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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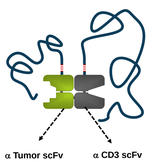
INTRODUCTION

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 **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 improve the eradication of solid tumors.

XPAT PLATFORM

XPATs Are XTENylated Protease-Activated T Cell Engagers



Tandem scFvs

- Binds CD3 and tumor target
- Short half-life in circulation
- Small, flexible design may foster immunological synapse formation

XTEN Polypeptides: Universal Masks

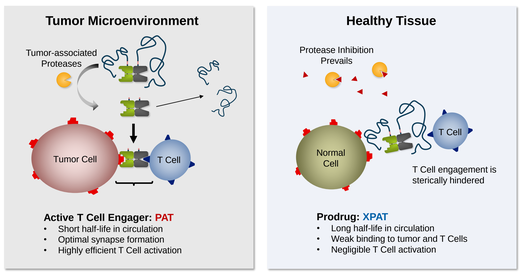
- Masks antigen binding and sterically blocks target engagement; no need to tailor by target
- Sequence comprising 6 amino acids designed to be unstructured and have low immunogenicity
- Typically >200 amino acids in length

Tandem Protease Release Sites (RS) RS Site 1 | RS Site 2 | RS Site 3

- Proprietary linker consisting of 3 tandem cleavage sequences for 3 different classes of proteases dysregulated in tumors
- Designed for maximal breadth of anti-tumor activity through unmasking by proteases active in all stages of cancer

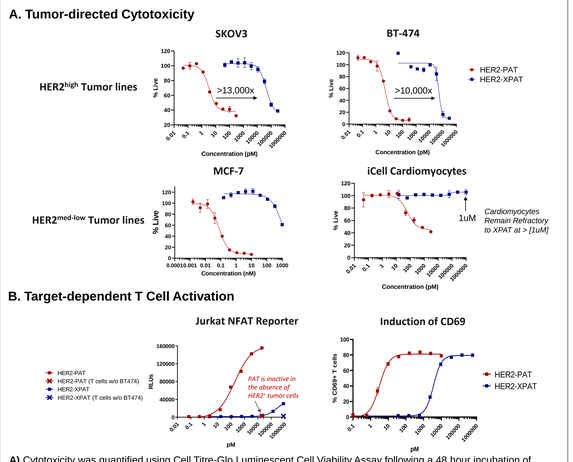
Expressed from a single cDNA in *E. coli*

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



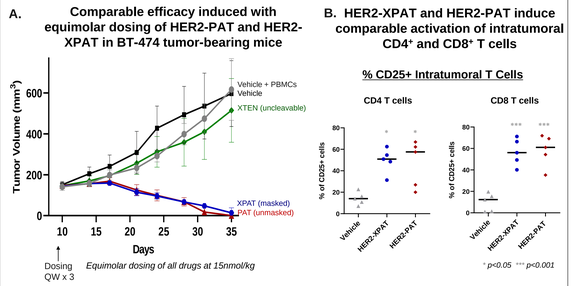
RESULTS

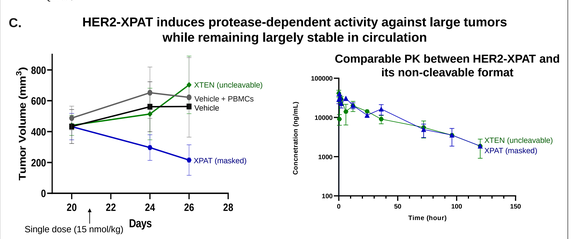
Figure 1. XTEN Polypeptide Masks on HER2-XPAT Significantly Reduce T Cell–Mediated Cytotoxicity and T Cell Activation *in vitro*



A) Cytotoxicity was quantified using Cell Titre-Glo Luminescent Cell Viability Assay following a 48 hour incubation of huPBMCs and the indicated tumor cell lines or human iCell Cardiomyocytes at a 1:1 Effector:Target ratio. Co-cultures were treated with HER2-XPAT or HER2-PAT at the concentrations shown. **B)** Jurkat reporter T cells were incubated with or without BT-474 cells at a 5:1 E:T ratio for 6 hours and NFAT-induced Luciferase activity quantified in response to the test articles. Surface CD69 expression was evaluated on T cells by flow cytometry following a 72 hour co-incubation of PBMCs and SKOV3 cells at a 5:1 E:T ratio with test articles at the indicated concentrations.

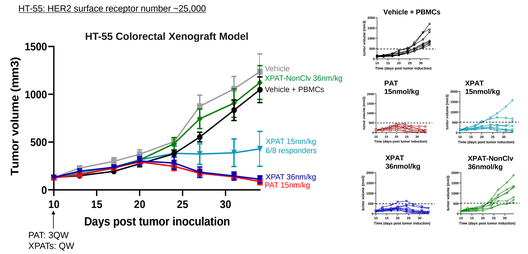
Figure *2.* HER2-XPAT Induces Robust Tumor Regressions in Mice That Are Dependent on the Protease Release Site





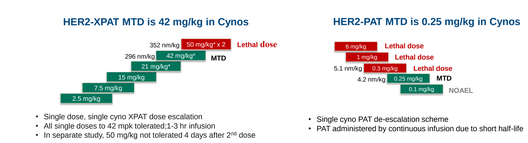
A) NOG mice were inoculated SC with $2x10^7$ BT-474 tumor cells, engrafted with $1x10^7$ huPBMCs on Day 8 and treated 2 days later at MTV of 147 mm^3 with the indicated test articles at equimolar doses QW for 3 weeks. Lack of tumor growth inhibition by the Non-cleavable HER2-XTEN demonstrates the requirement 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)** PBMCs were implanted on Day 8 post-BT-474 tumor inoculation, with a single dose administered on Day 21 when tumors averaged ~444 mm^3. 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

Figure 3. HER2-XPAT is Efficacious in HER2^{low} HT-55 Colorectal Xenograft Model

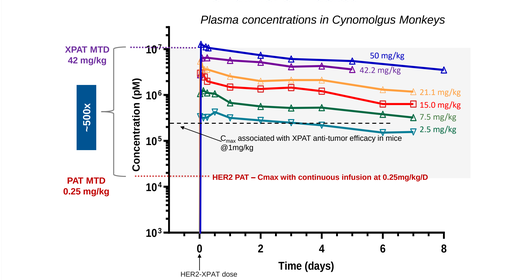


NPSG mice were inoculated SC with 5×10^6 HT-55 tumor cells, engrafted with $1x10^7$ huPBMCs on Day 6 and treated on D10 (at MTV of 129 mm3) with XPAT and XPAT-NonClv at 15 and 36 nm/kg doses QW for 3 weeks. PAT was administered at 15nm/kg 3QW. Lack of tumor growth inhibition by the non-cleavable XPAT format demonstrates the requirement of protease cleavage for XPAT efficacy

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



Masked HER2-XPAT Provides ~500-fold Higher Tolerated Cmax vs. Unmasked PAT



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 XTEN mask was used. HER2-PAT was administered by continuous infusion due to its short half-life. Plasma concentrations of HER2-XPAT were measured by ECLIA* using recombinant HER2 capture and an antibody directed against the XTEN mask 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. *ECLIA = Electrochemiluminescent Immunoassay.

Figure 5. HER2-XPAT Induces T cell Margination at >2.5mpk but Does Not Activate Peripheral T Cells or Induce Cytokine Release Syndrome Even at 50mg/kg

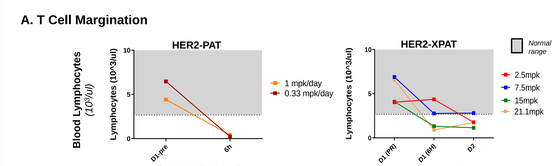
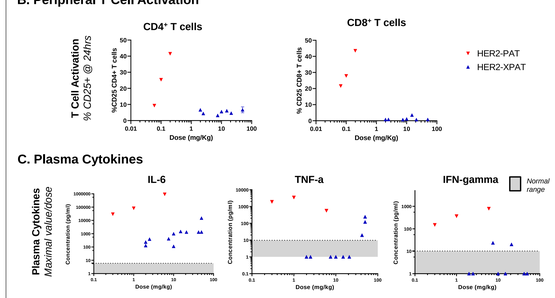
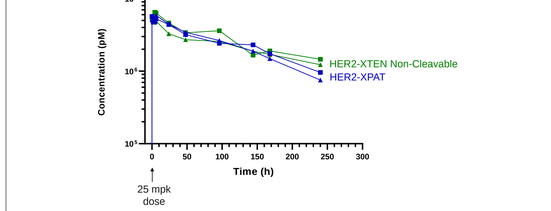


Figure 5B &C. HER2-XPAT Does Not Activate Peripheral T Cells or Induce Cytokine Release Syndrome Even at 50mg/kg



A) Blood Lymphocyte counts decline sharply at 6 or 24 hours post-dose, consistent with T cell margination **B)** Peripheral T cell activation (%CD25+) was evaluated by flow cytometry 24 hours post-HER2-XPAT treatment. **C)** 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 6. HER2-XPAT is Largely Stable in Circulation of Cynomolgus Monkeys at 25 mg/kg, Consistent With its Strong Safety Profile



HER2-XPAT or its non-cleavable counterpart, HER2-XTEN were administered as a single IV dose of 25 mg/kg. 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

SUMMARY/CONCLUSIONS

- *In vitro*, proteolytically-unmasked HER2-XPATs demonstrate potent cytotoxicity against tumor lines with EC50s in the single-digit pM range. XTEN masking reduces target-directed T cell cytotoxicity and T cell activation by up to 13,000-fold
- In the established BT-474 xenograft model, HER2-XPAT induced protease-dependent tumor regressions at equimolar doses as the unmasked (active) T cell engager while remaining stable in circulation. HER2-XPAT was also efficacious in the HER2^{low} HT-55 colorectal xenograft model that expresses only ~25,000 surface HER2 receptors, opening the clinical potential to treat HER2$^{med\text{-}low}$ tumors
- In cynomolgus monkeys, HER2-XPAT demonstrated a high safety margin, supported by its protease stability in circulation and a maximum tolerated exposure that was ~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
- 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