Novel drug-responsive domain (DRD)-based regulation technology enables tightly controlled activity of potent membrane-bound IL-12 in adoptive cell therapies

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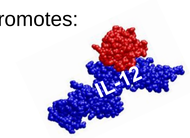
Introduction

Interleukin-12 (IL-12)

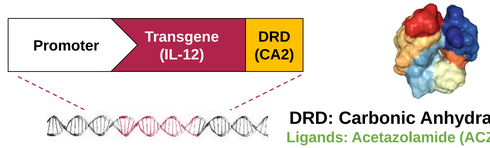
IL-12 is a promising candidate for armoring cellular therapies such as chimeric antigen receptor T cells (CAR-Ts) or tumor infiltrating lymphocytes (TILs) if its concentration, localization, and toxicities can be controlled

- Hallmark Th1, proinflammatory cytokine promotes:
  - IFNγ and TNFα production
  - T cell and NK cell proliferation and activation
  - Adaptive cell-mediated immunity
  - Repolarizes suppressive myeloid cells
  - Enhances antigen presentation
- Preclinical efficacy in multiple solid tumor models
- **Potential clinical utility limited by toxicity** at even moderate systemic concentrations



The Obsidian cytoDRiVE® platform

Obsidian's cytoDRiVE® platform can be used to control protein expression, acting as a titratable and reversible rheostat for on demand activity



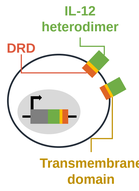
DRD: Carbonic Anhydrase 2 (CA2)
Ligands: Acetazolamide (ACZ), Celecoxib

- Drug responsive domains (DRDs)
  - Off-state = in the absence of ligand, the DRD is unfolded and degraded by the proteasome along with the target (IL-12)
  - On-state = in the presence of ACZ the DRD is stabilized allowing for target protein (IL-12) expression and function
- Carbonic Anhydrase DRD is fully human
- The stabilizing small molecule ligand, Acetazolamide (ACZ) is
  - Orally bioavailable
  - FDA approved

Membrane bound IL-12 (mbIL-12)

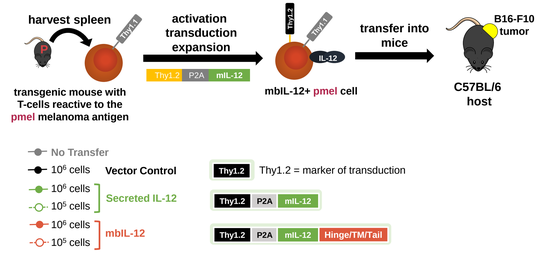
Tethering IL-12 to the membrane of cellular therapies may control the localization of IL-12

- Spatially constrain IL-12 to the cell product
  - Retain cell intrinsic and extrinsic function
  - Limit systemic exposure of the DRD-IL-12 fusion protein

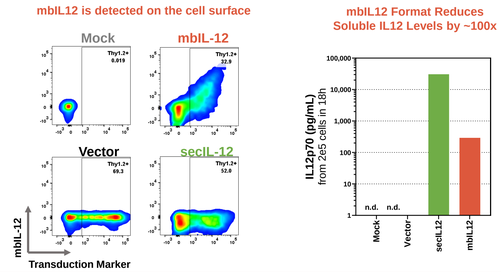


Membrane bound IL-12 has more localized effects and reduced toxicity compared with secreted IL-12

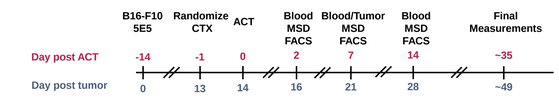
The pmel model as a system for testing mbIL-12 for cell therapy



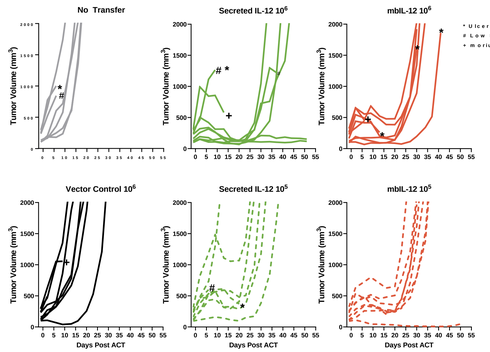
Less IL-12 is detected in cell supernatants from pmel cells expressing mbIL-12 instead of secreted IL-12



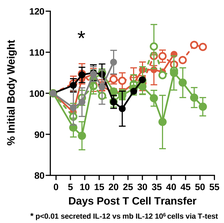
mbIL-12 enhances anti-tumor efficacy in the syngeneic B16/pmel model and is better tolerated than secreted IL-12



Both mbIL-12 and systemic IL-12 delay tumor outgrowth in the pmel model

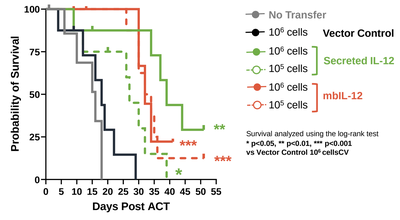


Unlike secreted IL-12, mbIL-12 does not cause bodyweight loss



* p<0.01 secreted IL-12 vs mb IL-12 10^6 cells via T-test

Both mbIL-12 and secreted IL-12 extend survival. However, toxicities with secreted IL-12 result in more animal losses in the first 2 weeks than occur with mbIL-12

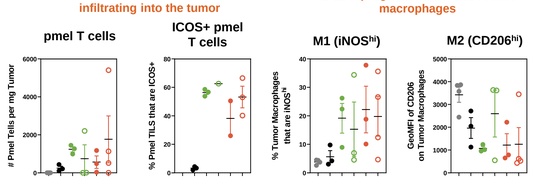


Survival analyzed using the log-rank test
* p<0.05, ** p<0.01, *** p<0.001 vs Vector Control 10^6 cells/CV

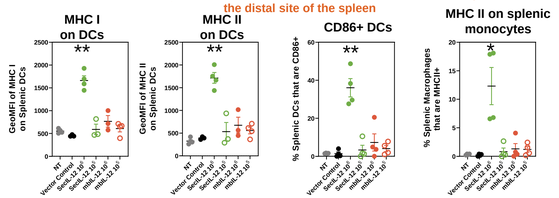
mbIL-12 remodels the tumor microenvironment while driving less systemic effects than secreted IL-12

IL-12 increases Th1 pmel T-cells infiltrating into the tumor

IL-12 reprograms tumor associated macrophages



Secreted IL-12, but not mbIL-12, activates dendritic cells and monocytes in the distal site of the spleen

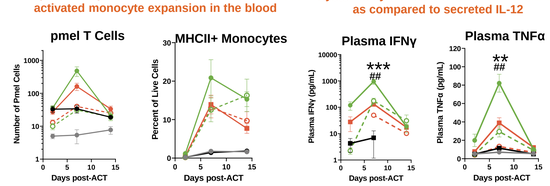


Tumors and spleens were analyzed on day 7 post adoptive cell therapy (ACT)

Both mbIL-12 and secreted IL-12 elicit similar PD profiles

IL-12 promotes pmel T cell expansion and increases activated monocyte expansion in the blood

Systemic cytokines are lower with mbIL-12 as compared to secreted IL-12



These data show that constitutively expressed membrane bound IL-12 increases the potency of tumor-specific T-cells and remodels the B16 tumor microenvironment, while reducing toxicity. Accordingly, these data suggest that Obsidian's cytoDRiVE® technology could be used to further tune the efficacy and safety of mbIL-12 in human cellular therapies by tightly regulating IL-12 expression

Statistics

All statistics were analyzed in Graphpad Prism using an unpaired t-test with two-stage linear step-up of Benjamini, Krieger, and Yekutieli

*** p<0.01, ** p<0.001 sec v mb IL-12 10^6 cells**
 # p<0.01, ## p<0.001 sec v mb IL-12 10^6 cells

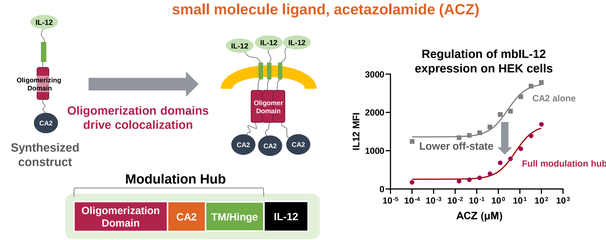
Details

Gating

Blood Monocytes: CD11b+ MHCII+ CD11c-
PMEL T-Cells: CD3+ Thy1.1+, Thy1.2+
Tumor Macrophages: CD45+ CD11b+ CD11c- F4/80+
Splenic DCs: CD45+ CD11b+ CD11c+ MHCII+
Splenic Monocytes: CD45+ CD11b+ CD11c- Ly6G- Ly6C High

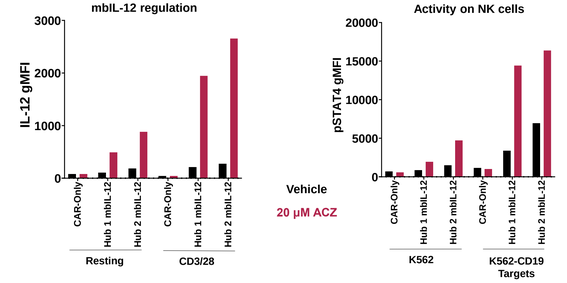
DRD modulation hubs enhance mbIL-12 regulation in human T cells

Oligomerizing drug responsive domains (DRDs) lower the off-state level of mbIL-12 and increases the dynamic range for regulation with the small molecule ligand, acetazolamide (ACZ)

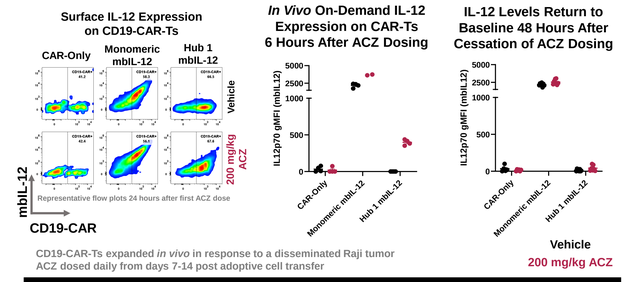


mbIL-12 regulation on human CD19-CAR-Ts *in vitro* and *in vivo*

T cell activation state and ACZ drive CA2 modulation hub mbIL-12 expression and activity *in vitro*



ACZ drives reversible CA2 modulation hub mediated mbIL-12 expression *in vivo*



CD19-CAR-Ts expanded *in vivo* in response to a disseminated Raji tumor ACZ dosed daily from days 7-14 post adoptive cell transfer

Conclusions

- These data indicate that attaching IL-12 to the membrane maintains anti-tumor efficacy while reducing toxicity associated with secreted IL-12
  - Reduced systemic cytokine levels in the plasma
  - IL-12 effects localized to the tumor and away from the spleen
- Regulating mbIL-12 using cytoDRiVE®-paired modulation hubs could be used to enhance the safety of IL-12-engineered cell therapies for patients with cancer
  - The data demonstrate low-off states and a high dynamic range for regulation of IL-12 on human T-cells *in vitro* and *in vivo*
  - The *in vivo* pharmacokinetics study highlights the ACZ dose-dependent titratable expression of mbIL-12

For questions, please reach out to Sean Smith (ssmith@obsidiantx.com)

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