Regulatory T cells and Living Donor Kidney Transplantation

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Disclosures

I have financial/advisory relationship(s) with:
ImmunoFree, Scientific Advisory Board
TRACT Therapeutics – Founder
Singulera Therapeutics – SAB, Medical Director
Cellvie – Advisory Board and Grant support
eGenesis – Advisory Board

AND

My presentation includes discussion of the investigational use of TRK-001

Organ Transplant: Challenges of Chronic Immunosuppression

Immunosuppression is not disease-modifying; requires lifelong chronic immunosuppression

Kidney toxicity

~35% of living donor transplants and ~50% of deceased donor transplants fail within 10 years[1]

Significantly increased risk of cancer[2]

Hypertension, diabetes, high cholesterol, weight gain[3]

Cardiovascular (CV) issues are leading cause of post-transplant mortality

Increased risk of serious infection[4]

High cost, pill burden (>20 pills/day for life) and decreased QoL

- Cost ~$25K in first year and $5K-$10K annually for life of organ[1]
- Poor compliance can lead to rejection or organ loss
- Sleep disturbance, CNS issues, depression, and other AEs affecting QoL
- **Transplant dysfunction, loss, return to dialysis, retransplant … ALL increase costs and reduce QoL**

Sources: patientslikeme; The Voice of the Patient FDA Meeting (Sep 2016): Patients who Have Received an Organ Transplant

1. USRDS 2020 Annual Data Report, Fig 6.16: https://adr.usrds.org/2020/end-stage-renal-disease/6-transplantation
2. Engels et al JAMA 2011; 306(17): 1891-1901
3. Nankivell BJ et al, Lancet 2011, 378:1428-37
4. Karuthu et al, Clin J Am Soc Nephrol. 2012 Dec;7(12):2058-70

QoL: Quality of life; CNS: Central Nervous System

What Is Immune Tolerance?

Post-transplant immune tolerance denotes a state in which the recipient's immune system

Accepts donor organs, tissues, and cells

Responds normally to foreign antigens/pathogens

Establishment of post-transplant immune tolerance may allow transplant recipients to discontinue chronic immunosuppression

Exner BG et al. *Acta Haematol*. 1999;101(2):78-81.

Cell Therapies being considered for Tolerance Induction

- HSC to induce chimerism
- HSC to induce immunomodulation
- **Regulatory T cells (polyclonal, Ag specific, CARTreg)**
- Dendritic cells (DC)
- Mesenchymal Stem Cells (MSC)
- Apoptotic Cell Delivery (ECDI, Mitomycin C, ECP)
- ? Combination of cell types (HSC + Treg)
- ? Single vs multiple infusions

Special Feature

OPEN

Meeting Report: The Sixth International Sam Strober Workshop on Clinical Immune Tolerance

Helen Stark, MBBS, DPhil,[1] Quan Yao Ho, MRCP, MMed,[1,2] Amy Cross, PhD,[1] Alessandro Alessandrini, PhD,[3] Alice Bertaina, MD, PhD,[4] Daniel Brennan, MD, FACP,[5] Stephan Busque, MD, MSc, FRCSC,[6] Anthony Demetris, MD,[7] Luke Devey, PhD,[8] Gilbert Fruhwirth, PhD,[9] Ephraim Fuchs, MD,[10] Peter Friend, PhD, FRCS,[11] Ed Geissler, MD, PhD,[12] Carole Guillonneau, PhD,[13] Joanna Hester, PhD,[1] John Isaacs, PhD, FRCP,[14,15] Elmar Jaeckel, MD, PhD,[16] Tatsuo Kawai, MD, PhD,[17] Fadi Lakkis, MD,[18] Joseph Leventhal, MD, PhD,[19] Megan Levings, MD,[20] Josh Levitsky, MD,[21] Giovanna Lombardi, PhD,[22] Marc Martinez-Llordella, PhD,[8] James Mathew, PhD,[23] Aurélie Moreau, PhD,[24] Petra Reinke, MD, PhD,[25] Leonardo V. Riella, MD, PhD,[3,26] David Sachs, MD,[27,28,29] Alberto Sanchez Fueyo, MD, PhD,[30] Katharina Schreeb, MD,[31] Megan Sykes, MD,[32] Qizhi Tang, PhD,[33] Angus Thomson, PhD, DSc,[34] Timothy Tree, PhD,[35] Piotr Trzonkowski, PhD,[36] Koichiro Uchida, MD, PhD,[37] Jeffrey Veale, MD,[38] Josh Weiner, MD[39] Thomas Wekerle, MD,[40] and Fadi Issa, DPhil, FRCS[1]

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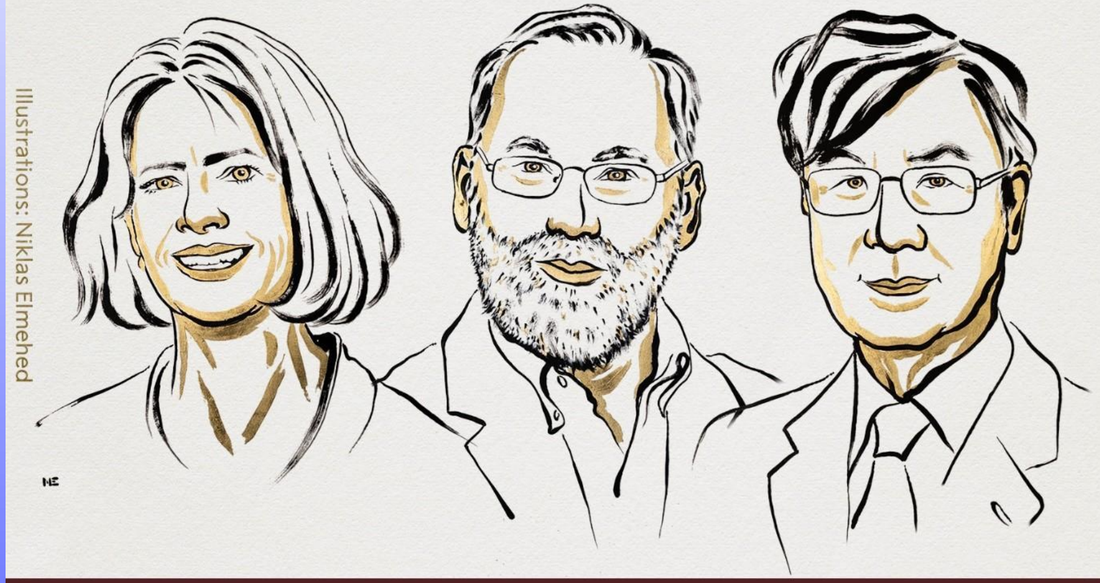
THE NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE 2025



Illustrations: Niklas Elmehed

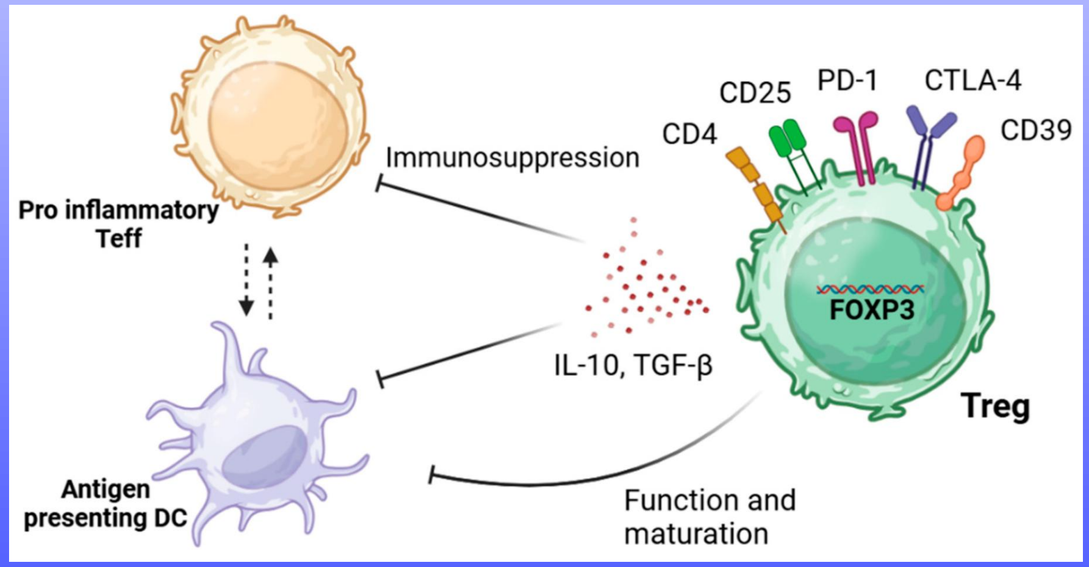
Mary E. Brunkow

Fred Ramsdell

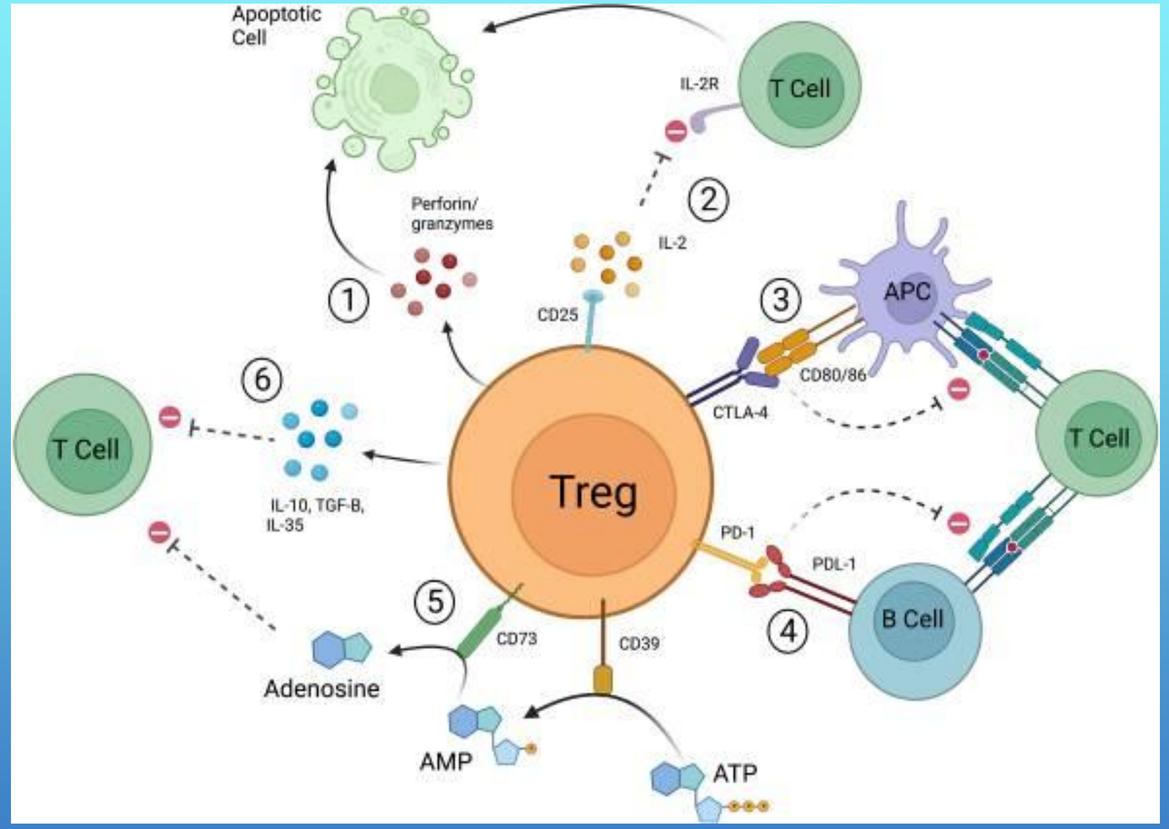
Shimon Sakaguchi

"for their discoveries concerning peripheral immune tolerance"

THE NOBEL ASSEMBLY AT KAROLINSKA INSTITUTET



Tregs Restore Balance to the Immune System



Sanders J, et al Frontiers in Immunology 2022

Mechanisms of Action

Interacting and suppressing activity of “effector cells”

Converting naïve, uncommitted T cells into Tregs, a process known as infectious tolerance

Influencing other cells in the immune system to reduce inflammation and promote tolerance

Need sufficient numbers of Tregs for clinical application: **ex vivo expansion**

Issa F, Chandrasekharan D, Wood KJ. Regulatory T cells as modulators of chronic allograft dysfunction Curr Opin Immunol. 2011 Oct;23(5):648-54.

Tregs: Clinical Evidence in Transplantation

Higher circulating numbers of Tregs in tolerant liver transplant recipients.

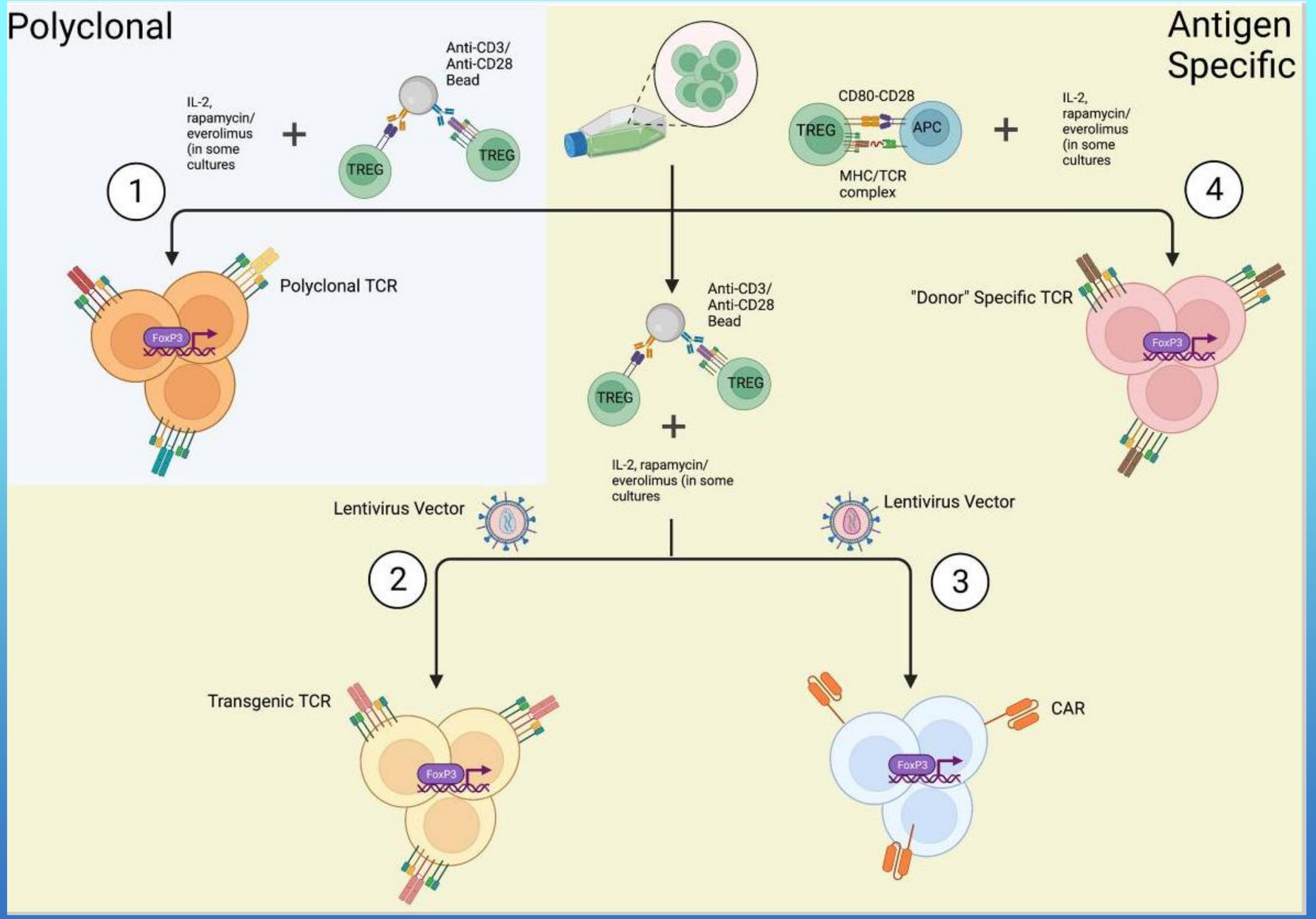
Increased numbers of Tregs in tolerant kidney transplant recipients.

Improved outcomes in stem cell transplant patients receiving infusion of expanded Tregs.

IS withdrawal in LDLT recipients receiving Treg like cell infusions (Todo et al)

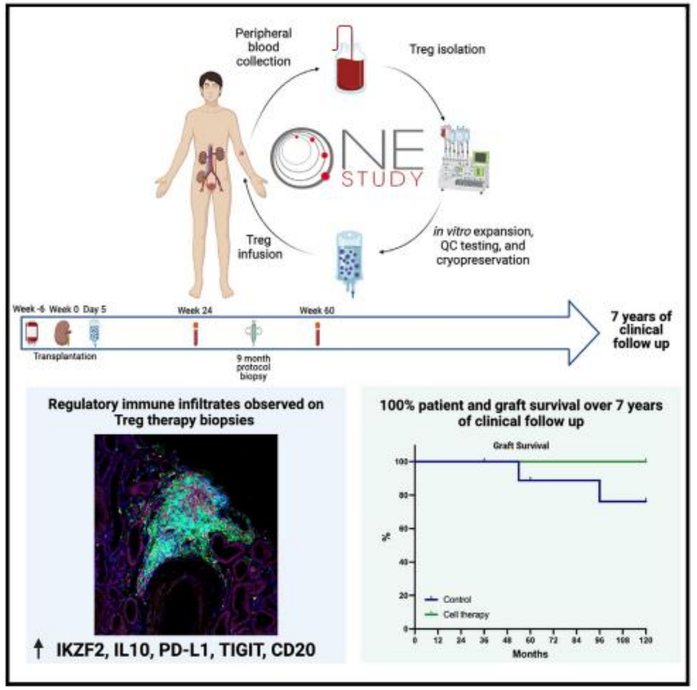
Safe reduction of IS to monotherapy in LDK transplant recipients receiving autologous expanded Tregs (Harden and Issa et al, ONE Study)

Sagoo P, Perucha E, Sawitzki B, et al. Development of a cross-platform biomarker signature to detect renal transplant tolerance in humans. J Clin Invest 2010; 120:1848.
Wood KJ, Regulatory T. cells in transplantation. Transplant Proc 2011; 43: 2135.
Leventhal JR, Mathew JM, Salomon DR, et al. Am J Transplant. 2016 Jan;16(1):221-34. Nonchimeric HLA-Identical Renal Transplant Tolerance: Regulatory Immunophenotypic/Genomic Biomarker.
Zowitzkey B, Brunstein C, Meisel C, et al. Prevention of graft-versus-host disease by adoptive T regulatory therapy is associated with active repression of peripheral blood Toll-like


Polyclonal
Antigen Specific
IL-2, rapamycin/ everolimus (in some cultures
Anti-CD3/ Anti-CD28 Bead
TREG
TREG
1
CD80-CD28
TREG
APC
MHC/TCR complex
IL-2, rapamycin/ everolimus (in some cultures
4
Polyclonal TCR
FoxP3
Anti-CD3/ Anti-CD28 Bead
TREG
TREG
IL-2, rapamycin/ everolimus (in some cultures
"Donor" Specific TCR
FoxP3
Lentivirus Vector
Lentivirus Vector
2
3
Transgenic TCR
FoxP3
CAR
FoxP3

Regulatory T cell therapy is associated with distinct immune regulatory lymphocytic infiltrates in kidney transplants

Graphical abstract



Highlights

- Patients receiving Treg therapy have excellent long-term outcomes
- Immune infiltrates within the transplant are observed after Treg therapy

Authors

Oliver McCallion, Amy R. Cross, Matthew O. Brook, ..., Paul N. Harden, Joanna Hester, Fadi Issa

Correspondence

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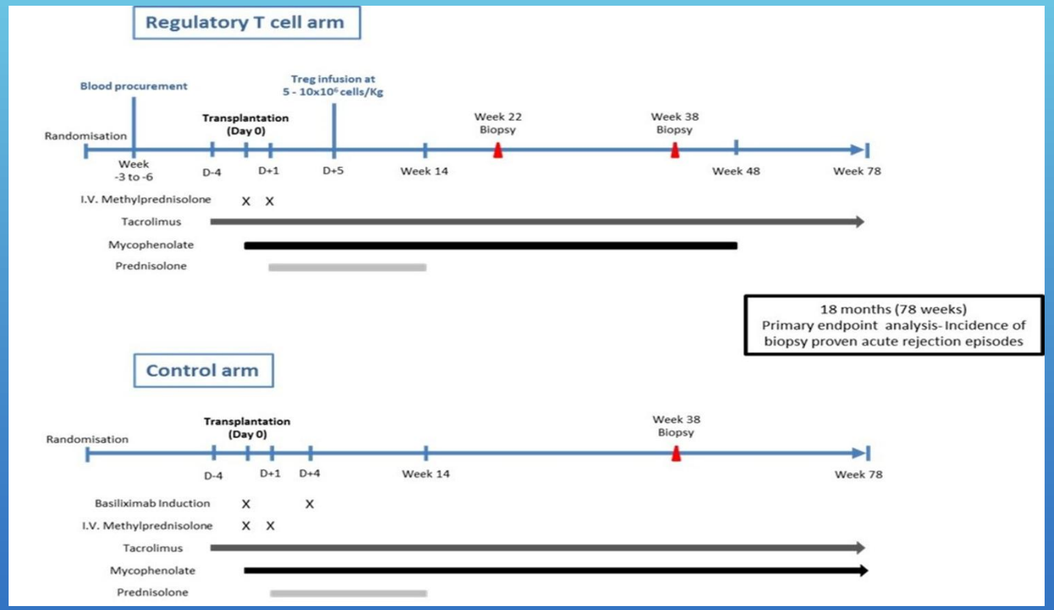
In brief

McCallion et al. report that kidney transplant recipients treated with Treg therapy exhibit prominent immune infiltrates within the transplanted organ. Infiltrates occur in patients with excellent long-term outcomes and exhibit immunoregulatory and B cell signatures, supporting the notion that local regulation is a prominent mechanism of Treg therapy.

The TWO Study: Oxford UK

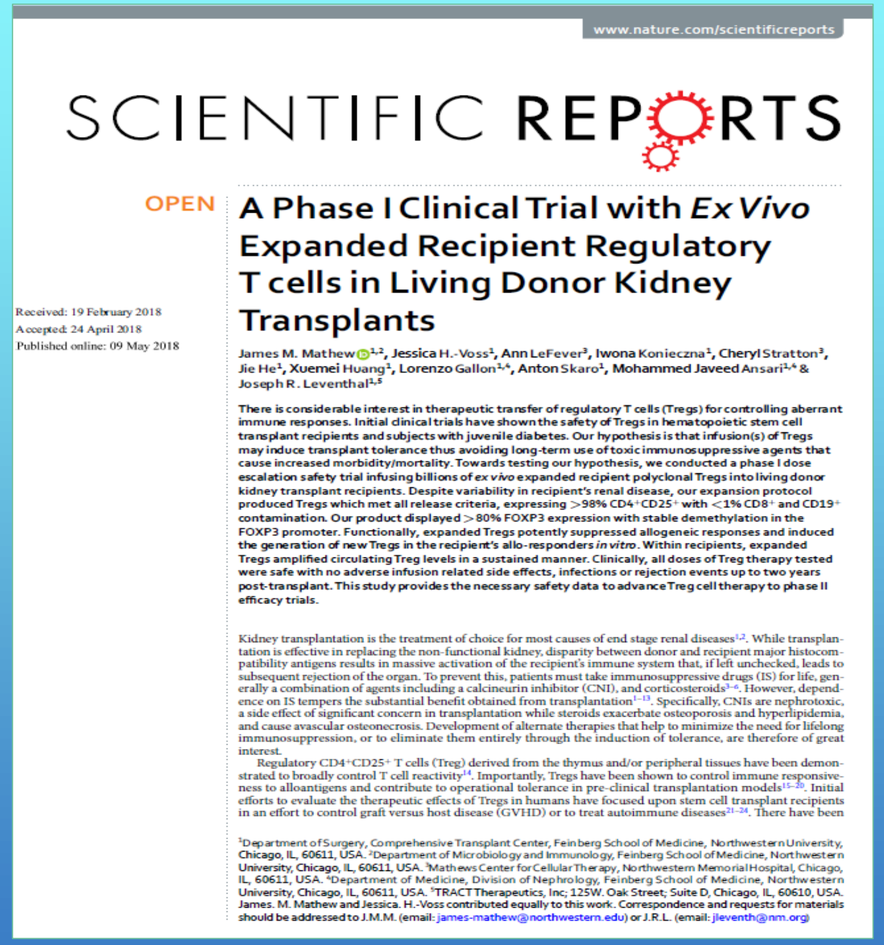
- Single center, phase IIb randomized controlled trial of autologous regulatory T cell therapy in renal transplantation (N=68)
- Recipient Tregs immunomagnetically selected pretransplant, polyclonal expansion and cryopreserved.
- Pandemic related modifications to protocol – no induction in the Treatment arm
- Tregs infused on Day+5 post-KTx. **MMF weaned by one year.** Primary endpoint at 18 months is incidence of BPAR



TRK-001 Phase 1

Trial Design and Clinical Results

Completed First in Human Phase 1 Trial: TRACT



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SCIENTIFIC REPORTS

OPEN

A Phase I Clinical Trial with *Ex Vivo* Expanded Recipient Regulatory T cells in Living Donor Kidney Transplants

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There is considerable interest in therapeutic transfer of regulatory T cells (Tregs) for controlling aberrant immune responses. Initial clinical trials have shown the safety of Tregs in hematopoietic stem cell transplant recipients and subjects with juvenile diabetes. Our hypothesis is that infusion(s) of Tregs may induce transplant tolerance thus avoiding long-term use of toxic immunosuppressive agents that cause increased morbidity/mortality. Towards testing our hypothesis, we conducted a phase I dose escalation safety trial infusing billions of *ex vivo* expanded recipient polyclonal Tregs into living donor kidney transplant recipients. Despite variability in recipient's renal disease, our expansion protocol produced Tregs which met all release criteria, expressing >98% $CD4^+CD25^+$ with <1% $CD8^+$ and $CD19^+$ contamination. Our product displayed >80% FOXP3 expression with stable demethylation in the FOXP3 promoter. Functionally, expanded Tregs potently suppressed allogeneic responses and induced the generation of new Tregs in the recipient's allo-responders *in vitro*. Within recipients, expanded Tregs amplified circulating Treg levels in a sustained manner. Clinically, all doses of Treg therapy tested were safe with no adverse infusion related side effects, infections or rejection events up to two years post-transplant. This study provides the necessary safety data to advance Treg cell therapy to phase II efficacy trials.

Kidney transplantation is the treatment of choice for most causes of end stage renal diseases[1,2]. While transplantation is effective in replacing the non-functional kidney, disparity between donor and recipient major histocompatibility antigens results in massive activation of the recipient's immune system that, if left unchecked, leads to subsequent rejection of the organ. To prevent this, patients must take immunosuppressive drugs (IS) for life, generally a combination of agents including a calcineurin inhibitor (CNI), and corticosteroids[3–6]. However, dependence on IS tempers the substantial benefit obtained from transplantation[1–13]. Specifically, CNIs are nephrotoxic, a side effect of significant concern in transplantation while steroids exacerbate osteoporosis and hyperlipidemia, and cause avascular osteonecrosis. Development of alternate therapies that help to minimize the need for lifelong immunosuppression, or to eliminate them entirely through the induction of tolerance, are therefore of great interest.

Regulatory $CD4^+CD25^+$ T cells (Treg) derived from the thymus and/or peripheral tissues have been demonstrated to broadly control T cell reactivity[14]. Importantly, Tregs have been shown to control immune responsiveness to alloantigens and contribute to operational tolerance in pre-clinical transplantation models[15–20]. Initial efforts to evaluate the therapeutic effects of Tregs in humans have focused upon stem cell transplant recipients in an effort to control graft versus host disease (GVHD) or to treat autoimmune diseases[21–24]. There have been

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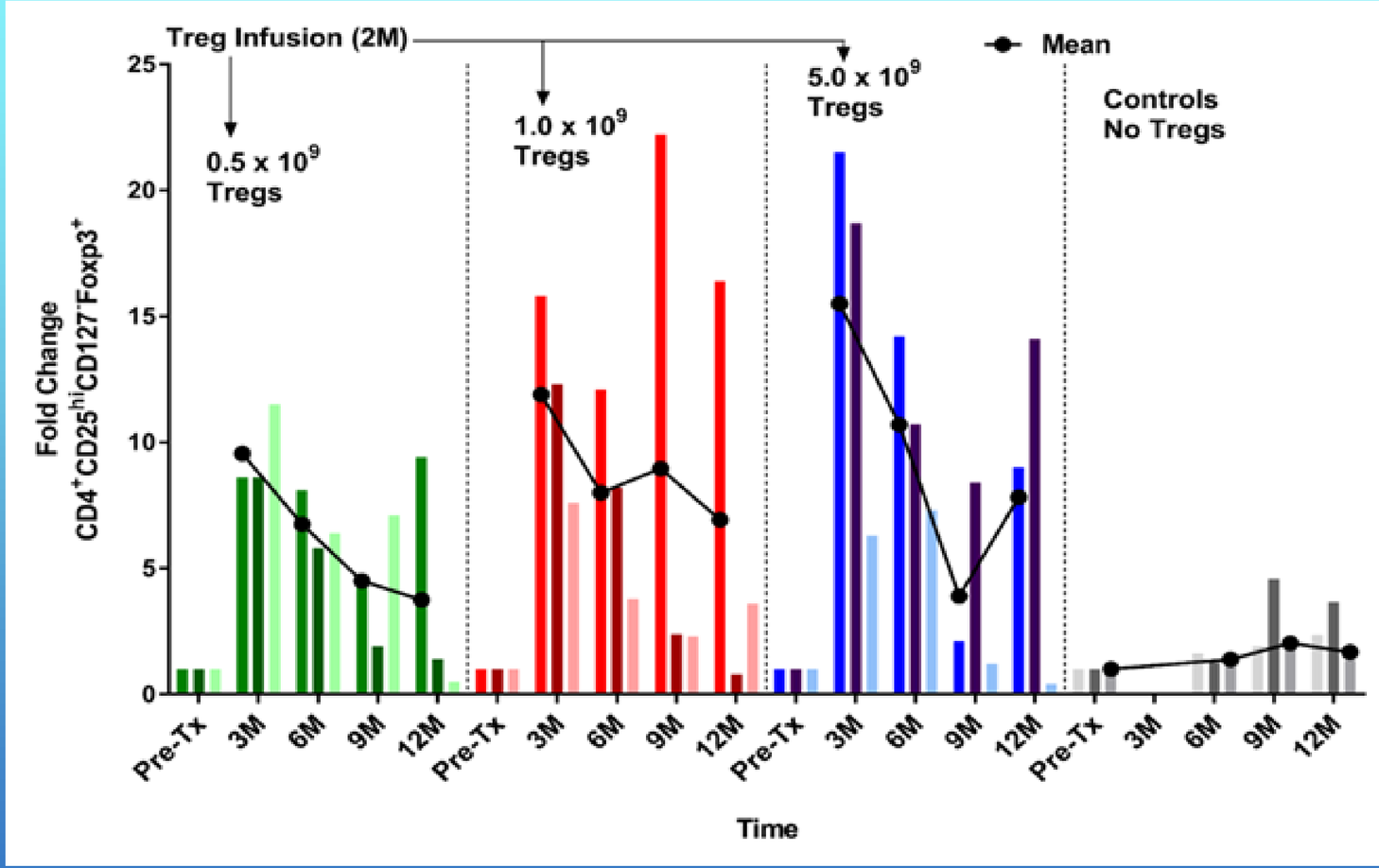
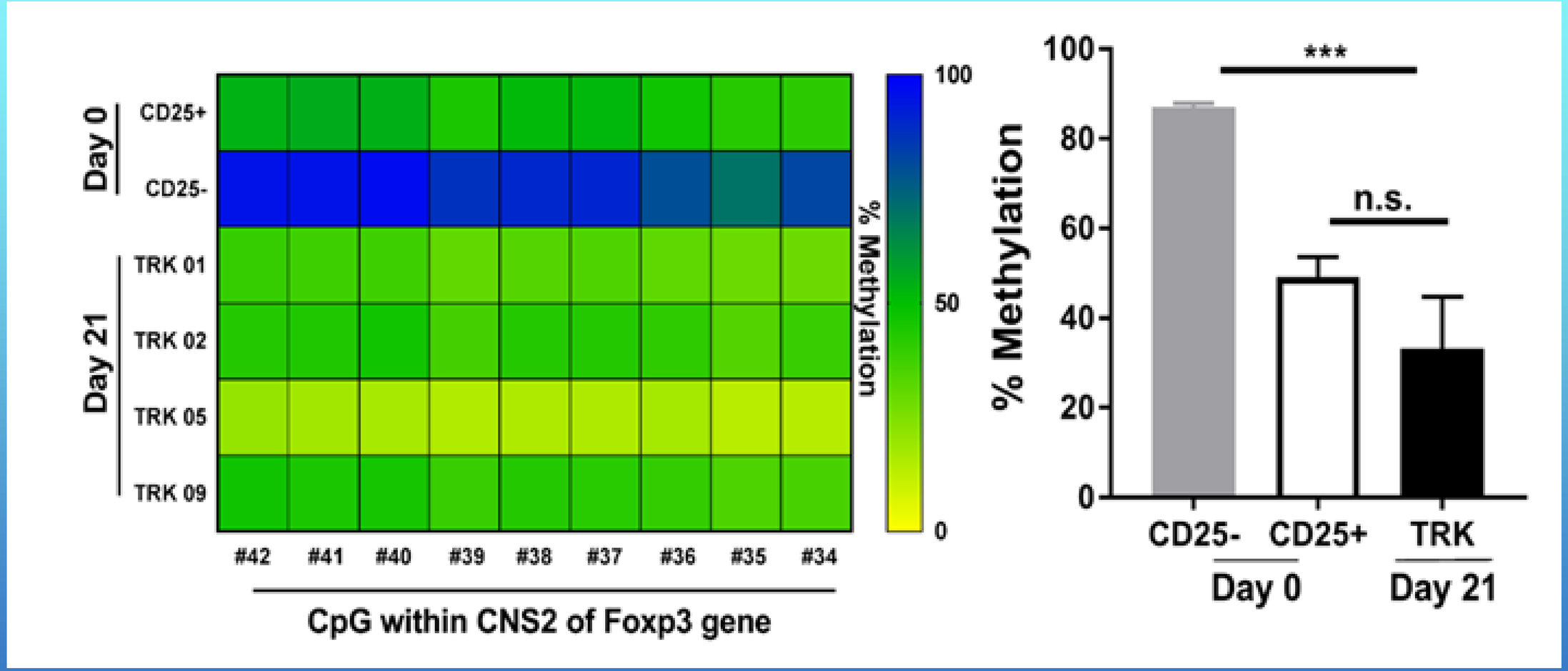
- Infusion with polyclonally expanded Tregs up to 5 billion cells is safe.
- No infusion related serious adverse events.
- Two-year biopsies were normal with no rejection.
- TRK-001 was associated with an increase in circulating numbers of Tregs in the immune system: this biomarker has been linked to development of tolerance in kidney and liver transplant recipients.
- High-level long-term subject data shows promising efficacy signals with a survival rate of 88.8% and death-censored graft survival of 75% at 8 to 9 years post-transplant.

Phase 1: Kidney Transplant Study Design

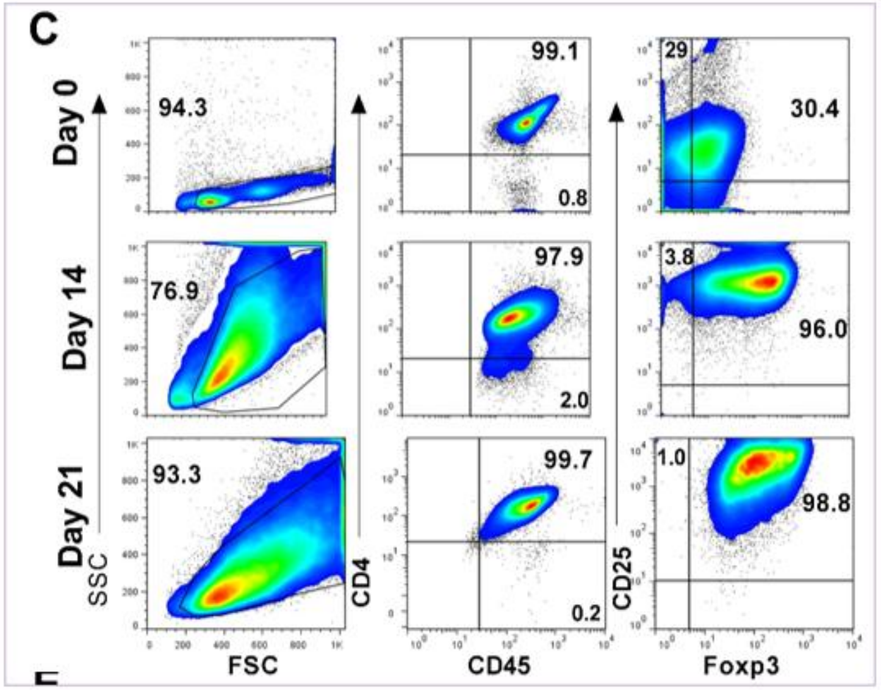
Phase 1 Trial Subjects Show Increased and Persistent Tregs Following TRK-001 Infusion



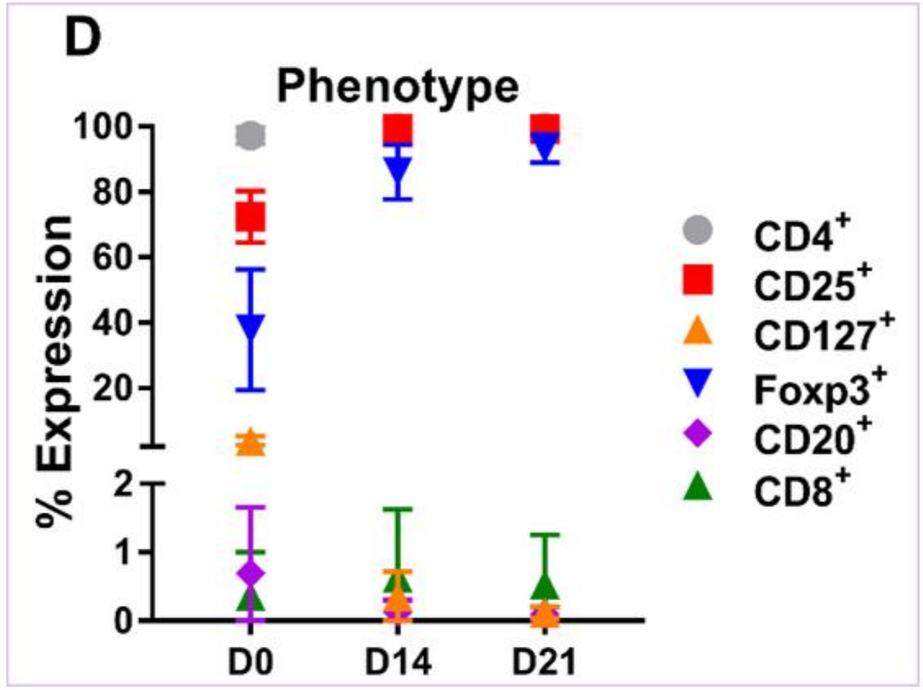
Phase 1: TRK-001 Demonstrates Preserved Demethylation of Foxp3 Promoter Region



Phase 1: Profiling of Expanded Treg Products

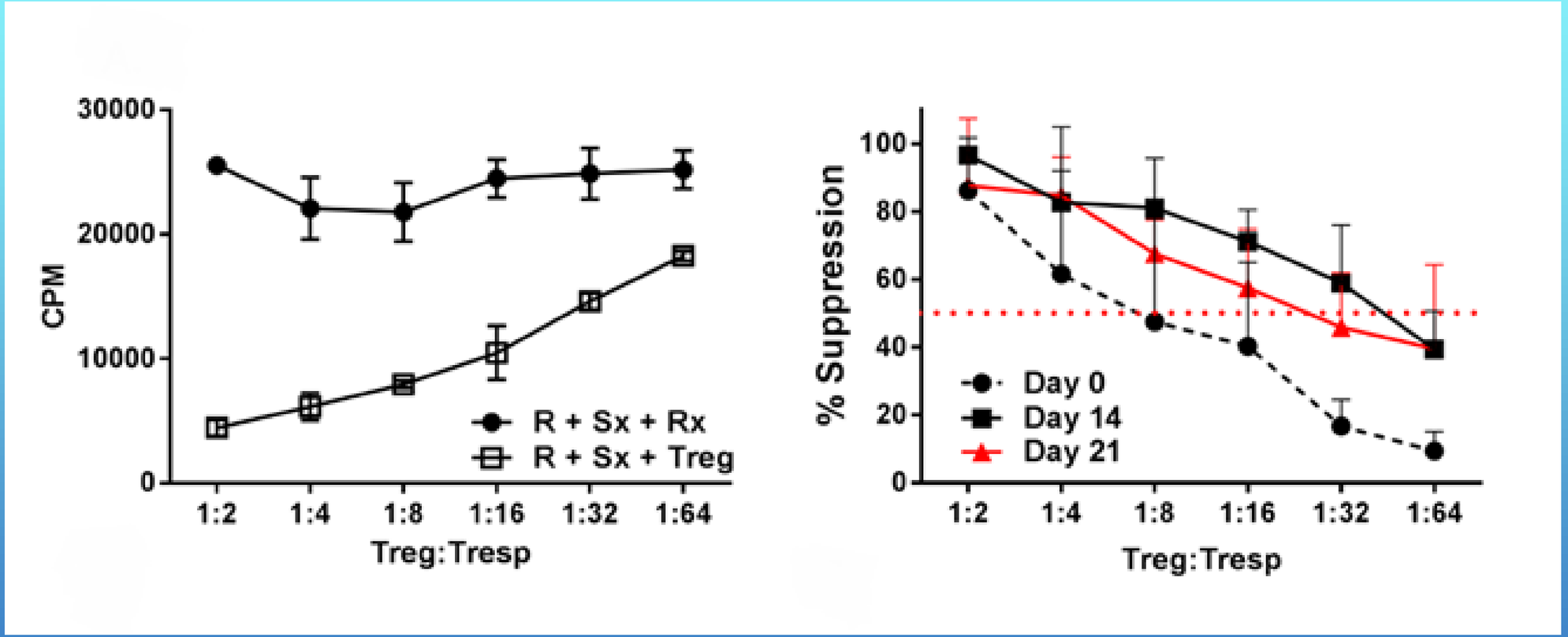


(C) Phenotyping Scheme for $CD4^+CD25^+FOXP3^+$ cells.



(D) Mean (±SD) expression of Treg (CD4, CD25 & FOXP3) and non-Treg (CD8, CD20 & CD127) markers (n=9).

Phase 1: Immunoregulatory Capabilities of Expanded Tregs



TRK-001 Phase 2: RETIRE TRIAL

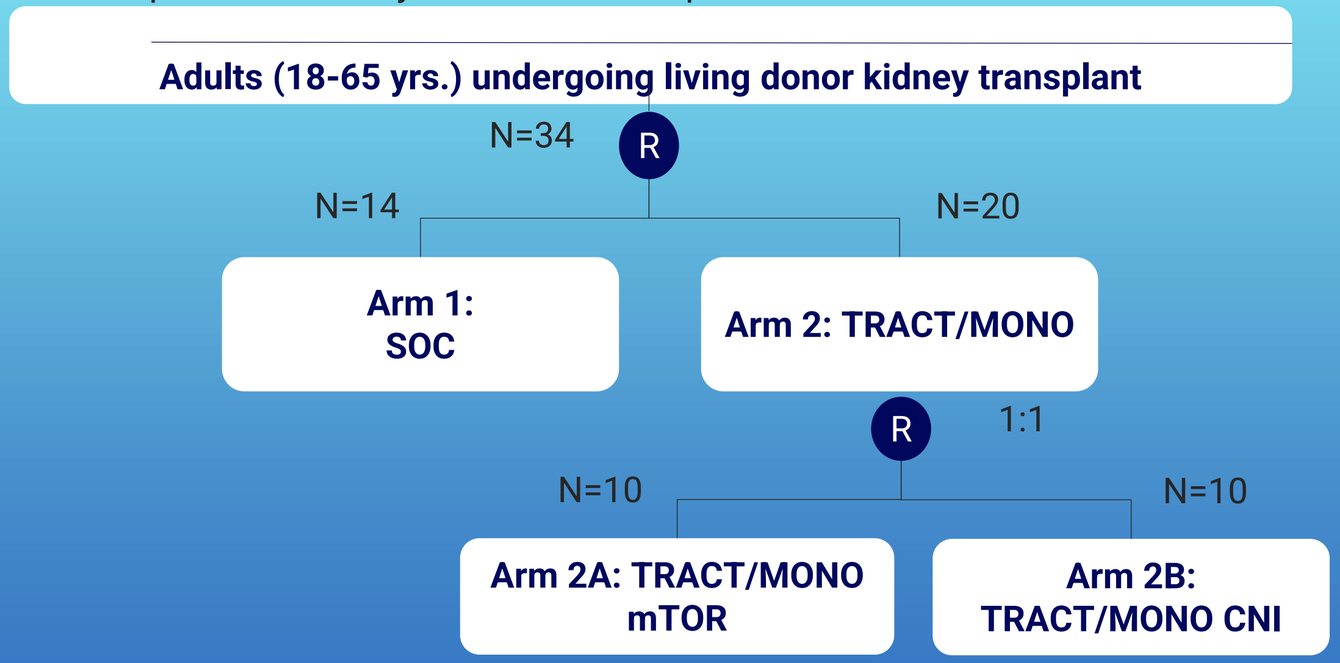
Singulera Therapeutics

Phase 2 Clinical Sites

Phase 2 TRACT-KD-101: Reduction of Immunosuppression CTP v9

Study Methodology: Prospective, multi-center, open-label, randomized clinical trial, **analyses after all subjects complete Month 12 and Month 24**

Study Duration: Five years including a 2-year post-transplant follow-up period and a 3-year surveillance period



Primary Endpoints at 1 Year

- Successful taper to monotherapy
- Composite endpoint for immunological failure including evidence of *de novo* DSA, BPAR, biopsy-proven subclinical rejection, IFTA

Secondary Endpoint at 2 Years

- Successful maintenance of monotherapy

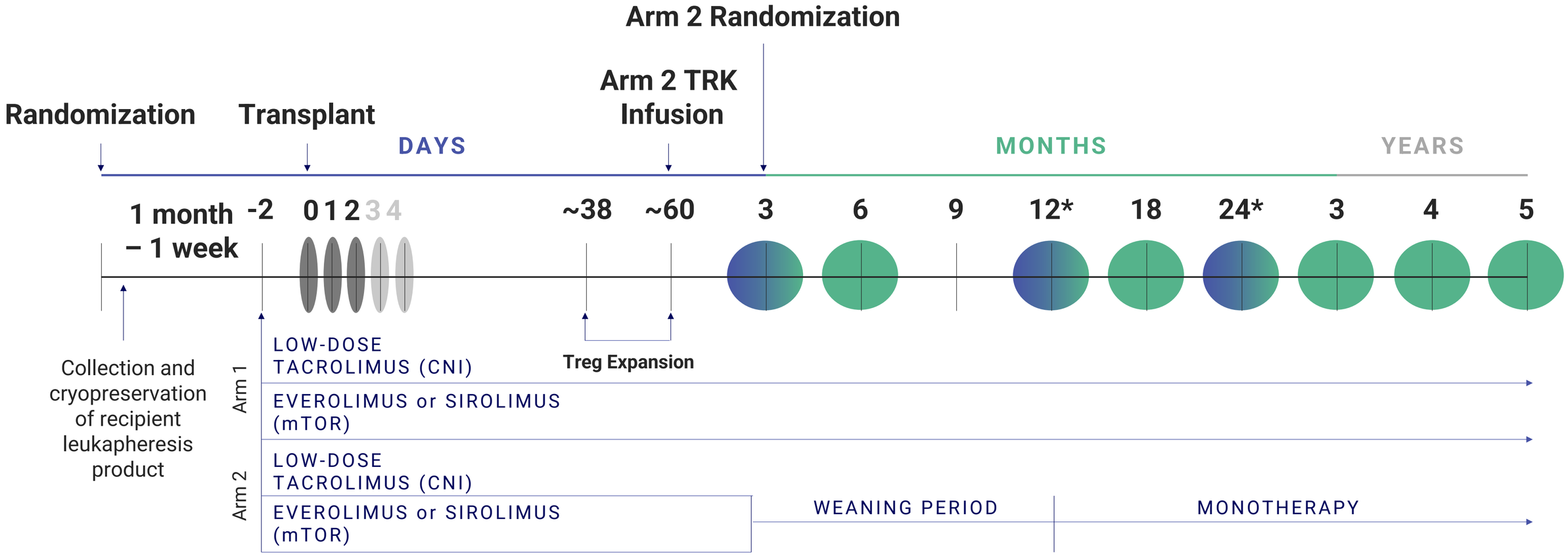
Other Key Endpoints

- Changes in metabolic function, renal function, transplant biomarkers (gene expression and donor-derived cell free DNA assays)
- Patient reported outcomes
- Incidence of adverse events
- Absolute number of Tregs

Singulera

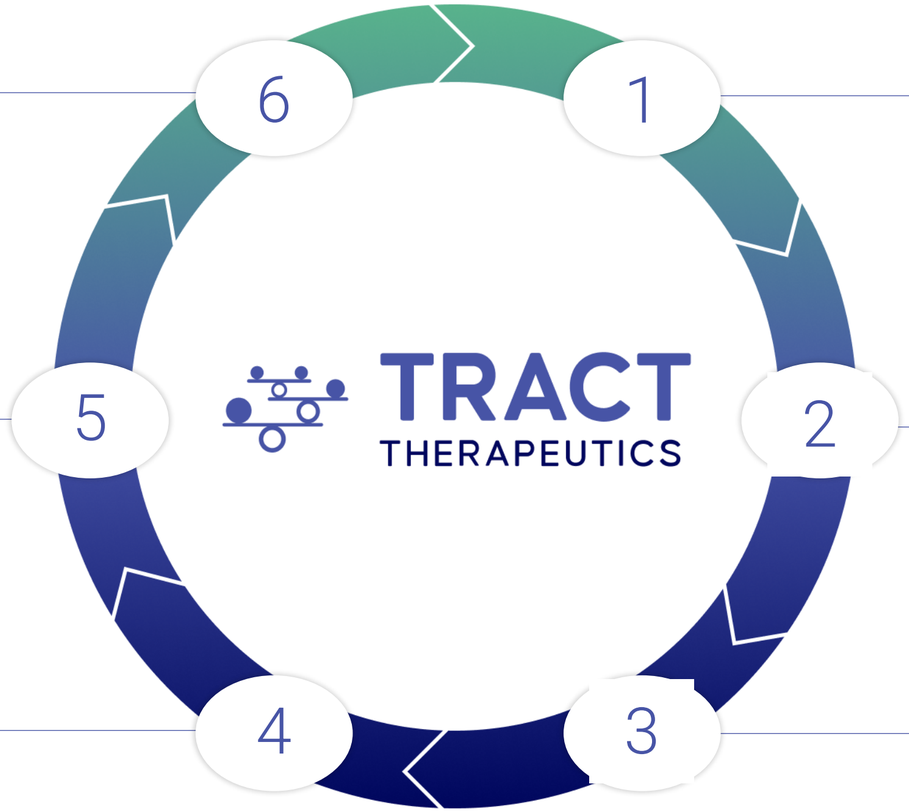
TRACT-KD-101 Study Design CTP v9

Thymoglobulin (Arms 1 & 2)
Biopsy (Month 3: Arm 2 only)
Immune Monitoring & Transplant Biomarkers
*Interim Analyses



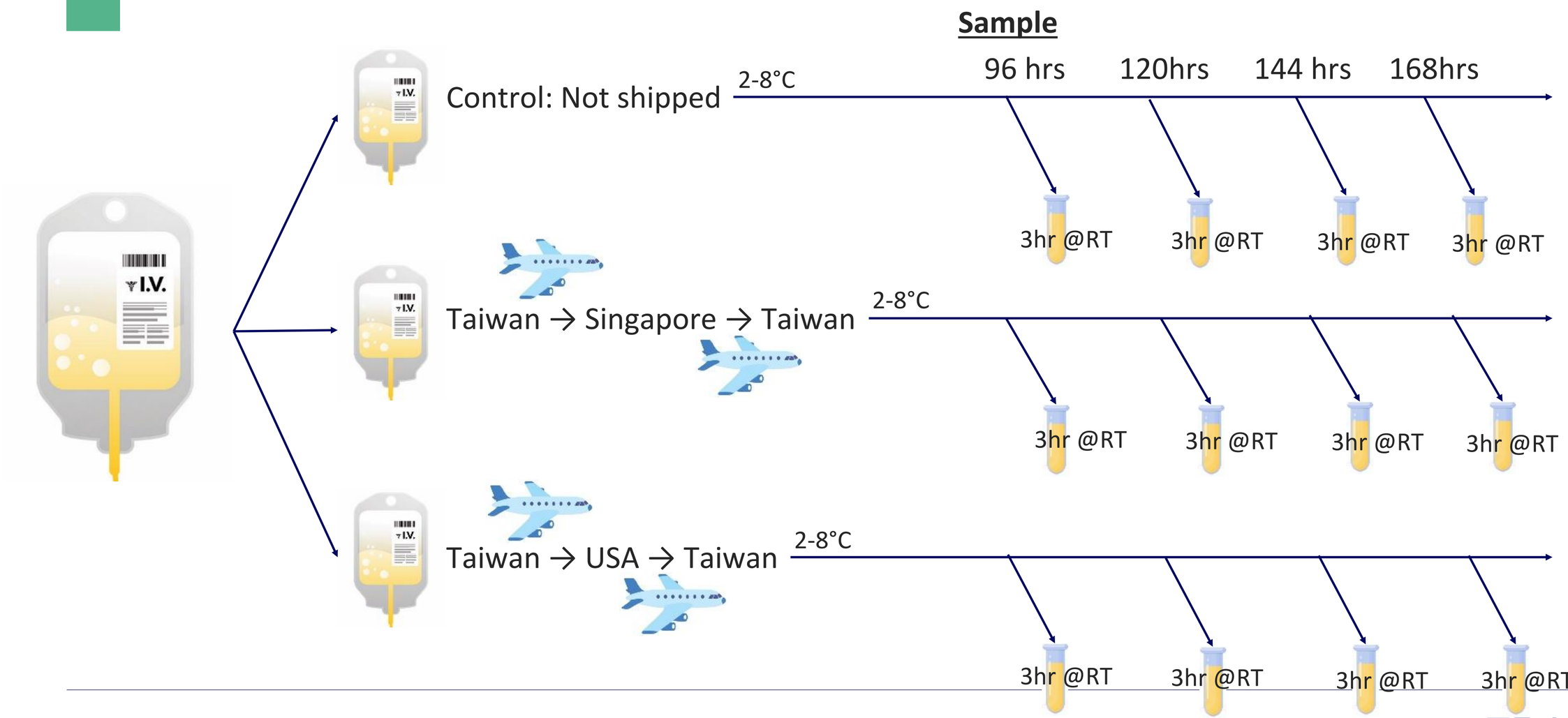
Singulera

TRK-001 cGMP Manufacturing Process



1. Leukapheresis, Cryopreservation
2. Thaw and Isolate CD25 + Enriched Cells
3. Activate and Expand T- Reg Cells
4. Formulate Cells
5. Quality Control & Release
6. Reinfuse Cells

Shipping and Product Use Study Design



TRACT THERAPEUTICS

Shipping and Product Conclusions for Phase 2 Use

Conclusions:

- **TRK-001 is able to be shipped/ flown without loss of viability or function**
- Product Expiration is **120 hrs**
- Product can be used up to **3 hours** after placed at room temperature

Lymphodepletion: The Importance of Conventional T Cell Depletion in Treg Therapy

Lymphodepletion using T cell-depleting monoclonal (alemtuzumab) or polyclonal antibodies (thymoglobulin) is essential to the clinical application of Tregs.

- A **1:1 or 1:2 ratio of Tregs to conventional CD4+ T cells** (Tconv) is control Tconv immune responses
- A **high prevalence of Tregs** is needed to establish a dominant tolerogenic milieu through bystander suppression that is later maintained locally by infectious tolerance
- A drastic **change of Tconv to Treg balance** is needed due to small percentage of nTregs (<5% of circulating CD4+ T cells) in peripheral blood
- Leveraging clinically available strategies for T cell depletion with alemtuzumab or thymoglobulin can reduce the CD4+ Tconv pool by 95-99% to approximately 4.6 x 10^9 cells.

Hara M, Kingsley CI, Niimi M, et al. IL-10 is required for regulatory T cells to mediate tolerance to alloantigens in vivo. J Immunol 2001; 166:3789–3796

Graca L, Thompson S, Lin CY, et al. Both CD4+CD25+ and CD4+CD25- regulatory cells mediate dominant transplantation tolerance. J Immunol 2002; 168:5558–5565

Francis RS, Feng G, Tha-In T, et al. Induction of transplantation tolerance converts potential effector T cells into graft-protective regulatory T cells. Eur J Immunol 2011; 41:726–738

Kendal AR, Chen Y, Regateiro FS, et al. Sustained suppression by Foxp3+ regulatory T cells is vital for infectious transplantation tolerance. J Exp Med 2011; 208:2043–2053.

Tang Q, Lee K. Regulatory T-cell therapy for transplantation: how many cells do we need? Curr Opin Organ Transplant 2012; 17:349–54.

TRACT-KD-101: Eligibility CTP v9

Key Inclusion Criteria

1. **Males or females** aged **18-65 years** as of the date of informed consent who will undergo a **single organ, living donor kidney transplant**.
2. **Donor aged 18-65 years** as of the date of organ donation. A certain degree of HLA matching between the donor and the recipient is not required.
3. **Blood type compatibility** between recipient and donor must be established as follows.

Recipient Blood Type	Acceptable Donor Blood Type
A	A or O
B	B or O
AB	A, B, AB, or O
O	O

4. **No prior organ transplant** of any kind.

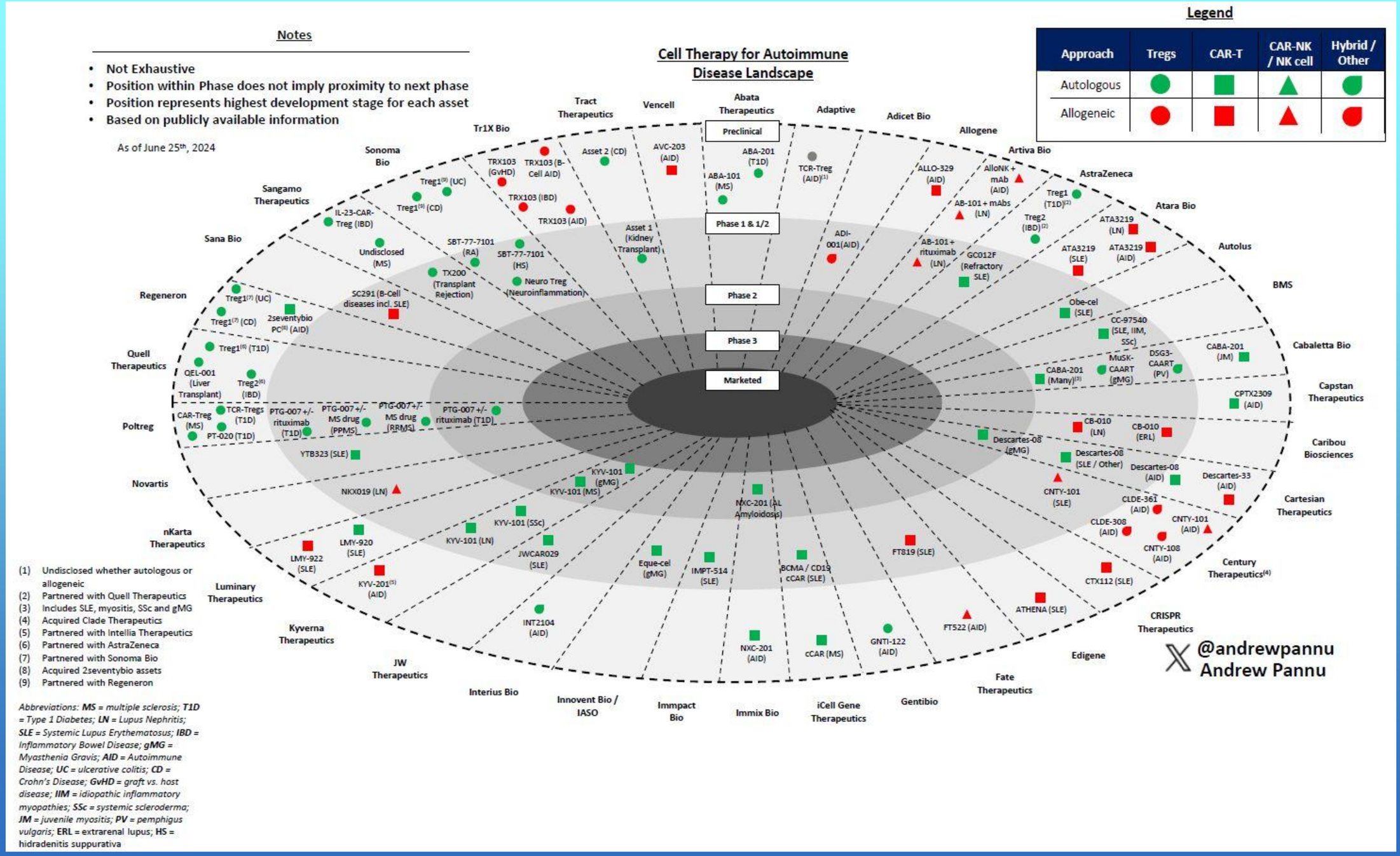
Key Exclusion Criteria

1. Known **sensitivity or contraindication** to thymoglobulin, everolimus, sirolimus, or tacrolimus or other **immunosuppression medication** prescribed.
2. Subjects with an **active infection** considered clinically significant by an investigator that has not resolved prior to transplant
3. Subjects with **a positive flow cytometric crossmatch** using donor lymphocytes and recipient serum.
4. Subjects with **PRA >80%** per SOC pre-transplant assessment. PRA must be repeated prior to transplant if patient receives a blood product transfusion after the initial assessment.
5. Subjects with **current or historic donor specific antibodies**.
6. Body Mass Index **(BMI) of < 16 kg/m^2 or > 38 kg/m^2** per SOC pre-transplant evaluation.

Summary and Conclusions

- Nearly all clinical trials of tolerance induction in kidney transplantation have been conducted in living donor kidney transplantation
- LDKTx offers logistical and immunological advantages for early phase trials of Tregs as compared to deceased donor transplantation
- Questions remain re: best IS reduction strategy when using Tregs, role of lymphodepletion, biomarkers to be used to guide long term patient management
- TRK-001 expansion data suggests “world-wide” distribution of a fresh product is possible….

Dynamic Biotech Landscape of Cellular Therapies for Immune Indications



QEL-001 LIBERATE Study Expansion Cohort
CAR-Treg engraftment and expansion following rATG conditioning in liver transplantation

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

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

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.

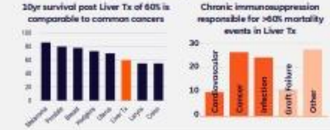
GMP manufacturing experience & process maturity:

- 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

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)



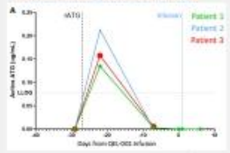
LIBERATE Study

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.

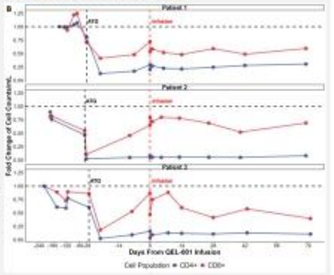
This single-arm, open-label, multi-centre trial focuses on HLA-A2-negative adult liver transplant recipients who have received a graft from an HLA-A2-positive donor.

Low dose ATG effectively depletes CD4 and CD8 T-cells with minimal & transient AEs and is cleared from the circulation within 3 weeks

ATG is cleared 3 weeks post dosing



ATG depletes CD4 and CD8 T-cells



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

Low dose ATG administration was well tolerated with short term and transient mild/moderate AEs.

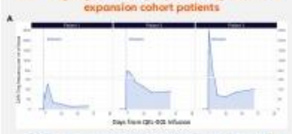
Adverse events (AEs) either directly associated with or occurring contemporaneously with administration of rATG are listed in Table 1. Adverse events encompassed: pyrexia (n=2); headache (n=2); infusion site swelling (n=1), phlebitis (n=1) and leukocytosis (n=1, driven by a neutrophilia). AE severities were all mild to moderate with minimum or no intervention. All adverse events recovered or resolved within 5 days or less of rATG administration. Patients 1 & 2 had no AEs within the first 24 hours of infusion of QEL-001. Patient 3 had one adverse event of hyperglycaemia (severity: mild) reported that occurred on the day of QEL-001 infusion and was resolved the following day. There were no reports of cytokine release syndrome (CRS) and no reports of immune effector cell-associated neurotoxicity syndrome (ICANS).

Table 1: Summary of Adverse Events associated with ATG administration

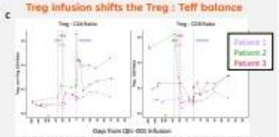
Subject	AE	Severity	Duration (days)	Outcome
Patient 1	Pyrexia	Moderate	1	Resolved
	Phlebitis	Mild	1	Resolved
Patient 2	Headache	Mild	1	Resolved
	Headache	Mild	<1	Resolved
	Leukocytosis	Mild	1	Resolved
Patient 3	Pyrexia	Mild	1	Resolved
	Infusion site swelling	Mild	1	Resolved

CAR-Tregs persist in the circulation for at least 12 months and their engraftment is promoted by low-dose ATG conditioning

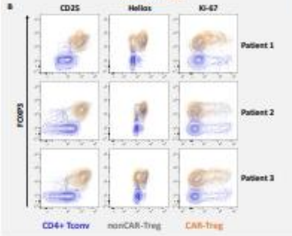
CAR-Treg engraft in the peripheral Treg niche in all expansion cohort patients



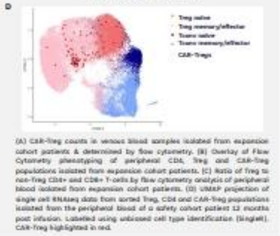
T-cell reconstitution following ATG and CAR-Treg infusion shifts the Treg : Teff balance



CAR-Treg express canonical markers of stable & functional Tregs 10 weeks following infusion
(expansion cohort)



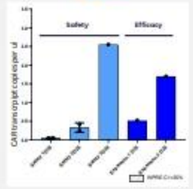
CAR-Tregs 12m post infusion share similar transcriptional profile to non-transduced Tregs
(scRNAseq data – Safety Cohort)



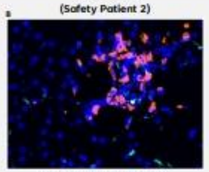
(A) CAR-Treg counts in venous blood samples isolated from expansion cohort patients & determined by flow cytometry. (B) Overlay of Flow Cytometry phenotyping of peripheral CD4, Treg and CAR-Treg populations isolated from expansion cohort patients. (C) Ratio of Treg to non-Treg CD4+ and CD8+ T-cells by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

QEL-001 tCAR Tregs accumulate in the liver accounting for a large proportion of the liver Treg niche

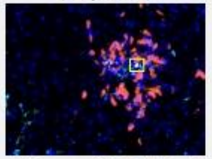
CAR-Treg detection in the liver by dPCR



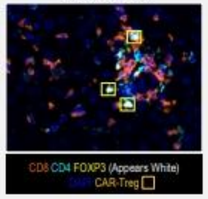
Pre-infusion (Safety Patient 2)



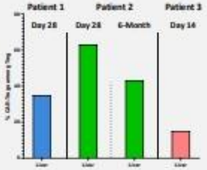
1 month post-infusion (Safety Patient 2)



6 months post-infusion (Safety Patient 2)



CAR-Treg represent 15 – 65% of all Tregs in the liver of infused patients while limited frequencies are observed in the periphery



(A) Liver CAR Tregs detected 1 month post infusion. cDNA dPCR analysis of liver biopsies taken 4 weeks post-infusion of QEL-001. RNA was extracted from biopsy samples, cDNA was retro-transcribed and dPCR was performed to quantify CAR transcript.
(B) 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 Qtrend (not shown).
(C) The proportion of CAR-Treg and Treg from (B).

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.

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Meeting Report: The Sixth International Sam Strober Workshop on Clinical Immune Tolerance

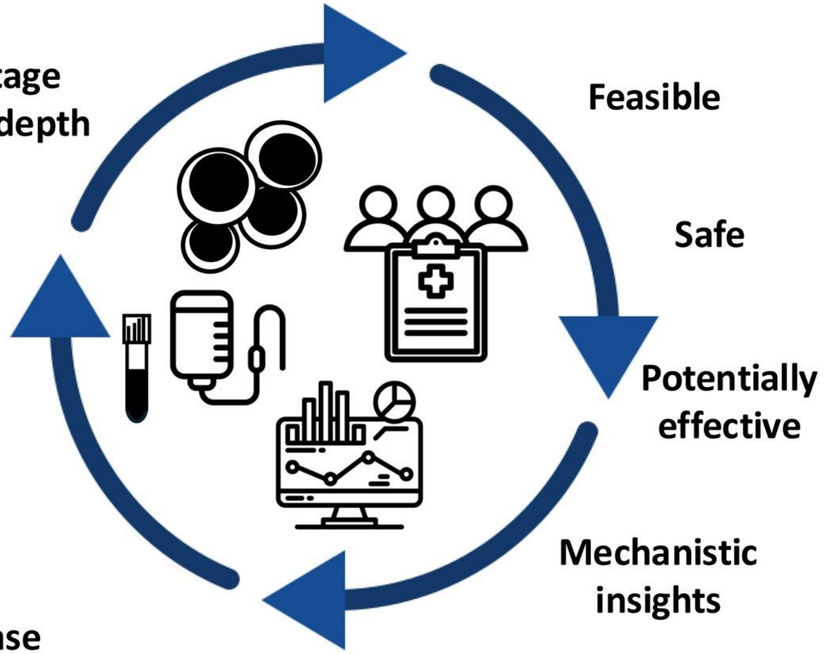
Progress so far

Pre-clinical & early-stage clinical trials with in-depth immune monitoring

Using cellular therapies to modify the immune response

For different clinical indications

- solid organ transplantation
- autoimmune disease



Future Directions

- Complete late-stage clinical trials & longer-term follow-up
- Refine patient selection & clinical protocols
- Develop global collaboration, consensus & standards
- Improve cell product manufacturing
- Elucidate intra-tissue immune mechanisms & discover novel biomarkers

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