LIBERATE: A Phase I/II Clinical Trial of QEL-001 CAR-Treg Cell Therapy to Induce Tolerance and Allow Removal of Immune Suppression in Liver Transplantation

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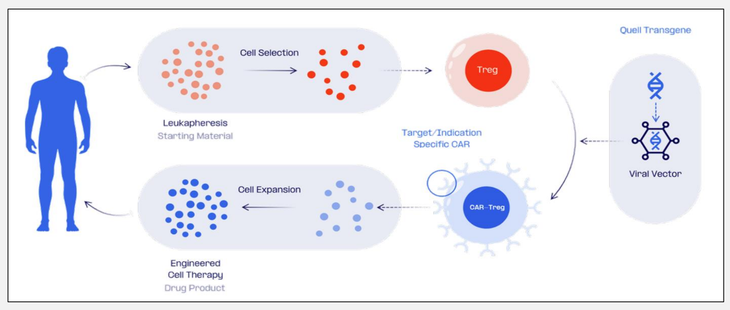
LIBERATE clinical trial: CAR-Treg therapy to modulate immune responses in Liver Transplantation patients

Regulatory T cells (Tregs) play a critical role in maintaining immune tolerance and controlling inflammatory responses. Preclinical models of transplantation have demonstrated the capacity of Tregs to control donor-specific immune responses and promote allograft acceptance. These findings support the clinical exploration of donor antigen-specific Tregs as therapeutics to mediate transplantation tolerance and eliminate the need for lifelong pharmacological immunosuppression.

A proprietary GMP manufacturing process has been developed to engineer recipient-derived Tregs to express an anti-HLA-A2 targeted CAR, a FOXP3 phenotype lock, and a safety switch. QEL-001 CAR-Tregs demonstrated consistent expression of these three transgenes while retaining the transcriptional and protein profile characteristics of unmodified Tregs. Key Treg-associated markers, including FOXP3, HELIOS, and CTLA4, alongside a demethylated TSDR region of the FOXP3 gene and low pro-inflammatory cytokine expression, confirm the stable suppressive phenotype of QEL-001.



CAR-Treg GMP manufacturing experience & process maturity:

- Significant experience with >100 therapeutic scale runs performed
- GMP manufactured patient doses of QEL-001 for LIBERATE study
- Scaled therapeutic Treg manufacturing platform with ability to manufacture doses >1Bn CAR-Tregs

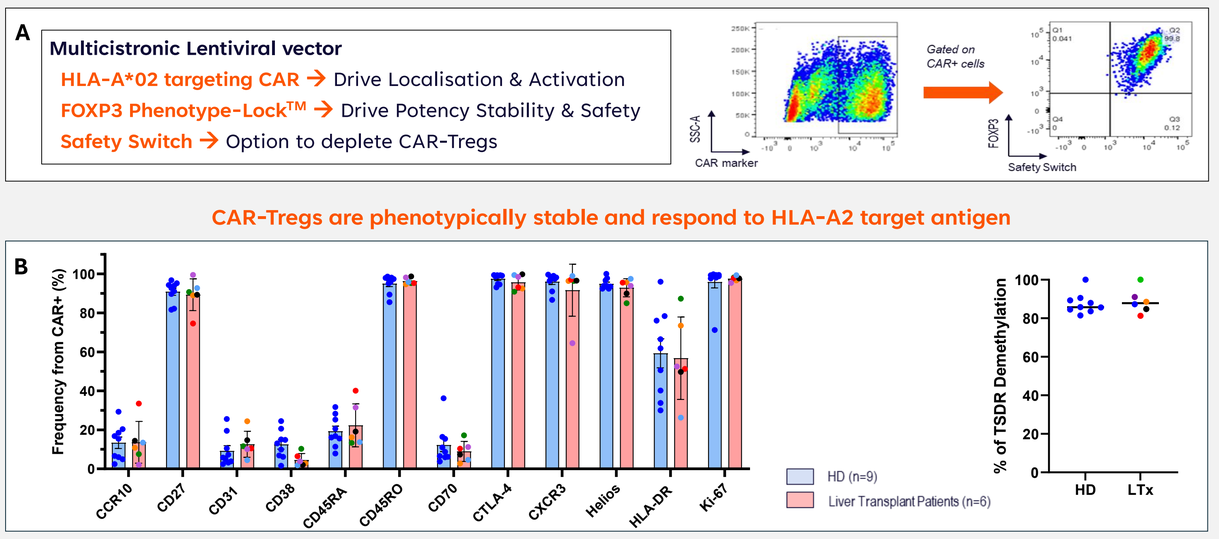
LIBERATE Study

The LIBERATE study is a first-in-human Phase I/II clinical trial (NCT05234190) designed to evaluate the safety and activity of autologous CAR-Tregs directed to HLA-A2 (QEL-001) in promoting operational liver allograft tolerance.

This single-arm, open-label, multi-centre trial focuses on HLA-A2-negative adult liver transplant recipients who have received a graft from an HLA-A2-positive donor.

Clinical Scale GMP Manufacturing of stable, durable and targeted specific CAR-Treg

QEL-001 Product: Multi-modular construct that ensures co-expression of 3 transgenes

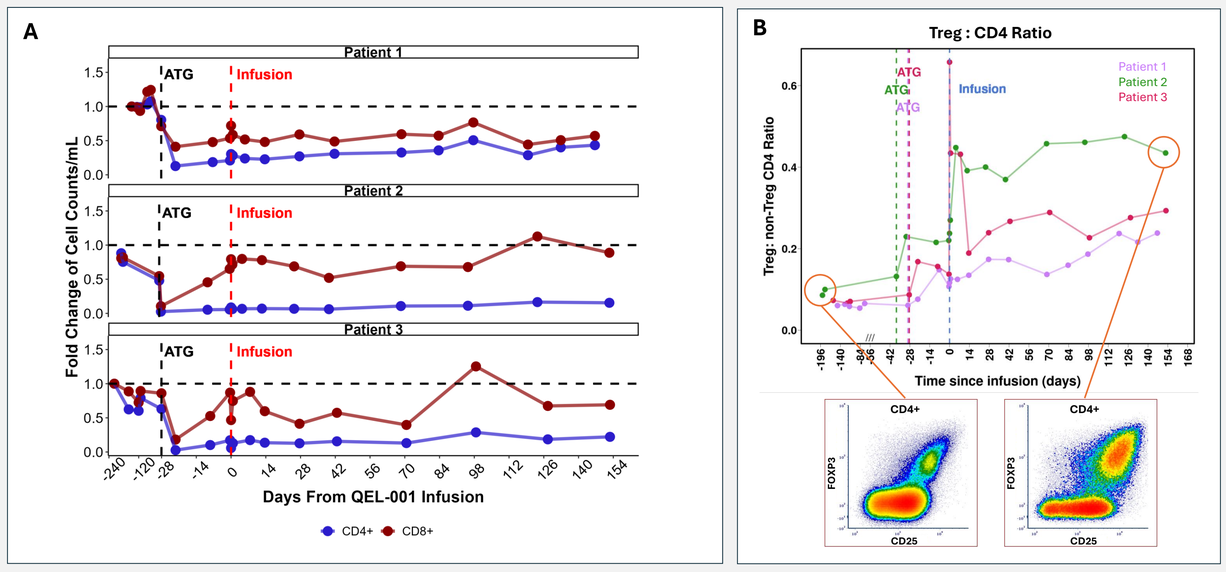


(A) Description of QEL-001 multi-modular product and characterization of the expression levels of the 3 gene components. (B) Phenotypic characterization and methylation analysis of FOXP3 locus in CAR-Tregs generated from Healthy Donors (HD) and Liver transplant patients (LTx). (C) Analysis of activation markers after in vitro stimulation with polyclonal (anti-CD3/CD28 beads) or CAR-specific stimulation (K562.HLA-A2+ cells). (D) Production of pro-inflammatory cytokines by CAR-Tregs after the in vitro culture with Th1 and Th17 instability cocktails.

Low-dose ATG conditioning depletes circulating CD4 and CD8 T cells and promotes expansion of Tregs

ATG depletes circulating CD4 and CD8 T-cells

Increasing frequency of circulating Tregs after ATG and QEL-001 CAR-Treg infusion



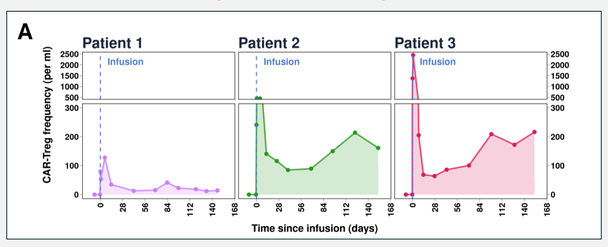
Flow cytometry analysis of peripheral blood CD4+ Tconv and CD8+ T cell frequencies following ATG treatment
(A) Whole blood is stained for T cell lineage markers in Trucount tubes. Fold change is calculated based on baseline circulating cells.

Flow cytometry analysis of peripheral Tregs
(B) Ratio of Treg to non-Treg CD4+ T cells by flow cytometry analysis of peripheral blood isolated from 3 patients of the expansion cohort.

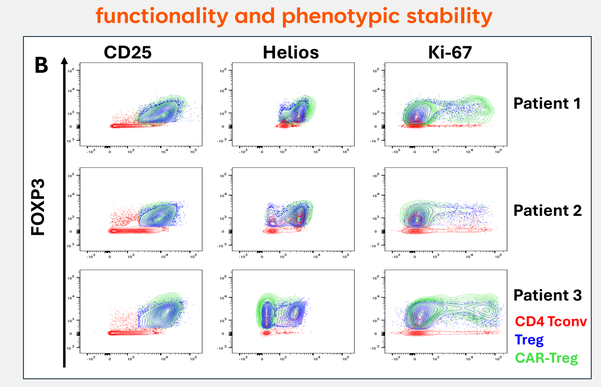
CAR-Tregs engraft and persist in circulation for >1yr, whilst maintaining Treg phenotypic stability

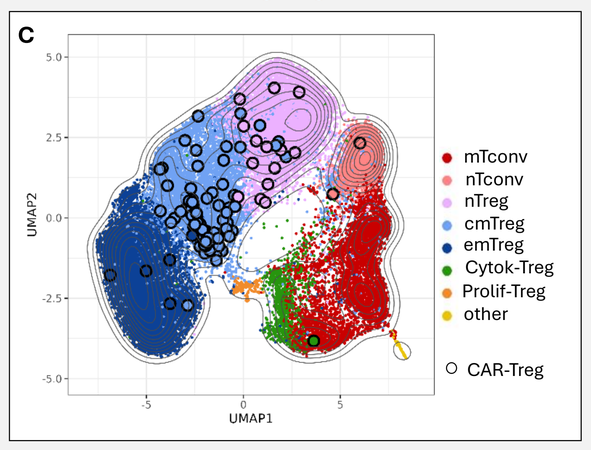
CAR-Treg engraft in the peripheral Treg niche in all expansion cohort patients

CAR-Treg shares the transcriptional profiling of circulating Treg subsets



Circulating CAR-Treg express canonical markers of functionality and phenotypic stability

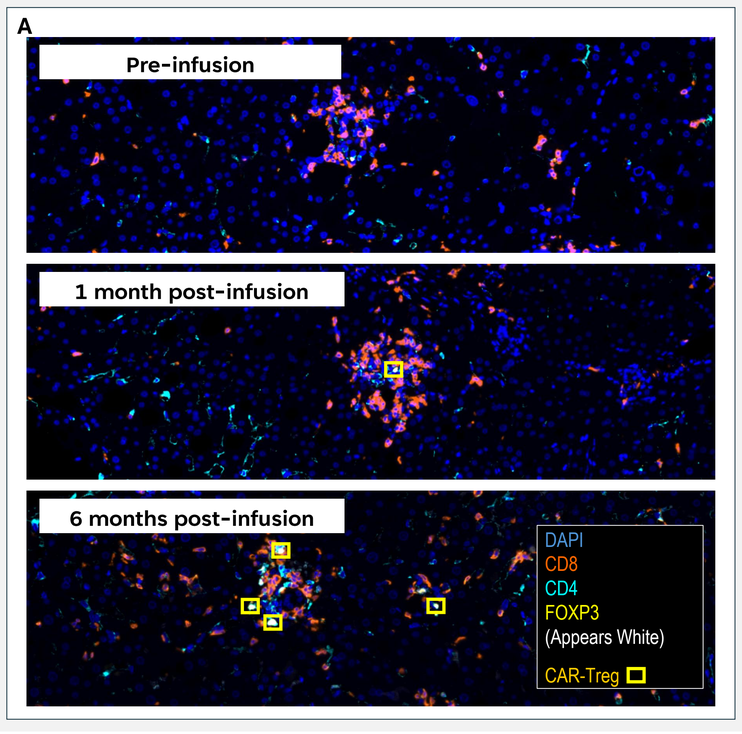




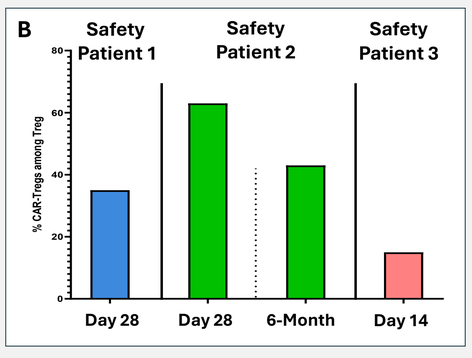
(A) CAR-Treg counts determined by flow cytometry from venous blood samples isolated from expansion cohort patients. (B) Overlay of Flow Cytometry phenotyping of peripheral CD4, Treg and CAR-Treg populations isolated from the 3 expansion cohort patients 150 days post-infusion. (C) UMAP projection of single cell RNAseq data from sorted Treg, CD4 and CAR-Treg populations isolated from the peripheral blood of a safety cohort patient 12 months post infusion. Labelled using unbiased cell type identification (SingleR). CAR-Treg are highlighted with a black circle.

QEL-001 CAR Tregs traffic to the liver tissue and occupy a significant proportion of the liver Treg niche

Detection of CAR-Tregs in patient liver biopsies (safety chohort)



CAR-Tregs preferentially enrich the intrahepatic Treg niche



(A) CAR-Tregs detected in liver biopsy at 1- & 6-months post infusion. Multiplex immuno-histochemistry analysis of liver biopsies taken prior to the infusion, at 1 month and at 6 months post infusion of QEL-001 from a representative patient of the Safety Cohort. Slides were incubated with consecutive rounds of antibody staining for targets indicated in the legend with HRP-tagged secondary antibodies followed by fluorescently bound tyramides. CAR-Treg are identified by their positive staining for CD4, FOXP3 and QBEND (staining of CD34 epitope in the safety switch).

(B) CAR-Tregs detection in liver biopsies after QEL-001 infusion in the Safety Cohort patients. The frequency of CAR-Treg represent 15-65% of the total intrahepatic Treg pool, demonstrating enhanced engraftment in the target tissue.

Conclusions and Acknowledgments

Conclusions

1. QEL-001 was well tolerated in a safety cohort consisting of three patients, supporting progression to the expansion phase of the LIBERATE clinical trial. No instances of CRS or ICANS were noted.
2. Low-dose ATG conditioning was safe and well tolerated with only transient mild/moderate AEs. No patients exhibited serum sickness.
3. ATG effectively depleted CD4 and CD8 T-cell populations and was cleared from the blood after 3 weeks (1 week prior to CAR-Treg infusion).
4. T-cell reconstitution following ATG and CAR-Treg infusion favored the expansion of circulating Treg subsets and shifted the Treg:Teff ratio in favor of the regulatory compartment.
5. CAR-Tregs persisted in the circulation for the duration of sampling (ongoing) exhibiting a stable regulatory immunophenotype including canonical markers of Treg lineage such as FOXP3 and HELIOS. Furthermore, their transcriptome overlapped with that of native Tregs.
6. Liver biopsies collected at 28 days and 6 months post-infusion provided evidence of CAR-Treg graft trafficking leading to substantial intra-hepatic enrichment and confirmed their phenotypic stability.
7. LIBERATE expansion cohort recruitment is ongoing with ATG pre-conditioning before QEL-001 infusion.

Acknowledgements & Thanks

Patients and investigators participating in LIBERATE study, Quell Clinical Operations, Manufacturing & QA teams, Precision For Medicine (study clinical operations CRO) and Phastar (clinical data management)

The Translational Team for generation and analysis of data: Alicia Roden, Anastasia Voitovich, Christina Burke, Coral Smith, Dafne Franz Demane, Florence Mehtar, Jacob Tree, Marco Romano, Paola Colaco Osorio, Sara Seshadri, Sean Holm, Sophie Howson, Yasaman Shahrabi