HER2-XPAT, A Novel Protease-Activatable Prodrug T Cell Engager (TCE), Engineered to Address On-Target, Off Tumor Toxicity and Provide Large Predicted Safety Margins in Non-Human Primates

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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, XPAT or XEYNlated 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 (XPTEN) are attached to the core that stochastically reduce target engagement and extend protein half-life. Protease cleavage sites at the base of the XTN 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, XPTENs should remain predominantly inactive as intact prodrugs. In addition to localized activation, the short half-life of the unmasked PAT form should further widen the therapeutic index while providing the potency of T-cell immunity to improve the eradication of solid tumors.

XPAT PLATFORM

XPATs Are XEYNlated Protease-Activated T Cell Engagers


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

Tumor Microenvironment

Healthy Tissues

Activated T Cell Engagers


Patient-derived tumor

Highly penetrant and persistent

Healthy target cell

Prodrug


In vitro, proteolitically-unmasked HER2-XPATs demonstrate potent cytotoxicity against tumor lines with EGFRs in the single digit range. XEYN masking reduces target-directed T cell cytotoxicity and T-cell activity by up to 13,500-fold. HER2-XPAT, demonstrated a high safety margin, supported by protease stability in circulation and a maximum tolerated exposure that is 500-fold higher than that of its active form (PAT). No CRS or systemic T-cell activation was observed even at 500 mg/kg supportive of minimal CRS risk for XPATs vs standard TCEs.

Negligible clearance of XPAT to fully active PAT occurred in vitro following extended incubation at 37 degrees C in plasma from patients with cancer and healthy humans. XPAT degradation was strongly inhibited by the plasma from the XYPAT+ mice. The degree of PAT generation was determined by size exclusion chromatography and confirmed by flow cytometry in human lymphocytes.

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 the potent modality.

SUMMARY/CONCLUSIONS

RESULTS

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

A. Tumor-directed Cytotoxicity

B. Target-directed T Cell Activation


Figure 2. HER2-XPAT Induces Robust Tumor Regressions in Mice That Are Dependent on the Protease Release Site

A. Comparative efficacy induced with equivalent dosing of HER2-PAT and HER2-XPAT in BT-474 xenograft murine model

B. HER2-PAT and HER2-XPAT Induce comparable activation of intratumoral CD4+ and CD8+ T cells


Figure 3. XEYN Masks Significantly Expand Safety Margin of HER2-XPAT vs. PAT in Cynomolgus Monkeys

A. HER2-XPAT MTD vs 45 mg/kg in Cyns

B. HER2-XPAT MTD vs 2.25 mg/kg in Cyns


Figure 4. HER2-XPAT Induces T Cell Margination at Doses $>2.5$ mg/kg But Does Not Activate Peripheral T Cells or Induce Cytokine Release Syndrome Even at 50mg/kg

A. T Cell Margination

B. Peripheral T Cell Activation


Figure 5. HER2-XPAT is Largely Stably in Circulation of Cynomolgus Monkeys at 25 mg/kg. Consistent With Its Strong Safety Profile


Figure 6. Negligible Amounts of Fully Active PAT Are Generated in vitro in Plasma Samples from Patients with Cancer and Inflammatory Diseases


Table: XPATs demonstrate significant reduction in T-cell cytotoxicity and activity compared to unmasking proteolytic release.

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