The GAMECHANgER-1 Trial

A randomised controlled trial of reGulatory T cells in sensitised pAtients to iMprovE outComes after HLA-Ab iNcompatiblE Renal transplantation

Statistical Analysis Plan
Version 2
Version 2 started: 10/10/2023

ISRCTN: 14582152
EudraCT: 2021-001664-23

Approval signatures

M. Douiri _____ 02/02/2022

Dr Abdel Douiri, Senior Statistician — Date (dd-mmm-yyyy)

_____ _____

Prof Anthony Dorling, Chief Investigator — Date (dd-mmm-yyyy)

This document contains up to date statistical analysis plans (with version numbers and dates).

A) Quantitative Analysis Plan
B) Economic Analysis Plan
C) Schedule of Assessments and Measures

Contents

A) QUANTITATIVE ANALYSIS PLAN 4
1. Description of the trial 5
1.1 Principal research objectives to be addressed 5
Outcome measures 5
1.2 Trial design including blinding 6
Part 1: Observational Study: 6
Part 2: Interventional Study: 6
Figure 1. Trial design flow diagram 7
1.3 Eligibility Criteria 8
Part 1: Observational Study 8
Part 2: Interventional Study 8
Definition of females of childbearing potential and contraception 9
1.4 Method of treatment allocation 9
1.5 Trial medication and treatment protocol 10
1.6 Frequency and duration of follow-up 10
Part 1: Observational Study 10
Part 2: Interventional Study 11
1.7 Visit windows 11
1.8 Data collection 11
Part 1: Observational Study 11
Part 2: Interventional Study 12
1.9 Sample size estimation (including clinical significance) 13
Part 1: Observational Study 13
Part 2: interventional Study 14
1.10 Brief description of proposed analyses and any pre-analysis statistical checks required 14
2. Data analysis plan – Data description 14
2.1 Recruitment and representativeness of recruited patients 14
2.2 Baseline comparability of patient subpopulations 18
2.3 Adherence to allocated treatment and treatment fidelity 18
2.4 Loss to follow-up and other missing data 18
2.5 Adverse event reporting 18
2.6 Descriptive statistics for outcome measures 18
3. Data analysis plan – Inferential analysis 19
3.1 Main analysis 19
Analysis of primary outcome 19
Analysis of secondary outcomes 19
3.2 Statistical considerations 20
Missing outcome data 20
Missing baseline data 20
Method for handling multiple comparisons 20

Model assumption checks 20
3.3 Sensitivity analyses 20
3.4 Planned subgroup analyses 20
3.5 Exploratory analyses 21
3.6 Interim analysis 21
4. Software 21
B) ECONOMIC ANALYSIS PLAN 21
C) SCHEDULE OF ASSESSMENTS AND MEASURES 22

(MAY ALSO HAVE OTHERS SUCH AS QUALITATIVE ANALYSIS PLAN)

A) QUANTITATIVE ANALYSIS PLAN

Sponsor Contact
Name: Amy Holton, King's College London and Guy's and St Thomas' NHS Foundation Trust.
Address: King's Health Partners Clinical Trials Office, Guy's Hospital, Great Maze Pond, London SE1 9RT
M: 07703469925
Email: amy.holton@kcl.ac.uk

Chief investigator
Name: Professor Anthony Dorling
Address: MRC Centre for Transplantation, King's College London, Guy's Hospital, Great Maze Pond, London, SE1 9RT
Telephone: 020 7188 5880
Fax: 020 7188 5660
Email: anthony.dorling@kcl.ac.uk

Co-Investigators
Name: Professor Nizam Mamode
Address: Transplantation, Renal and Urology Directorate, 6th Floor, Borough Wing Guy's Hospital, Great Maze Pond, London, SE1 9RT
Telephone: 020 7188 1543
Email: Nizam.Mamode@gstt.nhs.uk

Name: Dr David S Game
Address: Transplantation, Renal and Urology Directorate, 6th Floor, Borough Wing Guy's Hospital, Great Maze Pond, London, SE1 9RT
Telephone: 020 7188 5665
Email: David.Game@gstt.nhs.uk:

Name: Professor Giovanna Lombardi
Address: King's College London, Guy's Hospital, Great Maze Pond, London, SE1 9RT
Telephone: 020 7188 7674
Email: Giovanna.Lombardi@kcl.ac.uk

Name: Professor Alberto Sanchez-Fueyo
Address: King's College London, King's College Hospital, London, SE5 9RS
Telephone: 07774 554731
Email: sanchez_fueyo@kcl.ac.uk

Trial manager
Name: Dr Izabela Pilecka
Address: King's College London, Guy's Hospital, Great Maze Pond, London, SE1 9RT

Telephone: 07706374559
Email: izabela.pilecka@kcl.ac.uk

Trial statisticians
Name: Dr Abdel Douiri
Address: King's College London, School of Life Course & Population Sciences (SLCPS), Addison House, Guy's Hospital, London SE1 1UL
Telephone: 020 7848 8224
Email: abdel.douiri@kcl.ac.uk

Name: Katherine Phillips
Address: King's College London, School of Life Course & Population Sciences (SLCPS), Addison House, Guy's Hospital, London SE1 1UL
Telephone: 020 7848 6620
Email: katherine.phillips@kcl.ac.uk

1. Description of the trial

1.1 Principal research objectives to be addressed.

The primary objective is:
To investigate whether in vitro anti-HLA T & B cell responses in sensitised patients can be inhibited by adoptive transfer of Tregs.

The secondary objectives are to determine:
1. The proportion of sensitised dialysis patients who may be eligible for a future trial based on patterns of IFNg/IL-17 responses to HLA on Fluorospot assays.
2. The duration of Treg suppression of HLA-specific Fluorospot assays.
3. The adverse events associate with Treg therapy.

The secondary exploratory mechanistic objectives are to determine:
4. How adoptive Treg therapy changes the number and phenotype of circulating Tregs.
5. How adoptive Treg therapy changes HLA Ab profiles measured by Luminex analysis.

Outcome measures

Primary outcome

The primary outcome is the proportion of patients showing suppression of a defined HLA specific-Fluorospot response for 2-months post treatment, compared to the proportion of patients undergoing baseline Immunomonitoring in Part 1 who show the same changes at any time during baseline Immunomonitoring.

Secondary outcomes

The secondary outcomes are:

1. The proportion of sensitised dialysis patients with unregulated T & B cell anti-HLA responses.
2. The duration of suppression of HLA-specific responses by Tregs

3. The adverse events associated with Treg therapy.

The secondary exploratory mechanistic outcomes are:

4. Changes in Treg number and phenotype comparing baseline to post-Treg treatment.
5. The changes in HLA Ab profiles measured by Luminex.

1.2 Trial design including blinding.

The trial design is represented in a flow diagram (Figure 1).

This trial consists of two parts: an observational study examining in vitro anti-HLA T & B cell responses and an adaptive unblinded two stage interventional study examining the effect of adoptive transfer of Tregs on the anti-HLA T & B cell responses.

Part 1: Observational Study:

100 end stage renal failure patients awaiting transplantation will be assessed for HLA specific patterns of responsiveness in Fluorospot assays at a single timepoint. These assays will either be collected retrospectively if patients have participated in a comparative observational study in the past 12 months (REC ref 16/WM/0370), or prospectively through the withdrawal of up to 80ml of blood for testing.

From these patients, those with either positive IL-17 or IFN gamma anti-HLA reactivity without evidence of regulation by CD25+ cells will be identified as eligible for Part 2 of this trial. The first 9 patients identified as having eligible Fluorospot patterns will undergo baseline immunomonitoring over 2 months, providing data on the natural variability of HLA specific patterns of responsiveness necessary for the primary endpoint. Immunomonitoring will begin once it is clear that the funder's milestone 1 has been met (either after 75 patients have been recruited or 12 months, whichever occurs first) OR, once we have >21 recruits with eligible fluorospot patterns for Part 2.

Part 2: Interventional Study:

This is two stage, adaptive, open labelled, trial using a Simon's two stage design aimed at assessing the effectiveness of TR001 in suppressing memory T and B cell responses against specific HLA antigens. **TR001 is a single use named patient therapy containing autologous Tregs isolated from the peripheral blood of the patient by leukapheresis. The Treg cells are then expanded ex vivo and administered to the patient in question.**

Within this study, 12 patients will be treated in stage 1. This will include the 9 patients who underwent baseline immunomonitoring in Part 1. The treatment will then be assessed for futility before commencement of stage 2 which involves the treatment of 9 patients. Futility is defined as 2 or fewer patients in stage 1 demonstrating suppression of anti-HLA responses for at least two months post treatment (the primary endpoint). If 6 or more patients reach this endpoint, the treatment will be deemed non-futile. In the case that 3-5 patients out of the 12 patients in stage 1 demonstrating suppression of anti-HLA responses for at least two months post treatment, the trial will be paused, and the sample size recalculated after discussion with the funders.

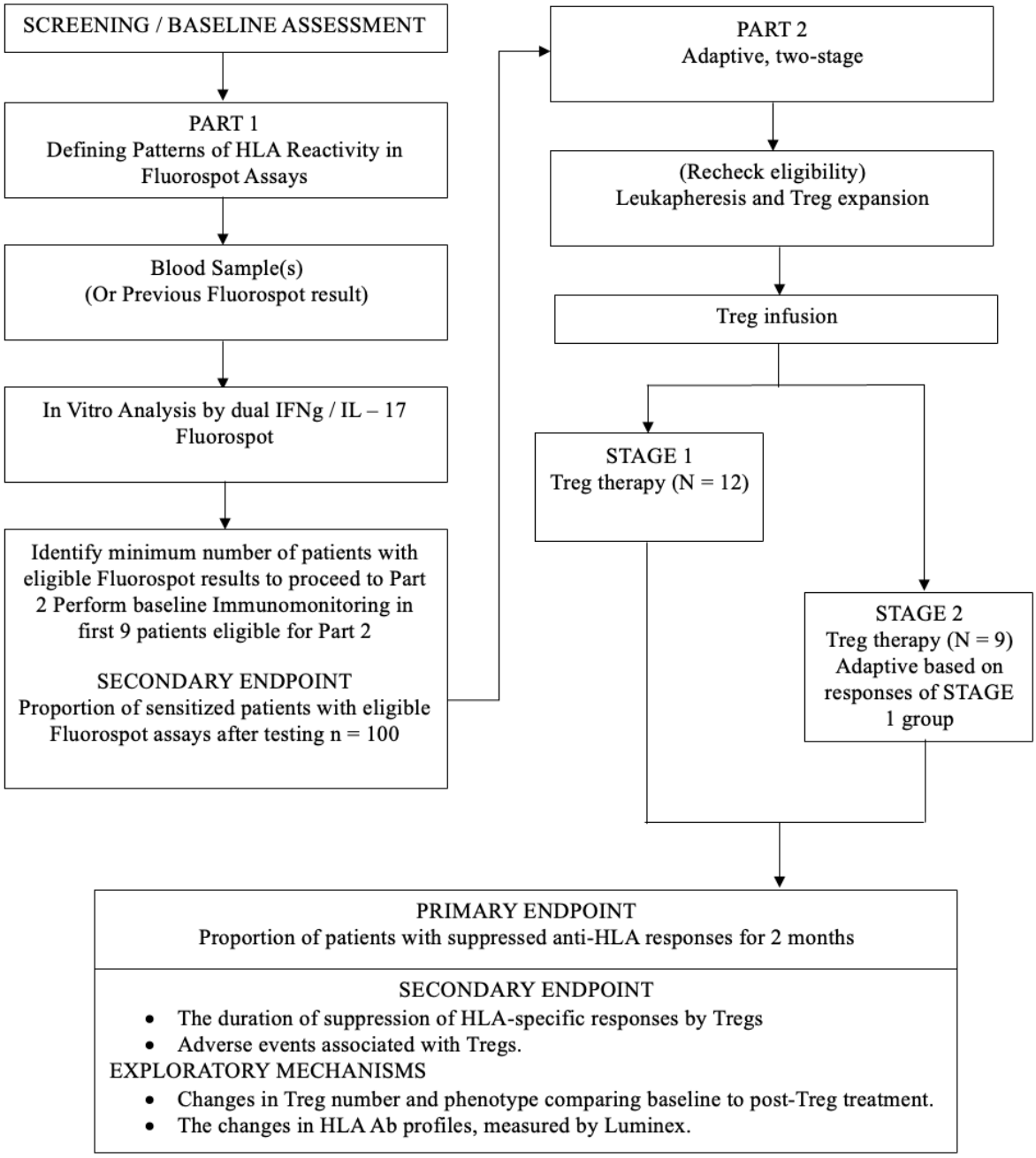

Figure 1. Trial design flow diagram

1.3 Eligibility Criteria

Patient eligibility for this trial will be confirmed against an initial list of inclusion/exclusion criteria in Part 1 followed by an additional list of criteria at the start of Part 2. The criteria list for Part 2 may contain items from Part 1 which need to be reconfirmed.

Part 1: Observational Study

Inclusion criteria:

1) Adult (≥18yrs) dialysis patients on the renal transplant/deceased donor waiting list with HLA Ab and a CRF ≥50%.
2) HLA Ab specificities corresponding to available PURE HLA protein.
3) Able to give written informed consent.
4) Female participants of childbearing potential* and male participants whose partner is of childbearing potential must be willing to consent that they or their partner use highly effective** contraception during Part 2 of the trial.

Exclusion criteria:

1) Living donor kidney transplant planned/
2) Listed as recipient of multi-organ transplants (i.e. combined kidney and pancreas)/
3) Known HIV+ or previous HCV or HBV. If no HIV, HCV or HBV tests within 5 years, these will be performed post-consent.
4) Patient involved in other clinical trials of investigational medicinal products.
5) Active infection or history of recurrent infection. Recurrent infection defined as more than 2 confirmed infections requiring either antibiotics, antivirals, antifungals or hospitalisation in 6 months prior to consent.
6) Female patients of childbearing potential with a positive pregnancy test at enrolment.
7) Female patients who are breastfeeding.
8) Hypersensitivity to IMP or to any of the excipients.
9) Known contraindication to the protocol-specified treatments or procedures
10) Severe liver impairment, defined as ≥Grade 3 or severely elevated ALT, AST or total bilirubin, on bloods done within the last 3 months
11) ECG abnormalities suggesting active myocardial ischaemia or (potentially) malignant ventricular arrhythmia: ECG to have been performed within the last 3 months.
12) Patients, who in the opinion of the PI, have a medical condition, or other relevant psychological, familial or social factor that may jeopardise their health, compliance, or influence the trial integrity in any way.

Part 2: Interventional Study

Inclusion criteria:

1) Dual fluorospot assay result to PURE HLA proteins that indicates anti-donor reactivity without evidence of suppression by CD25+ cells – the assay will most likely have been performed as part of part 1 assessment but may have been performed as part of a separate observational study, within the time frame of this study (REC ref 16/WM/0370).
2) Female participants of childbearing potential* and male participants whose partner is of childbearing potential must be willing to reconfirm that they or their partner use highly effective** contraception during Part 2 of the trial.

Exclusion criteria:

1) Living donor kidney transplant planned

2) Patient involved in other clinical trials of investigational medicinal products.
3) Active infection or history of recurrent infection. Recurrent infection defined as more than 2 confirmed infections requiring either antibiotics, antivirals, antifungals or hospitalisation in 6 months prior to entering into Part 2.
4) Female patients of childbearing potential with a positive pregnancy test in the week prior to leukapheresis
5) Female patients who are breastfeeding.
6) Hypersensitivity to IMP or to any of the excipients.
7) Known contraindication to the protocol-specified treatments or procedures
8) Severe liver impairment, defined as ≥Grade 3 or severely elevated ALT, AST or total bilirubin on bloods done within the last 3 months
9) ECG abnormalities suggesting active myocardial ischaemia or (potentially) malignant ventricular arrhythmia: ECG to have been performed within the last 3 months.
10) Patients, who in the opinion of the PI, have a medical condition, or other relevant psychological, familial or social factor that may jeopardise their health, compliance, or influence the trial integrity in any way.

Definition of females of childbearing potential and contraception

*Female patients of childbearing potential are female patients who have experienced menarche and who are not post-menopausal or permanently sterilised (eg. By tubal occlusion, hysterectomny, bilateral salpingectomy). 'Postmenopausal' 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 post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement is required.
**Highly effective methods of birth control are those with a failure rate <1% per year when employed consistently and correctly (e.g. hormonal contraception, some intrauterine devices, vasectomised partner, total abstinence). Hormonal contraception must be associated with inhibition of ovulation. Abstinence will be evaluated in the context of the usual lifestyle of the recipient. Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) are not acceptable methods of contraception. A female participant with a vasectomised partner must confirm that the vasectomised partner is the only sexual partner, and that surgical success of the vasectomy has been medically confirmed.
All females of childbearing potential and males whose partner is of childbearing potential must be willing to use such methods if going into Part 2 and continue to use to the end of phase 2 follow-up.

1.4 Method of treatment allocation

Only patients with either positive IL-17 or IFN gamma anti-HLA reactivity without evidence of regulation by CD25+ cells will be eligible for Part 2 of the trial. The first 9 patients who are eligible will proceed to baseline immunomonitoring (Part 1) and be offered TR001 treatment at the start of Part 2 as long as they are still eligible for treatment (based on exclusion criteria, e.g. have not undergone a transplant in the intervening period).

Treatment will be offered to patients in each of the three study sites in a ratio proportionate to the number of patients found to be eligible at each site. This ratio will be calculated as follows:

$$\frac{\text{Num. patient eligible from site}}{\text{Total num. eligible patients}} \times \text{Sample size for stage*} = \text{Patient allocation for study site}$$

will be 12 for stage 1 and 9 for stage 2

For example, after 75 patients have been analysed in Part 1, if the number found eligible at each site is 10 (site 1), 20 (site 2) and 15 (site 3), then in stage 1 3 patients from site 1, 5 patients from site 2 and 4 patients from site 3 will be treated. In stage 2 another 2 patients from site 1, 4 patients from site 2 and 3 patients from site 3 will be treated.

Once each site is given their allocated number of patients to be treated, patients from each site will be offered treatment in the order that they were deemed eligible for Part 2 of the trial until the quota is filled.

There is a slim chance that the 9 patients included in baseline immunomonitoring and subsequently offered TR001 treatment will cause one study site to exceed their allotted number of treated patients. In this scenario, the remaining patients offered TR001 treatment will come from the remaining two study sites.

In the small chance that a study site has not enough patients with either positive IL-17 or IFN gamma anti-HLA reactivity without evidence of regulation by CD25+ cells who remain eligible for treatment to fulfil their patient allocation, all eligible patients from that site will be offered treatment and the remainder of the allocation will be evenly divided between the other two sites.

1.5 Trial medication and treatment protocol

The intervention (TR001) to be used in this trial is a cell suspension formulated for each patient. Cells will be derived from the patient in question through a process of leukapheresis. T-regulatory cells will be expanded in vitro until a dose of 5-10x106 cells/Kg is reached. This will be administered to the patient from which the cells are derived.
The administration of the treatment involves a one-time administration via intravenous infusion. The infusion will take 30 minutes and the clinical condition of the recipient will be observed every 15 minutes during the infusion and for 1 hour after.

1.6 Frequency and duration of follow-up

Part 1: Observational Study

Blood will be collected for Fluorospot assays at a single timepoint unless a similar assay has been completed in the past 12 months in which no procedures will occur. Patients will be invited back for a second visit where they will be informed of their Fluorospot results and whether they are eligible for Part 2 of the trial.
Patient who undergoes baseline immunomonitoring in this phase of the trial will have blood samples collected at 0, 1, 2, 4 and 8 weeks after a specified start point. This will only commence once it is clear that the funder's milestone 1 has been met (either after 75 patients have been recruited or 12 months, whichever occurs first) OR, once we have >21 recruits eligible for Part 2.

Part 2: Interventional Study

Participants will be followed up for 12 months after TR001 treatment. Specifically, follow-up appointments will be at 1, 2, 4, and 8 weeks after TR001 treatment as well as 6 and 12 months after treatment. At each of these timepoints blood will be collected for Fluorospot assays to measure HLA specific responses. Biochemistry results from the patient medical files will also be collected at every timepoint bar 2 weeks post-treatment. At 1, 4, and 8 weeks after treatment additional blood will be collected to assess changes in Treg number and phenotype and changes in HLA antibody profile (as required for the exploratory objectives). All measures will also be collected prior to treatment to provide a baseline measure for comparison.

1.7 Visit windows

Visits will primarily take place during patient's dialysis sessions with blood being taken while patients are on dialysis were possible to minimise hospital visits and to mitigate the risks to the patient.

1.8 Data collection

Part 1: Observational Study

Visit 1: After consent, the initial visit will be a recruitment visit and consist of a registration and eligibility check. Any extra eligibility checks needed (ECG, LFTs, virology, pregnancy) will be arranged at this timepoint and completed post-consent.
Once eligibility has been confirmed, the following information will be recorded as soon as convenient (for instance at next dialysis): age, date of birth, sex, race, cause of kidney failure, significant past medical history, time on dialysis, potential routes of sensitisation, CRF. A blood sample (up to 80mls) will be taken for non-routine baseline Fluorospot to study patients' pattern of the memory responses against HLA (see below) if the patient has not had the same assay performed as part of a separate ongoing observational study in the past 12 months. Where possible, blood will be collected while patients are on dialysis.

Visit 2: All participants will be invited to attend the second visit to inform them of results of Fluorospot test and whether they are eligible for Part 2 of the trial. Any AE's related to study procedures (i.e blood taking) will be recorded at this visit.

If recruit is not eligible for Part 2, this is the end of their involvement in the study and all study procedures finish at this visit.

If recruit is eligible for Part 2, then they will be informed about additional visits and blood sampling relating to immunomonitoing. Immunomonitoring will only begin once it is clear that the funder's milestone 1 has been met (either after 75 patients have been recruited or 12 months, whichever occurs first) OR, once we have >21 recruits eligible for Part 2.

In those 9 patients found to be eligible for Part 2 who also undergo baseline immunomonitoring, further blood (up to 80ml) for fluorospot analyses will be obtained at regular intervals matching the timepoints of blood samples collected post-treatment. This blood samples will be collected at the following time points:

- ***Visit 3:*** First baseline immunomonitoring sample, deemed week 0.
- ***Visit 4:*** 1 week (or closest dialysis session) after visit 3

- ***Visit 5:*** 2 weeks (or closest dialysis sessions) after visit 3 (±1 session)
- ***Visit 6:*** 4 weeks (or closest dialysis session) after visit 3 (±1 session).
- ***Visit 7:*** 8 weeks (or closest dialysis session) after visit 3 (± 2 sessions).

These visits will only proceed once it is clear that the funder's milestone 1 has been met (either after 75 patients have been recruited or 12 months, whichever occurs first) OR, once we have >21 recruits with eligible fluorospot patterns for Part 2.

Pre-Part 2 eligibility re-check

All patients identified as being eligible for Part 2 will be seen prior to the start of Part 2 to re-check eligibility for Part 2. This may involve repeating ECG and LFTs (if no clinical test results are available within the last 3 months) and will involve re-confirming a negative pregnancy test in all women of childbearing age.

Demographic data will be updated, more specifically, details of intercurrent illnesses since enrolment to part 1, potential sensitisation events and concomitant medication will be collected. All females of childbearing potential and males with partners of childbearing potential must re-confirm their commitment to using highly effective methods of contraception in Part 2 of the study. Any AE's related to study procedures up to that point (i.e blood taking) will be recorded.

Part 2: Interventional Study

Visit 1 Leukapheresis: This will take place at a time point specified by the order of recruitment and at a time convenient for the patient. Vital signs (pulse BP and temperature) will be monitored during leukapheresis.

As per the EU Tissues and Cells Directive, a sample for serology testing will also be collected at the time of leukapheresis or, if not possible, within 7 days of leukapheresis. Mandatory serological tests (Anti-HIV-1, 2, HBsAg, Anti HBc, Anti-HCV-Ab, T. pallidum-specific test) are supplemented with HTLV-I/II antibody testing.

Pre-TR001 infusion, AE's reported to be related to previous blood taking or leukapheresis will be recorded.

Visit 2 Treg infusion: The first sample of blood (up to 80mls) for fluorospot analysis will be taken ideally within 24 hours prior to administration of TR001 or, if not possible, at the dialysis session immediately before. Where possible, to avoid the risk of fluid overload, TR001 will be administered within 24-48 hours of the next routine dialysis. Vital signs such as pulse, temperature, BP will be monitored immediately prior to administration of TR001 (see section 5.2.3). The patient will be monitored closely for 1 hour after the infusion and will be kept in the CRF for adverse event monitoring for 6 hours post-TR001 administration. A symptoms-directed physical examination will be conducted prior to discharge.

Full adverse event monitoring will begin with the administration of TR001 (Visit 2)

Post-TR001 infusion follow up visits

In all the post-TR001 infusion visits, the need for a symptoms-directed physical examination and/or symptom-specific tests will be determined according to standard medical care.

Further blood (up to 80ml) for fluorospot analyses will be obtained at each of the following time points:

Visit 3*:* 1 week after Treg infusion +/- 1 dialysis session
- Perform symptoms-directed physical examination if needed and record vital signs (pulse BP, temp) in patient notes
- Blood sample for treatment immunomonitoring – Fluorospot, HLA Ab, Treg numbers and phenotype
- Send sample for FBC, U&E, LFTs and CRP unless results available from last dialysis session

Visit 4*:* 2 weeks after Treg infusion +/- 1 dialysis session
- Perform symptoms-directed physical examination if needed and record vital signs (pulse BP, temp) in patient notes
- Blood sample for treatment immunomonitoring – Fluorospot only
- Send sample for FBC, U&E, LFTs and CRP unless results available from last dialysis session

Visit 5*:* 4 weeks after Treg infusion +/- 1 dialysis session
- Perform symptoms-directed physical examination if needed and record vital signs (pulse BP, temp) in patient notes
- Blood sample for treatment immunomonitoring – Fluorospot, HLA Ab, Treg numbers and phenotype
- Send sample for FBC, U&E, LFTs and CRP unless results available from last dialysis session

Visit 6*:* 8 weeks after Treg infusion +/- 2 dialysis sessions
- Perform symptoms-directed physical examination if needed and record vital signs (pulse BP, temp) in patient notes
- Blood sample for treatment immunomonitoring – Fluorospot, HLA Ab, Treg numbers and phenotype
- Send sample for FBC, U&E, LFTs and CRP unless results available from last one or two dialysis sessions

Visit 7*:* 6 months after Treg infusion +/- 3 dialysis sessions
- Perform symptoms-directed physical examination if needed and record vital signs (pulse BP, temp) in patient notes
- Blood sample for treatment immunomonitoring – Fluorospot, HLA Ab
- Send sample for FBC, U&E, LFTs and CRP unless results available from last 1-3 dialysis sessions

Visit 8*:* 12 months after Treg infusion +/- 3 dialysis sessions
- Perform symptoms-directed physical examination if needed and record vital signs (pulse BP, temp) in patient notes
- Blood sample for treatment immunomonitoring – Fluorospot, HLA Ab
- Send sample for FBC, U&E, LFTs and CRP unless results available from last 1-3 dialysis sessions
- Confirm negative pregnancy test on all women of child-bearing potential

1.9 Sample size estimation (including clinical significance)

Part 1: Observational Study

For Part 1 we will recruit up to 100 patients. This number is based on availability of the clinical population in the study sites which is feasible in the study timeframe.

Part 2: interventional Study

Within Part 2, Simon's two-stage design will be used, with 5% significance level and 90% power. Clinical consensus amongst the expert team is that if fewer than 20% meet the primary endpoint there is no merit in further investigating Tregs. Clinical consensus amongst the expert team is that provided ≥50% respond to Tregs, they will clearly merit further investigation in the form of a UK clinical trial comparing use of Tregs against standard of care. Based on these parameters a sample size of 21 patients is required, all of whom will be monitored for at least 2 months post-treatment to meet the data collection for the primary endpoint. Of these 21 patients, 12 will be allocated to stage 1 and 9 to stage 2, based on the order of recruitment.

Data from stage 1 will be used to assess for futility of the trial. If 2 or less patients show suppression of a defined HLA-specific Fluorospot response for at least 2 months post-treatment (the primary endpoint) the trial will be stopped, with patients in stage 2 not receiving TR001 and a conclusion will be reached that Treg therapy has no efficacy. If 6 or more patients show suppression of a defined HLA-specific Fluorospot response, we will proceed with dosing patients in stage 2. In the event that 3, 4 or 5 out of the 12 patients in stage 1 respond, the estimates of efficacy are below our hoped for 50 % but are not so low as to be regarded as evidence of futility. We will therefore pause the trial and re-estimate the number of patients that we need to recruit, after discussion with the funders.

1.10 Brief description of proposed analyses and any pre-analysis statistical checks required

Analyses will be carried out by the junior trial statistician, Katherine Phillips. In the first instance data from patient who receive a complete or a partial dose will be included in the data analysis (an intention-to-treat principle). The only exception to this principle is patients who agree to treatment and then withdrawn from the study before receiving treatment due to no longer meeting the eligibility criteria (e.g. they undergo a kidney transplant between patient consent and TR001 infusion). These patients will be replaced during the course of the study, maintaining patient numbers and power.

Analysis will be primarily descriptive and focus upon estimation with confidence intervals. Area under curve measures or mixed effects regression models will be used to describe and analyse outcomes that are measured repeatedly over time, with log or other transformation made where necessary to meet model assumptions.

2. Data analysis plan – Data description

2.1 Recruitment and representativeness of recruited patients

A CONSORT flow chart will be constructed – see Figure 2. This will include the number of eligible patients, number of patients consenting to take part in the trial and the number of patients refusing participation.

Out of those who consent and undertake Part 1 of the trial, the number of patients deemed eligible for Part 2 (have positive IL-17 or IFN gamma anti-HLA reactivity without evidence of regulation by CD25+ cells), split into those who remain eligible based on the other criteria and those who are not longer eligible, will be reported along with the number of patients ineligible for Part 2.

The number of patients offered treatment in Part 2 will be reported followed by the number of patients who refuse treatment, the number who received the complete dose of TR001 and the number who received a partial dose of TR001 (any patient for whom the infusion was ended prematurely). The reasons for the premature stopping of treatment infusion will be recorded. The number of patients lost

to follow-up over the 12 month observation period will be reported, as will the number of patients who withdraw from the trial.

As this treatment is a single-dose therapy administered by intravenous infusion, compliance/non-compliance will not be an issue and so will not be reported. It is thought that a partial dose of TR001 may still have beneficial effects and so this dose cannot be considered inadequate at this stage. Instead of the terms ‘adequate’ and ‘inadequate’, the terms ‘complete’ and ‘partial’ dose shall by used.

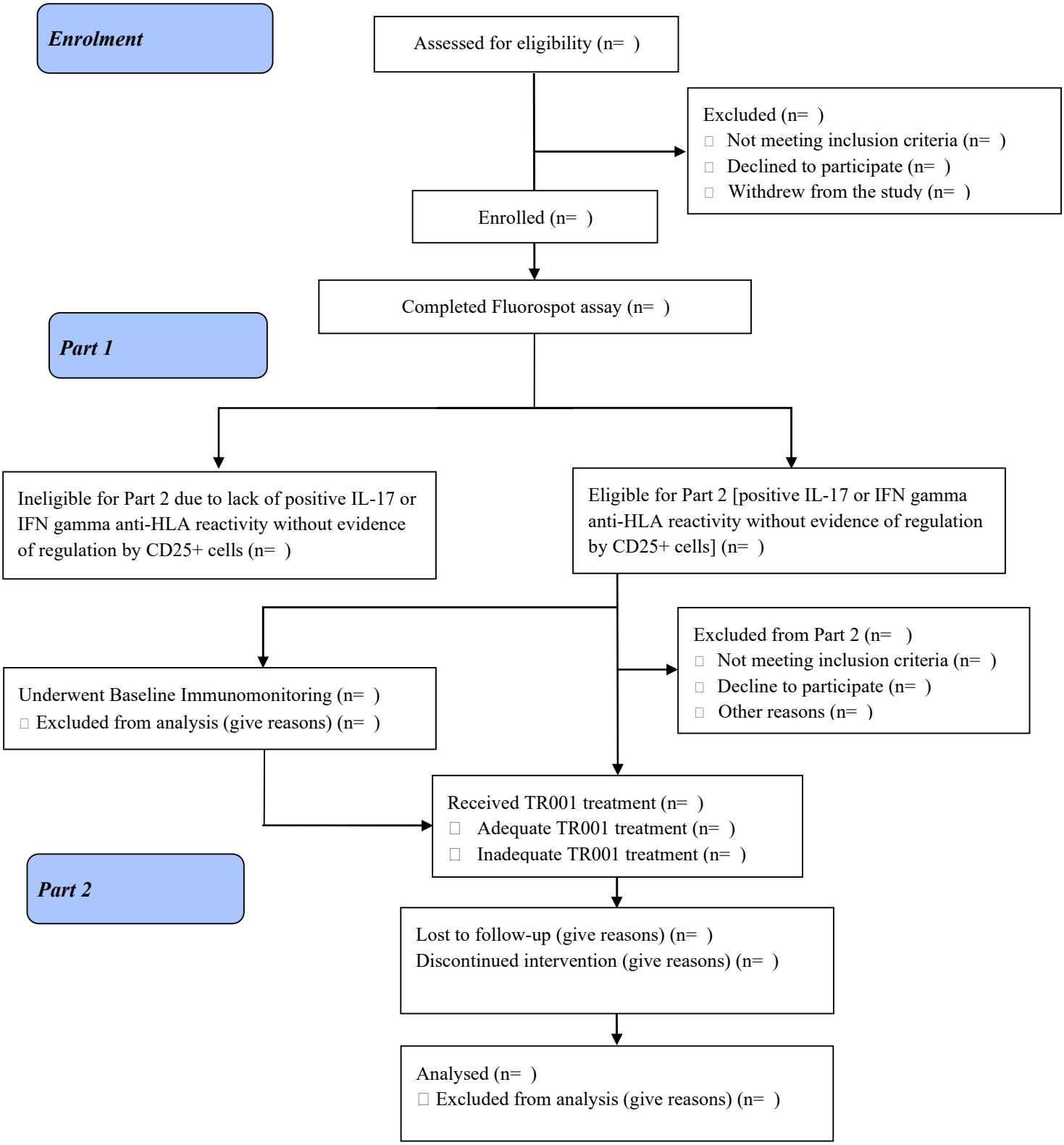

Figure 2. Template CONSORT diagram for GAMECHANgER-1 trial.

2.2 Baseline comparability of patient subpopulations

Baseline descriptions of different patient subpopulations will be reported as means and standard deviation or numbers and proportions as appropriate. These subpopulations include: patients eligible for Part 2 compared to patients not eligible for Part 2 (based on the positive IL-17 or IFN gamma anti-HLA reactivity without evidence of regulation by CD25+ cells), eligible patients administered TR001 compared to eligible patients who were not administered TR001. No significance testing will be used.

2.3 Adherence to allocated treatment and treatment fidelity

The reasons for withdrawal from treatment will be summarised. No comparison of compliant vs. non-compliant will be made as this treatment is a single dose treatment administered by intravenous infusion.

2.4 Loss to follow-up and other missing data

The proportions of participants missing each variable at each timepoint will be summarised for patient undergoing baseline immunomonitoring and patients who received treatment in Part 2. The baseline characteristics of those missing follow up will be compared to those with complete follow up. Reasons for withdrawal from the trial will be summarised. This includes patient who are deemed no longer eligible for the trial at Part 2.

2.5 Adverse event reporting

Adverse events (AE), adverse reactions (AR), serious adverse events (SAE) and serious adverse reactions (SAR) will be summarised. Partial adverse event monitoring (AE's that reports as related to study procedures (i.e blood taking)) will be reported in Part 1 of the study. Full adverse event monitoring will begin with the administration of TR001.

2.6 Descriptive statistics for outcome measures

Primary Outcome

The primary outcome will be reported as the number and percentage (with confidence interval) of treated patients show suppression of a defined HLA-specific-Fluorospot response for at least 2 months post-treatment. That is, the point estimate of suppression, 2 sided 95% confidence interval and p-value using exact methods will be calculated as the primary analysis. To achieve a clinically significant results it is required to have at least subjects ≥50% achieve suppression after treatment with Treggs for at least 2 months.

In addition, the number and percentage of treated patients demonstrating suppression of a defined HLA specific-Fluorospot response at each time point will be reported. Point estimates, 2 sided 95% confidence intervals and p-values using exact methods will be calculated as per the primary endpoint. The comparative values from the patients undergoing baseline immunomonitoring (defined HLA specific-Fluorospot response suppression for 2 months and at each timepoint) will also be reported.

Secondary outcomes

The number and percentage (with confidence interval) of patients who show positive IL-17 or IFN gamma anti-HLA reactivity without evidence of regulation by CD25+ cells in Part 1 of this trial will be reported. No significance testing will be used.

The duration of defined HLA specific-Fluorospot response suppression for each treated patient will be calculated and reported as median and interquartile range. The same calculation will be made for patient undergoing baseline immunomonitoring.

The number of adverse events related to study procedures reported in Part 1 of the trial and until Treg administration in Part 2 will be reported. No significance testing will be used.

The number of adverse events reported from Treg administration until the following time intervals will be reported: within 2 weeks of treatment, within 2 months of treatment, within 6 months of treatment, until study end. The number and percentage of these events that are deemed to be associated with Treg treatment will also be reported. No significance testing will be used.

For the exploratory mechanistic endpoints:
The recorded number of Treg cells at each timepoint post-treatment will be reported. These numbers will be further broken down by phenotype.

The number and percentage of treated patients at each timepoint with each predominant HLA Ab profile will be reported.

3. Data analysis plan – Inferential analysis

3.1 Main analysis

The main statistical analyses will assess the biological activity of TR001 in terms of the suppression of defined HLA specific responses and its duration.

Analysis of primary outcome

In addition to the primary analysis, an exact test of the proportions of patients showing suppression of a defined HLA specific-Fluorospot response for 2-months post treatment with the proportion of patients undergoing baseline immunomonitoring showing suppression of a defined HLA specific-Fluorospot response for 2-months.

Analysis of secondary outcomes

The duration of suppression of HLA-specific responses by Tregs caused by TR001 treatment will be examined using a generalised linear mixed model examining the binary outcome ‘suppressed HLA-specific response’ measured at each timepoint. This will demonstrate whether there is a change in this outcome over the course of the observation period. Patient-level covariates will be accounted for in these analyses, if possible, as will the route of HLA sensitisation.

A regression model incorporating data from both treated patients and patients who underwent baseline immunomonitoring will also be explored, allowing the natural variability of HLA specific responses to be incorporated into the model. This model will investigation whether suppression of HLA-specific responses change over time and after treatment.

3.2 Statistical considerations

Missing outcome data

It is expected that approximately 50% of patients over 12 months will have to withdraw from the study to receive transplants. As such there is likely to be a large degree of missing data within this study, particularly at the last timepoints (6 and 12 months post-treatment). To avoid an inability to complete the statistical analysis, the analysis will focus on the first 2 months post-treatment (as specified by the primary outcome). The secondary analyses will use generalised linear mixed models which allows valid inferences to be made under the assumption that the missing data mechanism is ignorable (or MAR). If the data loss at 6 and 12 months post-treatment is deemed substantial, the analyses will focus on the first 2 months post-treatment and specify that the inferences are only made until 2 months post-treatment.
If possible, methods to account for missing values, such as multiple imputation, will be used – provided data collected within the trial is predictive of patient withdrawal.

Missing baseline data

Missing baseline data should not be an issue for the primary analysis. Some extensions to this analysis may use baseline variables as covariates. If these contain missing data, the number of patients with complete data will be reported and they will be imputed using a method suitable to the variable as per the recommendations of White and Thompson (lost ref).

Method for handling multiple comparisons

Bonferroni adjustment for multiple comparisons will be used where necessary.

Model assumption checks

The generalised linear mixed models used for the secondary outcomes assume normally distributed residuals; these will be plotted to check for normality and inspected for outliers.

3.3 Sensitivity analyses

N/A

3.4 Planned subgroup analyses

Analyses will be repeated as described above for patients who received a complete dose of TR001 and patients who received a partial dose of TR001 separately (unless all patients received a complete dose of TR001 in which case this will not be necessary).

In addition, the outcomes relating to suppression of HLA specific responses in comparison with data from baseline immunomonitoring will be analysed using only data from patient who underwent

baseline monitoring and treatment (if applicable), with the methods refined to allow for paired comparisons.

3.5 Exploratory analyses

The changes in Treg number and phenotype (post-treatment compared to baseline) and the changes in HLA Ab profiles are considered exploratory endpoints. Therefore, these outcomes will be measured in exploratory analyses assessing how Treg number/phenotype and HLA ab profiles change over time. These analyses will be completed using generalised linear mixed models with exact details of the model dependent on the outcome in question and the natural variance seen with the data. Patient-level covariates will be accounted for in these analyses, if possible, as will the route of HLA sensitisation.

3.6 Interim analysis

After the first 12 patients have been treated with TR001 (completion of stage 1 in Part 2), the trial will pause for an interim analysis assessing futility. The number of patients demonstrating suppression of anti-HLA responses for at least two months post treatment will be determined. If 2 or fewer patients reach this endpoint, the trial will be stopped for futility. It 6 or more patients reach this endpoint, the treatment will be deemed non-futile and continue, recruiting a further 9 patients for treatment (stage 2). In the case that 3-5 patients out of the 12 patients in stage 1 demonstrating suppression of anti-HLA responses for at least two months post treatment, the trial will be paused, and the sample size recalculated after discussion with the funders.

4. Software

Data management: Describe the systems used and mechanism for requesting data extracts (include randomisation systems, outcome data, safety data plus any ancillary datasets used such as direct patient data entry systems, laboratory systems or others where data will be exported and passed directly to the statisticians for analysis)

Data relating to the primary outcome, the three main secondary outcomes, and safety is being hosted and managed by KCTU using a KCTU Elsevier Macro 4 EDC system dataset. Source data worksheets will also form part of the participants NHS medical notes.
All requests for access to the data entry system must be authorised by the trial manager. All requests for data exports must be authorised by the trial statistician.
No data will be entered unless a participant has signed a consent form to participate in the trial.
Data relating to the exploratory mechanistic endpoints will be treated differently. A copy of the raw unmanipulated data, labelled with data, PIN and type of analysis will be stored securely as soon as possible after obtained. Descriptive analyses will be prepared from cleaned data by the trial statisticians. Outlying data will be compared to raw data files for validation.

Analyses of this data will be complete in R. Scripts used to perform this analysis will saved in a secure location and clearly described to allow analyses to be reviewed as necessary.

B) ECONOMIC ANALYSIS PLAN

Economic measures will not be assessed as part of this trial.

C) SCHEDULE OF ASSESSMENTS AND MEASURES

Amendments to version XXX

LIST HERE ANY AMENDMENTS TO THE SAP THAT WERE MADE AFTER THE SAP WAS SIGNED OFF BY THE TSC

D) APPENDIX

1. Table Shells

1.1 Summary of Patient Status

Protocol No/Study ID:
Page x of x

Table XX.X.X Summary of Patient Status

Population Subset	n (%)
Number of Patients Screened	xxx (100.0)
Number of Screen Failure	xxx (xx.x)
Number of Patients Entered into Part 1	xxx (xx.x)
Number of Patients Discontinued During Part 1	xxx (xx.x)
Completers for Part 1(a)	xxx (xx.x)
ITT Population Part 1 (b)	xxx (xx.x)
Number of Patients Not Eligible for Part 2	xxx (xx.x)
Number of Patients Considered for Part 2	xxx (xx.x)
Number of Patients Progressing to Part 2	xxx (xx.x)
Number of Patients Entered into Part 2	xxx (xx.x)
Number of Patients Discontinued During Part 2	xxx (xx.x)
Completers for Part 2 (a)	xxx (xx.x)
ITT Population Part 2 (b)	xxx (xx.x)

(a) <The ITT population defined as per SAP>.
(b) <The completers for study are defined as per SAP>.

Source Listing: XX.X.X
Program ID: Study xxxxxx.SAS. Source Data: xxxx File ID: xxxxxx.HTM. Transfer Date: DDMMMYYYY Runtime ID: DDMMMYYYY HH:MM

<Include the appropriate footnote for population and completers as per SAP>

1.2 Demographic and Baseline Characteristics

Protocol No/Study ID:
Page x of x

Table XX.X.X Summary of Demographic and Baseline Characteristics by Treatment in Part 1 of the Study

Characteristic	(N=xxx)
Age (years)	
n	xxx
Mean (SD)	xx.x (xx.x)
Median	xx.x
Min, Max	xx, xx
Sex n (%)	
Male	xxx (xx.x)
Female	xxx (xx.x)
Race n (%)	
White	xxx (xx.x)
Asian	xxx (xx.x)
Black	xxx (xx.x)
Mixed	xxx (xx.x)
Other	xxx (xx.x)

Abbreviation: SD = Standard Deviation.
Note: N = number of patients in the treatment group analysis set, n = Number of subjects in the specified category with non-missing values.

Source Listing: XX.X.X
Program ID: Study xxxxxx.SAS. Source Data: xxxx File ID: xxxxxx.HTM. Transfer Date: DDMMMYYYY Runtime ID: DDMMMYYYY HH:MM

Repeat Table for Part 2 of the Study: Table XX.X.X Summary of Demographic and Baseline Characteristics by Treatment in Part 2 of the Study

1.3 Primary Outcome

Protocol No/Study ID:
Page x of x

Table XX.X.X Primary Endpoint: Suppression of an HLA-Specific Fluorospot Response – Visit 8

	Proportion of Suppressed Responses (N=21)	(Confidence Interval) P-Value
Stage 1	xx/12 (n%)	
Stage 2	xx/9 (n%)	
Total	xx/21 (n%)	(xxx, xxx) xxxx

Note: N = number of patients in the treatment group analysis set, n = Number of subjects in the specified category with non-missing values.
HLA Specific Fluorospot Responses is Defined as <To be added>

Source Listing: XX.X.X
Program ID: Study xxxxxx.SAS. Source Data: xxxx File ID: xxxxxx.HTM. Transfer Date: DDMMMYYYY Runtime ID: DDMMMYYYY HH:MM

**Repeat Table for other Visits: Table XX.X.X Primary Endpoint: Suppression of an HLA-Specific Fluorospot Response – Week (Treg Infusion, 2, 3, 4, 5, 6, 7) **

1.4 Secondary Outcomes

Secondary outcomes table shell to be added.

1.5 Adverse Events

Protocol No/Study ID:
Page x of x

Table XX.X.X Summary of Adverse Events in Part 1 of the Study

	(N=xxx) n (%)
Patients with any Adverse Event	xx (xx.x)
Patients with any Serious Adverse Event	xx (xx.x)
Patients with any Treatment Emergent Adverse Event (TEAE)	xx (xx.x)
Patients Withdrawn from Treatment due to Adverse Event	xx (xx.x)

Note: Percentage (%) based on number of patients in the row category/N within the column category.
Treatment emergent adverse event is defined as any event that occurs on or after the first dose of study drug administration or any pre-existing event which worsened in severity after dosing.

Source Listing: XX.X.X
Program ID: Study xxxxxx.SAS. Source Data: xxxx File ID: xxxxxx.HTM. Transfer Date: DDMMMYYYY Runtime ID: DDMMMYYYY HH:MM

Repeat Table for Part 2 of the Study: Table XX.X.X Summary of Adverse Events in Part 2 of the Study

Protocol No/Study ID:
Page x of x

Table XX.X.X Number (%) of Patients Reporting Treatment Emergent Adverse Events (TEAE) by System Organ Class and Preferred Term in Part 1 of the Study

System Organ Class (a) Preferred Term	(N=xxx) n (%)
Patients with Any TEAE	xx (xx.x)
System Organ Class 1	xx (xx.x)
Preferred Term 1	xx (xx.x)
Preferred Term 2	xx (xx.x)
Preferred Term 3	xx (xx.x)
System Organ Class 2	xx (xx.x)
Preferred Term 1	xx (xx.x)
Preferred Term 2	xx (xx.x)
Preferred Term 3	xx (xx.x)

Note: Treatment emergent adverse event is defined as any event that occurs on or after the first dose of study drug administration or any pre-existing event which worsened in severity after dosing.
Totals for the number of patients at a higher level are not necessarily the sum of those at the lower levels since a patient may report two or more different adverse events within the higher-level category.

Source Listing: XX.X.X
Program ID: Study xxxxxx.SAS. Source Data: xxxx File ID: xxxxxx.HTM. Transfer Date: DDMMMYYYY Runtime ID: DDMMMYYYY HH:MM

<Programming note: SOC and preferred terms are sorted in alphabetical order>

Repeat Table for SAE: Table XX.X.X Number (%) of Patients Reporting Serious Adverse Events (SAE) by System Organ Class and Preferred Term in Part 1 of the Study

Repeat Table for Patients Withdrawn due to AE: Table XX.X.X Number (%) of Patients Withdrawn from Treatment due to Adverse Event by System Organ Class and Preferred Term in Part 1 of the Study

Repeat Table for Part 2 of the Study: Table XX.X.X Number (%) of Patients Reporting Treatment Emergent Adverse Events (TEAE) by System Organ Class and Preferred Term in Part 2 of the Study

Repeat Table for Part 2 of the Study: Table XX.X.X Number (%) of Patients Reporting Serious Adverse Events (SAE) by System Organ Class and Preferred Term in Part 2 of the Study

* Repeat Table for Part 2 of the Study: Table XX.X.X Number (%) of Patients Withdrawn from Treatment due to Adverse Event by System Organ Class and Preferred Term in Part 2 of the Study*

Protocol No/Study ID:
Page x of x

Table XX.X.X Number (%) of Patients Reporting Treatment Emergent Adverse Events (TEAE) by CTC Severity and Treatment Group in Part 1 of the Study

System Organ Class (a) Preferred Term	Mild n (%)	Moderate n (%)	Severe n (%)	Total n (%)
Patients with Any TEAE	xx (xx.x)	xx (xx.x)	xx (xx.x)	xxx
System Organ Class 1	xx (xx.x)	xx (xx.x)	xx (xx.x)	xxx
Preferred Term 1	xx (xx.x)	xx (xx.x)	xx (xx.x)	xxx
Preferred Term 2	xx (xx.x)	xx (xx.x)	xx (xx.x)	xxx
Preferred Term 3	xx (xx.x)	xx (xx.x)	xx (xx.x)	xxx
System Organ Class 2	xx (xx.x)	xx (xx.x)	xx (xx.x)	xxx
Preferred Term 1	xx (xx.x)	xx (xx.x)	xx (xx.x)	xxx
Preferred Term 2	xx (xx.x)	xx (xx.x)	xx (xx.x)	xxx
Preferred Term 3	xx (xx.x)	xx (xx.x)	xx (xx.x)	xxx

Note: Treatment emergent adverse event is defined as any event that occurs on or after the first dose of study drug administration or any pre-existing event which worsened in severity after dosing.

If a patient experiences adverse events within the same SOC and PT at different severity levels, only the worst severity will be presented for that SOC and PT.

(a) Totals for the number of patients at a higher level are not necessarily the sum of those at the lower levels since a patient may report two or more different adverse events within the higher-level category. ‘n’ is the total count of all grades.

Source: Listing XX.X.X

Program ID: Study xxxxxx.SAS. Source Data: xxxx File ID: xxxxxx.HTM. Transfer Date: DDMMMYYYY Runtime ID: DDMMMYYYY HH:MM

<Programming note: SOC and preferred terms are sorted in alphabetical order>

*Repeat Table for Part 2 of the Study: Table XX.X.X Number (%) of Patients Reporting Treatment Emergent Adverse Events (TEAE) by CTC Severity and Treatment Group in Part 2 of the Study *