LOW-DOSE IL-2 TO EXPAND ENDOGENOUS REGULATORY T CELLS AND ACHIEVE TOLERANCE IN LIVER TRANSPLANTATION

End of Study Report

Acronym: LITE

Short title: Treg Liver Trial

TRIAL IDENTIFIERS

EudraCT Number – 2017-000177-37

REC Number – 14/LO/1014

IRAS Number - 215874

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1 Synopsis

Title of clinical trial	Low-dose IL-2 to expand endogenous regulatory T cells and achieve tolerance in liver transplantation
Protocol Short Title/Acronym	**L**ow-dose **I**L-2 for **T**reg **E**xpansion and tolerance (LITE)
Study Phase if not mentioned in title	IV
Medical condition or disease under investigation	Liver Transplantation
Chief Investigator	Professor Alberto Sanchez-Fueyo
Sponsor name	King's College London and King's College Hospital NHS Foundation Trust
Eudra CT number	2017-000177-37
Purpose of the trial	The purpose of the trial is to assess the capacity of low doses of IL-2 to expand endogenous regulatory T cells and promote the development of operational tolerance following liver transplantation.
Primary objective	The primary objective is to determine the capacity of a short course of low-dose IL-2 to facilitate the complete discontinuation of immunosuppressive drugs in liver recipients 2-6 years after transplantation.
Secondary objectives	(1) To determine the capacity of low-dose IL-2 to expand endogenous regulatory T cells (Tregs) in patients under calcineurin inhibitor immunosuppression; (2) to assess the safety of low-dose IL-2 administration in liver transplantation; (3) to investigate the sequential changes in Treg function, immunophenotype, and molecular profile following low-dose IL-2 treatment and calcineurin inhibitor discontinuation; (4) to determine if the baseline Treg function, immunophenotype, and molecular profile predict the response to low-dose IL-2; (5) to assess the changes in the blood and liver tissue inflammatory microenvironment; (6) to evaluate the development of anti-HLA antibodies.
Trial Design	Phase IV, open-label, activity, safety and efficacy, prospective, single-arm clinical trial in which liver recipients <50 years old and 2-6 years after transplantation will receive IL-2 and gradually discontinue their immunosuppressive medication. Based on the IL-2 activity and safety after 1 month of treatment in the first 8 participants, the study will continue or it will terminate. In participants with a satisfactory increase in circulating Tregs after 1 month of IL-

	2 therapy, treatment will be maintained for 5 additional months while immunosuppressive drugs are gradually discontinued over a 3-month period. Efficacy will be determined by assessing the proportion of subjects who achieve successful immunosuppression withdrawal defined by the absence of rejection and a rejection free biopsy at 1 year following discontinuation of immunosuppression.
Endpoints	Primary: Proportion of subjects who achieve successful immunosuppression withdrawal defined by the absence of rejection and a rejection free biopsy at 1 year following discontinuation of immunosuppression. Secondary: rejection, graft loss, patient survival, IL-2-related adverse events, effect of IL-2 on blood and liver Treg numbers, sequential immunological changes in blood and liver tissue, development of serum anti-HLA antibodies.
Sample Size	25 participants
Eligibility criteria	**Inclusion criteria:** 1. Adult liver transplant recipients 2-6 years post-transplant and age ≤50 years; 2. Recipient of single organ transplant only; 3. Liver function tests: direct bilirubin and alanine aminotransferase (ALT) <2 x upper limit of normal (ULN) at the screening visit; 4. On calcineurin inhibitor (CNI) based IS; with or without one of the following: Low dose mycophenolic acid (≤ 1080 mg daily), mycophenolate mofetil (MMF ≤ 1500 mg daily), or azathioprine (≤ 150 mg daily); 5. Provision of written informed consent. **Exclusion criteria:** 1. Serum positivity for HCV-RNA at screening; 2. Serum positivity for HIV-1 infection, HBV surface antigen or HBV-DNA at screening; 3. Active liver or systemic immune-mediated disease in which IS discontinuation is inadvisable (autoimmune hepatitis, primary sclerosing cholangitis, primary biliary cirrhosis); 4. Acute or chronic rejection within the 52 weeks prior to screening; 5. eGFR <40 mL/min (to mitigate the risk of worsening renal failure should rejection occur and high level of CNI be required); 6. The need for chronic anti-coagulation that cannot be safely discontinued to safely perform for a liver biopsy;

	7. Screening liver biopsy showing signs of clinically significant histological damage will preclude continuation in the trial; 8. Maintenance immunosuppressive therapy with a mTOR inhibitor (sirolimus or everolimus); 9. Active infection or malignancy; 10. Inability to comply with study directed treatment; 11. Any medical condition that in the opinion of the principal investigator would interfere with safe completion of the trial (including severe cardiac disease, severe respiratory disease with O2 blood saturation <92%, any other major organ dysfunction, and Eastern Cooperative Oncology Group (ECOG) performance status of ECOG > 1). 12. Participation in another Investigational medicinal product (IMP) study within 3 months from consent; 13. Any known allergy or intolerance to the IMP components; 14. Any contraindication to Proleukin administration as per SmPC; 15. Pregnancy or lactation; 16. Lack of effective methods of contraception for women and men of childbearing potential (see section 7.6); 17. Hypersensitivity to Proleukin or to any of the excipients.
IMP, dosage and route of administration	IL-2 (Proleukin, Novartis). 1 million international units (MIU) sub-cutaneous injections per day for a total of 180 days. Temporary or permanent adjustment allowed down to 0.5 MIU/day according to patient's tolerability and side effects, and dose increase up to 2 MIU/day allowed depending on IL-2 biological activity.
Active comparator product(s)	NA
Project timetables	Enrolment phase (29 months); patient follow-up (16 months). Total study duration: 47 months.

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3 List of Abbreviations

ADR	Adverse drug reaction
AE	Adverse event
ALT	Alanine aminotransferase
CI	Chief Investigator
CNI	Calcineurin inhibitor
CRF	Case report form
DMEC	Data Monitoring and Ethics Committee
ECOG	Eastern Cooperative Oncology Group
GCP	Good clinical practice
CTCAE	Common terminology criteria for adverse events
GVHD	Graft-versus-host disease
HBV	Hepatitis B virus
HIV	Human immunodeficiency virus
HCV	Hepatitis C virus
ICF	Informed consent form
IEC	Independent Ethics Committee
IL-2	Interleukin 2
IMP	Investigational medicinal product
IRB	Institutional Review Board
IS	Immunosuppression
i.v.	Intravenous
KHP-CTO	King's Health Partners Clinical Trials Office
eGFR	Estimated glomerular filtration rate
MHRA	The Medicines and Healthcare Products Regulatory Agency
MIU	Million international units
MMF	Mycophenolic acid
MRC	Medical Research Council
mTOR	Mammalian target of rapamycin
NIMP	Non-investigational medicinal product
NFAT	Nuclear factor of activated T-cells
PBMC	Peripheral blood mononuclear cell
PI	Principal Investigator
PIS	Patient information sheet
REC	Research Ethics Committee
S/c	Subcutaneous
SAE	Serious adverse event
SDV	Source data verification
SLE	Systemic lupus erythrematosus
SmPC	Summary of product characteristics
SUSAR	Suspected unexpected serious adverse reaction
Teffs	Effector lymphocytes
TMF	Trial master file
Tregs	Regulatory T cells
TSC	Trial Steering Committee
ULN	Upper limit of normal

4 Ethics

4.1 Independent Ethics Committee (IEC) or Institutional Review Board (IRB)

The study and all amendments were reviewed by the National Research Ethics Service (NRES) Committee South Central – Oxford A, with Chair Dr Karen Melham.

4.2 Ethical Conduct of the Study

The trial was conducted according to the protocol and in compliance with the principles of the Declaration of Helsinki (1996) as amended, the principles of Good Clinical Practice (GCP) and in accordance with Medicines for Human Use (Clinical Trials) Regulations 2004, as amended, the Research Governance Framework for Health and Social Care, the Data Protection Act 1998 and other regulatory requirements as appropriate. The trial protocol and substantial amendments were reviewed by the United Kingdom Medicines and Healthcare products Regulatory Agency (MHRA)

4.3 Subject Information and Consent

A master copy of the patient information sheet and consent form were prepared by the trial team and approved by the Research Ethics Committee (REC). It is the responsibility of the trial team to ensure that any changes to these documents are approved by the REC before being used in the study.

4.4 Consent Procedure

All trial Investigators seeking consent have received specific training in the taking of consent for donors of tissues and cells for patient treatment and was up-to-date on their annual refresher training. For each trial participant, an authorised trial Investigator obtained written informed consent prior to conducting a trial-related procedure involving the subject. Each subject was provided with a copy of the completed informed consent form (ICF) and patient information sheet (PIS) and store the original in the trial master file (TMF).

Signed, original consent forms were retained in the TMF at all times and made available (for review) to study monitors, auditors and inspectors, upon request. A copy of the signed patient consent form was kept in the patient notes.

A comprehensive verbal explanation was given by the Investigator in addition to the written information sheet provided to potential trial participants. The Investigator would explain the aims, methods, anticipated benefits and potential risks of the study, including any discomfort it may entail. They were informed of the serological tests that must be undertaken and their right to receive the results. Subjects were given sufficient time to read the information sheet thoroughly and the opportunity to clarify any points that they do not understand. At the end of the discussion, the subject was granted as long as they feel necessary to digest the information provided and to consider their involvement in the trial. They were free to discuss their participation with others outside the clinical trial team (e.g. family, friends, general practitioner) and must not feel pressure to provide an immediate decision. Subjects were also allowed a second opportunity to ask the

Investigator and/or research nurse questions regarding their participation, after the initial interview.

4.5 Consent Withdrawal

A trial participant has the liberty to withdraw their consent at any time and for any reason, without penalty or loss of benefits to which the individual would otherwise be entitled. Prior to giving consent, recipients were informed that they are able to request the destruction of stored biological samples (e.g. blood/urine for immuno-monitoring assays upon withdrawal, and that this will only be possible for samples that have not been tested at the time of withdrawal. Patients will not be able to request the deletion of immune-monitoring data generated from tested samples.

4.6 Personal Data Privacy Protection

To protect the identities of trial patients, each transplant recipient was assigned a unique patient trial identifier upon enrolment in the order in which they are enrolled in the study. All electronic and paper records containing patient data generated by the study were encoded with the appropriate patient trial identifier. Patient names were not be used to report or record trial data. Only Investigators and authorised staff at the trial centre have access to documents that link patient names to patient trial identifiers (i.e. IS/ICF and Patient Identification Log). It is the responsibility of the PI to ensure that these documents are treated in a confidential manner and stored securely. Safety reports transmitted by the Sponsor to the responsible authorities and RECs will use encoded subject identifiers. All data collected by the study was regarded as strictly confidential. Access to the trial eCRF platform was password protected.

5 Investigators and Study Administrative Structure

Protocol: LITE

Name	Role
Professor Alberto Sanchez-Fueyo	Chief Investigator
Jurate Wall	Clinical Trial Manager
Mike Lyne	Translational Research Manager
Dr Tiong Yeng Lim	Co-Investigator
Graeme Alexander	Trial Steering Committee (TSC) Chair – Consultant Hepatologist
Irene Rebollo-Mesa	Data Monitoring and Ethics Committee (DMEC) Chair
Abdel Douiri	DMEC Independent Statistician
Clare Flatch	Trial Statistician
Josep Maria Grinyó	TSC Member
Gilbert Bensimon	TSC Member
Elmar Jaeckel	TSC Member

6 Trial Summary

6.1 Summary

This is a phase IV activity, safety and efficacy, open-label, prospective, single arm clinical trial in which liver transplant recipients more than 2 years post transplantation will undergo low-dose IL-2 immunotherapy and immunosuppression withdrawal. Trial participants will receive low-dose IL-2 together with their conventional doses of immunosuppressants for one month in order to determine the safety and biological activity of IL-2. Only those participants with at least a 2-fold increase in the proportion of circulating Tregs will remain in the trial. Based on the response of the first 8 patients receiving IL-2 and who have a Treg measurement at 4 weeks the trial will continue, or it will terminate. In participants with a satisfactory increase in circulating Tregs IL-2 therapy will be maintained for 5 additional months while immunosuppressive drugs will be gradually discontinued over a 3 month period.

Efficacy will be determined by assessing the proportion of subjects who achieve successful immunosuppression withdrawal defined by the absence of rejection and a rejection free biopsy at 1 year following discontinuation of immunosuppression. Ancillary studies will include investigation into the effects of low-dose IL-2 on the homeostasis of regulatory lymphocytes and alloimmune responses.

6.2 Rationale for low-dose IL-2 use in Liver Transplant Recipients

Evidence that the expansion of the endogenous Treg pool achieved through IL-2 administration promotes allograft tolerance are derived from a stringent MHC-mismatched murine islet transplant model in which the combination of IL-2 with a calcineurin inhibitor (CNI) induced allograft tolerance in the majority of recipients. Of note, in the same animal model adoptive transfer of ex vivo expanded Tregs failed to induce tolerance unless recipient Teffs had been aggressively depleted beforehand. These data go along with the recent observation that in humans, adoptive transfer of a large number of Tregs only increases the pool of circulating Tregs by <20%, with only 25% of them persisting in the circulation >3 months. Evidence that low-dose IL-2 is also efficacious in an alloimmune setting in humans comes from a report describing striking beneficial effects in chronic GVHD described above, and from an additional study in which low-dose IL-2 prevented acute GHVD. It is noteworthy that in the GHVD studies patients were receiving a variety of adjuvant immunosuppressive agents, which did not prevent the expansion of circulating Tregs.

Whether transient expansion of endogenous Tregs by means of low-dose IL-2 is capable of inducing (or re-establishing) immunological tolerance in humans is very difficult to formally assess in autoimmune diseases, and has never been attempted in transplantation. The intentional discontinuation of immunosuppression in stable liver transplant recipients >2 years after transplantation provides a unique experimental setting to do so, as patients can be carefully selected to avoid confounding factors, the withdrawal of immunosuppression can be timed so that the biological efficacy of IL-2 can be confirmed before immunosuppression discontinuation is initiated, and a non-ambiguous clinical endpoint (rejection or tolerance) can be reached in a short period of time.

A key aspect in the clinical development of a tolerance-promoting regimen in transplantation is the choice of adjunctive immunosuppression. CNIs are the mainstay immunosuppressants in liver

transplantation and the most effective agents in controlling alloreactive Teffs in humans. Calcineurin inhibitors, however, inhibit nuclear factor of activated T-cells (NFAT) nuclear translocation, which is required for IL-2 production by Teffs, and, according to some reports, for the expression of Foxp3 by Tregs. Indeed, CNIs reduce the number of circulating Tregs both in animals and in humans, and block Treg-dependent allograft tolerance induced by co-stimulation blockade in mice. For this reason, tolerance-promoting regimens often include an mTOR inhibitor and not a CNI as adjunctive immunosuppression. The use of mTOR in combination with low-dose IL-2 remains contentious, however, as it can counteract the beneficial effects of IL-2 in type-1 diabetes. On the other hand, as described above, CNIs can synergise with IL-2 in inducing allograft tolerance in mice.

The unpublished novel findings described here indicate that although CNIs reduce Treg survival and impair their function, these effects are the result of decreased IL-2 availability rather than consequence of impaired NFAT signalling. Hence, they can be completely reversed by administration of low doses of exogenous IL-2.

Notably, in addition to restoring the impaired Treg phenotype induced by CNIs, low-dose IL-2 also increases the total number of Tregs, both in vitro and in vivo, and confers stronger suppressive function upon them. Furthermore, Tregs not only expand in blood but also in peripheral tissues, with the largest relative increase being observed in the liver, and acquire the capacity to migrate into the allograft and to improve graft survival. Thus, we anticipate that administration of low-dose IL-2 to liver transplant recipients on CNI will: i) restore the homeostatic abnormalities induced by CNI on Tregs; ii) directly increase the number of Tregs; iii) shift the balance between Teff and Treg in the liver allograft.

These data indicate that, as tolerance-promoting agents, CNIs are a double-edged sword (i.e. they effectively control Teffs but at the same time severely disrupt Tregs). In contrast, the combination of CNI and low-dose IL-2 is capable of favourably tipping the balance between Teffs and Tregs, and can constitute therefore a potent immunomodulatory regimen. We hypothesize that in a non-highly immunogenic setting such as human liver transplantation, administration of low-dose IL-2 to patients in whom Teffs are adequately suppressed by CNI treatment will constitute a simple and effective tolerance-promoting regimen capable of facilitating the complete discontinuation of immunosuppression.

Low-dose IL-2 could also synergize with *ex vivo* expanded alloantigen-specific Treg immunotherapy, by prolonging Treg survival following adoptive transfer. Such a combined immunotherapeutic strategy could achieve tolerance in more stringent settings such as kidney transplantation. Before this is attempted, however, a clear understanding of the safety, efficacy and biological effects of low-dose IL-2 in transplantation is required. This is particularly important, given a recent report in monkeys describing the breakdown of kidney allograft tolerance induced by mixed hematopoietic chimerism following IL-2 administration.

6.3 Trial objectives

Primary objective: The primary objective is to determine the capacity of a short course of low-dose IL-2 to facilitate the complete discontinuation of immunosuppressive drugs in liver recipients 2-6 years after transplantation.

Secondary objective:

1) To determine the capacity of low-dose IL-2 to expand endogenous regulatory T cells (Tregs) in patients under calcineurin inhibitor immunosuppression;
2) To assess the safety of low-dose IL-2 administration in liver transplantation;
3) To investigate the sequential changes in Treg function, immunophenotype, and molecular profile following low-dose IL-2 treatment and calcineurin inhibitor discontinuation;
4) To determine if the baseline Treg function, immunophenotype, and molecular profile predict the response to low-dose IL-2;
5) To assess the changes in the blood and liver tissue inflammatory microenvironment;
6) To evaluate the development of anti-HLA antibodies.

6.4 Study Endpoints

6.4.1 Primary Endpoints

The primary endpoint is defined as the proportion of subjects who achieve successful immunosuppression withdrawal as defined by the absence of rejection and a rejection free biopsy at 1 year following discontinuation of immunosuppression.

6.4.2 Secondary endpoints

Clinical endpoints

- Rejection (incidence, severity, timing, steroid resistant rejection, chronic rejection);
- Patient survival;
- Graft loss.

Mechanistic Endpoints

- IL-2-related adverse events;
- Effect of IL-2 on blood and liver Treg numbers;
- Sequential immunophenotypic changes in blood and liver tissue;
- Sequential changes in Treg function, immunophenotype, and molecular profile following low-dose IL-2 treatment and calcineurin inhibitor discontinuation;
- Sequential changes in the inflammatory microenvironment in blood and liver tissue;
- Development of serum anti-HLA antibodies.

6.5 Trial Duration

The study planned to enroll 25 liver transplant recipients followed-up at King's College Hospital.
Estimated recruitment period: 29 months.
Individual patient follow-up following enrolment: 16 months.
Total estimated study duration: 47 months. End of trial will be defined by the database lock.

7 Eligibility Criteria

7.1 Recruitment

Participants who underwent liver transplantation at Kings College Hospital and who are 6 to 12 months post liver transplantation will be selected according to the criteria stated below. The trial specifically excludes transplant recipients that are at increased risk of acute cellular rejection and, recurrent disease. The criteria will be assessed at the time of enrolment.

Inclusion Criteria:

1. Adult liver transplant recipients 2-6 years post-transplant and age ≤50 years;
2. Recipient of single organ transplant only;
3. Liver function tests: direct bilirubin and ALT <2 x ULN at the screening visit;
4. On calcineurin inhibitor (CNI) based IS; with or without one of the following: Low dose mycophenolic acid (≤ 1080 mg daily), mycophenolate mofetil (MMF ≤ 1500 mg daily), or azathioprine (≤ 150 mg daily);
5. Provision of written informed consent.

Exclusion Criteria:

1. Serum positivity for HCV-RNA at screening;
2. Serum positivity for HIV-1 infection, HBV surface antigen or HBV-DNA at screening;
3. Active liver or systemic immune-mediated disease in which IS discontinuation is inadvisable (autoimmune hepatitis, primary sclerosing cholangitis, primary biliary cirrhosis);
4. Acute or chronic rejection within the 52 weeks prior to screening;
5. eGFR <40 mL/min (to mitigate the risk of worsening renal failure should rejection occur and high level of CNI be required);
6. The need for chronic anti-coagulation that cannot be safely discontinued to safely perform for a liver biopsy;
7. Screening liver biopsy showing signs of clinically significant histological damage will preclude continuation in the trial;
8. Maintenance immunosuppressive therapy with a mTOR inhibitor (sirolimus or everolimus);
9. Active infection or malignancy;
10. Inability to comply with study directed treatment;
11. Any medical condition that in the opinion of the principal investigator would interfere with safe completion of the trial (including severe cardiac disease, severe respiratory disease with O2 blood saturation <92%, any other major organ dysfunction, and Eastern Cooperative Oncology Group (ECOG) performance status of ECOG > 1).
12. Participation in another IMP study within 3 months from consent;
13. Any known allergy or intolerance to the IMP components;
14. Any contraindication to Proleukin administration as per Summary of product characteristics (SmPC);
15. Pregnancy or lactation;
16. Lack of effective methods of contraception for women and men of childbearing potential (see section 7.6);
17. Hypersensitivity to Proleukin or to any of the excipients.

8 Study Medication

Formulation: Vials of powder for parenteral injection at 22 MIU of aldesleukin per vial, 18 MIU/ml.

Preparation and delivery: For 14 days treatment, 14 tuberculin syringes containing 1MIU aldesleukin in 1ml D5% water was prepared by Guy's & St Thomas' NHS Foundation Trust (GSTFT) Pharmacy Manufacturing Unit (MIA(IMP)11387) under aseptic condition. In contrast to the information provided in Proleukin SmpC, data is available demonstrating the stability of reconstituted IL-2 up to 21 days when provided to patients for home administration in pre-filled capped tuberculin syringes and stored under refrigeration at 2°C – 8°C. This information, together with the IL-2 reconstitution instructions can be found in the Ceplene® SmPC. The manufactured doses were supplied to Kings College Hospital Pharmacy for named patient dispensing. Appropriately labelled boxes of 14 syringes were provided for patients for home administration by KCH pharmacy. The dispensation log was kept by the KCH Pharmacy according to the local procedures. The IMP labelling complied with Eudralex Volume 4 annex 13 for the purposes of the trial. Patients were specifically trained in self-injection and received written instructions and a medication diary to monitor compliance.

Route of administration: subcutaneous (s/c).

Dose regimen: 1 MIU s/c per day for a total of 180 days.

- Throughout the study, patients developing grade 2 or higher toxic effects (as defined by the *National Cancer Institute's Common Terminology Criteria for Adverse Events*; CTCAE v4) can reduce the daily dose by half from 1 to 0.5 million IU/day. If toxicity persists at a dose of 0.5 million IU/day, IL-2 will be discontinued and patients will be withdrawn from the study.

IMP: Proleukin (Novartis; commercial stock will be used for trial purposes)
NIMPs (Non-investigational medicinal products): Tacrolimus, cyclosporine A, azathioprine, mycophenolate mofetil/mycophenolic acid (administered in any combination as per standard of care)

8.1 IL-2 dosing regimen

Flexibility: Yes, temporary or permanent adjustment allowed down to 0.5 MIU/day according to patient's tolerability and side effects, and dose increase up to 2 MIU/day allowed depending on IL-2 biological activity (i.e. increase in circulating Tregs), as follows:

- All patients initiated treatment at an IL-2 dose of 1 million IU/day.
- One week following initiation of treatment the number of circulating Tregs was assessed and in those patients in whom the increase in Tregs is <2-fold as compared with baseline, the dose of IL-2 were increased to 1 million IU twice daily provided no grade 2 or higher toxic effects are observed (as defined by the CTCAE v4). This is based on a recent report in which the Treg expansion achieved after 1 week of IL-2 treatment was very similar to what was observed at 4 weeks.
- Following 4 weeks of IL-2 treatment, only those patients exhibiting at least a 2-fold increase in circulating Tregs as compared to baseline (regardless of the those they are receiving) were

considered for immunosuppression withdrawal. Patients not reaching this cut-off would discontinue IL-2 and will be withdrawn from the study.

8.2 Definition and grading of Dose Limiting Toxicities

Dose Limiting Toxicity (DLTs) or Adverse Events (AEs) were determined from data collected during trial duration. Patients with no undue toxicity was managed and continued on therapy. DLTs and AEs were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE – Version 4.0) on a 1 to 5 sale as follows:
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.

9 Immunomonitoring studies

9.1 Specimens

Sequential peripheral blood samples were collected at various timepoints between enrolment and the end of follow-up. Blood was collected into EDTA vacutainers and employed fresh in flow cytometric experiments or used to isolate peripheral blood mononuclear cells (PBMCs) by density gradient sedimentation on Ficoll-Paque (GE Healthcare) that were then cryopreserved. Serum specimens were isolated from blood samples collected into Z Serum Separator Clot Activator vacutainers (Greiner bio-one) that were left upright for 45 minutes at room temperature (RT) and then centrifuged at 1300 x g for 10 min followed by removal of the top layer fluid and cryopreservation.

9.2 Flow cytometry immunophenotyping

Sequential peripheral blood samples were collected during each patient visit. PBMCs were isolated employing a Ficoll-Hypaque gradient (Amersham Pharmacia Biotech, Ltd., United Kingdom) and cryopreserved in liquid nitrogen. Tregs were defined as $CD4^+CD25^+FoxP3^+$, whilst Tcons were defined as $CD4^+CD25^-FoxP3^-$. $CD4^+Foxp3^+$ T cells were further classified into 3 functionally distinct sub-populations: (i) resting Treg cells ($CD45RA^+FOXP3^{lo}$); (ii) effector Treg cells ($CD45RA^-FOXP3^{hi}$), both of which are known to be suppressive in vitro; and (iii) cytokine-secreting non-suppressive T cells ($CD45RA^-FOXP3^{lo}$). All flow cytometry experiments were performed on a BD LSRFortessa™ cell analyser (BD Bioscience) and analysed using FlowJo software (TreeStar Inc).

Absolute quantification of each immune cell type was measured using BD Trucount™ tubes. In short, 200µl of whole blood and the appropriate monoclonal antibodies were added directly to the Trucount™ tube. The lypophilised pellet in the tube would dissolve, releasing a known number of fluorescent beads. During flow cytometry analysis, the absolute number of positive cells were determined by comparing the cellular events to bead events.

10 Study Patients

10.1 Patient flow

Between December 2017 and June 2018, a total of 18 patients with liver transplantation were approached to participate in the trial, 10 were interested and 6 were consented. All 6 patients were eligible to participate in the trial, whilst 4 others had agreed to undergo screening later in the year. The main reason for non-consent (4/8) was to have more time to think about it, 1 could not take time off work, and 2 did not provide any reason for refusal. The first individual was screened on 18/12/17 and the latest screened on 28/03/17. Patient characteristics are summarised in Table 1.

Figure 1. Patient recruitment

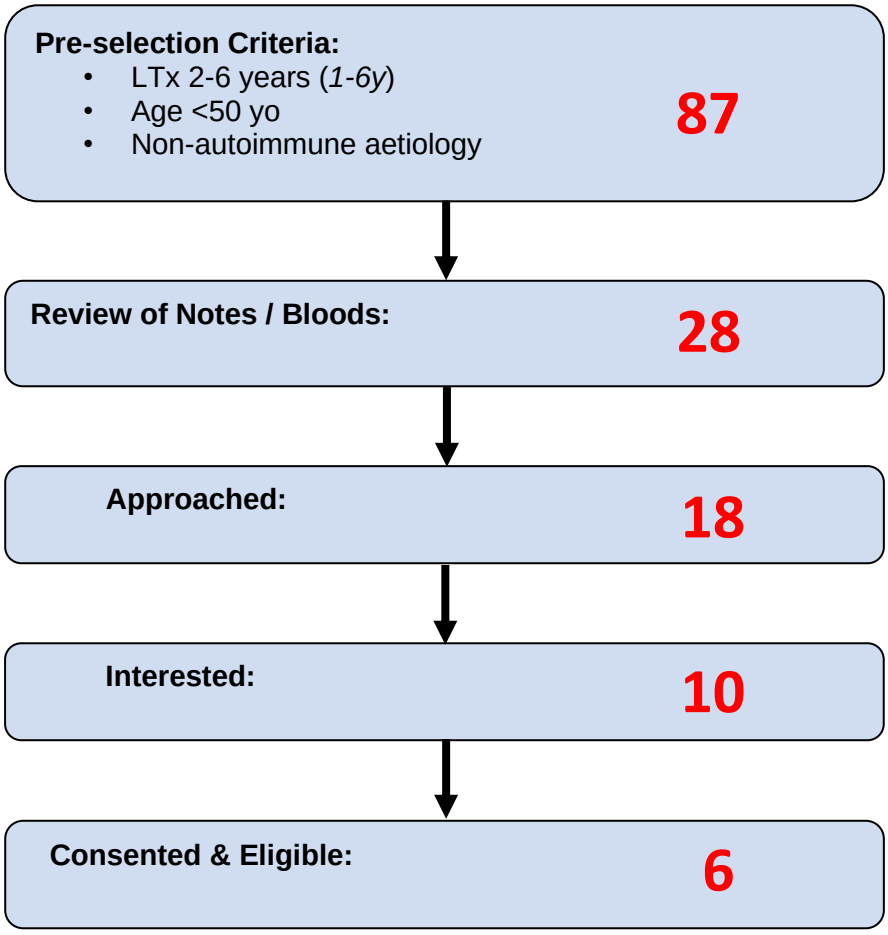

Table 1. Visit completion.

Visit	Number expected	Number completed (%)
Screening 1	6	6 (100%)
Screening 2	6	6 (100%)
Baseline	6	6 (100%)
Visit 1	6	6 (100%)
Visit 2	6	6 (100%)
Visit 3	6	5 (83%)
Visit 4	6	1 (17%)
Visit 5	6	0 (0%)

10.2 Baseline Demographics

Table 2. Baseline demographics of enrolled patients

		Enrolled patients N=6
Gender	Male	3 (50%)
	Female	3 (50%)
Ethnicity	Caucasian	4 (67%
	Black	0
	Mixed	0
	Asian	2 (33%)
	Other	0
Age *	(years)	34.3 (22-43)
BMI (Body mass index) *	(kg/m2)	27.1 (20.5-35.1)
Months since LT*	(months)	37.2 (29-48)

Number (percentage from total number in group) is shown for all, except for continuous measures (*), summarised with mean (minimum - maximum).

11 Criteria for Evaluation: Endpoints

11.1 Efficacy

Primary efficacy outcome measure was successful IS withdrawal as defined by the absence of rejection and a rejection free biopsy at 1year following IS discontinuation. This outcome was not achieved in any patient Both primary and secondary outcome measures are summarised in Table 3.

The biological activity of LDIL-2 was effective at expanding the target cells, i.e. CD4+CD25+FoxP3+ Tregs. All 6 patients showed a >= 2-fold increase in absolute number of Tregs from baseline (Table 4Table 5).

Table 3. Summary of outcome measures

Outcome Measure	Number achieved (n, %)
1º: Successful IS withdrawal at 1 year	0
2º: Allograft dysfunction	5 (83%)
2º: Allograft rejection	6 (100%)
2º: Patient graft survival	5 (83%)
2º: Patients underwent IS withdrawal	5 (83%)
2º: Failure of IS withdrawal	3 (50%)
2º: Operational Tolerance	0

Table 4. Summary of Treg Measurements.

Timepoint	**Patient count**	**# patients with >= 2-fold increase in Tregs**	**% of patients with Treg measurements**	**% of patients eligible**
Baseline	6	-	-	-
Visit 1 (1wk)	6 (100%)	6	100%	100%
Visit 2 (4-6wks)	6 (100%)	6	100%	100%

Table 5. Detailed Treg Measurements.

Absolute Treg Quantification			
Patient ID	**Baseline**	**Week 4**	**Fold-increase**
P01	70.6	142.3	2.0
P02	23.5	213.6	9.1
P03	31.2	131.8	4.2
P04	60.9	119.5	2.0
P05	146.5	877.7	6.0
P06	26.8	103.5	3.9

11.2 Adverse Events

During the trial period, a total of 25 AEs, including 2 severe AEs (SAEs), were identified. Summary tables for AEs and SAEs are shown below (Table 6-Table 8). Overall, all 6 patients developed at least one AE, and one patient (17%) experienced 2 SAEs.

Incidence of adverse drug reactions (ADRs): 19/25 AEs (44%) were assessed as possible/likely/definitely related to the IMP, and all 6 patients experienced at least one ADR. There was one episode of serious ADR, which was also a suspected unexpected serious adverse reaction (SUSAR).

Table 6. Adverse events.

Adverse events	**Total Patients**	**Total new events**	**Total cumulative events**
Adverse events	6	23	23
Serious adverse events	1	2	2

Table 7. Listing of adverse events for all patients.

Subject	Adverse Event	Start date	Stop date	Intensity	Related to study drug	Dose Limiting Toxicity?	Serious Adverse Event?
1	Injection site reaction	01/02/18	30/07/18	Mild	Definitely	No	No
1	Coryzal symptoms - runny nose	18/03/18	20/03/18	Mild	Possibly	No	No
1	Hypereosinophilia	06/02/18	03/08/18	Mild	Likely	No	No
2	Teeth extraction x 5	18/01/18	18/01/18	Mild	NA	No	No
2	Upper respiratory tract infection	08/01/18	15/01/18	Mild	NA	No	No
2	Injection site reaction	13/02/18	18/06/18	Mild	Definitely	No	No
2	Pruritis	21/03/18	18/04/18	Moderate	Likely	No	No
2	Flu-like symptoms including sore throat, cough, fever)	09/03/18	11/03/18	Mild	Possibly	No	No
2	Severe Rejection Episode	18/06/18	08/08/18	Severe	Possibly	No	Yes
2	Hypereosinophilia	27/02/18	06/06/18	Moderate	Likely	No	No
2	Hepatic artery pseudoaneurysm	02/08/18	08/08/18	Severe	Unlikely	No	Yes
3	Flu-like symptoms	04/05/18	11/05/18	Mild	Possibly	No	No
3	Injection site reactions	01/06/18	24/08/18	Mild	Definitely	No	No
3	Headaches (occasionally)	15/06/18	24/08/18	Mild	Possibly	No	No
3	Hypereosinophilia	11/05/18	01/06/18	Mild	Likely	No	No
4	Injection site reaction	04/07/18	01/08/18	Mild	Definitely	No	No
4	Hypereosinophilia	23/07/18	13/08/18	Mild	Likely	No	No
5	Loose Stools with mild abdominal pain	11/05/18	12/05/18	Mild	Possibly	No	No
5	Flu-like symptoms (fever, headache, loose stool, arthralgia)	18/05/18	02/06/18	Mild	Possibly	No	No
5	Hypereosinophilia	14/05/18	23/07/18	Mild	Likely	No	No
6	Injection site reaction	02/05/18	01/10/18	Mild	Definitely	No	No
6	Loose stools	18/05/18	25/05/18	Mild	Possibly	No	No
6	Hypereosinophilia	08/05/18	10/07/18	Mild	Likely	No	No

Table 8. Summary of treatment-emergent AEs.

System Organ Class	Preferred Term	No. of Subjects Experiencing the AE	Total No. of Occurrences of the AE
Blood and lymphatic system disorders	Hypereosinophilia	6	6
Gastrointestinal disorders	Loose stools	2	2

Injury, poisoning and procedural complications	Severe rejection episode	1	1
Infections and infestations	Upper respiratory tract infection	3	3
Injury, poisoning and procedural complications	Hepatis artery pseudoaneurysm	1	1
Nervous system disorders	Headache	1	1
Respiratory, thoracic and mediastinal disorders	Coryzal symtpoms	1	1
Skin and subcutaneous tissue disorders	Injection site reactions Pruritis	5 1	5 1
Surgical and medical procedures	Teeth extraction	1	1

12 Early Termination

Following the episode of SAEs as described above, we notified the Sponsor, regulators and governance committees (DMEC & TSC) as appropriate. After detailed discussions, further recruitment was halted pending interim data analysis. The data derived from the first 6 patients treated with the 1 million IU IL-2/daily regimen indicates that this dose of IL-2 is highly effective at expanding circulating Tregs >2-fold but also induces sub-clinical inflammatory changes that can be detected 1 month after initiating treatment. Therefore, a proposal was made for an amendment to the protocol design and interleukin-2 regimen. However, MRC concluded that the proposed changes in the amended protocol were beyond the scope of the original study, and the trial should be terminated.