

Development of conditional T cell engagers (INDUCER™ molecules) with a highly effective masking approach to reduce dose-limiting cytokine release and off-target peripheral toxicity

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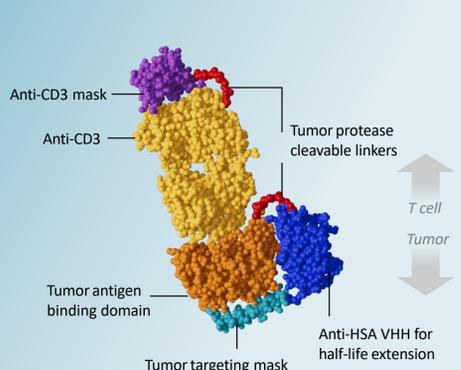
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Abstract

T cell engagers (TCEs) are a promising therapeutic modality that redirects cytotoxic T cells to recognize tumor cells for destruction. By binding both cytotoxic T cells and target antigen-expressing tumor cells, TCEs allow for the activation of bystander T cells and the directed release of cytotoxic proteins, such as perforin and granzymes, thereby leading to antitumor activity. While TCEs are effective in the treatment of hematological malignancies, achieving clinical antitumor activity in solid tumors has been more difficult. Challenges for TCE molecules targeting solid tumors include induction of cytokine release syndrome (CRS) through peripheral activation of T cells, as well as on-target/off-tumor toxicity. These challenges are due to the systemic activity of these molecules, which could potentially be mitigated by effective masking and selective activation in the tumor microenvironment. Here, we describe the development of a TCE platform to target solid tumors that is designed to overcome the limitations of existing unmasked and prodrug TCEs. Our conditionally activated molecules employ a differentiated approach to masking the anti-CD3 domain that is intended to render INDUCER molecules nearly completely inactive until activation in the tumor microenvironment restores their functional activity. Our data demonstrate that this masking approach eliminated targeted killing of antigen-expressing cells in vitro and peripheral cytokine release in mouse models. Furthermore, when coupled with novel and proprietary linker substrates, the mask was efficiently removed in a tumor-selective, protease-dependent manner across a variety of primary human tumor patient samples. INDUCER molecules that target tumor-associated antigens exhibited potent cytotoxicity in vitro, as well as antitumor efficacy in vivo. Together, these data demonstrate the promise of INDUCER molecules for the development of effective and better tolerated TCEs for the treatment of solid cancers.

Inducible T Cell Engager (INDUCER) Platform

Key Features of INDUCER Molecules

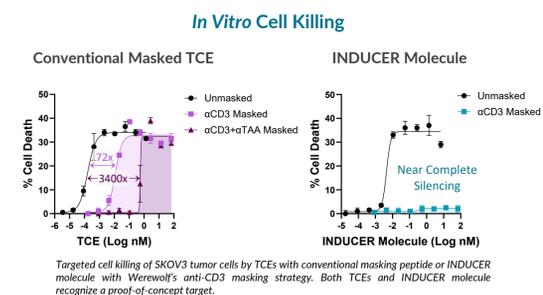


- Proprietary and optimized anti-CD3 masking technology to reduce systemic toxicity and off-tumor effects
- Novel pan-cancer linker acts as tumor-specific switch, enabling precise unmasking and conditional activation in the TME
- Novel anti-CD3 antibody optimized for developability
- Tumor-associated antigen (TAA) binding domain selected for optimal immune cell engagement
- Tumor targeting mask applicable for targets with extensive expression in normal tissues, to limit off-target toxicity and/or target-mediated drug disposition
- Proprietary anti-HSA VHH for half-life extension and increased tumor exposure

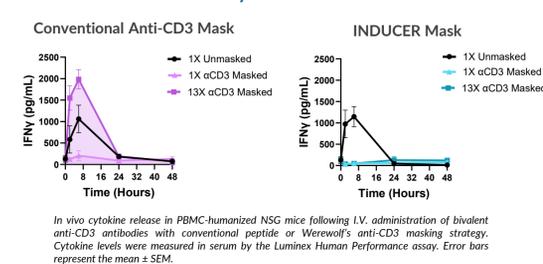
Differentiating Components of Our INDUCER Platform

Optimized CD3 Masking Strategy

Unique CD3 Masking Strategy Is Highly Effective In Vitro and In Vivo

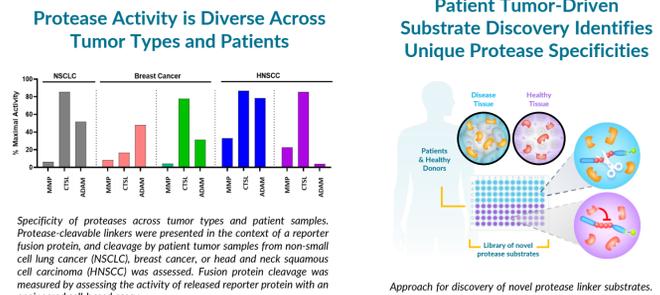


In Vivo Cytokine Release

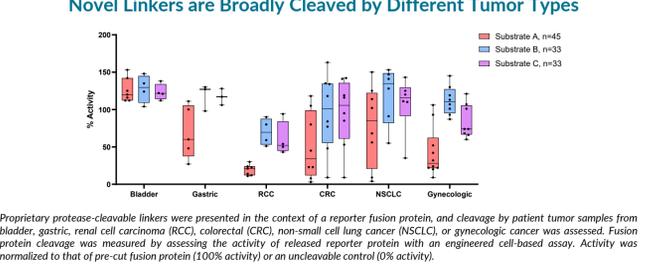


Novel Tumor Protease Cleavable Linkers

Selection of Linker Substrates Based on Cleavage by Patient-Derived Tumors and Stability in Healthy Tissues



Novel Linkers are Broadly Cleaved by Different Tumor Types



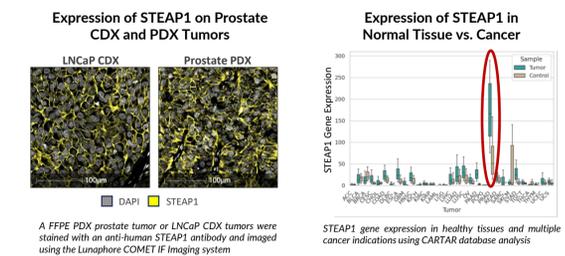
WTX-1011

A Potential First-in-Class Masked Anti-STEAP1 T Cell Engager with an Improved Therapeutic Index

Key Opportunities

- Due to highly effective silencing and selective release of active INDUCER molecule in tumors, WTX-1011 is designed to avoid the toxicities associated with anti-STEAP1 T cell engager therapy.
- Potential to provide STEAP1 T cell engager therapy broadly to patients with metastatic castration-resistant prostate cancer (mCRPC)
- Potential opportunities in other solid tumor types, including lung and colorectal

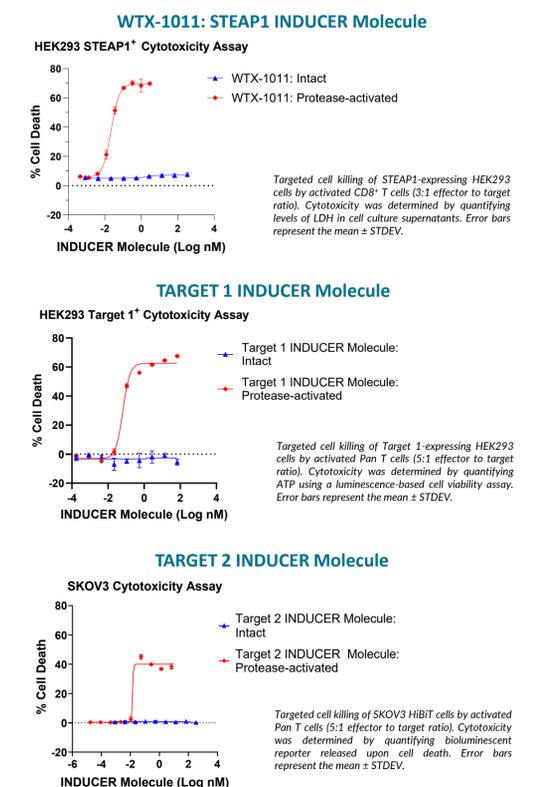
STEAP1 is a Promising Prostate Cancer Target with Limited Expression in Normal Tissues



- Overexpressed in >80% of metastatic castration-resistant prostate cancer with bone or lymph node involvement, and expressed at various stages of disease.^{1,2}
- Expressed in subpopulations of other cancers, including colorectal, hepatocellular carcinoma, gastric, lung, Ewing sarcoma, bladder, pancreatic, glioblastoma, ovarian, leukemia, lymphoma, and head and neck cancer.^{2,3}
- In normal tissue, mainly expressed in prostate, with low to moderate expression reported in adipose tissue, breast, and bladder.^{2,3}

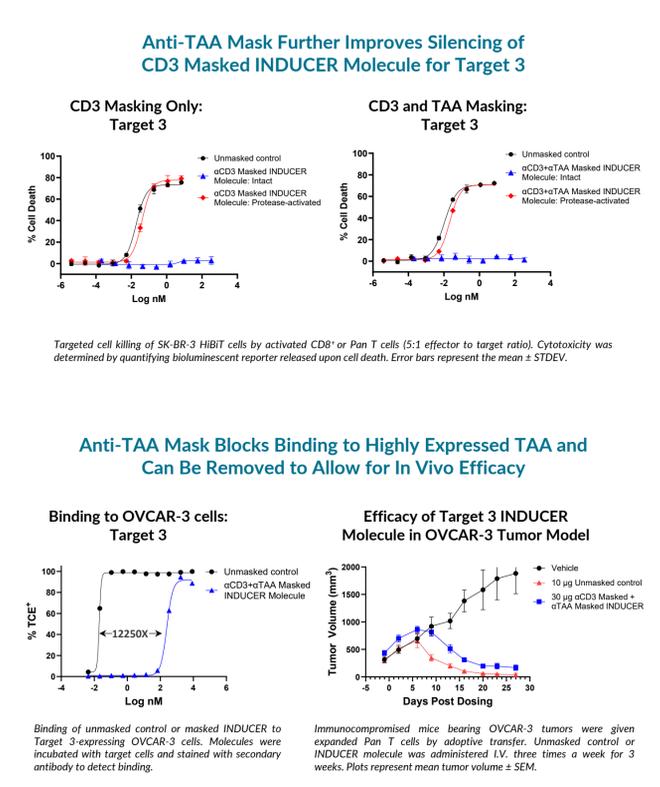
Masking Efficiency Across Targets

CD3 Masking Is Reversible and Silences INDUCER Molecules that Recognize a Broad Range of Targets

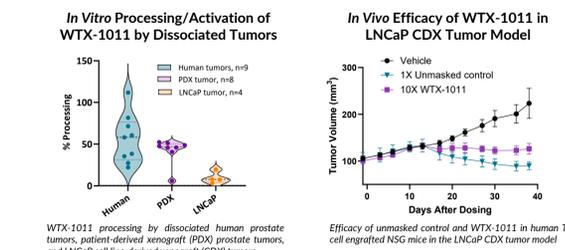


Addition of Anti-TAA Domain Mask

Masking Anti-TAA Domain Further Silences INDUCER Molecules For Targets with Normal Tissue Expression

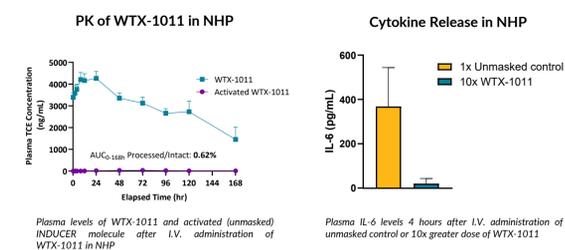


WTX-1011 is Unmasked by Human Tumors and Demonstrates Anti-tumor Activity



- WTX-1011 was broadly unmasked by human prostate tumor specimens and prostate PDX and CDX tumors
- WTX-1011 controlled tumor growth in the LNCaP CDX tumor model

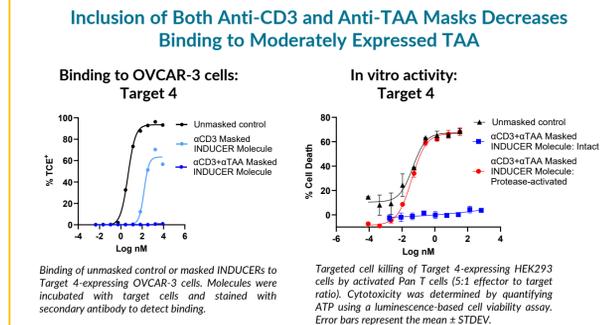
WTX-1011 Exhibits Highly Effective Silencing in the Periphery, with a Favorable PK Profile and Negligible Cytokine Release in NHP



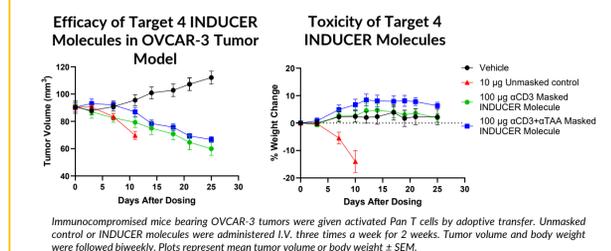
- WTX-1011 showed beneficial exposure in cynomolgus monkeys with a half-life close to 100 hours
- WTX-1011 was stable in the periphery, with less than 0.7% of active INDUCER detected
- Effective masking of WTX-1011 was confirmed by low levels of cytokine production compared to a 10-fold lower dose of unmasked control

Application of INDUCER Platform to Other Targets

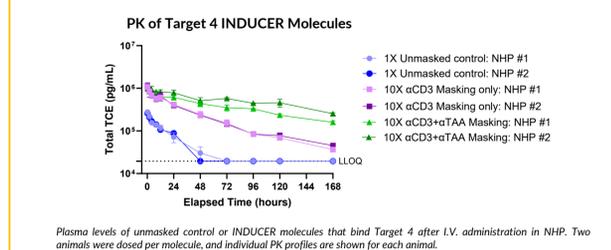
Development of a Potent INDUCER Molecule for a Tumor-Associated Antigen with Moderate Normal Tissue Expression



Toxicity Observed in Mice for Unmasked Control is Mitigated by Masking the Anti-CD3 Domain



Masking Both the Anti-CD3 and Anti-TAA Domains Improves Exposure in NHP



CONCLUSIONS

- While the development of T cell engagers for solid tumor indications has been challenging, we describe a platform that aims to prevent cytokine release syndrome and on-target/off-tumor toxicity.
- INDUCERs utilize a novel, highly effective masking strategy for inactivation of the anti-CD3 domain, as well as selective and efficient activation in the tumor via unique protease-sensitive linkers.
- The INDUCER masking design can be applied to TCEs that bind a diverse set of tumor-associated antigens.
- Our first development candidate generated with this platform, WTX-1011, targets STEAP1, a promising target for prostate and other human cancers.
- WTX-1011 is activated by dissociated human prostate tumors and patient-derived xenograft prostate tumors, and demonstrates in vivo efficacy in mice using an LNCaP tumor model.
- WTX-1011 is stable in the periphery when administered to non-human primates and does not induce cytokine release in its prodrug form.
- This platform is being extended to additional targets, with promising data for other solid tumor target antigens.