QEL-005: CD19 CAR-Treg Cell Therapy, a Novel Approach for the Treatment of Complex Immune Mediated Inflammatory Diseases Including Rheumatoid Arthritis and Systemic Sclerosis

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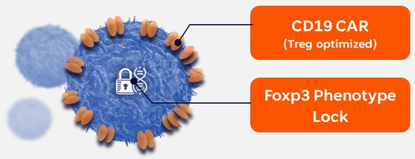
Introduction: Clinical Opportunity

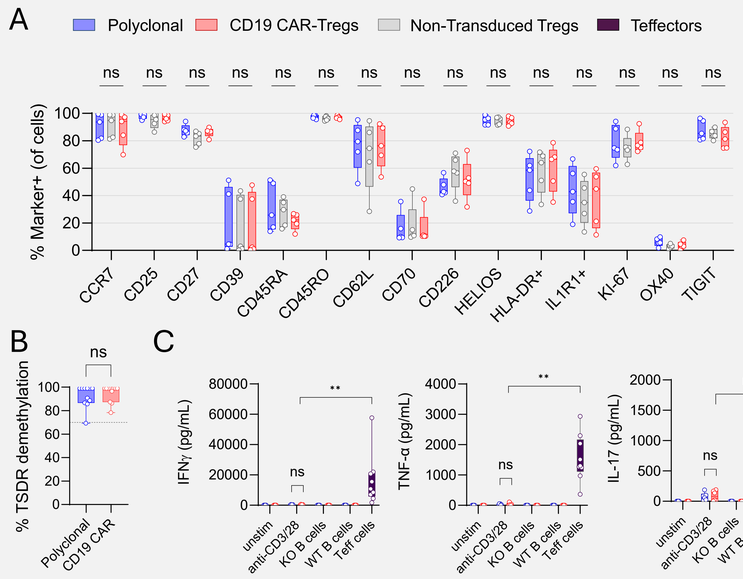
- Rheumatoid Arthritis (RA) and Systemic Sclerosis (SSc) are autoimmune diseases where a complex interplay of activated immune cells drives a chronic loop of inflammation and subsequent tissue damage
- Regulatory T cells (Tregs) have an essential role in restraining autoimmunity and are key mediators of immune and tissue homeostasis through the modulation of an array of immunological pathways
- B cell depletion approaches highlight the contribution of B cells in rheumatological diseases but are limited to targeting a single cell type and carry safety and tolerability risks in some patient populations
- We hypothesise that in SSc and RA, a CD19 CAR-Treg cell will be activated by CD19+ B cells in secondary and tertiary lymphoid organs to control chronic inflammatory responses driven by B cells, T cells, Macrophages and others to restore tissue homeostasis

QEL-005 CAR-Treg Product Candidate



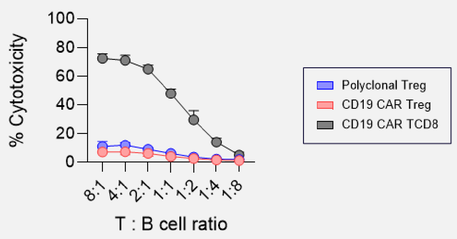
Naïve Tregs were transduced with the QEL-005 lentivirus, driving expression of a CD19 CAR and constitutive FOXP3. QEL-005 CAR-Tregs were expanded for 14 days in the presence of IL-2 before assessment for phenotype and function.

QEL-005 CAR-Tregs demonstrate a highly functional Treg phenotype



(A) Treg markers as determined by flow cytometry n=5. **(B)** Demethylation status of the Treg specific demethylated region (TSDR) determined by PCR n=10. **(C)** Cytokine secretion by Tregs or Teffs after 24hour stimulation under indicated conditions. Determined by multiplex ELISA (n=10).

QEL-005 CAR-Tregs show no cytotoxic function



CD19 CAR T CD8+ (grey), CD19 CAR Tregs (red) or polyclonal Tregs (blue) were co-cultured with CD19-expressing B LCL cell lines for 4 days. Analysis of the absolute number of viable B cells was determined by flow cytometry and percentage cytotoxicity was calculated

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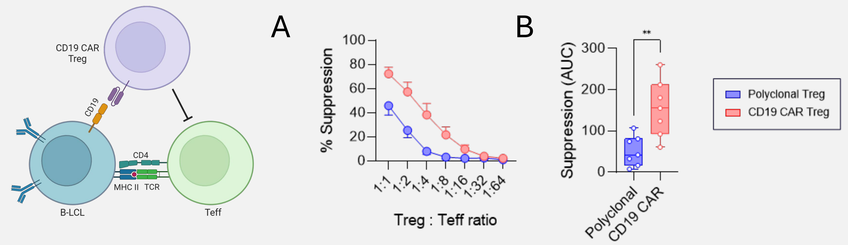
References: Cheng et al. *Nat Commun* **13**, 6110 (2022) https://doi.org/10.1038/s41467-022-33787-8

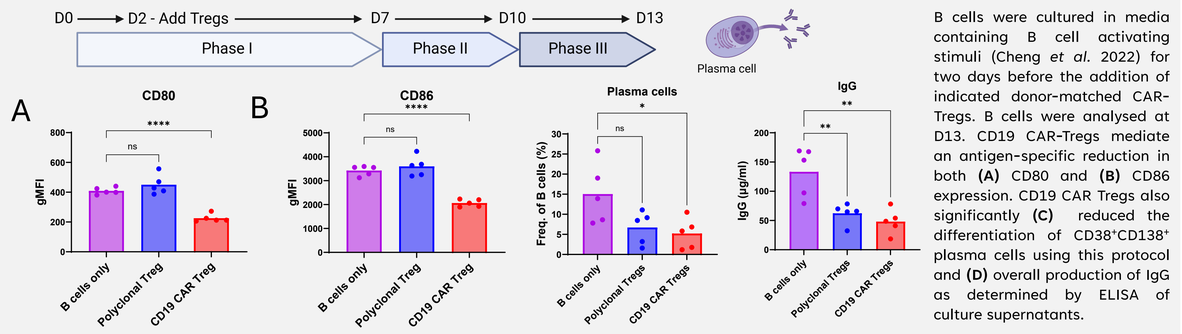
QEL-005 CAR Tregs: A broad Mechanism of Action Across Pathogenic Immune Cell Types

T cells:
QEL-005 activation by B cells restrains T cell responses



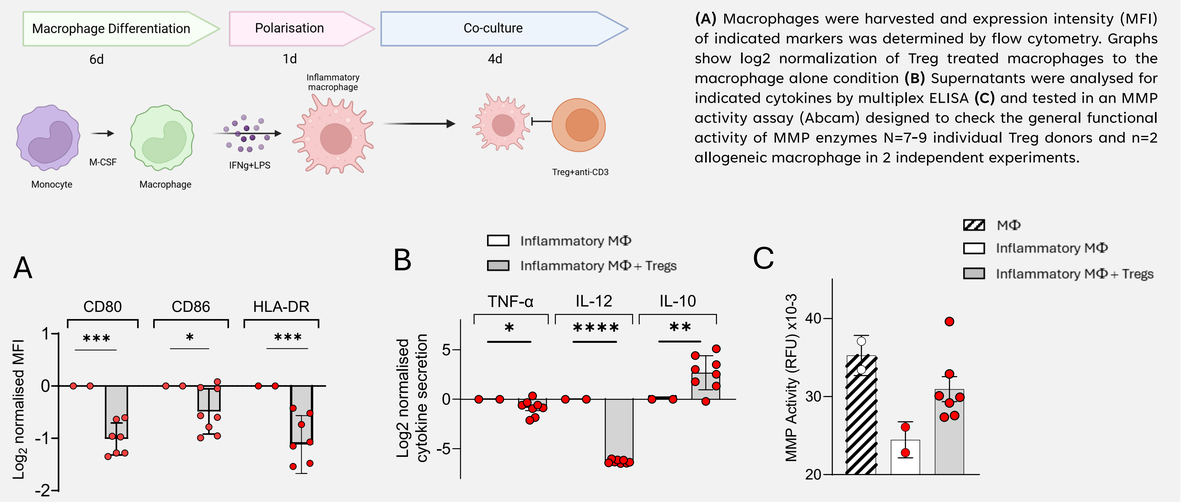
CD19-expressing B-Lymphoblastoid Cell Line cells (B-LCLs) were co-cultured with primary Teffectors and polyclonal or CD19 CAR-Tregs at indicated ratios. Suppression of Teffector proliferation was assessed after 5 days. (A) suppression curves across serial Treg:Teff ratios (B) area under the curve (AUC) values summarising overall suppressive capacity.

B cells:
QEL-005 modulates antigen presentation, plasma cell differentiation and antibody production



B cells were cultured in media containing B cell activating stimuli (Cheng *et al.* 2022) for two days before the addition of indicated donor-matched CAR-Tregs. B cells were analysed at D13. CD19 CAR-Tregs mediate an antigen-specific reduction in both **(A)** CD80 and **(B)** CD86 expression. CD19 CAR Tregs also significantly **(C)** reduced the differentiation of $CD38^{+}CD138^{+}$ plasma cells using this protocol and **(D)** overall production of IgG as determined by ELISA of culture supernatants.

Macrophages:
Activated Tregs dampen inflammatory macrophages



(A) Macrophages were harvested and expression intensity (MFI) of indicated markers was determined by flow cytometry. Graphs show log2 normalization of Treg treated macrophages to the macrophage alone condition **(B)** Supernatants were analysed for indicated cytokines by multiplex ELISA **(C)** and tested in an MMP activity assay (Abcam) designed to check the general functional activity of MMP enzymes N=7-9 individual Treg donors and n=2 allogeneic macrophage in 2 independent experiments.

Conclusions

- QEL-005 CD19 CAR-Tregs can elicit broad immune regulation without depleting B cells
- In patients with autoimmune diseases driven by complex, interconnected immune pathways CD19 CAR Tregs have the capacity to control not just B cell responses but also T cells and macrophages
- CD19 CAR-Tregs have the unique potential to reduce inflammation and restore tissue homeostasis in SSc, RA and other complex immune mediated diseases