A Pilot Study to Evaluate the Efficacy and Safety of Budesonide as an Alternative to Prednisone for Liver Transplant Immune Suppression

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Synopsis:

This is a pilot study that investigates the efficacy and safety of budesonide as an immune suppressing agent for liver transplant recipients in the early post-transplant period.

The primary end-point is rates of acute cellular rejection within first 24 weeks post-liver transplant. Secondary end points include rates of new onset diabetes after transplant and safety of budesonide.

The study is structured as a prospective clinical trial. After receiving 4 days of intravenous corticosteroids on liver transplant post-operative days 0 through 3, subjects will be started on standard immunosuppression plus enteric coated budesonide (study drug) in place of standard immune suppression plus prednisone (standard of care). Study drug will be tapered over 12

weeks in accordance with the existing standard of care immune suppression protocol. Subjects will be followed in outpatient transplant clinic for 24 weeks. The purpose of the study is to conduct a pilot study to generate rates and effect size that can be used in a subsequent equivalent trial. A total of 20 subjects will be enrolled to receive the standard immunosuppression plus budesonide and their outcomes will be compared to 20 controls receiving standard immunosuppression plus prednisone (standard of care). The use of controls is to generate rate and variability that can be compared with the rate obtained from patients that receive study drug by examining the 95% confidence band.

1- BACKGROUND:

1.1: Statement of Problem

Corticosteroids are an important part of liver transplant (LT) immune suppression. They act at multiple targets to prevent acute cellular rejection (ACR) and are the mainstay for the treatment of ACR [1]. Corticosteroids constitute an important part of immune suppression regimen in the early post LT period and up to 80% of LT centers in United States use systemic corticosteroids for the first three to twelve months after LT [2]. Multiple studies have looked at the safety and efficacy of corticosteroid free immune suppression regimens after LT. Results of a meta-analysis which included 21 randomized trials comparing regimens with and without corticosteroids revealed higher rates of ACR in the non-corticosteroid group when corticosteroids were not replaced with another immunosuppressant such as antibody induction or an antimetabolite preparation [3].

Use of systemic corticosteroids like prednisone is associated with many well-known adverse effects including elevated blood glucose, weight gain, increased rate of infections and metabolic bone disease. New onset diabetes after transplantation (NODAT) is an important long term complication affecting graft and patient survival. Incidence of NODAT after LT is estimated to be 10-20% [4]. A retrospective review of a cohort of LT recipients at the University of Cincinnati Transplant Center over the last 3 years revealed rates of NODAT of 10-12%. Immunosuppressant agents, especially corticosteroids, are a major contributor to altered glucose regulation. LT recipients with diabetes have higher rates of death due to infections, graft failure, late onset hepatic artery thrombosis and cardiovascular events [4]. In addition management of corticosteroid induced hyperglycemia requires significant clinical resources, monitoring and additional clinic visits in the early post LT period. Although corticosteroids are typically only used during the first few months post LT, their adverse effects are significant, leading to numerous hospital readmissions and reduced patient quality of life. Corticosteroid free regimens have shown to decrease the rates of NODAT, infectious complications and elevated cholesterol levels but are associated with higher rates of graft rejection [3].

1.2: Budesonide Role in Immune Mediated Liver Conditions

Budesonide, a synthetic corticosteroid undergoes complete intestinal absorption after oral intake and extensive first-pass hepatic metabolism with only 10% systemic bioavailability. Budesonide is approved by Food and Drug Administration (FDA) for treatment of Crohn's disease. Multiple studies have shown it to be effective in the treatment of immune mediated liver conditions such as primary biliary cirrhosis and autoimmune hepatitis while having minimal adverse effects likely due to its limited systemic bioavailability [5,6,7]. In a phase IIB randomized clinical trial, combination of budesonide and azathioprine was found superior to combination of prednisone and azathioprine to induce biochemical remission in patients with newly diagnosed autoimmune hepatitis while showing significantly lower rates of steroid specific side effects [8]. Incidence of new onset diabetes was 1% in the budesonide group. In another study of patients with Crohn's disease, average reduction in bone mineral density was significantly lower (1.04%) for patients who used long term budesonide as compared to patients on prednisone (3.84%) [9]. Use of budesonide in immune mediated liver conditions is shown to be safe for up to 2 years [6].

1.3: Role of Budesonide in Transplant Immune Suppression

The role of budesonide as an immunosuppressant post LT is limited to animal models where it has shown to prevent ACR [10,11]. Budesonide has also been shown to be effective as a single agent immunosuppressant in a case series of 3 patients with severe sepsis [12]. When used in place of systemic corticosteroids like prednisone, budesonide has the potential for fewer systemic adverse effects including lower rates of NODAT, infections and metabolic bone disease while providing adequate liver specific immune suppression to prevent ACR in the early period post LT.

2- STUDY OBJECTIVES

2.1: Hypothesis

We hypothesize that use of standard immune suppression (SIS) with budesonide in the early post LT period will provide comparable efficacy and will result in similar rates of ACR while significantly reducing the steroid related adverse effects of NODAT compared to the use of SIS with prednisone.

2.2: Primary Aim

To estimate with 95% confidence interval, the incidence rate of ACR during the first 24 weeks post liver transplantation amongst recipients receiving SIS plus budesonide and compare it to the estimate obtained from controls that received SIS plus prednisone. This outcome will be used to assess efficacy of the study drug.

2.3: Secondary Aims

Will be used to assess the safety of the study drug

a) To estimate with 95% confidence interval, the rate of new onset diabetes after transplant (NODAT) at 24 weeks post liver transplant amongst recipients receiving SIS plus budesonide and compare it to the estimate obtained from controls receiving SIS plus prednisone.

c) To estimate the rate of adverse events and severe adverse events during the first 24 weeks post liver transplant amongst recipients receiving standard immunosuppression plus budesonide.

d) To estimate the magnitude of adrenal suppression by measuring serial serum cortisol levels during first 12 weeks post liver transplant and one time adrenocorticotropic hormone (ACTH) stimulation test at week 12 amongst recipients receiving SIS plus budesonide.

3- STUDY DESIGN AND PROCEDURES

3.1: Study Design

This will be a pilot, phase IIA, prospective trial of SIS plus budesonide (off label) in liver transplant recipients during early post LT period. Due to limitations of pilot funds available, we have chosen to compare these to controls rather than include a randomized control group.

3.2: Sample Size

Sample size will be based on primary outcome measure of efficacy (rate of ACR). Review of data from historic cohort reveals rate of ACR ~ 10%. We hypothesize that similar rates of ACR will be observed using budesonide. Since we are not directly comparing the ACR rate between those on budesonide and those on standard treatment (due to the small sample size), our plan is to estimate this rate with a given confidence in those who are on budesonide group. As such with the proposed sample size of 20, a two sided 95% confidence interval will be able to estimate this rate within 0.13 range.

3.3: Study Duration

Based on the 90-100 LT performed annually at our center, enrollment of 20 subjects will be achieved over a 6-9 month time frame. Duration of study per subject is 6 months; thus total study duration of 12-15 months.

3.4: Study Subjects

Subjects undergoing a LT for any indication will be eligible for enrollment

Inclusion criteria

a) Female or male subjects aged 21-75 years old
b) Received a primary liver transplant within 4 days of enrollment

Exclusion criteria

a) Received previous organ transplants
b) Undergoing multiple organ transplants
c) Recipients with advanced fibrosis in graft
d) Treatment plan for subject includes receiving immunosuppressant therapy other than standard immune suppression (SIS) as per University of Cincinnati LT immune suppression protocol (UC-ISP).
e) Inability to take enteral (orally or by tube feed) medications by day 4 post-transplant
f) Subjects with diabetes mellitus prior to transplant (diabetes mellitus defined as use of hypoglycemic agents or HbA1c > 6.4 prior to transplant)
g) Subjects who have any severe medical condition requiring acute or chronic treatment that in the investigator's opinion would interfere with study participation.
h) Subjects who have been exposed to an investigational therapy within 30 days prior to enrollment or 5 half-lives on the investigational product, whichever is greater.
i) Subjects in which concomitant use of medications which are inhibitors of CYP3A4 (such as ketoconazole, itraconazole, ritonavir, indinavir, saquinavir, erythromycin) cannot be avoided during the study period.
j) Pregnant females
k) Diminished mental capacity to consent for the study as determined by attending on the record.

3.5: Screening and Enrollment of study subjects

Upon institutional review board (IRB) approval, all consecutive patients undergoing LT at the University Of Cincinnati Transplant Center will be screened for enrollment upon admission for transplant surgery. Subjects will be approached after permission from attending on record by one of the members of research team. Study rationale, procedures, different interventions, potential benefits and risks as well as alternatives to study participation will be explained to the subjects in their native language in non-medical terms, as much possible. For non-English speakers, certified interpreter services will be used. Subjects meeting all of the inclusion and none of the exclusion criteria will be educated about the study and written informed consent will be obtained either 12 hours prior to undergoing LT or within 72 hours of completion of a successful transplant surgery. This time frame is proposed so that subjects have sufficient time to go over the study and are not pressured to sign the consent within a short time frame prior to LT surgery. In addition, all patients being placed on liver transplant list at our center will be provided with a copy of the consent form in the LT clinic at the time of listing for review only so that this study proposal is not completely novel to them at the time of LT surgery. A pregnancy test will be performed on all the females of childbearing potential.

3.6: Enrollment of Controls:

Each study subject will be matched with a control subject. Patients undergoing LT at University of Cincinnati that are not part of the study will serve as controls. Confounding and effect modifiers will be accounted for by matching the controls on multiple variables which may affect the primary outcome of ACR. These variables include: age less than 55 years, serum creatinine greater than 1.5 ng/mL, the use of antibody therapy at the time of transplant and a history of autoimmune hepatitis. To minimize the selection bias, the matched control will be selected in a manner that he/she would have undergone liver transplantation within a 24 week period (8 weeks prior or 16 weeks after) of the liver transplantation of the matched study subject. This means that data from controls will also be collected prospectively. Since outcomes are being measured at week 24 of liver transplantation, this will ensure that the investigators have no influence on selecting the controls with a known outcome. Controls will be identified through LT clinic. They will not undergo any study specific procedures, interventions, testing or evaluations. A written and informed consent will be obtained from all controls prior to their enrollment in the study. University of Cincinnati transplant program has performed 90-100 LTs each year over last 2 calendar years. Application of inclusion and exclusion criteria of our study to this database estimates that 65% of all LTs will be potential candidates for participation either as study subject or control. Based on this estimate we are confident that we can achieve goal enrollment of 20 subjects and 20 controls in 6-9 month period.

3.7: Study Drug

After LT surgery, subjects will be started on SIS including intravenous corticosteroids, calcineurin inhibitor (CNI), mycophenolate mofetil (MMF) +/- thymoglobulin as per University of Cincinnati LT immune suppression protocol (UC-ISP) [13].

On post-operative day 4, intravenous corticosteroids will be discontinued and replaced by the study drug; budesonide. Based on pharmacokinetic and bioavailability studies, 3 mg of budesonide is equivalent to 10 mg of prednisone [14]. Starting dose of budesonide will be 9 mg by mouth daily which will be equivalent to 30-40 mg of prednisone used as standard of care. Study drug will be tapered over the next 3 months as detailed in Table 1 and in line with UC-ISP [13]. At 3 months post LT, study drug will be discontinued. Subjects with autoimmune hepatitis as an etiology for LT will be initiated on prednisone 5 mg daily in addition to SIS as per UC-ISP.

Table 1: Study Drug Taper

Time post Liver Transplantation	Immunosuppressive therapy
Days 0-3	Standard immune suppression (SIS) + Intravenous corticosteroids
Days 4-30	SIS + Budesonide 9 mg
Days 31-45	SIS + Budesonide 6 mg
Days 46-90	SIS + Budesonide 3 mg
Days 90 onwards	SIS[1]

[1]Subjects with h/o autoimmune hepatitis may be initiated on prednisone 5 mg

3.8: Study Assessments (Visits 3-7)

Subjects will undergo study visits in the LT clinic or inpatient transplant unit at post-LT weeks 2,4,6,8 and 12 (Figure 1). At each visit, data regarding history and physical, vital signs, concomitant medications, use of hypoglycemic agents or insulin, adverse event assessment, laboratory blood work including blood counts, chemistries, blood glucose, liver function test, and tacrolimus trough level will be recorded. Blood samples for early morning cortisol level (between 6 AM and 9 AM) will be collected at week 4 and week 8 visits only. These samples will be centrifuged at 3600 rpm for 15 minutes, appropriately labelled and stored in – 80 C freezer at Schubert Research Clinic. All the samples from the study will be analyzed for serum cortisol in 1-2 batches to ensure uniform and standardized testing conditions and reagents. No other testing will be performed on these samples. Hemoglobin A1c will be checked at week 12 (visit 7). Additionally, at each visit, the study team will perform a pill count for the study drug. Study drugs will be dispensed every 4 weeks at study visits 2, 4 and 6. Decisions regarding obtaining a liver biopsy for evaluation of abnormal liver tests will be at the discretion of treating physician. Biopsy proven ACR will be treated according to UC-Rejection protocol (UC-REJ) [13].

Study drug will be discontinued at week 12 (visit 7) and subjects will continue routine post-LT follow up as per the current standard of care.

A low dose ACTH stimulation test will be performed at Schubert Research Clinic at week 12 visit. This test comprises of intravenous injection of cosyntropin at a dose of 1 micrograms per 1.73 m^2 of body surface area and checking the serum cortisol levels at baseline and 30 minutes after cosyntropin injection. This test is used to assess adrenal insufficiency with a high sensitivity.

Figure 1: Study visits

Study Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

	Screening and Enrollment	Study Drug	Week 2	Week 4	Week 6	Week 8	Week 12	Week 18	Week 24
Post-Operative Day (POD)	0	4	14 ± 5	28 ± 5	42 ± 5	56 ± 5	84 ± 5	126 ± 14	168 ± 14
History and Physical	X		X	X	X	X	X	X	X
Laboratory			X	X	X	X	X	X	X
Vital Signs	X		X	X	X	X	X	X	X
Informed consent	X								
Dispense study drug		X		X		X			
Study drug pill count			X	X	X	X	X		
Concomitant meds			X	X	X	X	X	X	X
Use and dose of Insulin/ Hypoglycemic	X		X	X	X	X	X	X	X
HbA1c	X						X		X
Adverse event/Rejection assessment			X	X	X	X	X	X	X
ACTH stimulation test (Schubert Research Clinic)							X		

3.9: Study Assessments (Visits 8-9)

Subjects will undergo 2 additional study visits once study drug has been discontinued at post-LT weeks 18 and 24 (Figure 1). At these visits data regarding history and physical, vital signs, concomitant medications, use of hypoglycemic agents or insulin, adverse event assessment, laboratory blood work including chemistries, blood glucose, liver function test and tacrolimus trough level will be recorded. Blood samples will not be stored for future analysis or testing. Hemoglobin A1C will be checked and recorded at weeks 24.

3.10: Removal of Subjects

Subjects will be removed from the study under any of the following circumstances:

a) Completion of study
b) Biopsy proven ACR
c) Severe or life threatening illness or infection requiring changes in SIS as per discretion of treating physician
d) Undergoing a repeat LT
e) Subject's or legally authorized representative's refusal to continue in the study
f) Death
g) Development of an adverse event which, in the investigator's opinion, requires termination of the study medication
h) Substantial non-compliance with study requirements or if subject is lost to follow-up
i) Request by the patient or a legal representative/relative that the subject discontinue the study treatment or be withdrawn from the study

In the case of early withdrawal or removal of a subject from the study, SIS will be continued as per UC-ISP or at the discretion of treating physician.

3.11: Study Aims

Primary Aim:

To estimate with 95% confidence interval, the incidence rate of acute cellular rejection (ACR) during the first 24 weeks of liver transplant amongst recipients receiving SIS plus budesonide and compare it to the estimate obtained from controls that received SIS plus prednisone. This outcome will be used to assess efficacy of the study drug.

The definition of ACR is based on widely accepted Banff criteria [15] of liver histology. A Banff score of 3 or more on liver histology as interpreted by local liver pathologist will be considered as ACR. Rates of ACR during first 24 weeks after LT will be calculated for the study subjects and compared to controls. This outcome will provide the assessment for the efficacy for study drug.

Secondary Aims:

a) To estimate with 95% confidence interval, the rate of new onset diabetes after transplant (NODAT) at 24 weeks post liver transplant amongst recipients receiving SIS plus budesonide and compare it to the estimate obtained from controls that received SIS plus prednisone.

Variable definitions of NODAT have been used in liver and kidney transplant literature. Expert recommendations from an international consensus meeting suggest that NODAT

should be differentiated from transient hyperglycemia during corticosteroids use since in majority of cases transient hyperglycemia resolves once corticosteroids are stopped [16]. For our study, subjects will be assessed for NODAT at week 24 of LT which will be 12 weeks after discontinuation of study drug or prednisone while subjects are on stable doses of other immune suppression. NODAT will be defined as glycosylated hemoglobin (HbA1c) of 6.4 % or more or requirement of insulin or any hypoglycemic agent.

c) To estimate the rate of adverse events and severe adverse events during the first 24 weeks post liver transplant amongst recipients receiving SIS plus budesonide.

Adverse events and severe adverse events will be recorded at each study visit and at any time that these are being reported by the subject to the study team. The data will be used in final analysis to determine the safety of the study drug as well as by the medical monitor at interim analyses to ensure that appropriate determination can be made regarding continuation, modification, or termination of the study.

d)) To estimate the magnitude of adrenal suppression by measuring serial serum cortisol levels during first 12 weeks post liver transplant and one time ACTH stimulation test at week 12 in recipients receiving SIS plus budesonide.

Early morning serum cortisol level is used as a screening test for adrenal suppression due to exogenous use of corticosteroids. The blood sample will be collected between 6 AM and 9AM. A serum cortisol value of 3 micrograms/deciliter or below will be used as a cutoff for adrenal suppression. Serial measurements of serum cortisol will help observe the effect of study drug on adrenal suppression over a 12 week period. One time ACTH stimulation test at week 12 (when study drug is stopped) will further assess for any adrenal suppression with high sensitivity.

All the secondary outcomes will help in assessing the safety of the study drug.

4-SAFETY INFORMATION

4.1: Budesonide

For the most recent safety update please refer to Budesonide (Entocort®) prescribing information [17].

4.2: Contraindications

Budesonide is contraindicated in patients with known severe hypersensitivity to budesonide or any of the excipients.

4.3: Special Warnings and Precautions

Graft Rejection: Budesonide has not been used as an immunosuppressant for LT recipients and its use in this setting can lead to increased risk of graft rejection.

Hypercorticism and adrenal suppression: When corticosteroids are used are used chronically, systemic effects such as hypercorticism and adrenal suppression may occur. Corticosteroids can reduce the response of the hypothalamus-pituitary-adrenal (HPA) axis to stress. Since budesonide is a corticosteroid, general warnings concerning corticosteroids should be followed.

Transferring patient from systemic corticosteroid therapy: Care is needed in patients who are transferred from long term corticosteroid treatment with high systemic effects with lower systemic availability like budesonide, since symptoms attributed to withdrawal of steroid therapy, including those of acute adrenal suppression or benign intracranial hypertension, may develop.

Immunosuppression: Patients who are on drugs that suppress immune system are more susceptible to infection than healthy individuals. Corticosteroids should be used with caution in patients with active or quiescent tuberculosis infection, untreated fungal, bacterial or systemic viral or parasitic infections. Replacement of systemic corticosteroids with budesonide may unmask allergies (such as rhinitis and eczema) which were previously controlled by systemic drugs.

Increased systemic corticosteroid susceptibility: Reduced liver function affects the elimination of corticosteroids, and increased systemic availability of oral budesonide has been demonstrated in patients with liver cirrhosis.

Other corticosteroid effects: Caution should be taken in patients with hypertension, diabetes mellitus, osteoporosis, peptic ulcer, glaucoma or cataracts or with any other condition where corticosteroids may have unwanted effects.

Pregnancy and lactation: Budesonide is pregnancy category C. Budesonide was teratogenic and embryocidal in rabbits and rats. There are no adequate and well controlled studies in humans. Budesonide should be used in pregnancy only if potential benefits outweigh the potential risk.

Budesonide is secreted in breast milk and breast feeding should be discontinued during budesonide use.

Pediatric use: Safety and effectiveness of budesonide in pediatric patients have not been established. Systemic and inhaled corticosteroids, including budesonide, may cause a reduction in growth velocity in pediatric patient population

Geriatric use: Clinical studies of budesonide did not include sufficient numbers of subjects aged 65 or more to determine whether they respond differently from younger subjects. Other reported clinical experience has not identified differences in response in elderly and younger patients.

Interaction with other medications: Concomitant oral administration of ketoconazole (a known inhibitor of CYP3A4 in the liver and intestinal mucosa) caused an eight-fold increase in the systemic exposure to oral budesonide. If treatment with inhibitors of CYP3A4 activity (such as ketoconazole, itraconazole, ritonavir, indinavir, saquinavir, erythromycin, etc.) is indicated, reduction in budesonide dose should be considered. After extensive intake of grapefruit juice (which inhibits CYP3A4 activity, mainly in intestinal mucosa), the systemic exposure for oral budesonide increased about two times. Ingestion of grapefruit or grapefruit juice should be avoided in connection with budesonide administration.

Common adverse effects: Based on data from five clinical trials (520 patients), the common adverse events occurring in 5% or more patients from budesonide use are as follows

a) Headache (21%)

b) Acne (15%)

c) Easy bruising (15%)

d) Moon face (11%)

e) Respiratory infection (11%)

f) Nausea (11%)

g) Diarrhea (10%)

h) Swollen ankle (7%)

i) Back pain (7%)

j) Dyspepsia (6%)

k) Dizziness (7%)

l) Abdominal pain (6%)

m) Flatulence (6%)

n) Vomiting (6%)

o) Fatigue (5%)

p) Pain (5%)

q) Hirsutism (5%)

Adverse events occurring in less than 5% of patient using budesonide but more than placebo group are leukocytosis, palpitation, tachycardia, vision abnormality, malaise, fever, flu-like symptoms, dependent edema, epigastric pain, gastrointestinal fistula, glossitis, hemorrhoids, intestinal obstruction, tongue edema, ear infection, bronchitis, urinary tract infection, thrush,

weight gain, increased appetite, hypokalemia, arthritis, cramps, myalgia, hyperkinesia, paresthesia, tremor, vertigo, somnolence, amnesia, confusion, agitation, nervousness, dysuria, micturition frequency, nocturia, intermenstrual bleeding, dyspnea, alopecia, dermatitis, eczema, increased sweating, purpura, flushing and hypertension.

5-SAFETY AND MONITORING PLAN

5.1: Risk Assessment

The risks associated with the current study are deemed moderate for the following reasons:

We do not view the risks associated with the administration of budesonide as minimal due to the target population and the relatively novel nature of this agent. Given that budesonide is FDA approved for treatment of mild to moderate Crohn's disease we do not believe this study is high risk.

5.2: Monitoring

Although we have assessed the proposed study as one of moderate risk, the potential exists for an anticipated and/or unanticipated adverse event(s), serious or otherwise, to occur. To ensure close monitoring of adverse events and unanticipated events, adverse event monitoring will occur at each study visit (Figure 1)

5.3: Adverse Events

An adverse event is defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure. Adverse events will be monitored and assessed for each subject at each study visit (Figure 1). In addition to the adverse events reported by subjects, each subject will be asked "Yes/No" questions regarding the common side effects of study drugs listed above. For each adverse event the principal investigator will grade, determine if it meets criteria for serious adverse event and attribute to study procedure as specified below.

5.4 Adverse Event Grading

All adverse events occurring during the study will be graded by the primary investigator according to the following criteria:

- Mild adverse event defined as discomfort noticed, but no disruption of normal daily activity
- Moderate adverse event defined as discomfort sufficient to reduce or affect normal daily activity

- Serious adverse event defined as incapacitation, with inability to work or perform normal daily activity

5.5: Serious Adverse Event (SAE) Criteria:

All adverse events occurring during the study will be assessed by the primary investigator to determine if they meet the following criteria for a serious adverse event:

- is life-threatening
- results in hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- results in a congenital anomaly or birth defect OR
- results in death
- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition, or adversely affects the risk/benefit ratio of the study

It is noted that an adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. The principal investigator will consider the grade of the event as well as its "seriousness" when determining whether reporting to the IRB is necessary.

5.6: Attribution of Adverse Events

All adverse events occurring during the study will be attributed as related to the study procedure by the primary investigator according to the following:

- Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).
- Probable: Adverse event is likely related to investigational procedures(s)/agent(s).
- Possible: Adverse event may be related to investigational procedures(s)/agent(s).
- Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).
- Unrelated: Adverse event is clearly not related to investigational procedures(s)/agent(s).

5.7: Reporting Serious and Unanticipated Adverse Events

The following types of adverse events will be reported within 48 hours of becoming known to the study team to the Institutional Review Board (IRB) using the event reporting form.

a) Serious AND unanticipated AND possibly or definitely related events

b) Anticipated adverse events occurring with a greater frequency than expected

c) Other unanticipated problem involving risks to subjects or others.

An isolated laboratory abnormality, is not reportable as an SAE; unless the investigator assesses that the event meets standard IRB criteria for an SAE. Baseline laboratory abnormalities that are part of the disease profile should not be reported as an SAE, specifically when they are allowed or not excluded by the protocol inclusion/exclusion criteria.

6-DATA COLLECTION, SAFETY AND STATISTICAL CONSIDERATIONS

6.1: Data Handling and Quality Assurance

All of the data will be collected on case report forms (CRF) at each study visit and then entered directly in to electronic research database (Research Electronic Data Capture ;REDCap). Case report forms will be prepared by the study team. It is the expectation that all data has source documentation available. It will be ensured that all study personnel receive the required trainings and comply with the rules and regulations of data safety and security. All attempts will be made to collect the relevant data in a timely and accurate manner. All the paper records will be kept under lock and key. Only authorized study personnel will have access to electronic research data.

6.2: Statistical Considerations

Sample Size:

Sample size calculation and rationale has been discussed above.

Controls:

Control for each study subject will be identified in a manner that he/she would have undergone LT either 8 weeks prior or 16 weeks after the LT of the study subject. This will ensure that investigators are blinded to the outcomes (measured at week 24) in the controls and these are measured prospectively. Based on existing literature, predictors of ACR (primary outcome) are age less than 55 years, normal kidney function with serum creatinine of less than 1.5 ng/ml, history of autoimmune liver disease and lack of antibody therapy at the time of transplant. Using a directed acyclic graph, autoimmune hepatitis appears to be a confounding factor whereas age, kidney function and antibody therapy are effect modifiers. Each historical control will be matched to the study subject on all of these four factors.

Primary Outcome:

Assessment of the primary end point will be performed by measuring the rates and 95% confidence intervals of ACR in budesonide group as compared to historical controls that used

prednisone. Overlapping 95% confidence intervals may suggest similar rates of ACR between groups and will provide preliminary data regarding efficacy of budesonide.

Secondary Outcomes:

Assessment of the secondary outcome of the rate of NODAT will also be performed in similar manner by estimating the incidence rates and 95% confidence intervals in study subjects and controls. A non-overlapping confidence interval may indicate that the rate of NODAT between the two groups might be different.

Effect of budesonide on hypothalamic-pituitary-adrenal axis will be assessed by calculating proportion of subjects with serum cortisol levels dropping below the threshold of 3 microgram/deciliter during the first 12 weeks of LT OR subjects with positive ACTH stimulation test defined as serum cortisol level of < 18 micrograms/dl at any of the three times points (baseline, 30, 60 minutes). Graphic representation of serial cortisol levels will be depicted to show any trend towards lower cortisol levels while using budesonide. There will be no comparison group for this outcome.

The proportion of subjects experiencing adverse events and severe adverse events will be tabulated, graded and evaluated to determine degree of relationship to study drug. Rates will be calculated and reported for the study group.

6.3: Data Safety, Monitoring and Planned Interim Analyses

Medical monitoring committee will comprise of Drs. Tayyab Diwan and James Heubi. Dr. Heubi is a Professor of Medicine in Pediatrics Hepatology at Cincinnati Children's Hospital and Medical Center and Dr. Diwan is an assistant professor of transplant surgery at University of Cincinnati. Both have extensive experience in clinical care of post liver transplant patients and basic and translational research. The committee will review all the adverse and unanticipated events of the study every 6 months and make recommendations about modifying or continuing the study to prevent or reduce the frequency of these effects. In addition any study related serious adverse events will be reported to the committee within 1 week of becoming known to the study team. A planned interim analysis of study data will occur under the committee's supervision after the first 10 subjects have completed the study. This will allow an interim evaluation of the data to ensure that the study is not overwhelming negative or positive. Table 2 provides a guideline regarding stopping rules for the trial based on number of ACR or SAE. This table is formulated using sequential probability ratio testing. Following assumptions and statistical cutoffs were used to formulate this table.

- Baseline rate of ACR in historic controls = 10%
- Ceiling rate of ACR in study subjects $\leq$ 15%
- 2-sided significance level = 0.05
- Power = 0.2

Table 2: Stopping rules based on ACR/SAE

Patients enrolled	Stop if cumulative number of ACR/SAE reach
-16	4
-8	5
1	6
8	7
16	8
24	9

"-"indicates that these numbers will not result in stopping the trial

6.4 Premature Study Termination

The study will be terminated prematurely if the risk-benefit ratio becomes unacceptable due to any circumstance such as:

a) Results of the safety assessments

b) Results of any interim analysis

c) Results of parallel clinical studies

d) Results of parallel animal studies

e) If the study conduct (e.g. recruitment rate; drop-out rate; data quality; protocol compliance) does not suggest a proper completion of the trial within a reasonable time frame.

6.5: Institutional Review Board (IRB) and Informed Consent

This protocol, the informed consent document, and relevant supporting information must be submitted to the IRB for review and approval before study initiation. The principal investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the study protocol, as well as any SAE. Prior to participation/randomization in the study, the IRB approved informed consent statement must be obtained from a competent subject or legal representative. The process for obtaining consent must be in compliance with the IRB guidelines and policies for obtaining informed consent for research participation. Waiver of consent (exception of informed consent) will not be allowed in this study and a subject will not be enrolled if consent cannot be obtained from the subject or legal representative. Informed Consent will be obtained by a study investigator or their appointed designee. A copy of the signed and dated informed consent document must be provided to the subject. Signed and dated consent forms must remain in each subject's study file and must be available for verification at any time.

6.6: Recruitment of Minorities and Women

This study will encourage enrollment of all eligible subjects regardless of gender, race, or ethnicity. Any gender, racial or ethnic disparity in recruitment detected will be the result of differences in the pattern of referral to the participating institutions. Budesonide is pregnancy category C. Budesonide was found harmful to fetus in rabbits and rats studies. There are no adequate and well controlled studies in humans. Budesonide should be used in pregnancy only if potential benefits outweigh the potential risk. Pregnant females will be excluded from this study since they are unable to undergo a liver transplant surgery. Subjects will be recommended not to become pregnant or father a baby while they are on study drug.

Budesonide is secreted in breast milk. Breastfeeding will be contraindicated while on study drug.

Only persons 21 to 75 years of age will be enrolled in the study.

6.7: Drug Distribution and Pharmacy Support

Study drug will be arranged and dispensed by The Department of Pharmacy Services at The University of Cincinnati Medical Center using the study funding. The pharmacy coordinated Investigational Drug Service (IDS) which has more than 20 years of experience in industry pharmaceutical research. IDS has successfully facilitated, dispensed and provided accountability and control of medications used in multiple drug studies.

6.8: Cost to Subjects

This study will not infer any additional costs to the subjects or their insurance companies. The study drug will be provided to the subjects at no cost. All other study related procedures including SIS, clinic visits, and routine blood work are part of routine post-LT follow up as per the current standard of care.

6.9: Benefit to Subjects:

Participation in this study will not be represented as providing any direct benefit to the study participants. It is not known whether the study drug will differ from prednisone in efficacy for this specific clinical setting. The data obtained from this study will help conduct larger trials focusing at improving the medical care of LT recipients as explained in next section.

6.10: Future Perspectives:

If budesonide is found to be safe and effective in LT recipients, large multi-center trials with larger sample size can be proposed to evaluate for less sensitive outcomes including rates of infection, overall glycemic control and other steroid specific side effects. In addition, a large double blind, head to head comparison of budesonide and prednisone will also be warranted to

establish budesonide as a novel, liver-specific, immune suppression agent with minimal systemic toxicity to help improve the outcomes of LT. Data from this study will help estimate the sample size for larger trials. Results and data from this study will form the basis of career development award (K23) application for the principal investigator.

Long term use of budesonide in liver related conditions like primary biliary cirrhosis is safe for up to two years. If safety and efficacy of budesonide is established in LT recipients, future studies will be targeted at exploring the potential of budesonide as a long-term immune suppression agent. This can lead to significant reduction in the use of other highly toxic immunosuppressant agents such as calcinuerin inhibitors (i.e. tacrolimus and cyclosporine) and mycophenolate acid products which are currently the mainstay therapies in the majority of LT recipients but are associated with significant long term adverse effects of renal failure, diabetes and metabolic syndrome.

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