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

Fiore Cattaruzza, Ayesha Nazeer, Zachary Lange, Caitlin Koski, Mikhail Hammond, Trang Dao-Pick, Angela Henkensielken, Daniel Hostetter, Mika K. Derynck, Volker Schellenger and Bryan A. Irving

Amunix Pharmaceuticals, Inc. Mountain View, CA

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 toxicity in healthy tissue. To address this challenge, Amunix has developed conditionally active TCEs, XPATs or XENylated Protease-Activatable bispecific T Cell Engagers targeting HER2 and EGFR that exploit 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 stericly 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 rigorously regulated, XPATs should remain predominantly inactive as intact prodrugs. In addition to localized activation, the short half-life of the unmasked TAT form should further widen the therapeutic index while providing the potency of T-cell immunity to eradicate solid tumors.

RESULTS

Figure 1. XTEN Polypeptide Masks on HER2-XPAT 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-XPAT Induces Robust Tumor Regressions in Mice That are Dependent on the Protease Release Site

A. Comparative efficacy induced with equivalent levels of HER2-PAT and HER2-XPAT in BT-474 tumour-bearing mice

B. HER2-XPAT is effective against target tumours with a single dose

C. HER2-XPAT and HER2-PAT induce comparable activation of intratumoral CD8+ and CD4+ T cells

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

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. T1 Cell Margination

B. Peripheral T Cell Activation

C. Plasma Cytokines

Figure 5. HER2-XPAT is Largely Stable in Circulation of Nonhuman Primates at 25 mg/kg, Consistent With Its Strong Safety Profile

Comparison PK Observed Between HER2-XPAT and Non-Cleavable Form in NHPs

Table 1. Plasma Samples incubated with HER2-PAT for 7 days at 37 Degrees C.

Table 2. 20 patients with metastatic melanoma

SUMMARY/CONCLUSIONS

In vitro, proteolytically-optimized HER2-XPATs demonstrate potent cytotoxicity against tumor lines with EC50s in the single-digit mM range. XTEN masking reduces target-directed T cell cytotoxicity and T cell activity by up to 10,000-fold.

In the established 9T4.4a xenograft model, HER2-XPAT induced protease-dependent tumor regressions at equimolar doses as the unmasked (active) T cell engager.

In cynomolgus monkeys, HER2-XPAT demonstrated a high safety margin, supported by its protease stability in circulation and a maximum tolerated exposure of 500-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.

Negligible clearance of XAT to fully active PAT occurred in vivo following extended incubation at 37 degrees C in plasma from patients with cancer. This may be due to a significant decrease in the abundance of protease inhibitors in circulation even in disease states.

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