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Abstract # 4074

Discovery of WTX-518, an IL-18 Pro-drug That is Conditionally Activated Within the Tumor Microenvironment and Induces Regressions in Mouse Tumor Models

Kristin Morris, Heather Brodkin, Kyriakos Economides, Daniel J. Hicklin, Julie LePrevost, Celesztina Nagy-Domonkos, Christopher Nirschl, Andres Salmeron, Cynthia Seidel-Dugan, Cierra Spencer, Zoe Steuert, and *William M. Winston*

Werewolf Therapeutics Inc., Watertown, MA

Werewolf THERAPEUTICS

Shifting the Balance In Cytokine Therapeutics

BACKGROUND

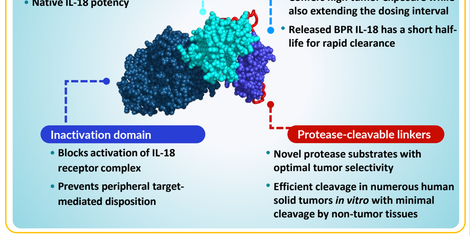
Development of an IL-18 pro-drug for cancer immunotherapy

Systemic administration of proinflammatory cytokines is a promising approach to treat cancer. IL-18 has been shown to promote activation of both innate and adaptive anti-tumor responses in pre-clinical models. Specifically, IL-18 has been shown to impact macrophage suppressive function as well as driving CD4+ T cells towards a T_H1 phenotype. However, IL-18 therapies have been hampered by a lack of efficacy due to the inhibitory activity of IL-18 binding protein (IL-18BP), which binds to IL-18 and prevents its interaction with its receptor complex. This negative regulation can be overcome by engineering IL-18 to not interact with IL-18BP. However, unopposed IL-18 signaling is toxic and thereby presents a challenge to engineered IL-18 therapy.

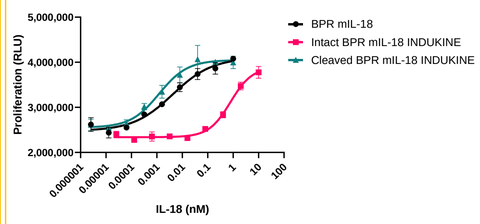
To explore the therapeutic benefit of delivering a conditionally-activated IL-18 to the tumor microenvironment, we have developed inducible polypeptides (INDUKINE™ molecules) consisting of wild-type mouse (WT) IL-18 (mIL-18) or binding protein resistant (BPR) mIL-18 tethered via protease-sensitive linkers to a high affinity antibody blockade domain and a half-life extension (HLE) domain which improves exposure in the tumor. IL-18 INDUKINE molecules are inactive until reaching the tumor microenvironment, where the linkers are cleaved by dysregulated intra-tumoral proteases, releasing active IL-18. Intraperitoneal (i.p.) administration of a BPR mIL-18 INDUKINE molecule led to complete tumor regression in the MC38 tumor model. In contrast, equimolar dosing of wild-type mIL-18 INDUKINE protein was not as efficacious. Moreover, BPR mIL-18 INDUKINE molecule treatment led to increased activation and frequencies of NK cells and tumor specific CD8 T cells in MC38 tumors.

WTX-518 is a novel INDUKINE molecule that is designed to selectively deliver active human BPR IL-18 to the tumor microenvironment. WTX-518 is inducible in vitro, either when tested using a reporter assay or primary immune cells. Critically, the active BPR IL-18 payload is resistant to IL-18BP as evidenced by the lack of IL-18BP mediated inhibition of BPR IL-18 activity in primary immune cell assays.

Key Features of WTX-518

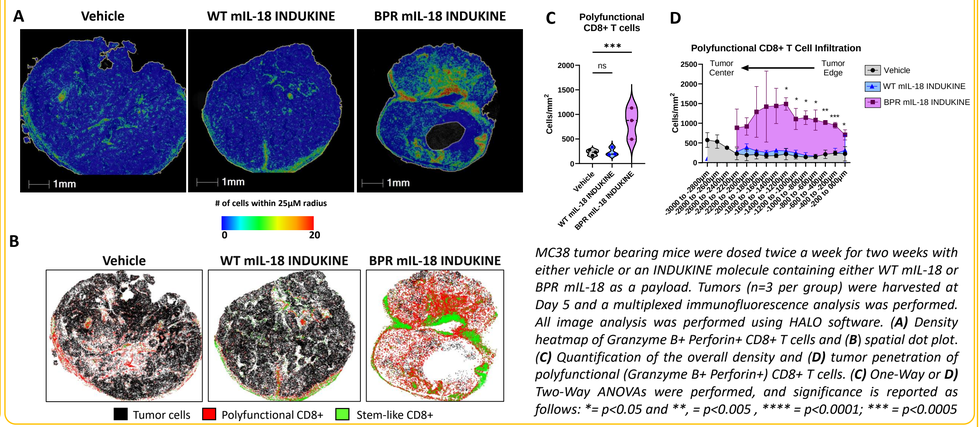


BPR Mouse IL-18 INDUKINE Molecule is Inducible in Cell-Based Assays



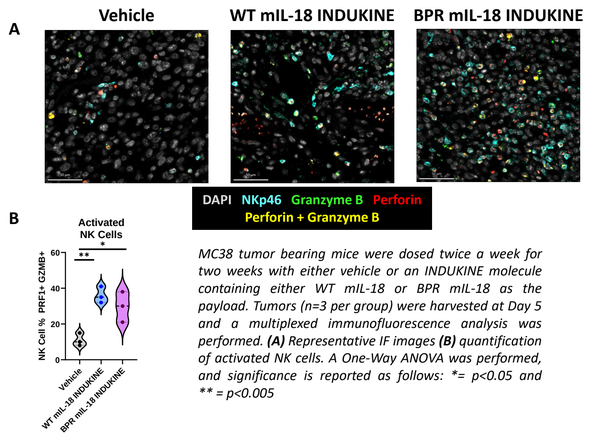
The activity of the BPR mIL-18 INDUKINE molecule was assessed in a murine splenocyte assay. Murine splenocytes were pre-activated for 3 days with plate-bound anti-CD3 (10 µg/mL) and soluble anti-CD28 (5 µg/mL), followed by stimulation with 2 ng/mL mIL-12 for 24 hrs. Cells were then stimulated with BPR mIL-18, intact BPR mIL-18 INDUKINE molecule, or cleaved BPR mIL-18 INDUKINE molecule in combination with 0.1 ng/mL mIL-12. After 24 hours cell proliferation was measured using Cell Titer Glo.

BPR Mouse IL-18 INDUKINE Molecule Drives More Infiltration of Activated CD8+ T Cells



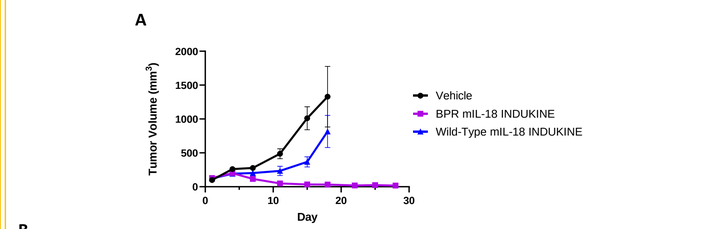
*MC38 tumor bearing mice were dosed twice a week for two weeks with either vehicle or an INDUKINE molecule containing either WT mIL-18 or BPR mIL-18 as a payload. Tumors (n=3 per group) were harvested at Day 5 and a multiplexed immunofluorescence analysis was performed. All image analysis was performed using HALO software. (**A**) Density heatmap of Granzyme B+ Perforin+ CD8+ T cells and (**B**) spatial dot plot. (**C**) Quantification of the overall density and (**D**) tumor penetration of polyfunctional (Granzyme B+ Perforin+) CD8+ T cells. (**C**) One-Way or (**D**) Two-Way ANOVAs were performed, and significance is reported as follows: *= p<0.05 and **, = p<0.005 , **** = p<0.0001; *** = p<0.0005*

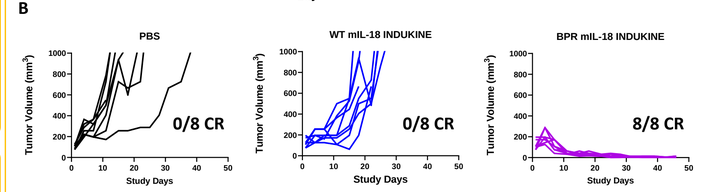
BPR Mouse IL-18 INDUKINE Molecule Increases Activated NK Cell Frequencies



*MC38 tumor bearing mice were dosed twice a week for two weeks with either vehicle or an INDUKINE molecule containing either WT mIL-18 or BPR mIL-18 as the payload. Tumors (n=3 per group) were harvested at Day 5 and a multiplexed immunofluorescence analysis was performed. **(A)** Representative IF images **(B)** quantification of activated NK cells. A One-Way ANOVA was performed, and significance is reported as follows: *= p<0.05 and ** = p<0.005*

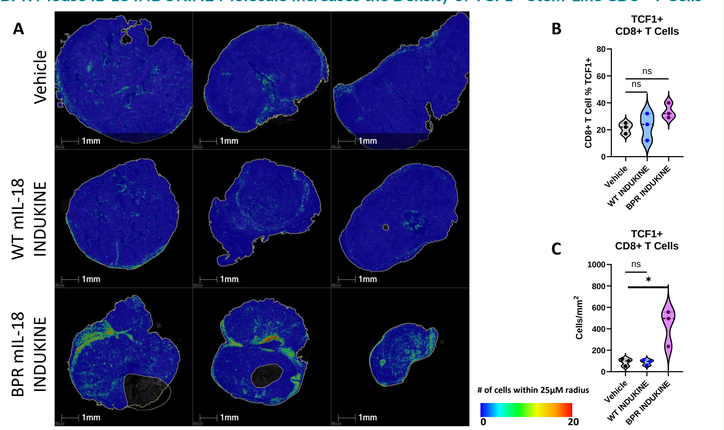
BPR Mouse IL-18 INDUKINE has More Robust Anti-Tumor Activity Than WT INDUKINE





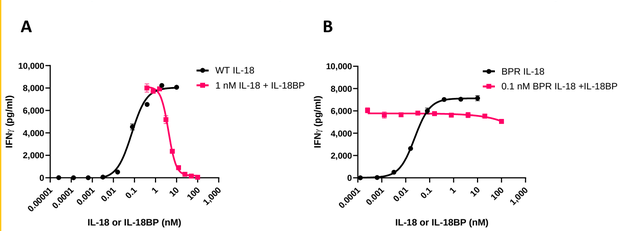
*MC38 tumor bearing mice were randomized into treatment groups (n=8 per group) and dosed with 37.5 µg of WT mIL-18 INDUKINE or BPR mIL-18 INDUKINE molecules twice weekly for two weeks. Tumors were measured twice weekly. **(A)** Average tumor volumes and **(B)** individual spider plots. CR = complete regression.*

BPR Mouse IL-18 INDUKINE Molecule Increases the Density of TCF1+ Stem-Like CD8+ T Cells



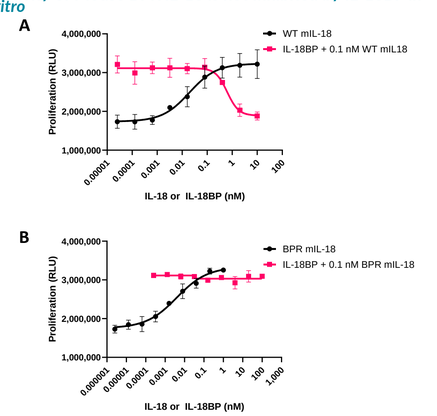
*MC38 tumor bearing mice were dosed twice a week for two weeks with either vehicle or an INDUKINE molecule containing either WT mIL-18 or BPR mIL-18 as a payload. Tumors (n=3 per group) were harvested at Day 5 for analysis and a multiplexed immunofluorescence analysis was performed. Shown above are **(A)** representative IF images of polyfunctional Granzyme B+ Perforin+ CD8+ T Cells interacting with SOX2+ tumor cells, **(B)** quantification of the frequency of tumor cells with an adjacent polyfunctional CD8+ T cell and **(C)** average distance between tumor cells and polyfunctional CD8+ T cells. One-Way ANOVAs were performed, and significance is reported as follows: **= p<0.005.*

The Activity of BPR Human IL-18 Payload for WTX-518 is Not Inhibited by IL-18BP



*The activity of WT IL-18 **(A)** or BPR IL-18 **(B)** was assessed by IFNγ production from PBMCs in the presence of 10 ng/mL IL-12 +/- IL-18BP at various concentrations for 48 hours. Cell culture supernatants were harvested, and IFNγ production was measured using a hIFNγ AlphaLISA.*

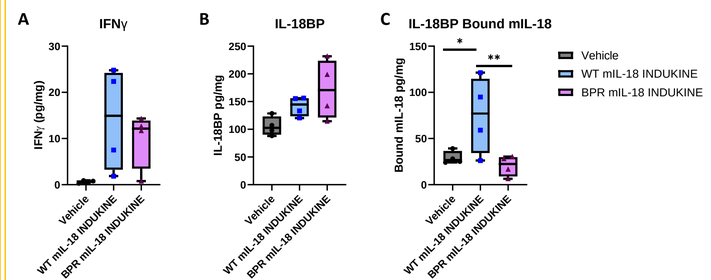
Activity of Mouse BPR IL-18 is not Inhibited by IL-18BP *in vitro*



*The activity of wild type (WT) or BPR mIL-18 was assessed in a murine splenocyte proliferation assay. Murine splenocytes were pre-activated for 3 days with plate-bound anti-CD3 (10 µg/mL) and soluble anti-CD28 (5 µg/mL), followed by stimulation with 2 ng/mL mIL-12 for 24 hrs. Cells were then stimulated with 0.1 ng/mL mIL-12 in addition to either **(A)** WT mIL-18 or **(B)** BPR mIL-18 in the presence or absence of IL-18BP at various concentrations. After 24 hours, proliferation was measured using Cell Titer Glo.*

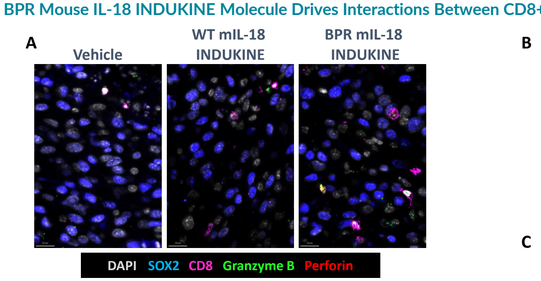
Released BPR Mouse IL-18 Does Not Bind IL-18BP in the Tumor Microenvironment (TME)

Increased levels of WT mouse IL-18 complexed with IL-18BP, but not BPR, despite similar IL-18BP levels in tumors



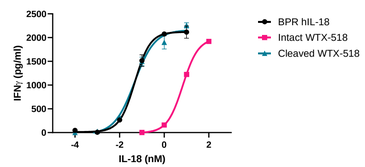
*MC38 tumor bearing mice (n=4 per group) were dosed with WT mIL-18 or BPR mIL-18 INDUKINE molecules twice weekly. Tumors were harvested on Day 5, 24 hours after the second dose. **(A)** IFNγ and **(B)** IL-18BP were measured in tumor lysates using commercially available or custom MSD ECL assays, respectively. **(C)** The levels of mIL-18 bound to IL-18BP were assessed in a custom MSD ECL assay where the complex was captured using an anti-mIL-18 antibody and detected using an anti-IL-18BP antibody. Two-way ANOVA analysis was performed, and significance is reported as follows: *= p<0.05 and ** = p<0.005.*

BPR Mouse IL-18 INDUKINE Molecule Drives Interactions Between CD8+ T Cells and Tumor Cells



*MC38 tumor bearing mice were dosed twice a week for two weeks with vehicle or an INDUKINE molecule containing either a WT mIL-18 or a BPR mIL-18 payload. Tumors (n=3 per group) were harvested at Day 5 for analysis and multiplexed immunofluorescence analysis was performed. Shown above are **(A)** representative IF images of polyfunctional Granzyme B+ Perforin+ CD8+ T Cells interacting with SOX2+ tumor cells, **(B)** quantification of the frequency of tumor cells with an adjacent polyfunctional CD8+ T cell and **(C)** average distance between tumor cells and polyfunctional CD8+ T cells. One-Way ANOVAs were performed, and significance is reported as follows: **= p<0.005.*

WTX-518, a Human INDUKINE Molecule Containing BPR IL-18, is Inducible



The inducibility of WTX-518 was assessed by incubating indicated concentrations of BPR IL-18, intact WTX-518, or cleaved WTX-518 with human PBMCs in the presence of 10 ng/mL IL-12. After 48 hours, cell culture supernatants were harvested, and IFNγ production was measured using a hIFNγ AlphaLISA.

SUMMARY and CONCLUSIONS

- BPR IL-18 molecules are resistant to IL-18BP in vitro and in vivo.
- The BPR mIL-18 INDUKINE molecule and WTX-518 demonstrate in vitro inducibility.
- Systemic administration of BPR mIL-18 INDUKINE molecule drives more robust anti-tumor immune responses than the WT mIL-18 INDUKINE molecule resulting in complete tumor regressions.
- The BPR mIL-18 INDUKINE molecule promotes the activation and infiltration of polyfunctional of CD8+ T cells into the MC38 tumors.
- The BPR mIL-18 INDUKINE molecule leads to more direct interactions between activated CD8+ T cells and tumor cells.

Werewolf Therapeutics
200 Talcott Avenue
Watertown, MA 02472

media@werewolftx.com
info@werewolftx.com
https://werewolftx.com/

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