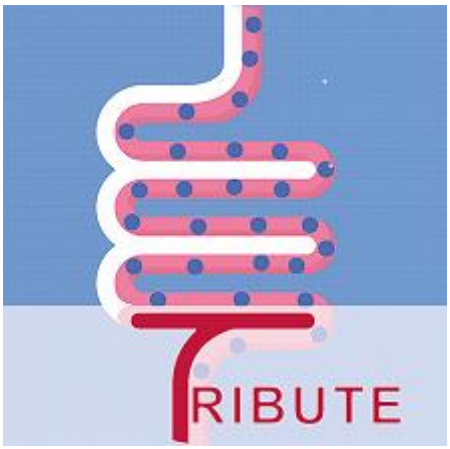

A first in human feasibility study of T regulatory cells (TR004) for Inflammatory Bowel disease Using (ex vivo) Treg Expansion

Study Acronym - **TRIBUTE**
Short Title - Treg Immunotherapy in Crohn's Disease
Trial Protocol
Version 1 14 March 2022

Trial Identifiers

EudraCT Number: 2017-000170-11

IRAS Number: 1003824

REC Number: 22/NE/0062

ISRCTN Number: TBC

ClinicalTrials.gov: TBC

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SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

Chief Investigator:

Signature:  Date: 16/03/2022

.....

Name (please print):
Dr Peter Irving

Lead Statistician:

Signature: Date:

..../...../.....

Name (please print):

.....

Table of Contents

1 Study Synopsis and General Information

Title of clinical trial	A first in human feasibility study of T regulatory cells (TR004) for Inflammatory Bowel disease Using (ex vivo) Treg Expansion
Protocol Short Title	Treg Immunotherapy in Crohn's disease
Protocol Acronym	TRIBUTE Feasibility
Trial Phase	IB
Medical condition or disease under investigation	Inflammatory Bowel Disease – Crohn's Disease (CD)
Purpose of clinical trial	A feasibility study to explore the safety profile of a single dose of TR004 in patients with moderate to severe Crohn's Disease and assess feasibility for a larger phase IB/IIA trial
Primary Clinical Objective	• To explore the preliminary safety and tolerability of a single dose administration of TR004 in patients with moderate to severe CD who are refractory or intolerant to standard treatment
Feasibility Objective	• To pilot the operation of the clinical protocol and inform the design of a subsequent larger trial
Secondary objectives	• To investigate the clinical and immunological responses to TR004 administered intravenously in patients with moderate to severe CD • To assess the lifespan and tissue localisation of infused T regulatory cells (TR004) • To investigate the timing of DLTs and other safety events from the time of infusion
Endpoints	Primary Clinical Endpoint: • Occurrence and nature of Dose-Limiting Toxicities (DLTs) occurring within 5 weeks post-infusion Feasibility Endpoints: • Amount of TR0004 manufactured per patient • Number of participants recruited within the duration of the trial • Number of study visits completed

	• Responses to items in questionnaires or surveys exploring the experience of the participants, trial team and DSMB members
	
	Secondary Endpoints:
	• Assessment of clinical response:
	  ○ Disease Activity Score (CDAI / PRO-2),
	  ○ Biomarkers analysis (CRP, FCP),
	  ○ Mucosal healing response(SES-CD)
	• Immunological response in blood and intestinal lamina propria (LP): numbers and functions of Tregs, measurement of deuterium-enriched cells, cytokine levels, comparison of circulating and localised cells to determine differences and similarities
	• Description of non-DLT adverse events and those occurring beyond week 5

Trial Design	A first-in-human, single dose, open label, single centre feasibility study
Sample Size	4 patients
Summary of eligibility criteria	Main Inclusion Criteria: • Able to give written informed consent • Diagnosis of moderate to severe Crohn's disease • Active CD including ulceration (as assessed by colonoscopy at screening) • Failure to tolerate or respond, or lost response, to at least 2 prior lines of standard CD medications Main Exclusion Criteria: • Diagnosis of ulcerative colitis or IBD-unclassified • CD treatment-naïve patients • Ileostomy or colostomy • History of tuberculosis unless fully treated • Severe congestive heart failure • History of dysplasia of gastrointestinal tract within 12 months of consent • Previously received stem cell transplant • History of malignancy within last 5 years excluding non-melanoma skin cancer, successfully treated squamous cell

	or basal cell carcinoma without metastases, or localised carcinoma in situ of the cervix. • Hb < 100g/L or WBC < 3.5 x 10^9/L or Plt < 100 x 10^9/L • Creatinine > 1.5 ULN • Total bilirubin > 34µmol/L or ALT > 2ULN or GGT > 2ULN
ATIMP, dosage and route of administration	3.0 – 5.0 x 10^6 TR004/kg
Comparator product(s)	No comparator
Study duration from FPFV to database lock	32 months (24 month follow up per patient plus 8 month recruitment period)
Maximum trial duration per patient	24 months (including safety follow up)
Number of trial sites	One – GSTT

2 Abbreviations and Glossary

ALT	Alanine Transaminase
AST	Aspartate Transaminase
ATIMP	Advanced Therapy Investigational Medicinal Product
BSG	British Society of Gastroenterology
C&S	Culture and Sensitivity
CD	Crohn's Disease
CDAI	Crohn's Disease Activity Index
CI	Chief Investigator
CRA	Clinical Research Associate
CRF	Clinical Research Facility
CRM	Continual Reassessment Method
CRP	C-Reactive Protein
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CXR	Chest X-Ray
DLT	Dose-Limiting Toxicity
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Form
FBC	Full Blood Count
FCBP	Female of Child Bearing Potential
FCP	Faecal Calprotectin
GCP	Good Clinical Practice
GSTFT	Guy's and St Thomas' NHS Foundation Trust
GMP	Good Manufacturing Practice
GvHD	Graft Vs Host Disease
Hb	Haemoglobin
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
Hp	Haptoglobin
HSA	Human Serum Albumin
IBD	Inflammatory Bowel Disease
IBDQ	Inflammatory Bowel Disease Questionnaire
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IM	Immune Monitoring
IMP	Investigational Medicinal Product
IMPD	IMP Dossier
IV	Intravenous
LDH	Lactate Dehydrogenase

LP	Lamina Propria
MED	Minimum Effective Dose
MTD	Maximum Tolerated Dose
MRI	Magnetic Resonance Imaging
nTreg	natural Treg
PB Treg	Peripheral Blood Treg
PI	Principal Investigator
PIS	Participant Information Sheet
RN	Research Nurse
rTreg	Resting Treg
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SES-CD	Simple Endoscopic Score for Crohn's Disease
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Tuberculosis
TCR	T Cell Receptor
Tcon	Conventional T cell
Teff	Inflammatory Effector T-Cell
Treg	Regulatory T Cell
tTreg	"Thymus-derived" regulatory T Cell
TMF	Trial Master File
TSC	Trial Steering Committee
TSH	Thyroid Stimulating Hormone
U/S	Ultrasound

3 Background and Rationale

3.1 Background

Crohn's disease (CD) is a chronic, immune-mediated inflammatory bowel disease (IBD) with no known cure, resulting in significant morbidity. Goals of therapy include resolution of symptoms and mucosal healing. However, many patients have sub-optimal responses to currently available therapies. This represents a significant unmet medical need.

"Thymus-derived" regulatory T Cells (tTreg) are effective in modulating immune responses and when expanded to therapeutically relevant numbers *in vitro* they show promise in pre-clinical models of IBD. This has led to interest in this therapeutic approach in Crohn's disease. Building on our expertise in developing GMP-grade Treg manufacturing to reduce immunosuppressant burden in renal (ONE study) and liver transplantation (ThRIL study), we propose to translate these recent discoveries into a novel therapy for CD patients.

Our published data (Canavan *et al.*, 2016, Golberg *et al*., 2019, Clough *et al*., 2020) show that the starting population for tTreg expansion from the peripheral blood (PB) of CD patients has a critical effect on the phenotype of the expanded cell population. In CD patients, tTregs expanded from a highly pure FACS-sorted "naïve" $CD4^{+}CD25^{hi}CD127^{lo}CD45RA^{+}$ tTreg precursor population have substantial advantages over tTregs expanded from FACS-sorted $CD4^{+}CD25^{hi}CD127^{lo}CD45RA^{-}$, or MACS-enriched $CD8\text{-}CD25^{+}$ precursor populations.

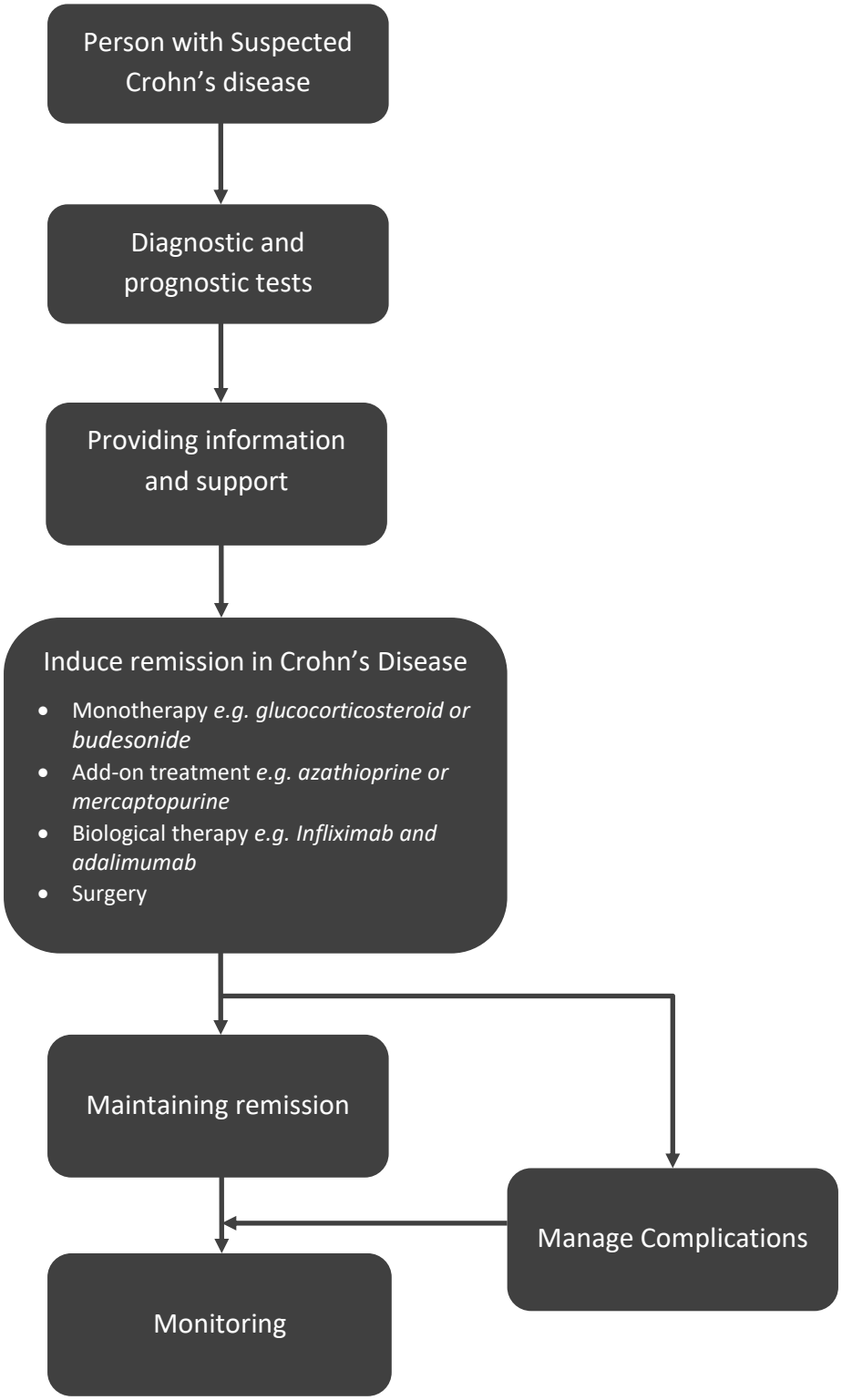
These include:

1. Median 98.2% (IQR 97.1-98.4%, n=12) $CD4^{+}CD25^{hi}CD127^{lo}$ purity after 24d expansion, allowing precise definition of each clinical preparation, and increased *in vitro* suppressive ability in comparison to the other cell preparations described above.
2. 100% FOXP3 TSDR demethylation and reduced Th17 plasticity *in vitro*, in comparison to other cell preparations, reducing the risk of in vivo conversion to an effector phenotype.
3. A demonstrated ability to traffic to human intestine in vivo, in a humanised mouse model.
4. A demonstrated ability to suppress activation of mucosal T cells *in vitro*, obtained from inflamed CD intestinal resections.

TR004 addresses this unmet need by translating our pre-clinical and patient studies into a clinical-grade GMP flow cytometric sorting solution for the enrichment of highly pure $CD45RA^{+}$ tTregs from CD patients with GMP cell expansion.

3.2 Current Clinical Management Pathway

According to NICE guidelines, this is a summary of the clinical management pathway for Crohn's disease (National Institute for Health and Clinical Excellence 2019).



Crohn's disease clinical diagnosis incorporates medical history and physical findings with objective data from imaging, endoscopy and laboratory studies (Baumgart & Sandborn 2012). Important differential diagnoses need to be excluded, for example irritable bowel syndrome or infection (Baumgart & Sandborn 2012). Patients are given information and advice about Crohn's disease, as well as information about treatment options and monitoring (National Institute for Health and Clinical Excellence 2019)
Two main treatment paradigms exist for CD:

1) "Bottom–up" therapy: inducing remission with steroids, with subsequent introduction of immunomodulators (azathioprine/mercaptopurine/methotrexate); corticosteroids induce remission in 58% (with response in a further 26%), but one year later, 28% are steroid-dependent and 1/3 require surgery (Faubion *et al.* 2001). Further escalation to biological therapy is used when immunomodulators fail to induce remission, and
2) "Top-down" therapy: inducing remission with biologics +/- azathioprine. In theory, withdrawal of biologics might be possible in such cases.

One study compared "Top-down" infliximab/azathioprine to induce or maintain remission vs. conventional "bottom-up" therapy, finding 60% vs. 36% steroid-free remission rates at 26 weeks, $p<0.006$ (D'Haens *et al.* 2008). In another study, 26 week remission rates for infliximab/azathioprine vs. infliximab alone were 57% vs. 44%, $p<0.02$ (Colombel *et al.* 2010). Therefore, even with early aggressive therapy, a substantial proportion of patients have sub-optimal therapeutic responses.

CD is also associated with the need for surgery: 12% of patients have undergone surgery 1 year after diagnosis (Vind *et al.* 2006) and 44% at 9 years (Peyrin-Biroulet *et al.* 2011). The advent of azathioprine has been associated with only a 10 – 15% reduction in 10 year surgical rates (45% to 35%) (Bernstein *et al.* 2012).
CD medications may also be associated with side-effects such as anaemia, pancreatitis lymphoproliferative disease (thiopurines (Beaugerie *et al.* 2009) and infection (infliximab/steroids (Lichtenstein *et al.* 2012).

It is hoped that the product under investigation in this trial, TR004, will prove to be a suitable option for inducing remission in Crohn's disease, without the side effects seen with current therapies or the need for surgery.

3.3 Rationale

The current requirement for novel therapies in Inflammatory Bowel Disease

With an annual cost to the NHS of over £900 million, a rising incidence and a prevalence approaching 0.5%, inflammatory bowel disease (IBD) is a prominent and costly cause of chronic morbidity in the UK.
IBD comprises two conditions characterised by chronic, idiopathic inflammation of the intestine: ulcerative colitis (UC) and Crohn's disease (CD), both of which result in considerable morbidity, reduced quality of life and significant occupational loss. Goals of therapy include resolution of symptoms and induction of mucosal healing. Many patients have a sub-optimal or a non-sustained response to current therapies used to induce or maintain remission, or develop side effects from

medications. Despite a modest reduction in surgery rates in the era of azathioprine, CD continues to be associated with increased mortality in cohort studies. All of these factors indicate a pressing need for novel therapeutic modalities.

Regulatory T Cells and Mucosal Inflammation in Inflammatory Bowel Disease

There remains an unmet need to develop novel therapies for CD, as current drug treatments frequently fail to maintain long-term remission and may be complicated by significant side effects. Currently available CD therapies (e.g. corticosteroids, anti-TNF, vedolizumab, ustekinumab, azathioprine and methotrexate) seek to reduce immune activation in the gut by targeting effector immune mechanisms.

Cellular therapies are emerging as potentially attractive therapeutic strategies (Canavan *et al.* 2015). We will use autologous, *in vitro* expanded Tregs as a parenteral cell-based therapy to augment regulatory immune responses in the gut. We recently identified the optimum cell population from which to expand Tregs from CD peripheral blood (PB) for potential therapeutic use (Canavan *et al.* 2015). We have also optimised *in vitro* expansion of Tregs using GMP grade reagents (Canavan *et al.* 2015) (Sagoo *et al.* 2011) (Scotta *et al.* 2013).

The Rationale for *In Vitro* Expanded Regulatory T Cells as a therapy for Crohn's Disease

The maintenance, or indeed loss, of intestinal homeostasis hinges on the balance between inflammatory effector T-cells (Teff), which have been implicated in auto-immunity and transplant rejection and a population of immunoregulatory T cells (Treg)(Di Ianni *et al.* 2011; Valencia *et al.* 2007; Trzonkowski *et al.* 2009). Tregs are a unique subset of CD4+ T-cells with powerful immunosuppressive action. They are defined by expression of the master transcriptional regulator FOXP3 and a set of key surface markers (Sakaguchi *et al.* 2010; Miyara *et al.* 2009; Sagoo *et al.* 2008). Tregs serve to limit immune-mediated pathology; mice or humans lacking functional Tregs develop severe multisystem inflammatory disease, including chronic intestinal inflammation (IPEX syndrome) (Katoh *et al.* 2013).

We have established a clinical protocol for GMP compliant large-scale expansion of regulatory T cells from patients on the waiting list for liver and kidney transplants. Two clinical trials headed by our BRC centre completed in 2018 (ONE Study and ThRIL). Mirroring efforts have been made by other groups in diseases such as graft vs host disease and Type 1 Diabetes (Di Ianni *et al.* 2011; Trzonkowski *et al.* 2009; Brunstein *et al.* 2011; Marek-Trzonkowska *et al.* 2012).

Lamina propria (LP) Tregs are increased in the mucosa of patients with active Crohn's disease and decreased in blood, compared to healthy controls (Maul *et al.* 2005; Saruta *et al.* 2007; Reikvam *et al.* 2011). LP Tregs taken from inflamed CD mucosa suppress proliferation of conventional $CD4^{+}CD25^{lo/int}$ T cells (Tcon) obtained from blood but not LP Tcons (Fantini *et al.* 2009), suggesting that mucosal Tcon in active CD may be resistant to Treg-mediated suppression. However, we and others have shown that *in vitro* expanded Tregs generated from blood in the presence of rapamycin are more potently suppressive than freshly isolated Tregs (Afzali *et al.* 2013; Cao *et al.* 2010). Thus, *in vitro* expanded Tregs from blood may overcome mucosal Tcon resistance to suppression. Consequently, parenteral therapy with autologous *in vitro* expanded Tregs generated from Crohn's blood may be an attractive approach to address defects in, or resistance to, mucosal Treg function in CD.

The possibility of harnessing Tregs to treat IBD represents an exciting new era in cell mediated therapy. We, and others, have shown that Tregs can be infused into animals with IBD, which results in prevention or reversal of intestinal inflammation (Izcue *et al.* 2008; Coombes *et al.* 2005; Garrett *et al.* 2007; Mottet *et al.* 2003). The scene is now set to determine whether this exciting novel therapy can be effective for the treatment of human IBD.

In our pre-clinical studies, we have built on work to formulate a GMP-compatible method for isolation and expansion of Tregs from Crohn's blood that addresses barriers to Treg therapy for CD (Canavan *et al.* 2015).

These barriers include:

- The stability of Treg expanded from Crohn's blood

In vitro, expanded Tregs retain the ability to suppress proliferation of autologous $CD4^+CD25^-$ Tcons. In preclinical studies both $CD45RA^+$ and $CD45RA^-$ Tregs demonstrated specific suppression of Tcon proliferation above an 8:1 Tcon:Treg ratio (Fig 1D Canavan). $CD45RA^+$ and $CD45RA^-$ Tregs suppressed Tcon proliferation to an equivalent degree (fig 1E Canavan) and reduced IL-2 expression in 96h co-culture supernatants (fig. S2A Canavan). $CD45RA^+$ Tregs also suppressed IFN-γ expression in 96h co-culture supernatants (fig S2A Canavan).
Our data have shown that it is feasible to select and expand *in vitro* Tregs obtained from Crohn's blood, including patients receiving thiopurines or anti-TNF medications to clinically useful numbers under GMP compatible conditions (Hoffmann *et al.* 2004; Golovina *et al.* 2011; Scotta *et al.* 2013; Brunstein *et al.* 2011; Battaglia *et al.* 2006; Tresoldi *et al.* 2011; Trzonkowski *et al.* 2009; Marek-Trzonkowska *et al.* 2012; Afzali *et al.* 2013). Even after prolonged culture, these Tregs maintained FOXP3 expression and suppressed activation of autologous T cells.

- The potential for adoptively transferred Tregs to express IL-17 and exacerbate Crohn's lesions

Pro-inflammatory cytokines (IL-2, IL-1, IL-6 and TGF-β or IL-2, IL-21, IL-23 and TGF-β) failed to induce IL-17 production by $CD45RA^+$ Tregs. In contrast, IL-17 production by $CD45RA^-$ Tregs was three-fold higher than $CD45RA^+$ Tregs in neutral conditions (IL-2 alone) and ten-fold higher in skewing conditions ($p<0.001$ each comparison).

- Mucosal T cells may be resistant to Treg-mediated suppression in active CD

Significant dose-dependent supression of CD154 expression in mesenteric lymph node (MLN) and LP T cells has been observed (fig 4d-E Canavan), demonstrating that *in vitro* expanded D24 $CD45RA^+$ Tregs not only suppress proliferation of MLN Tcons, but also supress early activation of MLN and LP Tcons *in vitro*. These results indicate that *in vitro* expanded $CD45RA^+$ Tregs may overcome Tcon resistance to Treg-mediated suppression seen in previous studies (Fantini *et al.* 2009; Monteleone *et al.* 2005) and may be biologically active in the immune niches directly relevant to the pathogeneis of CD.

- The capacity for expanded Tregs to express homing receptors for inflamed gut and lymphoid tissue

The expression of gut homing receptors by *in vitro* expanded Tregs has been examined by Fluorescence Activated Cell Sorting (FACS) (fig 3a–b Canavan). D24 $CD45RA^+$ Tregs modestly expressed $\alpha_4\beta_7$ integrin and CCR6 (20.8%±7.8% and 12.2%±7.9%, respectively) and did not express CCR9. Both CD62L (84.8%±20.6%; p=0.04 vs. $CD45RA^-$) and CCR7 (92.1%±12.8% p=0.03), required for lymph node homing were more highly expressed in $CD45RA^+$ Tregs than $CD45RA^-$ Tregs. CCR4 (95.4%±4.2%) was also highly expressed.
$CD4^+CD25^{high}CD127^{low}CD45RA^+$ Treg subset cultured in the presence of RAR568 expressed significantly more integrin $\alpha_4\beta_7$ than those cultured under standard conditions (rapamycin and IL-2 only) (95.9% ±1.93% vs 5.95% ±3.18% p<0.0001, Goldberg *et al*., 2019).

Safety Profile of *In Vitro* Expanded Regulatory T Cells: Side effects seen in recent clinical trials

Treg therapy is safe and well-tolerated in other conditions. Preliminary data shows that *in vitro* expanded $CD45RA^+$ Tregs are phenotypically stable and lack potentially pathogenic plasticity *in vitro*. Trzonkowski reported the delivery of flow-sorted, expanded nTreg to two HSC-transplant patients with GVHD (Trzonkowski *et al.* 2009). They treated one patient with chronic GvHD and one patient with acute GvHD with Tregs expanded from allogeneic CD4+CD25hiCD127lo precursors.

In the first patient, life-threatening drug-refractory acute GVHD, prompted the administration of expanded nTreg isolated from the HSC donor. Unfortunately, although administered nTreg could be detected in the circulation and a temporary improvement in symptoms was observed, the patient succumbed to graft-versus-host disease 112 days post-transplant. No further nTreg were available for additional infusions. In the second patient, chronic GVHD developed four months post-transplant and although this condition was managed with conventional immunosuppressive therapy for two years, the consequences of drug toxicity prompted a cell therapy approach. The patient received a single dose of $1x10^5$ expanded nTreg from the HSC donor. The patient experienced a significant improvement. PB Tregs, as a proportion of CD4+ cells, doubled from 2.5% to 5% six months later. Treg infusion was associated with an improvement in bronchiolitis obliterans, allowed a subsequent reduction in the dose of steroids (from 60mg per day to 5mg per day) and a complete cessation of mycophenolate mofetil. Importantly, the delivery of the nTreg was not associated with any detectable adverse events.

Brunstein *et al.* treated 23 patients with Tregs expanded *in vitro* from umbilical cord blood (UCB) after double UCB transplantation (Brunstein *et al.* 2011). 74% received the target dose. Infusion-related toxicities included hypertension (n=2) and neurologic changes that were attributed to pre-existing medications (n=2). Adoptively transferred Tregs were detectable in PB for up to 14 days following infusion. Engraftment, disease-free survival and opportunistic infections were comparable to historic controls, while the incidence of grade II-IV GvHD in UCB Treg recipients was 41%, compared with 61% for historical controls (p=0.05).

Marek-Trzonkowska *et al.* treated 10 patients with type 1 diabetes mellitus (T1DM) diagnosed in the preceding 2 months with Tregs expanded *in vitro* from autologous CD4+CD25hiCD127lo Tregs (Marek-

Trzonkowska *et al.* 2012). One patient was diagnosed with influenza the day after infusion. No other infusion-related toxicities were reported. 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.

Desreumaux *et al.* treated 20 patients with refractory Crohn's Disease in a 12-week, open-label, multicentre, single-injection, escalating-dose, phase Ib/IIa clinical study. Ovalbumin-specific Treg cells (ova-Tregs) were isolated from patients' peripheral blood mononuclear cells (PBMCs), exposed to ovalbumin, and administrated intravenously. Safety and efficacy were assessed using clinical and laboratory parameters. Proliferation of PBMCs in response to ovalbumin was evaluated.
Injections of ova-Tregs were well tolerated, with 54 adverse events (2 related to the test reagent) and 11 serious adverse events (3 related to the test reagent, all recovered). Overall, a response based on a reduction in Crohn's Disease Activity Index (CDAI) of 100 points, was observed in 40% of patients at weeks 5 and 8. Six of the 8 patients (75%) who received doses of 10(6) cells had a response at weeks 5 and 8, with a statistically significant reduction in CDAI. In this group, remission (based on CDAI ≤150) was observed in 3 of 8 patients (38%) at week 5 and 2 of 8 patients (25%) at week 8.
The conclusion was that administration of antigen-specific Tregs to patients with refractory CD was well tolerated and had dose-related efficacy. The ovalbumin-specific immune response correlated with clinical response, supporting immune-suppressive mechanisms of ova-Tregs. The consistency of results among different assessment methods supports the efficacy of ova-Tregs; this immune therapy approach warrants further clinical and mechanistic studies in refractory CD.

In a study published in 2015, Bluestone et al conducted a phase 1 dose escalation study of Treg therapy for T1DM. They treated 14 adult patients recently diagnosed with T1DM in four dosing cohorts with autologous $CD4^{+}CD25^{hi}CD127^{lo}$ polyclonal ex-vivo expanded Tregs. Doses ranged from 5×10^{6} to 2.6×10^{9} cells which were given in a single infusion. There were no infusion reactions. No opportunistic infections or malignancies occurred after a mean follow up of 31 months.

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. There were too few patients to draw conclusions about efficacy in this study.

In a study published in 2019, Sanchez-Fueyo *et al.* conducted an open-label, dose-escalation, Phase I clinical trial of autologous Treg adoptive transfer in liver transplantation at GSTT. Nine patients were treated in two cohorts with a single infusion of TR002. Three patients received 0.5-1 million Tregs/kg while awaiting liver transplantation and 6 patients were treated post-transplant with 3-4.5 million Tregs/kg. Adoptive transfer of Tregs was found to be safe; there was no increased incidence of infections or cancer. One patient receiving the higher dose experienced an infusion reaction, classed as a DLT. The patient developed high grade pyrexia 16 hours after the infusion, along with transient neutropenia, lymphopenia and mild liver graft dysfunction. Serum cytokine levels increased 1 day post infusion, with a gradual decrease by day 3 and total normalisation by day 7. As a result, the cohort was expanded from 3 to dose 6 patients. No other adverse events were observed. Circulating Tregs

were increased post infusion for up to 1 month. A gradual decrease of T cell responses against donor-type cells was observed at the higher dose.

Griessler *et al.* (2020) treated 12 participants with recipient-derived naturally occurring regulatory T cells (nTreg) as part of the international ONE study consortium – a group of six, non-randomised phase I/IIA studies of regulatory cell-based medicinal products in kidney transplantation. Participants received cell therapy alongside standard of care immunosuppression. Studies were compared with a reference group trial of participants receiving standard of care only. Living-donor kidney transplant recipients were recruited across two sites in the UK into a 3+3 design, testing four dose levels ranging from 0.5 – 10 million Tregs/kg. Participants received a single infusion of the cell therapy, five days post transplantation.

Results of the UK study were pooled and published as part of the ONE study, with a detailed paper of the Treg trial to follow. Analysis of the adverse event data showed no safety issues when compared 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.

Dong *et al.* (2021) treated 7 patients in a dose escalation study with a single infusion of autologous polyclonal Tregs in combination with two 5-day courses of recombinant human low-dose IL-2. Treg doses up to $20x10^6$/kg were used in combination with IL-2 which is a survival and growth factor for Tregs; in mouse models it has been shown to expand Tregs and treat autoimmune disease and is currently being used in multiple trials to treat autoimmune disease.

The trial was abandoned early as it did not show any sign of benefit. A drop in C-peptide was noted and possibly felt to be related to IL-2 administration but, on further investigation was felt to be disease-related (being found in a matched placebo-treated cohort from another trial). No safety signals were reported with 3 years of follow up other than an injection site reaction related to IL-2 administration.

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.

DOSING RATIONALE

Based on the safety signals seen in previous studies of ex-vivo expanded autologous Tregs (some of which have been deuterated) we propose to use a dose of $3.0\text{-}5.0x10^6$ Tregs/kg

Hypothesis

Tregs will “reset” the balance of the immune system and thus provide a safe, well-tolerated treatment for further investigation in patients with moderate to severe Crohn’s Disease.

4 Trial Objectives and Design

4.1 Trial Aims

- To explore the safety and tolerability of TR004 in participants with CD
- To assess the feasibility of the clinical trial protocol to inform future trial design

4.2 Trial Objectives

Primary Clinical Objective:

- To explore the preliminary safety and tolerability of a single dose of TR004 in patients with moderate to severe CD who are refractory or intolerant to standard treatment

Feasibility Objectives:

- To pilot the operation of the clinical protocol and inform the design of a subsequent larger trial
    - To assess the feasibility of manufacturing the IMP at the doses for this study and at higher doses to inform future trial design
    - To assess the feasibility of recruiting to time and target
    - To assess the feasibility of retaining participants for the duration of the study and completion of study visits and assessments
    - To understand the experience of the participants, trial team and DSMB members, exploring any barriers and challenges to trial performance, recruitment and retention

Secondary Objectives:

- To investigate the clinical and immunological responses to TR004 administered intravenously in patients with moderate to severe CD
- To assess the lifespan and tissue localisation of infused T regulatory cells (TR004)

To investigate the timing of DLTs and other safety events from the time of infusion

4.3 Trial Endpoints

Primary Clinical Endpoint:

- Rate of dose-limiting toxicities (DLTs) occurring within 5 weeks post-infusion of TR004

Feasibility Endpoint:

- Amount of TR0004 manufactured per patient
- Number of participants recruited within the duration of the trial
- Number of study visits completed
- Responses to items in questionnaires or surveys exploring the experience of the participants, trial team and DSMB members. This will not form part of the final trial report. Qualitative data collection and any data analysis will be separate from the trial.

Secondary Endpoints:

- Assessment of clinical response:
    - Disease Activity Score (CDAI / PRO-2),
    - Biomarkers analysis (CRP, FCP),
    - Mucosal healing response(SES-CD)

- Assessment of immunological response – in blood and intestinal lamina propria (LP):
    - numbers and functions of Tregs
    - measurement of deuterium-enriched cells
    - cytokine levels
    - comparison of circulating and localised cells to determine differences and similarities

- Description of non-DLT adverse events and those occurring beyond week 5

4.4 Trial Design

This study is an open label, first-in-human feasibility study of a single dose of TR004 in patients with moderate to severe CD.

Four participants will receive a single dose of TR004. Participants will be dosed singly. Safety data will be collected for five weeks post administration and reviewed by the DSMB before proceeding to dose

the next participant. All participants will be followed up to week 21 to collect further safety and exploratory efficacy data, with additional safety monitoring at 1 and 2 years post dose.

5 Trial Treatment

Regulatory T cells (Tregs) are naturally present in low numbers in peripheral blood (5-10% of peripheral $CD4^+$ T cells) but, for therapeutic use, a large number of these cells are required.

TR004 is a natural regulatory T cell (nTreg) product derived from autologous T cells isolated from PBMCs obtained by leukapheresis. PBMCs are then CD25 enriched. The resulting naïve $CD4^+CD25^+CD127^{low}CD45RA^+$ T cells are FACS sorted in order to isolate them. These cells will undergo polyclonal expansion to achieve the required doses. The resulting expanded $CD4^+CD25^+CD127^{low}CD45RA^+$ T cells are predominantly FOXP3 positive, a marker although not completely unique to nTreg, necessary to their function. TR004 will be manufactured as a frozen product in a CryoMACS Freezing Bag (50 ml ± 5%).

Refer to section 5.3 for detailed information about TR004 Manufacture.

5.1 TR004 Dosing Regimen and Administration

Eligible patients will receive a single dose of TR004 at 3.0 – 5.0 x 10^6 TR004/kg.

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 DSMB and statisticians should be informed.

There may even be instances where the patient cannot be dosed if TR004 manufacture fails. In such a situation, the PI would have to decide whether it is appropriate to re-screen the patient and repeat the leukapheresis. The patient's consent to be re-screened would have to be sought. Leukapheresis can be performed on a maximum of two occasions, at least six weeks apart.

Patients will be dosed in the CRF, 15th Floor, Tower Wing, at Guy's Hospital.

The TR004 IMP will be thawed by the GMP team, and the thawed 50 mL (3.0 – 5.0 x 10^6 TR004/kg) IMP bag will be spiked to a giving set for infusion. The procedure will be detailed in the TR004 Handling Instructions. The infusion will be administered intravenously by a suitably trained and delegated member of the study team.

5.2 TR004 Risk Assessment

All patients administered TR004 will be monitored as in-patients for 24 hours post infusion to enable close monitoring in case of acute toxicity and to facilitate ease of regular sample collection for safety monitoring.

The risk-benefit profile of this clinical trial essentially balances the expected clinical benefits of preventing or reversing intestinal inflammation against the potential clinical complications that may result from the infusion of the Treg cell product. Other risks can be attributed to certain trial specific procedures (e.g. colonoscopies, biopsies, additional blood collection).

The potential risks associated with Treg therapy can be categorised as immunological, physiological, infectious, or due to adverse interactions with other treatments. While the adaptive dose finding design combined with a minimum observation period of five weeks between patients should minimise these risks, it is nonetheless necessary to consider the potential risks in turn.

- 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 isotonic 3.5-5% human serum albumin and for this reason known allergies to components of the infusion are listed as exclusion criteria.

Patients will be admitted to the Clinical Research Facility for administration of Tregs, 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 anti-histamine.

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, may be responsible for adverse transfusion-associated responses, 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.

However, 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 will be treated prophylactically with paracetamol, orally, prior to infusion.

Type III hypersensitivity reactions:

Although once a common sequela during 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 the Treg ATMP 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; however, febrile reactions have not been reported in patients treated with expanded nTreg (Trzonkowski 2009, Brunstein 2011 and Marek-Trzonkowska 2012). Mild febrile responses do not constitute a major clinical concern and should be largely prevented by treatment of recipients with an antipyretic and anti-histamine prior to cell infusion.

- Unintended immune sensitisation

The Treg cell product in this trial is autologous and will be subject to stringent quality control testings 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 basiliximab. This is a monoclonal antibody to CD25 which blocks IL2 signalling in T cells and therefore ameliorates T cell activation. Basiliximab antibody therapy is part of many immunosuppression protocols in kidney transplantation and notably, the similar anti-CD25 antibody daclizumab has been used off-license in unintended immune activation.

If this is not effective or the clinical team wish, for any reason, to eliminate the circulating Tregs, then anti-thymocyte globulin (ATG) or alemtuzumab will be administered according to local hospital guidelines. Both agents deplete T cells effectively and are in routine use in the treatment of severe rejection in kidney transplant recipients. Other treatments may be administered on a case by case basis in line with Trust/CRF SOPs.

- Transfusion – related acute lung injury

A well-recognised 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 nTreg. 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.

- 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 major 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, the Treg Cell Product 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.

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 tumour lysis, rhabdomyolysis or haemolytic transfusion reactions. Nevertheless, measuring LDH levels pre- and post-cell infusion may be useful in assessing the fate of Tregs. It is conceivable that lysis of Tregs could cause bystander injury to recipient erythrocytes, resulting in a rise in free Hb and Hp, but this is unlikely to manifest clinically.

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. The Treg cell product 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).

Malignancy

Malignant disease after treatment with the nTreg 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.

5.3 TR004 Production

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

TR004 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, PBMCs, will be obtained by leukapheresis for each patient. In order to isolate naïve Tregs, a CD25 enrichment process will be performed on the CliniMACS Prodigy. From these enriched cells naive Tregs will be isolated by sorting on CD4+CD25+CD127lowCD45RA+ cells using the MACSQuant Tyto. The isolated naïve Tregs are then expanded through up to three cycles of stimulation using anti-CD3 and CD8 expansion beads which will provide polyclonal stimulation and drive cell expansion. High doses of IL-2 will also be included in the expansion phase as a T cell growth factor, in addition to a retinoic acid agonist with the aim to up-regulate expression of the homing marker α4β7 to encourage the cells to home to the gut. Approaches used during expansion tend to favour the proliferation of non-suppressive T cells. To prevent this, rapamycin will be added during expansion in order to favour the proliferation of Tregs. Following the expansion phase any residual expansion beads are removed and the cells are then formulated to the required cell concentration in a final formulation buffer containing the cryoprotectant DMSO (10%).

Deuterium-enriched glucose (labelled with 6,6-$^{2}H_{2}$-glucose) will be incorporated into the feeding medium, resulting in the TR004 final product to be partially deuterium-labelled. This will enable an assessment of T cell trafficking in whole blood/plasma/serum and to the colon, as well as pharmacokinetic profiling and half-life evaluation. Mass spectrometry analysis will allow us to differentiate adoptively transferred Tregs from endogenous Tregs and identify their location within the intestine and peripheral pools. This method will also allow us to determine if any effector T cells contain deuterium, which would indicate that these potentially pathogenic cells derive from the adoptively transferred cells. Although this scenario is considered unlikely, this provides a very sensitive measure of cells 'flipping' which we will be able to detect at the lowest dose of infused Tregs.

The manufacturing process will take between 16-23 days, depending on the number of stimulations required to achieve the required dose level. Once the cells have been harvested, the final product

will be formulated to the correct cell concentration for the patient dose in 4.5% (v/v) HSA, Plasmalyte 148 (86%v/v) and 10%(v/v)DMSO and frozen in a cryoprotective freezing bag.

5.4 TR004 Storage

Once manufacture is completed TR004 will be frozen in a controlled rate freezer to -100^{o}C before being transferred to the vapour phase of a liquid nitrogen storage tank (-196^{o}C). It will be stored until administration to the patient.

5.5 TR004 Traceability

The following documents will allow for accurate reconstruction of the ATIMP manufacture and dispensing. They will be kept in the GMP File and maintained by the GMP team:

- Notification Form for Batch Manufacture
- Order Form for commencing production
- Product Batch Release Record (will include trial name, product name, batch number, volume/number of cells, expiry date, total quantity received)
- Certificate of Analysis (CoA) for each batch
- QP certification for each batch
- TR004 trial prescriptions

The label content will be in accordance with the applicable national and local regulations for GMP manufacture. Both the IMP label and the IV bag label will be Annex 13 compliant.

The IV bag containing the reconstituted product will also be appropriately labelled by the GMP team.

As TR004 will be provided in an infusion bag, it will get discarded as per local destruction guidelines as soon as the product has been administered. Therefore physical reconciliation won't be performed. Dispensing records will be verified by the local Clinical Research Associate (CRA).

5.6 Concomitant Medication

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 related to Crohn's Disease will be recorded in the eCRF at screening. All concomitant medications (including over-the-counter or prescription medicines, vitamins and/or herbal supplements) taken by a patient during their participation in the trial must be recorded in the relevant section of the eCRF.

The following concomitant medications will be permitted:

- Azathioprine, mercaptopurine, tioguanine and methotrexate will be permitted as long as the patient has been on treatment for at least 12 weeks prior to dosing, including 8 weeks at a stable dose

- Corticosteroid treatment will be permitted from two weeks prior to dosing (week -2) to week 16 but the dose must be tapered down to 20mg of prednisolone (or equivalent) two weeks prior to dosing. Any alterations during the study must be reviewed by the CI, and the dose must not exceed 20mg of prednisolone (or equivalent). Corticosteroid treatment can not be initiated after week -2.
- 5-ASA medications will be permitted but the dose must be stable two weeks prior to dosing.
- Antibiotics for the treatment of Crohn's disease will be permitted but the dose must be stable two weeks prior to dosing.

In the event of accidental or unintentional changes, clinical review is required. Significant modifications defined by CI review are not permitted.

NIMPs

Medications that will be given as prophylaxis prior to TR004 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 – 4mg
- Paracetamol, administered orally 1g

In the event of cytokine storm post IMP administration, the following rescue medications may be administered:

- Basiliximab
- Anti-thymocyte globulin (ATG)
- Alemtuzumab
- Other medications may be administered on a case by case basis in line with Trust/CRF SOPs.

These medications are designated as NIMPs (Non-Investigational Medicinal Products). They will be dispensed from the CRF stock and according to the local hospital guidelines. All the above have marketing authorisation and are routinely used in accordance with their license.

6 Selection and Withdrawal of Participants

6.1 Inclusion Criteria

1. Able and willing to provide written informed consent and able to comply with the protocol requirements
2. Male or female aged between 18 and 80 (inclusive) years of age at date of consent
3. A diagnosis of Crohn's disease (CD) established ≥12 weeks prior to date of consent by standard clinical, radiological, endoscopic and histological criteria
4. Documented moderate to severe CD with a Crohn's Disease Activity Index (CDAI) $\geq$ 220 within 3 months of date of consent

5. Active CD (mucosal inflammation) including ulceration, as assessed by colonoscopy at screening
6. Failure to tolerate or to respond, or lose response to at least 2 prior lines of standard CD medication intended to induce or maintain remission, as determined by the referring gastroenterologist. Examples of such medications include, but are not limited to, azathioprine, mercaptopurine, methotrexate, vedolizumab, ustekinumab or anti-tumour necrosis factor antibody therapy. This does not include steroids and 5-ASA medications
7. Stable doses of concomitant medications, as defined in Section 5.6
8. Normal or non-clinically significant electrocardiogram (ECG), as assessed by the Investigator at screening
9. Negative stool test for Clostridium difficile and faecal culture for standard pathogens at screening. For non-pathogenic organism, inclusion will be at the discretion of the Principal Investigator (PI).
10. Negative serology for HIV – 1/2, Hepatitis B (cAb and sAg), Hepatitis C, HTLV and Syphilis at screening
11. Patient is judged by the Chief Investigator to be in otherwise good health based upon the results of all screening investigations in combination with medical history and physical examination

6.2 Exclusion Criteria

1. A diagnosis of ulcerative colitis or IBD-unclassified
2. CD treatment-naïve patients, defined as patients who have never received or have refused standard CD treatment
3. History of clinically significant drug or alcohol abuse in the last 12 months prior to date of consent.
4. Any history of major immune deficiency disorder, except Crohn's disease
5. Patients with a history of pulmonary embolism or deep vein thrombosis. Current or recent history (within 1 year prior to screening) of major organ or system failure or condition, acute or chronic that in the opinion of the investigator should preclude enrolment, except Crohn's disease
6. History of intestinal resection or intra-abdominal surgery within 6 months prior to date of consent.
7. Requirement for immediate or imminent surgical, endoscopic or radiological intervention for indications including (but not limited to) toxic megacolon, obstruction, massive haemorrhage, perforation, sepsis, or intra-abdominal or perianal abscess
8. Patients with ileostomy or colostomy
9. Patients with short bowel syndrome (less than 1.5m of small bowel)
10. Complication of Crohn's disease such as strictures/stenosis, penetrating disease, or any other condition that might require gastrointestinal surgery
11. Patients receiving therapeutic enema or suppository, other than required for endoscopy, within 14 days prior to date of consent and/or during the screening period
12. Patients who are currently using anticoagulants including but not limited to warfarin, heparin, enoxaparin, dabigatran, apixaban, rivaroxaban (note that anti-platelet agents such as aspirin up to 325mg daily or clopidogrel are permitted)

13. Use of corticosteroids on the day of leukapheresis sampling, prior to the procedure. Dosing should be delayed until after the procedure has been completed. This must be checked prior to the appointment and rescheduled if use is confirmed.
14. Current medically significant infection(s) requiring treatment with intravenous (IV) anti-infectives within 30 days prior to consent or oral anti-infectives for non-Crohn's disease related infections within 14 days prior to consent
15. Participant with an active systemic viral infection or any active viral infection that based on the investigator's clinical assessment makes the patient unsuitable for the study
16. History of tuberculosis (TB), unless there is documented evidence of completion of a full course of anti-TB treatment prior to screening. For patients with latent TB, as defined by a physician specialised in TB, they must have received prophylactic treatment for 4 weeks minimum prior to dosing
17. History of severe congestive heart failure (NYHA class III or IV), recent cerebrovascular accident (within 6 months of screening) and any other condition which, in the opinion of the investigator, would put the patient at risk by participation in the study
18. Patient with a previous history (within 12 months of consent) of dysplasia of the gastrointestinal tract, or found to have dysplasia during the screening endoscopy unless this is deemed to be a sporadic adenoma and has been completely removed

19. Significant laboratory abnormalities, assessed on Day-1 at Week 0:
    Hb < 100g/L or WBC < 3.5 x 10^9/L or Plt < 100 x 10^9/L
    Creatinine > 1.5x ULN
    Total bilirubin > 34 µmol/L or ALT > 2x ULN or GGT > 2xULN. Elevated unconjugated bilirubin related to Gilbert's syndrome is allowed

    These bloods will be initially reviewed at screening and if considered clinically significant, the patient will be excluded. If the CI considers it appropriate to proceed, any abnormalities identified at screening will be corrected as part of routine care before assessment prior to dosing at day -1, Week 0.

20. Anti-TNF or ustekinumab therapy within 8 weeks of dosing (day 0). Vedolizumab therapy within 5 half-lives (15 weeks) of dosing. Exposure to cyclosporine or tacrolimus within 2 weeks of consent
21. Patient currently receiving total parenteral nutrition (TPN) or plan to receive TPN at any time during the course of the study
22. Received another investigational drug within 60 days of anticipated study date of consent or 5 half-lives whichever is greater
23. Patient who previously received stem cell transplantation
24. Current evidence of dysplasia or history of malignancy within the last 5 years of consent (except non-melanoma skin cancer, successfully treated squamous cell or basal cell carcinoma, without metastases or localised carcinoma in situ of the cervix)
25. Pregnant and lactating patients (females of childbearing potential with a positive serum pregnancy test at screening visit 1 or day -1 at week 0).

26. 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 consent to W21 visit) 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, e.g.

- 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 consent until the week 21 visit. 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.

27. Male patients who are not willing to use an effective method of contraception (condoms), for the duration of the study (consent to W21 visit), when engaging in sexual activity with a female of childbearing potential
28. Allergy to any component / excipients used for the manufacture of TR004
29. Patient is considered by the Principal Investigator, for any reason, to be an unsuitable candidate for the study

6.3 Selection of Participants

Suitable patients for the clinical trial will be identified from Gastroenterology clinics at GSTT by the direct care team. Patients may be referred from other centres as clinical referrals. The participants' medical history and results will be reviewed by the clinical team at GSTT 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. A screening and enrolment log and study ID log will be maintained by the site.

Potentially eligible participants 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.

6.4 Consent

It is the responsibility of the PI or delegate at physician level in line with GSTT consent policy to obtain written informed consent for each patient prior to performing any trial related procedures. All trial investigators seeking consent must have received Human Tissue Act training for the taking of consent involving tissues and cells used for human application as part of the trial, be up-to-date with their GCP training and delegated for the task on the Delegation Log.

The PIS is provided to facilitate the informed consent process. Investigators must ensure that they adequately explain the aim, trial treatment, potential risks and benefits of taking part in the trial. The patient should be given ample time (at least 24 hours) to read the PIS and to discuss their participation with others outside of the clinical research team. The patient must be given the opportunity to ask questions which should be answered to their satisfaction. The right of the patient to refuse to participate without giving a reason must be respected.

If the patient decides to participate in the trial they must be asked to sign and date the latest approved version of the Informed Consent Form (ICF). The form must also be signed and dated by the PI or delegate. Details of the informed consent discussions should be recorded in the patient's medical notes. A copy of the signed ICF and PIS should be provided to the patient, and a copy will be kept in their medical records. The original signed consent forms will be kept in the Investigator Site File. The written informed consent will be obtained prior to any study related procedures including screening.

Every patient will be issued with a patient card providing the contact details and telephone numbers for office hours and out of hour emergencies. Patient will be requested to carry this card with them at all times whilst participating in the trial and to present this card to their attending healthcare professional.

6.5 Patient Confidentiality

To protect the privacy and identity of trial patients, patients will be assigned a unique patient trial identifier upon enrolment. Only the direct care team can identify and approach potential participants without prior consent (whether at GSTT or their local site). Potential participants will be asked to provide verbal consent for a member of the research team to contact them to discuss the study further.

Only Investigators and authorised staff at the trial site will be in possession of documents that link patient names to patient trial identifiers (i.e. 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.
In case any medical records containing patient data (e.g. laboratory results, medical reports) have to be sent to the Sponsor or external parties, the trial team will remove the patient's name as well as any other potential identifiers (e.g. hospital number, date of birth) and encode the documents with the appropriate patient trial identifier. Safety reports transmitted to the Sponsor and to the regulatory

authorities and ECs will also be pseudo-anonymised with this unique patient number. All data collected as part of the study will be regarded as strictly confidential.

6.6 Withdrawal of Participants

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, participants who wish to withdraw from trial medication (IMP) 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 will be recruited and dosed if:

- They withdraw prior to the start of their 5-week active period
- They withdraw during their 5-week active period, and have not yet experienced a DLT

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

The DSMB 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 5-week active safety period
- If they experience a DLT during their 5-week active safety period, which may or may not be the reason for withdrawal (as described in section 9.1)

6.7 Expected Duration of Trial

Total Estimated Duration from FPFV: 33 months
Estimated Recruitment Period: 8 months
Individual Patient Follow-up Duration: 24 months from TR004 infusion

The end of the trial will be defined as the date of the final database lock.

7 Procedures

7.1 Schedule of Assessments

Please refer to Table 1: Study Procedures and Table 2: Translational Research Samples Collection and Clinical Response Assessments.

Table 1: Study Procedures

Table of Assessments

Visits / Assessments	Screening 10 weeks (+/- 2 weeks)	Week 0			W1 +/-1 day	W2 +/-1 day	W3 +/-1 day	W5 +/-2 days	W8 +/-2 days	W16 +/-3 days	W21 +/-3 days	Safety FUP W52 +/- 2 weeks	Safety FUP W104 +/- 2 weeks
		Day -1[11]	Day 0[12]	Day 1									
Informed Consent	X												
Inclusion/Exclusion Criteria Review[1]	X	X											
Medical History (including demographic data)	X												
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X		
Adverse Events[2]	X	X	X	X	X	X	X	X	X	X	X	X	X
Doctor review including Physical Examination	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs[3]	X	X	X	X	X	X	X	X	X	X	X		
12- lead ECG*	X		X										
Colonoscopy	X								X				
Biopsy (Ileum and Colon)[4]	X								X				

Assessments \ Visits	Screening 10 weeks (+/- 2 weeks)	Week 0			W1 +/-1 day	W2 +/-1 day	W3 +/-1 day	W5 +/-2 days	W8 +/-2 days	W16 +/-3 days	W21 +/-3 days	Safety FUP W52 +/- 2 weeks	Safety FUP W104 +/- 2 weeks
		Day -1[11]	Day 0[12]	Day 1									
MRI or CT abdomen/pelvis or U/S small bowel	X												
Screening TB (chest x-ray and IGRA blood test)	X												
Leukapheresis for TR004 manufacturing[5]	X												
Clinical Blood Tests[6]	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood samples for Translational Research[7]	X	X			X	X	X	X	X	X	X		
Serum Pregnancy Test	X	X							X				
Stool samples collection[8]	X	X			X	X	X	X	X	X			
QoL questionnaire		X						X		X			
Dispense patient diary[9] [10]	X							X		X			
TR004 Dosing			X										
Participant Experience Assessment											X		

[1] For inclusion criteria prior to dosing (Day-1 at Week 0) please see exclusion criteria 19 in section 6.1. Screening will be performed in three stages due to the number and complexity of the assessments involved. Eligibility will be reviewed at each stage and confirmed at day -1, prior to dosing.

[2] Please refer to section 10.4 Safety Reporting for further details.

[3] Vital signs: Blood Pressure (BP), Heart Rate (HR), Respiratory Rate (RR), Temperature, Oxygen Saturation (0_2 Sats), weight (excluding day 0 and day 1), height (screening only). On dosing day (Day 0 at W0), vital signs, excluding height and weight, will be performed pre-dose (within 1 hour of infusion start) and post-dose: 15min, 30 min, 45 min, 1 hr, 1.5 hr, 2 hr, 3hr, 4hr, 6 hr.

[4] Ileum and Colon 24 punch biopsy will be performed at same time as colonoscopy. Biopsy Organ Culture Cytokine Array sample to be collected at Screening, and Week 8.

[5] Leukapheresis will be performed only once all the screening tests have confirmed patient's eligibility up to this point.

[6] Haematology (FBC and differentials), Liver Profile (albumin, alkaline phosphatase, total bilirubin, ALT, AST), Renal Profile (sodium, potassium, creatinine, urea, uric acid, chloride, bicarbonate), Bone Profile (calcium, phosphate), LDH, total protein, glucose, CRP, Thyroid Profile at screening only (TSH, free T3, free T4), Virology at screening and on leukapheresis day only (HIV-1/2, HBsAg, HBC, HCV, HTLV, syphilis) – Total blood volume approximately 25mls. On dosing day (Day 0 at W0) clinical blood tests will be performed pre-dose and 1 hour and 6 hours post-infusion.

[7] Translational research analysis: Immune Cell Phenotyping, Cytokine Analysis, level of circulating regulatory T cells. Total blood volume: 80-100mls. Please refer to Table 2

[8] Stool Testing for C&S, O&P, C Difficile (at screening only), Calprotectin testing (all visits), The stool collection instructions and stool container(s) should be dispensed to the patients at the visit falling before the collection visit

[9] At study entry (consent), 2 patient diaries must be dispensed: 1 at the beginning of the screening period and 1 at the end of the screening period. Only 1 diary to be dispensed at the other visits.

[10] Patient diary will be collected at end of screening period, Week 8, and Week 21, to enable CDAI score calculation.

[11] Day -1 visit will take place in the CRF as a day visit

[12] Patients will be admitted to the CRF on day 0 for an overnight stay

*ECG pre dose and 6hrs post dose on day 0

Table 2: Translational Research Samples and Clinical Response Assessments

Visits / Assessments	Screening 10 weeks (+/- 2 weeks)	Week 0			W1 +/-1 day	W2 +/-1 day	W3 +/-1 day	W5 +/-2 days	W8 +/-2 day	W16 +/-3 days	W21 +/-3 days	Safety FUP W52 +/- 2 weeks	Safety FUP W104 +/- 2 weeks
		Day -1	Day 0	Day 1									
CDAI / PRO-2 Score	X	X							X		X		
IBD-Control Questionnaire		X							X		X		
SES-CD Score	X								X				
Immune Cell Phenotyping	X	X			X	X	X	X	X	X	X		
Cytokine Analysis	X	X			X	X	X	X	X	X	X		
Circulating Regulatory T-Cells	X	X			X	X	X	X	X	X	X		
Biopsy organ culture cytokine array	X								X				

7.2 Clinical Trial Visits

Please refer to section 7.4 for trial assessments description

Screening Period (out-patient visits): within 10 weeks +/- 2 weeks prior to dosing

- Informed Consent
- Inclusion/Exclusion criteria review
- Medical History
- Clinical blood tests
- Serum pregnancy test
- Vital signs
- ECG
- Concomitant medication collection
- Adverse events collection
- Doctor review including physical examination
- Distribution of patient diary (x2)
- CDAI/PRO-2 calculation
- SES-CD calculation
- Colonoscopy
- Biopsy (ileum and colon)
- MRI or CT abdomen/pelvis (with contrast) or Ultrasound small bowel
- Tuberculosis screening
- Leukapheresis for TR004 manufacturing
- Translational research blood samples
- Stool samples collection

Dosing (single infusion, in-patient visit): Week 0 – Day -1, Day 0 and Day 1

- Concomitant medication collection (Day -1, Day 0, Day 1)
- Adverse events collection (Day -1, Day 0, Day 1)
- Doctor review (Day -1, Day 0, Day 1)
- Vital signs (Day -1, Day 0, Day 1)
- Clinical blood tests (Day -1, Day 0 pre-dose and 1 and 6 hours post infusion-, Day 1)
- ECG (Day 0 only)
- CDAI/PRO-2 calculation (Day -1 only)
- Inclusion/Exclusion criteria review (Day -1 only)
- Translational research blood samples (Day -1 only)
- Serum Pregnancy test (Day -1 only)
- Stool samples collection (Day -1 only)
- Quality of Life Questionnaire completion (IBD control questionnaire) (Day -1 only)

- TR004 Dosing (Day 0)

Follow Up: W1, W2, W3, W5, W8, W16, W21 (out-patient visits)

- Concomitant medication collection
- Adverse events collection
- Doctor review
- Vital signs
- Clinical blood tests
- Translational research blood samples
- Stool samples collection (except at W21)
- Serum pregnancy test (W8 only)
- Colonoscopy (W8 only)
- Biopsy (W8 only)
- Quality of Life Questionnaire completion (IBD control questionnaire) (W5 and W16)
- Distribution of patient diary (W5, W16)
- CDAI/PRO-2 calculation (W8 and W21)
- SES-CD calculation (W8 only)

Safety Follow Up: Week 52 and Week 104 (out-patient visits)

- SUSAR collection
- Doctor review
- Clinical blood tests

7.3 Clinical Trial Assessments Description

Please refer to Table 1: Schedule of Assessments and Table 2: Translational Research Samples and Clinical Response Assessments

Clinical blood tests and a serum pregnancy test (if applicable) will be repeated on Day-1 at Week 0 to ensure there are no abnormal results preventing the patient from being dosed.

- Medical History

Medical history information, including demographic data, will be collected at Screening.
All the relevant medical events will be recorded and should include start date and stop date if applicable.

- Adverse Events

Safety Events will be recorded and reported as necessary throughout the study as detailed in section 10.

- Doctor Review / Physical examination

The patients will be reviewed and assessed by the CI or delegate throughout the trial, from Screening to Week 104.

- Vital Signs

Vital signs will be performed from Screening to Week 21.
The following parameters will be measured:

- Height – at screening only
- Weight – every visit except Day 0 and Day 1 at W0
- Blood pressure (BP)
- Heart rate (HR)
- Respiratory rate (RR)
- Temperature
- Oxygen saturation (O2 Sats)

On the day of dosing (Day 0 at W0), vital signs, excluding height and weight, will be performed pre-dose (within 1 hour of infusion start) and post-dose: 15min, 30min, 45min, 1hr, 1.5hr, 2hr, 3hr, 4hr, 6hr.

Day 0 (Week 0)	**Vital Signs**	**Clinical Bloods**	**ECG**
Pre-Dose (within 1 hour)	X		X
Post Dose: 15 mins	X		
Post Dose: 30 mins	X		
Post Dose: 45 mins	X		
Post Dose: 1 hr	X	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	X

- 12-Lead ECG

12-Lead ECG will be performed at screening in order to exclude any severe heart diseases, including congestive heart failure. An ECG will be repeated pre dose and 6hrs post dose on day 0, week 0, and may be repeated as unscheduled tests throughout the study, at the physician's discretion.

- Colonoscopy

Colonoscopy will be performed at screening and Week 8. Instructions for colonoscopy preparation will be provided to the patient by the clinical team. All results will be retrospectively verified by an independent and appropriately qualified central reader.

- Biopsy

Biopsy will be performed at screening and week 8. 24 punch biopsies will be taken from a combination of the ileum and the colon. The procedure will take place at the time of the colonoscopy.

- Radiological Tests

MRI or CT abdomen/pelvis or U/S abdomen will be performed at screening, in order to exclude intra-abdominal sepsis. The CI will decide on the method of assessment on a case-by-case basis, as per standard of care.

- TB Screening

Tuberculosis testing will be performed at screening. It will involve a chest x-ray and a blood test (IGRA).

- Leukapheresis

Leukapheresis will be performed in the Haematology Day Unit during the screening period, once all the screening tests results are available and the patient's eligibility has been confirmed by the CI or delegate. Every effort should be made to allow for the procedure to take place as early as possible during the screening process; this will enable the TR004 manufacture to start without delays and will minimise the time between study entry and dosing. Trial participants will receive prophylactic continuous IV administration of low dose calcium-gluconate during the procedure to mitigate for hypocalcaemia.

- Clinical Blood Tests

Clinical blood tests will be performed throughout the study, from screening to W104.
On the dosing day (Day 0 at W0), clinical blood tests will be obtained 1hr and 6hr post-infusion. The pre-dose tests will be done on admission to the CRF Unit the day before (Day-1).
All the samples will be analysed by the local hospital laboratory.

Clinical blood tests will include:

- Haematology: full blood count (FBC) and differentials
- Liver profile: albumin, alkaline phosphatase, total bilirubin, ALT, AST
- Renal profile: sodium, potassium, creatinine, urea, uric acid, chloride, bicarbonate
- Bone profile: calcium, phosphate
- Others: LDH, total protein, glucose, CRP
- Thyroid profile (at screening only): TSH, free T3, free T4
- Virology (at screening and on leukapheresis day only): HIV-1/2, HBsAg, anti-HBC, anti-HCV, HTLVI/II, Syphilis
- Blood Sampling for Translational Research

Please refer to section 8 for information about collection time points and sample handling.

Detailed information about the research samples collection, analysis, storage and shipping (if applicable) will be described in the TRIBUTE Laboratory Manual.

- Serum Pregnancy Test

A serum pregnancy test will be performed at screening, prior to dosing (Day-1) at Week 0 and Week 8 on female patients of childbearing potential. The test will be performed by the nursing team.

- Stool sample collection

Stool samples will be collected throughout the trial, from screening to Week 16. The stool collection instructions and stool container(s) should be dispensed to the patients at the visit prior to the collection visit.

The following tests will be performed:

- Culture and Sensitivity (C&S) – at screening only
- Ova and Parasites (O&P) – at screening only
- Clostridium Difficile – at screening only
- Faecal Calprotectin (FCP) – from screening to Week 16 (except Day 0 and Day 1 at Week 0 and Week 8)
- Microbiome analysis – this will be performed at the end of the study

- Treatment Allocation

Treatment in this study is unblinded. The dose level administered to each participant will be recorded by the GMP Unit.

- TR004 Dosing

TR004 dosing will be administered in the CRF on Day 0 at Week 0.
Trial patients will be discharged 24 hours post-infusion (Day 1) except in case of safety concerns.

- Clinical Symptoms Assessment

Clinical symptoms will be assessed by calculation of CDAI and PRO-2 scores at screening, Week 0 (Day -1), Week 8, and Week 21.
Patients will be provided with a diary in which they will record the following information for 7 consecutive days prior to the trial visit: number of liquid or soft stools, abdominal pain self-assessment and general well-being assessment. Weeks in which a patient is having a bowl preparation (Week 0, 8), will require 10 days of data collection to allow for exclusion of the day before, the day of and the day after colonoscopy.

The patient diary will be given at the following visits:

- Study entry: 2 diaries will be dispensed – 1 for completion start 7 days prior to the next visit in the screening period and 1 for completion start 7 days prior to Week 0 Day-1
- Week 5 – 1 diary to be dispensed for completion start 7 days prior to Week 8
- Week 16 – 1 diary to be dispensed for completion start 7 days prior to Week 21

On the trial visit day, the information recorded in the diary will enable the clinical team to calculate the CDAI and PRO-2 scores. The calculated scores will be recorded in the patient's medical notes.

- QoL Questionnaire

The IBD-Control questionnaire will be completed by patients at Week 0 (Day-1), Week 5 and Week 16. The completed questionnaire will be filed in the patient's medical notes.

- Disease Activity Assessment

Disease activity will be assessed by calculation of the SES-CD score at Screening and Week 16. The calculated scores will be recorded in the patient's medical notes.

- Participant Experience

At the last follow up visit participants will be asked a series of questions to obtain their views on the acceptability of the trial design and procedures, with the aim of identifying any barriers to recruitment, follow up and compliance with study procedures.

8 Laboratory Tests

8.1 Clinical Blood Tests

All clinical blood tests will be taken as per standard of care in the appropriate vacutainers and analysed by Viapath, Guy's and St Thomas NHS Foundation Trust, with results available on the electronic patient record (EPR).

Please refer to section 7.4 for detailed information about Clinical Blood Tests.

8.2 Translational Research Analysis

The translational research samples will be analysed at two main laboratories depending on the type of samples:

- Lord Lab, School of Immunology and Microbial sciences , 5th Floor Bermondsey Wing, Guy's Hospital
- Institute of Infection and Immunity, St Georges University London

External collaborators may also be involved with the analysis of some of the research samples. Appropriate material transfer agreements and contractual agreements will be put in place before any transfers take place. Consent for further analysis will be sought from participants using the PIS and consent form.

Stool samples collected during the trial will be sent for microbiome analysis after the end of the study. Participants will be asked to provide consent for their samples to be used for future research.

Please refer to the TRIBUTE Laboratory manual for detailed information on sample collection and sample analysis.

8.3 Immune Cell Phenotyping and Cytokine Analysis

Objectives

1) To analyse the populations of circulating lymphocytes in the blood of patients undergoing TR004 immunotherapy
2) To analyse levels of cytokines (IFNγ, TNFα, IL-5, IL-10,IL-12, IL-13, IL-17, IL-18, IL-22, IL-23, IL-33, GM-CSF, oncostatin) in the peripheral circulation of patients undergoing TR004 immunotherapy

Materials Collected
40ml of whole blood will be collected into a clotted sample collection tubes by peripheral venous puncture.

Timing of blood sampling for immune cell phenotyping and cytokine analysis

Sample No.	1	2	3	4	5	6	7	8	9
Trial Visit	SCR	W0 (D-1)	W1	W2	W3	W5	W8	W16	W21

Sample Handling
Blood will be collected into a clotted sample collection tube by peripheral venous puncture. Blood will then be appropriately transported to the immunology laboratory for downstream processing and analysis.

8.4 Biopsy Organ Culture Cytokine Array

Objectives

1) To analyse the populations of lymphocytes localised in the ileum and colon of patients undergoing TR004 immunotherapy
2) To analyse levels of cytokines produced by lymphocytes in biopsies of patients who undergo TR004 immunotherapy.
3) Compare this data with that obtained in aim 9.1 to determine differences/similarities between circulating and localised cells.

Materials Collected
24 punch biopsies will be taken from a combination of the ileum and the colon from each patient undergoing TR004 immunotherapy.

Timing of biopsy sampling for immune cell phenotyping and cytokine analysis
Biopsy samples will be collected for immune cell phenotyping and cytokine analysis at screening, Week 8.

Sample Handling
Biopsies will be taken directly from the patient and placed in x-vivo cell culture medium during endoscopy. Samples will then be transported on ice to the immunology lab for downstream analysis.

8.5 Treg Kinetics and Distribution

Objectives
To analyse the levels of circulating regulatory T cells labelled with Deuterium.

Materials Collected
20ml of whole blood will be collected into clotted sample tubes by peripheral venous puncture.

Timing of blood sampling for analysis of Treg kinetics and distribution

Sample No.	1	2	3	4	5	6	7	8	9
Trial Visit	SCR	W0 (D-1)	W1	W2	W3	W5	W8	W16	W21

Sample Handling
Blood will be collected into a clotted sample collection tube by peripheral venous puncture. Blood will then be appropriately transported to the appropriate immunology laboratory for downstream processing and analysis.

9 Assessment of Safety and Efficacy

9.1 Definition of Dose-Limiting Toxicities (DLT)

Dose-Limiting Toxicities (DLT) will be assessed from W0 to W5. DLTs will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4.03 as described below:

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 dose-limiting toxicity is a toxicity considered to be related to the TR004 infusion.

DLTs will be assessed from data collected between W0 to W5.

Causality to TR004 infusion will be classified as follows:

- Definite – clearly related to ATIMP
- Probable – likely related to ATIMP

- Possible – may be related to ATIMP
- Unlikely – doubtfully related to ATIMP
- Unrelated – clearly not related to ATIMP

The following events will be considered DLTs:

Occurring in the first 24 hours post-infusions:

- CTCAE ≥ Grade 2 Cytokine Release Syndrome (CRS)
- CTCAE ≥ Grade 2 Injection Site Reaction
- CTCAE ≥ Grade 3 Hypoxia
- CTCAE ≥ Grade 3 Bronchospasm
- CTCAE ≥ Grade 3 Fever and/or Chills

Occurring in the 5 week-period post-infusions:

- CTCAE ≥ Grade 3 Infection
- CTCAE ≥ Grade 3 Haematological Toxicities. Anaemia, thrombocytopenia, leucopenia and Neutropenia will be included. Lymphopenia will be excluded given that most patients will be taking thiopurines which result in lymphopenia
- Any other CTCAE ≥ Grade 3 Toxicities not clearly related to CD. Fatigue, diarrhoea and weight gain will be excluded

In addition, all serious adverse reactions will be considered as DLTs up to week 21.

9.2 Clinical Assessments for Toxicity

Patients will undergo regular clinical examination, blood tests and other trial specific assessments as indicated at scheduled visits in order to identify potential toxicities.

9.3 Determination of Primary Endpoint

Determination of Safety and Tolerability of TR004

- Rate of Dose-Limiting Toxicities (DLTs) occurring within 5 weeks post-infusion.

9.4 Determination of Secondary Endpoints

Determination of the Clinical Response to TR004

Efficacy will be assessed using the CDAI and PRO2 scoring at screening, prior to infusion at Week 0, Week 8, Week 16 and Week 21. In line with current EMEA guidance on the development of new medicinal products for the treatment of Crohn's Disease, disease activity at weeks 8 will be used as the main assessment points

Definition of Clinical Response

A decrease in CDAI of ≥ 70 points from baseline

Definition of Remission

An absolute CDAI of < 150

Definition of Mucosal Improvement / Healing

Baseline and follow-up endoscopies will be reported and the validated endoscopic disease activity score, the Simple Endoscopic Score for Crohn's Disease (SES-CD), will be calculated.
Mucosal healing is defined as an absence of ulceration in the colon and terminal ileum. Endoscopic response is defined as either a 25% decrease in SES-CD from baseline or a 50% decrease in SES-CD from baseline.

Assessment of the Immunological Response to TR004

The data obtained from the analysis of the translational research samples will provide the basis for the immunological response assessment to TR004. These results will be available at the end of the trial, once all the research samples have been analysed and the results interpreted. These results will be presented in publications/reports about the trial findings.

10 Safety Assessments

All patients will be admitted to the Clinical Research Facility (CRF) at Guy's Hospital prior to dosing and observed for 24 hours following Treg infusion. The infusion will be administered by a clinical member of the research team and supported by one-to-one nursing cover in the post-infusion period. Resuscitation facilities and anaesthetic cover are available in the CRF.

Based on available data we anticipate that TR004 is likely to be safe in patients with CD.
The definition and management of DLTs is discussed in Section 9.1.

10.1 Specification, Timing and Recording of Safety Parameters

Crohn's disease patients require monitoring due to the potential complications associated with their condition and the adverse effects of systematic immunosuppression. Patient safety will be monitored closely and regularly, using the data collected during scheduled trial visits. The data collected will be recorded contemporaneously in the eCRF, enabling safety information to be interpreted as they emerge.

10.2 Safety parameters

The clinical trial visits and assessments have been designed to incorporate thorough safety evaluations for all trial patients.

The following measures will be taken to ensure that the maximum safety for the patients participating in the trial is assured:

- Stringent eligibility criteria to exclude patients with underlying health issues that could potentially put them at increased risk of developing serious adverse events or reactions

- Overnight admission to CRF unit where highly specialised staff care for patients taking part in first in human clinical trials. Emergency procedures in place to deal with potential cytokine release syndrome and serious adverse reactions
- As primary endpoint in the trial, the incidence of toxicities will be assessed over a five-week period, from Week 0 to Week 5. Patients will be carefully monitored for signs of toxicities using a variety of parameters collected at the trial follow-up visits.
- Regular and thorough patients' monitoring during trial visits:
    - Vital signs, clinical blood tests and physical examination will be performed at W0, W1, W2, W3, W5, W8, W16 and W21.
    - Colonoscopies and biopsies will be performed during the screening period and W8 visits.
    - AEs (including SAEs) will be reported from consent to the Week 21 visit.
    - SUSARs will be reported from the end of Week 21 to Week 104
    - On the day of dosing (W0, Day 0) vital signs and clinical blood tests will be monitored at regular intervals throughout the day in order to identify any potential reactions to the infusion.

10.3 Procedures for Recording and Reporting Adverse Events

The Medicines for Human Use (Clinical Trials) Regulations 2004 and Amended Regulations 2006 gives the following definitions:

Adverse Event (AE): Any untoward medical occurrence in a patient to whom a medicinal product has been administered including occurrences which are not necessarily caused by or related to that product.

Adverse Reaction (AR): Any untoward and unintended response in a patient to an investigational medicinal product which is related to any dose administered to that patient.

Unexpected Adverse Reaction (UAR): An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in the Investigator's Brochure (IB) for the Investigational Medicinal Product.

Serious adverse Event (SAE), Serious Adverse Reaction (SAR) or Unexpected Serious Adverse Reaction (USAR): Any adverse event, adverse reaction or unexpected adverse reaction, respectively, that:

- Results in death;
- Is life-threatening;
- Required hospitalisation or prolongation of existing hospitalisation;
- Results in persistent or significant disability or incapacity;
- Consists of a congenital anomaly or birth defect.

As TR004 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.

Important Medical Events (IME) & Pregnancy

Defined as events that may not be immediately life-threatening or result in death or hospitalisation, but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above and should also be considered serious.

Although not a serious adverse event, any unplanned pregnancy will also be reported via the SAE reporting system.

10.4 Safety Reporting Period

AEs (including SAEs) will be recorded from study entry (once the patient has consented) to Week 21 visit. During the safety follow-up period (from end Week 21 to Week 104), only SUSARs will be reported.

As stated in section 9.1, DLTs will be assessed from week 0 to week 5 of the study.

10.5 Reporting Responsibilities

The delivery of the co-sponsors' responsibility for pharmacovigilance (as defined in Regulation 5 of the Medicines for Human Use (Clinical Trials) Regulations 2004) has been delegated to the King's Health Partners Clinical Trials Office (KHP-CTO).

All SAEs, SARs and SUSARs will be reported immediately (and certainly no later than 24hrs after becoming aware of it) by the CI or person delegated by the CI to KHP-CTO.

The KHP-CTO will report SUSARs to the regulatory authority, the MHRA.

The Chief Investigator will report to the relevant ethics committee. Reporting timelines are as follows:

- SUSARs which are fatal or life-threatening must be reported not later than 7 days after the sponsor is first aware of the reaction. Any additional relevant information must be reported within a further 8 days.
- SUSARs that are not fatal or life-threatening must be reported within 15 days of the sponsor first becoming aware of the reaction.
-

The Chief Investigator and KHP-CTO (on behalf of the co-sponsors), will submit a Development Safety Update Report (DSUR) relating to this trial IMP, to the MHRA and REC annually.

The CI will submit annually to the main REC an Annual Progress Report.

All SAEs, SARs and SUSARs (including any follow-up information), will be reported using the KHP-CTO SAE report form.

10.6 Treatment Stopping Rules

The study may be terminated prematurely by the Sponsor, Chief Investigator or Regulatory Authority on the basis of new safety information or for other reasons given by the Data Safety Monitoring Board, regulatory authority or ethics committee concerned. The Sponsor and CI reserve the right to stop the trial at any time, for any justifiable reason. In the event of premature termination, the Sponsor will notify the regulatory authorities within 15 days by providing a detailed written explanation. The CI will inform the REC. The affected trial participants will also be informed promptly and appropriate follow-up visits will be arranged. No further participant data will be collected.

The clinical trial may be prematurely terminated for the following reasons:

- Serious and/or persistent non-compliance with trial protocol
- Non-compliance with ethical standards, regulatory requirements or GCP compliance
- Findings uncovered during monitoring visits, audits or inspections that compromise patient safety or suitability of the site to act as a trial centre
- Recommendation from DSMB
- Failure to meet recruitment targets

During the course of the study, any of the following events will trigger a pause in patient recruitment and an emergency review by the DSMB:

- Death
- DLT (as defined in section 9.1) observed in two patients out of four

In the event of emergency review by the DSMB, a substantial amendment will be submitted to the MHRA for approval to restart the study.

11 Statistics

11.1 Sample Size

A sample size of four participants will be recruited to assess the safety and tolerability of TR004 for further investigation in a larger clinical trial.

11.2 Analysis

During the study, data will be made available from the database to the DSMB. In view of the feasibility design and the numbers of patients, the statistical analysis approach will be a descriptive presentation of each patient's data rather than the calculation of summary statistics. The primary endpoint of this Phase I feasibility study is to determine the safety and tolerability of a single dose of TR004 in four participants. Safety and tolerability of the single dose of TR004 will be represented as the occurrence and nature of any DLTs, and other non-DLT adverse events, stratified according to those that are SAEs or not, whether or not related to IMP, and by whether they fall into the initial 5

week period from the TR004 injection or arising as longer term effects. The dose range manufactured for each patient will be presented. Adherence to visit windows will be assessed. Markers of efficacy, including evidence of mucosal healing, and continuous variables such as quality of life and clinical and immunological responses, will be tabulated by patient. Those measures that are continuous-valued and are repeated measures over several study weeks will also be presented graphically over time per patient. The completeness of data will be assessed across measures, noting whether missing data is due to any withdrawal or other causes. The DMC will be asked for qualitative feedback on the adequacy of data provided for their role, and how the process could be improved. The DLT results will be used to better inform a safe starting dose for any subsequent trial. The results from the four patients will be included as prior information to aid in investigating the use this prior information in obtaining a smaller sample size for the main trial through the use of simulations.

Treg deuterium enrichment will be modelled by fitting to a dual exponential disappearance model as used by Bluestone et al, giving disappearance rates for fast and slow turnover subpopulations. Only data to week 21 will be used for such modelling as beyond that timepoint, enrichments are predicted to be at or around the lower limit of detection and unlikely to add to the accuracy of the curve fits.

12 Trial Management

12.1 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 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.

12.2 Trial Management Group

This group is led by Dr Peter Irving, the CI for this study. The group will include the CI, statisticians, clinical trial manager, project manager and representatives of the other teams involved in the delivery of the trial, including the GMP unit, laboratories, data management and the CRF. 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.

12.3 Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) will be constituted prior to study opening. The DSMB charter will detail membership and terms of reference. The DSMB members will be independent and supported by the trial statistician. In order to ensure patient safety throughout the conduct of the trial, the DSMB will review and evaluate accumulated safety data, study conduct and progress. The DSMB will make the decisions about the continuation, modification or termination of the study.
The DSMB members will convene at defined time points stated in the DSMB charter.
A DSMB meeting would also be triggered if specific safety events occurred (see below).

The DSMB meeting will take place:

- If a DLT is observed in two patients out of four
- If death of a patient infused with TR004 occurs
- Or at any other time deemed necessary by the DSMB chair

13 Ethics and Regulatory Approval

13.1 Ethical Conduct of Study

All parties involved in this study should conduct the trial in accordance with the ethical principles that have their origin in the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects, adopted by the General Assembly of the World Medical Association (1996), and are consistent with GCP and applicable local regulatory requirements including but not limited to the Research Governance Framework and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments.

13.2 Ethics Committee Approval

This protocol and related documents will be submitted for review to a chosen NHS Research Ethics Committee (REC), the Health Research Authority (HRA) and to the Medicines and Healthcare products Regulatory Agency (MHRA) for Clinical Trial Authorisation.

The Chief Investigator will submit a final report at conclusion of the trial to the KHP-CTO (on behalf of the co-sponsors) and the REC within the timelines defined in the Regulations. The KHP-CTO or delegate will upload the final report to a publicly registered database on behalf of the co-sponsors

14 Quality Assurance

The trial will be monitored to ensure compliance with Good Clinical Practice and scientific integrity of data reported. This will be managed by the KHP-CTO Quality Team who will retain oversight throughout the study.

15 Data Handling

The PI 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 King's Health Partners Clinical Trials Office Archiving SOP (and all amendments to follow).

An electronic Case Report Form (eCRF) will be designed using the MedSciNet 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.

15.1 Direct Access to Source Data and Documents

The PI must allow the Sponsor, designated trial monitors, and when necessary, members of the EC or representatives of the regulatory authorities to review, monitor, audit and/ or inspect the trial by providing direct access to source data and other documents (e.g. patients' case sheets, blood test reports, X-ray reports, histology reports etc.). During such activities, the confidentiality of personal data will be respected at all times. By signing the ICF, the recipient will specifically consent to direct access to his/her medical records and source documentation for the purpose of source data verification (SDV) and regulatory inspection.

16 Data Management

A specific data management plan will be created for the trial.

16.1 Data Collection

The site staff responsible for data entry will be trained in the use of the eCRF system. Data entered in the eCRF system must be consistent with source data. All applicable fields in an eCRF page should be completed and if data are not available, this should be clearly indicated on the form. The eCRF platform automatically creates a protected audit trail for all data entries and changes. Amendments to eCRF data will be recorded in the audit trail with a time and date stamp, along with a user-specified reason for the implemented change.

The CI or delegate is responsible for submitting a complete set of eCRFs for each enrolled patient.
Any supportive paper documentation (including details of any SAE) transmitted from the investigators to the Sponsor should be clearly marked with the trial name, patient trial identifier and patient age. Any personal information, including the name of the patient, should be removed or rendered illegible to preserve individual confidentiality.

16.2 Specification of Source data

Source data are defined as all the information in original records (and certified copies of original records) of clinical findings, observations, or other activities that are necessary for the complete reconstitution and evaluation of the trial.

Source data must be available at the trial centre, to authenticate the existence of the study participants and substantiate the integrity of the data in the trial database. An eCRF is a data entry screen and does not constitute source data, unless otherwise specified in the Data Management Plan. The data entered into an eCRF should be verifiable with original source records.
Source documentation for the study includes, but is not limited to:

- Informed consent forms
- Medical records/clinical reports/laboratory reports/hospital correspondence/patient

questionnaires

The PI or delegate is responsible for producing a clean data set for the final statistical analysis. Inconsistencies between the source data and eCRF entries will be raised using data queries; this will prompt the trial site to clarify, correct or confirm discrepancies. At the end of the study, once all the data reported on the database has been monitored and cleaned, the eCRF will be locked.

16.3 eCRF Database Access

Access to the trial eCRF platform will be password protected and electronic login credentials will be issued only to authorised individuals. It is a legal requirement that passwords to the eCRF are not shared, and that only those authorised to access the system are allowed to do so. If new staff members join the study, a personalised username and password should be requested via the Trial Manager.

17 Publication Policy

All data and results generated from this trial are confidential. Agreement from the co-sponsors will be required prior to the disclosure of any trial related data.
It is intended that the results of the trial will be submitted for publication in a peer-reviewed scientific journal. Results will also be reported and disseminated at International conferences.

The outcomes of this trial will be communicated at regular intervals during the course of the study via the following channels:

1. Local academic, clinical and patient meetings.
2. National and International conferences.
3. High Impact Journals.

This trial is subject to an external communications strategy which makes patients and healthcare providers aware of the study and to encourage recruitment.

We have partnered with patient groups (e.g. Crohn's & Colitis UK), GI societies (e.g. British Society of Gastroenterology) and have direct communication with lead clinicians in research-active clinical gastroenterology departments.

18 Insurance / Indemnity

King’s College London, as the main sponsor, will provide cover under its no fault compensation insurance. It will cover for payment of damages or compensation in respect to any claim made by a research patient for bodily injury arising out of participation in the clinical trial. Guy’s and St Thomas’ NHS Foundation Trust as co-sponsor provide cover under the NHS Indemnity Scheme.

19 Financial Aspects

19.1 Funding

The clinical trial is funded by the Medical Research Council (MRC) by means of a research grant awarded by the MRC Industry Collaboration Agreement (MICA) grant.

19.2 Patient Travel Expenses

Participants will be reimbursed for reasonable travel expenses incurred to attend trial visits.

20 Archiving

At the end of this trial, all trial data will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the 2018 Data Protection Act and archived in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 as defined in the (Co)-Sponsor(s) Archiving Standard Operating Procedure (SOP).

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Appendices

Appendix I – Summary of Clinical Trials using In Vivo Expanded Tregs

Table A: Summary of trials in ClinicalTrials.gov

Study name/PI	Leading Sites	ClinicalTrials.gov identifier	Context
Baron/Beguin	University Hospital of Liege	NCT01903473	Treatment of chronic GvHD.
Bluestone/Feng/ Tang/Kang	University of California, San Francisco	NCT02188719	Antigen-specific expanded Tregs. Reduction in acute or chronic rejection following liver transplantation.
Bluestone/Herold	University of California, San Francisco	NCT01210664	Polyclonally expanded Tregs. Safety in paediatric T1DM.
Brunstein/Blazar	U Minnesota Masonic Cancer Centre, Minneapolis	NCT02118311	Phase II clinical trial of polyclonally expanded UCB Tregs. Prevention of acute GvHD with double UCB HSCT.
Bykovskaia/Kaabak	Russian State Medical University, Moscow	NCT01446484	Polyclonally expanded Tregs. Prevention of renal transplant rejection in children.
Dong	Parker Autoimmune Research Laboratory and 2 Diabetes Center, UCSF, San Francisco, California, USA	NCT01210664	Phase I trial of polyclonally expanded Tregs2 and low dose IL-2. Type 1 Diabetes.
The “ONE Study” Lombardi/Hilton	King’s College London Oxford University	NCT02129881	Polyclonally expanded Tregs. Prevention of renal transplant rejection.
Lu	Nanjing University	NCT01624077	Antigen-specific expanded Tregs. Improved graft survival following liver transplantation.
The “ThRIL” study Sanchez-Fueyo	King’s College London	NCT02166177	Antigen-specific expanded Tregs. Reduction in immunosuppressive burden following liver transplantation.

Study name/PI	Leading Sites	ClinicalTrials.gov identifier	Context
Pidala	Lee Moffitt Cancer Centre, Tampa, Florida	NCT01795573	Polyclonally expanded Tregs. Prevention in acute GvHD following UCB transplantation.
Skaro/Stare	Northwestern University, Chicago	NCT02145325	Autologous polyclonally expanded Tregs. Prevention of renal transplant rejection.
Vinceti/Chandran	University of California, San Francisco	NCT02088931	Autologous polyclonally expanded Tregs. Pilot study (n=3) to determine the safety of a single infusion of autologous Tregs in renal transplantation.
Lacerda	Instituto de Medicina Molecular Portugal	NCT02385019	Phase 1/2 clinical study for the treatment of steroid-refractory chronic graft versus host disease after an allogeneic transplant of hematopoietic progenitors with donor CliniMACS-selected regulatory T cells
Feng/Bluestone/Kang/Tang	University of California San Francisco / Mayo Clinic	NCT02188719	The purpose of this study is look at the safety of: Taking a specific combination of immunosuppressant drugs after liver transplantation and receiving one of three different doses of donor-alloantigen-reactive regulatory T cells (darTregs).
Wang	Nanjing Medical University	NCT02704338	Phase 1/2a trial of safety and efficacy in Autoimmune Hepatitis. Separated and expanded the CD4+CD25+CD127- Tregs from peripheral blood of autoimmune hepatitis patients and administrate the cells (5 x 10^6 cells/kg) into patients.

Study name/PI	Leading Sites	ClinicalTrials.gov identifier	Context
Dall'Era/Haemel/Bluestone/Rosenblum/Wofsy	University of California San Francisco	NCT02428309	Autologous polyclonal Tregs for cutaneous Lupus. *Ex vivo* Expanded Autologous CD4+CD127lo/-CD25+ Polyclonal Regulatory T Cells

Table B: Published Phase 1/2a clinical trials of Treg-based cell therapy in humans

Study	Indication	Cell Type	Enrichment Protocol	Expansion Protocol	Dose
Trzonkowski *et al.* (2009)	Treatment of acute and chronic GvHD. N=2	*In vitro* expanded Tregs	Tregs from allogeneic buffy coat. $CD4^+$ negative MACS selection, followed by FACS-based sorting of $CD4^+CD25^{hi}CD127^{lo}$ cells.	RPMI 1640 with 10% autologous plasma. IL-2 (1000 IU/ml). Anti-CD3/anti-CD28 beads (1:1). 3 weeks.	Acute GvHD: 1 x 10^5/kg Chronic GvHD: 3 x 10^6/kg
Brunstein *et al.* (2011)	Prevention of GvHD following UCB transplantation. N=23	*In vitro* expanded Tregs	$CD25^+$ bead positive selection	X-VIVO15 with 10% human AB serum. IL-2 (300 IU/ml). Anti-CD3/anti-CD28 beads (1:2). 18 ± 1 days.	0.1-30 x 10^5/kg after double UCB transplantation
Marek-Trzonkowska *et al.* (2012)	Safety of autologous *in vitro* expanded Tregs in paediatric T1DM . n=10	*In vitro* expanded Tregs	FACS-based sorting of $CD3^+CD4^+$ $CD25^{hi}CD127^{lo}$ cells	CellGro medium with 10% autologous plasma. IL-2 (1000 IU/ml). Anti-CD3/anti-CD28 beads (1:1). Up to 2 weeks	10-20 x 10^6/kg

Di Ianni *et al.* (2011)	Prevention of GvHD and improved reconstitution in HLA-haploidentical HSCT. N=28	Freshly isolated $CD4^+CD25^+$ Tregs	Donor apheresis, followed by $CD4^+CD25^+$ enrichment using Miltenyi CliniMACS	No expansion.	2-4 x 10^6/kg 4d prior to HSCT
Desreumaux *et al (8).* (2012)	Treatment of Crohn's Disease N=29	Ovalbumin specific Tregs	Ovalbumin specific Tregs were enriched from peripheral blood and exposed to ovalbumin	X-VIVO 15 in the presence of IL2 and IL4. Activated by anti CD3, CD80 and CD58 for 7/7.	Up to 10^9 Tregs per infusion(8)
Bluestone *et al.* (2015)	T1DM Immunotherapy N=14	Polyclonal Tregs	*Ex vivo*-expanded autologous CD4(+)CD127(lo/-) CD25(+) polyclonal Tregs	X-VIVO 15 in the presence of IL2/Rapamycin	Up to 26 x 10^8 Tregs
Brunstein *et al.* (2016)	GVHD Prevention N=11	Umbilical cord blood derived Tregs	CD25+ cell CliniMacs enrichment	Stimulated with anti-CD3 mAb loaded KT64/86 artificial antigen-presenting cells (aAPCs) in the presence of IL2	Up to 300 x 10^6 Treg / kg(9)
Sanchez-Fyeyo *et al.* (2020)	Liver transplant N=9	*Ex vivo* expanded autologous polyclonal Tregs	Donor apheresis, followed by $CD4^+CD25^+$ enrichment using Miltenyi CliniMACS	TM/HS in the presence of IL2/Rapamycin. Activated with ExpAct beads	0.5-1 million Tregs/kg or 3-4.5 million Tregs/kg

Giessler *et al.* (2020)	Renal transplant N=12	Recipient-derived naturally occurring regulatory T cells	Not specified	Not specified	0.5-10 x 10^6 Tregs/kg
Dong *et al.* (2021)	Type 1 Diabetes N=7	Autologous polyclonal Tregs in combination with two 5-day courses of recombinant human low-dose IL-2.	Described as per Bluestone *et al.* 2015	14 day ex vivo expansion	Up to 20x106/kg
All studies are phase I/IIA except Trzonkowski *et al.* which is a case series.					

Table C: Safety profile and signal of efficacy in published clinical trials of Treg-based cell therapy in humans

Study	Indication	Evaluable patients (n)	Signal of efficacy	Observed toxicities
Trzonkowski *et al.* (2009)	Treatment of acute and chronic GvHD.	2	1 patient with bronchiolitis obliterans was able to reduce prednisone and discontinue MMF.	None reported.
Brunstein *et al.* (2011)	Treatment of steroid-resistant GvHD	28	Reduced incidence of grade II-IV acute GvHD in treated cohort compared with historic controls	"No deleterious effect on risks of infection, relapse or early mortality."
Marek-Trzonkowska *et al.* (2012)	Safety of autologous *in vitro* expanded Tregs in paediatric T1DM	10	Remission at 6 months: 8/10 (treated) *vs.* 6/10 (untreated, p=0.04). Plasma C-peptide levels higher in treated group (p=0.01).	None reported.
Di Ianni *et al.* (2011)	Prevention of GvHD and improved reconstitution in HLA-haploidentical HSCT.	26	Only 2/26 (7.6%) patients developed ≥ grade II GvHD. Earlier appearance of pathogen-specific $CD4^+$ and $CD8^+$ lymphocytes *vs.* standard haploidentical HSCT.	None reported.
Desreumaux *et al.* (2012)	Treatment of Crohn's Disease	29	40% of patients had a reduction in CDAI at 5 and 8 weeks post treatment	11 SAEs

Study	Indication	Evaluable patients (n)	Signal of efficacy	Observed toxicities
Brunstein *et al.* (2016)	Prevention of GVHD	11	In the context of sirolimus, mycophenolate mofetil immunosuppression, adoptive transfer of Tregs resulted in low risk of acute GVHD	None reported
Bluestone *et al.* (2016)	Treatment of T1DM	14	C-peptide levels persisted out to 2+ years after transfer in several individuals.	None reported
Abbreviations: GVHD: Graft *vs.* Host Disease. HSCT: Haematopoietic stem cell transplantation. MMF: Mycophenolate mofetil. T1DM: Type 1 diabetes mellitus. UCB: Umbilical cord blood.				