Armoring T-cells with regulatable, membrane tethered cytokines using improved drug-responsive domain cytoDRiVE™ technology

Dhruv K. Sethi, Dan Jun Li, Shabnam Davoodi, Dexue Sun, Sean G. Smith, Michael Schebesta, Michelle Ols, Jan ter Meulen

Obsidian Therapeutics, Inc. 1030 Massachusetts Avenue, Cambridge, MA 02138

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Abstract

Solid tumors are the next frontier in adoptive cell therapy (ACT) and cytokines such as IL12 and IFNα can amplify its efficacy by modulating the tumor microenvironment. Indeed, combinations of these and other immunomodulators have shown great promise in pre-clinical studies when expressed from genetically modified T-cells, however, they are generally too toxic for systemic exposure. Regulatory systems that allow precise control over the level and timing of their expression are therefore required for their safe use in ACT.

To this end we have developed our regulation platform, which enables post-translational control of protein abundance through modulation of protein stability. Proteins of interest are fused to a Drug-Responsive Domain (DRD) which acts as a degron, allowing for dose-dependent increase in protein levels in the presence of a stabilizing drug and decrease in the absence of the drug. DRDs are typically derived from human proteins, e.g. enzymes such as carbonic anhydrase 2, for which FDA approved drugs as the stabilizing agents (e.g. the CA2 inhibitor acetazolamide) are readily available.

Advances in our regulation platform based on multimerization of DRDs with homologous or heterologous oligomerization domains to increase their degron effect enable tight regulation with extremely low cell surface abundance in the absence of drug, and robust induction (10-20 fold) in the presence of drug. In addition, membrane tethering of the cytokines further reduces the risk of systemic toxicity, whereas engineering of protease sites allows for controlled shedding, if desirable. Combining these design elements enables full control of the abundance of IL12, IL23, IL18, IL2, and IFNα in genetically modified T-cells using a small molecule drug as the on/off switch for precise control of cytokine activity.

Introduction

The tumor microenvironment (TME) creates significant barriers to the success of adoptive cell therapy in solid tumors. The TME is immunosuppressive and supports tumor survival, growth and metastasis. Armoring cell therapies with cytokines is a strategy being developed to overcome immunosuppression and mediate anti-tumor efficacy. Toxicity limits the constitutive expression and use of many of the most potent cytokines in armored cellular therapies. Cytokines such as IL-12 and IFNα have profound immunomodulatory effects on the TME. Their clinical use, however, is limited by toxicity. They would be strong candidates for armoring cellular therapies such as chimeric antigen receptor T cells or tumor infiltrating lymphocytes (TILs) if their concentration and localization can be effectively regulated.

The Obsidian cytoDRiVE® platform

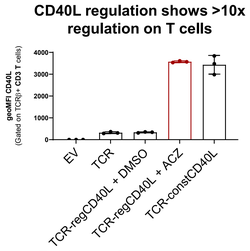
Obsidian's cytoDRiVE® platform can be used to control protein abundance, acting as a titratable and reversible rheostat for on demand activity

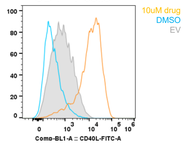
- Drug responsive domains (DRDs)
  - Off-state = in the absence of ligand, the DRD is unfolded and degraded by the proteasome along with the target.
  - On-state = in the presence of ACZ the DRD is stabilized allowing for target protein expression and function in a dose-dependent manner
- Our lead DRD carbonic anhydrase is fully human
- The stabilizing small molecule ligand, Acetazolamide (ACZ) is
  - Orally bioavailable
  - FDA approved

Target protein is expressed as a DRD fusion

Enhancing the Obsidian cytoDRiVE® platform

Trimeric TNFRSF member CD40L is one of the best DRD regulated payloads

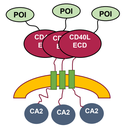




- Evidence for trans-degradation of endogenous CD40L in CD4+ T cells

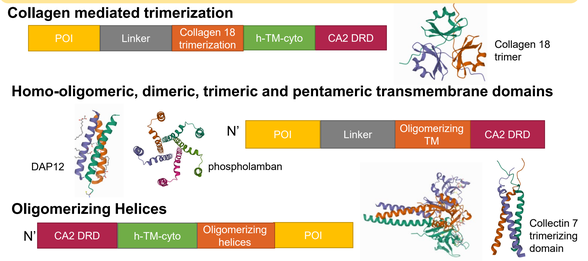
Hypothesis:
Trimerization enforces a lower basal protein abundance

Appending cytokines to extra-cellular domain of CD40L might enable better regulation of other cytokines

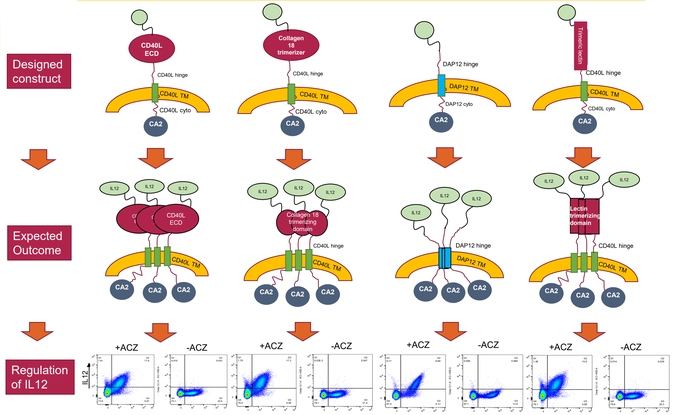


- Enable co-regulation of CD40L with other cytokines
- Multiple members of TNFR superfamily trimerize, several combinations possible
- Might be bulky and introduce unwanted function
  - Mutation of receptor binding sites possible to modulate function

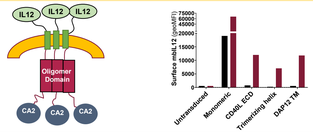
Replacing TNFSF extracellular domains with alternate oligomerizing domains will limit construct size and reduce unwanted functionality



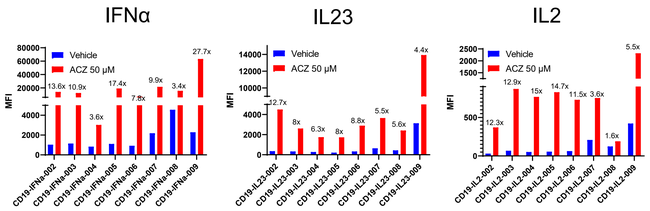
Oligomerizing DRD modulation hubs enable membrane bound IL-12 regulation in human T cells



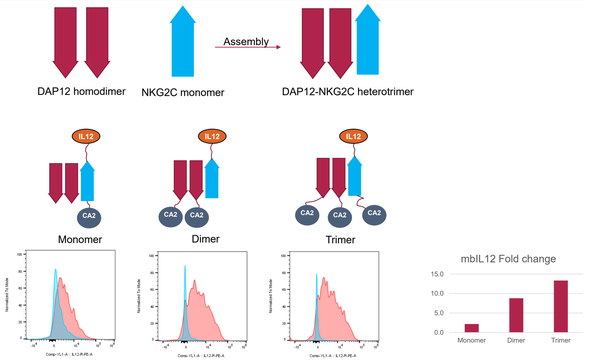
Intracellular relocation of oligomerization domains maintains regulation while lowering immunogenicity risk



Oligomeric modulation hubs incorporating a multiplicity of DRDs can be used to regulate multiple different cytokines



Hetero-oligomerization provides mechanistic insights and can form the basis of cytokine co-regulation



Conclusions

- **Obsidian's next-generation regulation platform enables robust regulation of multiple cytokines**
- **We have demonstrated that multiple non-covalent homo-oligomerization approaches can lower basal abundance and increase fold change of fusion protein levels**
- **Using hetero-oligomerization we demonstrate that increasing multiplicity of DRDs reduces basal abundance and enhances fold change**
- **Trans-degradation is proposed as the mechanism for the lower protein abundance seen with DRD oligomerization**

For questions, please reach out to Dhruv Sethi (dsethi@obsidiantx.com)

obsidiantx.com