Discovery and Optimization of XTX601, a Masked Claudin 18.2-Targeting T Cell Engager

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SCAN ME

Abstract 1619

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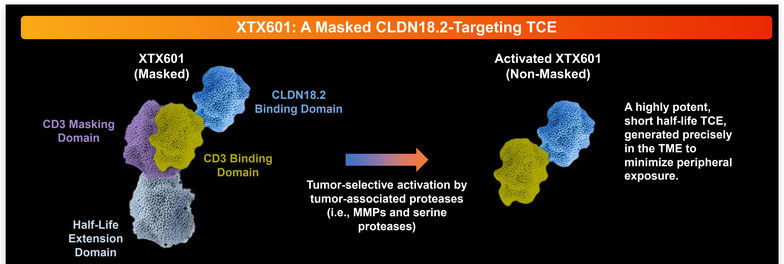
BACKGROUND

- T cell engagers (TCEs) are multi-specific molecules that are engineered to bind to tumor-associated antigens (TAAs) on cancer cells and simultaneously engage T cells via CD3 interaction, inducing an immunological synapse that results in T cell-mediated killing of tumor cells.
- TCE-based therapies have been highly efficacious in the treatment of hematological malignancies.[1] However, success in solid tumor indications has been limited due to:
  - Systemic toxicities including cytokine release syndrome (CRS)
  - On-target off-tumor toxicity driven by TAA expression in normal tissues
- To address these limitations, Xilio has leveraged its expertise in masking technology to develop masked T cell engagers including XTX601, a masked CLDN18.2-targeting TCE.
- XTX601 is designed to be activated by tumor-associated proteases present in the tumor microenvironment (TME), thereby maximizing activity in the tumor while minimizing T cell engagement in healthy tissue, to limit the severe side effects typically associated with unmasked TCEs.

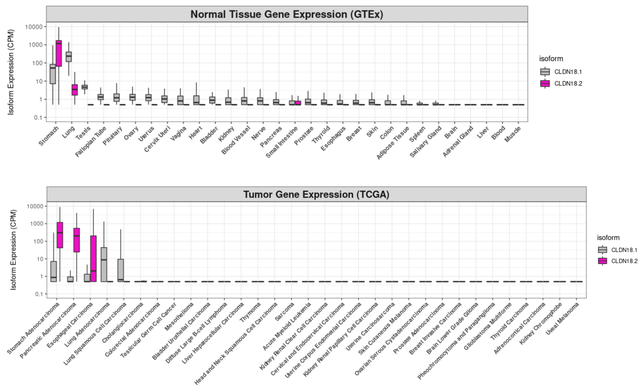
XTX601 IS A MASKED TCE DESIGNED USING XILIO'S MODULAR ARCHITECTURE AND CLINICALLY-VALIDATED MASKING TECHNOLOGY



- *Masked T cell Engager Design:* XTX601 is designed in the ATACR format and incorporates a masked CD3 binding domain, half-life extension domain, and CLDN18.2 targeting domain.
- *Conditional Half-Life Modulation:* Designed to release mask and half-life extension domains upon activation in the TME resulting in antibody-like half-life in masked state, short half-life once activated.
- *Modular TCE Architecture:* Enables flexibility to evaluate additional designs that incorporate masking the CLDN18.2 binding domain and/or adding a co-stimulatory domain (SEECR format) in parallel with advancing the current XTX601 molecule design.

CLDN18.2 IS A CLINICALLY-VALIDATED TARGET

- Claudins are a family of transmembrane proteins that play critical roles in cell-to-cell adhesion and intracellular signaling in epithelial cells.
- Under normal physiological conditions, individual claudins generally have a limited expression profile. However, expression of claudins is commonly dysregulated in various solid tumors, making them favorable candidates for targeted immunotherapy strategies.
- Amongst the claudins, one particularly promising therapeutic target is claudin18 isoform 2 (CLDN18.2). In healthy tissues, CLDN18.2 is primarily restricted to gastric mucosal cells lining the stomach. However, expression of CLDN18.2 is often significantly upregulated in gastric, esophageal and pancreatic cancers.

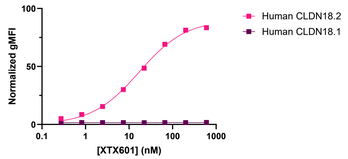


Top panel: Gene expression count data from The Genotype-Tissue Expression (GTEx) database was analyzed to assess the expression of both CLDN18 isoforms in normal tissue, with selected tissue types indicated.
Bottom panel: Gene expression from The Cancer Genome Atlas (TCGA) database was analyzed to assess the expression of both CLDN18 isoforms in cancer indications, with relevant indications indicated. Results were visualized via boxplots with CLDN18.1 and CLDN18.2 indicated by blue and yellow boxes, respectively.

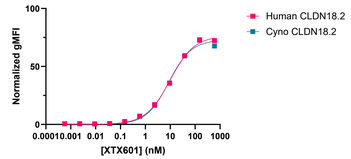
- The first FDA approved CLDN18.2-targeting immunotherapy is the monoclonal antibody zolbetuximab, which was approved in 2024 for the treatment of gastric or gastroesophageal junction (GEJ) adenocarcinoma in combination with chemotherapy.[3]
- Building on the success of zolbetuximab, the landscape of novel CLDN18.2-targeting modalities has continued to proliferate in order to address the remaining unmet needs for the treatment of gastric, esophageal, and pancreatic cancers.
- Current clinical-stage CLDN18.2-targeting TCEs are not masked and have been observed to cause severe adverse events in patients, with Grade 3 or greater TRAEs being commonly reported. By masking the anti-CD3 domain of the TCE to limit systemic toxicity, XTX601 has the potential of a first-in-class masked CLDN18.2-targeting TCE with an improved therapeutic window for the treatment of solid tumors.

XTX601 DEMONSTRATED HIGH SELECTIVITY FOR CLDN18.2

XTX601 Contains a High Affinity CLDN18.2 Isoform Selective Binding Domain



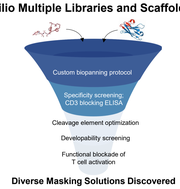
XTX601 Binds to Human and NHP CLDN18.2 with Equivalent Affinities



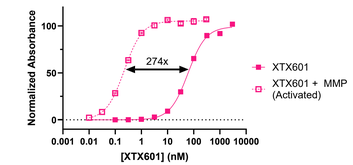
Left panel: Binding of XTX601 to Chinese Hamster Ovary-K1 (CHO-K1) cells that were engineered to overexpress either human CLDN18.2 or human CLDN18.1. **Right panel:** Binding of XTX601 to CHO-K1 cells that were engineered to overexpress CLDN18.2 from either human or *Macaca fascicularis*, a non-human primate (NHP). In both panels, the geometric mean fluorescence intensity (gMFI) generated from XTX601 binding was normalized to that of a control anti-CLDN18.2 antibody which was analyzed in parallel.

XILIO'S OPTIMIZED MASKED ANTI-CD3 DOMAIN REQUIRES PROTEOLYTIC ACTIVATION FOR FULL ACTIVITY

Xilio Multiple Libraries and Scaffolds



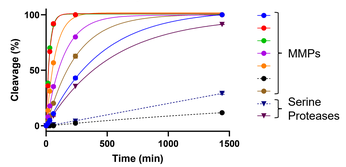
XTX601 Demonstrated a 274-Fold Increase in CD3 Binding After Activation



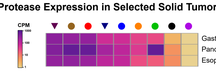
Left panel: Comprehensive mask discovery campaign yielded multiple CD3 masking solutions. **Right panel:** Masking of the anti-CD3 domain of XTX601 was assessed using intact XTX601 or XTX601 that was pre-activated with MMP in an enzyme-linked immunosorbent assay (ELISA) using immobilized human CD3 epsilon and CD3 delta heterodimeric protein as the target. The ELISA readout was normalized using a control unmasked T cell engager which was analyzed in parallel.

XTX601 WAS ACTIVATED BY TUMOR-ASSOCIATED PROTEASES *IN VITRO* AND BY PRIMARY HUMAN TUMOR CELLS *EX VIVO*

XTX601 Was Activated by Recombinant Human Proteases *In Vitro*

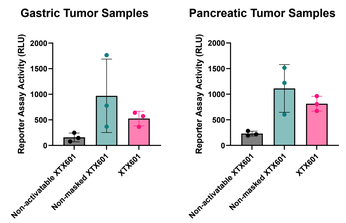


Protease Expression in Selected Solid Tumor Indications



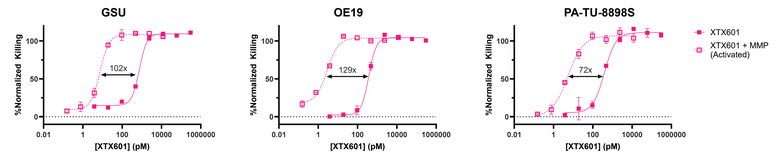
Top left panel: Kinetic cleavage/activation of XTX601 by selected recombinant proteases over time. Analysis of proteolytic cleavage of XTX601 performed using capillary electrophoresis.
Bottom left panel: Heatmap of protease expression in selected solid tumor indications. Individual proteases tested in vitro have been indicated by the symbol.

XTX601 Demonstrated Protease-Dependent Activity in Primary Human Tumor Co-Culture *Ex Vivo*

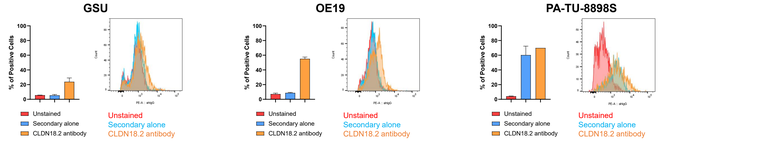


Left Panel: Primary human gastric tumor cells from three independent donors were incubated with test molecules and a JNL-reporter cell line for 24 hours. **Right Panel:** Primary human pancreatic tumor cells from three independent donors were incubated with test molecules and a JNL-reporter cell line for 24 hours. The signaling activity of the indicated test articles at 1 nM was assessed using a luciferase-based readout.

XTX601 EXHIBITED POTENT ACTIVITY AGAINST MULTIPLE HUMAN TUMOR CELL LINES WITH VARYING CLDN18.2 EXPRESSION LEVELS



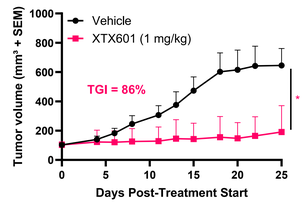
T cell-dependent cytotoxicity (TDCC) assays with three human cancer cell lines were utilized to assess the masking and potency of XTX601 using either intact XTX601 or XTX601 which was pre-activated using recombinant MMP.
Left panel: Primary human T cells were co-cultured with GSU gastric carcinoma cells. **Middle panel:** Primary human T cells were co-cultured with OE19 esophageal adenocarcinoma cells. **Right panel:** Primary human T cells were co-cultured with PA-TU-8898S pancreatic adenocarcinoma cells. For all three panels, tumor cell killing in response to the indicated titrations of XTX601 was evaluated using a luciferase-based cell viability readout.



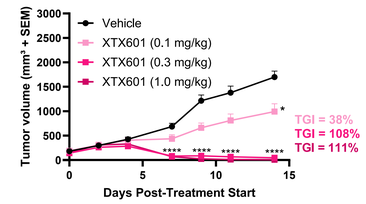
The expression level of CLDN18.2 on the human tumor cells plated for the above TDCC was assessed using flow cytometry. Comparison of the background signals to that of the signal with the CLDN18.2 antibody present.
Left panels: Bar graphs depicting the level of CLDN18.2 expression in each of the three human tumor cell lines tested. **Right panels:** Single variable flow cytometry histograms depicting the data used to generate the bar graphs in the left portion of the panel.

XTX601 DEMONSTRATED ROBUST, DOSE-DEPENDENT ANTI-TUMOR ACTIVITY IN MULTIPLE *IN VIVO* MOUSE CDX MODELS

XTX601 Demonstrated Robust Anti-Tumor Activity in a GSU *In Vivo* Model



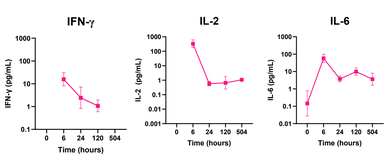
XTX601 Demonstrated Robust Anti-Tumor Activity in an OE19 *In Vivo* Model



Left panel: GSU cells were inoculated in NSG mice engrafted with human T cells. Anti-tumor activity change in response to a dose of 1.0 mg/kg XTX601 was evaluated over time. A two-way ANOVA followed by Dunnet's multiple comparisons test was used for statistical analysis: p<0.05(*). **Right panel:** OE19 cells were inoculated in NSG mice engrafted with human T cells. Anti-tumor activity change in response to the indicated dose levels of XTX601 was evaluated over time. A two-way ANOVA followed by Šidák's multiple comparisons test was used for statistical analysis: p<0.05(*), p<0.0001(****).

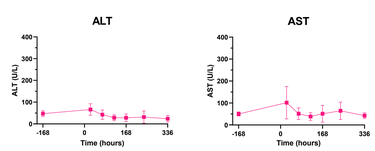
XTX601 WAS WELL TOLERATED IN NHP, WITH NO CRS OR CHANGES TO LIVER ENZYMES OBSERVED

XTX601 Caused Low Levels of Pro-Inflammatory Cytokines in NHP and Did Not Induce CRS in NHP



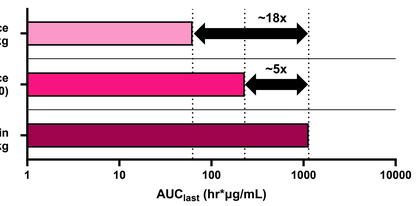
Analysis of interferon-γ (IFN-γ) levels (**left panel**), interleukin-2 (IL-2) levels (**middle panel**), and interleukin-6 (IL-6) levels (**right panel**) in the plasma at the indicated timepoints following the administration of a 1.0 mg/kg dose of XTX601 to NHP. Cytokine levels were determined using a U-PLEX Assay kit from Meso Scale Discovery (MSD). The levels of six other pro-inflammatory cytokines were below the detection limit of the assay.

XTX601 Did Not Cause Changes to Liver Enzymes in NHP



Analysis of alanine aminotransferase (ALT) levels (**left panel**) and aspartate aminotransferase (AST) levels (**right panel**) in the serum at the indicated timepoints following the administration of a 1.0 mg/kg dose of XTX601 to NHP.

INTEGRATION OF DRUG EXPOSURE DATA FROM MURINE AND NHP MODELS INDICATES A FAVORABLE, POSITIVE THERAPEUTIC INDEX FOR XTX601, CONSISTENT WITH MASKED DESIGN



XTX601 concentrations after administration of a single intravenous dose of XTX601 in either naïve NHP or tumor-bearing mice were quantified using mass spectrometry and used to generate drug exposure parameters. The area under the curve (AUC) for total XTX601 (intact XTX601 + cleaved XTX601) were determined and used to calculate the therapeutic index of XTX601 (indicated by green arrows).

CONCLUSIONS

- XTX601 is a potential first-in-class masked TCE designed to selectively target CLDN18.2, with the ability to achieve a wide therapeutic index.
  - XTX601 demonstrated protease-dependent activation and tumor cell killing in high and low expression settings for CLDN18.2, with effective masking of the anti-CD3 domain.
  - XTX601 exhibited robust, dose-dependent anti-tumor activity in multiple murine models.
  - Consistent with the masked design, XTX601 was well tolerated in NHP with no evidence of CRS and no changes to liver enzymes.
- Xilio's modular architecture for TCEs enables flexibility to evaluate additional design that incorporate masking the CLDN18.2 binding domain and/or adding a co-stimulatory domain (SEECR format) in parallel with advancing the current XTX601 molecule design.

ABBREVIATIONS: ANOVA: analysis of variance; ATACR: advanced tumor-activated cell engager; CD: cluster of differentiation; CDX: cell line-derived Xenograft; ELISA: enzyme-linked immunosorbent assay; GTEx: The Genotype-Tissue Expression database NSG: NOD Scid Gamma; IL: Interleukin; IFN-γ: Interferon gamma; NHP: non-human primate; SEECR: selective effector-enhanced cell engager; SEM: standard error of the mean; TGCA: The Cancer Genome Atlas; TGI: Tumor Growth Inhibition; TNF-α: tumor necrosis factor-alpha; TRAEs: treatment-related adverse events;

REFERENCES: 1.) Tian, Z., Liu, M., Zhang, Y. et al. *J. Hematol Oncol.*, 2021 2.) Uli Bialucha (2025). Design Illustration [AI-generated image]. Luma-Photon and Playground_V3 3.) Zolbetuximab-clzb, USPI: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/761365s000lbl.pdf

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