STU00215838

PROTOCOL TITLE: Erythropoietin Therapy to Induce Regulatory T cells in Liver Transplant Recipients

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VERSION NUMBER: 3
VERSION DATE:
4.6.2022

STUDY SUMMARY:

Investigational Agent(s) (Drugs or Devices)	
IND / IDE / HDE #	
Indicate Special Population(s)	☐ Children ☐ Children who are wards of the state ☐ Adults Unable to Consent ☐ Cognitively Impaired Adults ☐ Neonates of Uncertain Viability ☐ Pregnant Women ☐ Prisoners (or other detained/paroled individuals) ☐ Students/Employees
Sample Size	20 patients
Funding Source	The Dr. Michael M. Abecassis Transplant Innovation Endowment
Indicate the type of consent to be obtained	X Written ☐ Verbal/Waiver of Documentation of Informed Consent ☐ Waiver of HIPAA Authorization ☐ Waiver/Alteration of Consent Process
Site	☐ Lead Site (For A Multiple Site Research Study) ☐ Data Coordinating Center (DCC)
Research Related Radiation Exposure	☐ Yes X No

DSMB / DMC / IDMC	☐Yes X No
Protocol Changes v3 (4.6.2022)	Exclusion Criteria: Pg 6: Removing "history of venous thromboembolism including deep vein thromboses or pulmonary emboli" Per team meeting and on the advice from the Pharmacy and Nephrologist, thromboembolic events are not contra-indications for ESA use of EPO except in the case of active malignancy.
	COMPENSATION FOR PARTICIPATION IN RESEARCH ACTIVITIES: Pg 6: Administrative error correction

OBJECTIVES:

Due to the adverse effects associated with immunosuppressive therapy (IST), strategies to increase the success of IST withdrawal are of significant interest in improving the outcomes and lives of liver transplant recipients (LTR). The goal of this study is to test the safety of erythropoietin (EPO) and its effects on regulatory T cell induction and function in liver transplant recipients on different IST regimens. To achieve this, we will:

1. Define EPO effects on Treg induction in liver transplant recipients *in vivo.*
2. Delineate the regulatory functional capabilities of EPO induced Tregs in liver transplant recipients *in vivo.*
3. Confirm the safety of EPO in liver transplant recipients

The hypothesis of this proof-of-concept study is that EPO increases the frequency, stability and/or function of Tregs in liver transplant recipients. We also hypothesize that EPO will have a greater effect in everolimus vs. tacrolimus treated LTR, thus providing the rationale for a subsequent clinical trial to utilize EPO in combination with everolimus as a more successful pathway toward tolerance.

BACKGROUND:

While advances in immunosuppressive therapy have lowered the rate of acute rejection following LT, the consequence has been an increase in morbidity and mortality related to the lifelong need for maintenance IST [1]. These complications include an increased risk of malignancy, infection, metabolic disorders, and chronic kidney disease, as well as high health care costs associated with these therapies and their required monitoring. Given these issues, most clinicians attempt trial and error dose minimization with variable success rates, and there has been significant interest in full drug withdrawal in highly select patients. These strategies would however be more successful and broadly applicable if immunomodulatory therapies could be developed to promote IST withdrawal earlier after LT. Based on the increased recognition that Treg play an essential role in maintaining tolerance to self- and allo-antigens, significant effort is being directed to *ex vivo* expansion and reinfusion of Treg in patients [2,3]. However, as the effectiveness of these costly, more invasive strategies remains largely unproven, alternative, safer approaches to increase Treg need to be explored.

EPO and EPO receptors: EPO is predominantly produced by the kidney in response to hypoxia[4]. EPO binds to a homodimer EPO receptor (EPOR) responsible for erythropoietic effects and a heterodimer complex composed of one chain of the EPOR and the ubiquitous β-common receptor.[5,6] EPO activation of the EPOR/CD131 heterodimer does not mediate erythropoiesis but promotes TGF-β production by APCs that leads to conversion of naïve CD4 T cells into Tregs.

EPO promotes Treg in vitro and in vivo in mice: Our data show that human T cells express the EPOR and that EPO inhibits human T conventional cell (Tconv) proliferation.[7] In contrast, EPO promotes *in vitro* Treg induction by increasing release of active TGF-β by monocytes. We also showed that EPO has immune regulatory functions *in vivo* at physiological concentrations and is responsible for kidney grafts acceptance from B6 into BALB/c mice.[7] We also treated MRL-*lpr* mice - a strain that spontaneously develops lupus associated with impaired Tregs - with EPO or vehicle control and found that EPO increased Tregs and reduced disease severity.

EPO induces Tregs in vivo in humans: To test the hypothesis that therapeutic doses of EPO used to treat anemia increase Treg in humans *in vivo*, we performed a prospective-cohort study quantifying circulating Treg[8,9] in 28 advanced chronic kidney disease patients who were given standard therapeutic doses of EPO to treat anemia [8,9]. Flow cytometric analyses showed significant increases in the frequencies of Treg in subjects given EPO, yet no changes in controls. We next performed a proof-of-concept, mechanistic study of EPO in patients with autoimmune hepatitis (AIH), a liver disease that is managed with IST similar to LT and can progress to and recur after LT [10]. We treated six stable AIH patients with a single 10,000 IU dose of EPO and characterized changes in Treg before and at 4 and 12 weeks after treatment, using mass cytometry (CyTOF) combined with an unbiased clustering approach (Phenograph) based on 22 Treg-relevant cell-surface markers. EPO was well tolerated and no patients had significant changes in hematological parameters, liver enzymes, or IgG levels. Total Treg and Treg/CD8 T cell ratios significantly increased at 4 weeks and returned to baseline levels at 12 weeks after EPO injection. We identified 17 Treg subsets of which $CD4^{+}CD25^{HI}CD127^{NEG}$ $HLADR^{+}$ Treg had the highest increase and the most favorable Treg/$CD8^{+}$ ratio after EPO treatment. *Ex vivo* assays documented that Treg were functional and those isolated at 4 weeks after EPO were significantly more suppressive than those isolated at baseline. These results provide the rationale for studies testing the hypothesis that EPO improves outcomes of AIH patients by increasing Treg, as well as LTR proposed in this RFP, for similar reasons.

Everolimus induces Tregs in vitro and in humans in vivo. Calcineurin inhibitors, tacrolimus in particular, have been the mainstay IST after LT for several decades. Although CNIs are highly efficacious in preventing rejection, they may ironically hinder the development of tolerance by reducing the number and function of Tregs. Alternative IS drugs, such as mTOR-Is sirolimus and everolimus, inhibit T effector cells but spare Tregs and may favor successful IS withdrawal[11]. We have performed several studies demonstrating that peripheral blood Tregs are reduced in LT recipients on tacrolimus compared to sirolimus and increased with conversion from tacrolimus to sirolimus[12-14]. We have more recently shown success with sirolimus withdrawal in LTR, as well as protolerogenic effects of tacrolimus to everolimus (the current approved mTOR-I in LTR) conversion early after LT [15,16].

These findings indicate that clinically used doses of EPO augment human Treg frequencies and may enhance tolerance to ultimately promote IST withdrawal success. Our overarching goal is to test this approach specifically in liver transplant recipients and assess the impact of different IST regimens (CNI vs. mTORi) on EPO-induced Treg.

STUDY ENDPOINTS:

Our primary endpoint is to define EPO effects on Treg induction in liver transplant recipients *in vivo.* We will use flow cytometry to analyze the phenotype of peripheral blood mononuclear cells (PBMC) collected before and 4 and 12 weeks after EPO administration. Data will be analyzed to extract changes in lymphocyte subset frequencies, including Tregs.

Our secondary endpoint is to delineate the regulatory functional capabilities of EPO induced Tregs in liver transplant recipients *in vivo.* We will determine T_{REG} function at baseline and after treatment in both arms by suppression assays.

Finally, we will confirm the safety of EPO in liver transplant recipients by monitoring CBC and liver parameters, as well as any clinical symptoms, at weeks 4 and 12 visits.

STUDY INTERVENTION(S) / INVESTIGATIONAL AGENT(S):

We will enroll stable liver transplant recipients on either tacrolimus or everolimus immunosuppression monotherapy to receive a single dose of erythropoietin 10,000 IU and have blood samples for Treg and other immune assessments before and up to 12 weeks after EPO. As described above, EPO has been shown to induce Tregs in humans in previous studies.

Patient will receive a single dose of erythropoietin 10,000 units which will be given subcutaneously at the initial clinic visit. The medication is available a single-dose vial and stored in a refrigerator between 36°F and 46°F prior to use. Prior to injection, name, date of birth, and medical record number of the participant will be verified. Consent and willingness to participate in the study will also be reviewed prior to administration of EPO

PROCEDURES INVOLVED:

Study Design will be as follows:

Visit 0 – recruitment

- Patients will be contacted by the study coordinator to discuss the study and provide information regarding the purpose of the study and the potential risks and benefits from participation.
- If the patient has access to the internet, an electronic copy of the informed consent will be provided via email.
- If the patient does not have access to the internet, a copy of the informed consent may be mailed to his/her house.

Visit 1 – enrollment (week 0)

- Informed written consent will be obtained.
- Medical history will be reviewed to ensure electronic medical records are accurate and confirm the patient meets all inclusion and exclusion criteria
- Physical examination including vital signs.
- 70 mL of blood samples will be collected Peripheral blood mononuclear cells (PBMCs) and serum samples will be isolated and stored at the Northwestern University Transplant Immunology Core/Biorepository.
- Confirm lab results prior to administering erythropoietin to confirm inclusion/exclusion criteria
- Administer a single dose of erythropoietin 10,000 units for research purposes (this dose

has been decided based on safety/efficacy study in other studies and our prior study with AIH patients)

Visit 2 – follow-up (week 4)

- 50 mL of blood samples will be collected (Repeat CBC, hepatic function panel, tacrolimus and everolimus levels). Peripheral blood mononuclear cells (PBMCs) and serum samples will be isolated and stored at the Northwestern University Transplant Immunology Core/Biorepository.

Visit 3 – follow-up (week 12)

- 50 mL of blood samples will be collected (Repeat CBC, hepatic function panel, tacrolimus and everolimus levels).

DATA AND SPECIMEN BANKING

Participants will have a total of three blood draws over the course of this study. Each participant will be assigned a study number and that will be the only information located on the corresponding blood vial. Blood specimens will be stored in a locked facility.

Data obtained from this study will be stored in Redcap database which is password protected. Only study investigators listed on this IRB will have access to this data.

Aliquots of PBMCs and serum samples will be isolated and froze for batched phenotypic and functional analyses. During storage, the blood sample will be identified only by the patient's study number. There will be no patient identifying information available on each individual blood sample tube.

Blood specimens will be frozen at -80°C and stored in a locked area at the Gastroenterology Biorepository freezers

These samples will be stored at the Northwestern University Transplant Immunology Core/Biorepository. At the end of the study (12 weeks), cryopreserved samples will be thawed and the flow cytometric analysis and functional studies will be performed in the Immune Monitoring Core by Dr. Mathew and team. No other team members will have access to the collected serum samples once stored at the biorepository. Once the study is completed, these samples will be banked for future use only if further larger trials are ensued. If larger trials are planned, only the PI's will have access to the stored specimens. Laboratory data will remain de—identified in the Redcap database.

SHARING RESULTS WITH PARTICIPANTS

The data obtained will be accessible only to the study investigators. The results of the investigation will not be released to each individual patient. If requested, final publication can be shared with participants.

STUDY TIMELINES

Participants enrolled in the study will be followed for a total of 12 weeks. Blood samples will be obtained at each visit (week 0 before administration of erythropoietin and weeks 4 and 12 after erythropoietin). Erythropoietin will be administered per this protocol. It will be given once at the

first visit after the initial blood sample has been obtained to confirm inclusion/exclusion criteria are met. We anticipate completion of the study in 12-24 months (Up to 12 months for enrollment, 3 months for data/serum collection, 6 months to complete data analysis).

INCLUSION AND EXCLUSION CRITERIA

Patients will be identified from the liver transplant program at NMH.

Inclusion Criteria

- Male or female
- Age 18-74 years
- History of liver transplantation > 2 years prior for non-immune causes
- Use of immunosuppressive monotherapy (either tacrolimus or everolimus) for treatment of liver transplantation
- Stable immunosuppression regimen at least 3 months prior to enrollment.
- Ability to provide verbal and written informed consent

Exclusion Criteria

- Hgb above average normal value (15.7 g/dL in men, 13.8 g/dL in women); ALT > 2 times upper limit of normal; uncontrolled hypertension with SBP≥160 or DBP≥100; end-stage renal disease on hemodialysis; stroke, heart failure, seizure disorder, significant cardiovascular disease including a history of myocardial infarction, pure red cell aplasia, intolerance or allergy to erythropoietin; Active malignancy (untreated or undergoing therapy); known hypersensitivity to mammalian cell-derived products; known hypersensitivity to human albumin; presence of vascular access; prior recipient of erythropoietin within 12 weeks of the study; and pregnancy
- Patient unable to provide consent including infants, children, teenagers, prisoners, cognitively impaired adults.
- Prisoners and other vulnerable populations will also be excluded

RECRUITMENT METHODS

Patients will be identified from the liver transplant program at NMH during clinic visits in the post-transplant clinic. During the clinic visit, any patient that fits the inclusion criteria will be identified, consented and enrolled in the study. No advertisements will be used to recruit patients. To ensure no coercion, discussion of the study will occur at the end of the medical clinic visit.

COMPENSATION FOR PARTICIPATION IN RESEARCH ACTIVITIES

Patient compensation will include $50 per patient per session x 3 sessions via check following the last patient visit. Payment will be adjusted if patients do not complete all three sessions. Parking vouchers will also be made available, if needed ($6 per patient per session x 3 sessions).

WITHDRAWAL OF PARTICIPANTS

Participant involvement in the study includes medical record review on the first visit and a total of 4 blood draws. As a result, we anticipate low likelihood of patient desire to withdraw from the study after enrollment. However, participants will be made aware of the option for withdrawal if he/she does not want to return for subsequent blood draws.

Patients will be withdrawn from the research without their consent if any of risk or threat to his/her medical health is detected (rise in hemoglobin above normal value, rise in ALT greater than two times the upper limit of normal, sudden uncontrolled hypertension with SBP≥160 or DBP≥100; stroke, heart failure, seizure, or myocardial infarction, active malignancy, pregnancy or newly detected allergy to erythropoietin.)

Termination from the study will occur as soon as a medical threat is detected and any side effects will be reported to the IRB.

RISKS TO PARTICIPANTS

Erythropoietin is a medication with extensive use clinically and rare side effects or adverse events. However, the following warnings and precautions are advised to prescribers when used long-term:

- Uncontrolled hypertension
- Lower seizure threshold
- Development of antibodies to erythropoietin
- Tumor progression or recurrence of malignancy
- Vascular access thrombosis
- Increased risk of stroke
- Increased risk of venous thromboembolism
- Pure red cell aplasia
- Anaphylactic reaction

Since the patients in this study will receive a single dose of erythropoietin, the risk of these occurring is minimal. Patient will be monitored closely after receiving the medication to ensure a severe allergic reaction does not occur. Milder side effects related to single drug administration may include injection site reactions i.e. pain and erythema. Other reported side effects including arthralgias, myalgias, fever, cough, rash, nausea, vomiting, itching, and headache.

To minimize this risk, at every visit, we will record vital signs and we will monitor hemoglobin to test erythropoietic effects of EPO. Given such close monitoring, the risk of such events is rare. In addition, major issues such as uncontrolled hypertension, seizures, red cell aplasia, anaphylaxis, etc were not seen in our previous EPO/AIH study and rarely in other EPO studies, but need to be assessed in the liver transplant population herein.

Adverse events reported spontaneously by subjects outside the above-indicated time points will also be documented. The causality of AEs with EPO will be assessed by the PI and submitted to the IRB.

Risks of Study Procedures

Risks associated with blood sampling will include minimal discomfort and/or bruising. Infection, excess bleeding, clotting, and/or fainting also are possible, although unlikely.

Blood sampling will be performed at several visits. Sometimes the blood sampling may be a little uncomfortable and subject may experience pain, some redness or swelling in the area where blood has been taken. There may be risk of infection. Some people also experience faintness.

Loss of Confidentiality

Anytime information is collected, there is a potential risk for loss of confidentiality. Every effort will be made to keep all subject information confidential.

POTENTIAL BENEFITS TO PARTICIPANTS

Participants recruited for this study will have a history of liver transplantation requiring long-term immunosuppression. Though this particular study will not withdraw immunosuppression, the overall goal of this study is to determine the possibility of weaning immunosuppression in the future if this is successful in showing the ability of erythropoietin to selectively augment regulatory T cells. Thus, benefit will not be immediate but will require a future study that would include close monitoring with withdrawal of immunosuppression.

DATA MANAGEMENT AND CONFIDENTIALITY

We will compare changes in Tregs between month 0 (pre-EPO treatment) versus week 4 and 12 (after EPO treatment) within each subject, as a whole and between tacrolimus vs. everolimus treated patients. Our previous data indicate that EPO, at the doses used to treat anemia, increases Tregs in 92% of patients vs. 50% of untreated ones. Therefore, we estimated a sample size of 20 patients would achieve 80% power to detect a difference of 30% in the percentage of Treg increase between baseline and after EPO treatment. It must be recognized that 10 patients in each group are considered pilot numbers to more adequately power future larger trials utilizing this protocol.

To test the hypothesis that EPO increases Treg induction in humans *in vivo*, we will compare percentages of $CD4^+CD25^+CD127^{low}$ Tregs before and serially at 4 and 12 weeks after EPO treatment. We will also comprehensively characterize the effects of EPO on the immune system (including naïve, memory, exhausted, senescent, and anergic T cells) by flow cytometry. We will also determine Treg function at baseline and after treatment in both arms by suppression assays. Briefly, CFSE-labeled third-party PBMC will be stimulated with anti-CD3 mAb (1ug/ml; BD Biosciences) in the presence of increasing ratios of Miltenyi magnetic microbead-sorted $CD25^+$ and PKH26-labeled modulator cells (enriched for Tregs) in complete RPMI media. The modulator $CD25^+$ cells are purified from the baseline, 4 weeks and 12 weeks collections from the recipient, and are added at responder: modulator ratios of 1:1, 2:1, 4:1, 8:1, 16:1 and 32:1. After 5 days, $CD4^+$ and $CD8^+$ T cell proliferation will be quantified by CFSE dilution using a flow cytometer. We will use the same third-party PBMC as target cells to reduce variability associated with changes in conventional T cell responses.

Laboratory data collected during clinics will be de-identified and stored in a password protected database, called Redcap. Only those who are responsible for collection and analysis of the labs will have access to the database. Serum samples stored for analysis will also be de-identified and only Dr Matthew's team will have access to the samples. Data will be available for the length of the study and will include the information listed on the attached data collection sheet.

Aliquots of PBMCs and serum samples will be collected during clinic visits and transported by the research nurse to the Northwestern University Transplant Immunology Core/Biorepository, where they will be isolated and froze for batched phenotypic and functional analyses. At the end of the study (12 weeks), cryopreserved samples will be thawed and the flow cytometric analysis and functional studies will be performed in the Immune Monitoring Core by Dr. Mathew and team

PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF PARTICIPANTS

Blood draws incur only minimal risk. The administration of a single dose of erythropoietin has

low risk but patients will be monitored closely for adverse events. If a patient has a symptom that is related to the administration of erythropoietin, he/she will be followed until complete resolution.pr

PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF PARTICIPANTS

During the consent process, the study team will review all of the steps and procedures required by the participant throughout the 12 week study timeline. Participants will be able to state any limits on whom they provide personal information to and whom they interact with. Members of the study team will be introduced to the participants during the consent process and specific team members will be designated for each step of the process. This will help ensure participants feel at ease during any examinations and/or procedures.

Members of the research team will enter de-identified data into redcap. Personal information will only be available to the PI's while the rest of the research team will have access to deidentified data only.

COMPENSATION FOR RESEARCH-RELATED INJURY

Compensation for injury is not available. This study is expected to be low risk with minimal harm.

ECONOMIC BURDEN TO PARTICIPANTS

Patient will not incur any costs for participation in the study. Phlebotomy will be performed by clinic staff who also perform routine blood draws in the routine clinical setting. The blood samples will be run at the Northwestern research laboratory; thus, the patients' insurance companies will not be made aware of this study. As the patient will be required to return at a greater frequency than typical clinic visits, patients will be provided with $50/visit to cover the cost of travel, parking, and other additional expenses.

CONSENT PROCESS

Consent will be obtained by the principal investigator or coinvestigators. Patients may be contacted by telephone up to 90 days prior to the first clinic visit to provide information about the study, but informed written consent will be obtained in person at the initial visit. Patients will be able to withdraw from the study at any point by contacting the study coordinator.

Only patients fluent in English will be enrolled in the study. Patients unable to provide their own consent (i.e. children, cognitively impaired adults, prisoners) will not be eligible for enrollment.

Comprehension of the consent and study requirements will be assessed by a member of Dr Levitsky's team by asking the participant to explain his/her understanding of the risks and benefits of the study

NON-ENGLISH SPEAKING PARTICIPANTS

Only English speaking participants will be involved in this study.

WAIVER OR ALTERATION OF CONSENT PROCESS

No waiver or alteration of the consent process is required for this study

PROTECTED HEALTH INFORMATION (PHI AND HIPAA)
The study will involve collection of protected personal health information (Medical record number and age). However, after enrollment into the study, all data will be de-identified and stored using password protected, encrypted software (i.e. Redcap).

QUALIFICATIONS TO CONDUCT RESEARCH AND RESOURCES AVAILABLE
At Northwestern's Transplant Institute, there are an estimated 500 patients who are two years post transplants on monotherapy for immunosuppression. Therefore, we only need to recruit 4% of these patients for this particular study. As mentioned above, the consent process, EPO administration and collection of blood will take place in clinic with highly trained nurses. Peripheral blood mononuclear cells (PBMCs) and serum samples will be isolated and stored at the Northwestern University Transplant Immunology Core/Biorepository and utilized by Dr Mathew's team only.

MULTI-SITE RESEARCH
This is a single site study.