


BMJ Open Safety and tolerability of a single infusion of autologous ex vivo expanded regulatory T cells in adults with ulcerative colitis (ER-TREG 01): protocol of a phase 1, open-label, fast-track dose-escalation clinical trial

Caroline J Voskens ,^{1,2} Diane Stoica,^{1,2} Susanne Roessner,^{1,2} Francesco Vitali,^{3,2} Sebastian Zundler,^{3,2} Marita Rosenberg,^{1,2} Manuel Wiesinger,^{1,2} Jutta Wunder,^{3,2} Britta Siegmund,⁴ Beatrice Schuler-Thurner,^{1,2} Gerold Schuler,^{1,2} Carola Berking,^{1,2} Raja Atreya,^{3,2} Markus F Neurath^{3,2}

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For numbered affiliations see end of article.

Correspondence to

Dr Caroline J Voskens;
caroline.bosch-voskens@uk-erlangen.de

ABSTRACT

Introduction Accumulating evidence suggests that the adoptive transfer of ex vivo expanded regulatory T cells (Treg) may overcome colitogenic immune responses in patients with inflammatory bowel diseases. The objective of the ER-TREG 01 trial is to assess safety and tolerability of a single infusion of autologous ex vivo expanded Treg in adults with ulcerative colitis.

Methods and analysis The study is designed as a single-arm, fast-track dose-escalation trial. The study will include 10 patients with ulcerative colitis. The study intervention consists of (1) a baseline visit; (2) a second visit that includes a leukapheresis to generate the investigational medicinal product, (3) a third visit to infuse the investigational medicinal product and (4) five subsequent follow-up visits within the next 26 weeks to assess safety and tolerability. Patients will intravenously receive a single dose of 0.5×10^6 , 1×10^6 , 2×10^6 , 5×10^6 or 10×10^6 autologous Treg/kg body weight. The primary objective is to define the maximum tolerable dose of a single infusion of autologous ex vivo expanded Treg. Secondary objectives include the evaluation of safety of one single infusion of autologous ex vivo expanded Treg, efficacy assessment and accompanying immunomonitoring to measure Treg function in the peripheral blood and intestinal mucosa.

Ethics and dissemination The study protocol was approved by the Ethics Committee of the Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany (number 417_19 Az). In addition, the study was approved by the Paul-Ehrlich Institute, Federal Institute for Vaccines and Biomedicines, Langen, Germany (number 3652/01). The study is funded by the German Research Foundation (DFG, KFO 257 project 08 and SFB/TransRegio 241 project C04). The trial will be conducted in compliance with this study protocol, the Declaration of Helsinki, Good Clinical Practice and Good Manufacturing Practice. The results will be published in peer-reviewed scientific journals and disseminated in scientific conferences and media.

Strengths and limitations of this study

- This fast-track dose-escalation study will provide evidence on safety and tolerability of a single infusion of ex vivo expanded autologous Treg cells in patients with ulcerative colitis.
- Ex vivo expanded Treg cells might suppress intestinal inflammation, and thereby contribute to effective treatment of ulcerative colitis.
- Due to its single-arm design, this study does not allow direct outcome comparisons with a respective control group.

Trial registration number NCT04691232.

INTRODUCTION

Together with Crohn's disease (CD), ulcerative colitis (UC) is one of the major forms of inflammatory bowel diseases (IBD). The disorder is characterised by episodes of inflammatory flares and periods of remission and poses a substantial socioeconomic burden to the healthcare system. Patients typically suffer from abdominal pain, diarrhoea, rectal bleeding, anaemia, weight loss and fatigue. These symptoms severely affect quality of life and also negatively impacts work ability. UC typically affects young people, with a peak onset between 15 and 30 years of age. Currently, no curative therapy is available, since the pathophysiology of this disease is incompletely understood.^{1–3} In addition, patients with UC are at increased risk of developing colorectal cancer.⁴ The current therapeutic armamentarium in UC consists of

anti-inflammatory substances like mesalazine and steroids, immunosuppressants such as thiopurines or calcineurin inhibitors, biologicals like antitumour necrosis factor antibodies or the interleukin (IL)-12/IL-23 antibody ustekinumab and the Janus kinase-inhibitor tofacitinib.^{5 6} In addition, the anti- $\alpha 4\beta 7$ integrin antibody vedolizumab is approved for the treatment of IBD.⁷ By blocking the trafficking of circulating T cells into the intestinal mucosa, this antibody dampens inflammation in the gut without inducing systemic immunosuppression. Clinical practice demonstrates that current therapies induce lasting remission in subgroups of patients only. Importantly, many patients develop steroid-refractory or steroid-dependent disease courses and in therapy refractory cases, surgical intervention with colectomy is necessary.⁸

Accumulating evidence suggests that the adoptive transfer of ex vivo expanded regulatory T cells (Treg) may overcome colitogenic immune responses in patients with IBD. Specifically, T helper (Th) cells with an atypical Th2 profile, which excessively produce IL-5 and IL-13 but not IL-4, are abundant in the inflamed bowel wall in UC.^{9–11} Furthermore, Treg are also identified within the lamina propria and these cells normally control the effector T cell population mentioned above.^{12–15} However, previous studies demonstrated an insufficient expansion of mucosal Treg compared with a massive local expansion of effector T cells, which explains why Treg fail to control ongoing immune responses in the gut.¹⁶ Moreover, experimental colitis studies in mice have demonstrated that colitogenic immune responses can be controlled by increasing the number of mucosal Treg and highlight the potential use of Treg in cell-based therapies in UC.¹⁷ Therefore, Treg are likely candidates for cell-based immunotherapy, that is, to suppress ongoing T cell-mediated immunity in patients affected by autoimmune disease.

Until recently, clinical application of Treg was hampered by (1) the small number of Treg in the peripheral blood that could be isolated relative to the number of cells that would be required to be effective and (2) the difficulties associated with the isolation and ex vivo expansion of highly enriched Treg-specific cell populations in compliance with Good Manufacturing Practice (GMP). We have developed and established a method to generate large numbers of suppressive CD25⁺ cells under aegis of IL-2, rapamycin and anti-CD3/anti-CD28 expander beads from CD25⁺ precursors derived from adult peripheral blood of healthy individuals as well as patients with UC.¹⁸ We have further improved the method for its use with a leukapheresis product in order to collect a larger number of CD25⁺ cells and adapted the method for clinical use under GMP conditions.¹⁹ The clinical production protocol enables us to generate large numbers of autologous suppressive CD25⁺ cells under GMP conditions, and to cryopreserve them without loss of biological function.

We hypothesise that adoptively transferred ex vivo expanded autologous Treg migrate to the gut and reverse gut-specific inflammation in patients with UC. The aim of this study is to define the maximal tolerable dose (MTD)

of one single intravenous administration of autologous ex vivo expanded Treg according to the fast-track dosing principle in 10 patients with active UC. Safety and tolerability are assessed at a single patient level during dose-escalation to securely monitor possible side effects or disadvantages of the Treg therapy. If tolerability is evident, Treg therapy is offered to one patient at the next level of dose escalation. Once dose escalation is completed, five additional patients will be treated with the highest tolerated Treg dose to extend safety assessment.

METHODS AND ANALYSIS

Participants, interventions and outcomes

Study setting

This study is executed at the Department of Medicine 1, University Hospital Erlangen, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany. Treg are produced and released by the GMP facility of the Department of Dermatology, University Hospital Erlangen, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany.

Eligibility criteria

Patients must meet all of the inclusion criteria in order to be eligible to participate in the study:

- ▶ Patients must have an established diagnosis of UC, with minimum time from diagnosis of ≥ 3 months.
- ▶ Patients must be either in remission under the allowed concomitant therapy or must have received all the beneficial pharmacological treatment lines before enrolment and have moderately to severely active disease activity (endoscopic disease activity should extend 15 cm or more above the anal verge) determined by a modified Mayo score (excluding the friability at grade 1 for the endoscopic sub score) of 6–12 with an endoscopic subscore ≥ 2 and no other individual subscore < 1 .
- ▶ Patients must have a WHO performance status of 0, 1 or 2 and must be in stable medical condition.
- ▶ Patients must be between 18 and 75 years old and must be able and willing to give informed consent.
- ▶ Women of childbearing age must have a negative pregnancy test at enrolment in the study, must be willing to undergo monthly pregnancy tests until at least 3 months after adoptive Treg transfer and must oblige to use effective contraception until at least 3 months after adoptive Treg transfer. A highly effective method of birth control is defined as one that results in a low failure rate (ie, less than 1% per year) when used consistently and correctly, such as implants, injectables, combined oral contraceptives, some intrauterine devices (IUDs), sexual abstinence, or a vasectomised partner. For subjects using a hormonal contraceptive method, information regarding the product under evaluation and its potential effect on the contraceptive must be addressed.

- ▶ Male study patients, who are partners of women of childbearing age, must be willing to use effective contraception until at least 3 months after adoptive Treg transfer. A highly effective method of birth control is defined as one that results in a low failure rate (ie, less than 1% per year) when used consistently and correctly, such as sexual abstinence or vasectomy. The sole use of condoms is not considered as an effective method of birth control. Therefore, partners of childbearing age from male study patients should be willing to use implants, injectables, combined oral contraceptives or IUDs as highly effective method of birth control. Information regarding the product under evaluation and its potential effect on the contraceptive must be addressed.
- ▶ Patients must be willing to undergo leukapheresis.
- ▶ Patients must be willing to get hospitalised for at least 24 hours following adoptive Treg transfer, and to cooperate for the whole period of the trial.
- ▶ Accomplishment of a washout phase for biological therapy of at least 8 weeks or no detectable serum trough levels prior to screening in case of a washout phase less than 8 weeks. Of note, this criterion was amended during the course of the study after peer review of the protocol. At study initiation a wash out phase for biological therapy of at least 4 weeks or no detectable serum through levels was allowed. However, only patients with a wash out phase of minimum 8 weeks were included since initiation of the study.
- ▶ Concomitant therapy with oral corticosteroids (prednisone or equivalent up to 20mg/day, stable for 2 weeks at inclusion), budesonide (9mg/day, stable for 8 weeks at inclusion), 5-aminosalicylic acid (stable for 2 weeks at inclusion) and azathioprine (stable for 8 weeks, initiated at least 3 months ago) is permitted. Concomitant oral corticosteroids can be reduced at the investigator's discretion from visit 5 onwards.

All subjects meeting any of the exclusion criteria at baseline will be excluded from study participation:

- ▶ Any of the above-mentioned inclusion criteria are not met.
- ▶ Impaired haematological function (on repeated testing) as indicated by
 - Leucocyte count $\leq 2500/\text{mm}^3$
 - Neutrophils $\leq 1000/\text{mm}^3$
 - Lymphocytes $\leq 700/\text{mm}^3$
 - Platelets $\leq 75\,000/\text{mm}^3$
 - Haemoglobin $\leq 9\text{ g/dL}$
- ▶ Impaired hepatic or renal function as indicated by
 - Serum creatinine $\geq 2.5\text{ mg}/100\text{ mL}$
 - Serum bilirubin $\geq 2.0\text{ mg}/100\text{ mL}$
- ▶ Any other major serious illness (e.g. active systemic infections, immunodeficiency disease, clinically significant heart disease, respiratory disease, bleeding disorders, etc) or a contraindication to leukapheresis.
- ▶ Evidence for HIV-1, HIV-2, human T-lymphotropic virus (HTLV)-1, Treponema pallidum

hemagglutination assay (TPHA), hepatitis B virus (HBV), or hepatitis C virus (HCV) infection.

- ▶ Patients who have spent a cumulative period of 1 year or more in the UK between the beginning of 1980 and the end of 1996.
- ▶ Patients who have a family history, which places them at risk of developing Creutzfeldt-Jacob disease.
- ▶ Patients who have received a corneal or dura mater graft, or who have been treated in the past with medicines made from human pituitary glands.
- ▶ Other active autoimmune diseases (such as but not limited to lupus erythematosus, autoimmune thyroiditis, uveitis or multiple sclerosis).
- ▶ Previous splenectomy or radiation therapy to the spleen.
- ▶ Patients with organ allografts.
- ▶ Patients with coeliac disease. Of note, this criterion was added during the course of the study through a study amendment after peer review of the protocol. At study initiation patients with coeliac disease were allowed to participate in the study. However, none of the already included patients has a history of coeliac disease.
- ▶ Concomitant treatment with chemotherapy, immunotherapy, any investigational drug and paramedical substances.
- ▶ Existence or prior history of a malignant neoplasm.
- ▶ Organic brain syndrome or significant psychiatric abnormality which would preclude participation in the full protocol and follow-up.
- ▶ Positive pregnancy test/pregnancy or lactation. If pregnancy occurs during the course of the trial to female patients, the patient has to be excluded (not valid for partners of male patients treated).
- ▶ Known hypersensitivities to human serum albumin and/or dimethyl sulfoxide (DMSO).

Interventions

Twelve patients, including 10% patient loss, resulting in at least 10 treated and fully evaluable patients with UC, will be enrolled in this single-centre, open-label, fast-track dose-escalation study. Autologous ex vivo expanded CD4⁺CD25⁺CD127^{-/lo} Treg cells will be adoptively transferred in patients with UC with moderate to severe disease activity or in remission on the allowed concomitant therapy described under the last inclusion criterion at the time of enrolment. The MTD is defined as the dose that does not produce more than one dose-limiting toxicity (DLT) among a total of four treated patients at the particular dose level. A DLT is defined as a related grade III or higher toxicity according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) that prevents an increase to the next dose level.

The first enrolled patient will receive the starting dose of 0.5×10^6 Treg/kg body weight. Adoptive transfer is escalated to the next dose level (1×10^6 Treg/kg, 2×10^6 Treg/kg, 5×10^6 Treg/kg and 10×10^6 Treg/kg body weight) in each consecutive patient, respectively, if no DLT occurs.

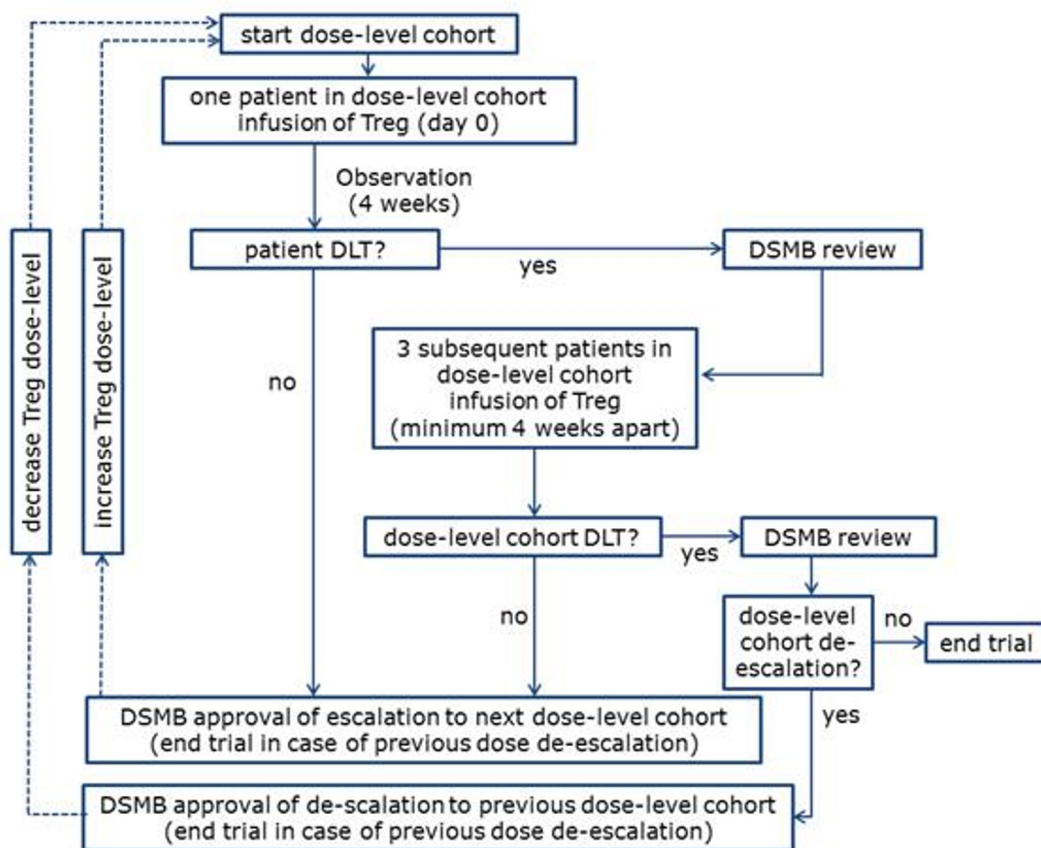


Figure 1 Flow chart of the ER-TREG 01 study. The initial starting dose is 0.5×10^6 Treg/kg bodyweight. Adoptive transfer is escalated to the next dose level (1×10^6 Treg/kg, 2×10^6 Treg/kg, 5×10^6 Treg/kg and 10×10^6 Treg/kg bodyweight), in a next patient, if no dose-limiting toxicity (DLT) occurs. Consecutive patients will be treated at least 4 weeks apart to monitor acute severe adverse events. If a DLT is noted, three additional patients will receive the same dose level. If two patients among a cohort of four patients experience a DLT, dose de-escalation to the highest previously tolerated dose-level will follow. DSMB, data safety monitoring board.

Patients will be treated at least 4 weeks apart to monitor acute severe adverse events (SAE). If a DLT is noted, three additional patients will receive the same dose level. Dose escalation continues until at least two patients among a cohort of four patients experience a DLT. If two patients among a cohort of four patients experience a DLT, dose de-escalation to the highest previously tolerated dose level will follow. Three additional patients will receive the highest previously tolerated dose. If a DLT is noted in at least two patients at the tested dose level, dose de-escalation will continue until less than two patients have experienced a DLT. After successful enrolment at the highest dose level, four additional patients will be enrolled at the highest dose level to extend safety assessment. If no DLTs or less than two DLTs are experienced at all dose levels tested, the MTD is not reached. In this case, a maximal administered dose (MAD) is defined. Due to Treg production limitations, no further dose-escalation is considered in this study. See also [figure 1](#).

Clinical evaluations will be performed initially and at week 2, 4, 8, 12 and 26 after adoptive Treg transfer. Evaluations include assessment of the clinical status and routine blood tests. In addition, blood will be drawn and processed (serum and peripheral blood mononuclear

cells) for later immunomonitoring analysis at all evaluation visits after treatment. Furthermore, imaging and tissue sampling through colonoscopy will be performed prior to enrollment and in week 4 and 12 after treatment.

Outcomes

The primary objective is to define the MTD of one single intravenous administration of autologous ex vivo expanded Treg in patients with UC. Primary endpoint is the assessment of the number of significant adverse events (AE) defined by any related National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) grade III or higher AE or any related SAE within 4 weeks after adoptive Treg transfer. Secondary endpoints include (1) all signs of negative impact on the course of UC as defined by an increase in the Mayo Clinic score of at least three points and an increase of at least 30% from baseline, with an accompanying increase in the rectal bleeding subscore of at least one point at week 4 (visit 5) after adoptive Treg transfer, (2) changes in disease activity score calculated by evaluation of the quality of life Inflammatory Bowel Disease Questionnaire (IBDQ) at week 4 (visit 5) after adoptive Treg transfer, (3) changes in Treg frequencies in peripheral blood at week 4 (visit 5)

after adoptive Treg transfer and (4) changes in effector T cell and Treg frequencies in the gut at week 4 (visit 5) after adoptive Treg transfer.

Participant timeline

The timing for the individual visits is outlined in [table 1](#). The time to complete study enrolment was estimated to be 10 months. Subject participation within the trial will be 7 months (1 month Treg drug production, 1 day adoptive Treg transfer and 6 months of observation after treatment). The time to complete the trial from enrollment of the first patient in until the last patient out of study should thus be 16 months. At the time of the last patient out, information on the clinical status of all patients who have regularly ended the trial or have been withdrawn will be collected.

Sample size

We anticipate to enrol 12 patients, including 10% patient loss, resulting in one fully evaluable patient at each dose tested, including 5 additional patients at the highest dose level. Only patients receiving Treg and performing the clinical evaluation at week 4 after treatment will be counted as fully evaluable for dose escalation. Patients not receiving Treg or not performing the evaluation at week 4 will not be evaluated in the per protocol analysis but in the intention-to-treat analysis and will be replaced.

Recruitment

The participants are actively recruited in the outpatient clinic of the Department of Medicine 1. Potential participants are informed about this clinical trial by their treating physician. If a participant is willing to participate in the study, he/she will be screened to determine if the inclusion and exclusion criteria are fulfilled. A potential participant will receive the patient information form. Afterwards, the potential participant is orally guided through the patient information form by one of the clinical investigators. When no questions remain by the potential participant, the informed consent form will be signed. The study will not recruit participants not able to give informed consent, nor concerns minors.

Data collection, management and analysis

Data collection methods

Patients' data are recorded in Case Report Forms (CRFs). CRFs have to be completed using a non-erasable black ball-pen, erasing or similar methods are not allowed. If corrections are necessary the original entry must remain visible, and the reason of the correction and the signature of the investigator or a delegated person correcting the original entry must be written on the respective CRF. A copy of the CRF stays with the investigator and will be safely stored for 30 years.

Data management

Data from the CRFs and data collected from immunomonitoring are transferred to a database accessible only

to members of the study team (study assistants and investigators of the clinical trial).

Statistical methods

Descriptive analysis will be performed comparing before (screening/visit 1) and 4 weeks after (follow-up 2/visit 5) Treg treatment. Continuous variables will be described using mean and SD or median and IQR; categorical variables will be described as counts and percentages. Safety analysis will be performed in all patients treated with Treg. All AEs, SAEs and AEs leading to treatment discontinuation will be recorded according to international guidelines. Differences in Mayo Score before and 4 weeks after Treg treatment will be statistically evaluated using (an approximation of) the exact Wilcoxon signed-rank test, for example, the `wilcoxsign_test` of the R package COIN. Treg-induced immunological differences will be analysed as the difference before and 4 weeks after Treg treatment. This will be calculated with the use of Treg data obtained at screening (visit 1) and follow-up 2 (visit 5). The mean values of Treg-specific surface receptors, gut homing receptors, cytokines and Treg-mediated suppression before and 4 weeks after Treg treatment will be compared. Mean differences will be calculated and compared using t test or Wilcoxon-Mann-Whitney test.

Monitoring

Data monitoring

Safety oversight for this study is under the direction of a data safety monitoring board (DSMB) composed of persons not involved in the conduct of the study. No member of the DSMB has certain financial, proprietary, professional or other interests that may affect impartial, independent decision-making by the DSMB. The DSMB will assess safety before escalation to the next dose-level. The principal investigator is responsible to ensure that the DSMB is apprised of all new safety information relevant to the study and the investigational medicinal product. DSMB meetings can have a public part, in which the principal investigator, the coordinating investigator and other investigators are allowed to participate. The DSMB will be provided with a written report in 3 months intervals containing a summary of patient recruitment and drop outs, summary of AEs/SAEs and overview of the clinical course of the patients. In the case of suspected unexpected serious adverse reactions or SAEs related to the study drug, the event will be reported to the DSMB within 2 weeks. Any decision to terminate or modify the trial before its regular end will be made by the DSMB. If a related grade III or IV toxicity (according to the NCI-CTC scale) is observed in one patient, three more patients will receive the same dose level. If no more patients experience a grade III or IV toxicity, dose escalation is continued to the next dose level. If at least two patients among a cohort of four patients experience a DLT, dose de-escalation to the highest previously tolerated dose level will follow. Three additional patients will receive the highest previously tolerated dose. If a DLT is noted in at least two

Table 1 Study visits

Visit	Screening		Leukapheresis		Adoptive Treg transfer		Follow-up 1		Follow-up 2		Follow-up 3		Follow-up 4		Follow-up 5	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Day	-56±14	-49±14	1±14	15±14	29±14	57±14	85±14	183±14								
Informed consent study	X															
Informed consent endoscopy	X				X											
Informed consent transfusion medicine	X															
Inclusion/exclusion criteria	X	X	X													
Medical history	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
WHO performance score	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Laboratory tests	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Leukapheresis			X													
Treg transfer					X											
Stool sampling	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood draw for immunomonitoring			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Infections-serology	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Calprotectin	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
IBDQ ^b	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Partial Mayo Clinic Score		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Mayo Clinic Score	X				X	X	X	X	X	X	X	X	X	X	X	X
Endoscopy	X				X	X	X	X	X	X	X	X	X	X	X	X
Adverse events			X	X	X	X	X	X	X	X	X	X	X	X	X	X

EOS, end-of-study; IBDQ, Inflammatory Bowel Disease Questionnaire.

patients at the tested dose level, dose de-escalation will continue until less than two patients have experienced a DLT. After successful enrolment at the highest dose level, five additional patients will be enrolled at the highest dose level to extend safety assessment. If no DLTs or less than two DLTs are experienced at all dose levels tested, the MTD is not reached. In this case, a MAD is defined. Due to Treg production limitations, no further dose escalation is considered in this study.

If any infection-related event, infusion reaction and/or laboratory changes from baseline within 24 hours after adoptive Treg transfer occur, the DSMB will be informed immediately and asked for advice how to proceed.

Harms

Safety of patients is the primary endpoint of this study and will be evaluated at each study visit from visit 3 to the end of study visit by medical history, evaluation of quality of life by IBDQ, physical examination (survey of skin reactions, vital sign measurement, including temperature, blood pressure, weight and pulse measurement, control for signs of autoimmunity), laboratory tests, assessment of faecal calprotectin and AE assessments. Moreover, at study visits 1, 5 and 7 an endoscopy including assessment of the full Mayo Clinic Score is performed.

Auditing

A Quality Assurance Plan will be implemented and all critical procedures will have to follow respective standard operating procedures (eg, for study initiation, archiving of documents etc).

Before the start of the trial an initiation visit will be performed at the Department of Medicine 1. Participating investigators will be supplied with the Clinical Trial Protocol, the Investigators Brochure and the CRFs and will be trained for Good Clinical Practice (GCP) compliance and correct completion of the CRFs.

The Clinical Monitor will check the Site Master File, required regulatory files and informed consents of each patient and will review the CRFs for completeness, verify the source data and instruct the responsible investigator to make any required corrections or additions.

The clinical monitor will check:

1. Regulatory files
 - Institutional Review Board Permission, Amendments
 - Signatures required
 - Patient Informed Consents (of each patient)
2. Patient records
 - Eligibility criteria
 - Concomitant medications
 - Adoptive Treg transfer
 - AE/SAE reporting
 - Missed visits and follow-up
 - Treatment discontinuation.

The informed consent forms and CRFs of each patient will be checked by the clinical monitor for accuracy and

completeness, any problems and questions will be clarified with the responsible investigator.

The team of investigators/study assistants responsible for documentation in the CRFs has to be present at the visit. Problems will be discussed between the team and the clinical monitor during the visit.

Quality indicators for the conduct of the study will be:

- ▶ Compliance with the inclusion and exclusion criteria
- ▶ Completeness and accuracy of the signed informed consent forms
- ▶ Compliance with the scheduled study timelines
- ▶ Accuracy of AE and SAE reporting.

The principal investigator will get a monitoring report and is responsible for sharing the information with the investigators/study assistants and ensuring that monitoring findings are addressed. The monitoring report contains location, date, names of persons involved, a summary of the items controlled and a summary of the findings collected.

Ethics and dissemination

The study protocol is approved by the German Federal Institute for Vaccines and Biomedicines (Paul-Ehrlich-Institute (PEI), document number 417_19 Az), Langen, Germany and the Institutional Review Board of the Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany (document number 3652/01). The study is funded by the German Research Foundation (DFG) with grants to Caroline Voskens and Markus Neurath (KFO 257 project 08 and SFB/TransRegio 241 project C04). The study will be conducted in compliance with this study protocol, in accordance with GCP and the Declaration of Helsinki. The laboratory producing the Treg drug product (Experimental Immunotherapy, Department of Dermatology, University Hospital Erlangen) has legal approval for GMP production of polyclonal CD4⁺CD25⁺ Treg cells (Certificate of GMP Compliance of a Manufacturer gem § 64 Abs. 3 AMG i.V. m § § 13, 72 AMG, 8 August 2007; approval of 20 September 2007, 53.2-ZAB-2671.1-H207, Regierung von Oberfranken, extended February 2017). The results will be published in peer-reviewed scientific journals, and nationally as well as internationally disseminated in scientific conferences and media.

Patient and public involvement statement

Patients were not and will not be involved in the research process.

Author affiliations

¹Department of Dermatology, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany

²Deutsches Zentrum für Immuntherapie (DZI), Erlangen, Germany

³Department of Medicine 1, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany

⁴Department of Medicine (Gastroenterology, Infectiology, Rheumatology), Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Campus Benjamin Franklin, Berlin, Germany



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Contributors Conception and design of the study: CJV, GS, RA, MFN. Clinical visits: FV, JW, RA, MFN. Data collection: SR, FV, JW, CJV, SZ. Production of the investigation's medicinal product Treg: DS, MR, MW, CJV, BS. Initial draft of the manuscript: CJV. Critical revision: CB, MFN, RA, GS, BS. All authors critically revised the manuscript for intellectual content and approved the final version.

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Competing interests GS is inventor of granted patents related to the manuscript (publication number EP 1379625; CD4⁺CD25⁺ regulatory T cells from human blood).

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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ORCID iD

Caroline J Voskens <http://orcid.org/0000-0001-9398-005X>

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