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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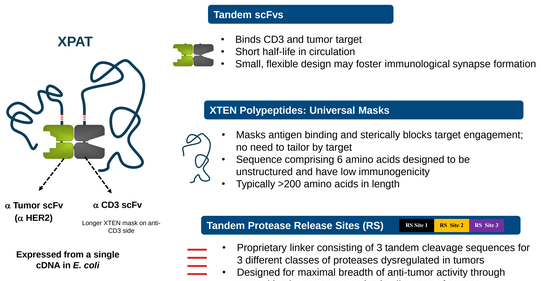
INTRODUCTION

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

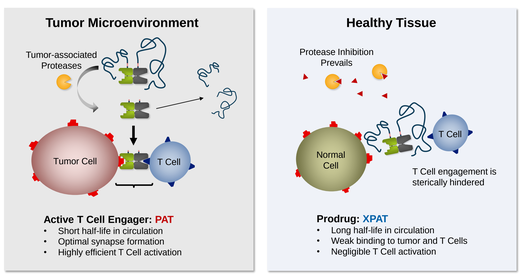
XTEN Polypeptides: Universal Masks

- 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

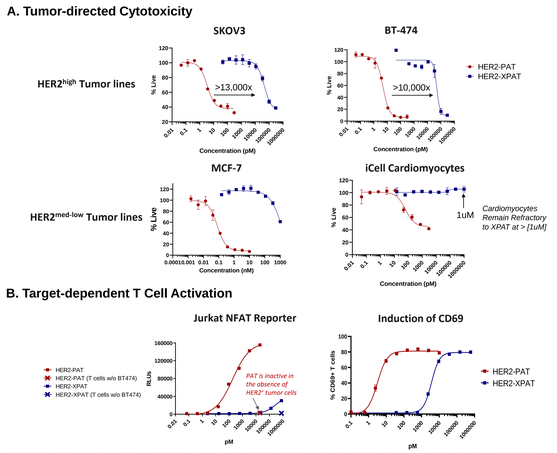
- Proprietary linker consisting of 3 tandem cleavage sequences for 3 different classes of proteases dysregulated in tumors
- Designed for maximal breadth of anti-tumor activity through unmasking by proteases active in all stages of cancer

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



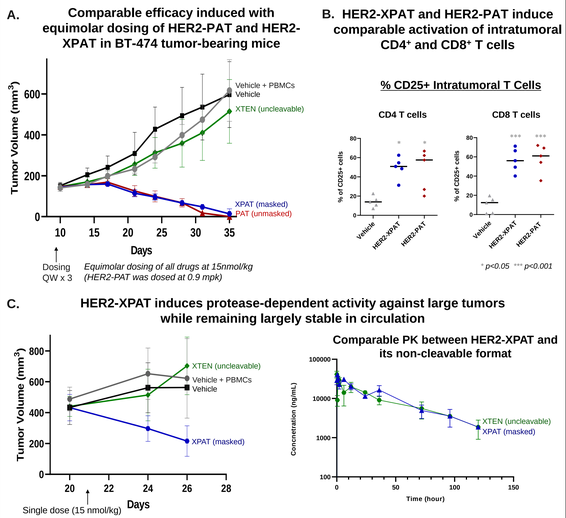
RESULTS

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



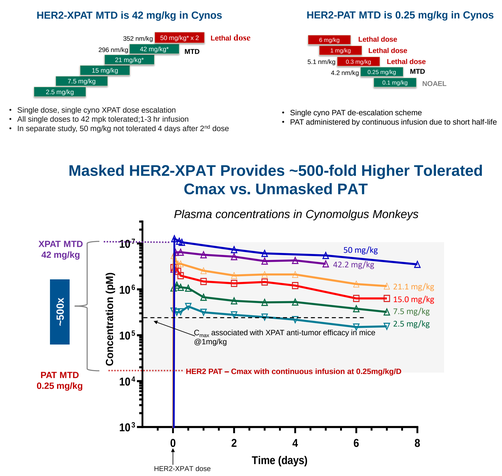
A) Cytotoxicity was quantified using Cell Titre-Glo Luminescent Cell Viability Assay following a 48 hour incubation of huPBMCs and the indicated tumor cell lines or human iCell Cardiomyocytes at a 1:1 Effector:Target ratio. Co-cultures were treated with HER2-XPAT or HER2-PAT at the concentrations shown. **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



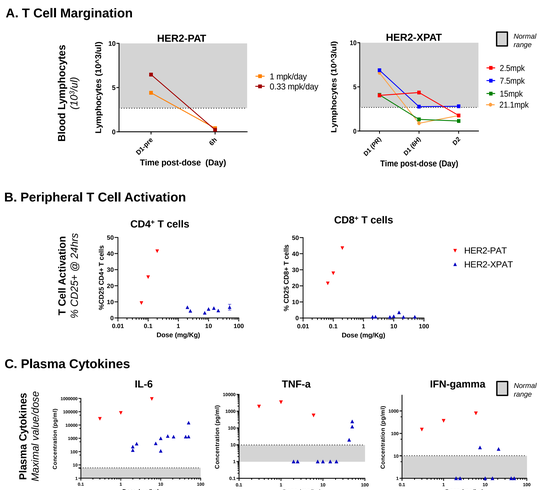
A) NOG mice were inoculated SC with $2x10^7$ BT-474 tumor cells, engrafted with $1x10^7$ huPBMCs on Day 8 and treated 2 days later at MTV of 147 mm^3 with the indicated test articles at equimolar doses QW for 3 weeks. Lack of tumor growth inhibition by the Non-cleavable HER2-XTEN demonstrates the requirement 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. XTEN Masks Significantly Expand Safety Margin of HER2-XPAT vs. PAT in Cynomolgus Monkeys



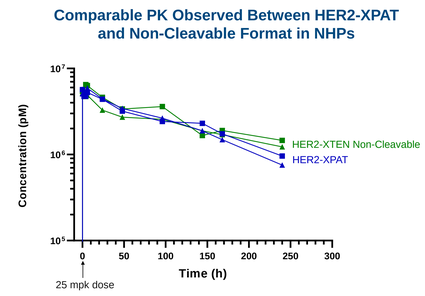
HER2-XPAT was administered IV, single dose/animal (doses 2.5-42mpk) and weekly x2 at 50mpk. *At doses 21mpk and above, a variant of HER2-XPAT with a shorter C-terminal XTEN mask was used. HER2-PAT was administered by continuous infusion due to its short half-life. Plasma concentrations of HER2-XPAT were 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 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



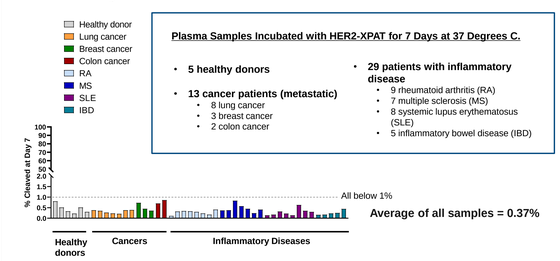
A) Blood Lymphocyte counts decline sharply at 6 or 24 hours post-dose, consistent with T cell margination **B)** Peripheral T cell activation (%CD25+) was evaluated by flow cytometry 24 hours post-HER2-XPAT treatment. **C)** Cytokine analysis was performed with a Luminex® suspension array system on plasma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

Figure 5. 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

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



A) A DyLight-labeled HER2-XPAT molecule was incubated for 7 days at 37 degrees C in plasma from healthy human donors or from patients with the indicated cancers or systemic autoimmune diseases. The degree of PAT generation was determined by size exclusion chromatography exploiting the fluorescence from the DyLight-labeling to increase sensitivity of detection.

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
- 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
- Negligible cleavage of XPAT to fully active PAT occurred in vitro following extended incubation at 37 degrees C in plasma from patients with cancer or systemic inflammatory disorders, consistent with 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 significant increase in the therapeutic index and expansion of target landscape for this potent modality