INTRODUCTION
Bispecific T cell Engagers (TCEs) can overcome common obstacles to immunotherapy such as an HLA bias 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/BBRAF-MSK, NSCLC, and pancreatic cancer. However, 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 (XPATylated Protease-Activated bispecific T Cell Engager), that exploits the dysregulated protein expression present in tumors vs. healthy tissues, enabling expansion of the therapeutic index (Ti). The core of EGFR-XPAT consists of 2 tandem sFv's targeting CD3 and EGFR. Attached to the core, two unstructured polyepitope masks (XPATs) stochastically reduce target engagement and extend protease half-life. Proteasome cleavages also encoded at the back of XPAT enable preferential release of XENAT in the tumor microenvironment, thereby utilizing 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.

EGFR-XPAT, A Novel Prodrug T Cell Engager (TCE) Engineered to Address On-Target, Off-Tumor Toxicity and an Oncolytic Approach for Cancer Immunotherapy in EGFR, KRAS/BRAF Cancers
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BIOSPECIFIC T CELL ENGAGERS (TCEs) CAN OVERCOME COMMON OBSTACLES TO IMMUNOTHERAPY SUCH AS AN HLA BIAS 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/BBRAF-MSK, NSCLC, AND PANCREATIC CANCER. HOWEVER, 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 (XPATYLATED PROTEASE-ACTIVATED BISPECIFIC T CELL ENGAGER), THAT EXPLOITS THE DYSREGULATED PROTEIN EXPRESSION PRESENT IN TUMORS VS. HEALTHY TISSUES, ENABLING EXPANSION OF THE THERAPEUTIC INDEX (TI). THE CORE OF EGFR-XPAT CONSISTS OF 2 TANDEM SFV'S TARGETING CD3 AND EGFR. ATTACHED TO THE CORE, TWO UNSTRUCTURED POLYPEPTIDE MASKS (XPATs) STOCHASTICALLY REDUCE TARGET ENGAGEMENT AND EXTEND PROTEASE HALF-LIFE. PROTEASOME CLEAVAGES ALSO ENCODED AT THE BACK OF XPAT ENABLE PREFERENTIAL RELEASE OF XENAT IN THE TUMOR MICROENVIRONMENT, THEREBY UTILIZING 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 XPATylated Protease-Activated T Cell Engagers

XPAT MASK: A FLEXIBLE, UNSTRUCTURED PROTEIN POLYMORPH BY DESIGN

Figure 1. XENAT Polyepitope Masks on EGFR-XPAT Significantly Reduce T Cell-Mediated Cytotoxicity Directed Against KRAS/MSK and BBAF/MSK Tumor Lines in vitro

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

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

Figure 4: Minimal Systemic Activity Observed with EGFR-XPAT at 0.46 mg/kg. At 8.9 mg/kg, MTD, XPAT induces Transient Tissue Toxicities, Activation of Peripheral T cells, and Release of Cytokines

SUMMARY/CONCLUSIONS

- In vitro, proteasemucolytically-unmasked EGFR-XPAT (XPAT) demonstrates potent cytotoxicity against tumor lines with CD30s in the single-digit range. XENAT masks reduce background T cell activation by up to 1000-fold.

- In the established HT-29 Braf-mutant model, EGFR-XPAT induced dose-dependent tumor regressions with efficacious doses within a 3-fold of the unmasked active T cell engager.

- In immunocompetent monkeys, masked EGFR-XPAT demonstrated ~190-fold higher tolerated exposures than that of the unmasked PAT, suggestive of favorable therapeutic index even for a target on broadly expressed as EGFR.

- At the MTD (8.9 mg/kg), cytokine spikes and peripheral organ toxicities were observed that resolved by Day 5.

- Preliminary results from in vivo primary human tumors indicate improved on-target, off-tumor safety compared to the preclinical release in cynomolgus monkeys. The preliminary results from the xenograft model using EGFR-XPAT following extended incubation in plasma and normal serum revealed no signs of toxicity.

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

Table 1: Pharmacokinetic and Tumor Development in Malignant Tissue

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cytokine Levels</th>
<th>Tumor Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTD</td>
<td>High</td>
<td>Significant</td>
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<tr>
<td>Low</td>
<td>Low</td>
<td>No regression</td>
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Figure 5: Cleavage-dependent Activation of EGFR-XPAT Detected in vitro in Primary Human Tumor Explant Cultures

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

Table 2: Cytokine Levels in Plasma After Treatment with EGFR-XPAT

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Baseline</th>
<th>After Treatment</th>
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<tbody>
<tr>
<td>TNF-alpha</td>
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<td>High</td>
</tr>
<tr>
<td>IL-6</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>IFN-gamma</td>
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<td>High</td>
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