HER2-XPAT, A Novel Protease-Activated Prodrug T Cell Engager (TCE) With Potent T Cell Activation and Efficacy in Solid Tumor Models and Large Predicted Safety Margins in Non-Human Primates

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INTRODUCTION

Bispecific T Cell Engagers (TCEs) have been effective at inducing immune responses in preclinical models, but their use in solid tumors has been limited by their extreme potency and on-target, off-tumor toxicities in healthy tissue. To address this challenge, Amunix has developed a clinically advanced TCE, XPAT or XEYlnated Protease-Activated bispecific T Cell Engager targeting HER2 that exploits the dysregulated protease activity present in tumors vs. healthy tissues, enabling expansion of the therapeutic index. The XPAT core consists of 2 single chain antibody fragments (scFvs) 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 XTEN masks enable proteolytic activation of XPAT in the tumor microenvironment, unleashing a small, highly potent TCE. In healthy tissues, where protease activity is tightly regulated, XPATs should remain predominantly inactive as intraprodugs. In addition to localized activation, the short half-life of the unmasked PAT form should further diminish the therapeutic index while providing the opportunity for T cell immunity to potentially improve the eradication of solid tumors.

XPAT PLATFORM

XPATs Are XENylated Protease-Activated T Cell Engagers

HER2-XPAT

- HER2-XPAT is a bispecific T cell engager with a variety of chemically stable bispecific T cell activators (TBs) that can be covalently linked to HER2

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XPAT PLATFORM

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

Tumor Microenvironment

Healthy Tissues

- In vitro, proteolytically-unmasked HER2-PAT demonstrates potent cytotoxicity against tumor lines with ECD50s in the single-digit pH range. Double XTEN masking reduces target-directed T cell cytotoxicity and T cell activation by up to 4 orders of magnitude, while singly-masked XPATs showed intermediate activity relative to unmasked HER2-PAT. Only minimal cleavage of XPAT is required to generate potent cytotoxicity.

SUMMARY/CONCLUSIONS

- In vitro, proteolytically-unmasked HER2-PAT demonstrates potent cytotoxicity against tumor lines with ECD50s in the single-digit pH range. Double XTEN masking reduces target-directed T cell cytotoxicity and T cell activation by up to 4 orders of magnitude, while singly-masked XPATs show intermediate activity relative to unmasked HER2-PAT. Only minimal cleavage of XPAT is required to generate potent cytotoxicity.

- In the established HER2™ BT-474 and HER2™ BT-55 xenograft models, HER2-XPAT induced protease-dependent tumor regressions comparable to the unmasked (active) T cell engager while remaining stable in circulation. In vivo, preferential cleavage of HER2-XPAT was demonstrated in tumors relative to healthy organs (average % HER2-PAT was 25.2% in tumors and 1.6% in combined other organs).

- In xenografts, HER2-XPAT demonstrated a high safety margin, supported by its protease stability in circulation and a maximum tolerated exposure that was ~45-fold higher than that of its active form (PAT). No CRS or systemic T cell activation was observed even at 50 mg/kg, supportive of minimal CRS risk for XPATs vs. standard TCEs. Only ~3% of singly-masked XPAT metabolites were detected in plasma from NHP administered high doses of HER2-XPAT (25 & 40mg/kg).

- XPATs represent a novel strategy to improve the toxicity profile of T cell engagers while maintaining their potency against solid tumors, thus enabling a significant increase in the therapeutic index and expansion of target landscape for this potent modality.

RESULTS

Figure 1. XEN Polyamide Masks on HER2-XPAT Significantly Reduce T Cell-Mediated Cytotoxicity and T Cell Activation in vitro

A. Tumor-directed Cytotoxicity

B. T Cell Activation

C. Only Minimal Clearance of HER2-XPAT is Required to Generate Potent Cytotoxic Activity

Figure 2. HER2-XPAT Induces Robust Tumor Regressions in Mice in Both HER2™ and HER2™ Xenograft Models That Are Dependent on the Protease Release

A. Comparable efficacy induced with equalizing dose of HER2-PAT and HER2-XPAT in BT-474 tumor-bearing mice

B. HER2-XPAT and HER2-PAT induce comparable activation of intratumoral CD3 and CD8 T cells

Figure 3. HER2-XPAT is Preferentially Unmasked in Tumor Tissues

A. A variant of HER2™ XPAT was constructed containing an additional cysteine between HER2 and the XTEN masks on HER2™ to sterically hindered Prodrug: Activated T Cell Engagers

B. HER2-XPAT MTD at 0.2 mg/kg/day.

C. HER-XPAT demonstrates efficacy in HER2™ BT-55 Colorectal Xenograft Model

D. HER2-XPAT demonstrates efficacy in HER2™ BT-474 Tumor-bearing mice

E. HER2-XPAT acts as an agonist for both HER2 and CD3

Figure 4. XEN Masks Significantly Expand Safety Margin of HER2-XPAT vs. PAT in Cynomolgous Monkeys

A. HER2-XPAT 2.0 μg/kg in Cynomolgous Monkeys

B. HER2-XPAT MTD in A 30 μg/kg in Cynomolgous Monkeys

C. Doses, Consistent With Its Strong Safety Profile

Figure 5. Masks on HER2-XPAT Provide Significant Protection from Peripheral T Cell Activations and Cytokine Release Syndrome in NHP Even at 50mg/kg