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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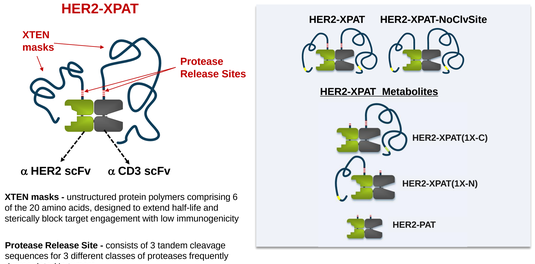
INTRODUCTION

Bispecific T Cell Engagers (TCEs) have been effective at inducing remissions in hematologic cancers, 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 conditionally-activated TCE, XPAT or **X**TENylated **P**rotease-**A**ctivated 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 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 potentially improve the eradication of solid tumors.

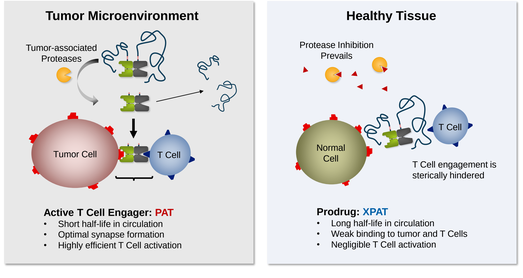
XPAT PLATFORM

XPATs Are XTENylated Protease-Activated T Cell Engagers



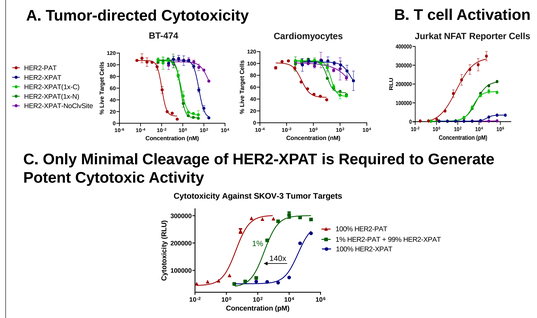
- **XTEN masks -** unstructured protein polymers comprising 6 of the 20 amino acids, designed to extend half-life and sterically block target engagement with low immunogenicity
- **Protease Release Site -** consists of 3 tandem cleavage sequences for 3 different classes of proteases frequently dysregulated in tumors

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



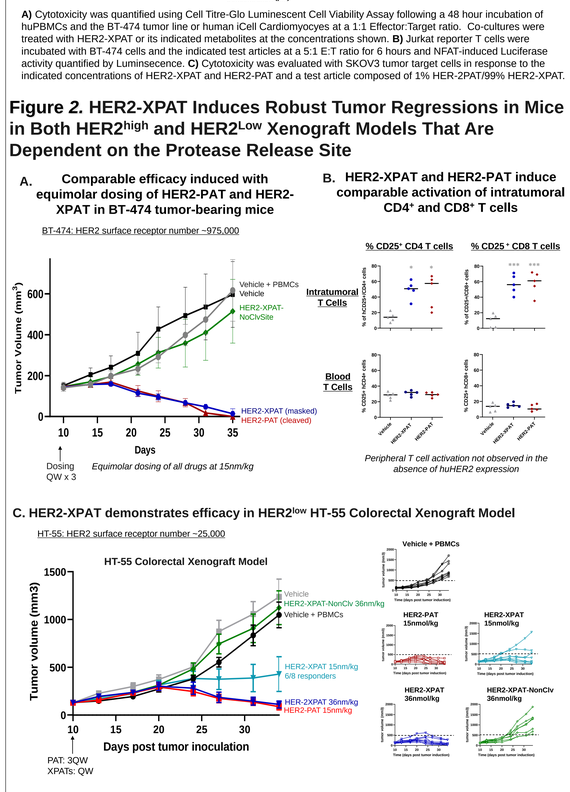
RESULTS

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



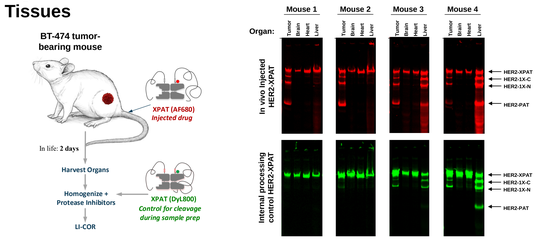
A) Cytotoxicity was quantified using Cell Titre-Glo Luminescent Cell Viability Assay following a 48 hour incubation of huPBMCs and the BT-474 tumor line or human iCell Cardiomyocytes at a 1:1 Effector:Target ratio. Co-cultures were treated with HER2-XPAT or its indicated metabolites at the concentrations shown. **B)** Jurkat reporter T cells were incubated with BT-474 cells and the indicated test articles at a 5:1 E:T ratio for 6 hours and NFAT-induced Luciferase activity quantified by Luminescence. **C)** Cytotoxicity was evaluated with SKOV3 tumor target cells in response to the indicated concentrations of HER2-XPAT and HER2-PAT and a test article composed of 1% HER-2PAT/99% HER2-XPAT.

Figure *2.* HER2-XPAT Induces Robust Tumor Regressions in Mice in Both HER2^{high} and HER2^{Low} Xenograft Models That Are Dependent on the Protease Release Site



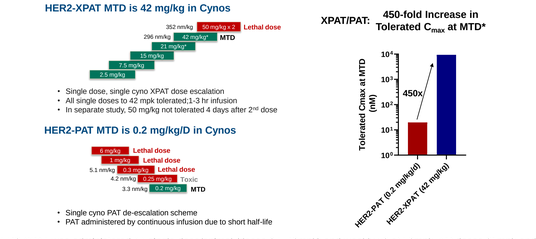
A) NOG mice were inoculated SC with 2x10^7 BT-474 tumor cells, engrafted with 1x10^7 huPBMCs on Day 8 and treated 2 days later at MTV of 147 mm^3 with the indicated test articles at equimolar doses QW for 3 weeks. Lack of tumor growth inhibition by the Non-cleavable HER2-XPAT-NoClvSite demonstrates the requirement of protease cleavage for XPAT efficacy **B)** From an independent BT-474 efficacy experiment conducted as in A), the activation status of tumor infiltrating T cells was evaluated by flow cytometry on Day 18 post-TIW dosing of HER2-XPAT and HER2-PAT at 15 nmol/kg. C. NPSG mice were inoculated SC with 5 × 10^6 HT-55 tumor cells, engrafted with 1x10^7 huPBMCs on Day 6 and treated on D10 (at MTV of 129 mm3) with HER2-XPAT and HER2-XPAT-NonClv at 15 and 36 nm/kg doses QW for 3 weeks. PAT was administered at 15nm/kg 3QW. Lack of tumor growth inhibition by the non-cleavable XPAT format demonstrates the requirement of protease cleavage for XPAT efficacy.

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



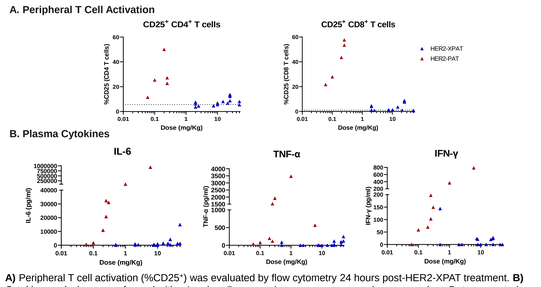
A variant of HER2-XPAT was constructed containing an additional cysteine between HER2-PAT and the C-terminal protease release site. The fluorescent dyes Alexa Fluor 680 and DyLight 800 were conjugated to this variant and the AF 680 form (red) injected into mice bearing BT-474 tumors for 2 days. The indicated organs were harvested and homogenized in the presence of protease inhibitors and the Dylight 800-labeled internal control (green). Homogenates were separated by SDS-PAGE and scanned on a LI-COR Odyssey imager. Bands corresponding to the indicated metabolites were quantified, subtracting the % cleavage obtained during processing from that obtained in vivo. An average of 25.2% cleavage to HER2-PAT was observed in tumor homogenates.

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



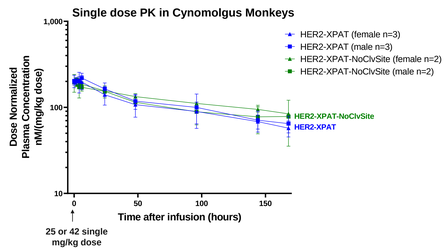
HER2-XPAT was administered IV, single dose/animal (doses 2.5-42mpk) and weekly x2 at 50mpk. *At doses 21mpk and above, a variant of HER2-XPAT with a shorter C-terminal mask was used. HER2-PAT was administered by continuous infusion due to its short half-life. Plasma concentrations of HER2-XPAT were measured by Electrochemiluminescent Immunoassay (ECLIA) using recombinant HER2 capture and an antibody directed against the XTEN masks for detection. The Cmax values for HER2 PAT were determined by ECLIA utilizing an a-idiotypic Ab directed against the a-CD3 scFv as capture and recombinant HER2 as detection. *The 450-fold value derives from an average value for Cmax for animals dosed with HER2-XPAT at 42mg/kg and the updated assessment for HER2-PAT MTD at 0.2 mg/kg/day.

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



A) Peripheral T cell activation (%CD25+) was evaluated by flow cytometry 24 hours post-HER2-XPAT treatment. **B)** Cytokine analysis was performed with a Luminex® suspension array system on plasma samples. Data presented are maximal values measured between 6-24 hours at each evaluated dose.

Figure 7. HER2-XPAT Protease Release Site Remains Largely Stable in Circulation of Cynomolgus Monkeys Even at High Doses, Consistent With its Strong Safety Profile



A) HER2-XPAT or its non-cleavable counterpart, HER2-XPAT-NoClvSite were administered as a single IV dose of 25 or 42 mg/kg. Dose normalized plasma drug concentrations were measured by ECLIA* using recombinant HER2 as capture and an antibody directed against the XTEN mask for detection. *ECLIA = Electrochemiluminescent Immunoassay **B)** Concentrations of singly-cleaved HER2-XPAT(1X-C) and HER2-XPAT(1X-N) were measured in plasma from cynomolgus monkeys administered HER2-XPAT. Quantitative Western was performed using an antibody recognizing the anti-HER2 scFv with standards prepared with recombinant versions of the singly-cleaved molecules. Results are expressed as a percentage of the total XTENylated species as measured by ECLIA as described above.

SUMMARY/CONCLUSIONS

- *In vitro*, proteolytically-unmasked HER2-PAT demonstrates potent cytotoxicity against tumor lines with EC50s in the single-digit pM 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^{high} BT-474 and HER2^{low} HT-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 cynomolgus monkeys, HER2-XPAT demonstrated a high safety margin, supported by its protease stability in circulation and a maximum tolerated exposure that was ~450-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 1-3% of singly-cleaved XPAT metabolites were detected in plasma from NHP administered high doses of HER2-XPAT (25 & 42mg/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