**INTRODUCTION**

Bispecific T Cell Engagers (TCEs) are effective at inducing remissions in hematologic cancers but their use in solid tumors has been challenging due to their extreme potency and on-target, off-tumor toxicities in healthy tissue. To address this challenge, Amunix has developed a conditionally activated TCE, X-PAT, or XENylated Protease-Activatable Bispecific T Cell Engager targeting HER2 that exploits the unregulated protease activity present in tumors vs. healthy tissues, enabling expansion of the therapeutic index. The X-PAT core consists of 2 single chain antibody fragments (scFv) targeting CD3 and the tumor target. Two unstructured polypeptide masks (XTEN) are attached to the core that sterically reduce target engagement and extend protein half-life. Protease cleavage sites at the base of the X-PAT masks enable proteolytic activation of X-PAT in the tumor microenvironment, unleashing a small, highly potent TCE. In healthy tissues, where protease activity is largely regulated, X-PATs should remain predominantly inactive as intact prodrugs. In addition to localized activation, the short half-life of the unmasked TAT arm should further widen the therapeutic index while providing the potency of T-cell immunity to improve the eradication of solid tumors.

**RESULTS**

**Figure 1.** XEN Polypeptide Masks on HER2-X-PAT Significantly Reduce T Cell-Mediated Cytotoxicity and T Cell Activation in vitro

A. Tumor-directed Cytotoxicity

B. Target-dependent T Cell Activation

**Figure 2.** HER2-X-PAT Induces Robust Tumor regressions in Mice That Are Dependent on the Protease Release Site

A. Comparative efficacy induced with equivalent masking of HER2-PAT and HER2-X-PAT in BT-474 tumor-bearing mice

B. HER2-X-PAT and HER2-PAT induce comparable activation of intramural CD4+ and CD8+ T cells

**Figure 3.** HER2-X-PAT is Efficacious in HER2+ HT-29 Colorectal Xenografts

A. HER2-XPAT achieves deeper tumor clearance

B. HER2-PAT achieves shallow tumor clearance

**Figure 4.** HER2-X-PAT Significantly Expand Safety Margin of HER2-X-PAT vs. PAT in Cynomolgus Monkeys

C. Plasma Cytokines

**Figure 5.** HER2-X-PAT Induces T cell Margination at ≥ 5.5µm but Does Not Activate Peripheral T Cells or Induce Cytokine Release Syndrome Even at 50mg/kg

A. T cell Margination

**Figure 6.** HER2-X-PAT is Large Stable in Circulation of Cynomolgus Monkeys at 25 mg/kg. Consistent With Its Strong Safety Profile

**X-PAT PLATFORM**

X-PATs are XENylated Protease-Activatable T Cell Engagers

- Bi-specific T cell engagers targeting HER2
- De-regulated proteasome activity provides therapeutic margin
- Proximal N-terminus masked by XTEN unmasking by proteases active in all stages of cancer
- Rapidly unmasking leading to increased potency
- Increased therapeutic index

**XPATs Enable Localized Tumor Killing, Limiting Toxicity Against Healthy Tissue Expressing the Target Antigen**