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Decoy-Resistant IL-18 enhances checkpoint inhibitor combinations beyond anti-PD-1 *in vitro* and *in vivo*

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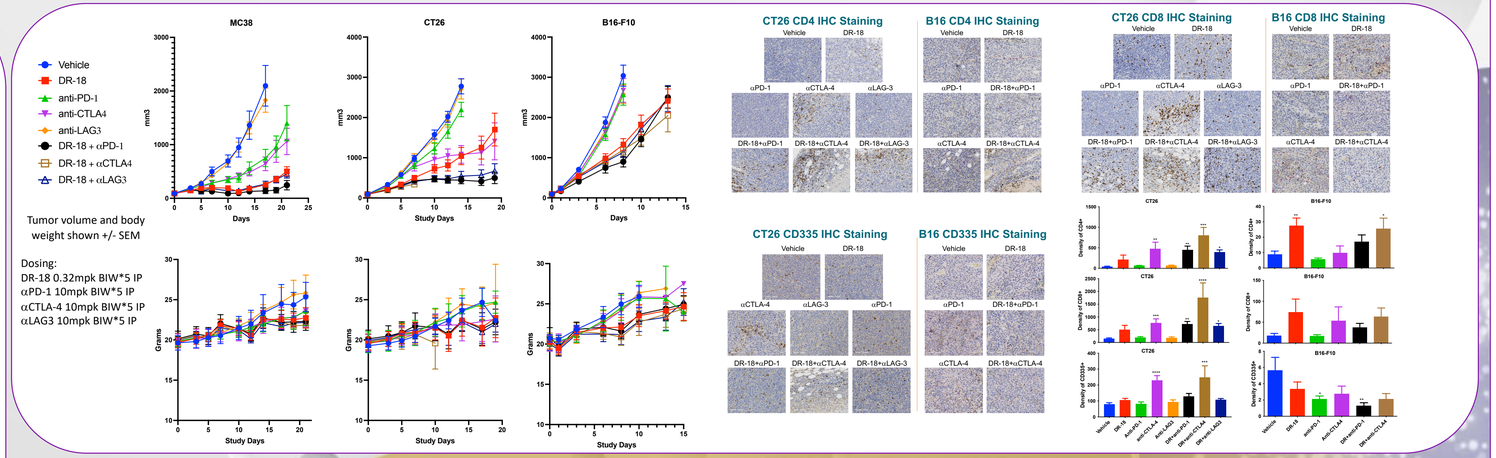
Abstract

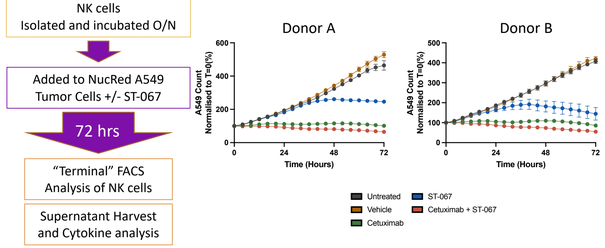
• Interleukin-18 (IL18) is a proinflammatory cytokine that modulates both innate and adaptive immune responses. Historically, wild-type recombinant IL-18 has shown limited anti-tumor efficacy in preclinical models and clinical trials, likely due to upregulation of IL-18 binding protein (IL-18BP), a negative regulator of the IL-18 signaling axis. Accordingly, an engineered IL-18 cytokine capable of interacting with the IL-18 receptor, but resistant to IL-18BP interactions (i.e., "Decoy-Resistant IL-18", DR-18), has demonstrated enhanced therapeutic potential in mouse tumor models, both as a single agent and in combination with anti-PD-1 [1, 2]. Here we evaluated murine DR-18 in combination with additional checkpoint inhibitors beyond anti-PD-1 in a set of mouse tumor models and tested human DR-18 activity *in vitro*/*ex vivo* for activation of human immune cells and anti-tumor activity.

• Results: Treatment with mDR-18 elicited strong efficacy as a single agent and combination activity with immune checkpoint inhibitors (ICIs) across diverse syngeneic mouse models of solid tumors. As a single agent, mDR-18 demonstrated robust single agent activity in tumor growth inhibition for MC38 (>80%), CT26 (>60%) and B16-F10 (>55%). Combination treatment of mDR-18 with anti-PD-1, anti-CTLA-4, or anti-LAG-3 increased the efficacy for the MC38 (>85-95%), CT26 models (>80-85%), while the B16-F10 (>60%) showed smaller efficacy enhancement with the combinations. *In vitro* assays revealed human DR-18 (ST-067) activated immune cells and enhanced A549 tumor cell killing over 72hrs. Furthermore, *ex vivo* studies of 3D patient-derived tumor spheroids demonstrated that ST-067 impaired growth and increased immune cell infiltration, demonstrating the potential of hDR-18 to enhance immune activity within the human tumor microenvironment.

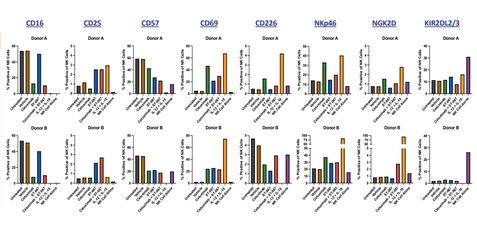
• Conclusion: These studies expand the breadth of IL-18/checkpoint synergism beyond anti-PD-1 and confirm enhanced human immune response *in vitro* with the clinical candidate ST-067. Taken together, these findings strengthen the rationale for clinical combination of ST-067 with ICI agents in patients with solid tumors.

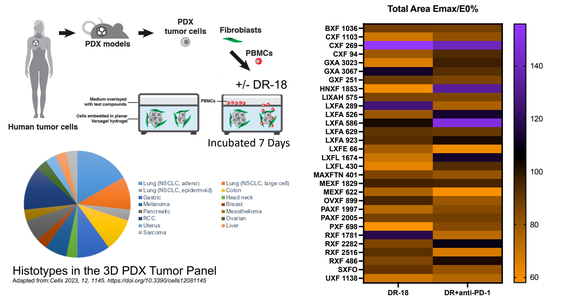
1. Zhou T, Damsky W, Weizman OE, et al. IL-18BP is a secreted immune checkpoint and barrier to IL-18 immunotherapy. *Nature*. 2020;583(7817):609-614. doi:10.1038/s41586-020-2422-6
2. Minnie SA, Waltner OG, Ensbey KS, et al. Depletion of exhausted alloreactive T cells enables targeting of stem-like memory T cells to generate tumor-specific immunity. *Sci Immunol*. 2022;7(76):eabo3420. doi:10.1126/sciimmunol.abo3420

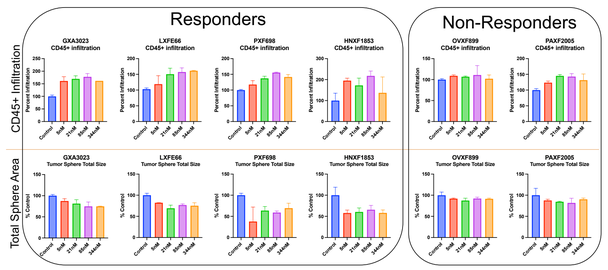




DR Expression Vs To Control		
Analyte	Donor A	Donor B
CD16	=	=
CD57	↓	↓
CD25	↑	↑
CD69	↑	↑
CD226	=	↓
NKp46	=	=
NGK2D	=	=
KIR2DL2/3	=	=







Methods:

In Vivo Pharmacology Studies: Performed at Crown Bioscience (Zhongshan) Inc.
Model: B16-F10; C57BL/6, 10 mice per group, each mouse inoculated subcutaneously in the right rear flank region with tumor cells (2 x 105) in 0.1 ml of PBS.
Model: CT26.WT; BALB/c, 10 mice per group, each mouse inoculated subcutaneously in the right rear region with tumor cells (5 x 105) in 0.1 ml of PBS.
Model: MC38; C57BL/6, 10 mice per group, each mouse inoculated subcutaneously in the right rear region with tumor cells (1 x 106) in 0.1 ml of PBS.
Antibodies:
BioXcell: Anti-PD-1 (RPMI1-14), Anti- CTLA4 (9H10), Anti-Lag3 (C9B7W)

For each model, the randomization started when the mean tumor size reached approximately 80-100 mm3.

Randomization and treatment initiated on Day 0. Animals checked daily for morbidity and mortality. Body weights measured three times per week after randomization. Tumor volumes measured three times per week after randomization in two dimensions using a caliper, and the volume expressed in mm3 using the formula: "V = (L x W x W)/2, where V is tumor volume, L is tumor length (the longest tumor dimension) and W is tumor width (the longest tumor dimension perpendicular to L).

Whole tumors snap frozen and FFPE samples were collected in Crown Bioscience (Zhongshan), IHC performed at Crown Bioscience (Taicang) Inc.

NK Tumor Cell Killing Study:
Study performed at Charles River Laboratories, Portishead, UK. Buffy coats from healthy donors used. PBMCs isolated over a density gradient and NK cells MACS purified and counted before being rested overnight. PBMC cells added to Nuclight Red labelled tumor targets (A549) at 3:1 E:T ratio. Tumor killing evaluated by counting viable Nuclight positive tumor cells over time. Caspase 3/7 dye used to identify apoptotic tumor cells.After the TKA finished, NK cells analyzed for receptor expression by flow cytometry. IL-18BP quantification by ELISA on supernatants.

PDX Tumor Spheroid Study: Study performed at Cypre, San Francisco / Charles River Laboratories Inc, Germany.
PDX Tumor cells were grown in 3D hydrogels co-embedded with human dermal fibroblasts (HDF), and later with PBMCs. ST-067 was dosed for 7 days in the 3D tumor model. After, 3D assays were stained with the Hoechst nuclear and DRAQ7 cell death markers and analyzed at endpoint for tumor size and killing using a high content imager and proprietary image analysis software.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10137152/

Conclusions:

DR-18 has single agent efficacy comparable to well known ICI agents in multiple mouse models.

DR-18 enhances anti-tumor efficacy of multiple ICI agents, including αPD-1, αCTLA-4 and αLAG-3 with combination treatment.

DR-18 activity alone, and in combination, increases CD4+ and CD8+, but not CD335+, immune cell infiltration into tumor area.

NK cells treated with DR-18 exhibit enhanced tumor cell killing and an activated NK-cell phenotype.

Screening a panel of 33 patient-derived tumor spheroids showed decreased area and increased immune cell infiltration with DR-18 in a subset of responder models.