Pharmacologically-controlled expression of membrane-bound IL-12 results in T-cell therapy with enhanced potency in preclinical solid tumor models

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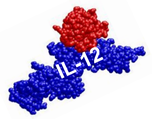
Abstract 278

Introduction

Interleukin-12 (IL-12)

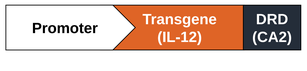
IL-12 is a promising candidate for armoring cellular therapies such as chimeric antigen receptor T cells (CAR-Ts) or tumor infiltrating lymphocytes (TILs) if its concentration, localization, and toxicities can be controlled

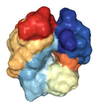
- Hallmark Th1, proinflammatory cytokine promotes:
  - IFNγ and TNFα production
  - T cell and NK cell proliferation and activation
  - Adaptive cell-mediated immunity
  - Repolarizes suppressive myeloid cells and tumor associated macrophages (TAMs)
  - Enhances antigen presentation
- Preclinical efficacy 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



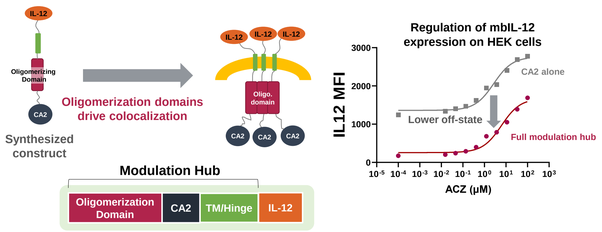


DRD: Carbonic Anhydrase 2 (CA2)
Ligands: Acetazolamide (ACZ), Celecoxib

- Drug responsive domains (DRDs)
  - Off-state = in the absence of ligand, the DRD is unfolded and degraded by proteases 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)
  - Orally bioavailable
  - FDA approved

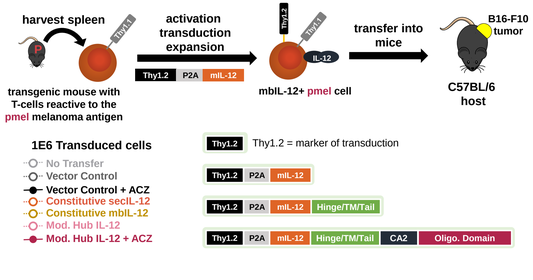
Modulation hubs enhance membrane bound regulation

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)

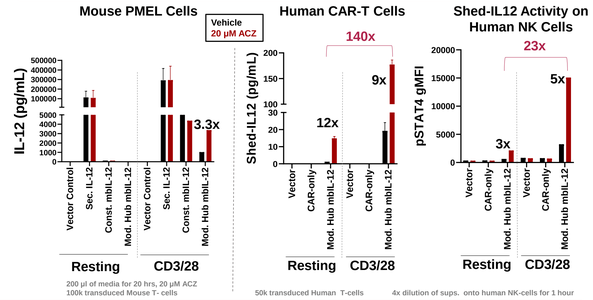


Membrane bound IL-12 has more localized effects and reduced toxicity compared with secreted IL-12

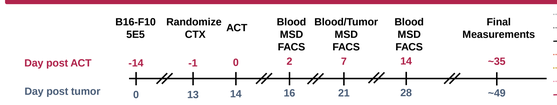
The pmel model as a system for testing mbIL-12 for cell therapy



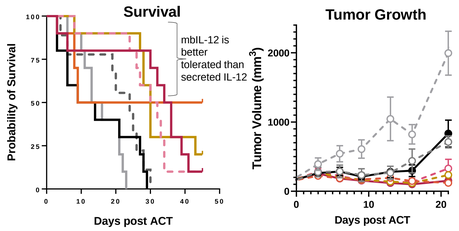
IL-12 regulation and activity in human and mouse cells is controlled by both T-cell activation and ACZ



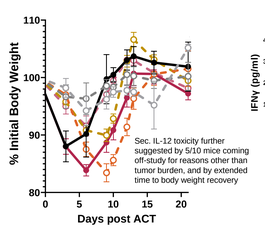
Regulated mbIL-12 enhances anti-tumor efficacy in the syngeneic B16/pmel model and is better tolerated than secreted IL-12



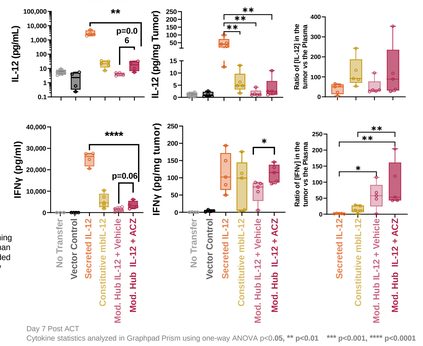
Both regulated and secreted IL-12 extend survival. However, toxicities with secreted IL-12 result in more animal losses in the first 2 weeks than occur with mbIL-12



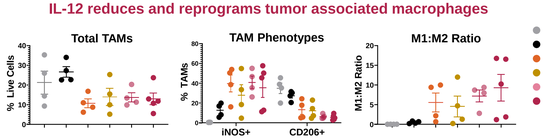
Modulation hub IL-12 does not cause weight loss beyond that anticipated by ACZ acting as a diuretic



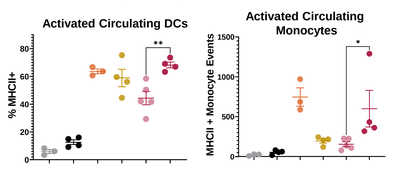
Modulation hubs regulate IFNγ in the tumor and plasma, while increasing the localization of cytokines within the tumor microenvironment (TME)



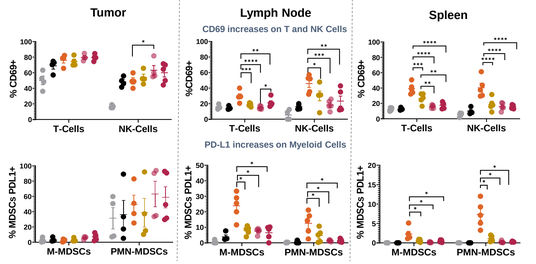
IL-12 reduces and reprograms tumor associated macrophages



Modulation hubs regulate myeloid cell activation in the blood



Modulation hubs minimize systemic impacts of IL-12 on myeloid cells



In the context of an intact immune system, modulation hubs control systemic and local levels of IL-12 and IFNγ with fewer toxic effects than secreted IL-12. Modulation hub IL-12 had similar impacts on the tumor microenvironment as secreted IL-12, but fewer systemic effects, suggesting the ability to preferentially deliver the potent cytokine to a solid tumor and alter its milieu.

Statistics

All phenotyping statistics were analyzed in Graphpad Prism using a one-way ANOVA with Tukey's multiple comparison's test.
*** p<0.05, ** p<0.01 *** p<0.001, **** p<0.0001**

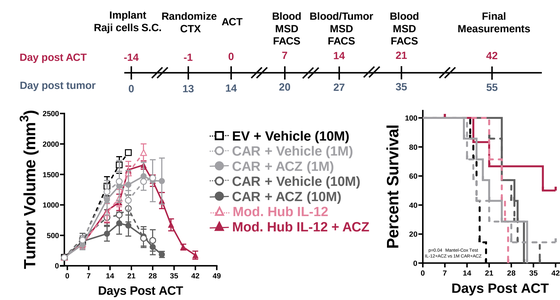
Results shown from day 7 (tumors, spleens, and TDLNs) and day 14 (blood) post adoptive cell transfer (ACT)

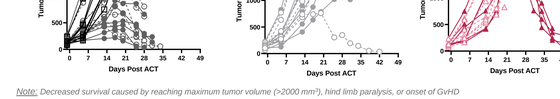
Gating

Blood Monocytes: CD45+ CD11b+ CD11c- Ly6G- Ly6C High
Tumor Macrophages: CD45+CD11b+F480+
Blood DCs: CD45+ CD11c+ MHCII+
M-MDSCs: CD45+ CD11b+ CD11c- Ly6G- Ly6C High
PMN-MDSCs: CD45+ CD11b+ CD11c- Ly6G+ Ly6Clow

DRD modulation hubs enable regulated efficacy against subcutaneous Raji tumors

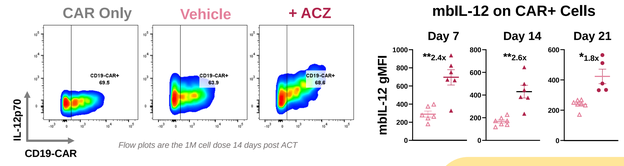
IL-12 modulation hub armored CAR-T cells control solid tumors



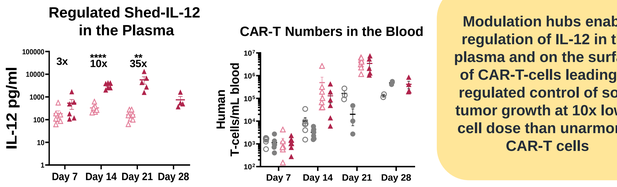


Note: Decreased survival caused by reaching maximum tumor volume (>2000 mm³), hind limb paralysis, or onset of GvHD

Modulation hubs regulate IL-12 on CAR-T cells *in vivo*



Flow plots are the 1M cell dose 14 days post ACT



Statistics analyzed in Graphpad Prism using unpaired T-Test *p<0.05, ** p<0.01 *** p<0.001, **** p<0.0001

Modulation hubs enable regulation of IL-12 in the plasma and on the surface of CAR-T-cells leading to regulated control of solid tumor growth at 10x lower cell dose than unarmored CAR-T cells

Conclusions

- **Modulation hubs showed regulated phenotypes in a syngeneic solid tumor model,** demonstrating that modulation hub mbIL-12 levels are within the linear range for impacting physiologic activities *in vivo*
  - Unlike secreted IL-12, modulation hub mbIL-12 promoted localized over systemic expression of IL-12 and IFNγ while limiting systemic activation of several cell phenotypes
  - Modulation hub mbIL-12 was better tolerated than secreted IL-12 at the tested cell doses
  - Elevated plasma cytokine levels were controlled by ACZ
  - Modulation hub mbIL-12 regulated the phenotypes of circulating antigen presenting cells
- Regulating mbIL-12 using cytoDRiVE®-paired modulation hubs could be used to enhance the safety and efficacy of IL-12-engineered cell therapies for patients with solid tumors
  - The data demonstrate very low off-states and a high dynamic range for regulation of IL-12 on human T-cells *in vitro* and *in vivo*
  - These data *show* regulated efficacy against solid Raji tumors at a 10x lower dose than unarmored CAR-Ts in mice

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

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