Werewolf THERAPEUTICS

Shifting the Balance In Cytokine Therapeutics

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The Combination of ACT and INDUKINE™ Therapy Leads to Improved Antitumor Immunity in Mouse Solid Tumors

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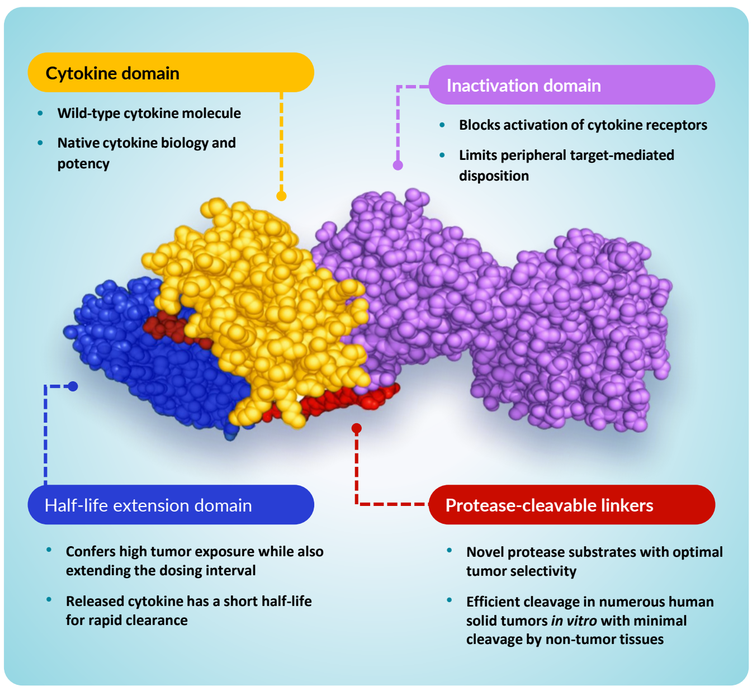
BACKGROUND

Adoptive cell therapy (ACT) utilizes tumor-specific T cells obtained from tumor biopsies or genetically engineered peripheral T cells to treat a patient's malignancy. Even though there has been clinical success with chimeric antigen receptor (CAR) T cells in hematological malignancies, limited success has been demonstrated with ACT in solid tumors. One of the main limitations of ACT is the high cell number required to treat patients. The cells require repeated *in vitro* restimulation and long-term expansion leading to an exhausted, terminally differentiated phenotype, which results in a short-lived antitumor response. This has warranted the need for novel strategies to improve engraftment and functionality of cellular therapies against solid tumors.

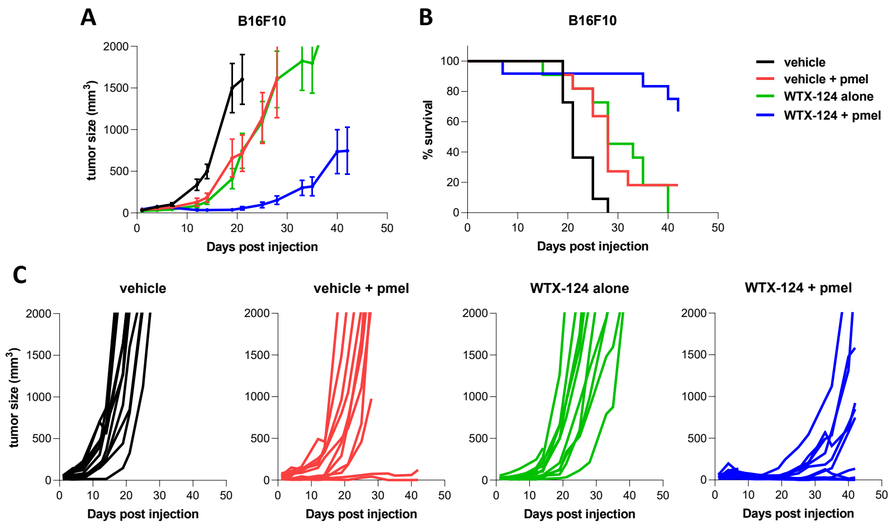
We have designed IL-2 and IL-12 INDUKINE™ polypeptides (cytokine prodrugs) masked from peripheral activation via an inactivation domain. They are designed to utilize the unique protease profile of tumors to deliver active cytokine preferentially to the tumor microenvironment. Additionally, our INDUKINE molecules are engineered with a half-life extension domain to improve exposure with less frequent dosing. In the following studies, we tested whether our INDUKINE molecules could improve the engraftment and antitumor activity of ACT. Our studies used the pmel-1 transgenic mouse model in which CD8⁺ T cells express a T cell receptor specific for gp100 expressed on melanocytes and melanoma and the human CD19 CAR T cell model against subcutaneous Raji tumors.

Key Features of INDUKINE Molecules



Systemic WTX-124 Enhances the Antitumor Activity of ACT

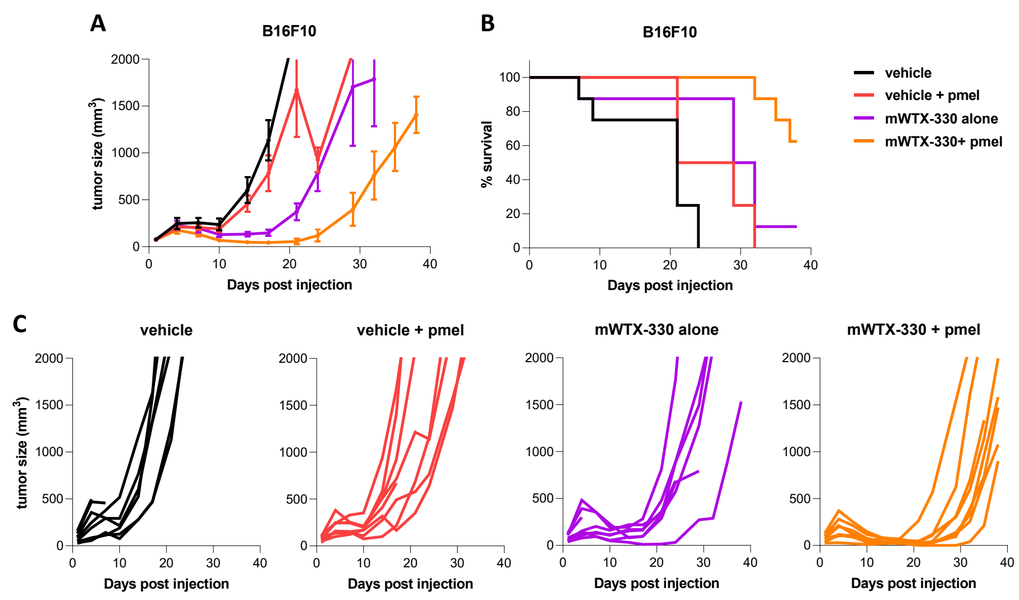
WTX-124 + pmel ACT Improves B16F10 Tumor Control and Survival



C57BL/6 mice were subcutaneously injected with B16F10 melanoma. Animals were intravenously infused with pmel CD8⁺ T cells that had been expanded for 7 days. Animals were treated intraperitoneally with either vehicle or IL-2 INDUKINE on Days 1, 4 and 8. Average tumor growth curves (A) and animal survival (B) of B16F10-bearing C57BL/6 mice treated with IL-2 INDUKINE and/or CD8⁺ pmel ACT. Individual tumor spider plots (C) in which each line represents an individual animal n=11-12 mice/group. Error bars represent standard error of the mean.

Positive Combination Effect of ACT and Systemic mWTX-330 Dosing

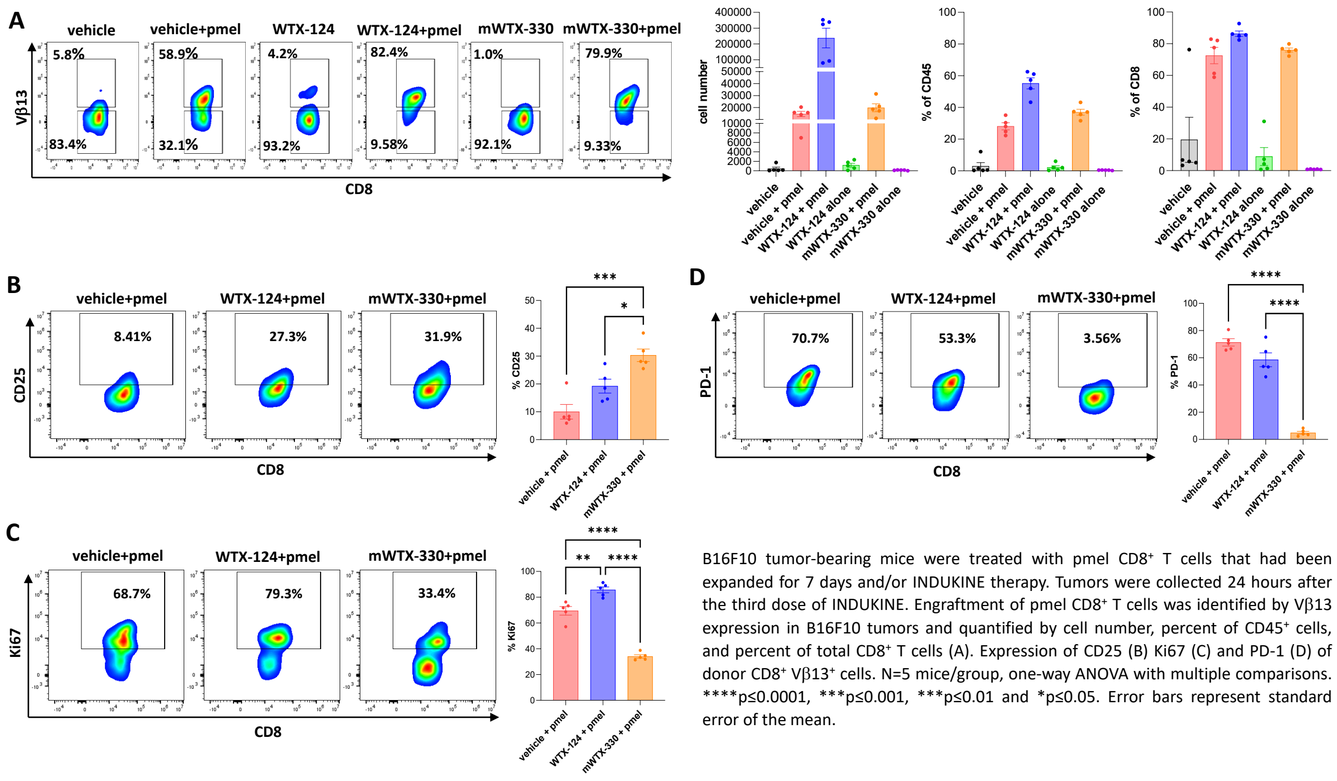
mWTX-330 Improves the Antitumor Efficacy of CD8⁺ pmel ACT Against B16F10



C57BL/6 mice were subcutaneously injected with B16F10 melanoma. Animals were intravenously infused with pmel CD8⁺ T cells that had been expanded for 7 days. Animals were treated intraperitoneally with either vehicle or IL-12 INDUKINE on Days 1, 4 and 8. Average tumor growth curves (A) and animal survival (B) of B16F10-bearing C57BL/6 mice treated with IL-12 INDUKINE and/or CD8⁺ pmel ACT. Individual tumor spider plots (C) in which each line represents an individual animal n=8 mice/group. Error bars represent standard error of the mean.

Improved Engraftment and Activation of ACT with Systemic INDUKINE Molecules Treatment

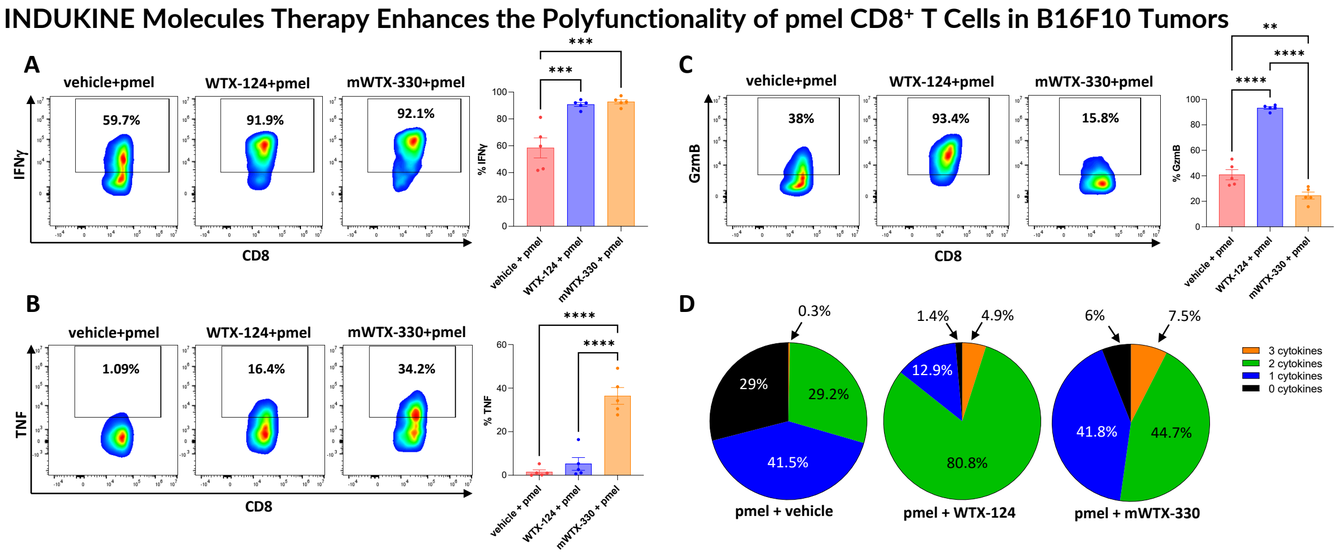
INDUKINE Molecule Therapy Increases the Engraftment and Activation of Donor CD8⁺ pmel in Tumors



B16F10 tumor-bearing mice were treated with pmel CD8⁺ T cells that had been expanded for 7 days and/or INDUKINE therapy. Tumors were collected 24 hours after the third dose of INDUKINE. Engraftment of pmel CD8⁺ T cells was identified by Vβ13 expression in B16F10 tumors and quantified by cell number, percent of CD45⁺ cells, and percent of total CD8⁺ T cells (A). Expression of CD25 (B) Ki67 (C) and PD-1 (D) of donor CD8⁺ Vβ13⁺ cells. N=5 mice/group, one-way ANOVA with multiple comparisons. ****p≤0.0001, ***p≤0.001, ***p≤0.01 and *p≤0.05. Error bars represent standard error of the mean.

INDUKINE Molecules Treatment Enhances Effector Functions of ACT

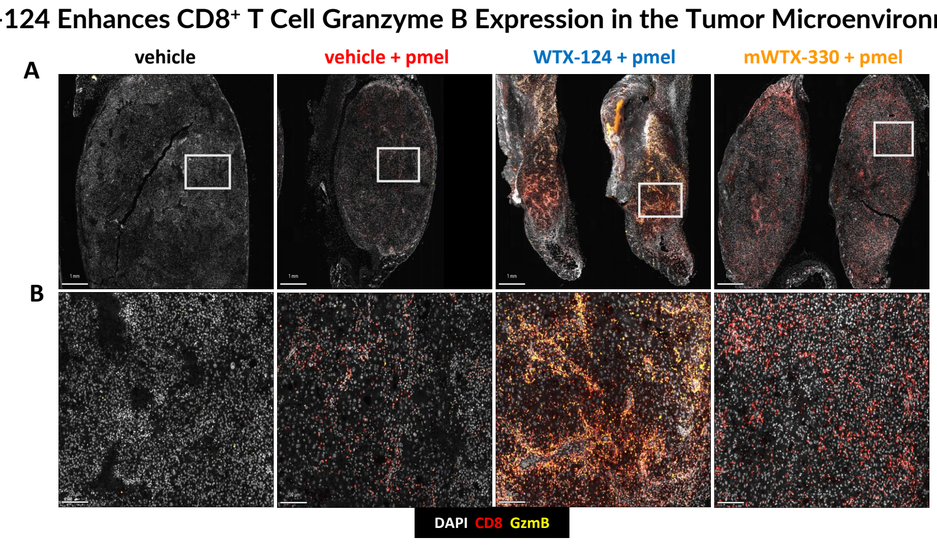
INDUKINE Molecules Therapy Enhances the Polyfunctionality of pmel CD8⁺ T Cells in B16F10 Tumors



B16F10 tumor-bearing mice were treated with pmel CD8⁺ T cells that had been expanded for 7 days and/or INDUKINE therapy. Tumors were collected 24 hours after the third dose of INDUKINE. Cells were restimulated with PMA, Ionomycin and Brefeldin A to assess cytokine production. Donor CD8⁺ pmel production of IFNγ (A) TNF (B) and Granzyme B (C) from B16F10 tumors. D) Frequencies of pmel CD8⁺ T cells co-expressing IFNγ, TNF and/or Granzyme B. N=5 mice/group, one-way ANOVA with multiple comparisons. ****p≤0.0001, ***p≤0.001, ***p≤0.01 and *p≤0.05. Error bars represent standard error of the mean.

INDUKINE Treatment Increases Tumor Penetrance and Activation of ACT

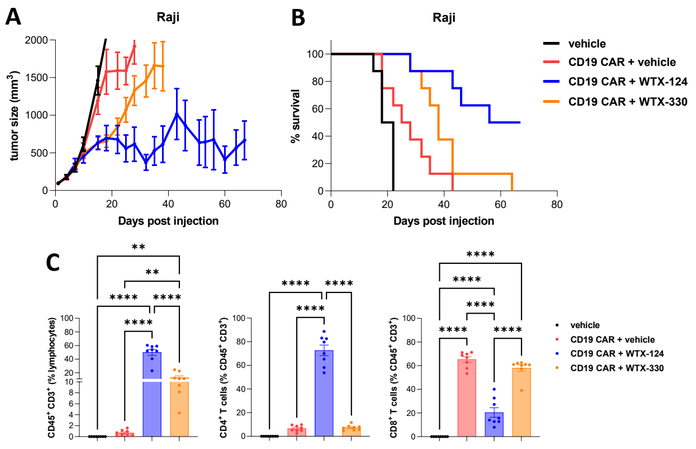
WTX-124 Enhances CD8⁺ T Cell Granzyme B Expression in the Tumor Microenvironment



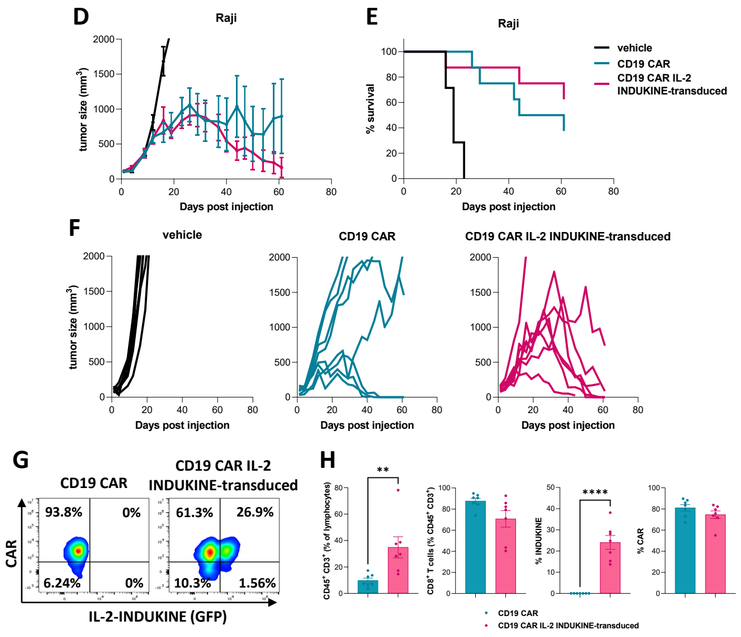
B16F10 tumor-bearing mice were treated with pmel CD8⁺ T cells that had been expanded for 7 days and/or INDUKINE therapy. Tumors were collected 24 hours after the third dose of INDUKINE. FFPE B16F10 tumors were analyzed using multiplex immunofluorescence staining performed with the Lunaphore COMET system. Markers are noted below for tumor sections at 1mm (A) and 200μm (B). White boxes indicate areas magnified to 200μm.

INDUKINE Molecules Improve the Antitumor Immunity of CAR T Cell Therapy

Systemic INDUKINE Molecule Therapy



Engineered CAR T cells with Membrane-Bound IL-2 INDUKINE



NSG mice were subcutaneously injected with Raji tumor cells. Raji tumor growth (A) and animal survival (B) with combination therapy of CD19 CARs and systemic INDUKINE on Days 1, 4 and 8. Day 17 peripheral blood analysis of donor CD19 CAR T cells in NSG mice (C). N=8 mice/group, one-way ANOVA with multiple comparisons. Raji tumor growth (D), animal survival (E), and tumor growth spider plots (F) with transfer of CD19 CAR or CD19 CAR IL-2 INDUKINE-transduced cells. Representative flow plots of CD8⁺ T cells from Day 29 peripheral bleed showing CAR and INDUKINE expression (G). Frequency of donor cells and expression of CAR and INDUKINE expression on Day 29 (H). N=7-8 mice/group, unpaired t test ****p≤0.0001, ***p≤0.001, ***p≤0.01 and *p≤0.05. Error bars represent standard error of the mean.

SUMMARY and CONCLUSIONS

- The combination of CD8⁺ pmel ACT and systemic IL-2 or IL-12 INDUKINE molecules therapy enhanced antitumor immunity against B16F10 melanoma
- WTX-124 enhanced donor cell engraftment of pmel cells in the tumor microenvironment
- mWTX-330 reduced the expression of PD-1 on donor pmel cells while increasing activation marked by CD25 expression
- Treatment with both INDUKINE molecules increased the pmel polyfunctionality in the tumor microenvironment where WTX-124 biased cells to produce IFNγ and Granzyme B, and mWTX-330 biased cells to produce TNF and IFNγ
- Systemic WTX-124 administration preferentially expanded CD4⁺ CAR T cells whereas WTX-330 expanded CD8⁺ CAR T cells
- CAR T cells engineered to express IL-2 INDUKINE polypeptides have enhanced antitumor immunity against Raji tumors and increased donor cell engraftment in NSG mice
- Current clinical ACT practices can result in short-lived immunity due to heavily differentiated and exhausted T cells in solid tumors. These data demonstrate that the administration of INDUKINE proteins with ACT could reinvigorate donor cell function leading to improved engraftment and long-term responses.

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