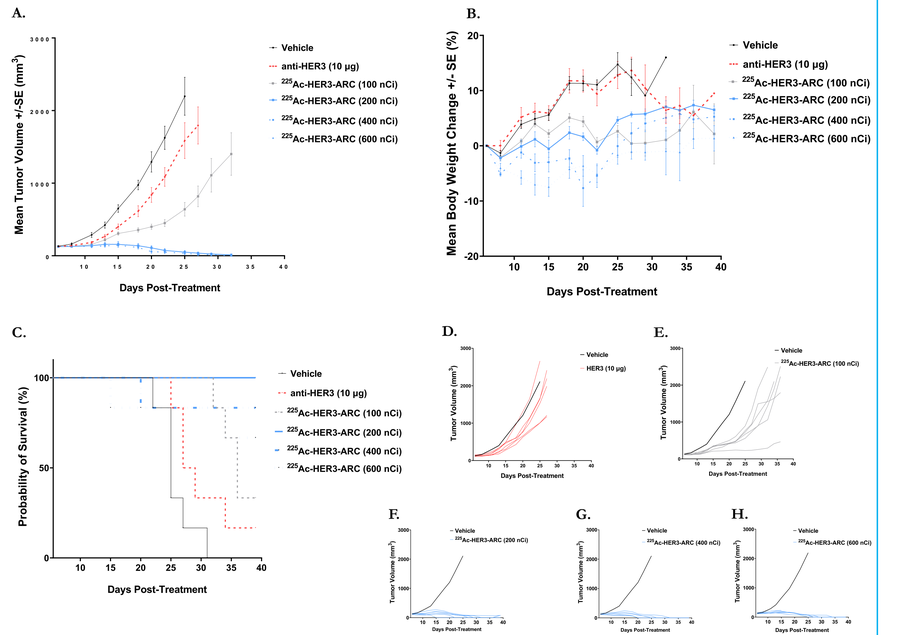



**Figure 4.** In vivo efficacy study of $^{225}$Ac-HER3-ARC in female BALB/c nu/nu mice bearing human NCI-H1975 NSCLC xenograft tumors. Single doses of $^{225}$Ac-HER3-ARC (100 - 600 nCi) were administered intravenously on day 0 (n = 6 per group). Mice injected with PBS and unmodified HER3 antibody (same mass as $^{225}$Ac-HER3-ARC) were used as controls. Tumor volume, body weight and survival was monitored over a period of 40 days. (A, D-H) $^{225}$Ac-HER3-ARC (200 – 600 nCi doses) inhibited-growth of NCI-H1975 tumors. (B) No significant loss of body weight was observed in mice treated with $^{225}$Ac-HER3-ARC (C) $^{225}$Ac-HER3-ARC treatment significantly increases survival in mice with NCI-H1975 tumors compared to vehicle and control groups while no statistical difference in survival was observed among the different administered doses of $^{225}$Ac-HER3-ARC. Log-rank (Mantel-Cox) test was used for statistical analysis of survival ($p < 0.0001$).

## CONCLUSIONS

- ✓ HER3 antibody was radiolabeled with $^{225}$Ac and the radioconjugate showed similar binding properties to those of the native antibody.
- ✓ Treatment with $^{225}$Ac-HER3-ARC was cytotoxic to HER3$^+$ tumor cells in a dose-dependent manner.
- ✓ Our findings demonstrate that targeting HER3 with a $^{225}$Ac-HER3-ARC results in potent anti-tumor response in a HER3$^+$ tumor xenograft model.
- ✓ This approach provides a promising therapeutic strategy for tumors expressing HER3 and warrants further investigation.

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