

**Abbreviated Title:** Biomarkers & ECP in Acute GVHD  
**Version Date:** October 02, 2018

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Title: Biomarkers in Acute Graft-Versus-Host Disease (GVHD) and Extracorporeal Photopheresis added to Investigator Chosen Therapies of Steroid Refractory Acute GVHD

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IDE Exempt Non-significant Device:	Extracorporeal photopheresis (ECP) Machine
Manufacture	Generic

Commercial Agent: Methoxsalen (Uvadex(R))

Study Exempt from IND Requirements per 21 CFR 312.2(b)

## PRECIS

### Background:

- Acute graft versus host disease (GVHD) remains a difficult to manage complication of allogeneic hematopoietic stem cell transplantation causing significant morbidity and mortality.
- Biomarkers have recently been described in acute GVHD that have the potential to better predict onset, severity, steroid failure, and non-relapse mortality.
- First line treatment of acute GVHD with high dose corticosteroids will fail in approximately 30% of patients and is associated with significant steroid related complications
- No second line treatment of acute GVHD after a failure of steroids has been established as a standard approach.
- Choice of second line therapy for acute GVHD is currently based primarily on physician familiarity, existing toxicities, and patient's ability to tolerate new potential toxicities.
- Extracorporeal photopheresis (ECP) is an attractive therapy to combine with other therapies for steroid refractory disease due to a unique mechanism of action involving immunomodulation as well as an extremely low rate of reported side effects and complications.
- Biomarkers may also prove useful in predicting the success or failure of specific treatments for steroid refractory disease, including those combined with ECP.
- This study will allow for collection of biomarker data in patients undergoing allogeneic transplantation on NCI protocols, including those who develop acute GVHD, and investigate their role in predicting outcomes in initial corticosteroid therapy as well as in currently used treatments in the management of patients with steroid refractory acute GVHD with or without the addition of ECP.

### Objective:

- To study biomarkers in patients undergoing allogeneic transplantation, with acute GVHD including their ability to predict steroid refractoriness and predict outcome of investigator chosen second line therapies with and without Extracorporeal Photopheresis (ECP).

### Eligibility:

- Adult patients on an NCI allogeneic transplantation protocol.

### Design:

- Non-randomized, single institution study.
- Research blood for biomarkers will be collected on all patients enrolled.
- ECP will be offered as an addition to investigator chosen treatments in patients who develop steroid refractory acute GVHD.
- The study will enroll a total of up to 450 patients.

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## **1. INTRODUCTION**

### **1.1 STUDY OBJECTIVES**

#### **1.1.1 Primary Objective**

To study biomarkers in patients undergoing allogeneic transplantation, with acute GVHD including their ability to predict steroid refractoriness and predict outcome of investigator chosen second line therapies with and without Extracorporeal Photopheresis (ECP).

#### **1.1.2 Secondary Objective**

To study steroid requirement, GVHD outcome, and overall survival at 6 months in patients with acute GVHD.

### **1.2 BACKGROUND AND RATIONALE**

#### **1.2.1 Acute GVHD**

Depending on the type of allogeneic transplant and the level of donor-recipient disparity, between 30%-60% of transplant patients will be diagnosed with acute GVHD despite strategies for prophylaxis<sup>1</sup>. Acute GVHD involves donor T cells causing damage in the target tissues of liver, bowel, and skin. This classically was defined as occurring in the first 100 days after transplant but it is increasingly recognized as occurring at later times after immunosuppression withdrawal and subsequent to donor lymphocyte infusions. Standard intervention is 1-2 mg/kg/day of methylprednisolone or equivalent steroid. Unfortunately, approximately 40%-50% of patients will be deemed steroid refractory and require some form of second line therapy.

#### **1.2.2 Biomarkers**

The role of biomarkers in acute GVHD has been the subject of several recent reviews<sup>2,3,4,5</sup>. Gander Lugt et al recently showed that the biomarkers, ST2 and REG3a have promise in predicting adverse outcomes of acute GVHD, including steroid refractoriness<sup>6</sup>. Haige et al demonstrated that a Lipopolysaccharide-Binding Protein (LPS-BP) level above 15,000 ng/ml at day 7 and 14 after transplant was independently associated with later acute GVHD.<sup>7</sup> Dietrich et al demonstrated that high levels of serum nitrate prior to transplant could independently predict for steroid refractory GVHD and that this risk could be abrogated by pre-transplant use of statins.<sup>8</sup> Jinhuan et al demonstrated that serum and mononuclear mRNA levels of Indoleamine 2,3 Dioxygenase (IDO), combined with interferon Gamma levels, was a powerful predictor of acute GVHD<sup>9</sup>. A summary of candidate biomarkers is shown in the table .

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Protein	Study	No. of patients in the study	Target organ	Diagnosis end point	Association direction*	Prognostic end point	Predictive end point
<b>Based on GVHD pathology</b>							
Albumin	Rezvani 2011 <sup>76</sup>	401	Systemic/GI tract/renal	ND	↓	2 y NRM, 6 mo OS in RIC patients	aGVHD 3-4 in RIC patients
CRP	Schots 2002 <sup>64</sup>	96	Systemic	All TRC	↑	Day 100 NRM	TRC
	Pihush 2006 <sup>66</sup>	350	Systemic	All TRC	↑	ND	TRC
CXCL10	Piper 2007 <sup>70</sup>	34	Systemic	aGVHD 0-4	↑	ND	aGVHD 0-4 by day 100
HGF	Okamoto 2001 <sup>71</sup>	38	Systemic	aGVHD 0-4	↑	ND	ND
	Harris 2012 <sup>78</sup>	826 + 128†	GI tract	aGVHD 0-4	↑	6 mo NRM	ND
IL-2R <sub>α</sub>	Miyamoto 1996 <sup>54</sup>	30	Systemic	aGVHD 0-4	↑	ND	ND
	Foley 1998 <sup>53</sup>	36	Systemic	aGVHD 0-4, sepsis, VOD	↑	ND	ND
	Shaiegan 2006 <sup>56</sup>	39	Systemic	aGVHD 2-3	↔	ND	ND
	August 2011 <sup>62</sup>	62	Systemic	ND	↑	ND	Prospective study aGVHD 3-4 by day 60
IL-6	Malone 2007 <sup>65</sup>	147	Systemic	aGVHD 0-4 by day 100	↑	ND	ND
	Schots 2003 <sup>61</sup>	84	Systemic	All TRC	↑	1 y NRM	ND
	Pihush 2006 <sup>66</sup>	350	Systemic	All TRC	↑	ND	TRC
IL-8	Uguccioni 1993 <sup>67</sup>	30	Systemic	aGVHD 0-4, rejection, liver fibrosis in thalassemic patients	↑	ND	ND
	Schots 2003 <sup>61</sup>	84	Systemic	All TRC	↑	1 y NRM	TRC
IL-10	Liam 1998 <sup>91</sup>	46	Systemic	aGVHD 2-4, cGVHD, infection, relapse	↑	ND	ND
IL-12	Mohy 2005 <sup>68</sup>	113	Systemic	aGVHD 2-4 in RIC patients	↑	ND	ND
IL-18	Fujimori 2000 <sup>90</sup>	37	Systemic	aGVHD 0-4	↑	ND	ND
	Shaiegan 2006 <sup>56</sup>	39	Systemic	aGVHD 2-3	↑	ND	ND
KRT18	Luft 2007 <sup>72</sup>	55	GI tract	GI GVHD vs non-GVHD-enteritis	↑	Response to GVHD treatment	ND
	Luft 2011 <sup>75</sup>	48	Systemic	Refractory GVHD	↑	ND	ND
	Harris 2012 <sup>78</sup>	826 + 128†	GI tract	GI GVHD	↑	6 mo NRM	ND
REG3 <sub>α</sub>	Harris 2012 <sup>78</sup>	826 + 128†	GI tract	GI GVHD	↑	6 mo NRM, response to GVHD treatment	ND
Syndecan-1	Seidel 2003 <sup>55</sup>	60	Systemic	aGVHD 0-4	↑	ND	ND
TNF <sub>α</sub>	Holler 1999 <sup>60</sup>	56	Systemic	All TRC	↑	ND	ND
	Abdallah 1997 <sup>58</sup>	80	Systemic	All TRC, aGVHD 0-1 vs 2-4	↔	ND	ND
	Schots 2003 <sup>61</sup>	84	Systemic	All TRC	↑	1 y NRM	ND
TNFR1	Choi 2008 <sup>69</sup>	438	Systemic	ND	↑	ND	aGVHD 2-4, 1 year NRM
	Kiko 2008 <sup>69</sup>	82 (children)	Systemic	ND	↑	ND	aGVHD 2-4, 1 year NRM
	August 2011 <sup>62</sup>	62	Systemic	ND	↑	ND	Prospective study aGVHD 3-4 by day 60
Tryptophan metabolites	Landfried 2011 <sup>74</sup>	51	Systemic	ND	↑	6 mo NRM	ND
<b>Based on proteomics discovery</b>							
Peptides set	Kaiser 2004 <sup>45</sup>	35	Systemic	aGVHD 0-4, sepsis	Presence vs Absence	ND	ND
	Srinivasan 2006 <sup>30</sup>	34	Systemic	aGVHD	Presence vs Absence	ND	ND
	Imanguli 2007 <sup>31</sup>	41	Systemic	1 month post-HSCT vs pre-HSCT	↑	ND	ND
	Weisinger 2007 <sup>77</sup>	33 + 141†	Systemic	aGVHD 2-4	Presence vs Absence	ND	ND
Proteins set	Wang 2005 <sup>34</sup>	9	Systemic	aGVHD vs pre-HSCT	Presence vs Absence	ND	ND
Albumin	Kaiser 2004 <sup>45</sup>	35	Systemic	aGVHD 0-4, sepsis	Presence vs Absence	ND	ND
	Imanguli 2007 <sup>31</sup>	41	Systemic	1 month post-HSCT vs pre-HSCT	↑	ND	ND
CCL8	Hori 2008 <sup>69</sup>	14	Systemic	Murine model discovery, Validation human serum: aGVHD 0-4	Presence vs Absence	ND	ND
Collagen α-1	Weisinger 2007 <sup>77</sup>	33 + 141†	Systemic	aGVHD 2-4	↓ while collagen chain III was ↑	ND	ND
Erlin	Paczesny 2010 <sup>84</sup>	20 + 492†	Skin	Skin aGVHD 0-4 vs non-GVHD rashes	↑	NRM, OS	ND
HGF	Paczesny 2009 (in a panel) <sup>2</sup>	42 + 282† + 142†	Systemic/GI tract	aGVHD 0-4 vs no GVHD	↑	NRM, OS	ND
IL-2R <sub>α</sub>	Paczesny 2009 (in a panel) <sup>2</sup>	42 + 282† + 142†	Systemic	aGVHD 0-4 vs no GVHD	↑	NRM, OS	ND
IL-8	Paczesny 2009 <sup>2</sup>	42 + 282† + 142†	Systemic	aGVHD 0-4 vs no GVHD	↑	NRM, OS	ND
Lactoferrin	Imanguli 2007 <sup>31</sup>	41	Systemic	1 month post-HSCT vs pre-HSCT	↑	ND	ND
Leukotriene A4 hydrolase	Kaiser 2004 <sup>45</sup>	35	Systemic	aGVHD 0-4, sepsis	↑	ND	ND
REG3 <sub>α</sub>	Ferrara 2011 <sup>38</sup>	20 + 871† + 143†	GI tract	GI-GVHD vs non-GVHD enteritis	↑	NRM, OS	ND
SLPI	Imanguli 2007 <sup>31</sup>	41	Systemic	1 month post-HSCT vs pre-HSCT	↑	ND	ND
TNFR1	Paczesny 2009 <sup>2</sup>	42 + 282† + 142†	Systemic	aGVHD 0-4 vs no GVHD	↑	NRM, OS	ND

ND indicates not done; OS, overall survival; RIC, reduced intensity conditioning; TRC, transplantation-related complications, major complications, including aGVHD, microangiopathic hemolytic anemia, VOD, pneumonitis, and infection; and VOD, veno-occlusive disease.

\* ↑, plasma protein increased; ↓, plasma protein decreased; and ↔, plasma protein unchanged.

†Validation on independent sets of patients.

(Figure from: Paczesny S. Discovery and validation of graft-versus-host disease biomarkers <sup>3</sup>)

It is hoped that biomarkers will improve future predictive and prognostic testing leading to better prophylaxis and treatment outcomes, especially in steroid refractory disease. None of these biomarkers has yet found its way into routine clinical decision making <sup>4,5</sup>. Prospective collection of biomarker data in patients remains an important goal of acute GVHD studies to further define their utility in understanding the biology and choosing effective treatments of acute GVHD. Patients enrolled on this study will have blood collected for biomarkers at enrollment and various time points throughout transplant. As this field is rapidly evolving, the exact panel of biomarkers that will be eventually be examined may change over time. Based on the current validation of candidate biomarkers in various studies, serum TIM3, IL-6, Reg-3-a, ST2, LPS-BP, Nitrate, TNFR1, IL-2Ra, CXCL10, and HGF will initially be studied.

### 1.2.3 Steroid Refractory GVHD

To date, there are no proven treatments based on Phase III trials for steroid-refractory acute GVHD <sup>10</sup>. The bulk of the data from treatment of these patients often rely on small Phase II trials with different eligibility criteria making comparisons and treatment decisions difficult. The choice of therapy when steroids fail is often based on pre-existing organ toxicity and prior GVHD prophylaxis of the patient <sup>11</sup>. In addition, treatment choices are further complicated by no universally accepted single definition of steroid refractoriness in acute GVHD, despite attempts at formalizing the criteria in recent reviews, and guidelines <sup>12, 13,14, 15,16</sup>. Dignan et al suggested that no response by day 5 of treatment with steroids is a reliable marker of poor outcome; patients who respond to 2 mg/kg/day of steroids by day 5 had a non-relapse mortality of 27%, compared to 49% non-relapse mortality for day-5 non-responders. A recent European review adopts this five day cutoff but also specifies that worsening of acute GVHD at day 3 days also constitutes steroid failure <sup>13,14</sup>. Guidelines recently put forth by American Society for Bone Marrow Transplantation used a criterion of lack of improvement despite 7 days of full dose steroids to define steroid refractoriness with poor outcomes in those so defined <sup>12</sup>. Stool volume, bilirubin levels, and area of skin rash are used as objective measures, although the decision to declare steroid refractoriness remains partly subjective and organ specific. For the purposes of instituting second line therapies, this study will utilize both objective and subjective measures to define steroid refractoriness as worsening despite 3 days, no improvement despite 7 days, or inadequate response despite 14 days of adequately dosed corticosteroids.

### 1.2.4 Current Therapy of Steroid Refractory Acute GVHD

It was hoped that recently conducted randomized trials of the most promising agents for the initial treatment of acute GVHD combined with steroids would provide some guidance as to which agents might also be used after steroids for the treatment acute GVHD. <sup>17</sup>The Bone Marrow Transplant Clinical Trials Network investigated the role of etanercept, mycophenolate mofetil (MMF), denileukin, or pentostatin plus corticosteroids for initial treatment of aGVHD. In this randomized Phase II trial, the day 28 complete response rates were 60%, 53%, 38%, and 26% for mycophenylate, denileukin, pentostatin, and etanercept, respectively. The combination of steroids and MMF was chosen for a Phase III trial for initial treatment of aGVHD. A follow up randomized Phase III trial which compared MMF/steroids with steroids alone for initial



treatment of acute GVHD was stopped by its futility end points<sup>18</sup>. Unfortunately, these trials failed to suggest an added benefit when further immunosuppressive agents are added to steroids for the initial treatment of aGVHD. Additionally, these efforts also did not point to any preferred agent for use as in second line therapy.

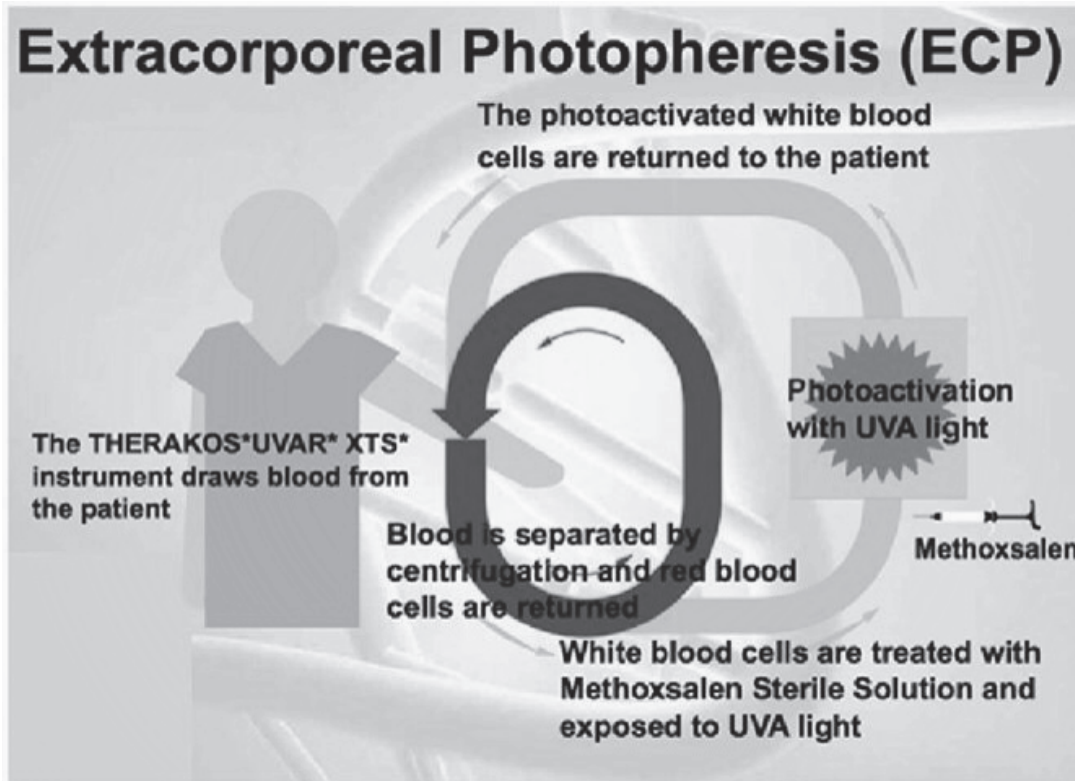
Despite the lack of a standard, all steroid refractory patients are offered second line and beyond therapies given the fatal nature of uncontrolled acute GVHD. In general, investigators have tended to use a single additional therapy added to some combination of calcineurin inhibitor and continued steroids in order to try and assess the balance of side effects and benefit. The choice and order of agents tried remains dictated largely by investigator experience and bias due to the lack of clear guidance from the available studies. Two broad classes of agents are used to treat GVHD; agents aimed at impairing cellular effectors and agents aimed at limiting cytokine activity in the effected tissues<sup>1</sup>. This study neither dictates the choice of secondary agents nor is designed to examine the activity or superiority of any particular agent or agents. This study will measure biomarkers in patients with acute GVHD and pilot the addition of extracorporeal photopheresis to current therapeutic choices in those patients with steroid refractory acute GVHD

#### 1.2.5 Extracorporeal Photopheresis

Extracorporeal Photopheresis (ECP) is currently approved by the FDA for the treatment of refractory cutaneous T-cell lymphoma (CTCL)<sup>19,20</sup>. Over the past two decades, ECP has also been further applied and studied in the treatment of several autoimmune diseases as well as for treatment for cardiac, renal and lung allograft rejection<sup>21, 22,23,24,10,25</sup>. Based on the presumed immunomodulation in these situations, ECP has been previously attempted in both acute and chronic GVHD<sup>26, 27, 28,29</sup>. The procedure involves separating the white blood cells (buffy coat) from the plasma and red blood cells, which are infused back to the patient. The buffy coat leukocytes are then exposed to UVA irradiation following pretreatment with 8-methoxypsoralen, which has been shown to result in apoptosis of the majority of cells within 48 hours<sup>30</sup>. Both in vitro and in vivo studies suggest that the subsequent clearance of these apoptotic cells can result in significant immunomodulation<sup>31</sup>. However, the specific immunologic mechanism by which ECP works has not been clearly elucidated<sup>10</sup>. Modulation of Th1/Th2 balance has been often invoked. In CTCL, where early stage disease is associated with Th2 skewing, ECP results in a shift toward Th1 cytokines and a restoration of the normal Th1/Th2 balance<sup>32</sup>. Alternatively Gorgun et al.<sup>33</sup> demonstrated that patients with cGVHD treated with ECP demonstrated an increase in DC2 dendritic cell subsets and concomitant shift in Th2 cytokine production by T cells. In addition, Silva et al.<sup>34</sup> demonstrated an increase in Th1 cells in patients with cGVHD. Other studies have showed ECP appears to alter cytokine profiles with increases in IL-10, a type 2 cytokine frequently associated with immunosuppression<sup>35, 34</sup>. These discrepancies suggest the complexity of the Th1/Th2 system and the biologic effects of ECP. Another important consideration when interpreting these studies is that many of the diseases treated with ECP, including GVHD, are associated with a Th1/Th2 shift which makes it difficult to know whether the restoration of this balance reflects a therapeutic mechanism of ECP or is a simply a secondary marker of disease improvement.

Another potential mechanism of ECP in the treatment of GVHD is the impact of ECP on dendritic cells. ECP has demonstrated reproducible decreases in circulating CD80+ and CD123+ dendritic cell subsets<sup>34,36</sup>. Further studies have suggested that ECP causes a shift toward immature dendritic

cell populations with lower levels of co-stimulatory molecule expression. This has several consequences including a decreased migratory response to MIP-3 $\beta$ , decreased T cell proliferation,



and increased production of IL-10 which further diminishes dendritic cell maturation and allostimulatory capacity<sup>37,38,39</sup>. Such changes have been importantly demonstrated to characterize tolerogenic dendritic cells<sup>40,41,42</sup>.

Meloni et al.<sup>43</sup> showed that there is an increase in central memory CD8 T cells and a decrease in central memory CD4 T cells in patients with cGVHD. It was demonstrated that the balance of memory T cells normalizes after ECP treatment and is associated temporally with improvement of clinical symptoms<sup>43</sup>. Recently, expansion of regulatory T cells has been associated with ECP treatment in humans<sup>43,44,45</sup> and in murine models<sup>46,29</sup>.

As noted above, the clinical application of ECP to a variety of disease processes has preceded a full understanding of the mechanisms by which this therapy might exert a therapeutic effect. Evidence continues to emerge from clinical studies, animal models and in vitro studies elucidating that significant immune modulation occurs with ECP. This, associated with the clinical safety of the procedure, has justified ECP's continued application even while knowledge of related areas such as apoptotic cell clearance, regulatory T cell biology, and tolerogenic dendritic cell manipulations are leading to better understanding of the mechanism of ECP in various disease states such as GVHD.

The safety of ECP has been extensively documented and the development of ECP arose, in part, from concerns with the safety of another commonly employed skin-directed treatment, PUVA. PUVA was introduced in the early 1970s as an effective treatment for many skin conditions but is

associated with a large number of adverse effects. PUVA is performed by giving patients oral 8-MOP capsules followed 1–2 hours later by exposure to a UVA light source. Unfortunately, the pharmacokinetics of oral 8-MOP has shown large variability as high as 18-fold. Variable GI absorption and extensive hepatic metabolism, including first-pass hepatic elimination, seem responsible for this variability. The unpredictable blood concentrations of oral 8-MOP likely underlie inconsistent UVA exposure leading to the toxicity or diminished therapeutic effect of PUVA. In contrast, with ECP, 8-MOP is mixed *ex vivo* with the patient's buffy coat cell layer, which is then re-infused back into the patient. The half-life of the drug administered in this fashion is extremely short resulting in substantially lower and much more predictable drug exposure.

ECP utilizing the Therakos system has an established safety profile with over 500,000 treatments performed to date (Therakos User Manual 2001). The only absolute contraindication for ECP is in patients who cannot tolerate extracorporeal volume loss (severe heart failure, hypotension, sepsis) or patients with coagulation disorders. In addition, methoxsalen (Oxsoresalen-Ultra®), the FDA-approved 8-MOP compound used with the Therakos device, is contraindicated in patients with idiosyncratic reactions to psoralen compounds, those with a history of light-sensitive disease (such as patients with systemic lupus erythematosus with photosensitive disease), or those with aphakia. Serious side effects with the procedure itself have rarely been reported, most of which are related to hypotension secondary to changes in extracorporeal volume. A large, multicenter survey focusing on immediate adverse effects of therapeutic apheresis reported no adverse events from a total of 79 ECP procedures with the majority of these procedures done using the Therakos system<sup>47</sup>. More recently, a phase II, randomized, controlled trial did not show significant differences in safety outcomes between the arm receiving ECP therapy for chronic graft versus host disease and the control-arm. The overall rates of infections (53.1% versus 44%), diarrhea (20.4% versus 20%), and nausea (18.4% versus 12%) were not significantly different between those receiving ECP and steroids versus steroids alone, while anemia occurred more frequently in those receiving ECP treatment (24.5% versus 6%;  $p = 0.02$ )<sup>29</sup>. In clinical practice, anemia in patients receiving ECP therapy can be seen and is most likely attributed to small volume blood loss over repeated procedures. In contrast to other GVHD therapies, studies have found that ECP therapy does not have immunosuppressive effects and therefore does not add to the risk for opportunistic infections<sup>26, 29, 48, 49</sup>. Secondary malignancies have not been associated with ECP; however, photosensitivity may occur and patients are instructed to take standard precautions as it relates to sun exposure. Fatigue, pruritus and fevers have also been reported during or after ECP treatment. Because intravenous (IV) access is required, patients are at risk for catheter-related complications including infection and thrombosis.

Because of the apparent safety, especially a reported lack increased infections, ECP is an attractive therapy to combine with other commonly used therapies of acute GVHD. ECP has been studied in acute GVHD with variable results (see Table below) leading to an inconsistent recommendation about the potential clinical role. In a pilot trial of 21 patients with steroid-refractory aGVHD by Greinix et al.<sup>49</sup>, 60% responded to ECP. However, none of the patients with acute GI GVHD responded to this intervention. Greinix et al.<sup>50</sup> reported the results of a follow-up Phase II trial of 59 patients treated with a more aggressive schedule of ECP with a complete response rate of 82%, 61%, and 61% in patients with skin, liver, and GI GVHD, respectively. A review, published in 2002, summarized the results on the use of ECP in aGVHD in 76 patients treated in 11 separate studies. Of the 76 patients, 59%, 47%, and 28% presented

Study	ECP regimen	Number of patients	Skin	GI	Liver	OS (%)
Greinix <i>et al.</i> [1998]	Twice every 2 weeks for 3 months, then twice monthly	6	OR=100% CR=67%		OR=100% CR=100%	100
Greinix <i>et al.</i> [2000]	Twice every 1–2 weeks to response then twice every 2–4 weeks	21	OR=81% CR=62%	OR=None	OR=67% CR=67%	57
Salvaneschi <i>et al.</i> [2001]*	Thrice weekly to response then twice every 2 weeks for 3 months	9	OR=89% CR=67%	OR=60% CR=60%	OR=20% CR=20%	67
Messina <i>et al.</i> [2003]*	Twice weekly for 1 month, then twice every 2 weeks for 2 months, then twice monthly for 3 months	33	OR=82% CR=76%	OR=75% CR=75%	OR=60% CR=60%	58
Perotti <i>et al.</i> [2010]*	Twice–thrice weekly to response then twice weekly for 1 week then twice every 2 weeks then twice monthly	50	OR=83%	OR=73%	OR=67%	44
Greinix <i>et al.</i> [2006]	Twice weekly to response then twice every 2–4 weeks	59	CR=82%	CR=60%	CR=61%	47
Perfetti <i>et al.</i> [2008]	Twice weekly for 1 months, then twice every 2 weeks for 2 months, then twice monthly until CR or stabilization	23	CR=66%	CR=40%	CR=27%	48

CR, complete response; ECP, extracorporeal photopheresis; GI, gastrointestinal; GVHD, graft-versus-host disease; OR, overall response (complete + partial response); OS, overall survival.  
 \*Study involving pediatric patients.

with skin, liver, and GI manifestations of aGVHD, respectively. Treatment duration ranged from 1 to 24 months. Regression of skin manifestations was observed in 83% of the patients with a complete response in 67%. A complete regression of liver and gut manifestations was reported in 38% and 54% of the patients, respectively. Despite these preliminary results, the optimal role of ECP in the management of steroid refractory acute GVHD remains undefined and there is little reported experience combining ECP with other therapies other than corticosteroids.

### 1.2.6 Background and Rationale: Summary

Current therapy of acute GVHD, especially steroid refractory disease, is unsatisfactory in a majority of patients due to both limited effectiveness and highly variable morbidity of many of the treatments. No single treatment regimen has been shown to consistently alter outcomes. Immunosuppression and subsequent infection contribute to the poor outcome steroid refractory disease. ECP is an attractive treatment modality due to its unique mechanism of action and low toxicity profile. Combining ECP with other treatments for GVHD is likely feasible, tolerable, and, based on prior experiences in the literature, not be expected to increase the toxicity of treatment. The potential of ECP to improve outcomes in this setting, either alone or in combination, has been incompletely explored. Recently described acute GVHD biomarkers have the potential to improve future prediction of refractory disease and therapeutic outcome of the varied treatments currently in use. Data will be collected on the procedure specific complications of ECP in combination with other therapies currently in use for acute GVHD.

## 2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

### 2.1 ELIGIBILITY CRITERIA

#### 2.1.1 Inclusion Criteria

2.1.1.1 Age  $\geq$  18 years.

- 2.1.1.2 Ability of subject to understand and the willingness to sign a written informed consent document.
- 2.1.1.3 Subject must be also enrolled on an NCI allogeneic transplant protocol.
- 2.1.1.4 Patients must agree to practice effective contraception (both male and female subjects, if the risk of conception exists). The effects of ECP on the developing human fetus are unknown. For this reason and as well as other Methoxsalen used in this trial is in a class of agents that is known to be teratogenic, men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation, and for 4 months after the completion of study treatment. Should a woman become pregnant or suspect she is pregnant while her partner is participating in this study, she should inform her treating physician immediately.

## 2.1.2 Exclusion Criteria

- 2.1.2.1 Any physical or mental condition that, in the opinion of the PI, would cause the risk/benefit ratio of participation to be unacceptable.
- 2.1.2.2 Inclusion of ECP in the treatment of any patient is contraindicated by any of the following:
  - Unstable hemodynamics requiring vasopressors or other support measures not amenable to or medically appropriate for continuation during the procedure.
  - Uncontrolled infection.
  - Inability to maintain acceptable venous access.
  - Uncontrolled or uncorrectable coagulopathy.
- 2.1.2.3 Pregnant women are excluded from ECP because methoxsalen, an agent utilized for the study procedure, may cause fetal harm. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with methoxsalen, breastfeeding should be discontinued if the mother is treated with methoxsalen. Pregnancy will be evaluated prior to initiation of ECP.
- 2.1.2.4 History of allergic or idiosyncratic/hypersensitivity reactions to 8-methoxypsoralen/psoralen compounds.
- 2.1.2.5 History of a light-sensitive cutaneous disease
- 2.1.2.6 Subjects with aphakia

## 2.2 SCREENING EVALUATION

- 2.2.1 The following will be documented prior to protocol entry.
  - Concurrent participation in an ETIB allogeneic transplant protocol.

## 2.3 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) must be completed and sent via

encrypted email to: NCI Central Registration Office [ncicentralregistration-l@mail.nih.gov](mailto:ncicentralregistration-l@mail.nih.gov). Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

### 2.3.1 Patient Assignment or Stratification Procedure

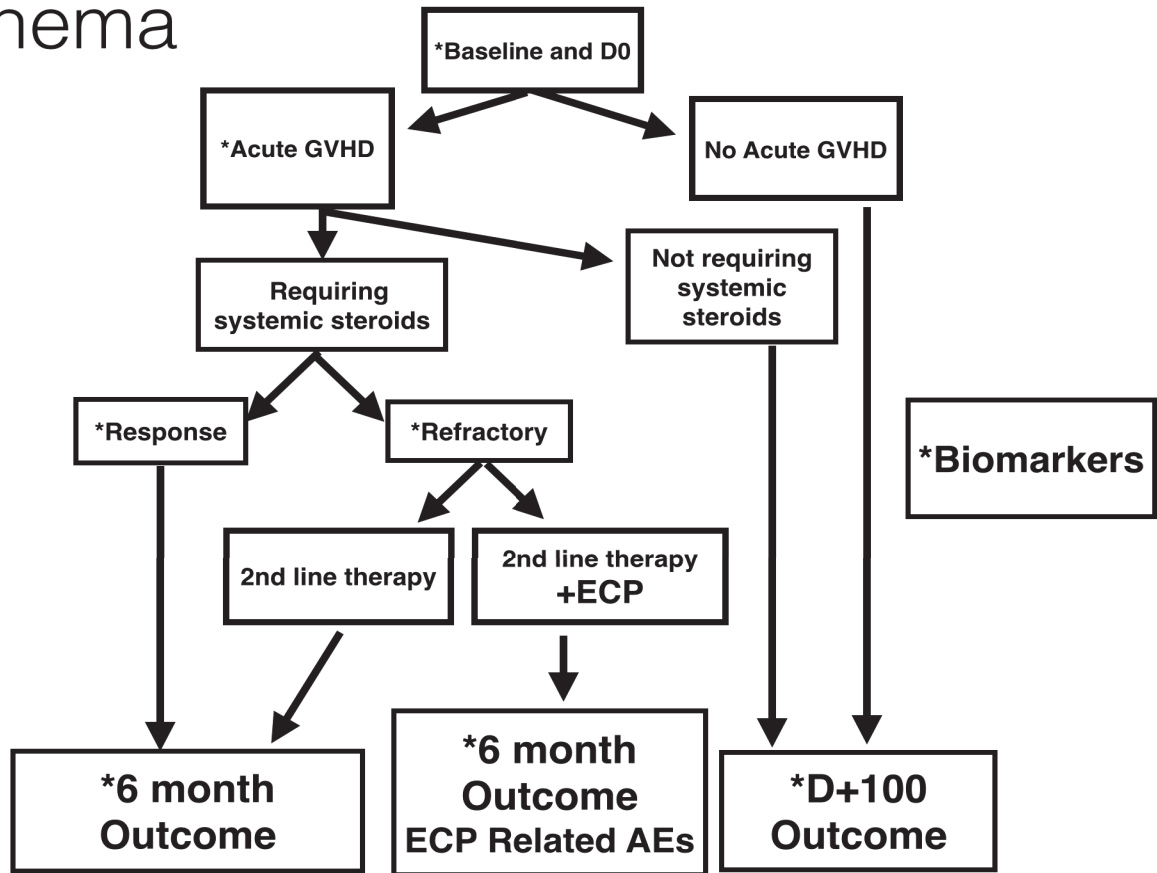
Patients will be enrolled based on their participation on any NCI allogeneic transplant protocol. Specific treatments for steroid refractory acute GVHD, including the use of ECP, will be at the discretion of the patient's primary transplant protocol PI (or designee) in consultation with the PI of this protocol. Patients will not be randomized or otherwise assigned. Patients may enter the protocol at any point during their participation on a separate NCI transplant protocol and absence of any biomarker time point(s) will be noted but will not constitute a deviation from this protocol.

## 3 STUDY IMPLEMENTATION

### 3.1 STUDY DESIGN

Please note that as of January 27, 2017 Accrual has been suspended on this protocol. Amendment D (version date: 10/02/2018) serves as the formal notification to the IRB of an Administrative Hold pending delivery of a new ECP device. Potential resources are being explored that would allow the protocol to begin to accrue in the future. No new patients will be enrolled. We enrolled total of 5 patients on this study, all 5 patients have completed the follow up period and are off study.

## Schema



Subjects will be followed according to the schedule outlined in section 3.3 for either three months post transplant (D+100), six months post transplant, or six months following the last ECP procedure. A final assessment will be completed prior to removing the subject from the study. Biomarker time points will commence when the patient is enrolled and enrollment at any point of the patient's transplant is allowed.

### 3.1.1 Diagnosis of steroid refractory acute GVHD

Diagnosis of steroid refractory acute GVHD in any patient will be defined at a minimum as clinical worsening despite 3 days, no clinical response despite 7 days, or an inadequate clinical response despite 14 days of methylprednisolone IV (or equivalent corticosteroid) at a minimum dose of 2mg/kg/day.

- Clinical responses are determined by measurements specific to the organ(s) affected.

- The determination of an inadequate clinical response to steroids will be made by the PI of the patient's primary transplant protocol (or their designee) in consultation with the PI of this protocol.
- General guidelines to be used for this determination by system will be as follows:
  - GI: failure to show at least a 50% reduction in stool volume compared to the highest daily stool volume.
  - SKIN: failure to show at least a 50% improvement of skin rash severity or distribution compared to the worst severity or extent of the rash.
  - LIVER: failure to demonstrate at least a 50% reduction in direct bilirubin compared to the peak preceding value.

### 3.2 EXTRACORPOREAL PHOTOPHERESIS (ECP)

#### 3.2.1 Schedule of ECP treatments

Patients who have ECP included in their treatment of steroid refractory acute GVHD will in general follow this recommended schedule:

- Twice weekly for 1 month
- Twice every other week for 2 months
- Twice during one week per month for 4 months for a total of up to 7 months of treatment

The frequency or duration of therapy can be individualized at PI discretion based on clinical risk and potential benefit of the variation. The rationale for the variation should be clearly documented in the study note of patient's medical record.

#### 3.2.2 ECP Procedure

Patients who will undergo ECP will be treated according to the standard operating procedure of the Clinical Center Department of Transfusion Medicine. The procedure may be done in a hospital room, an outpatient department, or in DTM

The UVAR will be used to perform therapeutic ECP under the direction of a Department of Transfusion Medicine (DTM) physician, in accordance with specific NIH Clinical Center Protocols, and the Medical Chief of Blood Services Section (BSS). Unless otherwise stated, the apheresis staff will adhere to the manufacturer's recommendations when performing ECP.

Registered Nurses will perform ECP as ordered by DTM physicians in the Clinical Research Information System (CRIS). The Operator's Manual for the UVAR and the directions for use with the apheresis kit will be followed at all times.

If the patient lacks adequate existing venous access for ECP, they may need to have a catheter placed by Interventional Radiology or the VAD Service.



### 3.3 STUDY CALENDAR

<i>Procedure</i>	<i>Baseline / Enrollment (+/- 2 days)</i>	<i>At D0 of transplant</i>	<i>If no acute GVHD or steroids, collect at D100 (+/- 1 week)</i>	<i>At diagnosis of acute GVHD (+/- 1 week)</i>	<i>At diagnosis of steroid refractory or responsive acute GVHD (+/- 1 week)</i>	<i>If acute steroid refractory GVHD with no ECP, collect at 6 months post transplant (+/- 1 month)</i>	<i>ECP Only</i>	
							<i>At first ECP of each month of treatment</i>	<i>At 6 months from last ECP (+/- 1 month)</i>
<i>Study Note</i>	X		X	X	X	X	X	X
<i>TBNK</i>	X	X	X	X	X	X	X	X
<i>Urine or serum <math>\beta</math>HCG in women of childbearing potential</i>							X	
<i>Biomarkers</i>	X	X	X	X	X	X	X	X
<i>GVHD Evaluation</i>				X	X	X	X	X
<i>ECP Adverse Events</i>							X	X
<i>GVHD Meds</i>		X		X	X	X	X	X

### 3.4 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from off study , effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

#### 3.4.1 Criteria for removal from protocol therapy.

- The patient refuses to continue therapy.
- At the principal investigator's discretion, if the PI deems the patient to be at an unacceptable risk/benefit ratio to remain on the study.
- PI of the patient's primary transplantation protocol requests removal.
- Participant becomes pregnant

#### 3.4.2 Off Study Criteria

- Completed either D+100 post-transplant evaluation, 6 month post-transplant evaluation, or 6 months evaluation after completion of ECP)
- Participant requests to be withdrawn from study
- Death
- PI decision to end this study

#### 3.4.3 Off Protocol Therapy and Off Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off-study. A Participant Status Updates Form from the web site (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) main page must be completed and sent via encrypted email to: NCI Central Registration Office [ncicentralregistration-1@mail.nih.gov](mailto:ncicentralregistration-1@mail.nih.gov).

## 4 CONCOMITANT MEDICATION

There are no specific restrictions on the use of any other agents or procedures deemed necessary for the ongoing care of the patients on this study.

## 5 BIOSPECIMEN COLLECTION

### 5.1 CORRELATIVE STUDIES FOR RESEARCH

#### 5.1.1 Biomarker Assays

One (1) 10 mL Sodium-Heparin/Green-top tube, and up to five (5) 8 mL CPT/Sodium-Heparin/Red+Green-top tubes will be sent via escort to ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF) (Bldg 10, Room 12C216). All enrolled patients will have this blood work drawn at time points as detailed in the study calendar (see Section **3.3**). TIM3, IL-6, Reg-3-a, ST2, LPS-BP, Nitrate, TNFR1, IL-2Ra, CXCL10, and HGF will initially be

studied. Assay will be either by Luminex Microbead (Luminex, Austin, TX) or enzyme linked immunosorbent assay (ELISA) using commercially available reagents and protocols.

## 5.2 STORAGE, TRACKING AND DISPOSITION

### 5.2.1 Storage/Tracking in the Preclinical Development and Clinical Monitoring Facility (PDCMF)

- Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without IRB notification and an executed MTA.
- Normal donor and patient blood and tissue samples, collected for the purpose of research under IRB approved protocols of the Experimental Transplantation and Immunology Branch, may be archived by the ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF). All data associated with archived clinical research samples is entered into the ETIB PDCMF's Microsoft Excel databases on frozen cells and plasma. These databases are stored on the NCI group drive in the ETIB 'PRECLINSERVICE' folder. Access to this folder is limited to PDCMF staff and ETIB clinical staff, requiring individual login and password. All staff in the PDCMF laboratory receive annually updated NIH/CIT training and maintain standards of computer security.
- The data recorded for each sample includes the patient ID, trial name/protocol number, date drawn, treatment cycle/post-transplant time point, cell source (e.g., peripheral blood, lymphapheresis, mobilized peripheral blood stem cells, marrow, pleural fluid) as well as box and freezer location. Patient demographics that correlate treatment outcomes and therapies with the samples can be obtained only through the NCI/ETIB clinical records. As of January 2007, all newly received samples will receive a unique bar code number, which is included in the sample record in the PDCMF database. Only this bar code is recorded on the sample vial and the vials will not be traceable back to patients without authorized access to the PDCMF database. All non-coded samples previously archived will be stripped of identifiers prior to distribution for any use other than as a primary objective of the protocol under which they were collected.
- Samples are stored in locked freezers. All samples will be labeled solely with a bar code (which includes the date, and serially determined individual sample identifier). The key will be available to a restricted number of ETIB investigators and associate investigators on the protocol. Coded samples will be stored frozen at -20, -80° or liquid nitrogen vapor phase to -180 C according to stability requirements in a single location under the restricted control of the PDCM Facility of ETIB.
- Access to samples from a protocol for research purposes will be by permission of the Principal Investigator of that protocol in order to be used (1) for research purposes associated with protocol objectives for which the samples were collected, or (2) for a new research activity following submission and IRB approval of a new protocol and consent, or (3) for use only as unlinked or coded samples under the OHSRP Exemption Form guidelines stipulating that the activity is exempt from IRB review. Unused samples must be returned to the PDCMF laboratory.

- Samples, and associated data, will be stored permanently unless the patient withdraws consent. If researchers have samples remaining once they have completed all studies associated with the protocol, they must be returned to the PDCMF laboratory.
- These freezers are located onsite at the Preclinical Service laboratory (12C216) or in ETIB common equipment space (CRC/3-3273).

### 5.2.2 Protocol Completion/Sample Destruction

- Once research objectives for the protocol are achieved, researchers can request access to remaining samples, providing they have both approval of the Principal Investigator of the original protocol under which the samples or data were collected and either an IRB approved protocol and patient consent or OSHRP approval.
- The PDCMF staff will report to the Principal Investigators any destroyed samples, if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container), lost in transit between facilities or misplaced by a researcher. The Principal Investigators will annually report this information to the IRB and the NCI Clinical Director, and the office of the CCR, NCI.
- The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

## 6 DATA COLLECTION AND EVALUATION

### 6.1 DATA COLLECTION

An enrollment log will be maintained in the regulatory binder/file which is the only location of personal identifiers with unique subject identification number.

No IND agents, equipment or procedures are being used in this study.

Study data will be maintained, entered, and audited for accuracy in the C3D Database by the research nurse(s) and data managers assigned to the study.

Only adverse events related to the ECP procedure will be collected as part of this study. All other AEs will continue to be collected on the patient's primary transplant protocol. Since AEs collected are only ECP related, baseline AEs will only be collected on patient's that proceed with ECP. The baseline will be considered the immediate time before their first ECP treatment.

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards.

Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for adverse events for at least 30 days after removal from study treatment or until off study, whichever comes first.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

**End of study procedures:** Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

**Loss or destruction of data:** Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

## 6.2 RESPONSE CRITERIA

### 6.2.1 Recording of Steroid Dose

Steroid dose will be recorded in prednisone mg equivalency.

### 6.2.2 Measurement of GVHD Outcome and Response

Patients will be followed for outcome using overall survival, GVHD free survival, and steroid free survival at either 3 months post-transplant (D+100), 6 months post-transplant, or 6 months after the last ECP procedure. The definitions of GVHD response that will be used are outlined in an American Society for Bone Marrow Transplantation consensus statement.<sup>51</sup>

#### 6.2.2.1 Complete Response (CR)

- No residual organ specific symptoms or findings.

#### 6.2.2.2 Very Good Partial Response(VGPR)

- Skin: Active erythematous rash involving less than 25% of the body surface area. Rash that is pink, fading or turning to brown is not included in the measurement, since these findings indicate resolving lesions.

- Liver: Persisting low-level hyperbilirubinemia that might be related to antecedent regimen-related hepatotoxicity, concomitant hemolysis or administration of hepatotoxic agents such as voriconazole, cyclosporine, or total parental nutrition (TPN), or other factors such as sepsis. A serum total bilirubin concentration of less than 2 mg/dL approximates normal values, and a reduction to less than 25% of the baseline concentration provides strong evidence of progression toward normal liver function among patients with levels greater than 2 mg/dL.
- Gut: Gastrointestinal function and water resorption in the colon are approaching normal. These criteria have some imprecision and rely heavily on patient recall, rather than measurement of stool volume. In certain cases, it might be necessary to make allowances for the effects of pre-transplant diseases that cause diarrhea.

#### 6.2.2.3 Partial Response (PR)

- Any improvement over baseline symptoms or findings not included in VGPR.

#### 6.2.2.4 Progressive Disease (PD)

- Stable or worsening organ specific findings requiring change of therapy.

### 6.3 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site ([http://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/ctc.htm#ctc\\_40](http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40)).

## 7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

### 7.1 DEFINITIONS

#### 7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

#### 7.1.2 Suspected Adverse Reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the procedure caused the adverse event.

#### 7.1.3 Unexpected Adverse Reaction

An adverse event or suspected adverse reaction is considered “unexpected” if it is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

#### 7.1.4 Unanticipated Adverse Device Effect

Any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

#### 7.1.5 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

#### 7.1.6 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death
- A life-threatening adverse experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

#### 7.1.7 Disability

A substantial disruption of a person’s ability to conduct normal life functions.

#### 7.1.8 Life-threatening adverse experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

#### 7.1.9 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

#### 7.1.10 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

#### 7.1.11 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to
  - (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator’s Brochure or other study documents, and
  - (b) the characteristics of the subject population being studied; AND
- Is related or possibly related to participation in the research; AND
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

## 7.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

### 7.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

### 7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review.

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
  - All Grade 2 unexpected events that are possibly, probably or definitely related to the research;
  - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
  - All Grade 5 events regardless of attribution;
  - All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported

## 7.3 DATA AND SAFETY MONITORING PLAN

### 7.3.1 Principal Investigator/Research Team



The clinical research team will meet on a regular basis, once a week, when patients are being actively treated on the trial to discuss each patient.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

## **8 STATISTICAL CONSIDERATIONS**

The primary objectives of this trial are to determine, in an exploratory fashion, if any information obtained from biomarkers in patients who undergo allogeneic transplantation may be used to predict whether patients will develop acute GVHD. In addition, it will be used to determine if biomarkers obtained from patients with acute GVHD may be used to predict steroid refractoriness and the outcome of investigator chosen therapies for steroid refractory disease with and without ECP. As secondary objectives, the study will evaluate acute GVHD outcome, overall survival at 6 months, and steroid use.

This study is intended to be a companion trial to other efforts in the ETIB by collecting information on a set of acute GVHD biomarkers at various times throughout the course of an allogeneic transplant. In order to allow for a large enough number of patients to conduct a series of analyses at several main points, the study will have an accrual ceiling of 450 transplanted, of which approximately 200 patients would be expected to develop acute GVHD.

It will be estimated that approximately 90% of 450 NCI patients who are transplanted over a 10 year period will enroll on this trial, and of that approximately 400 patients, 160 will develop aGVHD and 240 will not. As noted in the schema and sections 3.3 of the protocol, biomarkers will be collected on patients at various times, starting with collections at enrollment and just prior to transplantation. If a large set of biomarkers at baseline is able to predict 80% of those who develop aGVHD and 80% of those who do not develop aGVHD, then these estimates could be made with 95% two sided confidence intervals of 73.3% to 85.7 and 74.6% to 84.7% respectively. If we are able to actually obtain such a large enough number of patients, the results could also be divided into two groups to allow for 50% of patients to train the prediction classifier and 50% to test the prediction.

With up to 165 patients maximally available with acute GVHD, the following analyses could be undertaken.

1. A very large number of biomarkers could be obtained at baseline, and at the time a patient is declared to either have a steroid response or be determined to be steroid refractory. If there are at least 75-100 patients available overall for these analyses, for example, with 88 total patients, 44 with a response and 44 with refractory disease, there would be 80% power to detect a difference between the two groups, responsive vs. refractory, using a 0.01 two-tailed significance level two-group t-test for each biomarker evaluated with respect to either a baseline value or change from baseline, with an effect size of 0.75 (that is, if the difference in the biomarker values was equal to 0.75 SD of

either the marker itself or the change in marker, for each arm, assuming equal SD on both arms). With more patients, such as 50-75 per group, there could be over 85% power for this comparison.

2. From among those who are refractory to steroids, patients may be assigned by the investigator to receive a second line therapy with or without ECP. This assignment is not made by random selection but will be done by the investigator based on whatever criteria they choose, including availability of the equipment necessary to do the procedure at the time it would be needed. If there are 44 patients available with refractory disease and half of those are assigned to receive ECP, it would be of interest to determine if there are any differences in biomarkers between those who survive to 6 months or longer or do not live to 6 months after determined to be refractory to steroids, among those who receive ECP. With 22 patients receiving ECP, there would be 79% power to detect a difference in biomarkers between those who survive to 6 months or do not, with an effect size of 1.25 using a 0.05 two-sided significance test for a given biomarker. With sufficient patients to obtain 34 who have received ECP (perhaps from among 70 with refractory disease), there would be 80% power to detect a difference in biomarkers between those two groups, with an effect size of 1.0 using a 0.05 two-sided significance test for a given biomarker.
3. Similarly, from among all patients who develop acute GVHD and require systemic steroids, the association of biomarkers among those who respond or are refractory to steroids alone will be explored using all available patient data to determine if these outcomes could be predicted on the basis of the biomarker results. The association between biomarkers and outcomes among those not requiring steroids or not developing acute GVHD will also be considered for evaluation.

As this is entirely an exploratory study and patients are not randomly assigned to treatment, the results of the analyses above as well as any others undertaken must be considered entirely hypothesis generating and will be used to potentially guide future clinical decisions if the findings are able to be validated. The power results presented above are illustrations of what may be undertaken. Furthermore, as shown in the Schema, since this study may enroll several subsets for evaluation or comparison, any specific analyses of these subsets will be reported as being entirely exploratory. Additionally, any set of exploratory analyses felt to be reasonable by a statistician associated with this study may be performed and presented in the context of being exploratory.

The ETIB currently transplants approximately 45 patients per year and approximately 45% of these patients ordinarily develop acute GVHD. Thus, approximately 20 patients per year may develop acute GVHD. Approximately half of these will respond to steroids and half will be refractory to steroids. Thus, approximately 10 per year may be refractory patients and will be eligible to be assigned to receive ECP. As such, in order to have adequate patients to address any questions regarding the association between biomarkers and the development of acute GVHD, as well as biomarkers and ECP, the accrual ceiling will be set at 450 total patients in order to have up to 200 total patients with acute GVHD which may yield up to 100 patients who may be evaluable for any analyses restricted to patients who receive ECP.

## **9 HUMAN SUBJECTS PROTECTIONS**

### **9.1 RATIONALE FOR SUBJECT SELECTION**

Both men and women of all races and ethnic groups are eligible for this trial. The nature and size of this study does not make any specific predictions of diversity in enrolled patients possible. There is nothing to suggest that the results of the study will be affected by any specific level of participation of women or minorities.

## 9.2 PARTICIPATION OF CHILDREN

This study will be limited to subjects of age 18 years or older.

## 9.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section 9.5), all subjects  $\geq$  age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the “NIH Advance Directive for Health Care and Medical Research Participation” form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 and NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

## 9.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

### 9.4.1 Related to Blood Collection

Minor complications including bleeding, pain, and hematoma formation at the site of blood draws, or infections may rarely occur.

### 9.4.2 Related to Methoxsalen Administration and ECP Procedure

Potential risks of methoxsalen and the ECP procedure are described in Section 10 and the consent form. There may also be unexpected side effects. All subjects will be carefully monitored for side effects. In addition, if additional intravenous access is required, there will be risks associated with catheter insertion and maintenance including bleeding infection, pain, and rarely death.

### 9.4.3 Related to Pregnancy

Methoxsalen is in a class of agents that is known to be teratogenic. Women of child-bearing potential and men must agree to continue to adhere to methods of contraception and other fertility control measures as already prescribed by their primary transplant protocol.

## 9.5 RISKS/BENEFITS ANALYSIS (INCLUDING THOSE THAT DO AND DO NOT HAVE THE CAPACITY TO CONSENT)

Risks of participating in this trial if receiving ECP include the side effects of methoxsalen and the risk of the ECP procedure. Patients may receive benefit from the clinical monitoring or

potentially experience improvement in acute GVHD symptoms or quality of life. Therefore, this research represents more than minimal risk to subjects with prospect of direct benefit to individual subjects.

## 9.6 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

The investigational nature and research objectives of this trial, the procedure and its attendant risks and discomforts will be carefully explained to the subject. The potential subject will be educated regarding the nature of the condition, proposed intervention, and outcome measures. Study subjects will be informed that participation is entirely voluntary and that withdrawal from the study can be made at any time without penalty of benefits to which they may be entitled. Informed consent will be obtained by Dr. Gress or his designee. At any time during participation in the protocol if new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to all enrolled or prospective participants. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

### 9.6.1 Telephone re-consent procedure

Re-consent on this study may be obtained via telephone according to the following procedure: the informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent. The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone. A fully executed copy will be returned via mail for the subject's records. The informed consent process will be documented on a progress note by the consenting investigator.

### 9.6.2 Informed Consent of Spanish Speaking Subjects:

We anticipate the enrollment of Spanish speaking research participants into our study. The IRB approved full consent document will be translated into that language in accordance with the Clinical MAS Policy M77-2.

### 9.6.3 Short form consent process for other non-English speaking patients

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, and 45 CFR 46.117 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

## **10 PHARMACEUTICAL INFORMATION**

### **10.1 METHOXSALEN STERILE SOLUTION (UVADEX(R))**

Methoxsalen Sterile Solution is used in conjunction with THERAKOS Photopheresis.

Methoxsalen Sterile Solution is indicated for extracorporeal administration with the THERAKOS UVAR XTS® or THERAKOS CELLEX Photopheresis System Instrument.

Methoxsalen Sterile Solution is contraindicated in patients exhibiting idiosyncratic reactions to psoralen compounds. Patients possessing a specific history of a light-sensitive disease state should not initiate methoxsalen therapy.

Methoxsalen Sterile Solution is contraindicated in patients with aphakia, because of the significantly increased risk of retinal damage due to the absence of a lens.

Special care should be exercised in treating patients who are receiving concomitant therapy (either topically or systemically) with known photosensitizing agents.

Patients should be told emphatically to wear UVA absorbing, wrap-around sunglasses for twenty-four (24) hours after treatment. Patients should wear these glasses any time they are exposed to direct or indirect sunlight, whether they are outdoors or exposed through a window.

Methoxsalen Sterile Solution should be used only by physicians who have special competence in THERAKOS CELLEX Photopheresis Systems.

Safety in children has not been established.

### **10.2 THERAKOS PHOTOPHERESIS PROCEDURE**

The THERAKOS Photopheresis System Instrument / THERAKOS CELLEX® Photopheresis System Instrument is indicated for use in the ultraviolet-A(UVA) irradiation, in the presence of the photoactive drug 8-methoxypsoralen (Uvadex), of extracorporeally circulating leukocyte-enriched blood.

The THERAKOS or THERAKOS CELLEX Photopheresis Systems are not designated, sold or intended for use except as indicated. Certain underlying medical conditions contraindicate THERAKOS Photopheresis, including patients who cannot tolerate extracorporeal volume loss during the leukocyte-enrichment phase, patients exhibiting idiosyncratic or hypersensitivity reactions to 8-methoxypsoralen/psoralen compounds, and patients with uncontrolled coagulation disorders.

THERAKOS Photopheresis treatments should always be performed in locations where standard medical emergency equipment is available. Volume replacement fluids and/or volume expanders should be readily available throughout the procedure.

Hypotension may occur during any treatment involving extracorporeal circulation. Closely monitor the patient during the entire treatment for hypotension. Transient pyretic reactions, 37.7-38.90C (100-102 0F), have been observed in some patients within six to eight hours of reinfusion of the photoactivated leukocyte-enriched blood. A temporary increase in erythroderma may

accompany the pyretic reaction. Treatment frequency exceeding labeling recommendations may result in anemia. Venous access carries a small risk of infection and pain.

The ECP is not approved for the disease under investigation on this study. However, it will be used as a Non-Significant Risk Device. Therefore IDE from FDA is not required for ECP machine (Non Exempt NSR) use in this protocol per the below justification:

- Is not intended as an implant and does not present a potential for serious risk to the health, safety, or welfare of a subject.
- Is not purported or represented to be for a use in supporting or sustaining human life and does not present a potential for serious risk to the health, safety, or welfare of a subject.
- Is not for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and does not present a potential for serious risk to the health, safety, or welfare of a subject.

### 10.2.1 Warnings and Precautions

- 10.2.1.1 Patient-Related Precautions and Education Special care should be exercised in treating patients who are receiving concomitant therapy (either topically or systemically) with known photosensitizing agents.
- 10.2.1.2 Oral administration of methoxsalen followed by cutaneous UVA exposure (PUVA therapy) is carcinogenic. Because the dose of methoxsalen with UVADEX® therapy is about 200 times less than with PUVA therapy and the skin is not exposed to high cumulative doses of UVA light, the risk of developing skin cancer following UVADEX® therapy may be lower.
- 10.2.1.3 Patients exhibiting multiple basal cell carcinomas or having a history of basal cell carcinoma should be diligently observed and treated. Methoxsalen may cause fetal harm when given to a pregnant woman. Women undergoing photopheresis should be advised to avoid becoming pregnant.
- 10.2.1.4 Patients should be told emphatically to wear UVA absorbing, wrap-around sunglasses for twenty-four (24) hours after UVADEX® treatment (Cloud 1961). They should wear these glasses any time they are exposed to direct or indirect sunlight, whether they are outdoors or exposed through a window. The sunglasses will be provided to participants if they are necessary.
- 10.2.1.5 Patients who may not be able to tolerate the fluid changes associated with ECP should be monitored carefully. Procedures, such as renal dialysis, which might cause significant fluid shifts (and expose the patient to additional anticoagulation) should not be performed on the same day. If procedures on the same day are unavoidable, maximizing the time between procedures is recommended. Refer to UVAR-XTS Technical Bulletin #3 entitled, "Avoid Performing Extracorporeal Photopheresis and Procedures Such As Dialysis on the Same Day" (January 10, 2013). Consideration should be taken for each patient's clinical status. Unless otherwise medically indicated or instructed, DTM requires minimum platelet threshold of 20 K/uL.

- 10.2.1.6 Extracorporeal volume management is critically important. American Association of Blood Banking (AABB) guidelines recommend that extracorporeal blood volume does not exceed 15% of the estimated total blood volume. Members of the patient's medical care team will coordinate with DTM/BSS Nurses and DTM Physicians to ensure blood volume management needs are met prior to therapy initiation.
- 10.2.1.7 In clinical practice, anemia in patients receiving ECP therapy can be seen and is most likely attributed to small volume blood loss over repeated procedures. For this reason, DTM requires manual blood return of all fluid remaining within the procedural kit. When this requirement is met, there has been no observed decreasing of hemoglobin due to the procedure.
- 10.2.1.8 High lipids or triglycerides can cause plasma to have a milky or opaque appearance. This could potentially trigger the optic sensor prematurely and may result in collection of a large volume of plasma with little to no leukocyte content. To avoid this potential, DTM recommends the following per Therakos guidelines:
- Stop Lipids at least seven hours prior to ECP.
  - Encourage a low-fat diet for 24-hours prior to ECP
  - Re-schedule triglyceride-producing medication until after ECP
  - Administer a lipid-lowering agent

Refer to Therakos UVAR XTS Operators Manual Section Appendix B: Additional Troubleshooting (B-1) for further guidance.

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