Characterization of engineered CAR-Treg cells to induce immune tolerance in liver transplanted patients

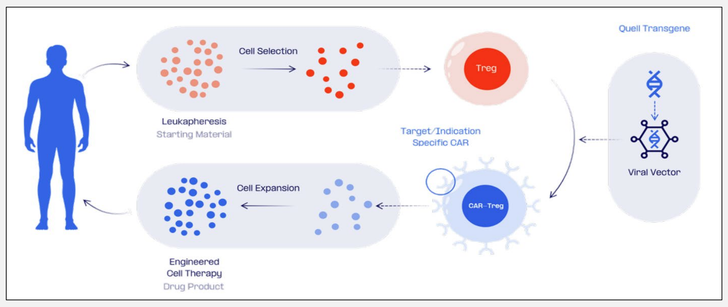
Marc Martinez-Llordella[1], Rupert Kenefeck[1], Sean Holm[1], Florence Mehtar[1], Alicia Roden[1], Anastasia Voitovich[1], Christina Burke[1], Sara Seshadri[1], Marco Romano[1], Alison Taylor [2], Kourosh Saeb-Parsy[3], Jacques Pirenne[4], Anthony J Demetris[5], Drew Lesniak[5], Nathalie Belmonte[1], Aaron Vernon[1], Chris Sharpe[1], John Tonkyn[1], Gareth Wright[1], Annie Woodburne[1], Laurie Baylor Curtis[1], Alberto Sanchez-Fueyo[2], Luke Devey[1]

(1) Quell Therapeutics Ltd, Translation & Innovation Hub, London, UK. (2) Institute of Liver Studies, King's College Hospital, King's College London University, London, UK. (3) Department of Surgery, University of Cambridge and NIHR Cambridge Biomedical Research Centre, Cambridge, UK. (4) Gasthuisberg Campus University hospital, UZ Leuven, Leuven, Belgium. (5) Department of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.

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

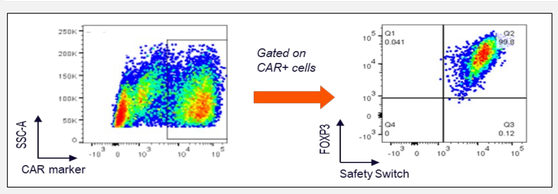
Regulatory T cells (Tregs) play a critical role in maintaining immune tolerance and controlling inflammatory responses. Preclinical models of transplantation have demonstrated the capacity of Tregs to control donor-specific immune responses and promote allograft acceptance. These findings support the 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.



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



Multicistronic Lentiviral vector

HLA-A*02 targeting CAR → Drive Localisation & Activation
FOXP3 Phenotype-Lock™ → Drive Potency Stability & Safety
Safety Switch → Option to deplete CAR-Tregs

LIBERATE clinical trial: CAR-Treg therapy to allow removal of toxic systemic immune suppression in Liver Transplantation patients

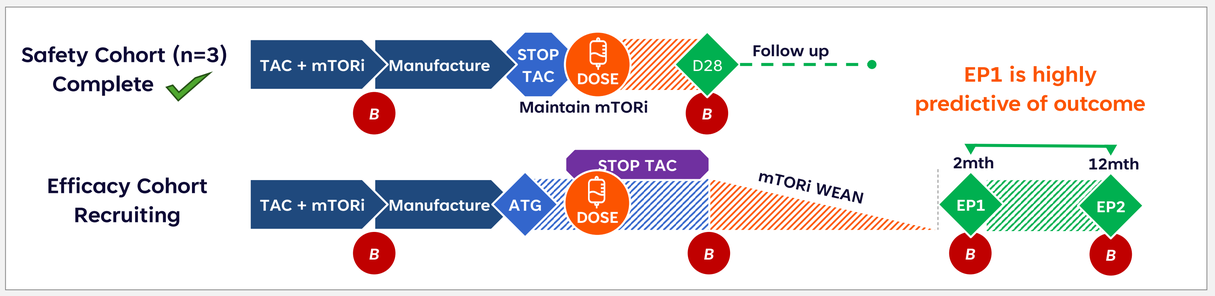
Patient Unmet Need

- **Transplant recipients experience substantial morbidity and mortality due to systemic immunosuppression**
- Reduced immune-surveillance -> increased rates of malignancies & infections
- Immunosuppression mediated Cardio & Nephro toxicity (which can result in dialysis and kidney transplantation)

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.

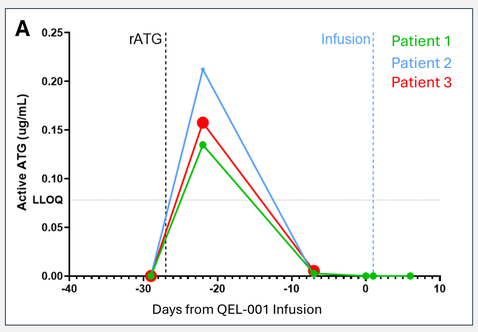


Safety – QEL-001 infusion and low-dose ATG was well tolerated

- There were no reports of cytokine release syndrome (CRS) and no reports of immune effector cell-associated neurotoxicity syndrome (ICANS) associated to QEL-001 CAR-Treg infusion.
- Adverse events (AEs) either directly associated with or occurring contemporaneously with administration of rATG were all mild to moderate with minimum or no intervention. All adverse events recovered or resolved within 5 days or less of rATG administration

Low dose ATG effectively depletes CD4 and CD8 T-cells and is cleared from the circulation within 3 weeks

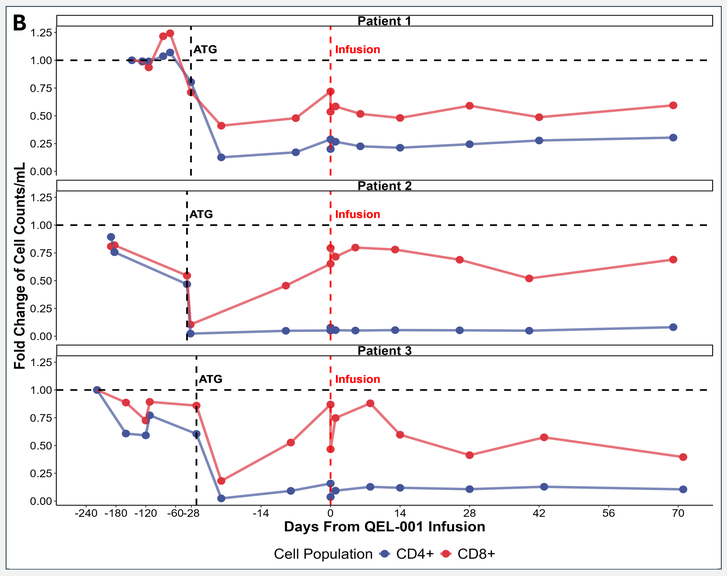
ATG is cleared 3 weeks post dosing



Active ATG detected in serum by Flow Cytometry (above)
(A) Active ATG is defined as the fraction of ATG able to bind to T-cells and is detected by Flow cytometric analysis of cells incubated with patient serum.

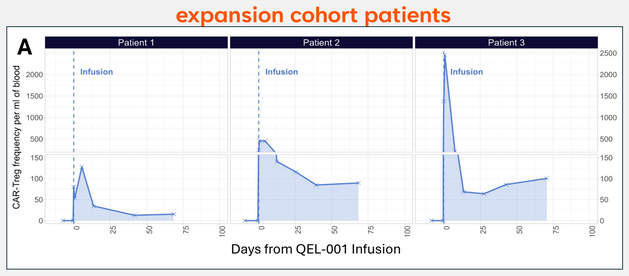
Flow cytometry analysis of peripheral blood CD4+ Tconv, CD8, frequencies following ATG treatment (right)
(B) Whole blood is stained for T cell lineage markers in Trucount tubes and the fold change from baseline calculated.

ATG depletes CD4 and CD8 T-cells

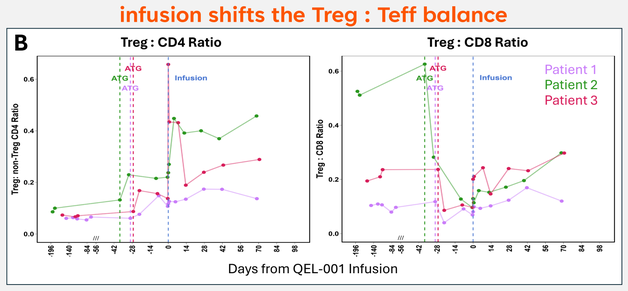


CAR-Tregs engraft and persist in circulation of Expansion Cohort patients maintaining phenotypic stability

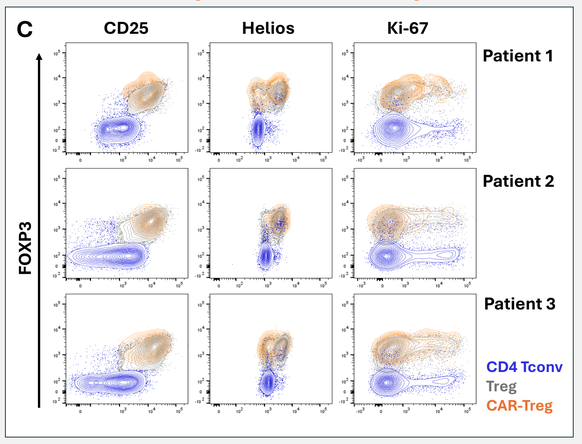
CAR-Treg engraft in the peripheral Treg niche in all expansion cohort patients



T-cell reconstitution following ATG and CAR-Treg infusion shifts the Treg : Teff balance

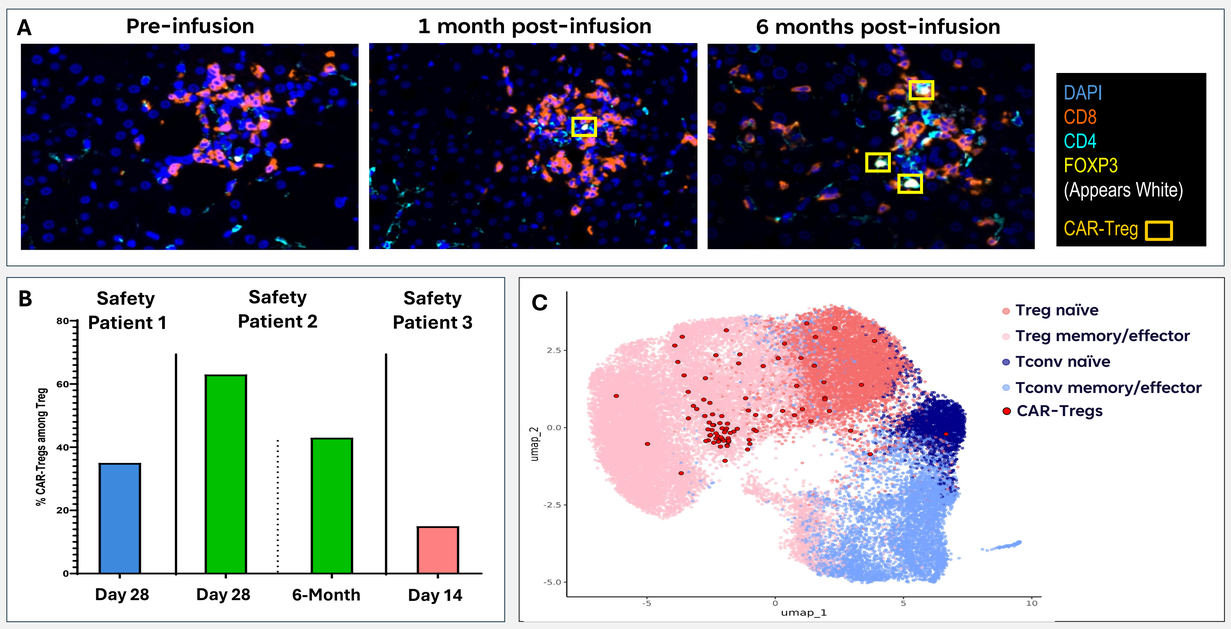


CAR-Treg express canonical markers of stable & functional Tregs 10 weeks following infusion



(A) CAR-Treg counts in venous blood samples isolated from expansion cohort patients & determined by flow cytometry. (B) Ratio of Treg to non-Treg CD4+ and CD8+ T-cells by flow cytometry analysis of peripheral blood isolated from expansion cohort patients. (C) Overlay of Flow Cytometry phenotyping of peripheral CD4, Treg and CAR-Treg populations isolated from expansion cohort patients.

QEL-001 CAR Tregs persist in circulation over 1-year in Safety Cohort and represent a significant proportion of the liver 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. 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 (not shown). **(B) CAR-Treg represent 15 – 65% of all Tregs in the liver of infused patients while limited frequencies are observed in the periphery**. The proportion of CAR-Treg and Treg from A figure. **(C) CAR-Tregs 12-months post infusion share similar transcriptional profile to non-transduced Tregs**. 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 highlighted in red.

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 administration 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 Treg populations and shifted the Treg:Teff ratio substantially in favor of Tregs.
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 clinical safety cohort is complete; recruitment of efficacy cohort is ongoing (with ATG pre-conditioning) to investigate full weaning of immunosuppression.

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