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. In contrast to natural Tregs (nTregs) that are present in small numbers and constitutively express CD25 (IL-2R alpha), induced Tregs (iTregs) are derived from the vastly larger component of the normal CD4+ T cell repertoire that is CD25-negative. While CD25-biased IL-2 variants/muteins are being developed to potentially expand nTregs, Cue Biopharma's approach incorporates both IL-2 and TGF-beta signals, which are needed for induction and expansion of iTregs. 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.

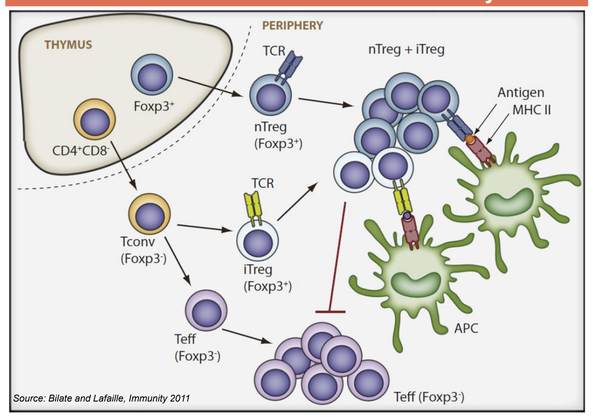
Harnessing iTregs over nTregs may have several key advantages from a therapeutic perspective: (i) the numbers of nTregs is limited since they come differentiated from the thymus with a fixed TCR repertoire, while iTregs can be readily generated from the vast majority of the conventional CD4+ T cell compartment that is diverse and adaptable to the local microenvironment; (ii) the ability to convert pathogenic autoreactive T cell into a regulatory phenotype is an attractive opportunity for immune re-set and restoration of immune balance; and (iii) the differentiation of iTregs can be achieved directly in the patient's body as long as the requisite two key modulators (IL-2 and TGF-beta) can co-localize to the same T cell. In chronic autoimmune diseases autoreactive T cells are constantly recognizing self-antigens (i.e. Signal 1 of TCR engagement is perpetual), which provides an attractive opportunity to co-deliver the IL-2 and TGF-beta to these T cells to potentially convert them into an iTreg phenotype.

In vitro data demonstrate CUE-401 effectively induces FOXP3-expressing iTregs from T cells isolated from healthy donors and donors with rheumatoid arthritis and inflammatory bowel disease, and that these iTregs suppressed effector T cell responses. In vivo studies demonstrate that a single dose of CUE-401 is effective at expanding Tregs in mice with active and ongoing autoimmunity. Further characterization of CUE-401 in preclinical disease models is ongoing.

Concept

Strategies to expand existing Tregs are being tested in the clinic, however strategies to generate new populations of induced Tregs (iTregs) are hampered by the inability to co-deliver the two signals, namely TGF-beta and IL-2, that are required to convert T cells during activation in vivo. We have exploited rational protein engineering to develop a first-in-class injectable biologic, CUE-401, that is comprised of an IL-2 variant and a TGF-β variant to induce iTregs. Induction of iTregs in vivo is an innovative and potentially effective means of suppressing chronic inflammatory diseases.

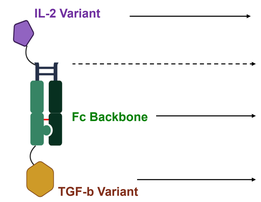
Immune Balance in Autoimmune & Inflammatory Disease



Biological considerations for iTregs

- **Numbers**: potentially large numbers of iTregs can be generated from the broader CD4+ T cell repertoire.
- **Diversity**: TCR specificity of nTregs is pre-determined and fixed, while iTregs can be generated from vastly diverse polyclonal CD4+ T cells.
- **Phenotype**: regulatory phenotype of iTregs can be achieved and sustained via IL-2 and TGF-beta signals.
- **Disease impact**: Conversion of pathogenic T cells into regulatory phenotype is an attractive therapeutic strategy for immune re-set.
- **Application**: Broad applications for iTregs in numerous autoimmune diseases, GVHD and transplantation.

CUE-401 Design



IL-2 Variant
Derived from CUE-101; Cue's lead IO asset with favorable activity/tolerability observed in clinic

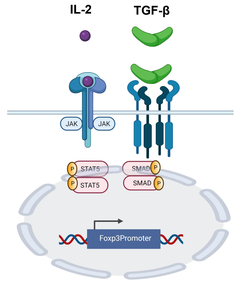
Free N-Terminus
Ability to localize to tissues or target cellular subsets through ScFv attachment (e.g., MadCAM, anti-CD4,others)

Fc Backbone
Provides structural stability and ease of manufacturability

TGF-b Variant
Improves TGF-β manufacturability and attenuates TGF-βR binding

CUE-401 has one molecule of attenuated IL-2 fused to an Fc along with an attenuated TGF-beta molecule. Importantly, the IL-2 variant in CUE-401 has already demonstrated tolerability in the clinic since it is the same IL-2 variant that is present in our current clinical candidate CUE-101, albeit in a different valency (CUE-101 harbors 4 molecules of an affinity attenuated IL-2 along with bivalent tumor-peptide-HLA molecules to activate tumor-specific T cells).

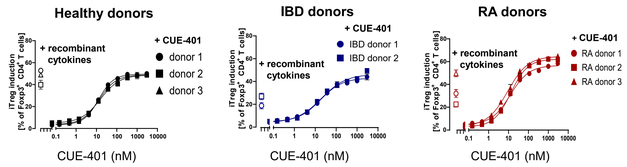
iTreg Differentiation: Key role for TGF-β & IL-2 signals



Made in BioRender

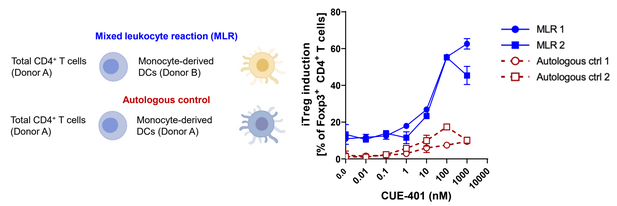
- **CD4+ T cells activated in the presence of TGF-β and IL-2 induce FOXP3 expression to generate iTregs that have suppressor activity**
- **Challenges for in vivo therapeutic applications:**
  - *To ensure TGF-β and IL-2 engage the same target T cell*
  - *Avoid systemic effects and safety liabilities associated with wildtype IL-2 and TGF-β*

CUE-401 induces iTregs as measured by expression of the master Treg transcription factor FOXP3



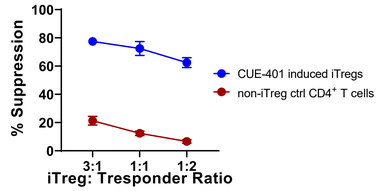
CUE-401 induces FOXP3 expression in CD4+ T cells from healthy and diseased donors. CD4+ T cells isolated from the indicated donors were stimulated with CD3 and CD28 in the presence of the indicated concentration of CUE-401. Positive control wells contained 5 ng/ml TGFβ3 and 100 U/ml IL-2. Cells were stimulated for 5 days and expression of FOXP3 was determined by flow cytometry. IBD, inflammatory bowel disease; RA, rheumatoid arthritis.

CUE-401 induces FOXP3+ iTregs in human mixed lymphocyte reactions



CUE-401 Induction of FOXP3 Expression in Allogeneic T cell Responses. Monocyte-derived dendritic cells (moDC) were differentiated with GM-CSF and IL-4 for 5 days, harvested, and incubated with freshly thawed allogenic or autologous CD4+ T cells. CD4+ T cell to moDC ratios were fixed at 5:1, and co-cultures incubated for 5 days in the presence of CUE-401 at the indicated concentration. At harvest, CD4+ cells were analyzed for FOXP3 expression by flow cytometry.

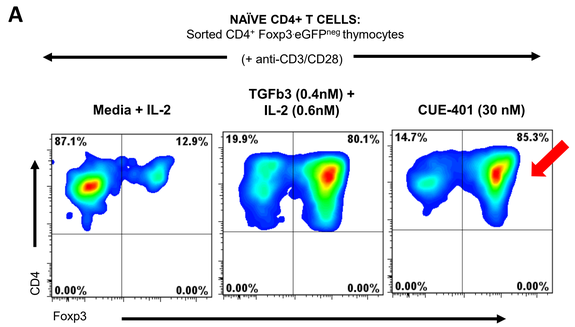
iTregs induced by CUE-401 suppress polyclonal T cell expansion

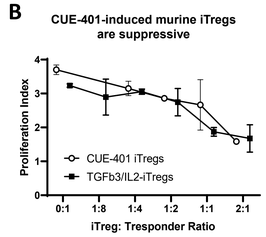


Average of 3 donors % suppression compared to T responder alone

Polyclonal stimulation of CD4+ T cells. CD4+ T cells were exposed to 300 nM CUE-401 and polyclonal (CD3/28) agonism for 5 days. Autologous, CTV labeled CD4+ T cells (responder) and iTregs were cultured at the indicated ratio with autologous mitomycin C treated PBMC and anti-CD3/28. Cultures were harvested at day 5 and proliferation of CTV labelled/diluted cells was used to determine % suppression.

CUE-401 induces Foxp3+ Suppressive iTregs From Naïve Murine CD4+ T Cells



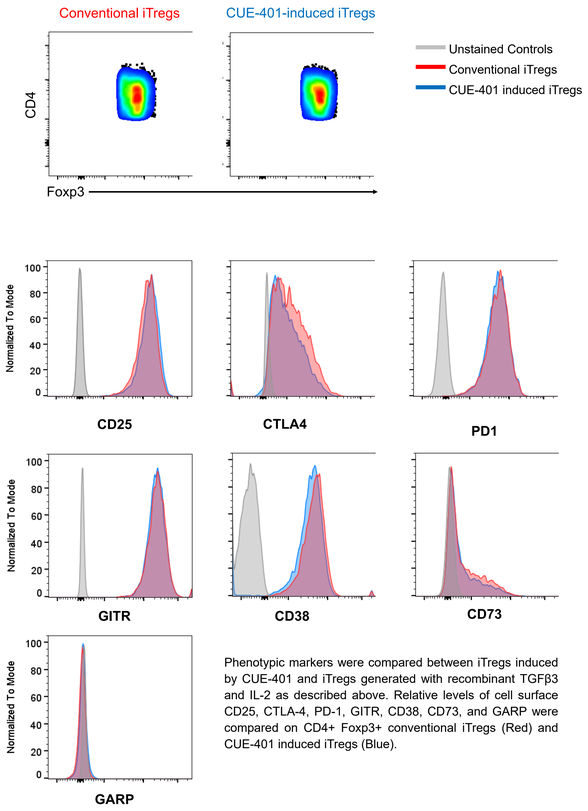


CUE-401 induces suppressive iTregs in vitro.

(A) Naïve CD4+ Foxp3 negative T cells sorted from the thymus of Foxp3-GFP reporter mice were stimulated with plate bound anti-CD3 and anti-CD28 in the presence of IL-2, recombinant TGFβ3 and recombinant IL-2, or CUE-401. Expression of CD4 and Foxp3 on Day 6 were determined by flow cytometry. CUE-401 and rTGFβ3 and rIL-2 induced Foxp3 expression in >80% of CD4+ T cells.

(B) The suppressive function of CUE-401 iTregs was measured by co-culturing Cell Trace Violet (CTV) labeled naïve CD4+ responder T cells with antigen presenting cells and iTregs at the indicated iTreg : T-responder ratios. Cells were co-cultured for 5 days and CTV dilution in proliferating CD4+ responder T cells was used to calculate the proliferation index.

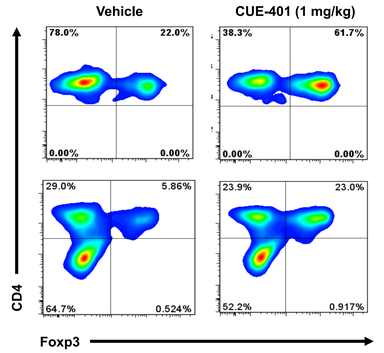
Conventional iTregs and iTregs induced by CUE-401 express similar levels of Treg-associated markers

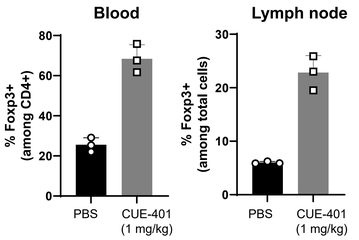


Phenotypic markers were compared between iTregs induced by CUE-401 and iTregs generated with recombinant TGFβ3 and IL-2 as described above. Relative levels of cell surface CD25, CTLA-4, PD-1, GITR, CD38, CD73, and GARP were compared on CD4+ Foxp3+ conventional iTregs (Red) and CUE-401 induced iTregs (Blue).

In vivo induction of Foxp3+ Tregs and inhibition of autoreactive T cells in the TxA23 model of autoimmune gastritis

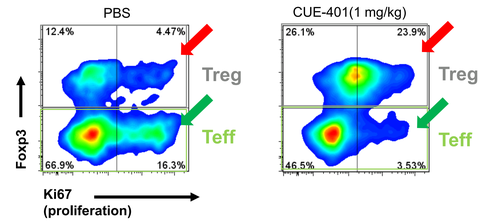
Increased Foxp3+ CD4+ T cells in blood and lymph nodes 7 days after treating TxA23 mice with CUE-401

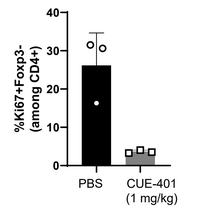




TxA23 mice with ongoing autoimmune gastritis were treated with PBS or with 1 mg/kg CUE-401. 7 days following treatment, Foxp3 expression in CD4+ T cells was analyzed by flow cytometry in the blood or gastric lymph node. FACS plots and graphs display frequencies of Foxp3+ cells among CD4+ T cells in the blood and CD4+ cells and Foxp3+ cells among total cells in the gastric LN.

Inhibition of autoreactive T cell proliferation in lymph nodes of CUE-401-treated mice





TxA23 mice with ongoing autoimmune gastritis were treated with PBS or with 1 mg/kg CUE-401. Seven days following treatment, proliferation of CD4+ Foxp3+ Treg and CD4+ Foxp3- effector T cells was measured in the gastric LN using Ki67 staining. FACS plots display Foxp3 and Ki67 expression among CD4+ T cells. The graph displays reduced frequencies of Ki67+, FoxP3-CD4+ effector T cells among total CD4+ cells in gastric lymph nodes of CUE-401 treated mice.

Conclusions

- Rational protein engineering was deployed to generate CUE-401, a single biologic that delivers the 2 key signals of IL-2 and TGF-beta for iTreg induction
- CUE-401 induces and expands FOXP3+ iTregs from healthy human PBMCs, and from PBMCs from IBD and RA patients
- CUE-401-induced iTregs demonstrate functional suppression of effector T cells with polyclonal stimuli (anti-CD3/CD28 signals) and antigen-specific stimuli (MLR assays)
- CUE-401-induced Tregs are phenotypically comparable to iTregs generated with soluble wildtype TGF-beta and IL-2
- CUE-401 expands Tregs *in vivo* in a preclinical model of autoimmune gastritis
- CUE-401-induced Tregs suppress proliferation of autoreactive T cells in gastric lymph nodes