UCL INSTITUTE OF CHILD HEALTH

Great Ormond Street Hospital for Children NHS Foundation Trust

Joint Research and Development Office
Division of Research and Innovation

KING'S College LONDON

Guy's and St Thomas' NHS Foundation Trust

ATT-Heart

Study Title:

ATT-Heart: An open label, single-centre dose escalation trial, investigating the safety and feasibility of **A**utologous **T**hymus derived regulatory **T** cell treatment for the prevention of cardiac allograft vasculopathy in children receiving **Heart** transplant.

Protocol Number/R&D number: 23HL27
IRAS Number: 1008875
REC Reference: XXXXX
EudraCT Number: XXXXX

Short title or acronym: ATT-Heart

Protocol Version: 1.0 (28-NOV-2024)

Chief Investigator:

Professor Michael Burch
Professor of Paediatric Cardiology at University College London (UCL) and Great Ormond Street Hospital for Children NHS Foundation Trust (GOSH),
Great Ormond Street Hospital,
Great Ormond Street,
London,
WC1N 3JH.

Telephone: 07818416502
Email: michael.burch@gosh.nhs.uk

Investigators:

Professor Giovanna Lombardi
Chief Scientific Investigator,
Professor of Human Transplant Immunology,

King's College London (KCL),
Peter Gorer Department of Immunobiology,
5th Floor Bermondsey Wing,
Guy's Hospital,
London,
SE1 9RT.

Telephone: 020 7188 7674
Email: giovanna.lombardi@kcl.ac.uk

Professor Robert Lechler
Scientific Investigator,
Emerita Vice President/Provost (Health),
King's College London,
Faculty of Life Sciences and Medicine,
Room 1.4 - Hodgkin Building,
Guy's Campus,
London,
SE1 1UL.

Telephone: 020 7188 7674
Email: robert.lechler@kcl.ac.uk

Professor Alberto Sanchez Fueyo
Clinical Investigator,
Professor of Hepatology and Head of Department of Liver Sciences,
King's College London,
Inflammation Biology,
James Black Centre,
Denmark Hill Campus,
125 Coldharbour Lane,
London,
SE5 9NU.

Telephone: 020 7848 5883
Email: sanchez_fueyo@kcl.ac.uk

Dr Marco Romano
Postdoctoral Research Associate,
Immunoregulation Laboratory,
Division of Transplantation Immunology and Mucosal Biology,
MRC Centre for Transplantation,
King's College London,
5th Floor Bermondsey Wing,
Guy's Hospital,
London,
SE1 9RT.

Email: marco.romano@kcl.ac.uk

Lead Statistician:	Dr Abdel Douiri Reader and Head of Medical Statistics, Public Health Sciences, Population Health and Environmental Sciences, King's College London, 4th Floor Addison House, London, SE1 1UL. Telephone: 0207 848 8224 Email: abdel.douiri@kcl.ac.uk
Funder:	British Heart Foundation
Sponsor:	Great Ormond Street Hospital for Children NHS Foundation Trust, Joint R&D Office GOSH/ICH based at UCL Institute of Child Health (ICH), 30 Guilford Street, London, WC1N 1EH. Clinical Trials Manager Telephone: +44 207 905 2346 Fax Number: +44 207 905 2201
Clinical Trial Management	Clinical Trial Management Team. Research & Development, 16th Floor - Tower Wing, Guy's Hospital, Guy's and St Thomas' NHS Foundation Trust, Great Maze Pond, London, SE1 9RT.
GMP Facility	Advanced Therapy Manufacturing (GMP) Unit. Clinical Research Facility, 15th Floor - Tower Wing, Guy's Hospital, Guy's and St Thomas' NHS Foundation Trust, Great Maze Pond, London, SE1 9RT.
Medical contact on site:	Office hours: 020 7405 9200 - Extension: 38532 Emergency contact details: GOSH Switchboard: 0207 405 9200 and ask for Dr Apoorva Aiyengar or Professor Michael Burch to be contacted.

Signatures

The Chief Investigator and Sponsor have discussed this protocol. All have agreed to perform the investigation as written and to abide by this protocol except in case of medical emergency or where departures from it are mutually agreed in writing.

Chief Investigator

_____

Signature

Date:

Sponsor

Great Ormond Street Hospital for Children NHS Foundation Trust
Clinical Trials Manager
Joint R&D Office

_____

Signature

Date:

Participating Site and Local Principal Investigator (PI)
Single Site:
Great Ormond Street Hospital for Children NHS Foundation Trust
Great Ormond Street Hospital
Great Ormond Street
London,
WC1N 3JH

Chief Investigator and Principal Investigator:
Professor Michael Burch

Abbreviations

AE	Adverse event
AR	Adverse reaction
ALT	Alanine Transaminase
CAV	Cardiac Allograft Vasculopathy
CI	Chief Investigator
CMV	Cytomegalovirus
CRA	Clinical Research Associate (Monitor)
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Clinical Trials
CTA	Clinical Trials Authorisation
DMC/DMSC	Data Monitoring Committee / Data Monitoring and Safety Committee
DSA	Donor-Specific Antibodies
DSUR	Development Safety Update Report
EBV	Epstein Barr Virus
EMEA	European Medicines Agency
EudraCT	European Clinical Trials Database
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice Facility
GOSH	Great Ormond Street Hospital
GP	General Practitioner
GTAC	Gene Therapy Advisory Committee
IB	Investigators Brochure
ICF	Informed Consent Form
ISF	Investigator Site File
ICH	International Conference of Harmonisation
IMP	Investigational Medicinal Product
IRB	Independent Review Board
ISHLT	International Society for Heart and Lung Transplantation
HIV	Human Immunodeficiency Virus
HTLV	Human T Lymphotropic Virus
MFI	Mean Fluorescent Intensity

MHRA	Medicines and Healthcare products Regulatory Agency
NHS	National Health Service
NRES	National Research Ethics Service
PI	Principal Investigator
PIL	Participant/Patient Information Leaflet
PIS	Participant/Patient Information Sheet
QP	Qualified Person for release of trial drug
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SMPC	Summary of Medicinal Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
TMG	Trial Management Group
Treg	Regulatory T cells
TSC	Trial Steering Committee
UCL	University College London

Study Synopsis

Title of clinical trial	An open label, single-centre dose escalation trial, investigating the safety and feasibility of autologous thymus derived regulatory T cell treatment for the prevention of cardiac allograft vasculopathy in children receiving heart transplant.
Sponsor name	Great Ormond Street Hospital for Children NHS Foundation Trust
Medical condition or disease under investigation	Cardiac allograft vasculopathy (CAV).
Purpose of clinical trial	Assess the safety of thymus derived Tregs in the treatment of paediatric heart transplant patients and assess the technical feasibility of generating Tregs from the thymus.
Primary objective(s)	To determine safety and optimal tolerated dose of expanded autologous thymus derived Tregs to prevent CAV in paediatric heart transplant patients in a Phase I study.
Secondary objective(s)	1. To assess the feasibility of generating expanded autologous thymus derived Tregs in the Good Manufacturing Practice facility. 2. To investigate the clinical and immunological responses to autologous thymic Tregs in paediatric heart transplant patients. 3. To assess the feasibility of retaining participants for the duration of the study and completion of study visits and assessments.
Study Design	A single ascending dose, 3+3, open label, single centre trial.
Study Endpoints	**Primary endpoint:** • Occurrence and nature of Dose-Limiting Toxicities (DLTs) occurring within 4 weeks post-infusion. **Secondary endpoints:** • Amount of thymic derived Tregs manufactured per patient. • Immunological response in blood: – Frequency of circulating leukocyte subsets – Quantity of alloantigen-specific conventional T cells, Tregs and CD8+ – Presence of anti-HLA to evaluate tissue infiltrating cells

	• Assessment of clinical response: – Development of CAV – Graft loss – Recipient mortality – Acute and chronic rejection – Opportunistic infections – Cardiac function – Renal function – Description of non-DLT adverse events and those occurring beyond week 4 post Treg infusion – Immunosuppressive doses • Number of study visits completed per participant. • Responses to items in questionnaires or surveys exploring the study and treatment experience of the participants and their parents/guardians
Sample Size	9 participants that could be expanded to 12 in case of DLTs
Summary of eligibility criteria	**Inclusion criteria:** 1. Male or female children aged between 0.5 and 16 years. 2. Written informed consent/assent. 3. Children receiving a heart transplant. 4. Single transplanted organ. 5. Willing and able to comply with the study visit schedule. **Exclusion Criteria:** 1. Active viral infection. 2. Age under 0.5 year or over 16 years. 3. Multi-organ transplant. 4. Highly-sensitised patients at high risk of rejection. 5. Allergy to any component/excipients used for the manufacture of the Treg product. 6. Previous recipient of any organ transplant. 7. History of previous sternotomy surgical procedure for congenital heart defect during which has had previous partial or full thymectomy. 8. Confirmed diagnosis of Di George Syndrome with absent thymus. 9. Participation in another interventional Clinical Trial of an Investigational Medicinal Product. 10. Pregnant and lactating patients. 11. Female patients of childbearing potential who are not willing to use a highly effective method

	of contraception. 12. Male patients who are not willing to use an effective method of contraception. 13. Patient is considered by the Chief Investigator to be an unsuitable candidate for the study.
Investigational medicinal product and dosage	Autologous thymus derived Tregs (TR006) infused at one of two doses in a 3+3 design: 1. Low dose: 1 - 3 x 10^6 Tregs/Kg or 2. High (and highest) dose: 5 - 10 x 10^6 Tregs/Kg
Active comparator product(s)	N/A
Route(s) of administration	Intravenous infusion.
Maximum duration of treatment of a subject	Each participant receives one infusion of autologous thymus derived Tregs at 3 - 6 months post transplantation and will be followed up for 24 months post infusion.
Procedures for safety monitoring during trial	DLT will be determined from data collected during the 4-week period post Treg infusion
Definition of end of trial	Database Lock

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1 Introduction

1.1 Background

Heart transplantation is a lifesaving procedure for some children. However, the long-term success of a heart transplant is often limited by inflammatory processes leading to progressive narrowing of blood vessels that supply oxygen to the heart, known as Coronary Allograft Vasculopathy (CAV).

CAV is a leading cause of death beyond 3 years after heart transplant and remains an important limitation to long term survival and graft longevity [1]. Once established, CAV is refractory to conventional medical or surgical approaches, and constitutes the most important predictor for all-cause mortality in this patient population. Data from 2017 derived from angiography reports found that 67% of patients (of all ages) were free from CAV 10 years after their transplant [2]. However, early onset of disease or the true burden of disease may be well underestimated as coronary angiography is not the most sensitive modality of diagnosis. Once a diagnosis of CAV has been confimed, graft survival is also significantly reduced across all age groups, with infants being the worst affected (with a median survival of 2 years after diagnosis) [2].

CAV can occur despite the use of immunosuppressive drugs that are also linked to morbidity and mortality themselves, with side effects including increased risk of infection, reactivation of viruses and Post-Transplant Lymphoproliferative Disorder (PTLD).

No effective treatments are available for established CAV. Revascularization has not shown to improve outcome and re-transplantation is limited by donor availability. Re-transplantation is rarely possible due to donor shortages and the presence of alloantibodies induced by the previous transplant.

Recent data from a multicentre study, involving GOSH, showed that impaired graft function associated with CAV was a poor prognostic sign [3]. Interestingly, the incidence of CAV in children has not declined over time. Therefore, the prevention of CAV, the main cause of graft damage, is still an unmet goal. The urgency is reflected by the clear directive from the Family Steering Group at Great Ormond Street Hospital.

The infusion of mesenchymal stem cells (MSC) or stem cell derived products (i.e induced pluripotent stem cells derived cardiomyocytes) represents an alternative to transplantation. This strategy aims to replace/regenerate damaged tissues. So far, different studies have shown safety of these products and new trials are ongoing, but only small improvements in ejection fraction have been shown [4,5].

The annual report on deceased donation and transplantation released by the NHS in May 2023 affirmed that 28 paediatric heart transplants were performed between 1 April 2022 and 31 March 2023 in the UK [6]. For these patients, CAV is the major barrier for long term post-transplant survival. As a result of CAV, for which there is currently no effective treatment, many recipients die or require re-transplantation as teenagers or young adults. CAV is also a major cause of death post-transplant in adults. There is therefore a pressing need for a therapy to prevent CAV in paediatric as well as in adult heart transplant recipients.

Cell therapy with regulatory T cells (Tregs) shows promise as potential treatment to generate immune tolerance and improve outcomes for patients in different therapeutic areas. If Tregs can also benefit heart transplant recipients and is successful in preventing CAV, all the paediatric

transplant recipients could receive this treatment in the future. Furthermore, Treg therapy could be translated to the 200 adults receiving a heart transplant every year in the UK.

To our knowledge there is currently only one ongoing clinical trial administering thymic derived CD25+ enriched cells (ClinicalTrials.gov Identifier: NCT04924491) [7]. This trial being run in Spain is aiming at recruiting 11 heart transplant paediatric patients.

Published clinical trials with Tregs thus far have used Tregs derived from blood and out of these studies only one in a paediatric population. Marek-Trzonkowska et al. treated 10 paediatric patients with type 1 diabetes mellitus (T1DM) diagnosed in the preceding 2 months with Tregs expanded in vitro from autologous CD4+CD25hiCD127lo Tregs [8]. One patient was diagnosed with influenza the day after infusion. No other infusion-related toxicities were reported.

Expanded autologous Tregs have been published and currently being trialled as treatment in the adult population for disorders such as GvHD, Crohn's disease, liver and renal transplant, multiple sclerosis, aplastic anaemia and systemic lupus erythematosus. Data from tens of patients so far indicate that they are safe and improve these diseases (see summary of clinical data below).

Tregs have shown great promise in the control of multiple inflammatory conditions including Type 1 diabetes, bone marrow and solid organ transplantation. At KCL and GSTT there have been two completed clinical trials using in vitro expanded Tregs obtained from the blood of patients receiving either kidney (The ONE study) or liver (ThRIL) transplants. Altogether, 21 patients were treated with Tregs (up to 1 x 10^7cells/Kg) with no safety concerns and some early signs of biological and clinical efficacy [9,10,11]. However, no such studies have been published in the context of human cardiac transplantation. In the paediatric population, obtaining enough blood to generate a sufficient number of Tregs for clinical use is not possible. However, the thymus, which is discarded during heart surgery, is a source of very stable and functional Tregs [12]. Based on the results from various clinical trials and the property of Tregs, we consider that adoptive Treg therapy with thymic-derived Tregs in paediatric heart transplant recipients will be feasible and safe. Furthermore, we believe that adoptive Treg therapy can prevent CAV in paediatric heart transplant recipients.

We are aiming to inject thymic-derived polyclonally expanded Tregs as a potential treatment to prevent CAV in 9 children receiving heart transplant.

1.2 Introduction to investigational treatment

Tregs, a subset of CD4+ T cells, are essential for immune-homeostasis and are critical mediators of immunological tolerance [13]. In preclinical animal models, we and others have shown that the adoptive transfer of Tregs can prevent transplant rejection, and most importantly inhibit CAV [14,15,16,17,18,19,20,21]. Mechanistically, Tregs are a fully differentiated autologous cell product which integrate into the endogenous immune system.

The technology involves generating immune tolerance in children receiving heart transplants through expanded autologous Tregs as an Advanced Therapy Investigational Medicinal Product (ATIMP). The process involves removal of the thymus during the heart transplantation or during the time of having a pump assist device fitted in (this can be up to a year before the transplant), transferring it to a Good Manufacturing Practice (GMP) facility for Treg isolation, expansion and cryopreservation for therapeutic administration at 3-6 months post-transplantation.

Tregs are obtained from the thymus by mechanical digestion followed by CD8+ cell depletion and CD25+ cell enrichment. Resultant cells are activated and expanded with a protocol involving

αCD3/αCD28 microbeads in Rapamycin-supplemented medium, with periodic recombinant-human IL-2 addition. At the end of the expansion (up to 23 +/-1 days), the ATIMP of purified and phenotypically defined Tregs will be cryopreserved as an individual dose for the infusion into patients.

Different immunosuppressive regimes may ameliorate some of the damaging side effects of transplantation, but all transplant patients will remain on life-long immunosuppression. Cell therapy with Tregs has the aim to reduce and/or stop immunosuppression by inducing the so called "operational tolerance".

Tregs offer distinct advantages over mesenchymal stem cells (MSC) or stem cells derived products. These can also provide some immune-modulatory effect, but MSC therapy is unlikely to have the fundamental or immune modulatory effect of Tregs, and requires a separate harvesting and manufacture process.

1.3 Data from non-clinical studies

Animal models indicate that Tregs are essential for immune-homeostasis and are critical mediators of immunological tolerance. We have previously shown in animal models of allograft vasculopathy how the adoptive transfer of Tregs can prevent CAV favouring organ acceptance [14,22].

In our previous work, we characterised Tregs from human thymus [23]. We confirmed that thymic Tregs, like Tregs in peripheral blood, are composed of CD45RA+ and CD45RA-.

In the literature it is well established that T cells egressing from the thymus are CD45RA+CCR7+ and that these naïve Tregs are the most stable in the periphery. In contrast, the role of thymic human CD45RA- Tregs is not well established. These Tregs might be thymic-resident cells under maturation or represent recirculating cells from the periphery. We have demonstrated that both total and CD45RA+ Tregs can be expanded *ex vivo*. High level of FOXP3, CTLA4 and CD25 expression together with a highly suppressive ability were found in both total and CD45RA+ Tregs. Our analysis on the methylation status in the FOXP3 promoter revealed no differences between total and CD45RA+ Tregs, suggesting that both preparations are safe to be used. We confirmed that the presence of rapamycin in culture is essential for Treg expansion as its presence inhibited IFN-γ production by Tregs [12].

In addition to this, and as part of our previous work, we developed a protocol for following the fate and localisation of the Tregs once injected *in vivo* by PET [24]. We demonstrated that thymic-Tregs can be successfully transduced with a lentivirus construct encoding for the human sodium iodide symporter (NIS). We were able to expand hNIS Tregs showing no differences with the un-transduced Tregs in terms of phenotype and suppressive ability. Overall, the results of the previous project have given us the opportunity to understand more about thymic Tregs and to establish a protocol for the expansion of thymic Tregs for clinical use that is directly relevant to the current trial.

1.4 Clinical data

Clinical trials of polyclonal expanded autologous Tregs to date have demonstrated the safety and clinical applicability of Tregs in different conditions.

Kidney and liver transplantation

Expanded autologous Tregs have now been prepared in GMP facilities and therapeutically used in Phase 1 clinical trials to reduce graft rejection following kidney and liver transplantation [10,11,25,26,27,28,29]. See also relevant reviews by our group [30,31,32,33].

The aim in this setting has been to determine whether recipient-derived Tregs can act as an adjunct to conventional immunosuppression, to permit accelerated drug minimisation without prejudicing transplant outcome.

In liver transplantation, the first-in-human, open-label, dose escalation Phase I clinical trial was performed at King's College Hospital/King's College London [11]. We evaluated the safety and immunological effects of purified, ex vivo expanded autologous polyclonal Tregs, in 9 adult liver transplant recipients (ThRIL study, NCT02166177). Infused cell doses were $1x10^6$/kg (n=3) and $4.5x10^6$/kg (n=6). Decreased donor-reactive T cell responses were observed as a marker of transplantation tolerance. The safety profile was good with only one infusion related Serious Adverse Event (SAE) reported (fever, rigors, CTCAE grade 2 or higher) without any haemodynamic compromise, and any transient neutropenia, lymphopenia and mild liver dysfunction [11].

In renal transplantation, the ONE Study is a harmonised design and analysis of regulatory cell therapy (Tregs or dendritic cells or macrophages) in 7 non-randomised, single arm phase I/II trials in Europe in living kidney transplant recipients [10]. Thus far, two of the trials have separately reported their outcomes:

1. The ONEnTreg13 phase I/IIa trial from Berlin (NCT02371434) comprised 11 renal transplant recipients and outcomes compared to 9 corresponding reference group trial patients. In this dose escalation study, patients received one dose of 0.5, 1.0 or 2.5-3.0$x10^6$/kg Tregs. No SAEs were reported and there were similar numbers of cytomegalovirus (CMV), polyoma and other viral infections to the reference group at 6 months and a lower incidence by 3 years. The Treg and reference groups had 100% three-year allograft survival and similar clinical and safety profiles [29].

2. The TR001 phase I/II study based at Oxford and King's College London (NCT02129881) comprised 15 patients who were enrolled into the trial; 2 patients were withdrawn due to failure to expand sufficient Tregs (n=2) and one due to bacterial contamination of the Treg product. Out of 12 remaining patients, there were no Serious Adverse Reactions (SARs) or episodes of graft rejection, and a lower incidence of opportunistic infections compared to the reference group. Participants received cell therapy alongside standard of care immunosuppression. Infused cell doses are shown in Table 1. In a new currently ongoing trial, TR001 is being administered to living-donor kidney transplant recipients as part of a single centre phase II trial - The TWO Study. The overall aim of this study is to evaluate the efficacy of Tregs in renal transplantation [9].

Results of the King's College London and Oxford study were pooled and published as with the ONE study, with a detailed paper of the Treg trial to follow [10]. Analysis of the adverse event data showed no safety issues when compared to the reference group trial. Infection rates were lower in the trials of cell-based medicinal products but the rejection rate was found to be similar across all trials.

Another phase I study (NCT02088931) from University of California treated 3 renal transplant recipients with an absolute Treg cell dose each of 319, 321 and $364x10^6$ (equivalent to $4.5x10^6$/kg, $4.6x10^6$/kg and $5.2x10^6$/kg assuming a body weight of 70kg). There were no opportunistic infections or SAEs, no CMV or polyoma neuropathy, no rejection, or cases of donor specific antibodies at one-month post infusion [26].

Autoimmune disorders

Clinical trials using autologous expanded Tregs have been conducted in specific autoimmune disorders, namely type I diabetes, and systemic lupus erythematosus, summarised in **Table 1** below).

In a phase I study of 10 children with type 1 diabetes (ISRCTN06128462), a single infusion of $10x10^6$/kg (n-4) or $20x10^6$/kg (n=6) was given with no serious infections or adverse events. A subsequent one year follow up was conducted that also included an additional 2 extra patients (2 other patients were lost to follow up). Of these patients, 6 received an additional dose of Tregs within 6-9 months of the first infusion, using doses of $10x10^6$/kg (n=3), $20x10^6$/kg (n=3) and $30x10^6$/kg (n=6). No serious infections were reported. Mild infections that all resolved were influenza, mild gastroenteritis of unknown origin (rotavirus and adenovirus negative) and repeat exacerbation of chronic sinusitis [**8**,**34**]. Signs of potential efficacy were noted. 4-5 months after Treg infusion, plasma C peptide levels were significantly higher in recipients, compared with an untreated control group, indicating preservation of insulin secretion. 8/10 recipients were in clinical remission at 4-5 months, compared with 4/10 in the control group.

In a Phase II randomised study (Sanford Project T-Rex Study, NCT02691247) using autologous polyclonally expanded Tregs in children and adolescent with recent onset Type 1 Diabetes was conducted in 14 sites across the US with a total of 2 year follow up period [**35**]. 40/ 110 patients received a single 'low' dose (1-7 x 10^6 cells/kg) and 24/110 received the 'high' dose 11-24 x 10^6 cells/kg with the rest of the children receiving a placebo treatment. Their clinical experience showed safety of both dosing regimens. There was no difference in clinical outcomes or decline in residual Beta cells function in the Treg dosing groups compared to placebo, however transient increase of activated memory Tregs was detectable one week after infusion in the 'high' dose cohort suggesting effective transfer of expanded Tregs [**35**].

In a phase I dose escalation trial to assess safety of Treg adoptive immunotherapy in type 1 diabetes (NCT01210664), fourteen adult patients, in four dosing cohorts, received ex vivo–expanded autologous CD4+CD127lo/−CD25+ polyclonal ex vivo expanded Tregs (a total of 0.05 × 10^8 to 26 × 10^8 cells) [**36**]. A subset of the adoptively transferred Tregs was long-lived, with up to 25% of the peak level remaining in the circulation at 1 year after transfer. Immune studies showed transient increases in Tregs in recipients and retained a broad Treg FOXP3+CD4+CD25hiCD127lo phenotype long-term. There were no infusion reactions or cell therapy–related high-grade adverse events, and no opportunistic infections or malignancies occurred after a mean follow up of 31 months. C-peptide levels persisted out to 2+ years after transfer in several individuals. The only SAEs were metabolic, related to diabetes (serious hypoglycaemia, day +14, +248, +463, and ketoacidosis, day+67). Preliminary signs of therapeutic benefit were reported [**36**].

In the above trial, Tregs were labelled with deuterium during expansion in this study and thus could be tracked in the circulation following infusion. Maximal percentage of Tregs was found in the circulation on days 7-14. Ninety days following infusion, 25% of the infused Tregs were still detectable in the circulation.

A phase I study of systemic lupus erythematosus had planned recruitment of 9 patients, but there were 8 screening failures or co-morbidity burden. This meant that only one patient was enrolled, and received an absolute dose of 10^8 cells (equivalent to $1.4x10^6$/kg assuming a body weight of 70kg). There was no documentation of any adverse reactions or safety issues (NCT02428309) [**25**].

The low rate of infusion-related toxicities and absence of Treg-mediated excess immunosuppression (e.g. excess infections) seen in these studies is promising safety data for in vitro expanded Tregs as a

therapeutic agent, and supports the further development of in vitro expanded Tregs as a cell-based therapy.

Table 1: Summary of clinical trial data

Reference/Study	Study Design & ID	Indication	Number of participants	Treg cell dose	SAEs
Sanchez-Fuyeyo, 2020 (ThRIL trial)	Phase I NCT02166177	Liver transplantation	9	$1x10^6$/kg (n=3) $4.5x10^6$/kg (n=6)	1 (infusion reaction)
Chandran, 2017 (TASK trial)	Phase I NCT02088931	Kidney transplantation	3	$4.5x10^6$/kg* (n=1) $4.6x10^6$/kg* (n=1) $5.2x10^6$/kg* (n=1)	0
Matthew, 2018 (TRACT trial)	Phase I NCT02145325	Kidney transplantation	9	$7.1x10^6$/kg (n=3) $14.3x10^6$/kg (n=3) $71.4x10^6$/kg (n=3)	0
Harden, 2020 (The ONE Study)	Phase I NCT02129881	Kidney transplantation	12	$1x10^6$/kg (n=3) $3x10^6$/kg (n=3) $6x10^6$/kg (n=3) $10x10^6$/kg (n=3)	0
Bluestone, 2015	Phase I NCT01210664	Type 1 Diabetes	14	$0.7x10^6$/kg (n=3) $0.57x10^6$/kg (n=3) $4.6x10^6$/kg (n=4) $37x10^6$/kg (n=4)	4 (metabolic related to diabetes, no infusion reactions or opportunistic infections)
Marek-Trzonkowska, 2012	Phase I	Type 1 Diabetes, paediatric	10	$10x10^6$/kg (n=4) $20x10^6$/kg (n=6)	0
Marek-Trzonkowska, 2014	1 year follow up of above study, plus 2 additional participants ISRCTN06128462	Type 1 Diabetes, paediatric	12	$10x10^6$/kg (n=3) $20x10^6$/kg (n=3) $30x10^6$/kg (n=6)	0
Dall'Era, 2019	Phase I NCT02428309	Systemic lupus erythematosus	1	$1.4x10^6$/kg (n=1)	0
Chwojnicki, 2021	Phase I EudraCT:2014-004320-22	Multiple sclerosis	14	$40x10^6$/kg (n=11 IV) $1.0x10^6$/kg (n=3 IT)	0
Bender et al, 2024 (Sanford Project T-Rex)	Phase 2 Study, NCT02691247)	Type 1 Diabetes	110	1-$7x10^6$/kg (40) 11-$24x10^6$/kg (24) Placebo treatment (46)	21 No SAEs were attributed as probably or definitely related to the treatment.
Esther Bernaldo-de-Quirós et al, 2023	Phase I/ II EduraCT 2018-003574-28	Heart transplantation	10 (6 dosed so far)	20 x 10^6/kg	Ongoing 2 year follow up from 1 patient reported so far. No SAE

* Studies where only absolute infused Treg cell dose provided; Treg cell dose/kg is calculated assuming body weight of 70kg

2 Study Rationale and Purpose

In humans, Tregs dysfunction play a critical role in autoimmune diseases in which this population of cells is defective in either number or function. Therefore, Tregs are attractive candidates for immunotherapy purposes given that they can be isolated and expanded in large numbers *ex vivo* without losing their immunoregulatory properties.

The goal of this immunotherapy technology is to reduce or completely remove the overall immunosuppression burden of paediatric cardiac transplant patients. The technology has the potential to be integrated into the current clinical care pathway, with the addition of the single ATIMP delivery step. The potential changes to clinical practice could include the following:

1. Introduce a minor step in the surgical transplantation procedure of thymus retrieval, to be sent for GMP processing.
2. Reduce the duration of standard immune-suppressive regimen to 3 months.
3. Introduce a single ATIMP administration at 3 - 6 months post-surgery.

This strategy holds promise as a novel potential treatment to prevent the development of CAV, prolong the life of the transplanted organ, reduce the immunosuppressive drugs required, and improve the quality of life and general health of transplant recipients.

If successful, our intervention will introduce a breakthrough new treatment for children who require heart transplants and can be applied to all paediatric heart transplant centres world-wide. This has the potential to improve the post-operative outcomes for patients, to reduce the severity or even the incidence of CAV, and improve on the intrinsic limitations of current pharmacological immune-suppression regimens. The proposed therapy also has the potential to improve the patient's quality of life, by replacing continual drug regimen dependency post recovery phase, by a singly administered 'curative procedure'. Furthermore, reducing or eliminating the clinical side effects of immunosuppressive regimens, such as life-long susceptibility to infection, renal damage and post-transplant malignancy, will reduce the resource commitment needed for long-term care.

The treatment has no ethical concerns regarding cell origin, reprogramming and differentiation protocols. If the strategy is successful in paediatric transplantation, it will establish a precedent for cellular Treg immune therapy and impact on the success of many types of other transplantation. Paediatric cardiac transplantation constitutes a unique opportunity to explore the capacity of Tregs derived directly from thymic tissue to prevent CAV.

2.1 Rationale for the study design

In this study we aim to conduct a Phase I clinical trial to assess the safety and feasibility of *in vitro* expansion of thymus-derived Tregs derived from explanted thymic tissue during surgery (or at the time of having a pump assist device fitted) and infusing them into 9 paediatric cardiac transplant recipients as a potential treatment to prevent CAV.

The first patient in Cohort 1 will receive TR006 and we will confirm the safety of the infused cell product following the treatment before infusing the next patient with at least a 4-week gap. Safety data that spans first four weeks post-dose will be made available from the database to the Data Monitoring Committee (DMC) who will assess this information to determine if the study can proceed with further recruitment and treatment.

After safety is confirmed for the first patient, the second and third patients will receive the same dose of TR006. A safety review will be done after the third patient to decide the dose for the next

cohort (Cohort 2). The same process will be done for the next cohorts (Cohort 2 and Cohort 3). A 3+3 strategy is employed to allow for expansion of the lower dose cohort in the event of any DLT or to allow for the higher dose cohort to begin sooner if there are no DLTs experienced at the lower dose.

In vitro cellular assays of anti-donor alloreactivity before and after Treg infusion will provide surrogate indicators of potential efficacy in promoting immune tolerance to the transplant antigens. The proposed research will yield valuable data on the applicability and safety of thymic Tregs for adoptive cell therapy in paediatric heart transplant patients. Results from this study will determine the optimal dose of injected Tregs. This will give the opportunity to test our strategy in a Phase II clinical trial where a larger cohort of patients will be tested. In addition, this will provide the opportunity to involve new national and international transplant centres for creating a consortium aiming to evaluate the efficacy of Treg therapy in children receiving heart transplant.

2.2 Rationale for dose and regimen selection

As part of Cohort 1, 3 patients will be treated with the first low dose of 1-3 x 10^6Tregs/kg. If well tolerated, a further 3 patients will be treated with the second higher dose of 5-10 x 10^6Tregs/kg as part of Cohort 2. If a DLT is observed, this second cohort of 3 will continue to be treated with the low dose. An additional 3 patients will form Cohort 3 and will be enrolled at the highest tolerated dose to test the biological activity of the Tregs. Should enrolment at the high dose only begin from the third cohort i.e. from participant 7 and 1/3 DLT is observed at this dose, further funding will be sought to expand this cohort to 6 and therefore treat a total of 12 participants.

The 2 doses were selected to match what we estimate to be the Minimal Anticipated Biological Effect doses based on the ThRIL and ONE studies [10,11]. This will allow us better to capture the safety of the product and biological activity at the selected dose in a more robust manner and also to provide information on the immunological effects of the Treg product.

In our work leading up to this study, we developed a protocol appropriate for translation of expanded thymic Tregs in our GMP facility at Guy's Hospital [12]. We established that 3 different steps are necessary to obtain the Treg product from the thymus; i) Tissue homogenisation, ii) Treg isolation and iii) Treg expansion.

Thymocytes were obtained from discarded thymi combining enzymatic and non-enzymatic procedures. We processed 12 thymi from children aged between 1 month and 13 years undergoing heart surgery. The medium yield of thymocytes isolated from a paediatric thymus was 0.64 x 10^9 cells/gr of tissue (range 0.35 - 1.08). Thymocytes obtained during this initial phase have been used for Treg isolation. CD3+CD4+CD8-CD25+ T cells were isolated by depleting CD8+ cells using immunomagnetic beads (CD8 microbeads, Miltenyi Biotec) followed by CD25 enrichment (CD25 microbeads, Miltenyi Biotec). The medium final cell yield was 2.19 x 10^6 Tregs/gr (range 0.59 - 4.03), indicating that from a single thymus, more than 10^7 Tregs can be isolated. The final Treg product had a high cell purity as the percentage of CD3+CD4+CD8-CD25+CD127low was above 80% (range 72-90.8%).

Isolated Tregs were expanded polyclonally using anti-CD3/CD28 coated beads (1:1 bead:cell ratio, MACS GMP ExpAct Treg Kit, Miltenyi Biotec) for 36 days in the presence of rapamycin (100nM) and IL-2 (1000 IU/ml; Proleukin, Novartis). The mean Treg fold expansion obtained from 7 different donors was 1589, range 221-3500 a number compatible with the infusion of more than 10^7 cells per kg (highest dose planned in this clinical trial).

The final product has been functionally and phenotypically tested and all the cells were CD4+CD25+CD127- and CD4+CD25+Foxp3+. In addition, we evaluated the expression of other markers essential for Treg homing and function. In detail, Tregs were all positive for CTLA4, CD62L, CXCR4 and Helios while homing receptors like CCR6, CCR7, CXCR3 and functional markers like CD39 were poorly expressed. We also evaluated the function of the final products showing the ability of expanded Tregs to suppress the proliferation of third-party effectors T cells (Teffs) in a dose-dependent manner. Stability was also evaluated by activating Tregs in the presence of two pro-inflammatory cocktails known to mediate Tregs conversion in detrimental T-helper 17 cells. However, no IL-17 producing cells were detected at the end of the culture. Altogether these data clearly demonstrate that Tregs obtained with this protocol are functional, stable and appropriate for their translation to the clinic.

3 Trial Objectives and Endpoints

3.1 Aim

We are aiming to inject thymic-derived polyclonally expanded Tregs as a potential treatment to prevent CAV in 9 children receiving heart transplant. The purpose of this Phase I trial is to assess the safety of thymus derived Tregs (TR006) in the treatment of paediatric heart transplant patients and assess the technical feasibility of generating Tregs from the thymus.

3.2 Objectives and endpoints

Objectives	Outcome Measures/Endpoints
Primary Objective: To determine safety and optimal tolerated dose of TR006 to prevent CAV in paediatric heart transplant patients in a phase I study.	**Primary Endpoint:** Occurrence and nature of Dose-Limiting Toxicities (DLTs) occurring within 4 weeks post-infusion of the expanded autologous thymus derived Treg product (ATIMP).
Secondary Objectives: 1. To assess the feasibility of generating TR006 in the Good Manufacturing Practice facility.	**Secondary Endpoints:** Amount of TR006 manufactured per patient.
2. To investigate the clinical and immunological responses to TR006 in paediatric heart transplant patients.	**Assessment of Immunological response:** • Frequency of circulating leukocyte subsets. • Quantity of alloantigen-specific conventional T cells, Tregs and CD8+. • Presence of anti-HLA to evaluate tissue infiltrating cells. **Assessment of clinical response:** • Diagnosis of CAV as measured by coronary angiography within 24 months post-infusion of TR006. • Graft loss as measured by inclusion of patient on the re-transplant waiting list through rejection (acute or chronic) within 24 months post-infusion of TR006. • Recipient mortality within 24 months post-infusion of TR006. • Acute graft rejection as measured by endomyocardial biopsy (EMB) within 24 months post-infusion of TR006. • Antibody-mediated rejection as measured by development of donor specific antibodies within 24 months post-infusion of TR006.

	• Infection related rehospitalisation events within 24 months post-infusion of TR006. • (Cardiac function) Left ventricular ejection fraction as measured by echocardiography monthly for 12 months and at 24 months post-infusion of TR006. • (Renal function) Mean calculated GFR at 24months post-infusion of TR006. • Description of non-DLT adverse events and those occurring beyond week 4 and up to 24 months post-infusion of TR006. • Immunosuppressive doses at 12 and 24 months post-infusion of TR006.
3. To assess the feasibility of retaining participants for the duration of the study and completion of study visits and assessments	• Number of study visits completed per participant. • Responses to items in questionnaires or surveys exploring the study and treatment experience of the participants and their parents/guardians.

4 Assessment and Management of Risk

This study is a Phase I, single-center clinical trial of treatment with autologous thymus-derived Tregs. The first patient in the Cohort 1 will receive the treatment and we will confirm the safety of the infused cell product following the treatment before infusing the next patient (with at least a 4-week gap). After safety is confirmed for the first patient, the second and third patients will receive the same dose of TR006. A safety review will be done after the third patient to decide the dose for the next cohort (Cohort 2). The same process will be done for the next cohorts (Cohort 2 and Cohort 3).

The first cohort (Cohort 1) of patients (n=3) will be treated with a low dose of 1-3 x 10^6 Tregs/Kg. Dose escalation to the higher dose (5-10 x 10^6Tregs/Kg) will only occur if the lower dose is considered well tolerated. In the event a DLT is observed, this will result in expansion of the lower dose cohort.

Participants will be monitored closely for 6 hours during and immediately after TR006 infusion and then also overnight for admission after the TR006 infusion in case of acute toxicity and to facilitate ease of regular sample collection for safety monitoring.

Following discharge, participants and/or their parents/legal guardians will receive daily phone calls for the first two weeks post TR006 infusion (except on day 14 post-dose) to monitor for any adverse events. Participants will be reviewed in person at days 14 and 28 post Treg infusion for safety assessments including clinical blood tests, ECG and echocardiography and continued adverse event monitoring. Thereafter, participants will be reviewed in person at months 2, 3, 6, 9 and 12 post-dose, with a final safety follow-up visit at 24 months post Treg infusion to monitor for any delayed or chronic complications.

4.1 IMP Risks

The Investigator's Brochure (IB) contains the reference safety information for TR006. For the Non-Investigational Medicinal Products (NIMPs) (details in **8.11 Non-Investigational Medicinal Products (NIMPs)** below), investigators should refer to the relevant summary of product characteristics (SmPC) for the possible adverse drug reactions that may be experienced by trial patients. The risk-benefit profile of this clinical trial essentially balances the expected clinical benefits of minimising maintenance immunosuppression against the potential clinical complications that may result from the infusion of the Treg product.

This clinical trial has been designed to reduce the level of foreseeable risk, wherever this is possible. The clinical assessments specified by this protocol are largely those suggested by normal clinical guidelines and will not present risks that extend beyond the normal hazards associated with the routine clinical follow-up of paediatric heart transplant recipients. Tests required prior to patient enrolment (to assess eligibility) are also largely within the boundaries of standard clinical practice.

4.2 Risks associated with the administration of the Treg cell product

The potential risks associated with Treg therapy can be categorised as immunological, physiological, infectious, or due to adverse interactions with other treatments. While the single ascending dose design combined with safety reviews after the first and last dose of each dosing cohort, should minimise these risks, it is nonetheless necessary to consider the potential risks in turn.

4.2.1 Immunological Complications

The possible immunological complications of Treg therapy are broadly similar to those associated with blood transfusions, but with the important difference that the cells to be administered are from the patient themselves and are thus autologous. However, it is not possible to rule out unintended outcomes such as non-specific cytokine release triggered by cell infusion.

Hypersensitivity Reactions

Type I hypersensitivity reactions

Antigenic challenge can drive the production of IgE which binds to the FcεRI receptor on mast cells and basophils leading to degranulation and the release of vasoactive and spasmogenic mediators, and proinflammatory cytokines. The clinical manifestation of such reactions varies in degree from relatively mild urticaria and pruritis to anaphylactic shock. Generalised pruritis and urticarial reactions can occur during blood transfusions, though typical incidence is of the order of 1 - 3%. These complications generally respond to parenteral antihistamines. Bronchospasm, laryngeal oedema, bradycardia and profound hypotension are uncommon occurrences during blood transfusions, but are potentially life-threatening and demand emergency treatment.

The possibility of hypersensitivity reactions against cellular antigens or excipients in Treg preparations cannot be discounted entirely but the fact that the infused cells are autologous indicates that the risk is low. The cell product will be infused in Cryostor CS10 medium (10% DMSO) and for this reason known allergies to components of the infusion are listed as exclusion criteria.

Patients will be admitted as an inpatient for administration of TR006, so that they can be closely monitored for signs of anaphylaxis or to receive emergency treatment should it be necessary. To prevent minor pruritic or urticarial reactions, patients will be treated prophylactically with an antihistamine 30 minutes before the planned infusion.

Type II hypersensitivity reactions

Antibodies against Treg cell-surface antigens, which may be intrinsic to the cell membrane or merely antigens adsorbed to the cell surface, can be responsible for a variety of adverse transfusion-associated reactions, including haemolytic transfusion reactions, post-transfusion purpura and some febrile reactions. A febrile transfusion reaction is defined as a 1°C rise in temperature during or within 3 hours of transfusion which cannot be attributed to sepsis or a haemolytic reaction. Febrile transfusion reactions may be accompanied by chills, rigors or pain. The incidence of febrile reactions to leuko-depleted blood products is approximately 1 in 330 for erythrocyte transfusions and 1 in 20 for platelet transfusions. Treatment consists of stopping the transfusion and providing supportive care, including antipyretic treatment.

Patients receiving TR006 will be prophylactically treated with paracetamol and an oral antihistamine prior to cell infusion. In the event of a 1°C rise in temperature, the rate of cell infusion will initially be slowed; if the febrile reaction persists, the infusion will be stopped.

Type III hypersensitivity reactions

Although once a common sequela to the administration of foreign serum for passive immunisation, serum sickness is now encountered infrequently. The pathogenesis of this systemic immune complex disease involves the deposit of antibody-antigen complexes in the tissues resulting in inflammation. Depending on their distribution, immune complexes cause vasculitis, glomerulonephritis or arthritis. Despite systemic immune complex disease not being a recognised complication of conventional transfusions, the possibility of Type III hypersensitivity responses after Treg administration cannot be completely excluded. However, since TR006 is autologous to the patient, such complications are

considered unlikely. Clinicians responsible for patient care will be informed of the theoretical possibility of immune complex disease so that it might be considered as a possible cause if post-infusion complications are seen.

Non-specific adverse reactions

Release of pyrogenic cytokines upon systemic infusion of cell products can cause febrile reactions. In tissue culture, human Tregs produce detectable amounts of IL-6 and TNF-α, so could potentially cause fever after administration. Mild febrile responses do not constitute a major clinical concern and could be largely prevented by treatment of recipients with an antipyretic and anti-histamine prior to Treg infusion.

Unintended immune sensitisation

The Treg cell product in this trial is autologous and will be subject to stringent quality control testing and QP release (refer to IMPD). Therefore, immune sensitisation is unlikely. However, in the event of a cytokine storm then supportive measures may be supplemented by the administration of then anti-thymocyte globulin (ATG). This is a highly effective treatment for the deletion of all T cells and is in routine use in the treatment of severe rejection in heart transplant recipients. Basiliximab will be avoided as repeat basiliximab increases the risk of allergic reaction. Other treatments may be administered on a case by case basis in line with Trust SOPs.

Transfusion-Related Acute Lung Injury (TRALI)

A well-recognised and clinically important complication of blood transfusion is non-cardiogenic pulmonary oedema, referred to as transfusion-related acute lung injury or TRALI, which occurs in roughly 1 in 5000 cases. The pathophysiology of TRALI is not completely understood, but neutrophil and platelet activation, and consequent damage to the pulmonary vascular endothelium are crucial components of the disease.

There is no evidence to suggest that Tregs should activate neutrophils in the lung and this complication has not been reported in published clinical trials of expanded naturally occurring Tregs. However, given the seriousness of the condition, all precautions will be taken to avoid it. A two-hit hypothesis has been advanced to explain the susceptibility of patients to TRALI, where neutrophil activation prior to transfusion predisposes to a detrimental neutrophil-mediated response when anti-leucocyte antibodies or other neutrophil-stimulating factors are subsequently administered.

4.2.2 Physiological Complications

Pulmonary embolism

The Treg cell product contains particulates, namely the Treg cells themselves, some of which may aggregate or clump, and in addition there may be particulates from the authorised container closure system (CellSeal vials). When a cell product is administered by venous infusion, the potential for embolism of cells, aggregates of cells or debris to impede the pulmonary vasculature is a concern.
Pulmonary vascular obstructions caused by cell infusion may be widespread and are more likely to affect small end-arteriolar branches or capillaries than larger vessels. Therefore, pulmonary embolism (PE) following cell infusion may not present with classical clinical signs of thromboembolic PE. Depending on its extent, PE may be a life-threatening condition and measures will be taken to avoid its occurrence. For this reason, TR006 will be administered through a standard IV administration set that contains a 200 µm filter plus an additional 40 µm filter to remove visible particulates.

Infusion-related circulatory overload

Fluid infusion may occasionally result in circulatory overload presenting as hydrostatic pulmonary oedema. Critically, the clinical features of fluid overload may be indistinguishable from non-cardiogenic pulmonary oedema, as might occur in a transfusion-related acute lung injury (TRALI)-like reaction. Fluid overload will be avoided by minimising the volume administered (maximum volume of cells will be 10ml) and using a slow rate of infusion over 30 minutes.

Biochemical disturbances

It is possible that immune-mediated lysis of Tregs may occur after infusion but as the cells are autologous this is considered unlikely. Furthermore, the cell dose delivered is negligible compared to the number of cells that must be lysed to cause biochemical disturbances associated with massive tumour lysis, rhabdomyolysis or haemolytic transfusion reactions. Nevertheless, measuring LDH levels pre- and post-Treg infusion may be useful in assessing the fate of Tregs. Patients treated with Treg will be investigated pre- and post-infusion for blood levels of LDH, haemoglobin and tumour lysis markers such as K+, Mg+ and bone profile. The blood pressure and ECG of patients will be closely monitored for hypotension and tachyarrhythmias.

4.2.3 Infectious Diseases

Transmission of infectious diseases through the application of cell products is a potential risk of cell therapy especially in immunosuppressed recipients. The two potential sources of infective contaminants are the consumables used during the manufacturing process or adventitious agents introduced during manufacture. All reagents and equipment used in the manufacturing process will be specified for clinical GMP use or CE-marked. TR006 will be produced using validated Standard Operating Procedures (SOPs) in a licensed GMP facility by fully trained, validated staff dedicated to Treg manufacture. The final cell product will be subject to defined release criteria (see IMPD for details).

4.2.4 Malignancy

Malignant disease after treatment with thymus-derived (Treg) cell product could, in principle, arise either as a consequence of transferring neoplastic cells or as a consequence of transferred cells promoting the growth of spontaneous tumours. Neoplastic cells within the cell product might arise during in vitro culture or after transfer into the recipient. Further, the infused regulatory T population might suppress immune responses against malignant cells. Although no long-term data on this aspect of Treg therapy are available, those that have been published did not report safety concerns of this type. Furthermore, the cell dose that will be infused represents approximately 2% of the patient's endogenous regulatory T cell population suggesting that an impact on malignancy will be small.

4.3 The Immunosuppression Regimen

We do not propose any change to our standard immunosuppression. We will continue to use tacrolimus and mycophenolate mofetil as immunosuppression. This is consistent with current standard of care treatment. This is similar to the policy in the recent study of Tregs in heart transplantation [7].

5 Trial Design

5.1 Description of study design

This is a dose escalation, 3+3, open label, Phase I, single centre-trial investigating the safety and feasibility of two doses of autologous thymus derived regulatory Tregs (TR006) infused into 9 paediatric heart transplant patients.

We will enrol 3 patients at the first low dose of 1-3 x 10^6Tregs/Kg (Cohort 1). The first patient in this cohort will receive the treatment and the safety of the infused cell product will be confirmed before infusing the next patient (with at least a 4-week gap). After safety of the treatment is confirmed for the first patient, the second and third patients of this cohort will receive the same dose of TR006 as and when this is possible. A safety review will be carried out after the third patient is treated to then decide to dose for the next cohort.

If safe, then another 3 patients (Cohort 2) will be treated with the second higher dose of 5-10 x 10^6Tregs/Kg. Similarly, the first patient in this cohort will receive the treatment and the safety of the infused cell product will be confirmed before infusing the next patient (with at least a 4-week gap). After safety of the treatment is confirmed for the first patient, the second and third patients of this cohort will receive the same dose of TR006.

A safety review will be performed after the third patient dosed as part of Cohort 2. If safe, a final 3 participants (Cohort 3) will be enrolled into the study to be treated at the highest tolerated dose (using the same dosing/safety review strategy as described for Cohort 1 and Cohort 2).

The study will determine the optimal dose of injected Tregs using a single ascending dose (SAD) design with groups of 3 patients in 3 cohorts (total of 9 patients that could be expanded to 12 in case of dose limiting toxicities). However, if a dose limiting toxicity (DLT) is observed in at least 1/3 in Cohort 1, this will result in expanding the low dose to Cohort 2 instead. A final 3 participants (Cohort 3) will be enrolled at the highest tolerated dose to test further the biological activity subject to there being no further DLTs observed at the low dose if expanded to Cohort 2 i.e. no more than 1/6 DLT or there are no more than 1/3 DLTs observed if Cohort 2 was escalated to the high dose.

If both doses are tolerated, there will be a total of n=9 patients in the study (3 at the low dose and 6 at the high dose or 6 at the low dose and 3 at the high dose). If the higher dose can only be assessed from Cohort 3 and there is 1/3 DLT observed, a further 3 patients will need to be treated at this dose to establish the highest tolerated dose. In this scenario, funding for a further 3 participants will be applied for and the total sample size will be 12 participants.

A DLT will be determined from data collected during the 4-week period post TR006 infusion. Patients with no undue toxicity will be managed and continued on therapy. A DLT will be graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) on a 1 to 5 sale as follows [37]:

Grade 1: Mild adverse event
Grade 2: Moderate adverse event
Grade 3: Severe and undesirable adverse event
Grade 4: Life-threatening or disabling adverse event
Grade 5: Death.

A DLT is a toxicity considered to be related to TR006. The following events will be considered DLTs:

Occurring in the first 72 hours post infusion:

- CTCAE ≥ grade 2 cytokine release syndrome
- CTCAE ≥ grade 2 injection site reaction
- CTCAE ≥ grade 2 fever and/or rigors
- CTCAE ≥ grade 3 bronchospasm
- CTCAE ≥ grade 3 hypoxia

Occurring in the first 4 weeks post TR006 infusion:

- CTCAE ≥ grade 3 infection
- Moderate or severe acute rejection (as defined by the International Society for Heart and Lung Transplantation (ISHLT) criteria grade II-III) [**38**]
- CTCAE ≥ grade 3 haematological complication (neutropenia, thrombocytopenia, anaemia).
- Any CTCAE ≥ grade 3 toxicity not clearly related to underlying disease

6 Population

9 paediatric patients on the heart transplant register at GOSH will be recruited to the study.

6.1 Inclusion Criteria

1. Male or female children aged between 0.5 and 16 (inclusive) years of age at date of consent/assent and date of transplant.

2. Written informed consent obtained from a parent/legal guardian.

3. Registered on the heart transplant list at date of consent and received a heart transplant on enrolment.

4. Receiving a single transplanted organ.

5. Willing and able to comply with the study visit schedule.

6.2 Exclusion Criteria

1. Active viral infection (with HIV (positive for HIV antibody type 1 or 2), hepatitis B (positive HBSAg), hepatitis C (anti-HCV antibody detection), Human T lymphotropic virus (HTLV antibody I/II detection) and syphilis (Treponema pallidum antibody detection)) at date of admission for transplant (Please refer to **Table 22** for more details).

2. Age under 0.5 year or over 16 years at date of consent and at date of transplant.

3. Multi-organ transplant.

4. Highly-sensitised patients at high risk of rejection assessed by HLA antibody measurement (cumulative risk of Mean Fluorescent Intensity (MFI) being >5000 of donor specific antibodies from HLA antibody testing).

5. Allergy to any component / excipients used for the manufacture of TR006.

6. Previous recipient of any organ transplant.

7. History of previous sternotomy surgical procedure for congenital heart defect during which has had previous partial or full thymectomy.

8. Confirmed diagnosis of DiGeorge Syndrome with absent thymus.

9. Participation in another interventional Clinical Trial of Investigational Medicinal Product during the study or within 28 days prior to date of transplant (at the Chief Investigator's discretion).

10. Pregnant and lactating patients (females of childbearing potential* with a positive urine pregnancy test at date of transplant).

11. Female patients of childbearing potential* who are not willing to use a highly effective method of contraception** for the duration of the trial (defined as from date of transplant to 12 months

post Treg infusion) to prevent pregnancy, or abstain from heterosexual activity.

*****Females of child-bearing potential are females who have experienced menarche and are not surgically sterilised (e.g., hysterectomy, bilateral salpingectomy or bilateral oophorectomy) or post-menopausal. Postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement is required.

****** Highly effective methods of contraception are those with a failure rate of < 1% per year when employed consistently and correctly. For example:

- combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation – oral, intravaginal, transdermal.
- transdermal progestogen-only hormonal contraception associated with inhibition of ovulation – oral, injectable, implantable.
- intrauterine device (IUD).
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomised partner, provided that partner is the sole sexual partner of the FOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success.

Sexual abstinence is considered to be a highly effective method only if defined as refraining from heterosexual activity from the date of transplant until 12 months post Treg infusion. The reliability of this method should be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.

12. Male patients who are not willing to use an effective method of contraception (condoms), for the duration of the study (date of transplant to 12 months post Treg infusion), when engaging in sexual activity with a female of childbearing potential.

13. Patient is considered by the Chief Investigator, for any reason, to be an unsuitable candidate for the study.

7 Trial Procedures

Please refer to **Appendix B** for trial Schedule of Events (and to

Table 3, **Table 4** and **Table 5** for a summary of the clinical procedures performed at the study visits).

Most of the assessments from sections **7.3 – 7.5** below all form part of the routine management of paediatric transplant patients with the exception of the translational research and cardiac biopsy samples being collected.

7.1 Recruitment

Potential participants will be identified by review of the paediatric heart transplant register at GOSH by the direct care team. The patients' medical history and results will be reviewed by the clinical team at GOSH to confirm potential eligibility for participation in the study before the PIS is given. A pre-screening log will be used to record the number of participants potentially eligible but not entered into the trial in order to fulfil CONSORT reporting guidelines [**39**]. A screening and enrolment log and study ID log will be maintained by the site. Potentially eligible participants and/or their parents/legal guardians that decline to take part will be asked if they are willing to provide a reason, which will be captured anonymously on the pre-screening log.

The Investigator will provide trial information to parents/legal guardians of children who are considered to meet the study eligibility criteria. If appropriate, children will also be given information about the trial with age-appropriate patient information sheets.

7.2 Informed Consent

Adequate time must be given for consideration by the patient and their parent/guardian before taking part. Due to the nature of transplantation, the date of transplant is often unpredictable and with little advance warning. As soon as a donor heart becomes available, patients and their parents/guardians will be notified and initiated on the heart transplantation clinical pathway which includes admission to hospital on the day of notification and clinical work-up for the procedure scheduled for transplantation the following day. Therefore, the PIS will be provided to patients and their parents/guardians whilst they are on the register and written informed consent sought ahead of this date due to the limited time available once the patient is admitted to hospital to receive the heart transplant. The date the PIS is provided and the date of written informed consent will be recorded on the study screening log.

As there may be a potential time lag between informed consent for the trial and date of transplantation, patients and their parents/guardians will be approached to provide their confirmation that they are still happy to participate in the trial. This will provide an opportunity for patients and their parents/guardians to be reminded about the study and to ask any questions about the trial. This can be performed verbally and the confirmation of continuation in the study will be recorded on the patient's medical records. Participant eligibility will also be rechecked prior to infusion of TR006.

Study participants who have a pump assist device fitted will be contacted regularly by the Study Team on at least a monthly basis to ensure that they are still happy to provide continued consent to remain part of the study. This regular contact will also provide the participant and their parents/guardians an opportunity to discuss the study and to answer any queries

In the interim time period between Informed Consent into the study and the Day of Transplant, participants and their parents/guardians will be regularly contacted by the Study Team (either as inpatient consultations, during routine outpatient clinic visits or by remote communication) to further discuss the study, answer any questions and to confirm that they are still happy to provide continued consent to remain part of the study.

If new safety information results in significant changes in the risk/benefit assessment, the informed consent form will be reviewed and updated if necessary and subjects will be re-consented as appropriate.

It is the responsibility of the Chief Investigator, or person to whom the Investigator delegates the responsibility, to obtain written informed consent for each parent/legal guardian prior to performing any trial related procedure in compliance with regulations. All trial investigators seeking consent must have received Human Tissue Act training for the taking of consent involving tissues and cells used as part of the trial, be up-to-date with their GCP training and delegated for the task on the Delegation Log.

Investigators must ensure that they adequately explain the patient information sheet outlining the aim, trial treatment, potential risks and benefits of taking part in the trial. The patient's parent/legal guardian should be given ample time to read the PIS and to discuss their child's participation with others outside of the clinical research team. The parent/legal guardian must be given an opportunity to ask questions which should be answered. The right of the parent/guardian to refuse to participate without giving a reason must be respected.

The trial includes both children and young adults $\leq$16 years and written assent will be obtained from the patient whenever it is possible to do so (as appropriate to age and legislation). If capable, and under appropriate circumstances, minors should be approached to provide assent by a delegated clinician. Depending on age and state of development, REC approved Patient Information Sheet and Assent forms, describing (in simplified terms) the details of the study intervention/product, study procedures and risks should be used. The minor should personally write their name (or initial) and date the assent form, which is then signed by the delegated clinician taking consent. Assent forms do not substitute for the consent form signed by the patient's legally acceptable representative. Assent should be taken where appropriate and documented in the patient notes, however the absence of assent does not exclude the patient, provided consent has been obtained from the parent/legal guardian.

If the parent/legal guardian decides for their child to participate in the trial they must be asked to sign and date the latest approved version of the Informed Consent Form. Details of the original informed consent and assent discussions should be recorded in the patient's medical notes. This should include date and content of the initial discussion, the date consent was obtained and trial name.

The patient (where applicable) and the parent/legal guardian should also provide verbal confirmation that they are happy for continuation in the trial at each subsequent study visit. Details of verbal confirmation of continuity in the study should also be recorded in the patient's medical notes at each study visit.

A copy of the PIS and signed consent forms and/or assent forms will be given to the patient/parent/guardian and a copy will be kept in their medical records. The original signed consent/assent forms will be kept in the Investigator Site File.

7.3 Screening and Eligibility Assessment

Screening/enrolment (prior to transplant).

- Informed Consent: Patient will provide written consent whilst on the transplant waiting list.
- Inclusion/Exclusion criteria review.
- Demographics.
- Medical History (including co-morbidity check).
- Concomitant Medication collection.
- Full blood borne infectious diseases testing.
- HLA antibody testing (includes panel reactive antibodies and donor specific antibodies).

Transplant (on admission for transplant): Day of Transplant (immediately before the surgery) and Transplant Day 1.

- Informed Consent: The study team will (verbally) confirm consent for participating in the study is still valid prior to the admission of the patient for the transplant. (Day of Transplant)
- Inclusion/Exclusion criteria review. (Day of Transplant)
- Concomitant Medication collection. (Day of Transplant and Transplant Day 1)
- Adverse Events collection. (Day of Transplant and Transplant Day 1)
- Doctor review (including physical examination). (Transplant Day 1)
- Vital signs. (Day of Transplant and Transplant Day 1)
- Height and weight. (Day of Transplant)
- ECG (12-Lead). (Transplant Day 1)
- Echocardiography. (Transplant Day 1)
- Clinical blood tests (Full blood count, liver profile, renal profile, LDH, EBV, CMV viral load, blood borne infectious diseases* and HLA antibody testing (includes panel reactive antibodies and donor specific antibodies)*). (Day of Transplant and Transplant Day 1)
- Urine pregnancy test (if applicable). (Day of Transplant)
- Translational research blood samples. (Day of Transplant and Transplant Day 1)
- Cardiac transplant. (Day of Transplant)
- Thymus extraction for TR006 manufacturing (Can be at the time of having a pump-assisted device fitted in; up to 1 year before surgery). (Day of Transplant)

* Tests for blood borne infectious diseases and HLA antibody testing (includes panel reactive antibodies and donor specific antibodies) on Day of Transplant (immediately before the transplant surgery happens) only.

Transplant follow-up: Transplant Follow-up Day 14, Transplant Follow-up Month 1, Transplant Follow-up Month 2 and Transplant Follow-up Month 3.

- Inclusion/Exclusion criteria review. (Transplant Follow-up Month 3)

- Medical History. (Transplant Follow-up Month 3)
- Concomitant Medication collection. (All visits)
- Adverse Events collection. (All visits)
- Doctor review (including physical examination). (All visits)
- Vital signs. (All visits)
- Height and weight. (All visits)
- ECG (12-Lead). (All visits)
- Echocardiography. (All visits)
- Intravascular ultrasound (only for children over 25kg). (Transplant Follow-up Month 3)
- Coronary angiography (only for children over 25kg). (Transplant Follow-up Month 3)
- Clinical blood tests (Full blood count, liver profile, renal profile, LDH, EBV, CMV viral load, full blood borne infectious diseases* and HLA antibody testing (includes panel reactive antibodies and donor specific antibodies)†). (All visits)
- Urine pregnancy test (if applicable). (Transplant Follow-up Month 3)
- Translational research blood samples. (Transplant Follow-up Day 14 and Transplant Follow-up Month 3)
- Clinical cardiac biopsy. (Transplant Follow-up Day 14 and Transplant Follow-up Month 3)

* Full blood borne infectious diseases testing on Transplant Follow-up Month 3 only.
† HLA antibody testing (includes panel reactive antibodies and donor specific antibodies) on Transplant Follow-up Day 14 and Transplant Follow-up Month 3.

Baseline (Day 0) can occur as soon as the results from the clinical cardiac biopsy (obtained at the Transplant Follow-up 3 Months study visit) have been reviewed by the Study Team. Biopsy results normally take 2-3 days to be reported.

If 30 days elapse after the Transplant Follow-up Month 3 study visit, then Transplant Follow-up Month 4 should be carried out. Similarly, if 30 days elapse after the Transplant Follow-up Month 4 study visit, then Transplant Follow-up Month 5 should be carried out.

If TR006 dosing is able to occur within 30 days after the Transplant Follow-up Month 3 study visit, then neither the Transplant Follow-up Month 4 or the Transplant Follow-up Month 5 study visits need to be carried out and the participant can proceed straight to Baseline (Day 0).

However, if the participant requires the Transplant Follow-up Month 4 study visit and then is able to receive the TR006 dose within 30 days after this visit, then the Transplant Follow-up Month 5 study visit does not need to be carried out and the participant can proceed straight to Baseline (Day 0).

Additional transplant follow-up (if required): Transplant Follow-up Month 4 and Transplant Follow-up Month 5.

- Inclusion/Exclusion criteria review. (All visits)
- Medical History. (All visits)
- Concomitant Medication collection. (All visits)
- Adverse Events collection. (All visits)
- Doctor review (including physical examination). (All visits)
- Vital signs. (All visits)

- Height and weight. (All visits)
- ECG (12-Lead). (All visits)
- Echocardiography. (All visits)
- Clinical blood tests (Full blood count, liver profile, renal profile, LDH, EBV, CMV viral load, blood borne infectious diseases and HLA antibody testing (includes panel reactive antibodies and donor specific antibodies)). (All visits)
- Urine pregnancy test (if applicable). (All visits)
- Translational research blood samples. (All visits)

7.4 Baseline Assessments

Dosing - TR006 single infusion (in-patient visit): Week 0: Baseline Day 0 and Baseline Day 1 (3-6 months post-surgery).

- Informed Consent: The study team will (verbally) confirm consent for participating in the study is still valid prior to the TR006 infusion. (Baseline Day 0)
- Inclusion/Exclusion criteria review. (Baseline Day 0)
- Medical History (including co-morbidity check). (Baseline Day 0)
- Concomitant Medication collection. (All visits)
- Adverse Events collection. (All visits)
- Doctor review (including physical examination). (All visits)
- Vital signs. (All visits)
- Height and weight. (All visits)
- ECG (12-Lead). (All visits)
- Echocardiography. (All visits)
- Clinical blood tests (Full blood count, liver profile, renal profile, LDH, EBV, CMV viral load, bone profile, magnesium and uric acid). (Baseline Day 0: pre-dose and 6 hours post-infusion and then Baseline Day 1)
- Urine pregnancy test (if applicable). (Baseline Day 0)
- Translational research blood samples. (Baseline Day 1)
- TR006 dosing. (Baseline Day 0)

Baseline: Day 0 (Week 0):

Please refer to **Table** for a summary of the assessments and procedures to be carried out on the day of TR006 dosing (Baseline: Day 0):

- Vital signs will be performed pre-dose (within 1 hour of infusion start), during dose (at: 15 mins, 30 mins (if applicable), 45 mins (if applicable) and at the end of the dose) and post-dose: 15 mins, 30 mins, 45 mins, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours and 6 hours.
- 12 Lead ECG should be performed pre-dose.
- Echocardiography should be performed pre-dose.
- Clinical blood tests (including additional blood tests*) will be obtained pre-dose (within 12 hours of infusion start) and 6 hours post-infusion.
- A urine pregnancy test will be performed pre-dose.
- There must be continuous clinical observation of the participant from the start of the TR006 infusion up until at least 1 hour after the dose has been fully administered.

*Blood tests for bone profile, creatinine kinase (CK), CRP, ferritin, magnesium, uric acid and coagulation screen are to be performed in addition to the standard protocol-mandated clinical blood tests. Any leftover blood remaining from clinical blood testing may be used for flow analysis (instead of being discarded).

Baseline Day 0 (Week 0)	Vital Signs	Clinical Blood Tests	ECG	Echocardiography	Pregnancy Test
Pre-Dose (within 12 hours)		X *	X	X	X
Pre-Dose (within 1 hour)	X				
During Dose: 15 mins	X				
During Dose: 30 mins (if applicable)	X				
During Dose: 45 mins (if applicable)	X				
At the end of Dose	X				
Post Dose: 15 mins	X				
Post Dose: 30 mins	X				
Post Dose: 45 mins	X				
Post Dose: 1 hr	X				
Post Dose: 1.5 hours	X				
Post Dose: 2 hr	X				
Post Dose: 3 hr	X				
Post Dose: 4 hr	X				
Post Dose: 6 hr	X	X *			

Table 6: Summary of assessments and procedures to be carried out on Baseline Day 0

Baseline: Day 1 (Week 0):

Please refer to **Table 7** for a summary of the assessments and procedures to be carried out on the day after TR006 dosing (Baseline: Day 1), prior to discharge:

- Vital signs.
- 12 Lead ECG.
- Echocardiography.
- Clinical blood tests (including additional blood tests*).
- Research blood samples (PBMC panel, Treg Panel and Transcriptomic/gene expression sample)

*Blood tests for bone profile, creatinine kinase (CK), CRP, ferritin, magnesium, uric acid and coagulation screen are to be performed in addition to the standard protocol-mandated clinical blood tests.

Baseline Day 1	Vital Signs	Clinical Blood Tests	Research Blood Sample	ECG	Echocardiography
Pre-discharge	X	X *	X	X	X

Table 7: Summary of assessments and procedures to be carried out prior to discharge on Baseline Day 1

7.5 Subsequent Visits

Safety Follow-up: Days 2 - 13 post TR006 infusion. (+/- 2 days)

- Concomitant medication collection. (All visits)
- Adverse Events collection. (All visits)
- Remote doctor review. (All visits)

Safety Follow-up: Days 14 post TR006 infusion. (+/- 2 days)

- Concomitant medication collection.
- Adverse Events collection.
- Doctor review (including physical examination).
- Vital signs.
- Height and weight.
- ECG (12-Lead).
- Echocardiography.
- Clinical blood test (Full blood count, liver profile, renal profile, LDH, EBV and CMV viral load).
- Translational research blood samples.

Safety Follow-up: Days 28 post TR006 infusion. (+/- 2 days)

- Concomitant medication collection.
- Adverse Events collection.
- Doctor review (including physical examination).
- Vital signs.
- Height and weight.
- ECG (12-Lead).
- Echocardiography.
- Clinical blood test (Full blood count, liver profile, renal profile, LDH, EBV and CMV viral load).
- Translational research blood samples.

Safety Follow-up: Month 2 post TR006 infusion. (+/- 1 week)

- Concomitant medication collection.
- Adverse Events collection.
- Doctor review (including physical examination).
- Vital signs.
- Height and weight.
- ECG (12-Lead).
- Echocardiography.
- Clinical blood tests (Full blood count, liver profile, renal profile, LDH, EBV and CMV viral load.

Safety Follow-up: Month 3 post TR006 infusion. (+/- 1 week)

- Concomitant medication collection.
- Adverse Events collection.
- Doctor review (including physical examination).

- Vital signs.
- Height and weight.
- ECG (12-Lead).
- Echocardiography.
- Clinical blood tests (Full blood count, liver profile, renal profile, LDH, EBV, CMV viral load, full blood borne infectious diseases screen and HLA antibody testing (includes panel reactive antibodies and donor specific antibodies)).
- Urine pregnancy test (if applicable).
- Translational research blood samples.
- Clinical cardiac biopsy.

Safety Follow-up: Month 6 post TR006 infusion. (+/- 2 weeks)

- Concomitant medication collection.
- Adverse Events collection.
- Doctor review (including physical examination).
- Vital signs.
- Height and weight.
- ECG (12-Lead).
- Echocardiography.
- Clinical blood tests (Full blood count, liver profile, renal profile, LDH, EBV and CMV viral load).
- Translational research blood samples.

Safety Follow-up: Month 9 post TR006 infusion. (+/- 2 weeks)

- Concomitant medication collection.
- Adverse Events collection.
- Doctor review (including physical examination).
- Vital signs.
- Height and weight.
- ECG (12-Lead).
- Echocardiography.
- Intravascular ultrasound (only for children over 25kg).
- Coronary angiography (only for children over 25kg).
- Clinical blood tests (Full blood count, liver profile, renal profile, LDH, EBV, CMV viral load and HLA antibody testing (includes panel reactive antibodies and donor specific antibodies)).
- Urine pregnancy test (if applicable).
- Translational research blood samples.

Safety Follow-up: Month 12 post TR006 infusion. (+/- 4 weeks)

- Concomitant medication collection.
- Adverse Events collection.
- Doctor review (including physical examination).
- Vital signs.

- Height and weight.
- ECG (12-Lead).
- Echocardiography.
- Clinical blood tests (Full blood count, liver profile, renal profile, LDH, EBV and CMV viral load).
- Translational research blood samples.

End of Study: Month 24 post TR006 infusion. (+/- 4 weeks)

- Concomitant medication collection.
- Adverse Events collection.
- Doctor review (including physical examination).
- Vital signs.
- Height and weight.
- ECG (12-Lead).
- Echocardiography.
- Clinical blood tests (Full blood count, liver profile, renal profile, LDH, EBV, CMV viral load and HLA antibody testing (includes panel reactive antibodies and donor specific antibodies)).
- Urine pregnancy test (if applicable).
- Translational research blood samples.

7.6 Laboratory tests

Refer to the Lab Manual for full details on collection, processing, storage of all research samples.

All laboratory investigations that are required are part of the routine management of heart transplant patients (desirable assessments only required if being collected as part of routine clinical care), except the following additional research samples:

- PBMC panel (2mL)
- Treg Panel (1mL)
- Circulating cytokines (3mL)
- Alloreactivity (2mL)
- Transcriptomic/gene expression (3mL)

These will be collected at the following study visits (as per **Table 8**):

	Screening	Transplant		Transplant Follow-up					
	Enrolment	Day of Transplant	Transplant Day 1	Transplant Follow-up Day 14	Transplant Follow-up 1 Month	Transplant Follow-up 2 Months	Transplant Follow-up 3 Months	Transplant Follow-up 4 Months [a]	Transplant Follow-up 5 Months [a]
				+/- 1 week	+/- 1 week	+/- 1 week	+/- 1 week	+/- 3 days	+/- 3 days
PBMC panel (2mL)		X	X	X			X	X	X
Treg Panel (1mL)		X	X	X			X	X	X
Circulating cytokines (3mL)		X		X			X	X	X
Alloreactivity (2mL)		X					X	X	X
Transcriptomic/gene expression (3mL)		X		X			X	X	X

	Baseline	
	Baseline Day 0 [b]	Baseline Day 1
PBMC panel (2mL)		X
Treg Panel (1mL)		X
Circulating cytokines (3mL)		
Alloreactivity (2mL)		
Transcriptomic/gene expression (3mL)		X

	Safety Follow-up (after TR006 Dosing)								End of Study
	Follow-up Days 2-13	Follow-up Day 14	Follow-up Day 28	Follow-up Month 2	Follow-up Month 3	Follow-up Month 6	Follow-up Month 9	Follow-up Month 12	Follow-up Month 24
	+/- 2 Days	+/- 2 Days	+/- 2 Days	+/- 1 week	+/- 1 week	+/- 2 weeks	+/- 2 weeks	+/- 4 weeks	+/- 4 weeks
PBMC panel (2mL)		X	X		X	X	X	X	X
Treg Panel (1mL)		X	X		X	X	X	X	X
Circulating cytokines (3mL)		X	X		X	X	X	X	X
Alloreactivity (2mL)					X		X		X
Transcriptomic/gene expression (3mL)		X	X		X	X	X	X	X

Key	Description
a	Visit is required if Baseline Day 0 cannot be carried out within 30 days of the preceding study visit. Otherwise proceed to Baseline Day 0.
b	TR006 dosing to occur 3-6 months post-cardiac transplant.

Table 8: Research Sample Schedule

A maximum of approximately 11mL of additional blood per study visit will be collected from participants for the processing of research samples.

Some of the cardiac biopsy samples that are collected as per routine clinical care will be transferred to labs at GOSH and/or Guy's Hospital and/or King's College London for analysis and storage. Additionally, if there are any leftover clinical blood samples (collected as per routine clinical care), these also may be sent to labs at GOSH and/or Guy's Hospital and/or King's College London for analysis and storage.

If there are any cells remaining from the TR006 manufacturing process, these may be retained by the study team to further investigate the IMP.

Appropriate material transfer agreements and contractual agreements will be put in place between GOSH, GSTT and KCL before any transfers take place.

Consent for optional samples will be sought from participants using the PIS and ICF. Samples will be labelled with the participant ID and initials so that the participant cannot be identified. Samples will either be destroyed at the end of the study or, may be used in future research studies with the participant's consent.

Comparison analysis may be performed with samples from a tissue bank from control patients for some of the samples collected from the study patients.

All samples will be processed, tracked and stored according to Human Tissue Act 2004 and the EU Directive Guidelines.

7.7 Research samples

Additional blood samples will be collected at different time-points to assess whether the Tregs have affected any of the immune responses in these patients. In detail, these samples will be collected at the following study visits (**Table 8**):

- Day of Transplant
- Transplant Day 1 (day after transplant)
- Transplant Follow-up Day 14 (14 days after heart transplant)
- Transplant Follow-up 3 Month (3 months after heart transplant)
- Baseline Day 1 (Day after the infusion with TR006)
- Safety Follow-up Day 14 (14 days after infusion with TR006)
- Safety Follow-up Day 28 (28 days after infusion with TR006)
- Safety Follow-up Month 3 (3 months after infusion with TR006)
- Safety Follow-up Month 6 (6 months after infusion with TR006)
- Safety Follow-up Month 9 (9 months after infusion with TR006)
- Safety Follow-up Month 12 (12 months after infusion with TR006)
- End of Study Follow-up (24 months after infusion with TR006)

Blood collected during the planned visits will be used for the following tests:

a) Immunophenotyping in the blood:

We will use spectral flow cytometry to assess the frequency of circulating leukocyte subsets in peripheral blood employing a new panel including up to 38 markers. This will be performed on fresh blood samples (3mL of blood) collected at specific time points. We will target CD8+, B and NK cells. We will fully characterize CD4+ lymphocytes focussing on the main Treg subsets described in the literature. Phenotypic markers of naïve versus memory, regulatory and effector, senescent/exhausted cells will be evaluated as well. All the data will be correlated with WBC count routinely executed in the clinic.

b) Evaluation of donor-specific responses:
We will employ an activation-induced marker assay as previously done in the Thril trial that simultaneously quantifies alloantigen-specific conventional T cells, Tregs and CD8+ through the co-expression of CD69, CD137 and CD154 molecules [11]. This will be accomplished through the use of surrogate donor and third-party PBMCs.

c) Evaluation of tissue resident T cells:
To evaluate tissue infiltrating cells and how Tregs modulate them after infusion we will collect biopsies (leftover from routinely collected tissue in the clinic) before and after Tregs infusion. We will employ both bulk RNASeq on cryopreserved tissue and employ the Nanostring GeoMx platform to conduct spatial protein/transcriptomics analysis of the endomyocardial biopsy (in collaboration with Dr Fadi Issa from Oxford, who has successfully characterized infiltrating Tregs in kidney biopsy following adoptive Treg transfer as part of the One Study).

d) Transcriptomic analysis:
PBMCs will be collected at specific time points to assess how Treg infusion modulates the transcriptomic profile at single cell level of the circulating T cells.

e) Evaluation of cytokines in serum:
Blood will be collected at specific time points to assess how Treg therapy modulates the circulating level of pro and anti-inflammatory cytokines. We will employ a commercially available MSD Kit multiplexing cytokines favouring organ rejection (IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, IL-13, TNF-α).

7.8 Treatment duration

This is a single infusion treatment.

7.9 Post treatment follow-up

Participants will be followed-up for 24 months post-treatment.

7.10 Discontinuation/Withdrawal of Participants from Trial Treatment

Each participant has the right to withdraw from the trial at any time. In addition, the Investigator may discontinue a participant from the trial at any time if the Investigator considers it necessary for any reason.

The participants and the parents/guardians of the participants who wish to withdraw from the study will be asked to confirm whether they are still willing to provide data collected as per routine clinical practice at visits. Their follow-up clinical care will be as per routine practice.

If the participant is withdrawn due to an adverse event, the Investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised.

If there is a significant reaction to the infusion of cells the infusion will be stopped and the patient will be withdrawn from the study.

If a patient has significant rejection (ISHLT grade III or above), then they will be treated in conjunction with the clinical team's usual practice. [38] This will likely include steroid medicines and potentially other agents as per GOSH policy. They will remain under careful follow up.

In the event that the participant withdraws from the trial, the appropriate withdrawal page in the eCRF should be completed. On the withdrawal page, the Investigator should record the date of the withdrawal, the person who initiated withdrawal and the reason for withdrawal. If a participant is withdrawn from the trial, data already collected for that participant prior to withdrawal will still be analysed and this will be made clear in the PIS and ICF.

7.11 Definition of End of Trial

The end of trial will be defined as the database being locked following all participants having completed their final follow-up visit, the data from all visits entered on the database and all queries resolved.

All SAEs will be followed up for a further 30 days after the last participant's final follow-up visit date or until resolution.

8 Treatment Regimens

The thymus (used to make TR006) will be collected from study participants at GOSH on the Day of Transplant (surgery) study visit. The Investigator (or the person to whom the Investigator has delegated this responsibility to) will attend the cardiac surgery and collect the thymus tissue explanted by the surgical team using aseptic technique. The thymus tissue will be put into a sterile pot containing MACS tissue storage solution. As the surgery may occur unpredictably out of hours, the tissue may need to be stored in a temperature-controlled fridge (at 2-8°C) prior to the CRF GMP Unit at Guy's and St Thomas' NHS Foundation Trust for processing. The thymus starting material may be need to be stored at GOSH for up to 48 hours prior to transfer to the CRF GMP Unit. Transport of the starting material will be carried out at 2 - 8°C in a validated transport box with a data logger that monitors the storage temperature.

Once the thymus is ready to be processed, initially, the thymus is mechanically digested to obtain a cell suspension of thymocytes. CD3+CD4+CD8-CD25+ T cells are isolated by depleting CD8+ cells using immunomagnetic beads (CD8 microbeads, Miltenyi Biotec) followed by CD25 enrichment (CD25 microbeads, Miltenyi Biotec). Isolated Tregs are expanded polyclonally using αCD3/αCD28 microbeads (1:1 bead:cell ratio, CTS Dynabeads Treg Xpander, ThermoFisher Scientific) for up to 23 (+/- 1) days in the presence of rapamycin (100nM) and IL-2 (1000 IU/ml).

The resulting product lacks of CD8 positive cells and is highly enriched in CD4+CD25+CD127-FOXP3+ cells. In addition, the product expresses a high level of CTLA4, CD62L, CXCR4 and Helios while homing receptors like CCR6, CCR7, CXCR3 and functional markers like CD39 are poorly expressed.

TR006 will be packed in a 10mL CryoMACS Freezing Bag (CE-Marked medical device).

8.1 Dosage schedules

Eligible patients will receive a single dose of TR006 at 1-3 x 10^6 TR006/Kg or at 5-10 x 10^6TR006/Kg.

Patients will be dosed at Great Ormond Street Hospital.

8.2 Route of Administration

The TR006 IMP will be thawed by the study team at GOSH, and the thawed 10 mL (1-3 x 10^6 TR006/Kg or at 5-10 x 10^6TR006/Kg) IMP bag will be spiked to a giving set for infusion. The procedure will be detailed in the TR006 Handling Instructions.

After the TR006 has been administered to a participant, up to 10mL of saline solution (equivalent to the volume of TR006 dose) will also be used to flush the dose to make sure that all the cells are given to the patient.

The infusion will be administered intravenously by a suitably trained and delegated member of the study team.

8.3 Maximum dosage allowed

5-10 x 10^6TR006/Kg is the highest dose being evaluated in this trial.

8.4 Maximum duration of treatment of a subject

The participants will only receive the treatment once at Baseline (3-6 months post-surgery).

The infusion should take around 30 minutes to complete for a single dose.

8.5 Dosage modifications

If the required dose cannot be manufactured the patient may have to be dosed at a lower dose than the dose originally planned. In such a situation, the Data Monitoring Committee and Statisticians should be informed.

8.6 Drug production

Please refer to IMPD for the full product specification and manufacturing process.

TR006 will be manufactured at:
CRF GMP Unit
15th Floor Guy's Tower
Guy's Hospital
Great Maze Pond
London
SE1 9RT

Site licence number: 11387. Site ID number: 6280106

The starting material, thymocytes, will be obtained from discarded thymus for each patient during surgery or during the time of having a pump assisted device fitted in (this can be up to 1 year pre-surgery). In order to isolate bulk Tregs, thymocytes will be depleted from the CD8+ cells and the remaining fraction will be enriched for CD25. After isolation, enriched Tregs will be activated with αCD3/αCD28 microbeads (1:1 beads to cell ratio) and cultured in the presence of IL-2 (1000IU/mL) and rapamycin (100nM).

The manufacturing process will take between 10-23 (+/- 1) days and cells will go through (up to) three rounds of stimulations (Day 0, Day 10+/-1 and Day 17+/-1). Once the cells have been harvested, activation beads will be removed and the final product will be formulated to the correct cell concentration for the patient dose in CryoStor CS10 Cell Freezing Medium and frozen in a cryoprotective freezing bag.

8.7 Drug storage

Once manufacture is completed, TR006 will be transferred to a controlled rate freezer, before transfer to the vapour phase of a liquid nitrogen storage tank (-150°C), where it will be stored prior to administration to the patient. The bag will be labelled with appropriate labels for the defined storage conditions.

Once the patient is ready to receive the TR006 IMP, it will be shipped in a liquid nitrogen vapour phase dry shipper to GOSH and then thawed by the study team at GOSH immediately prior to administration.

8.8 Known drug reactions

As TR006 has never been extensively tested in humans before, there is currently no available list of medical events/reactions that are expected for the ATIMP. Hence any serious adverse events that are deemed related to the ATIMP (SARs) will be considered SUSARs.

8.9 Concomitant Medications

Data will be collected relating to concomitant medications at every visit. Concomitant medication covers all medications and significant non-drug interventions. A complete history of medication taken by the participant will be recorded in the relevant Concomitant Medication Log/electronic Case Report Form at screening.

All concomitant medications (including over-the-counter or prescription medicines, vitamins and/or herbal supplements) and the doses taken by a patient during their participation in the trial must be recorded in the relevant section of the Concomitant Medication Log/electronic Case Report Form.

Participants in this trial will receive the local standard treatment for those undergoing a heart transplant. This encompasses pre-surgery medication, general anaesthesia, recovery from anaesthesia, pain control medication, and anti-infective therapy (viral, fungal and bacterial).

Immunosuppression therapy should be given as per local standard protocol. Typically after transplant, the below immunosuppressive medications (in **Table 9**) are prescribed and will usually be routinely taken by all patients. Depending on each patient's clinical course and treatment plan, there may be variations of the dosing schedule and/or drug choice.

Table 9: Routine Post-Transplant Immunosuppression Therapy:

Drug	Regime	Dose
Basiliximab	• First dose within two hours prior to organ implantation. • Second dose on day 4 post-transplant.	• <35kg: 10mg iv slow bolus over 3-5 minutes. • >35 kg: 20 mg iv slow bolus over 3-5 minutes.
Corticosteroids	• IV methylprednisolone - 15 minutes prior to cross clamp release (peri-operative). • IV methylprednisolone given in intensive care post-operatively during recovery for first few days until orally taking medication when switched to oral prednisolone.	• Peri-operative dose 15mg/kg iv in theatre. • In intensive care post op: ▪ Day 1: 10mg/kg iv daily in the morning. ▪ Day 2: 2mg/kg iv daily in the morning. ▪ Day 3: 2mg/kg iv daily in the morning. ▪ Day 4: 1 mg/kg iv daily in the morning and continue until switched to oral medication. • When able to take oral medicines (and tacrolimus is in range) convert to oral prednisolone (1:1 on mg basis): once

		daily in the morning. • If no signs of rejection: aim to wean to 0.1 to 0.15mg/kg daily by discharge or 4 weeks. Wean off before 3 month biopsy date if no previous rejection.
Tacrolimus	Tacrolimus orally / nasogastric post operatively. Usual start dose: 0.05mg/kg (max 2mg initially) orally twice a day. Take trough level in the morning before second or third dose. Target range is 10-15 ng/mL immediately post-operation.	Tacrolimus continued orally post-operation (lifelong) as per blood levels (depending on time scale reached post- transplant): ▪ 0-6 months: 10 - 14ng/mL ▪ 6-12 months: 8 - 12ng/mL ▪ From 1 year: 5 - 8ng/mL
Mycophenolate Mofetil (MMF)	Mycophenolate mofetil: iv 600mg/m2 twice a day. First dose immediately post-operation.	When tolerating oral medications, patients will continue oral dosing for 600mg/m^2 (rounded up to the nearest whole tablet). This is remains a long term immunosuppressant medication unless clinical indication to reduce or stop as per Transplant team advice). • For children < 2 years old mycophenolate mofetil reduced to 300mg/m^2 orally twice a day when tacrolimus stable in range.

Alternative immunosuppressants (such as Sirolimus, Ciclosporin, Azathioprine and Anti-thymocyte globulin (Thymoglobuline®) infusion) may be considered in specialised treatment schedules to prevent organ rejection. This may be done at the discretion of the local Transplant Team in specific clinical scenarios. These are not first line treatments, therefore, the required dosing schedules have not been extensively detailed here and local SOPs will be consulted if these are deemed necessary.

As per the standard of care oral medications for post-heart transplant patients at GOSH, additional medications may be recommended to manage traditional cardiac risk factors and to also reduce the risk of opportunistic infections. This is summarised below (in **Table 10**) and, where required, will be maintained for the study participants:

Table 10: Routine Post-Transplant Therapy:

Indication	Drug and dose
Hypertension	Options of agents as below. Usually one agent is commenced to maintain blood pressure target range for age. • Amlodipine 0.1 - 0.2mg/kg (max 0.4mg/kg or 10mg) orally daily. If no renal impairment and persistent high blood pressure: • Captopril initially 0.1mg/kg orally tds increasing to 1mg/kg tds as tolerated (and adult hypertension dose 12.5 - 25mg tds) For older children: • Enalapril initially 0.05 mg/kg bd.
Dyslipidaemia and CAV	• No pravastatin under 1 year.

	• Pravastatin (if above 1 year old): ▪ 1-2 years: 2.5 to 5mg orally at night. ▪ 2 – 12 years: 5 to 20mg orally at night. ▪ >12 yearrs: 20 to 40mg (max 40mg) orally at night.
Cardiac Allograft Vasculopathy	Aspirin: 5mg/kg (max 75mg) orally daily with food.
Prophylaxis of Pneumocystis carinii Prophylaxis of Toxoplasmosis gondii	• Co-trimoxazole: all doses are given orally twice daily on Mondays, Wednesdays and Fridays (life-long): ▪ <4kg: 30mg/kg/day in 1 or 2 doses ▪ 4 -5kg: 60mg bd ▪ 6 -10kg: 120mg bd ▪ 11 -40kg: 240mg bd ▪ >40kg: 480 mg bd For the prophylaxis of Toxoplasmosis gondii in donor positive / recipient negative transplants, co-trimoxazole is given at the same above dose twice a day every day for 6 months.
Prophylaxis of Herpes Simplex virus	• Aciclovir orally: ▪ 1 month - 2 years: 100mg 8-hourly. ▪ 2 years - 18 years: 200mg 8-hourly. Aciclovir is started when renal function is adequate and able to tolerate oral medication. Prophylactic doses are given for 3 months. If infection occurs, change to intravenous aciclovir 500mg/m2 tds.
Symptomatic or (high viral titre CMV) infection	Ganciclovir or Valganciclovir: • Ganciclovir: IV 5mg/kg 12 hourly for 7-14 days then maintenance 5mg/kg once daily. • Valganciclovir: Dosing as per product literature and local protocols, guided by drug monitoring.

Anaesthesia related medication will not be regarded as concomitant medication.

Prohibited medications:
Other experimental drug therapies will not be allowed for the duration of the trial.

Of note, there are medications and vaccinations that are contraindicated for transplant patients because of interactions with tacrolimus immunosuppression (detailed in the British National Formulary for Children). [40]

8.10 Management of Cytokine Release Syndrome (CRS)

Every participant that is due to receive the TR006 infusion will receive a dose of paracetamol and chlorphenamine maleate 30 minutes before the infusion.

The dosing schedules (Table 11 and
Table 12) are included below (adapted from British National Formulary for Children - BNFC) and will also be prescribed on the child's local medical electronic chart to give (as required) after pre-medication to manage any symptoms. [41] [42]

Table 11: Paracetamol Pre-medication Regime:

Age Range of child	Dose of Paracetamol (Can be given every 4-6 hours, max 4 doses per day)
6-23 months	120 mg
2-3 years	180 mg
4-5 years	240 mg
6-7 years	240-250 mg
8-9 years	360- 375 mg
10-11 years	480- 500 mg
12-15 years	480-750 mg
16-17 years	0.5- 1 g

Table 12: Chlorphenamine Pre-medication Regime:

Age Range of child	Dose of Chlorphenamine
1-23 months	1mg up to twice daily
2- 5 years	1mg every 4-6 hours
6- 11 years	2 mg every 4-6 hours
12-17 years	4 mg every 4-6 hours

Continuous monitoring and clinical checking of the patient during and immediately after the TR006 infusion will be carried out during the Baseline Day 0 visit. Clinical status updates and management will be discussed and coordinated between the study team (delegated clinicians, physicians and the Chief Investigator) as well as with the on-call, on-site clinical teams at Great Ormond Street Hospital.

The following clinical features may alert the study team of possible CRS:

- Fever usually persistent > 38°C.
- Hypotension: Assessed relative to baseline values (may be systolic or isolated diastolic causing widening of pulse pressure.
- Hypoxia.
- Rash.
- Coagulopathy.
- Organ dysfunction including cardiac, renal, hepatic or respiratory.
- Derangement in vital signs (for example: increased heart rate, respiratory rate and/or elevated PEWS score).

Investigations to be considered if CRS is suspected (and further may be considered as clinically indicated):

- Haematology profile (including Full Blood Count and blood film).
- Coagulation profile (including D dimers and fibrinogen).
- Serum biochemistry (including renal, liver, bone profile).
- CRP.
- Ferritin.
- Blood, urine and stool cultures.
- Nasopharyngeal aspirate for bacteria and viruses.
- Chest X-ray / CT chest.
- Transthoracic echocardiogram.
- Daily assessment of neurological and cognitive function with Glasgow Coma Scale, gait assessment and age appropriate cognitive test (if possible).
- Cytokine analysis (if feasible: ideally at onset of CRS symptoms and one week after).

The study team may maintain close liaison with the Critical Care Outreach Team for further clinical support if required.

On suspicion of CRS, features will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) grading system (**Table 13**) as shown below: [37]

Table 13: Chlorphenamine Pre-medication Regime:

Immune system disorders					
CTCAE Term	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Cytokine release syndrome	Fever with or without constitutional symptoms	Hypotension responding to fluids; hypoxia responding to <40% O2	Hypotension managed with one pressor; hypoxia requiring ≥ 40% O2	Life-threatening consequences; urgent intervention indicated	Death
Definition: A disorder characterized by fever, tachypnea, headache, tachycardia, hypotension, rash, and/or hypoxia caused by the release of cytokines. **Navigational Note:** Also consider reporting other organ dysfunctions including neurological toxicities such as: Psychiatric disorders: Hallucinations or Confusion; Nervous system disorders: Seizure, Dysphasia, Tremor, or Headache					

Based on the severity of the clinical features during presentation, assessment and treatment for CRS will be initiated as follows (**Table 14**):

Table 14: CRS Management:

CRS Grade	Symptom or Sign	Management
1	Persistent fever with or without constitutional symptoms.	• Paracetamol for fever. • Ibuprofen 2nd line if not contraindicated. • Assess for infection with blood and urine cultures and chest x-ray. • Empiric broad spectrum antibiotics to cover for infection. • Maintenance IV fluids for hydration if patient unable to meet this requirement orally. • Symptomatic treatment of constitutional symptoms, as required.
2	Hypotension. (Consider if >20% drop in systolic or diastolic reading from baseline)	• IV fluid bolus with 10-20ml/kg of normal saline (0.9% sodium chloride). • Can give further fluid bolus if BP remains low. • Consider IV tocilizumab (anti-IL6 therapy)* for the treatment of hypotension that is refractory to IV fluid boluses. • Early involvement of Critical Care Outreach Team. • If hypotension persists despite IV fluid boluses and anti IL6 therapy: Perform transthoracic echocardiogram and start vasopressors with consideration of more invasive methods of haemodynamic monitoring. • If hypotension persists beyond 24 hours of anti-IL6 therapy: consider starting IV methylprednisolone+ and continue until symptoms have improved (for example: Grade 1 CRS or below; after which dose can be tapered over 3 days). • Manage fever and constitutional symptoms as in Grade 1 CRS.
	Hypoxia Need oxygen to maintain saturations >90%	Supplementary oxygen via nasal cannula (low flow up to 6L/min) or facemask (high flow).
	Organ toxicities	Symptomatic management as required clinically as per standard guidelines depending on system affected.
3	Hypotension	• IV fluid boluses as indicated for Grade 2 CRS. • Early involvement of Critical Care Outreach Team.

		• Consider IV tocilizumab* as for Grade 2 CRS (if not already initiated). • Vasopressors, as needed. • If hypotension persists despite IV fluid boluses and anti-IL6 therapy, consider transfer to intensive care, perform transthoracic echocardiogram, start vasopressors and initiate methods of invasive haemodynamic monitoring. • Close monitoring of patients receiving IV fluid boluses with strict monitoring input and output fluid balance. Repeat boluses may be considered if no improvement of blood pressure or signs of poor peripheral perfusion (e.g. capillary refill time >2 seconds or urine output <1ml/kg/hour). • If no response to 2 doses of tocilizumab: consider initiating IV methylprednisolone[+] and continue until symptoms have improved and when they are Grade 1 CRS or below, dose can be tapered over 3 days. • Manage fever and constitutional symptoms as in Grade 1 CRS.
	Hypoxia	Supplementary oxygen via facemask (high flow) and consider if escalation of respiratory support is required (such as mechanical ventilation).
	Organ toxicities	Symptomatic management as required clinically as per standard guidelines depending on system affected.
4	Hypotension	• IV fluids, consider anti IL6 therapy, inotropic support and transfer to intensive care. • Echocardiogram and initiate invasive haemodynamic monitoring as in CRS Grade 3. • IV methylprednisolone[+] to start if no response to IV tocilizumab: Continue until symptoms are Grade 1 CRS or below, after which dose can be tapered over 3 days. • For life threatening or CRS that is unresponsive to the measures given above: Higher doses of corticosteroids may be considered e.g. IV Methylprednisolone 10mg/Kg as well as further therapies as guided by the transplant, intensive care and specialist immunology teams.
	Hypoxia	Mechanical ventilation may be required in addition to supplementary oxygen delivery.
	Organ toxicities	Symptomatic management as required clinically, as per standard guidelines depending on system affected.

*Intravenous tocilizumab (anti IL6 therapy) dose for children with body weight up to 30kg: 12mg/kg (max per dose 800mg) and IV Tocilizumab dose for bodyweight >30kg: 8mg/kg (max per dose 800mg). Doses can be repeated up to 3 times a day.
[+]Intravenous methylprednisolone: Initial dose 2mg/kg followed by 1mg/kg IV twice a day.
Equivalent doses of other corticosteroids (eg dexamethasone) may be used if associated neurotoxicity. Dexamethasone 10mg IV stat (or 1mg/kg for children under 10 kg) followed by Dexamethasone 6mg IV (or 0.5mg/kg for children under 10 kg) if associated neurotoxicity.

<u>Triggers for urgent intensive care transfer:</u>

If CRS is suspected, the above are guidelines only and the study team will discuss the participant's case with the on-call Paediatric Intensive Care Consultant and the Immunology Lab to guide monitoring and further treatment for each individual case. The following should be considered key triggering factors for the transfer of the patient to intensive care:

- Hypotension refractory to fluid boluses whether or not Tocilizumab has been commenced.
- Oxygen requirement >6L/min (approximately 40% FIO2).
- Need for other organ support (such as renal failure).

8.11 Non-Investigational Medicinal Products (NIMPs)

Medications that will be given as prophylaxis prior to TR006 administration are commercially available and routinely prescribed to prevent potential infusion reactions. All participants will be given the following medications prior to infusion:

- Chlorphenamine or equivalent anti-histamine, administered orally (See
- **Table 12**).
- Paracetamol, administered orally (See **Table 11**).
- The Trust guidelines/Ward SOPs will be followed in the event of a cytokine release syndrome (CRS) for participants dosed with TR006 in Walrus Ward (See **Section 8.10 Management of Cytokine Release Syndrome (CRS)**).

The above medications will be prescribed in line with the British National Formulary for Children (BNFC) at doses appropriate to the patient's age and/or weight. These medications are designated as NIMPs (Non-Investigational Medicinal Products). They will be dispensed from the Trust pharmacy stock and according to the local hospital guidelines. All the above have marketing authorisation and are routinely used in accordance with their license.

8.12 Participant Withdrawal Criteria

Participants have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw patients from the study in the event of inter-current illness, AEs, SAE's, SUSAR's, protocol violations, cure, administrative reasons or any other reasons deemed appropriate. It is understood by all concerned that an excessive rate of withdrawals can render the study un-interpretable; therefore, unnecessary withdrawal of patients should be avoided. Should a patient decide to withdraw from the study, all efforts will be made to report the reason for withdrawal as thoroughly as possible.

As the IMP in this trial is an advanced therapy with a single infusion, participants who wish to withdraw from trial after IMP has been administered, will be asked to confirm whether they are still willing to attend the safety follow up visits as per protocol for the duration of the trial. If participants are not willing to do this, consent will be sought for the trial team to access routine data collected from hospital/GP visits for the duration of the safety follow up period.

In situations where a patient has to be withdrawn or decides to withdraw from the study, an additional patient may be recruited and dosed if:

- They withdraw prior to the TR006 infusion.
- They withdraw during their 4-week follow-up period, and have not yet experienced a DLT.

If a patient withdraws from the trial a withdrawal form in the study database will be completed.

The DMC will review all patients, including any withdrawn patients, to determine if a primary outcome has occurred (DLT, no DLT or DLT not determined in the active period).

In the following situations, the withdrawn patient will have contributed to the primary outcome and no additional patient will be recruited:

- If they withdrew after they completed their 4-week follow-up safety period.
- If they experience a DLT during their 4-week follow-up safety period, which may or may not be the reason for withdrawal.

9 Assessment of Safety

Definitions

9.1.1 Adverse event

Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product.

9.1.2 Adverse Reaction (AR) of an Investigational Medicinal Product

An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant.

The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.

9.1.3 Serious Adverse Event or Serious Adverse Reaction

A serious adverse event is any untoward medical occurrence that:

- results in death.
- is life-threatening.
- requires inpatient hospitalisation or prolongation of existing hospitalization.
- results in persistent or significant disability/incapacity.
- consists of a congenital anomaly or birth defect.

Other ‘important medical events’ may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

9.1.4 Serious Adverse Reaction (SAR)

An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.

9.1.5 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out:

- in the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product.
- in the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question.

9.1.6 Important Medical Events (IME) & Pregnancy

These are defined as medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definitions above (in **Section 9.1.3 Serious Adverse Event or Serious Adverse Reaction**) and such medical events should also be considered serious.

Although not a Serious Adverse Event, any unplanned pregnancy will also be reported via the SAE reporting system (as listed in **Section 9.4 Reporting Procedures for Serious Adverse Events**). Pregnancy reporting includes pregnancy of a female participant or a female partner of a male participant. Should a trial participant become pregnant during the trial or female partners of male participants who become pregnant, she will be followed up for safety until the birth of the child or until Safety Follow-up Month 24 (whichever is latest).

9.2 Expected Adverse Drug Reactions and Serious Adverse Events

As TR006 has never been tested in humans before, there is currently no available list of medical events/reactions that are expected for the ATIMP. Hence any serious adverse events that are deemed related to the ATIMP (SARs) will be considered SUSARs.

9.3 Recording and evaluation of adverse events

AEs (including SAEs) will be recorded from the Screening Visit to Month 12 visit. From Month 12-24 safety follow-up period, only SUSARs will be reported.

As stated in **Section 5.1**, DLTs will be assessed from week 0 to week 4 of the study.

9.4 Reporting Procedures for Serious Adverse Events

All SAEs (other than those defined in the protocol as not requiring reporting) must be reported on the SAE reporting form to R&D within 24 hours of the Site Study Team becoming aware of the event. The event will be recorded in the sponsor's SAE form (F44) and emailed to CTIMP.safety@gosh.nhs.uk or faxed to the Joint R&D office (0207 905 2201) within the reporting timelines. R&D will perform an initial check of the report, request any additional information if required. Additional and further requested information (follow-up or corrections to the original case) will be detailed on a new SAE Report Form and faxed/emailed to R&D.

AEs (including SAEs) will be recorded from the Screening Visit to Month 12 visit. From Month 12-24 safety follow-up period, only SUSARs will be reported.

As stated in **Section 5.1**, DLTs will be assessed from Week 0 to Week 4 of the study.

9.4.1 Assessment of severity

Mild: The subject is aware of the event or symptom, but the event or symptom is easily tolerated.

Moderate: The subject experiences sufficient discomfort to interfere with or reduce his or her usual level of activity.

Severe: Significant impairment of functioning; the subject is unable to carry out usual activities and / or the subject's life is at risk from the event.

9.4.2 Assessment of causality

Probable: A causal relationship is clinically / biologically highly plausible and there is a plausible time sequence between onset of the AE and administration of the investigational medicinal product and there is a reasonable response on withdrawal.

Possible: A causal relationship is clinically / biologically plausible and there is a plausible time sequence between onset of the AE and administration of the investigational medicinal product.

Unlikely: A causal relation is improbable and another documented cause of the AE is most plausible.

Unrelated: A causal relationship can be definitely excluded and another documented cause of the AE is most plausible.

9.4.3 Expectedness

Expectedness will be determined according to the Investigators' Brochure.

An adverse reaction, the nature, or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved investigational product or Summary of Product Characteristics (SmPC) for an authorised product).

When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected.

9.5 Reporting of Suspected Unexpected Serious Adverse Reactions (SUSARs)

All suspected adverse reactions related to an investigational medicinal product (the tested IMP and comparators) which occur in the concerned trial, and that are both unexpected and serious (SUSARs) are subject to expedited reporting.

9.5.1 Who should report and whom to report to?

The sponsor should report all the relevant safety information previously described to the concerned competent authorities and to the Ethics Committee concerned. The sponsor shall inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects.

9.5.2 When to report?

Fatal or life-threatening SUSARs

The MHRA and the Research Ethics Committee should be notified as soon as possible but no later than 7 calendar days after the sponsor has first knowledge of the minimum criteria for expedited reporting.

In each case relevant follow-up information should be sought and a report completed as soon as possible. It should be communicated to the MHRA and the Ethics Committee within an additional eight calendar days.

Non-fatal and non-life threatening SUSARs

All other SUSARs and safety issues must be reported to the competent authority and the Ethics Committee in the concerned Member States as soon as possible but no later than 15 calendar days after the sponsor has first knowledge of the minimum criteria for expedited reporting. Further relevant follow-up information should be given as soon as possible.

Follow-up reports of SUSARs

In case of incomplete information at the time of initial reporting, all the appropriate information for an adequate analysis of causality should be actively sought from the reporter or other available sources. The sponsor should report further relevant information after receipt as follow-up reports.
In certain cases, it may be appropriate to conduct follow-up of the long-term outcome of a particular reaction.

9.6 Development Safety Update Reports

The sponsor will submit (in addition to the expedited reporting above) a DSUR once a year throughout the clinical trial, or on request, to the Competent Authority (MHRA in the UK), Ethics Committee and the Host NHS Trust (if applicable).

9.7 Procedures used to assess participant safety

The research team will perform various clinical tests and procedures to assess participant safety during the whole duration of study. These include the following:

Medical history, Adverse Event review and Concomitant Medication review:
Systematic history will be taken from participant (and from the Parent/Guardian of the participant where necessary) to record any new and significant medical events and to review existing recorded medical history. Symptoms related to all body systems will be enquired about. Travel history, unwell contacts and allergy information will be recorded where appropriate. A complete listing of all concomitant medication received during the trial must be recorded in the relevant Concomitant Medication Log/electronic Case Report Form.

Remote review:
Telephone review conducted by a delegated study physician with full discussion with patients (and/or parents/guardians where appropriate) where any symptoms related to all body systems will be enquired about. As per the standard study review: medical history will be revisited and reviewed, An adverse events collection and concomitant medication review will be undertaken over the phone

and recorded in the relevant Adverse Event Log/Concomitant Medication Log/electronic Case Report Form. Travel history, unwell contacts and allergy information will be recorded where appropriate.

Physical examination:
Performed by a delegated study physician to include routine examination of the following systems:

- Respiratory system (including visual inspection of ear drum and throat).
- Cardiovascular system.
- Gastrointestinal system (including palpation for lymphadenopathy).
- Neurological system.
- Skin and wounds check.
- Height (cm), Weight (Kg) and Body Surface Area (recorded as per protocol requirements).

Vital signs checks:
Performed by an appropriate trained and delegated clinician to measure:

- Heart rate (beats/min).
- Respiratory rate (breaths/min).
- Oxygen saturations (%).
- Blood pressure (mmHg).
- Temperature (°C).

The normal values will depend on age of child and the following table can be referred to as a guide (Table 15) using the Advanced Paediatric Life Support Ranges [43]:

Table 15: Normal Vital Signs in Children:

Normal Vital Signs in Children (APLS Values)					
	Infant	**1-2 years**	**2-5 years**	**5-12 years**	**Adolescent**
Pulse rate (bpm)	110-160	100-150	95-140	80-120	60-90
Respiratory rate (rpm)	30-40	25-35	25-30	20-25	14-18
Blood pressure Systolic mmHg	80-90	85-95	85-100	90-100	100-140
Temperature (°C)	35-37	35-37	35-37	35-37	36-37.5
Saturations (%)	94-98				

ECG:
Performed by an appropriate trained and delegated clinician to measure:

- Ventricular rate.
- Rhythm.
- QRS axis.
- QRS duration.
- Corrected QTC interval.

Comparison with previous ECG in medical records to occur where appropriate.

Transthoracic Echocardiogram:
Performed by an appropriate trained and delegated clinician to measure:

- Standard Transthoracic Echocardiogram views for cardiac structures and anastomoses sites from apical, parasternal long axis, parasternal short axis and suprasternal windows.
- Systolic functional assessment: Left Ventricular Ejection Fraction (LVEF) calculated by Simpson's biplane and on m-mode (Ejection Fraction by Teichholz formula).
- Right Ventricular function- comment on visual assessment and measurement of Tricuspid Annular Plane Systolic Excursion (TAPSE).
- Diastolic function parameters using tissue doppler interrogation: Mitral E wave and A wave velocities and ratio. Septal and lateral E/E' ratios and TVS' wave velocity.
- Any significant valvopathy on 2D echo and colour interrogation with doppler measurements (as required).
- The presence of pericardial effusion.
- Interval change(s) from previous tests.

Coronary vessel imaging:
The following imaging procedures are to be performed only in children above 25kg by an appropriate trained and delegated clinician:

- Coronary angiogram.
- Intravascular Ultrasound.

Blood tests:
The following tests are to be performed by an appropriate trained and delegated clinician:

- Clinical blood tests to assess: Haematology (full blood count), liver profile (albumin, alkaline phosphatase, total bilirubin and ALT levels), renal profile (sodium, potassium, creatinine and urea levels), LDH levels, EBV and CMV.
- Additional clinical blood tests to assess: bone profile, creatinine kinase (CK), CRP, ferritin, magnesium, uric acid levels and to coagulation screen.
- HLA antibody testing (includes panel reactive antibodies and donor specific antibodies)'
- Blood borne infection screen (with further details in **Table 22** for the specific diseases and markers assessed).

Additional research blood tests will be collected at various study visits (as per **Table 8**) but will not be used for the safety assessment purposes.

Endomyocardial cardiac tissue biopsy:
Performed by a delegated study physician to as part of the routine post-transplantation examination.

10 Statistics

Since this study is assessing the toxicity of only two dose levels, adaptive designs for dose escalation based on statistical modelling which accelerate through lower doses were not deemed necessary.

10.1 Statistical methods to be employed (plan of analysis)

Biometric evaluation will be performed under the responsibility of Professor Abdel Douiri. Demographic data and baseline characteristics will be summarized to describe the populations analysed in this study (both all participants and participants administered each dose level). Efficacy parameters: clinical and immunological responses will be reported descriptively based on the per-protocol population. Time to first response will be summarized by Kaplan-Meier curve. Median time to event estimation as well as the associated 95% CI will be reported. All other secondary endpoints will be summarized by rates and corresponding 95% CIs.

All safety data will be analysed descriptively. Safety data will be reviewed between each patient cohort, with interim analyses provided to the DMC in order for decisions related to the next patient cohort to be made (decisions will be based on the 3+3 dose escalation design described in trial design section).

Statistical comparisons between patient populations are unlikely to be utilised, but if needed a 5% level of significance will be used.

A Statistical Analysis Plan will be written and will be signed off prior to database lock and will provide detailed information on all statistical analyses planned for the study.

10.2 Number of Subjects to be enrolled

The study will be a Phase I trial to assess the technical feasibility of generating Tregs from the thymus in the GMP facility and most importantly their safety in the treatment of paediatric heart transplant patients.

In this study we will determine the optimal dose of injected Tregs using a single ascending dose (SAD) design, with groups of three patients. We will enrol 3 patients with the first low dose of (1-3 x 10^6 Tregs/Kg) and if safe, then another 3 will be added with the second higher dose of (5-10 x 10^6 Tregs/Kg). If the previous dose was safe, an additional 3 patients will be enrolled with the highest tolerated dose to test further the biological activity.

The first patient in the first cohort (Cohort 1) will receive the treatment and we will confirm the safety of the infused cell product following the treatment before infusing the next patient with at least a 4-week gap. After safety is confirmed for the first patient, the second and third patients within Cohort 1 will receive the same dose of treatment. A safety review will be done after the third patient to decide the dose for the next cohort (Cohort 2). The same process will be done for the next cohorts (Cohort 2 and Cohort 3). This will allow us better to capture the safety of the product and biological activity at the selected dose in a robust manner and also to provide information on the immunological effects of the Treg product.

To determine the maximum tolerated dose (MTD) of the two potential doses, a 3+3 dose escalation design will be implemented. A 3+3 dose escalation design assesses cohorts of patients for dose-limiting toxicities (DLTs) in order to make a decision about maintaining the dose level, increasing the dose level or stopping the trial using a rule base approach. Initially a cohort of 3 patients will be administered TR006 with the dose level of 1-3 x 10^6 Tregs/Kg.

The diagram below (**Figure 1**) describes the decision-making process for proceeding with the next dosing cohort based on the DLTs experienced. If a DLT occurs in the Cohort 1, the next cohort of 3 patients will be administered to the same dose. If there is no DLT in Cohort 1, another cohort of 3 patients will be administered to next higher dose. From this study design, the trial will be stopped when 2 patients out of 6 receiving the same dose level experience a DLT. At this point, either a MTD will be identified (green endpoints in diagram) or the trial will be stopped for safety (red endpoints in **Figure 1**).

Based on this study design, a sample size of between 9 and 12 patients will be needed to identify the maximum tolerated dose. The 3+3 design requires that 6 patients are dosed at the selected dose level, this is often a requirement of adaptive design approaches as well (see **Figure 1** below). These 6 patients dosed at the final selected dose level will provide useful information on haematological activity which will be expanded upon with the inclusion of additional patients.

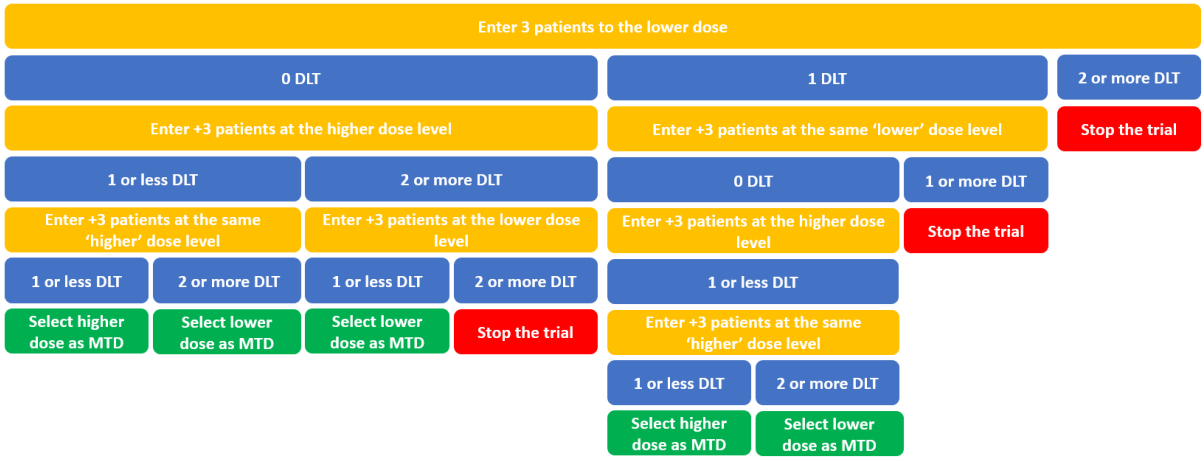

Figure 1: DLT decision-making process

A DLT is defined as grade ≥2 CRS, grade ≥2 injection site reaction, grade ≥2 fever and/or rigors, grade ≥3 bronchospasm or grade ≥3 hypoxia occurring within the first 72 hours post infusion OR a grade ≥3 infection, moderate or severe acute rejection (as defined by the ISHLT criteria grade II-III), grade ≥3 haematological complication considered to be related to Treg infusion or any grade ≥3 toxicity not clearly related to underlying disease occurring within 4 weeks post TR006 infusion (see **Section 5.1** for full definition). [**38**]

For the purposes of the primary endpoint regarding safety, any DLTs after the TR006 administration will be reported and compared.

10.3 Interim analyses

Safety data will be reviewed after dosing of the first and last patient of each cohort, with interim analyses provided to the DMC in order for decisions related to the next patient cohort to be made.

10.4 Criteria for the termination of the trial

The trial will be stopped when 2 patients out of 6 receiving the same dose level (2 cohorts) experience a DLT. At this point, either a MTD will be identified (green endpoints in **Figure 1**) or the trial will be stopped for safety (red endpoints in **Figure 1**).

10.5 Inclusion in Analysis

All eligible participants recruited will be analysed.

11 Data Management

11.1 Source Documents

Source documents are original documents, data, and records from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/code, not by name.

11.2 Direct access to source data / documents

Only members of the trial research team and authorised representatives from the sponsor will have direct access to the source data and trial documentation. All source data and trial documentation will also be available to external auditors if and when required, and inspectors in the event of regulatory inspection. Access to the final data set will remain with the Chief Investigator.

11.3 Data recording and record keeping

The Chief Investigator will act as custodian for the trial data. The following guidelines will be strictly adhered to:

- Patient data will be pseudo-anonymised.
- All trial data will be stored in line with the *Medicines for Humans Use (Clinical Trials) Amended regulations 2006* and the *Data Protection Act 2018 (and all amendments to follow).*
- All trial data will be archived in line with the Medicines for Humans Use (Clinical Trials) Amended regulations 2006 and as defined in the Sponsor's Archiving SOP (and all amendments to follow).

An electronic Case Report Form (eCRF) will be designed using the REDCap database which is fully validated and regulatory compliant. This is a web-based platform for electronic data capture. The eCRF will be designed in collaboration with the trial statisticians and trial team.

11.4 Archiving

Archiving will be authorised by the Sponsor following submission of the end of study report.
Essential documents will be retained for a **minimum** of 25 years after completion of the trial. These documents will be retained for longer if required by the applicable regulatory requirements.

For Advanced Therapy Medicinal Products:
Records related to traceability of the IMP at site along with the patient identifiers will be retained at site for at least 30 years after the expiry date of the product or longer if required by the clinical trial authorisation. This will include the relevant documentation contained in the sponsor and investigator files as well as the trial participants' medical records.

12 Oversight Committees Involved in Trial

12.1 Data Monitoring Committee (DMC)

This study will use an External Data Monitoring Committee (E-DMC).

The DMC will be responsible for on-going monitoring of the efficacy and safety of subjects in the study according to the DMC Charter. The DMC charter will detail membership and terms of reference.

In order to ensure patient safety throughout the conduct of the trial, the DMC will review and evaluate accumulated safety data, study conduct and progress. The DMC will make the decisions about the continuation, modification or termination of the study. The recommendations made by the DMC to alter the conduct of the study will be forwarded to the Sponsor for final a decision. The Sponsor will forward such decisions to regulatory authorities, as appropriate.

Outside of the planned reviews, a DMC meeting could be triggered if specific safety events occur (as stated below):

- If a DLT is observed in two patients within a dosing cohort.
- If death of a patient infused with TR006 occurs.
- Or at any other time deemed necessary by the DMC chair.

The DMC will develop its own operation procedures in consultation with the sponsor which will be documented in the DMC charter.

12.2 Trial Steering Committee

The Trial Steering Committee (TSC) will provide advice for the conduct of the trial. It will comprise an Independent Chair and at least two other members. Details of the TSC, its members and terms of reference will be described in the TSC charter.

The TSC provide overall supervision of the trial and ensure that it is being conducted in accordance with the principles of GCP and the relevant regulations. The TSC members will meet on an ad-hoc basis to discuss trial status, recruitment progress and any other relevant issues, and provide recommendations to the TMG/Sponsor.

12.3 Trial Management Group

This group is led by Professor Michael Burch, the CI for this study. The group will include the CI, statisticians, Clinical Trial Manager and representatives of the other teams involved in the delivery of the trial, including the GMP unit, laboratories and data management. The Trial Management Group will be responsible for the day-to-day management of the trial activities and will meet on a regular basis to discuss any trial related activities or issues.

12.4 Quality Control (Monitoring) and Quality Assurance (Audit)

The Sponsor is responsible for implementing Quality Control and Quality Assurance.
The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures. A Trial specific monitoring plan will be established and the trial will be monitored with the agreed plan.

Issues will be escalated to the Chief Investigator and Sponsor.

13 Ethical and Regulatory Approvals

13.1 Ethical Approval

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki, ICH Good Clinical Practice Guidelines and in accordance with the terms and conditions of the ethical approval given to the trial. A favourable opinion will be sought from the Research Ethics Committee before recruitment begins.

13.2 Regulatory Approval

A Clinical Trial Authorisation (CTA) will be sought from the MHRA before recruitment begins. The trial will be conducted at approved trial sites in accordance with the trial protocol and the terms of the CTA granted by the MHRA.

13.3 Patient Confidentiality & Data Protection

Patient identifiable data, including initials, date of birth and NHS number will be required for the registration process. The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participant ID number on the CRF and any electronic database. All documents will be stored securely and only accessible by trial staff and authorised personnel. The trial will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

Data will be stored in a secure manner and in accordance with the Data Protection Act 1998.

13.4 Serious Breaches

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the sponsor becoming aware of the breach.

A serious breach is defined as "A breach of GCP or the trial protocol which is likely to effect to a significant degree:
(a) the safety or physical or mental integrity of the subjects of the trial; or
(b) the scientific value of the trial".

In the event that a serious breach is suspected GOSH R&D must be contacted as soon as possible. GOSH R&D will notify the regulatory authority according to the SOP on 'Serious Breach Notification.'

14 Financial Information and Insurance

The clinical trial is funded by the British Heart Foundation (BHF) by means of a research grant awarded to King's College London.

Cover for negligent harm will be provided by the Great Ormond Street Hospital for Children NHS Foundation Trust through the Clinical Negligent Scheme for Trusts (CNST). No-fault compensation insurance cover for any non-negligent harm will be provided by University College London (UCL).

15 Publications Policy

All individuals who have made substantial intellectual, scientific and practical contributions to the trial and the manuscript should, where possible, be credited as authors; all individuals credited as authors should deserve that designation. It is the responsibility of the Chief Investigator and co-PI and, ultimately, the Sponsors to ensure that these principles are upheld. The status of manuscripts in preparation will be reviewed by the Chief Investigator and sponsor if requires. In all cases where journal policies permit, all investigators who contribute patients to the trial will be acknowledged.

The results of the study will be reported and disseminated as follows:

- Peer reviewed scientific journals.
- Internal report, plus possible article on Institute web pages (publicly accessible).
- Conference presentation(s).
- Written feedback to patient support groups.

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Appendix A: Trial Flow Chart

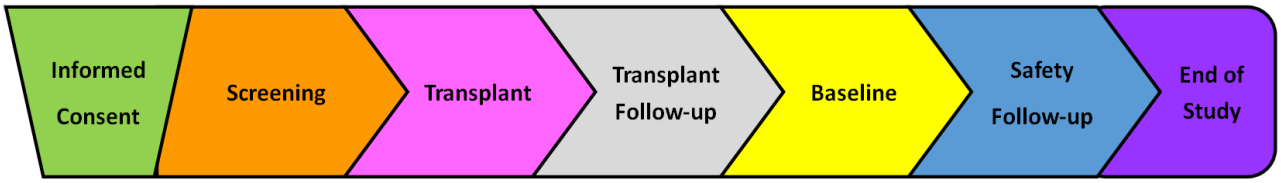

Figure 2: Trial Summary

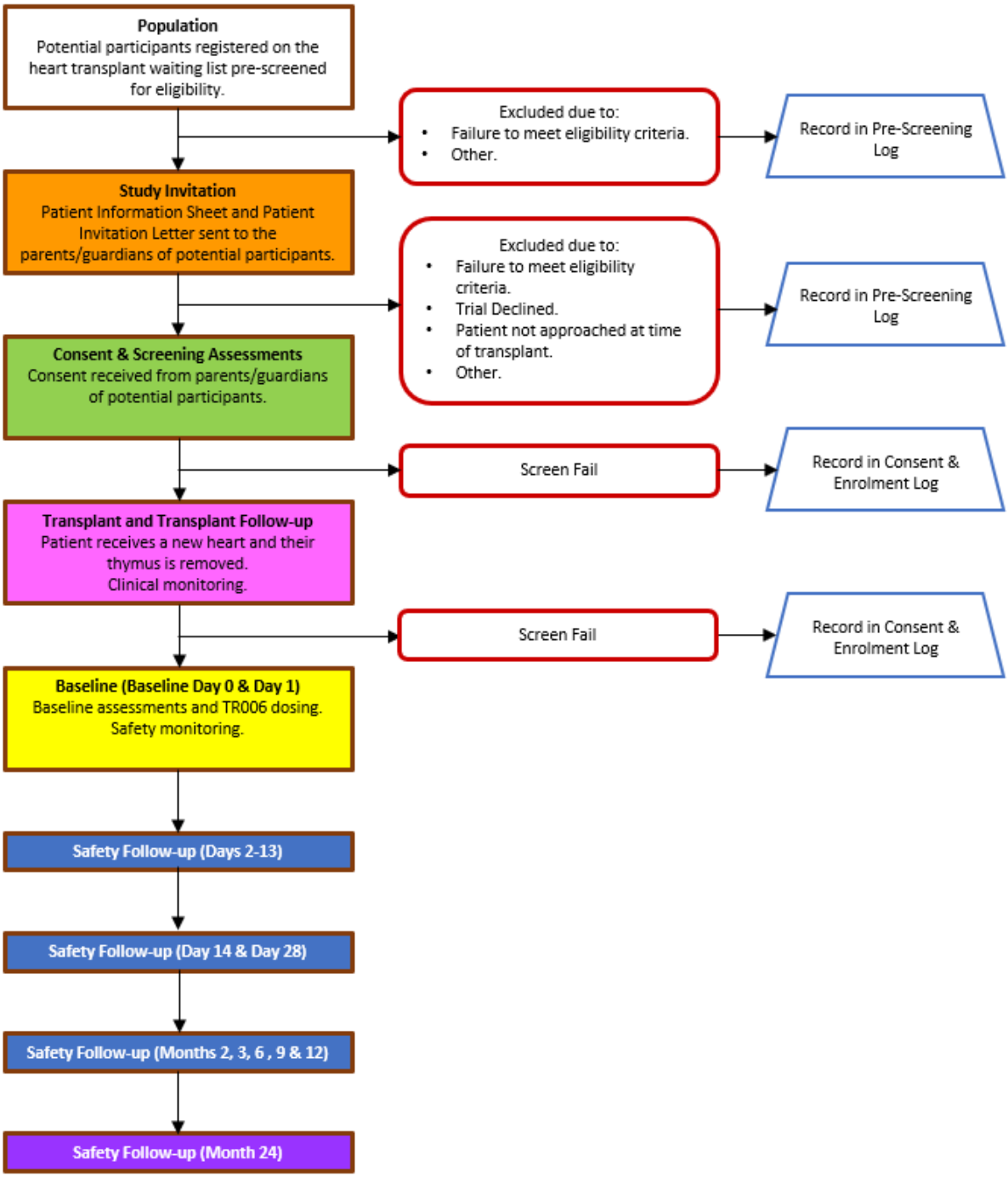

Figure 3: Trial Flowchart

Appendix B: Schedule of Procedures

Table 2: Blood Borne Infectious Diseases Information:

Disease	Serological Markers	Additional Information
HIV*	HIV antibody type 1+2	HIV antibody 1 or 2 positive suggests current infection. *Further testing of CD4 counts and viral antigen will be done if antibody positive.*
Hepatitis B*	Hepatitis B surface antigen (HBsAg) Antibody to core antigen (anti-HBc)	Positive HBsAg indicates current ongoing infection. Positive Anti-HB core indicates current or previous HBV infection. *Above is for initial screening test.* *Further testing such as hepatitis B viral PCR, hepatitis E antigen or antibody will be performed if above tests are positive* Antibody to HB surface antigen (anti-HBsAg) indicates recovery from and immunity to Hepatitis B virus *(Anti-HBsAg without anti-HBc is a marker of immunisation).*
Hepatitis C*	Hepatitis C antibody (Anti HCV)	Anti HCV antibody detected in HCV infection. *If positive, confirmatory testing performed internally for HCV virus.*
Syphilis *	Treponema pallidum (syphilis) antibody	Treponema pallidum antibody detected in positive cases of syphilis.
Human T lymphotropic virus*	HTLV I+II antibody	HTLV antibody I/II detected if positive.
Toxoplasmosis	Toxoplasma Gondii IgG antibody	Toxoplasma Gondii IgG antibody detected if positive. *If positive, further testing for toxoplasma IgM or DNA PCR in suspected infection.*
Epstein Barr Virus (EBV)	EBV IgG/IgM and Viral PCR	EBV antibody detected if positive with presence of viral DNA quantified.
Cytomegalovirus (CMV)	CMV IgG/IgM and Viral PCR	CMV antibody IgG detected with viral load quantified.

*Conditions for which if trial participants test positive, they must be excluded from the study as per the active viral infections listed in the exclusion criteria (in **Section 6.2 Exclusion Criteria**).
Testing for toxoplasmosis, Epstein Barr virus and cytomegalovirus is part of standard-of-care testing in post-transplant recipients and positivity for these will not necessarily exclude participants from the study. The Chief Investigator must be made aware of the positive cases of these infections and where appropriate the standard of care treatment can be given, or, the participant can be withdrawn from the study (if deemed necessary).

Table 3: Schedule of Events (Part 1 of 3: Screening, Transplant and Transplant Follow-up):

	Screening	Transplant		Transplant Follow-up					
	Enrolment	Day of Transplant	Transplant Day 1	Transplant Follow-up Day 14	Transplant Follow-up 1 Month	Transplant Follow-up 2 Months	Transplant Follow-up 3 Months	Transplant Follow-up 4 Months [a]	Transplant Follow-up 5 Months [a]
				+/- 1 week	+/- 1 week	+/- 1 week	+/- 1 week	+/- 3 days	+/- 3 days
Informed Consent	X	X							
Inclusion/Exclusion Criteria Review	X	X					X	X	X
Medical History (including demographic data)	X						X	X	X
Concomitant Medication Review	X	X	X	X	X	X	X	X	X
Adverse Events Review		X	X	X	X	X	X	X	X
SUSAR Review		X	X	X	X	X	X	X	X
Phone call Check-up (Doctor review)									
Doctor Review (including Physical Examination)			X	X	X	X	X	X	X
Vital Signs		X	X	X	X	X	X	X	X
Height and Weight		X		X	X	X	X	X	X
12 Lead ECG			X	X	X	X	X	X	X
Echocardiography			X	X	X	X	X	X	X
Intravascular Ultrasound							X [e]		
Coronary Angiography							X [e]		
Clinical Blood Tests [b]		X	X	X	X	X	X	X	X
Blood Borne Infectious Diseases Tests [c]	X	X					X	X	X
HLA Antibody Testing (includes panel reactive antibodies and donor specific antibodies)	X	X		X			X	X	X
Urine Pregnancy Test (for WOCP only)		X					X	X	X
Research Blood Samples		X	X	X			X	X	X
Clinical Cardiac Biopsy				X					
Cardiac Transplant		X [d]							
Thymus Sample Removal		X [d]							
TR006 Dosing									

SCHEDULE OF EVENTS KEY (FOR PARTS 1, 2 & 3)	
Key	**Description**
a	Visit is required if Baseline Day 0 cannot be carried out within 30 days of the preceding study visit. Otherwise proceed to Baseline Day 0.
b	Haematology (FBC), Liver Profile (albumin, alkaline phosphatase, total bilirubin and ALT), Renal Profile (sodium, potassium, creatinine and urea), LDH, EBV and CMV viral load (where required).
c	HIV-1/2, HBsAg, HBC, HCV, HTLV, syphilis, Toxoplasmosis Gondii IgG, EBV IgG and CMV IgG (+ viral load where positive).
d	Must occur within 24 hours of other procedures/tests performed as part of study visit.
e	Only for children over 25kg.
f	Clinical blood tests to additionally include testing for bone profile, creatinine kinase (CK), CRP, ferritin, magnesium, uric acid and coagulation screen.
g	TR006 dosing to occur 3-6 months post-cardiac transplant.

Table 4: Schedule of Events (Part 2 of 3: Baseline):

	Baseline	
	Baseline Day 0 [g]	Baseline Day 1
Informed Consent	X	
Inclusion/Exclusion Criteria Review	X	
Medical History (including demographic data)	X	
Concomitant Medication Review	X	X
Adverse Events Review	X	X
SUSAR Review	X	X
Phone call Check-up (Doctor review)		
Doctor Review (including Physical Examination)	X	X
Vital Signs	X	X
Height and Weight	X	X
12 Lead ECG	X	X
Echocardiography	X	X
Intravascular Ultrasound		
Coronary Angiography		
Clinical Blood Tests [b]	X [f]	X [f]
Blood Borne Infectious Diseases Tests [c]		
HLA Antibody Testing (includes panel reactive antibodies and donor specific antibodies)		
Urine Pregnancy Test (for WOCP only)	X	
Research Blood Samples		X
Clinical Cardiac Biopsy		
Cardiac Transplant		
Thymus Sample Removal		
TR006 Dosing	X	

SCHEDULE OF EVENTS KEY (FOR PARTS 1, 2 & 3)	
Key	**Description**
a	Visit is required if Baseline Day 0 cannot be carried out within 30 days of the preceding study visit. Otherwise proceed to Baseline Day 0.
b	Haematology (FBC), Liver Profile (albumin, alkaline phosphatase, total bilirubin and ALT), Renal Profile (sodium, potassium, creatinine and urea), LDH, EBV and CMV viral load (where required).
c	HIV-1/2, HBsAg, HBC, HCV, HTLV, syphilis, Toxoplasmosis Gondii IgG, EBV IgG and CMV IgG (+ viral load where positive).
d	Must occur within 24 hours of other procedures/tests performed as part of study visit.
e	Only for children over 25kg.
f	Clinical blood tests to additionally include testing for bone profile, creatinine kinase (CK), CRP, ferritin, magnesium, uric acid and coagulation screen.
g	TR006 dosing to occur 3-6 months post-cardiac transplant.

Table 5: Schedule of Events (Part 3 of 3: Safety Follow-up and End of Study):

	Safety Follow-up (after TR006 Dosing)								End of Study
	Follow-up Days 2-13	Follow-up Day 14	Follow-up Day 28	Follow-up Month 2	Follow-up Month 3	Follow-up Month 6	Follow-up Month 9	Follow-up Month 12	Follow-up Month 24
	+/- 2 Days	+/- 2 Days	+/- 2 Days	+/- 1 week	+/- 1 week	+/- 2 weeks	+/- 2 weeks	+/- 4 weeks	+/- 4 weeks
Informed Consent									
Inclusion/Exclusion Criteria Review									
Medical History (including demographic data)									
Concomitant Medication Review	X	X	X	X	X	X	X	X	X
Adverse Events Review	X	X	X	X	X	X	X	X	X
SUSAR Review	X	X	X	X	X	X	X	X	X
Phone call Check-up (Doctor review)	X								
Doctor Review (including Physical Examination)		X	X	X	X	X	X	X	X
Vital Signs		X	X	X	X	X	X	X	X
Height and Weight		X	X	X	X	X	X	X	X
12 Lead ECG		X	X	X	X	X	X	X	X
Echocardiography		X	X	X	X	X	X	X	X
Intravascular Ultrasound							X [e]		
Coronary Angiography							X [e]		
Clinical Blood Tests [b]		X	X	X	X	X	X	X	X
Blood Borne Infectious Diseases Tests [c]					X				
HLA Antibody Testing (includes panel reactive antibodies and donor specific antibodies)					X		X		X
Urine Pregnancy Test (for WOCP only)					X		X		X
Research Blood Samples		X	X		X	X	X	X	X
Clinical Cardiac Biopsy					X				
Cardiac Transplant									
Thymus Sample Removal									
TR006 Dosing									

SCHEDULE OF EVENTS KEY (FOR PARTS 1, 2 & 3)	
Key	**Description**
a	Visit is required if Baseline Day 0 cannot be carried out within 30 days of the preceding study visit. Otherwise proceed to Baseline Day 0.
b	Haematology (FBC), Liver Profile (albumin, alkaline phosphatase, total bilirubin and ALT), Renal Profile (sodium, potassium, creatinine and urea) LDH, EBV and CMV viral load (where required).
c	HIV-1/2, HBsAg, HBC, HCV, HTLV, syphilis, Toxoplasmosis Gondii IgG, EBV IgG and CMV IgG (+ viral load where positive).
d	Must occur within 24 hours of other procedures/tests performed as part of study visit.
e	Only for children over 25kg.
f	Clinical blood tests to additionally include testing for bone profile, creatinine kinase (CK), CRP, ferritin, magnesium, uric acid and coagulation screen.
g	TR006 dosing to occur 3-6 months post-cardiac transplant.

Table 16: Schedule of Clinical Procedures:

Study Procedure	Study Visit
Concomitant Medication Review	All visits.
Adverse Events Review	Day of Transplant, Transplant Day 1, Transplant Follow-up Day 14, Transplant Follow-up Month 1, Transplant Follow-up Month 2, Transplant Follow-up Month 3, Transplant Follow-up Month 4 [a], Transplant Follow-up Month 5 [a], Baseline Day 0, Baseline Day 1, Safety Follow-up Days 2-13, Safety Follow-up Day 14, Safety Follow-up Day 28, Safety Follow-up Month 2, Safety Follow-up Month 3, Safety Follow-up Month 6, Safety Follow-up Month 9, Safety Follow-up Month 12, Safety Follow-up Month 24.
SUSAR Review	Day of Transplant, Transplant Day 1, Transplant Follow-up Day 14, Transplant Follow-up Month 1, Transplant Follow-up Month 2, Transplant Follow-up Month 3, Transplant Follow-up Month 4 [a], Transplant Follow-up Month 5 [a], Baseline Day 0, Baseline Day 1, Safety Follow-up Days 2-13, Safety Follow-up Day 14, Safety Follow-up Day 28, Safety Follow-up Month 2, Safety Follow-up Month 3, Safety Follow-up Month 6, Safety Follow-up Month 9, Safety Follow-up Month 12, Safety Follow-up Month 24.
Vital Signs	Day of Transplant, Transplant Day 1, Transplant Follow-up Day 14, Transplant Follow-up Month 1, Transplant Follow-up Month 2, Transplant Follow-up Month 3, Transplant Follow-up Month 4 [a], Transplant Follow-up Month 5 [a], Baseline Day 0, Baseline Day 1, Safety Follow-up Day 14, Safety Follow-up Day 28, Safety Follow-up Month 2, Safety Follow-up Month 3, Safety Follow-up Month 6, Safety Follow-up Month 9, Safety Follow-up Month 12, Safety Follow-up Month 24.
Height and Weight	Day of Transplant, Transplant Follow-up Day 14, Transplant Follow-up Month 1, Transplant Follow-up Month 2, Transplant Follow-up Month 3, Transplant Follow-up Month 4 [a], Transplant Follow-up Month 5 [a], Baseline Day 0, Baseline Day 1, Safety Follow-up Day 14, Safety Follow-up Day 28, Safety Follow-up Month 2, Safety Follow-up Month 3, Safety Follow-up Month 6, Safety Follow-up Month 9, Safety Follow-up Month 12, Safety Follow-up Month 24.
12 Lead ECG	Transplant Day 1, Transplant Follow-up Day 14, Transplant Follow-up Month 1, Transplant Follow-up Month 2, Transplant Follow-up Month 3, Transplant Follow-up Month 4 [a], Transplant Follow-up Month 5 [a], Baseline Day 0, Baseline Day 1, Safety Follow-up Day 14, Safety Follow-up Day 28, Safety Follow-up Month 2, Safety Follow-up Month 3, Safety Follow-up Month 6, Safety Follow-up Month 9, Safety Follow-up Month 12, Safety Follow-up Month 24.
Echocardiography	Transplant Day 1, Transplant Follow-up Day 14, Transplant Follow-up Month 1, Transplant Follow-up Month 2, Transplant Follow-up Month 3, Transplant Follow-up Month 4 [a], Transplant Follow-up Month 5 [a], Baseline Day 0, Baseline Day 1, Safety Follow-up Day 14, Safety Follow-up Day 28, Safety Follow-up Month 2, Safety Follow-up Month 3, Safety Follow-up Month 6, Safety Follow-up Month 9, Safety Follow-up Month 12, Safety Follow-up Month 24.
Intravascular Ultrasound [b]	Transplant Follow-up Month 3, Safety Follow-up Month 9.
Coronary Angiography [b]	Transplant Follow-up Month 3, Safety Follow-up Month 9.
Clinical Blood Tests [c]	Day of Transplant, Transplant Day 1, Transplant Follow-up Day 14, Transplant Follow-up Month 1, Transplant Follow-up Month 2, Transplant Follow-up Month 3, Transplant Follow-up Month 4 [a], Transplant Follow-up Month 5 [a], Baseline Day 0 [d], Baseline Day 1 [d], Safety Follow-up Day 14, Safety Follow-up Day 28, Safety Follow-up Month 2, Safety Follow-up Month 3, Safety Follow-up Month 6, Safety Follow-up Month 9, Safety Follow-up Month 12, Safety Follow-up Month 24.
Blood Borne Infectious Diseases Tests [e]	Enrolment, Day of Transplant, Transplant Follow-up Month 3, Transplant Follow-up Month 4 [a], Transplant Follow-up Month 5 [a], Safety Follow-up Month 3.
HLA Antibody Testing (includes panel reactive antibodies and donor specific antibodies)	Enrolment, Day of Transplant, Transplant Follow-up Day 14, Transplant Follow-up Month 3, Transplant Follow-up Month 4 [a], Transplant Follow-up Month 5 [a], Safety Follow-up Month 3, Safety Follow-up Month 9, Safety Follow-up Month 24.
Urine Pregnancy Test (for WOCP only)	Day of Transplant, Transplant Follow-up Month 3, Transplant Follow-up Month 4 [a], Transplant Follow-up Month 5 [a], Baseline Day 0, Safety Follow-up Month 3, Safety Follow-up Month 9, Safety Follow-up Month 24.
Research Blood Samples	Day of Transplant, Transplant Day 1, Transplant Follow-up Day 14, Transplant Follow-up Month 3, Transplant Follow-up Month 4 [a], Transplant Follow-up Month 5 [a],

	Baseline Day 1, Safety Follow-up Day 14, Safety Follow-up Day 28, Safety Follow-up Month 3, Safety Follow-up Month 6, Safety Follow-up Month 9, Safety Follow-up Month 12, Safety Follow-up Month 24.

Key	Description
a	Only if study visit is required.
b	Only for children over 25kg.
c	Haematology (FBC), Liver Profile (albumin, alkaline phosphatase, total bilirubin and ALT), Renal Profile (sodium, potassium, creatinine and urea) LDH, EBV and CMV viral load (where required).
d	Clinical blood tests to additionally include testing for bone profile, creatinine kinase (CK), CRP, ferritin, magnesium, uric acid and coagulation screen.
e	HIV-1/2, HBsAg, HBC, HCV, HTLV, syphilis, Toxoplasmosis Gondii IgG, EBV IgG and CMV IgG (+ viral load where positive).