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AMX-818, a Protease-Activated T Cell Engager Targeting HER2 with Potent T Cell Activation, Proteolytic Cleavage and Efficacy in Xenograft Tumors, and Wide Safety Margins in Non-Human Primates

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I have the following financial relationships to disclose:

Stockholder in: Amunix Pharmaceuticals

Employee of: Amunix Pharmaceuticals

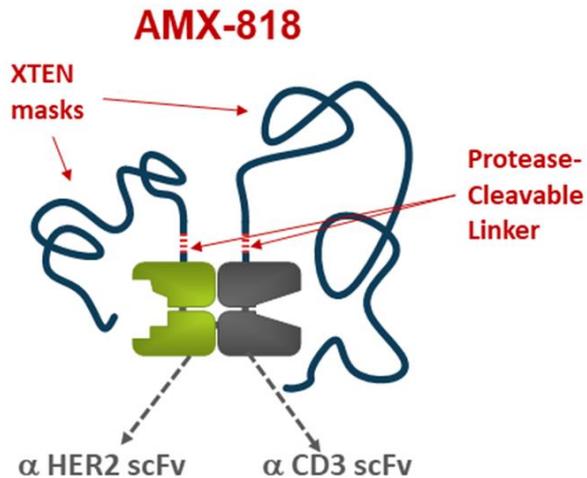
I will not discuss off label use and/or investigational use in my presentation.

Introduction

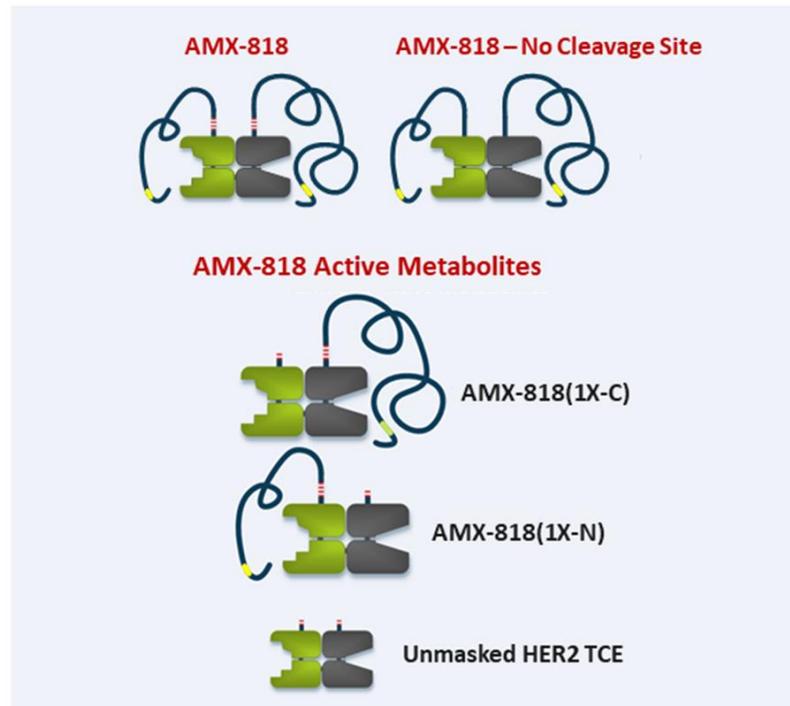


Bispecific T Cell Engagers (TCEs) have been effective at inducing remissions in hematologic cancers, but their use in solid tumors has been limited by their extreme potency and *on-target*, *off-tumor* toxicities in healthy tissue. Consequently, TCEs targeting HER2 have been unable to achieve a therapeutic index in clinical trials. To address this challenge, Amunix has developed a conditionally-activated TCE that targets HER2 using our proprietary XPAT (**X**TENylated **P**rotease-**A**ctivated **T** Cell Engager) platform that exploits the dysregulated protease activity present in tumors vs. healthy tissues, thereby enabling expansion of the therapeutic index. Two unstructured polypeptide masks (XTEN) that sterically reduce target engagement and extend protein half-life are attached to the core. These universal, tunable masks act by spatial shielding and can be customized based on number, length and position on the target protein. The protease-cleavable linkers at the base of the XTEN masks enable proteolytic activation of the XPAT in the tumor microenvironment, unleashing a small, highly potent TCE. In healthy tissues, where protease activity is tightly regulated, XPATs remain predominantly inactive. In addition to localized activation, the short half-life of the unmasked TCE form serves to further widen the therapeutic index while providing the potency of T cell immunity to potentially improve the treatment of solid tumors.

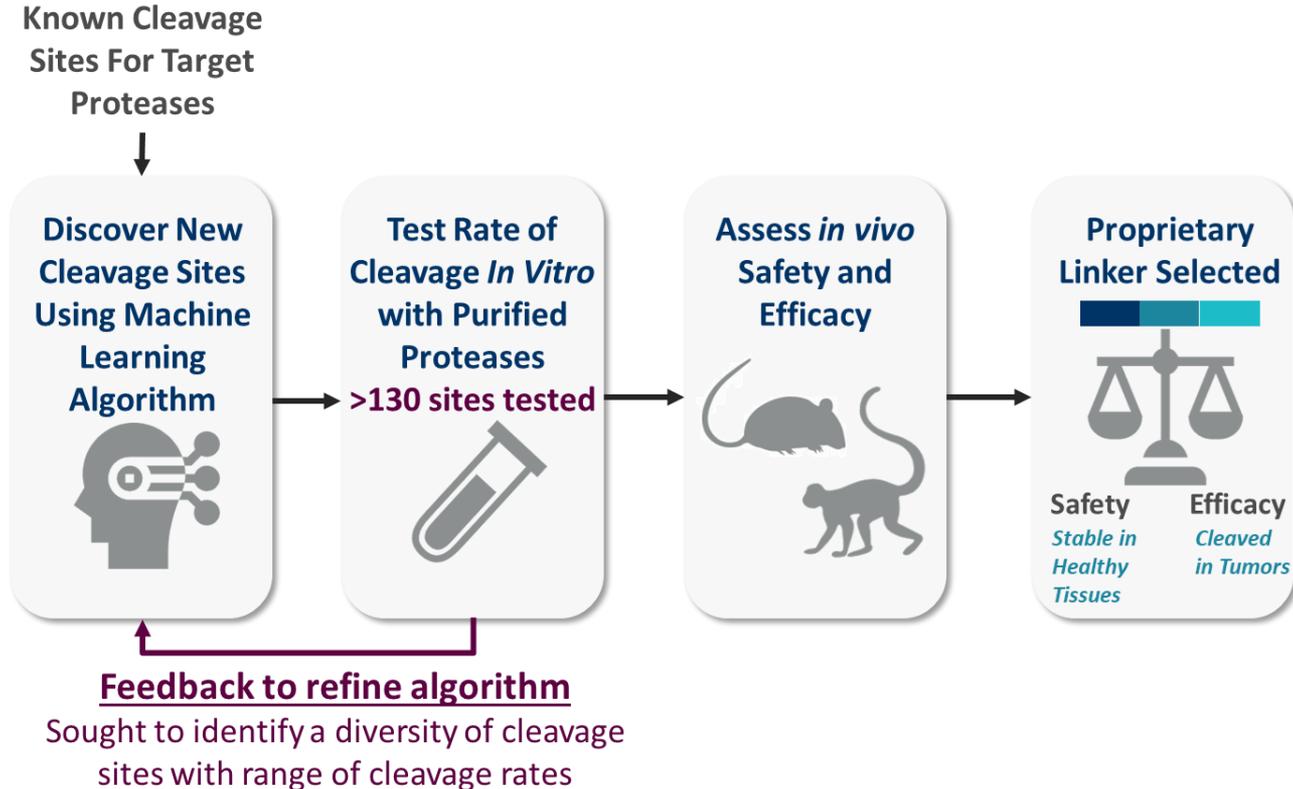
XPATs Are XTENylated Protease-Activated T Cell Engagers



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

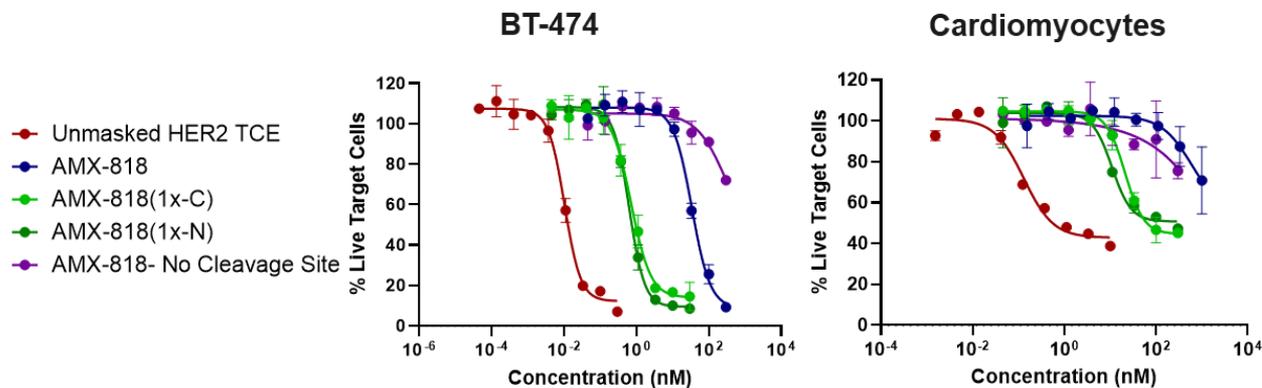


Robust Discovery Process Produces XPATs that Balance Cleavability in Tumor and Stability in Healthy Tissues

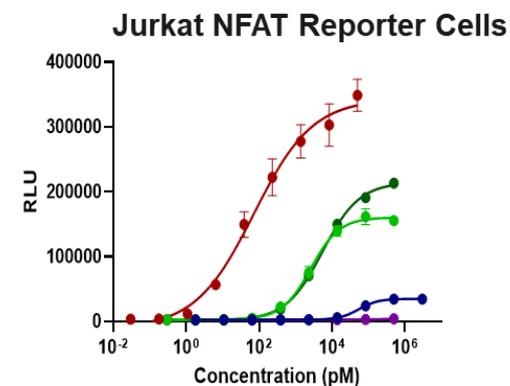


Double Masking using 2 XTEN Polypeptide Masks on AMX-818 vs. Single Masking Significantly Reduces T Cell-Mediated Cytotoxicity and T Cell Activation *in vitro*

A. Tumor-directed Cytotoxicity



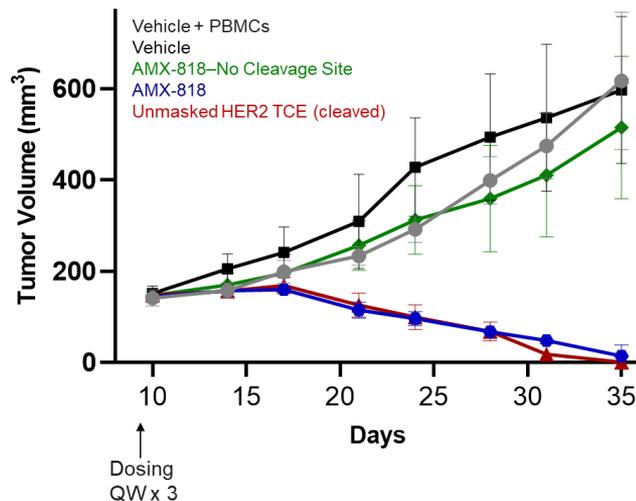
B. T cell Activation



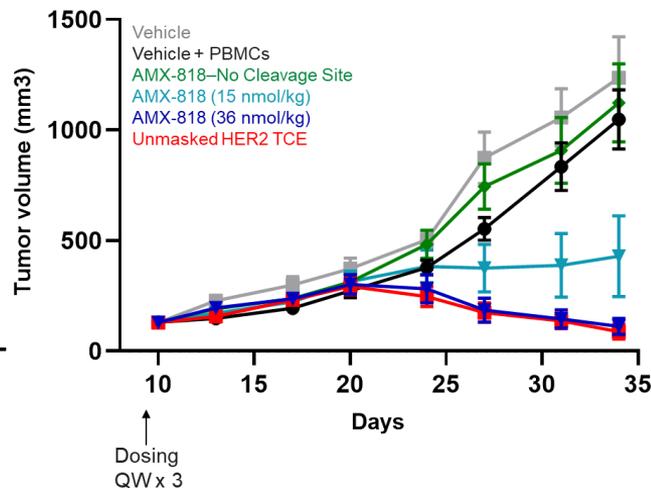
A) Cytotoxicity was quantified using the CellTiter-Glo® Luminescent Cell Viability Assay following a 48-hour incubation of huPBMCs and the BT-474 tumor line or human iCell Cardiomyocytes at a 1:1 Effector:Target ratio. Co-cultures were treated with AMX-818 or its indicated metabolites at the concentrations 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.

AMX-818 Induces Robust Protease Cleavage Dependent Tumor Regressions in Mice in Both HER2^{high} and HER2^{Low} Xenograft Models

A. BT-474 Breast Model:
(~975,000 HER2 receptors)

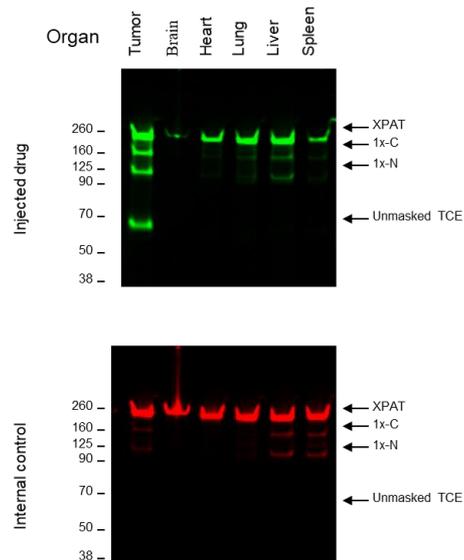
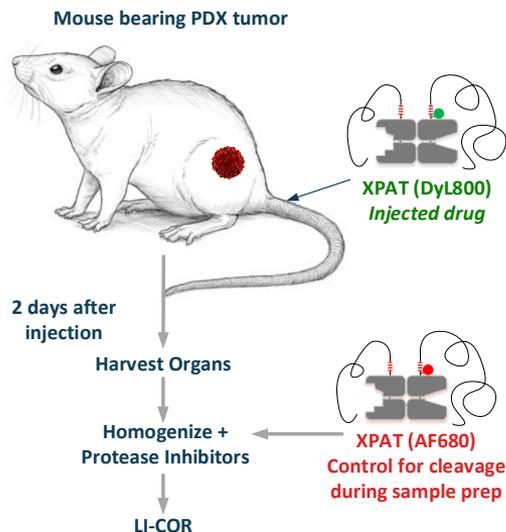


B. HT-55 Colorectal Model:
(~25,000 HER2 receptors)

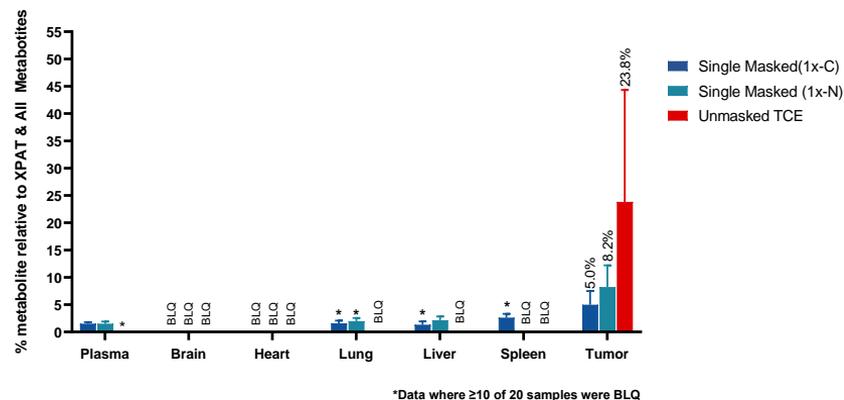


A) NOG mice were inoculated subcutaneously (SC) with 2×10^7 BT-474 tumor cells, engrafted with 1×10^7 huPBMCs on Day 8 and treated 2 days later at MTV of 147 mm³ with the indicated test articles at equimolar doses of 15nmol/kg QW for 3 weeks. Lack of tumor growth inhibition by the non-cleavable variant of AMX-818 demonstrates the requirement of protease cleavage for AMX-818 efficacy. **B)** NPSG mice were inoculated SC with 5×10^6 HT-55 tumor cells, engrafted with 1×10^7 huPBMCs on Day 6 and treated on D10 at MTV of 129 mm³ with AMX-818 at 15nmol/kg (light blue) and 36 nmol/kg (dark blue) doses QW for 3 weeks. Unmasked HER2 TCE was administered at 15nmol/kg TIW x 3. Again, lack of tumor growth inhibition by the non-cleavable AMX-818 variant, dosed at 36 nmol/kg, demonstrates the requirement of protease cleavage for AMX-818 efficacy.

Preferential Cleavage within Tumors to Unmasked TCE Observed *in vivo* in PDX Tumor-Bearing Mice with an Average of 24% Cleavage to Fully Active TCE after Two Days



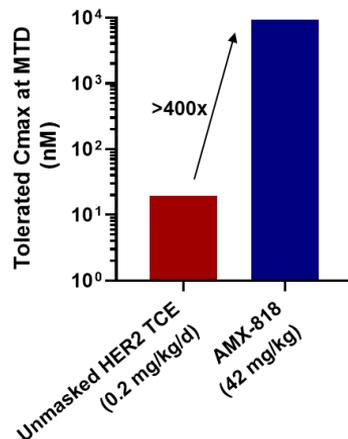
An Average of 24% Cleavage to Fully Active TCE in Tumors and Minimal to No Cleavage in Plasma and Healthy Tissues is Observed in PDX Tumor-Bearing Mice after Two Days



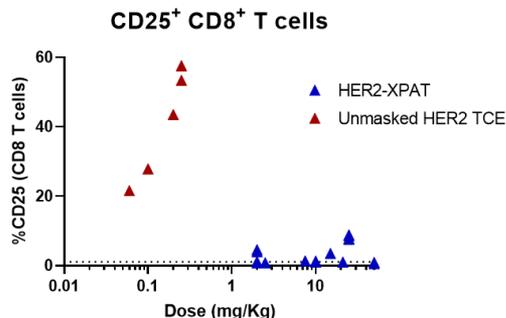
A variant of AMX-818 and an EpCAM-targeted XPAT with the identical protease cleavage linkers were constructed containing an additional cysteine between the TCE core and the C-terminal protease-cleavable linker. The fluorescent dyes Alexa Fluor 680 and DyLight 800 were conjugated to this variant and the DyLight 800 form (green) injected into mice bearing PDX tumors. The indicated tissues were harvested after 2 days and homogenized in the presence of protease inhibitors and the corresponding Alexa Fluor 680-labeled internal control (red). Homogenates were separated by SDS-PAGE and scanned on a LI-COR Odyssey imager. Bands corresponding to the indicated metabolites were quantified, and the internal control was used to correct for the amount of unmasking post-harvest. In plasma, 9/20 mice were BLQ for the unmasked TCE and thus the result $<0.06\%$ overestimates average cleavage. Samples with fewer than 3 quantifiable data points were treated as BLQ.

XTEN Masks Significantly Expand Safety Margin of AMX-818 vs. Unmasked TCE in NHPs

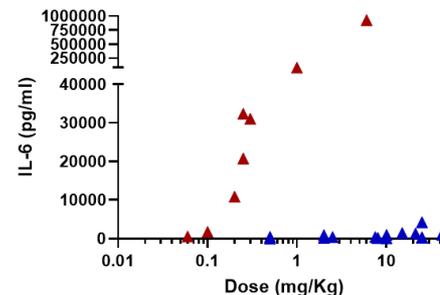
XPAT/uTCE: >400-fold Increase in Tolerated C_{max} at MTDs*



Peripheral T Cell Activation



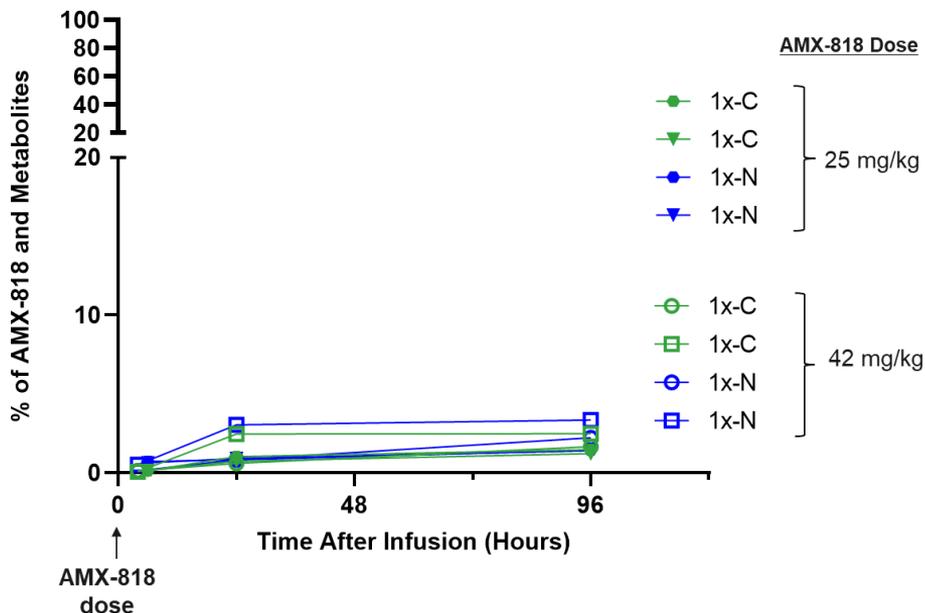
Plasma IL-6



AMX-818 was administered intravenously (IV), single dose/animal (doses 2.5-42mpk). *At doses below 21mpk, a variant of AMX-818 with a longer C-terminal mask was used. Unmasked HER2 TCE was administered by continuous infusion due to its short half-life. Plasma concentrations of AMX-818 were measured by Electrochemiluminescent Immunoassay (ECLIA) using recombinant HER2 capture and an antibody directed against the XTEN masks for detection. The C_{max} values for unmasked HER2 TCE were determined by ECLIA utilizing an α -idiotypic antibody (Ab) directed against the α -CD3 scFv as the capture Ab and recombinant HER2 as the detection Ab. *The >400-fold value derives from an average value for C_{max} for animals dosed with AMX-818 at 42mg/kg and the unmasked HER2 TCE MTD at 0.2 mg/kg/day.

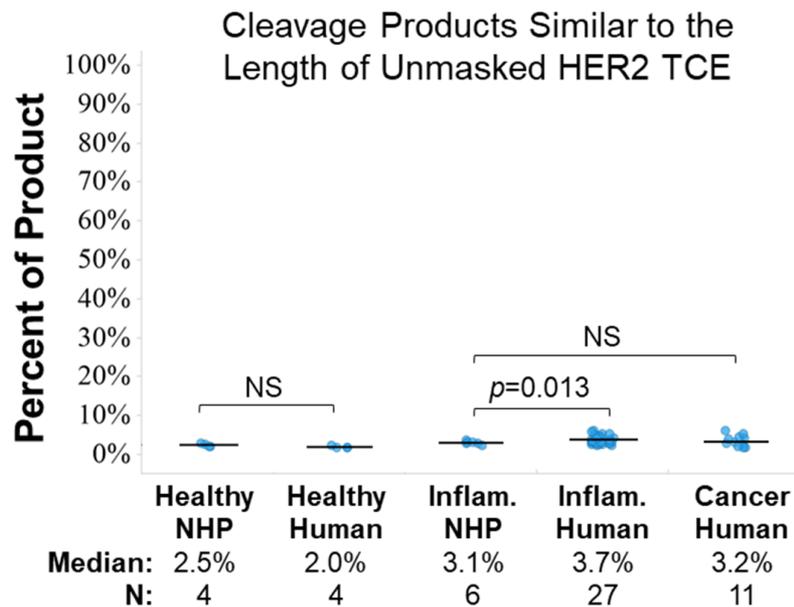
Peripheral T cell activation (%CD25⁺) was evaluated by flow cytometry 24 hours post-AMX-818 treatment. Cytokine analysis was performed with a Luminex® suspension array system on plasma samples. Data presented are maximal values measured between 6-24 hours at each evaluated dose.

AMX-818 is Stable in Circulation in NHPs, with Low Levels of Partially Unmasked Metabolites and Undetectable Levels of Fully Unmasked TCE



Concentrations of singly-cleaved metabolites of AMX-818 (1X-C and 1X-N) were measured by quantitative Western analysis using an antibody recognizing anti-HER2 scFv in plasma from NHPs treated with AMX-818. Results are expressed as % of total XTENylated species (AMX-818 + singly-cleaved metabolites) using molar concentrations. In the same studies, the fully unmasked TCE was below the limit of detection (3 nM), suggesting that it is <0.12% of total XTENylated material (based on observed molar concentrations 96 hours post dose [42 mg/kg]).

Peripheral Stability of AMX-818 in NHPs Translates to Humans Based on Ex Vivo Plasma Stability in Both Species Showing Minimal and Comparable Cleavage to Unmasked Form



Fluorescently-labelled AMX-818 was spiked into plasma, incubated for 7 days at 37°C and AMX-818 and its cleavage products were quantified by Quantitative Western analysis using LI-COR technology. Percent product is presented as relative to the total fluorescent signal of AMX-818-derived species present in the plasma samples. Plasma samples from NHPs with systemic inflammation were collected from NHPs with cytokine release syndrome (CRS), induced by a toxic dose of another TCE. Unpaired t-tests were used to evaluate the difference between healthy human and healthy NHP, humans with cancer and inflamed NHP, and humans with inflammation and NHPs with inflammation. $P < 0.05$ was defined as a statistically significant result. Horizontal black bars represent median values; dots represent individual observations. Given the absence of clearance mechanisms in the experiment, the percent of accumulated cleavage products observed here will overestimate their abundance in vivo.

Summary/Conclusions

- *In vitro*, proteolytically-unmasked AMX-818 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 variants of AMX-818 show intermediate activity relative to unmasked HER2 T cell engager (TCE). Only minimal cleavage of AMX-818 is required to generate potent cytotoxicity.
- In the established HER2^{high} BT-474 and HER2^{low} HT-55 xenograft models, AMX-818 induced protease-dependent tumor regressions comparable to the unmasked (active) TCE while remaining stable in circulation. *In vivo*, preferential cleavage of AMX-818 was demonstrated in patient-derived xenograft tumors relative to healthy organs (average was 24% unmasked TCE in tumors).
- In non-human primates, AMX-818 demonstrated a wide safety margin, supported by its protease stability in circulation and a maximum tolerated exposure that was >400-fold higher than that of its active form (unmasked HER2 TCE). 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 NHPs treated with high doses of AMX-818 (25 & 42mg/kg), while the fully unmasked TCE form was undetectable.
- *In vitro* studies in which AMX-818 was incubated for 7-days at 37°C (conditions which over-represent the accumulation of metabolites *in vivo*) in plasma from healthy and diseased NHPs and humans demonstrate that AMX-818 is stable in plasma with a similar amount of fully unmasked TCE form between species. The similar rates of cleavage between species and the strong safety margin observed with AMX-818 in NHPs predict limited systemic accumulation of active TCE in human patients.
- Overall, the collective data demonstrate a significant decrease in toxicity with AMX-818 compared to the potent, fully activated, unmasked HER2 TCE. Together with the data suggesting that AMX-818 is stable in plasma and healthy tissue and is preferentially cleaved in tumors, the nonclinical data package support the planned Phase I trial.
- AMX-818 represents a novel treatment option for patients with HER2-expressing tumors and is anticipated to enter the clinic in early 2022.

Contact Info



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