EGFR-XPAT, A Novel Prodrug T Cell Engager (TCE) Engineered to Address *On*-Target, *Off*-Tumor Toxicity and an Orthogonal Approach for Cancer Immunotherapy in EGFR, KRAS/BRAF Cancers

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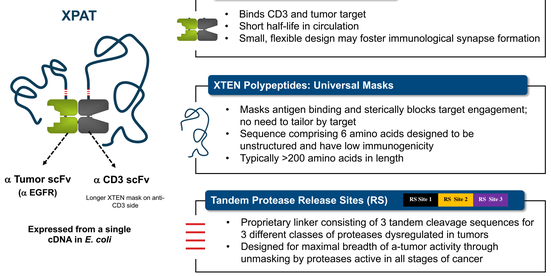
INTRODUCTION

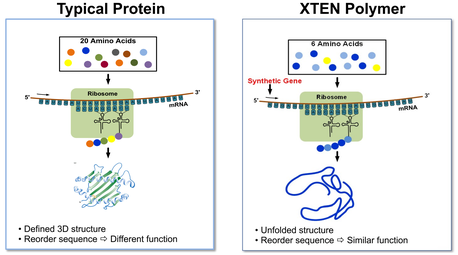
Bispecific T cell Engagers (TCEs) can overcome common obstacles to immunotherapy such as HLA loss or low tumor mutational burden, providing the potency of T cell immunity to patients with tumors commonly refractory to classic immunotherapies. These "cold" tumors can include EGFR-positive KRAS^{mut}/BRAF^{mut} CRC, NSCLC, and pancreatic cancer. However, given the extreme potency of TCEs, dose-limiting *on*-target, *off*-tumor toxicities have compromised therapeutic index in solid tumors. To address this challenge specifically for EGFR^{+} tumors, Amunix has developed a conditionally activatable EGFR-targeted TCE, EGFR-XPAT (**X**TENylated **P**rotease-**A**ctivated bispecific **T** Cell Engager), that exploits the dysregulated protease activity present in tumors vs. healthy tissues, enabling expansion of the therapeutic index (TI). The core of EGFR-XPAT consists of 2 tandem scFVs targeting CD3 and EGFR. Attached to the core, two unstructured polypeptide masks (XTEN) sterically reduce target engagement and extend protein half-life. Protease cleavage sites encoded at the base of XTEN enable preferential release of XTEN masks in the tumor microenvironment, thereby unleashing a small, highly potent TCE. 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

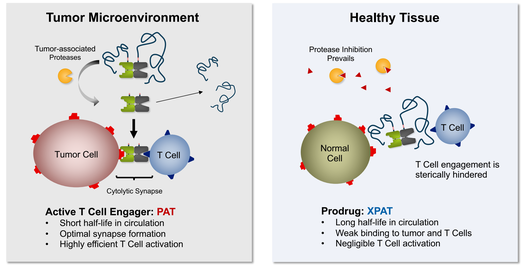


XTEN Mask: A Flexible, Unstructured Protein Polymer By Design



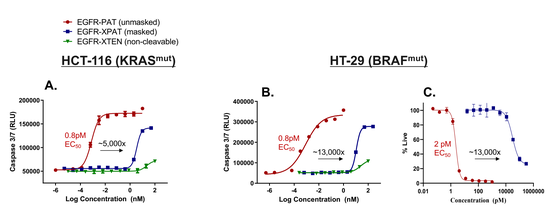
- Extends protein half-life
- Can provide position-dependent steric masking
- Low Immunogenicity Potential
  - Minimal diversity of amino acids for recognition - devoid of aromatic, hydrophobic, and positively-charged amino acids
  - Weak Ab binding potential due to lack of stable 3D structure or conformational epitopes
  - Minimal predicted T cell epitopes due to absence of strong MHC peptide anchor residues
- Clinical Validation of half-life extension and low immunogenicity in >200 patients in the context of human Growth Hormone (SC dosing) and Factor VIII

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



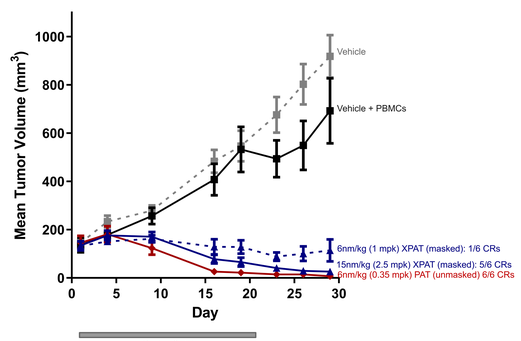
RESULTS

Figure 1. XTEN Polypeptide Masks on EGFR-XPAT Significantly Reduce T Cell–Mediated Cytotoxicity Directed Against KRAS^{mut} and BRAF^{mut} Tumor Lines *in vitro*



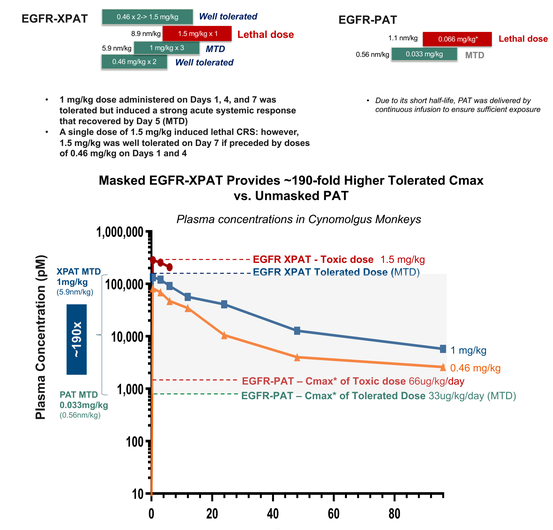
A & B) Cytotoxicity was quantified using Caspase Glo 3/7 Luminescent Cell Viability Assay following a 48 hour co-incubation of huPBMCs and the indicated tumor cell lines at a 10:1 ratio. **C.** Cell Titre-Glo Assay was used as an alternative method to measure cytotoxicity targeting HT-29 cells following a 48hr incubation at a 5:1 Effector:target cell ratio. Co-cultures were treated with increasing concentrations of EGFR-PAT or EGFR-XPAT as shown.

Figure 2. EGFR-XPAT Shows Dose-Dependent Tumor Regressions in BRAF Mutant HT-29 CRC Treatment Model



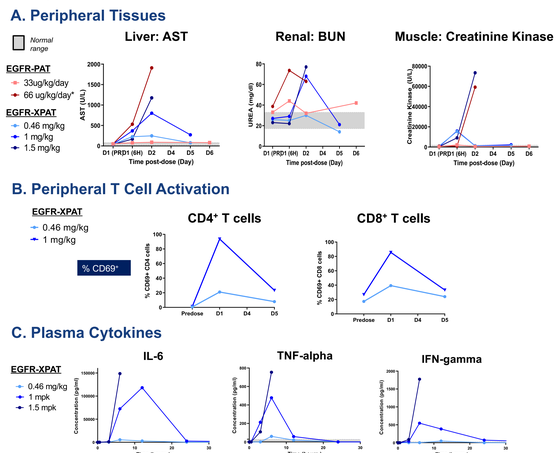
NOG mice were implanted SC with 1x10^{7} HT-29 tumor cells, engrafted with 1x10^{7} huPBMCs on Day 15 and treated with test articles at the indicated doses TIW for 3 weeks once tumors reached ~150mm3

Figure 3: XTEN Masks Significantly Expand Safety Margin of EGFR-XPAT vs PAT in Cynomolgus Monkeys



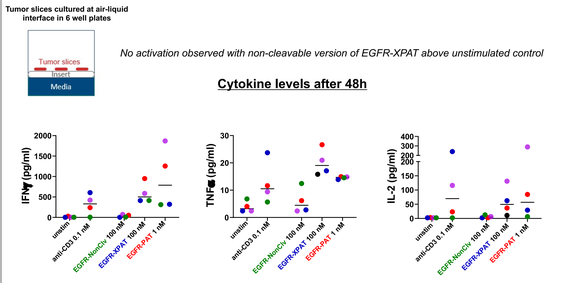
EGFR-XPAT was administered IV in NHPs at the indicated doses; XPAT dosing was scheduled for days 1, 4 and 7. *EGFR-PAT was administered by continuous infusion at 66ug/kg/day following a 1 hour bolus dose of 8ug/kg. Plasma concentrations of EGFR-XPAT were measured by ECLIA using recombinant EGFR as capture and an anti-XTEN antibody for detection. *The Cmax values for EGFR PAT were determined by ECLIA utilizing an a-idiotypic Ab for the a-CD3 scFv as capture and recombinant EGFR as detection. ECLIA = Electrochemiluminescent Immunoassay

Figure 4: Minimal Systemic Activity Observed with EGFR-XPAT at 0.46 mg/kg. At its 1 mg/kg MTD, XPAT Induces Transient Tissue Toxicities, Activation of Peripheral T cells, and Release of Cytokines



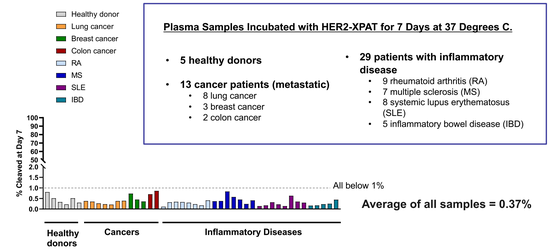
A) Serum chemistry results show dose-dependent increases in liver, kidney and skeletal muscle enzymes with EGFR-PAT and EGFR-XPAT. **B)** Peripheral T cell activation (%CD69+) was evaluated by flow cytometry 24 hours post-EGFR-XPAT dosing: dose-dependent upregulation of CD69 was observed. **C)** Cytokine levels in cyno plasma were measured using Luminex® suspension array

Figure 5: Cleavage-dependent Activation of EGFR-XPAT Detected *in vitro* in Primary Human Tumor Explant Cultures



Slices of primary human tumor explants from NSCLC and breast cancers were incubated for 48 hours at the air-liquid interface in 6 well plates in DMEM with the indicated test articles and concentrations. A positive control anti-CD3 antibody was included to directly activate cytokine release from tumor–resident T cells. After 48 hrs, supernatants were collected and the indicated cytokines measured by Meso Scale electrochemiluminescence. The cleavage dependence for cytokine secretion by XPAT is consistent with proteolytic release of the XTEN masks, leading to effective cross-bridging between tumor-expressed EGFR and CD3 on tumor-resident T cells. Methods are currently being developed to quantify the degree of cleavage of XPATs in human tumor tissue.

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



A DyLight-labeled HER2-XPAT molecule containing the identical protease release site present in EGFR-XPAT 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 EGFR-XPAT (PAT) demonstrates 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 HT-29 Braf^{mut} model, EGFR XPAT induced dose-dependent tumor regressions with efficacious doses within 2.5-fold of the unmasked (active) T cell engager
- In cynomolgus monkeys, masked EGFR-XPAT demonstrated ~190-fold higher tolerated exposures than that of the unmasked PAT, suggesting favorable therapeutic index even for a target as broadly expressed as EGFR
  - At the MTD (1mpk), cytokine spikes and peripheral organ toxicities were observed that resolved by Day 5
- Preliminary results from in vitro primary human tumor explant experiments demonstrate protease-dependent activation of EGFR-XPATs as measured by induction of cytokines from tumor-resident T cells. In contrast, minimal cleavage to active PAT was observed in vitro from the protease release site contained in EGFR-XPAT following extended incubation in plasma from patients with cancer or inflammatory diseases
- 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