CUE-401: A novel IL-2/TGF-beta fusion protein for the induction of CD4+ FOXP3+ regulatory T cells

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Introduction

Increasing the numbers of regulatory T cells (Tregs) is an attractive therapeutic strategy for treating autoimmune and inflammatory diseases. Conventional Tregs (Tregs) that are in low numbers and/or express only low levels of FOXP3 result in limited therapeutic benefit, and Tregs that are in large numbers and express high levels of FOXP3 can be toxic. CUE-401 is a novel IL-2/TGF-beta fusion protein comprising both IL-2 and TGF-beta signals, which are needed for induction and expansion of Tregs. Specifically, the IL-2 signal in our approach is biased to CUE-401 (IL-2R alpha) receptor subunit since the vast majority of the peripheral CD4+ T cell repertoire does not express CD25. Harnessing Tregs over Tregs may have several key advantages from a therapeutic perspective: (i) the numbers of Tregs that are biased to the fusion protein are expanded from the thymus and peripheral blood, while Tregs can be readily generated from the vast majority of the conventional CD4+ T cell repertoire that is diverse and expandable to local microenvironmental signals; (ii) the ability to convert naive murine CD4+ T cells that are naive to the local microenvironmental signals to functional suppressive Tregs. Importantly, the IL-2 signal in our approach is not biased to CD25 (IL-2R alpha), receptor subunit since the vast majority of the peripheral CD4+ T cell repertoire does not express CD25. Harnessing Tregs over Tregs may have several key advantages from a therapeutic perspective: (i) the numbers of Tregs that are biased to the fusion protein are expanded from the thymus and peripheral blood, while Tregs can be readily generated from the vast majority of the conventional CD4+ T cell repertoire that is diverse and expandable to local microenvironmental signals; (ii) the ability to convert naive murine CD4+ T cells that are naive to the local microenvironmental signals to functional suppressive Tregs. Importantly, the IL-2 signal in our approach is not biased to CD25 (IL-2R alpha), receptor subunit since the vast majority of the peripheral CD4+ T cell repertoire does not express CD25.

Concept

CUE-401 is a novel IL-2/TGF-beta fusion protein comprising both IL-2 and TGF-beta signals, which are needed for induction and expansion of Tregs. Specifically, the IL-2 signal in our approach is biased to CUE-401 (IL-2R alpha) receptor subunit since the vast majority of the peripheral CD4+ T cell repertoire does not express CD25. Harnessing Tregs over Tregs may have several key advantages from a therapeutic perspective: (i) the numbers of Tregs that are biased to the fusion protein are expanded from the thymus and peripheral blood, while Tregs can be readily generated from the vast majority of the conventional CD4+ T cell repertoire that is diverse and expandable to local microenvironmental signals; (ii) the ability to convert naive murine CD4+ T cells that are naive to the local microenvironmental signals to functional suppressive Tregs. Importantly, the IL-2 signal in our approach is not biased to CD25 (IL-2R alpha), receptor subunit since the vast majority of the peripheral CD4+ T cell repertoire does not express CD25.

Immune Balance in Autoimmune & Inflammatory Disease

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Biological considerations for iTregs

- Numbers: potential large numbers of iTregs can be generated from the broader CD4+ T cell repertoire
- Diversity: FOXP3 expression is pre-determined and fixed, while iTregs can be generated from vast numbers of naive T cells
- Phenotype: regulatory phenotypes of iTregs can be achieved and sustained with IL-2 and TGF-beta signals
- Disease impact: Conversion of pathogenic T cells into regulatory phenotypes is an attractive therapeutic strategy for immune-mediated diseases
- Application: Blood applications for iTregs in numerous autoimmune conditions, GVHD and transplantation

CUE-401 Design

- IL-2 Variant: Derived from CUE-101 (IL-2R alpha 5-fold over wild type)
- TGF-beta Variant: Derived from CUE-101 (TGF-beta 2-fold over wild type)
- FOXP3 Induction: CUE-401 is a novel IL-2/TGF-beta fusion protein comprising both IL-2 and TGF-beta signals, which are needed for induction and expansion of Tregs. Specifically, the IL-2 signal in our approach is biased to CUE-401 (IL-2R alpha) receptor subunit since the vast majority of the peripheral CD4+ T cell repertoire does not express CD25. Harnessing Tregs over Tregs may have several key advantages from a therapeutic perspective: (i) the numbers of Tregs that are biased to the fusion protein are expanded from the thymus and peripheral blood, while Tregs can be readily generated from the vast majority of the conventional CD4+ T cell repertoire that is diverse and expandable to local microenvironmental signals; (ii) the ability to convert naive murine CD4+ T cells that are naive to the local microenvironmental signals to functional suppressive Tregs. Importantly, the IL-2 signal in our approach is not biased to CD25 (IL-2R alpha), receptor subunit since the vast majority of the peripheral CD4+ T cell repertoire does not express CD25. Harnessing Tregs over Tregs may have several key advantages from a therapeutic perspective: (i) the numbers of Tregs that are biased to the fusion protein are expanded from the thymus and peripheral blood, while Tregs can be readily generated from the vast majority of the conventional CD4+ T cell repertoire that is diverse and expandable to local microenvironmental signals; (ii) the ability to convert naive murine CD4+ T cells that are naive to the local microenvironmental signals to functional suppressive Tregs. Importantly, the IL-2 signal in our approach is not biased to CD25 (IL-2R alpha), receptor subunit since the vast majority of the peripheral CD4+ T cell repertoire does not express CD25.

CUE-401 induces Foxp3+ iTregs in human mixed lymphocyte reactions

CUE-401 induces FOXP3 expression in CD4+ T cells from healthy and diseased donors. CD4+ T cells isolated from the indicated donors were stimulated with CUE-401 in the presence of the indicated concentration of CUE-401. Positive control wells contained 5 ng/ml TGFβ3 and 100 U/ml IL-2. Cells were harvested at 5 days and expression of FOXP3 was determined by flow cytometry. IBD, inflammatory bowel disease; RA, rheumatoid arthritis.

CUE-401 Differentiation: Key role for TGF-beta & IL-2 signals

- IL-2 TGF-beta variant
- FOXP3+ iTreg induction

CUE-401-induced iTregs demonstrate functional suppression of effector T cells with polyclonal stimuli (anti-CD3/CD28 signals) and antigen-specific stimuli (MLR). Conventional iTregs are suppressive while iTregs can be readily generated from the vast majority of the conventional CD4+ T cell repertoire that is diverse and expandable to local microenvironmental signals; (ii) the ability to convert naive murine CD4+ T cells that are naive to the local microenvironmental signals to functional suppressive Tregs. Importantly, the IL-2 signal in our approach is not biased to CD25 (IL-2R alpha), receptor subunit since the vast majority of the peripheral CD4+ T cell repertoire does not express CD25.