

**CLINICAL RESEARCH PROJECT**

**PROTOCOL #05-H-0206**  
**Drug Name:** Alemtuzumab (Campath®)  
**IND Number:** exempt  
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**To:** Richard Cannon, MD Chair, NHLBI IRB

**Title:** A Pilot Study of Alemtuzumab (Campath®) in Patients with Myelodysplastic Syndrome (MDS)

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**Subjects of Study:** Number 78 Sex Either Age-range 18-72 (inclusive)

**Project Involves Ionizing Radiation?** No  
**Off-Site Project?** No  
**Multi-Institutional Project?** No  
**DSMB Involvement:** Yes

## PRECIS

Many bone marrow failure syndromes in humans are now recognized to result from immunological mechanisms. These diseases include aplastic anemia, pure red cell aplasia, and some types of myelodysplasia. Patients with these conditions, who may suffer variable degrees of anemia, leukopenia, and thrombocytopenia, alone or in combination, have been shown to respond to a wide variety of immunosuppressive agents, ranging from corticosteroids to cyclosporine (CsA) and horse antithymocyte globulin (h-ATG). However, non-response and relapse continues to be a problem. Why some patients do not respond initially or others respond and then relapse is unclear. Autoreactive T cells may be resistant to the effect of h-ATG/CsA (non-responders), while in others, residual autoreactive T cells expand post-treatment leading to hematopoietic stem cell destruction and recurrent pancytopenia (relapse). Therefore, novel, less toxic immunosuppressive regimens that increase response rates and hematologic recovery and decrease relapse rates are needed.

One such novel therapy, alemtuzumab (Campath®) is a humanized IgG1 monoclonal antibody directed against the CD52 protein, which is highly expressed on all lymphoid cells and monocytes. Alemtuzumab (Campath®) produces profound and persistent lymphopenia, affecting predominantly the CD4+ T cell subset. This property has made it attractive in the treatment of a wide range of diseases including rheumatoid arthritis, multiple sclerosis, ocular inflammatory disease, lymphoid malignancies, organ allograft rejection, and in conditioning regimens in stem cell transplantation to prevent graft failure and graft-versus-host disease.

We therefore propose a non-randomized, off label, pilot, Phase I/II study of alemtuzumab (Campath®) in MDS patients who are likely to respond to immunosuppression.

Primary endpoints will be changes in peripheral blood counts (platelets, absolute neutrophil count, reticulocyte count, hemoglobin). Secondary endpoints (in transfusion-dependent patients) include improvement in the transfusion requirements (measured as decrease in the number of transfusion administered on "as needed basis"), duration of response, late effects of treatment, relapse and survival.

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## 1.0 OBJECTIVES

The primary objective of this clinical protocol is to assess the safety and efficacy of the genetically engineered humanized anti-CD52 monoclonal antibody, alemtuzumab (Campath®) on blood counts and transfusion requirements in patients with myelodysplastic syndromes.

## 2.0 BACKGROUND

### 2.1 Myelodysplastic Syndromes (MDS)

The myelodysplastic syndromes (MDS) are clonal stem cell disorders characterized by cytopenias (anemia, neutropenia, thrombocytopenia) due to ineffective hematopoiesis and dysplastic bone marrow morphology. As a result, patients with MDS are at risk for symptomatic anemia, infection, and bleeding, as well as a variable risk of progression to acute leukemia which is often refractory to standard treatment. These disorders may arise de novo or appear years after exposure to potentially mutagenic chemotherapy.

Symptoms that derive from low blood counts include: Anemia which leads to fatigue, weakness, lassitude, headaches, and in older patients, dyspnea and chest pain. These manifestations are most commonly responsible for the clinical presentation. Thrombocytopenia produces mucosal bleeding: petechiae of the skin and mucous membranes, epistaxis, and gum bleeding are frequent and early complaints. Bleeding can be brisk in the presence of accompanying physical lesions, as in gastritis and fungal infection of the lungs. The most feared complication of thrombocytopenia is intracranial hemorrhage. Bacterial and fungal infections in the setting of neutropenia are a major cause of morbidity and mortality, most often the cause of death in refractory cytopenia associated with MDS.

### 2.2 Prognostic Indicators

Analysis of previous data demonstrated that certain variables including the patient's age, whether or not they were HLA DR15, and days of red cell transfusion dependence prior to treatment were predictive of response.<sup>37</sup> Table 2 shows the relationship between these variables and the predicted probability of response in the previous study.

**Table 1: Predicted Probability of Response (PPR) to Immunosuppression in MDS**

<i>PATIENT'S AGE IN YEARS + DURATION OF RCTD IN MONTHS</i>		<b>Predicted Probability of Response</b>
<b>DR15 negative patients</b>	<b>DR15 positive patients</b>	
>58	>72	Low
50-58	64-72	Intermediate
<50	<64	High

**Key:** RCTD = red-cell transfusion dependence

**Table 2: The relationship between this status and the response to treatment.**

<b>Predicted Probability of Response</b>	<b>Responders</b>	<b>Non-Responders</b>
Low	1	14
Intermediate	0	2
High	8	1

When the predicted probability of response criteria were applied to evaluable patients in the current study (NHLBI protocol # 00-H-0169), fourteen of 15 (93%) patients with a low probability of response were non responders while 7 of 8 (88%) patients with a high probability of response were responders. All complete responders were in the high predicted probability of response category. None of the patients in the intermediate category responded to treatment.

### 2.3 FAB Classification

No single morphologic finding is diagnostic of myelodysplastic syndrome; rather, the combination of dysplastic features in the peripheral blood and/or bone marrow is necessary. The standard stains (hematoxylin and eosin, Romanowsky) will be done on the bone marrow sample as well as the Prussian blue stain for iron and the reticulin stain for fibrosis.

The common thread between the five subgroups below is dyspoiesis. **Quantitative abnormalities of myelodysplastic syndromes**

**Table 3. The World Health Organization (WHO) classification of myelodysplastic syndromes (MDS).**

Disease	Blood Findings	Bone Marrow Findings
Refractory anemia (RA)	Anemia No or rare blasts < 1 x 10 <sup>9</sup> /L monocytes	Erythroid dysplasia only < 10% grans or megas dysplastic < 5% blasts < 15% ringed sideroblasts
Refractory anemia with ringed sideroblasts (RARS)	Anemia No blasts	Erythroid dysplasia only < 10% grans or megas dysplastic ≥15% ringed sideroblasts < 5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenias (bicytopenia or pancytopenia) No or rare blasts No Auer rods < 1 x 10 <sup>9</sup> /L monocytes	Dysplasia in ≥10% of cells in two or more myeloid cell lines < 5% blasts in marrow No Auer rods < 15% ringed sideroblasts
Refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS)	Cytopenias (bicytopenia or pancytopenia) No or rare blasts No Auer rods < 1 x 10 <sup>9</sup> /L monocytes	Dysplasia in ≥10% of cells in two or more myeloid cell lines ≥15% ringed sideroblasts < 5% blasts No Auer rods
Refractory anemia with excess blasts - 1 (RAEB-1)	Cytopenias < 5% blasts No Auer rods < 1 x 10 <sup>9</sup> /L monocytes	Unilineage or multilineage dysplasia 5%–9% blasts No Auer rods
Refractory anemia with excess blasts - 2 (RAEB-2)	Cytopenias 5%–19% blasts Auer rods +/- < 1 x 10 <sup>9</sup> /L monocytes	Unilineage or multilineage dysplasia 10%–19% blasts Auer rods +/-
Myelodysplastic syndrome, unclassified (MDS-U)	Cytopenias No or rare blasts No Auer rods	Unilineage gran or mega dysplasia < 5% blasts No Auer rods

MDS associated with isolated del (5q)	Anemia < 5% blasts Platelets normal or increased	Normal to increased megakaryocytes with hypolobulated nuclei < 5% blasts No Auer rods Isolated del (5q)
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## 2.4 Evidence for Immune Mechanisms Causing Cytopenia of MDS

There is a growing body of clinical and experimental evidence suggesting that the cytopenia(s) in MDS is T-cell mediated in a proportion of cases.

### 2.4.1 Clinical evidence of immune mechanisms

- Twenty-one of sixty-one patients (33%) with MDS treated at the NIH with h-ATG immunosuppression (protocol 95-H-0189) became transfusion-independent.
- Three of 18 patients (17%) with MDS treated with CsA immunosuppression (96-H-0142, 99-CC-0021) became transfusion independent
- In a separate report, two patients with hypoplastic MDS responded to h-ATG and CsA either alone or in combination with durable responses. <sup>1</sup>
- In various other case reports, 8 of 13 patients with hypoplastic MDS have responded to h-ATG treatment <sup>2,3</sup>
- h-ATG treatment improved the cytopenia of MDS that had evolved from aplastic anemia. <sup>4</sup>
- In 17 cytopenic patients with MDS of the refractory anemia subtype, 82% responded to treatment with CsA alone <sup>5</sup>.
- Four patients with autoimmune diseases and myelodysplasia showed hematological responses to CsA. <sup>6</sup>
- In 30 patients with autoimmune diseases and MDS, 6 showed a hematological response to steroid treatment including one complete cytogenetic response. <sup>7</sup>
- CsA induced a remission in an infant with myelodysplastic syndrome <sup>8</sup>
- Our experience with 133 patients treated over a 10 year period with ATG or ATG/CsA demonstrating response rates of over 70% in patients in high or intermediate risk groups (see section 2.4)

### 2.4.2 Experimental evidence of immune mechanisms

- Patients with MDS have a high suppressor: helper lymphocyte ratio similar to that found in aplastic anemia, where the presence of cytotoxic CD8 cells producing interferon-gamma has been linked to the suppression of hematopoiesis. <sup>9,10,11,12</sup>
- In MDS, CD8 T cells suppress granulocyte progenitor cell growth, and, in patients responding to h-ATG, T cell mediated colony inhibition is lost after treatment <sup>13</sup>
- Immunohistochemical examination of the marrow of MDS patients showed increased staining for TNF-alpha and TGF-beta compared to controls <sup>14,15</sup>
- The percentage of T helper cells and T suppressor cells demonstrating an activated phenotype is increased in MDS
- Depletion of lymphocytes increased the in vitro hemopoiesis in long-term bone marrow cultures (LTBMC) from patients with myelodysplastic syndrome (MDS). <sup>16</sup>

In aplastic anemia, where a similar T-cell mediated marrow suppression is believed to occur, the response rate to immunosuppression with h-ATG alone or CsA alone is 40-50%. This response rate

was increased to 70-80% by combining the 2 agents.<sup>17,18</sup> Responders to immunosuppression have a major survival advantage over non-responders. As in aplastic anemia, more intensive immunosuppression may improve the response rate in MDS compared to h-ATG or CsA alone.

## **2.5 Treatment Options for MDS**

### ***2.5.1 Bone marrow transplantation***

The only definitive treatment is bone marrow transplantation; unfortunately, treatment-related mortality precludes the application of the procedure for patients older than 60 years and those lacking a suitable matched sibling donor. Our institution reported on 43 patients who received either a full myeloablative or reduced intensity (age >55) T-cell depleted peripheral blood stem cell for MDS. Actuarial 3 year overall survival was 64%, relapse 26% and treatment related mortality 23%<sup>80</sup>. While this is feasible option for younger patients with HLA matched siblings, treatment related mortality increases with age and degree of HLA mismatch. Recent data from the SEER database reports the median age of diagnosis of MDS to be 76 years old<sup>81</sup>.

### ***2.5.2 Growth factors***

Until recently, most patients are treated with transfusions and growth factors to improve blood counts. The disease transforms into acute myeloid leukemia (AML) in up to 40% of patients with MDS but an equal proportion of patients die from bleeding or infection resulting from cytopenias. The prognosis for patients with MDS/AML is very poor with high chemotherapy complication rates and a short duration of remission in responders. Additionally, symptoms of anemia, and frequent trips to the treating physician's office for blood and/or platelet transfusions significantly impact quality of life. In low risk MDS patients, erythropoietin provides a modest benefit for the treatment of anemia and many studies have shown a superior benefit when granulocyte stimulating factor (G-CSF) is added to erythropoietic<sup>78,79</sup>. Additionally, G-CSF alone can stimulate the production of neutrophils to prevent infectious complications from long-term neutropenia<sup>79</sup>. Unfortunately, growth factors work only for a finite period of time and patients eventually succumb to anemia requiring transfusion support and prolonged neutropenia. The prognosis for patients with MDS/AML is very poor with high chemotherapy complication rates and a short duration of remission in responders.

### ***2.5.3 Azacitidine (Aza-CR, 5-AZC) and Decitabine***

Azacitidine has been designated an orphan product for the use in the treatment of myelodysplastic syndromes but has shown only a 10-30% response rate.<sup>19</sup> Its use requires close monitoring as cytopenias and central nervous system toxicities (lethargy, somnolence, confusion) are a frequent complications. The drug was recently licensed and is widely available to those who wish to use it off study. 5-azacytidine, can reduce transfusion requirements, delay time to leukemic transformation, and improve quality of life when compared to supportive care<sup>9,10</sup> This trial will not exclude patients who have tried and failed Azacitidine nor does it preclude its use in patients who have failed treatment.

Decitabine, another hypomethylating agent, has shown to improve hematologic parameters (improve cytopenias and decrease blast counts) in some patients with MDS.<sup>83</sup> A study of 170 average and high risk MDS patients showed an overall response of 17% with a 9% complete response (Normal peripheral counts and  $\leq$  5% marrow blasts) and 13% hematologic improvement (improvement in peripheral cell counts). In this population there was a trend (although statistically non-significant) towards increased leukemia free progression time and death. While overall survival is improved with both drugs, some patients are limited in their ability to tolerate these medications secondary to

worsening cytopenias. Additionally, all patients eventually progress or die of complications of their disease despite treatment.<sup>82;83</sup>

#### **2.5.4 Revlimid® (lenolidomide)**

Revlimid® was approved by the United States Food and Drug Administration on December 27, 2005 for the treatment of low-or-intermediate risk MDS associated with deletion of 5q, a chromosomal (cytogenetic) abnormality, with or without other cytogenetic abnormalities. Revlimid is structurally similar to thalidomide, a drug known to cause severe birth defects. Additional studies are ongoing in animals to address whether there is a risk that Revlimid will also cause birth defects when taken during pregnancy. While these studies are under way, the company is marketing Revlimid under a risk management plan called RevAssist, designed to prevent fetal exposure.

Under RevAssist, only pharmacists and prescribers registered with the program will prescribe and dispense Revlimid. The program requires patients, including female patients undergoing mandatory pregnancy testing, to give informed consent before starting Revlimid. Physicians are to check pregnancy tests, limit prescriptions to a one-month mail supply, and report any pregnancies to FDA. FDA and the manufacturer will re-evaluate the risk management plan when results of further animal testing for birth defects are completed.

The labeling for Revlimid will include a Black Box Warning and a Medication Guide regarding the prevention of fetal exposure. Additional Black Box Warnings include the potential need to lower the dose due to suppressed blood counts and increased risk of blood clots. Common side effects reported with Revlimid include thrombocytopenia (low platelet count), neutropenia (low white blood cell count), diarrhea, pruritis (itch), rash, and fatigue.

#### **2.5.5 Antithymocyte globulin (h-ATG)**

h-ATG is currently approved for the treatment of aplastic anemia by the Food and Drug Administration. The mechanism by which h-ATG improves bone marrow failure in aplastic anemia and MDS is not fully known. Several modes of action are possible. After treatment with h-ATG, circulating levels of lymphocytes drop to 10% pretreatment level, through a variety of mechanisms; these include Fc receptor complement-dependent lysis, opsonization and phagocytosis by macrophages, and immunomodulation leading to long-term depletion via antibody dependent cell-mediated cytotoxicity and activation induced apoptosis.

While it is generally believed that h-ATG administration leads to depletion of immune competent cells, its exact mechanism of action remains unclear.<sup>20</sup> h-ATG preparations contain a variety of antibodies recognizing human T-cell epitopes, which are directed against activated T-cells or activation antigens.<sup>21,22</sup> After treatment with h-ATG, circulating levels of lymphocytes decline only transiently, but the number of activated T-cells is decreased for prolonged periods of time.<sup>23</sup> This effect is also reflected in the decrease of IFN- $\gamma$  and possibly TNF production after therapy with h-ATG.<sup>24</sup>

Susceptibility to h-ATG-induced apoptosis is restricted to activated cells, dependent on IL-2 and prevented by cyclosporine A, FK506 and rapamycin.<sup>25</sup> h-ATG includes antibodies against T-cell markers such as CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, HLA-DR, HLA Class I heavy chains, and beta-2-microglobulin. While absolute numbers of lymphocytes return to baseline after h-ATG, patients who respond to therapy show a decrease in the number of activated T cells at three months post treatment. Tissue culture preparations of peripheral blood lymphocytes treated with h-ATG produce hematopoietic colony stimulating factors, suggesting a possible stimulatory role

in vivo. h-ATG binds to numerous other cell types in addition to lymphocytes, including cells of the bone marrow.<sup>26,27,28</sup> The response to h-ATG may be mediated by circulating factors produced in this immunologically activated state, although response to h-ATG has not correlated with severity or presence of clinical serum sickness.

### **2.5.6 Cyclosporine (CsA)**

Cyclosporine (CsA) binds to intracellular receptors termed immunophilins, inhibiting in turn the activity of calcineurin, which results in the blocking of interleukin-2 production and T cell activation and proliferation.

In vivo, CsA inhibits the release of IL-2 from activated T-cells and consequently decreases T cell proliferation.<sup>29,30,31</sup> Production of gamma interferon is also reduced while growth factor production is unaffected.<sup>32</sup> In vitro, CsA inhibits transcription of IL-2 mRNA.<sup>33</sup> Cell culture studies have shown a predominant effect on helper/inducer lymphocytes at low dose, with loss of subtype specificity at higher doses.<sup>34</sup> B cells are also affected with an early direct inhibition of activation and a decrease in antibody production. In addition to inhibition of T-cell proliferation, CsA induces apoptosis of immune-competent cells.<sup>35</sup>

CsA is not cytotoxic. It has no direct stimulatory or suppressive effect on bone marrow cells in culture. To inhibit T cell activation, it must be present at the time of antigen stimulation. This is consistent with studies that show no improvement in colony formation in bone marrow cultures of patients with aplastic anemia after addition of CsA, even though improvement is shown in the same cultures with T cell depletion.<sup>36</sup>

### **2.5.7 Combination therapy with h-ATG and/or CsA**

The goal of immunosuppressive regimens applied to the treatment of aplastic anemia is inhibition of immune-mediated destruction of hematopoietic progenitor and stem cells. Although activated lymphocytes may be more susceptible to the action of CsA and h-ATG, these agents lack specificity for autoreactive T-cell clones.

Between 1998 and 2003, 108 patients with MDS and FAB classification RA, RARS or RAEB were enrolled in Immunosuppressive therapy (IST) treatment protocols at our institution: 11 received cyclosporine (CsA) to maintain levels above 100 ng/ml for up to 6 months; 66 received h-ATG (Pharmacia, 40mg/kg x 4 days); and 31 received a combination of h-ATG/CsA. Previously, we reported that responses to h-ATG alone could be predicted in individuals who were HLA DR15, of younger age and had a shorter duration of red cell transfusion dependence.<sup>37</sup> We used these factors to develop a score segregating patients into high, intermediate and low probabilities of response. We found that 90% high probability patients and 1% of low probability patients responded to h-ATG+CsA (P<0.001). The mean time to response to h-ATG/CsA was 6 weeks. The mean time to response to h-ATG alone was 3 months.

To analyze effectiveness of treatments of different immunosuppressive intensity, prognostic scores were assigned to 95 patients receiving CsA, h-ATG, or h-ATG+CsA. In patients with a low probability of response, no differences in responsiveness were seen between therapies (5% h-ATG alone, 5% h-ATG/CsA and 10% CsA alone responded to therapy).<sup>37</sup> However, in patients with a high probability of response, there was a statistically significant increase in the response rate of patients receiving h-ATG regimens compared to patients receiving CsA + h-ATG (p=0.02). Patients with cytogenetic abnormalities were more likely to be in the group of patients with a low probability of response with the exception of patients with trisomy 8; these patients were just as likely to respond

to treatment with immunosuppressive therapy as were patients with normal cytogenetics. These results confirm the predictive value of the immunosuppression-response score and suggest that h-ATG rather than CsA is the more effective agent inducing hematological responses in susceptible MDS patients (see Table 2, section 2.2)

## **2.6 Alemtuzumab (Campath®)**

### **2.6.1. Description**

Alemtuzumab (Campath®) is a humanized IgG1 monoclonal antibody directed against the CD52 protein, which is highly expressed on all lymphoid cells and monocytes.<sup>38</sup> Alemtuzumab (Campath®) is a recombinant DNA derived humanized monoclonal antibody that is directed against the 21-28 kD cell surface glycoprotein, CD52. CD52 is expressed on the surface of normal and malignant B and T lymphocytes, NK cells, monocytes, macrophages and tissues of the male reproductive system. Alemtuzumab (Campath®) is an IgG1 kappa with human variable framework and constant regions and complementarity-determining regions from a murine (rat) monoclonal antibody (Campath-1G). The alemtuzumab (Campath®) antibody has an approximate molecular weight of 150 kD. Alemtuzumab (Campath®) is produced in a mammalian cell (Chinese hamster ovary) suspension culture in a medium containing neomycin. Neomycin is not detectable in the final product. The rationale for using the humanized form of the monoclonal antibody is the decreased formation of antibodies against alemtuzumab (Campath®), which limit efficacy and the potential for re-administration of the drug.

### **2.6.2 Clinical pharmacology**

Alemtuzumab (Campath®) binds to CD52, a non-modulating antigen that is present on the surface of essentially all B and T lymphocytes, a majority of monocytes, macrophages and NK cells. Analysis of samples collected from multiple volunteers has not identified CD52 expression on erythrocytes or hematopoietic stem cells. The proposed mechanism of action is antibody-dependent lysis of cells following cell surface binding. Alemtuzumab (Campath®) Fab binding was observed in lymphoid tissues and the mononuclear phagocyte system. Significant binding was also observed in the skin and male reproductive tract (epididymis, sperm, seminal vesicle). Mature spermatozoa stain for CD52, but neither spermatogenic cells nor immature spermatozoa show evidence of staining.

Alemtuzumab (Campath®) pharmacokinetics displayed nonlinear elimination kinetics. In CLL patients treated with Campath®, mean half-life was 11 hours (range 2 to 32 hours) after the first 30 mg dose and 6 days (range 1 to 14 days) after the last 30 mg dose. Systemic clearance decreased with repeated administration due to decreased receptor-mediated clearance (i.e. loss of CD52 receptors in the periphery).

### **2.6.3 The CD52 antigen**

The CD52 antigen is a heavily glycosylated lymphocyte glycoprotein with a small protein sequence of only 12 amino acids, being attached to the cell membrane by a glycosylphosphatidylinositol (GPI) anchored protein. Other proteins on T and B lymphocytes like CD48, CD55 and CD59 also are expressed as GPI-anchored proteins. Mutations in the phosphatidylinositolglycan (PIG-A) gene in hematopoietic stem cell results in an acquired deficiency of the GPI anchor and is etiologic for PNH.<sup>39,40</sup> In patients treated with Campath-1H, emergence of CD52-, GPI-deficient lymphocytes have given rise to a transient PNH-like clone.<sup>41</sup> However, a classical PNH mutation in the PIG-A gene has not been identified in most studies, suggesting an alternate pathway for the presence of GPI-deficient

cells.<sup>42</sup> One study using ultra sensitive techniques detected mutations in the PIG-A gene prior to Campath-1H treatment in patients who develop CD52 deficient lymphocytes after therapy.<sup>43</sup> Despite the persistence of these cells, there have been no adverse reactions associated to their emergence with no clinical findings suggestive of PNH.<sup>44</sup> Emergence of a PNH cell clone population among lymphocytes will be monitored in our study, but we do not expect it to be of clinical significance. CD52 is not present on hematopoietic cells and therefore, Campath-1H does not influence proliferation and development of hematopoietic progenitor cells and should not impact on established PNH or the emergence of an expanded PNH clone.

## 2.7 Clinical Experience Alemtuzumab (Campath®)

Alemtuzumab produces profound and persistent lymphopenia, affecting predominantly the CD4+ T cell subset. This property has made it attractive in the treatment of a wide range of diseases including rheumatoid arthritis, multiple sclerosis, ocular inflammatory disease, lymphoid malignancies, and in conditioning regimens in stem cell transplantation to prevent graft failure and graft-versus-host disease.<sup>45,46,47,48,49,50,51,52,53,54,55,56</sup>

### 2.7.1 Rheumatoid arthritis

The mechanisms by which Campath-1H induces its lymphocytotoxic effect appear to be complement-mediated cytotoxicity, antibody-dependent cellular cytotoxicity and induction of apoptosis. Most data regarding the degree and duration of lymphopenia have been derived from patients with rheumatoid arthritis.<sup>57,58</sup> A decrease in lymphocyte and monocyte counts are seen immediately after administration. Initial cell recovery is noticed with CD16+ NK cells and CD14+ monocytes returning to normal levels within 1-2 months, and CD 19+ B cells within 3-6 months. Cytotoxic CD8+ T cell begin to recover at about 3 months after treatment and helper CD4+ T cells may remain suppressed for years. Metteson et al demonstrated that 22% of patients remained lymphopenic at 6 months after treatment, with CD4+ and CD8+ T cells being 14% and 13% pretreatment levels, respectively.<sup>59</sup> Others have shown that T cell subsets can remain suppressed for an even longer period, not rising above 20% of pretreatment levels at any time after 18 months of therapy.<sup>60</sup> Despite the prolonged and profound lymphopenia, infectious complications in practice have been minor, most occurring in the first 8 weeks and the majority being well controlled and self-limiting. Fatal opportunistic infections have been infrequent.

Campath-1H has been administered in rheumatoid arthritis by subcutaneous and intravenous routes. With the subcutaneous injection, clinical improvement was short-lived, with a high incidence of anti-Campath-1H antibodies.<sup>61</sup> In an intravenous dose-ranging study, 41 patients with RA who had failed at least one disease-modifying anti-rheumatic drug received a total of 100, 250 or 400 mg over 5 or 10 days.<sup>62</sup> Profound lymphopenia developed in all patients with clinical responses appearing more sustained in patients receiving 250 or 400 mg. Approximately 50% of patients achieved a 50% Paulus response at 31 days and 20% maintained a 50% Paulus response at least 6 months. There were 2 fatal infections. One patient died from infection with *Coccidioides immitis* (methotrexate was added after Campath-1H) and another of suspected hemolytic-uremic syndrome. Overall, infectious complications were minor (mainly herpetic) and all occurred within 8 weeks of therapy.

In rheumatoid arthritis and autoimmune diseases the half-life of Campath-1H has been estimated to be 5 - 9 days. It is likely that the half-life in aplastic anemia will be similar, even though no studies to date have evaluated the pharmacokinetics of Campath-1H in these patients.

Toxicities seen with Campath-1H are short-term infusion-related and long-term due to profound, sustained lymphopenia. Early symptoms include fever, rash, rigors, diarrhea, hypotension and nausea, probably related to cytokine release of TNF- $\alpha$ , interferon- $\gamma$ , and IL-6.<sup>63</sup> Treatment is mainly symptomatic with discontinuation of the infusion, restarting it as tolerated at a slower rate.

Opportunistic infections are seen most occurring within the first 8 weeks of treatment and have generally been mild. As expected, viral infections with herpes simplex and cytomegalovirus occur at a high frequency. Despite the long duration of CD4+ T cell lymphopenia after treatment with Campath-1H, few fatalities have been reported secondary to opportunistic infections, suggesting that the recovery of B cells and NK cells along with low but existing levels of CD4+ and CD8+ cells are adequate to avoid infectious catastrophes. However, serious, sometimes fatal, opportunistic infections do occur following treatment with Campath-1H.

### 2.7.2 Chronic lymphocytic leukemia (CLL)

The efficacy and safety of Campath-1H in CLL was evaluated in a prospective, non-comparative phase II trial conducted at 21 centers in the United States and Europe.<sup>64</sup> Ninety-three patients with high-risk CLL refractory to alkylating agents and fludarabine received Campath-1H 30mg, 3 times a week, up to a maximum of 12 weeks. Overall response was 33%, with 2% complete and 31% partial responses. Median time to progression was 4.7 months, 9.5 months for responders and overall median survival was 16 months, 32 months for responders.

The most common adverse event was infusion-related and was generally grade 1 or 2 in severity. They included rigors, fever, nausea, vomiting and rash. Infections occurred in 51 patients (55%) during the study, being mild to moderate in 26, and more severe (grade 3 or 4) in 25 patients. Septicemia occurred in 14 patients (15%), with grade 3 or 4 in 10 patients. Superficial candidiasis occurred in 9 patients; and viral reactivation with cytomegalovirus (n=7) or Herpes simplex (n=6) in 13 patients. A total of 18 patients developed opportunistic infections, 11 during treatment and 7 in the follow-up period. Opportunistic infections included *Pneumocystis carinii* (n=1); *Aspergillus* pneumonia (n=1); rhinocerebral mucormycosis (n=1); systemic candidiasis (n=1); cryptococcal pneumonia (n=1, fatal); herpes zoster (n=4, follow-up); pulmonary aspergillosis (n=1, follow-up, fatal); *Listeria meningitis* (n=1, follow-up). The most common opportunistic infection was CMV reactivation (n=7). Nine deaths occurred during treatment or within 30 days of the last administration of Campath-1H (3 progressive disease, 3 pneumonia, 1 pulmonary embolism, 1 sepsis, 1 rhinocerebral mucormycosis) and 19 deaths occurred between 30 and 180 days (11 progressive disease, 6 infections, 1 respiratory distress, 1 inanition). Patients who died during treatment were more likely to have advanced disease at study entry (Rai III or IV) and have failed to respond to Campath-1H.

Most patients experienced transient cytopenias during treatment, with neutropenia most commonly seen during weeks 5 and 6 (30% of patients) and thrombocytopenia during the first 2 weeks of therapy. Neutrophil and platelet counts recovered by 2 months follow-up.

Two smaller phase II trials in patients with chemotherapy and fludarabine refractory CLL (24 and 29 patients each) also showed similar response rates of about 30%.<sup>65,66</sup> Antimicrobial prophylaxis was not routinely recommended in these trials with infection rates somewhat higher compared to the larger pivotal study. Based on the data from these studies, Campath-1H received FDA approval in May 2001 for patients with B-cell chronic lymphocytic leukemia who have been treated with alkylating agents and have failed fludarabine therapy.

### 2.7.3 Transplant setting to prevent graft rejection and GVHD

Alemtuzumab has also been used as part of conditioning regimens in stem cell transplantation to prevent graft rejection and graft-versus-host disease.<sup>67,68</sup> In solid organ transplantation Campath-1H has been used with cyclosporine in renal allograft recipients to help prevent rejection and reduce further immunosuppressive therapy.<sup>69</sup> In bone marrow transplantation for aplastic anemia, Campath-1G (rat anti-human IgG2b monoclonal antibody directed against the same CD52 antigen) has been used in HLA-identical sibling donors as well as matched unrelated donors with low incidence of graft-versus host disease and graft failures.<sup>70,71</sup>

### 2.7.4 Immune cytopenias

There are no published studies of Campath-1H in patients with aplastic anemia, but a recent case series used Campath-1H in a diverse population of 21 patients with severe autoimmune cytopenias resistant to standard therapy.<sup>72</sup> The diseases included were: 4 with autoimmune neutropenia (AIN), 4 with autoimmune hemolytic anemia (AIHA), 4 with immune thrombocytopenia purpura (ITP), 3 with Evan's syndrome, 3 with autoimmune cytopenias, 1 with ITP and AIN and 1 with ITP and pure red cell aplasia (PRCA). Campath-1H was given as a daily dose of 10 mg intravenously for 10 days. CsA was given to 7 patients and was introduced following Campath-1H. Apart from first dose reactions, Campath-1H was well tolerated in all patients.

Of the 4 patients with AIN, a sustained response was seen in three, with the fourth patient requiring fewer G-CSF injections. Of the 4 patients with AIHA, 2 with warm type autoimmune hemolytic anemia responded and one with cold type hemolytic anemia had a partial response. In the 4 patients with PRCA, 2 responded with one having a relapse after the CsA level was found to be sub-therapeutic. Of the 3 patients with Evan's syndrome, 2 had an initial response but later one relapsed, and the third had a transient response only. A response was seen in 2/3 patients with autoimmune cytopenia, although one relapsed having responded again to a second course of Campath-1H. The one patient with ITP and AIN had a sustained response from the ITP, although he remained neutropenic.

Of the 7 patients who died, 2 had autoimmune cytopenia (1-Guillain Barre syndrome, 1-TTP), 2 had Evan's syndrome (1 – cerebral hemorrhage, 1 – recurrent bronchial carcinoma), 1 had ITP (cerebral hemorrhage), 1 with AIHA (intractable intravascular hemolysis and systemic venous thrombosis), 1 had PRCA (non-Hodgkin's lymphoma transformation). A profound and predictable lymphopenia developed in all patients. B lymphocyte recovery occurred before 3 months, CD8+ T cells recovered at variable times ranging from 1-36 months and the median CD4 T cell at last follow up visit was 140 (range 15-560). Despite the lymphopenia there was only one patient who developed a mild viral infection with scattered vesicular skin lesions treated with acyclovir. There was no correlation between CD4 counts and response.

In a case report, a 51 year old female with a 5 year history of autoimmune neutropenia failed treatment with G-CSF, azathioprine, cyclosporine, one course of horse ATG, one course of rabbit ATG and prednisolone.<sup>73</sup> She was then treated with Campath-1H 10 mg/d for 10 days. Two weeks later, the neutrophil count began to rise and normalized after 2.5 months, which was maintained eight months after treatment.

## 2.8 Use of Serial Immunosuppressive Therapies

Repeated courses of immunosuppression are commonly administered in human autoimmune diseases, including aplastic anemia and myelodysplastic syndromes. In patients who are refractory to initial

horse ATG/CsA, repeated courses of immunosuppression often are employed in order to improve marrow function.

## 2.9 CsA Therapy Following Alemtuzumab (Campath®)

Alemtuzumab (Campath®) is more immunosuppressive than ATG, but because of concern for potential relapse in the months after the effects of alemtuzumab wane, we will allow the institution of CsA in patients responding to alemtuzumab but relapsing following initial response at 3 months (see section 5.2.2). As the half-life of alemtuzumab is 12 days, the potential for increased toxicity should be minimal. The addition of CsA may rescue relapsed patients from worsening pancytopenia and all its complications. The rationale for using CsA in the relapse setting comes from the experience of improvement in the blood counts with addition of this agent in patients who relapse after h-ATG. <sup>74</sup>

## 2.10 Scientific and Clinical Justification of the Protocol

Selected MDS patients generally respond very well to h-ATG or CsA. However, there is substantial morbidity associated with h-ATG administration in this group of patients. In a previous trial using h-ATG/CsA to treat MDS, 7/38 patients died within a three month period of time following treatment; three deaths were directly attributable to h-ATG. In addition, 6/38 had a life threatening toxicity directly attributable to the h-ATG.

Our initial experience with alemtuzumab (Campath®) in severe aplastic anemia as salvage treatment for patients who have failed a first course of h-ATG have been encouraging. Infusion-related toxicities have been modest and immunosuppression-related toxicities, including infections, compare favorably to what is observed with h-ATG or r-ATG. In our preliminary experience of 12 patients, hematologic response rates have been as at least equivalent to r-ATG. Should alemtuzumab (Campath®) produce comparable responses to h-ATG but without the toxicity, alemtuzumab (Campath®) would be an attractive treatment, particularly in this group of patients who have substantial co-morbidities.

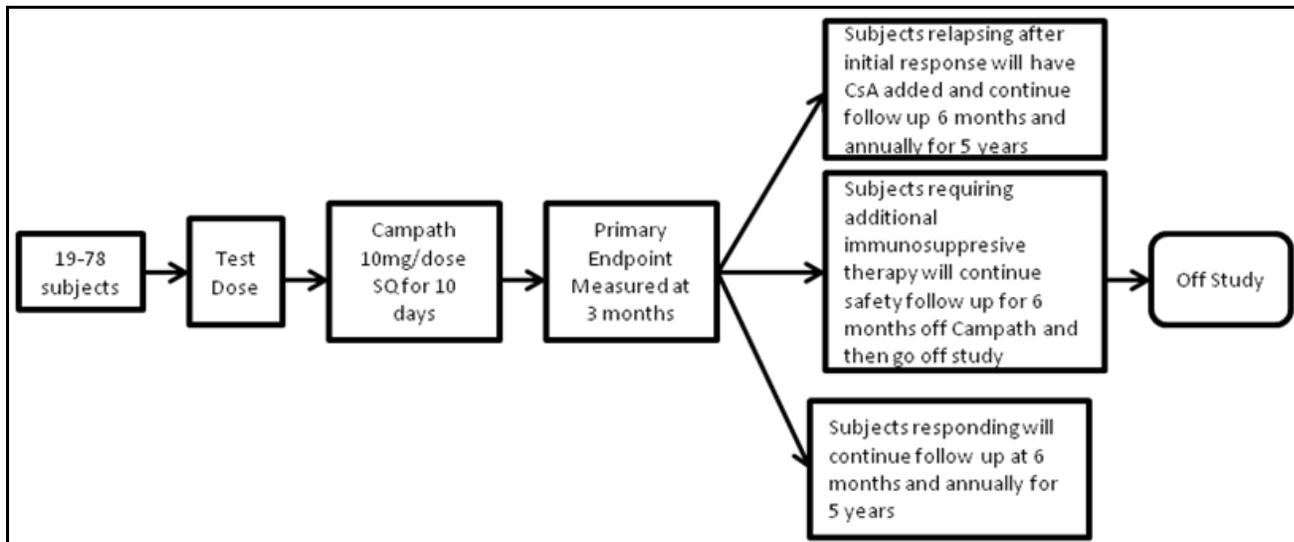
In our previous experience treating 133 patients with MDS with immunosuppression, we demonstrated the superiority of combination ATG and CsA over either drug alone ( $p=0.02$ ) (unpublished data). Furthermore, we noted that many patients relapsed following discontinuation of CsA, and required long-term continuation of the medication to maintain normal counts. Campath-1H is more immunosuppressive than ATG, but we are concerned about potential for relapse in the months after the effects of Campath-1H wane. Therefore we will allow institution of CsA in patients responding to Campath-1H but relapsing 3 months after initial response to Campath-1H measured at 3 months. As the half-life of Campath-1H is 12 days this would minimize the potential for increased toxicity. We will collect data on relapse rates and recovery of relapsing patients after institution of CsA.

We will treat patients who relapsed following Campath-1H with CsA regardless of prior response to CsA treatment. In patients who did not respond to CsA prior to Campath-1H, it is possible that CsA may have activity in the post Campath-1H setting due to a Campath-related alteration of the underlying disease.

We therefore propose to evaluate this new immunosuppressive therapy, alemtuzumab, to treat the cytopenias of MDS and to have CsA added to the treatment regimen in those subjects who relapse after initial response to Campath-1H.

### 3.0 STUDY DESIGN

We propose a non randomized, off label, Phase I/II study of Alemtuzumab (Campath®) in MDS subjects who are likely to respond to immunosuppression based in our previous model (see section 2.2 prognostic indicators)



### 4.0 ELIGIBILITY ASSESSMENT

All patients 18 years old or over with MDS who lack a suitable matched sibling marrow donor will be considered for enrollment. Patients who have a suitable matched sibling donor will be referred for consideration of allogeneic bone marrow transplantation. Patients not willing to undergo transplantation will be considered for protocol participation. Relimid treatment will be discussed particularly with patients diagnosed with 5q minus.

Patients who have responded to prior treatment with h-ATG or h-ATG/CsA will be eligible. Patients who have failed to respond to prior treatment with h-ATG or h-ATG/CsA will not be eligible for protocol participation.

#### 4.1 Inclusion Criteria

4.1.1 MDS with WHO classification of RA, RARS, RCMD-RS, and RCMD and RAEB-1

4.1.2 Anemia requiring transfusion support with at least one unit of packed red blood cells per month for greater than or equal to 2 months

OR

Anemia (hemoglobin <9 or a reticulocyte count <60,000)

OR

thrombocytopenia (platelet count less than 50000/ $\mu$ L)

OR

neutropenia (absolute neutrophil count less than 500/ $\mu$ L).

- 4.1.3 Off all other treatments for MDS (except filgrastim [G-CSF]), erythropoietin, and transfusion support and related medications) for at least four weeks. Filgrastim (G-CSF) can be used before, during and after the protocol treatment for patients with documented neutropenia (<500/Ul) as long as they meet the criteria for anemia and/or thrombocytopenia as stated above.
- 4.1.4 Ages 18-72 (inclusive)

## 4.2 Exclusion Criteria

- 4.2.1 Chronic myelomonocytic leukemia (CMML) , WHO RAEB-2 (see section 2.2)
- 4.2.2 Secondary MDS
- 4.2.3 Failure to respond to prior therapy with ATG or ATG/CsA
- 4.2.4 Prior therapy with combination chemotherapy
- 4.2.5 Transformation to acute leukemia (FAB sub-group RAEB-T, i.e., greater than 20% blasts in marrow aspirate)
- 4.2.6 Failure to discontinue the herbal supplements *Echinacea purpurea* or *Usnea barbata* (*Old Man's Beard*) within 2 weeks of enrollment
- 4.2.7 Active infection not adequately responding to appropriate therapy
- 4.2.8 HIV positive patients
- 4.2.9 Active malignant disease (excluding non-melanoma skin carcinoma)
- 4.2.10 Moribund status or concurrent hepatic, renal, cardiac, neurologic, pulmonary, infectious, or metabolic disease of such severity that it would preclude the patient's ability to tolerate protocol therapy or that death within 7-10 days is likely
- 4.2.11 Life expectancy less than 6 months
- 4.2.12 Low predicted probability of response (see section 2. 2)
- 4.2.13 Previous hypersensitivity to alemtuzumab (Campath®) or its components
- 4.2.14 Current pregnancy, or unwilling to take oral contraceptives or refrain from pregnancy if of childbearing potential
- 4.2.15 Not able to understand the investigational nature of the study or give informed consent

## 5.0 TREATMENT PLAN

## 5.1 Alemtuzumab (Campath®) Administration

All subjects will initially receive a test dose of 3 mg by subcutaneous injection the day before the first full dose.

If tolerated, alemtuzumab (Campath®) will be administered at 10 mg/dose for 10 days subcutaneously.

Subjects will be admitted to the Clinical Center hospital for study drug initiation only if necessary based on the adverse reactions they experience. If the following study drug injection is tolerated well (toxicity  $\leq$  grade 2) the subject may be discharged and receive the remainder of the treatment course as an outpatient.

Due to the subcutaneous administration of alemtuzumab subjects will be treated as outpatients without increasing the risk of side-effects<sup>85-88</sup>

## 5.2 Cyclosporine (CsA) Administration

### 5.2.1 Subjects who fail to respond to Campath-1H

Subjects who fail to respond to Campath-1H are not eligible to have CsA added to their treatment regimen, rather, will be taken off study per section 8.6.

### 5.2.2 Subjects who have responded to Campath-1H and then relapse

Subjects who relapse after initial response to Campath-1H measured at 3 months (see section 6.4) will have CsA added to the protocol therapy regimen unless otherwise contraindicated. CsA will be started at 10 mg/kg/d by mouth in divided doses q12 hr. Dosing will be based on ideal body weight and will be adjusted to maintain a target level of 200 – 400 ng/ml. If the blood counts improve, the CsA will be continued and tapered off gradually and the subject will continue to be followed on study. The CsA may be continued for 2-3 months or longer at the discretion of the PI to determine if immunosuppression with CsA will further improve the blood counts.

- If there is no improvement, the CsA will be discontinued and the subject will go off study.
- If further relapses occur while the subject is on therapeutic CsA (blood level of 200-400 ng.ml), then the subject will go off study.

## 5.3 Pre-medications and Management of -Adverse Reactions

Subjects will receive pre-medication with diphenhydramine 25 to 50 mg, orally or by IV, and acetaminophen 650 mg 30 minutes prior to the injection.

Adverse reactions will be treated symptomatically (e.g., anti-emetics, IV fluid hydration, acetaminophen, antihistamines, inhaled bronchodilators).

## 5.4 Supportive Treatment

- Transfusal supportive care (e.g., blood and platelets) as clinically indicated.
- Growth factors if deemed necessary by the investigator

## 5.5 Infection Prophylaxis and Monitoring

- **Pneumocystis prophylaxis:** Aerosolized pentamidine will be used as prophylaxis against *Pneumocystis carinii*, 300 mg every 4 weeks by inhalation beginning the first month of therapy and to continue for at least 6 months. If at 6 months CD4+ cells are  $< 200 /\mu\text{L}$ , *Pneumocystic carinii* prophylaxis will be continued until CD4+ cells  $> 200 /\mu\text{L}$ . Trimethoprim/ sulfamethoxazole, Dapsone or other prophylactic regimen against *Pneumocystic carinii* may be substituted at the discretion of the PI. This prophylaxis may be continued at the discretion of the PI.
- **Antiviral prophylaxis:** Valacyclovir 500 mg once daily will be given for at least 2 months to subjects regardless of HSV serology status. If at 2 months CD4+ cells are  $< 200 /\mu\text{L}$ , antiviral prophylaxis will be continued until CD4+ cells  $\geq 200 /\mu\text{L}$ . This drug may be continued at the discretion of the PI.
- **Antibacterial prophylaxis:** Ciprofloxacin 500 mg BID until ANC  $> 500/\mu\text{L}$ . This drug may be continued at the discretion of the PI. Potential drug interactions will be reviewed with the subject prior to initiating prophylactic therapy. In subjects who cannot tolerate ciprofloxacin, another drug may be substituted at the discretion of the PI.
- **EBV and CMV monitoring:** Subjects will be monitored for EBV and CMV PCR in the blood prior to treatment, then weekly for the first month, every other week in the second month, monthly for another 6 months and at 12 months. In case of a positive PCR for CMV, treatment will be instituted when clinical symptoms are attributed to CMV. EBV positivity by PCR will also be placed in context of clinical signs and symptoms.

## 5.6 Fever Management of Neutropenic Subjects (All subjects)

Subjects with a single temperature of  $38.5\text{ }^{\circ}\text{C}$  or two readings of  $38.0\text{ }^{\circ}\text{C}$  or greater will be evaluated for infection including cultures of blood and urine and any other suspicious sites prior to beginning empiric therapy. Antibiotics will be initiated following current infectious disease guidelines.

## 5.7 Instructions to Subjects

Regarding immunizations and Campath: Subjects who have recently received alemtuzumab (Campath®), should not be immunized with live viral vaccines due to the immunosuppression. In addition, other persons living in the household should not take oral polio vaccine since there is a chance they could pass the polio virus on to the subject. Subjects will be instructed to avoid persons who have taken oral polio vaccine within the last several months, not to get close to them, or stay in the same room with them for very long. If they cannot take these precautions, they will be instructed to consider wearing a protective face mask that covers the nose and mouth.

*Regarding subsequent monoclonal antibody therapy:* Subjects who develop hypersensitivity to alemtuzumab (Campath®) will be advised they may have an allergic or hypersensitivity reactions to other monoclonal antibodies.

*Regarding herbal sublement and Campath:* Subjects may not initiate or resume herbal supplements (*Echinacea purpurea* or *Usnea barbata* [*Old Man's Beard*]) while they are participating on this trial.

*Regarding concomitant medications and cyclosporine:* Certain other medications can change the level of cyclosporine in the blood. Some of these medications are erythromycin, ketoconazole, diltiazem, rifampin, phenytoin and phenobarbital. We will ask subjects to inform us of any medication taking concomitantly while on the study.

*Regarding prohibited food and cyclosporine:* Grapefruit and grapefruit juice may increase the effects of cyclosporine by increasing the amount of this medicine in the body. Subjects will be advised not to eat grapefruit or drink grapefruit juice while taking this medicine.

## 6.0 CLINICAL EVALUATION

Bone marrow aspirate will be read by a pathologist and used for diagnostic purposes. Samples will be ordered and tracked through the CRIS Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record.

### 6.1 **Pre-study Evaluation:** Subjects baseline status will be evaluated as follows:

- Medical history and physical examination
- MDS eligibility panel (done at screening or diagnostic workup, not repeated on study)
  - Folate level
  - B12 level
  - Coagulation screen (PT, PTT)
  - Iron panel (ferritin, transferrin, % saturation)
- Baseline laboratory studies
  - Complete blood count with differential
  - Reticulocyte count
  - DAT (direct antiglobulin test)
  - Type and Screen
  - Acute Care (Na, K, Cl, CO<sub>2</sub>, Creatinine, Glucose, and Urea Nitrogen), Mineral (Phosphorus, Magnesium, Albumin, and Calcium), Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin), and Other (Total Protein, CK, Uric Acid, and LD) panel.
  - Pregnancy test (urine HCG in women of child bearing potential)
- Thyroid function tests
- Viral serologies for hepatitis A, B, C, HIV, HSV, VZV
- EBV and CMV PCR in the blood prior to treatment
- Bone marrow aspirate and biopsy with cytogenetic analysis (morphology, cellularity, percentage of blast cells, and/or chromosomal analysis by PCR) as appropriate to stage and classify underlying disease
- Flow cytometry of the peripheral blood for GPI-cells
- HLA typing (if not already available)
- Chest X-ray
- Serum troponin-I before first dose of alemtuzumab (Campath®),
- ECHO, 24 hour Holter monitor before first dose of alemtuzumab (Campath®)
- EKG on the day of admission
- Lymphocyte phenotyping

## 6.2 On Study Drug Monitoring

On treatment monitoring will consist of:

- Physical examination
- CBC with differential (daily)
- Acute care, mineral, hepatic and other panel (every other day)
- Reticulocyte count (weekly +/- 3 days)
- Vital signs (daily)
- ECHO and 24 hour Holter monitor after last dose alemtuzumab (Campath®)
- Serum troponin-I at day 5 and after the last dose of alemtuzumab (Campath®)
- EBV and CMV PCR in the blood (weekly +/- 3 days)
- Chest x-ray, CT scans or appropriate imaging studies (only as medically indicated)

## 6.3 Post Treatment Monitoring Discharge Home Through 3 Months

After completing treatment, subjects will be followed by their home physician or at the Clinical Center and have blood work done as detailed below. Progress notes and laboratory results from home physicians will be faxed to the Research nurse, Barbara Weinstein, 301 402-3088. Subjects must be evaluated at the Clinical Center at the 1 month (+/- 7 days) and 3 month (+7 days) time points

- Physical examination
- Complete blood counts with differential (weekly +/- 3 days)
- DAT (direct antiglobulin test) (months 1 and 3 +/- 2 weeks)
- Type and screen (at 1 month and 3 months only +/- 2 weeks or if clinically indicated)
- Acute care, mineral, hepatic and other panel (Home MDs: electrolytes, transaminases, urea nitrogen (BUN), serum creatinine, total bilirubin) (weekly +/- 3 days)
- EBV and CMV PCR in the blood (weekly +/- 3 days) for the first month, then every other week +/- 3 days) in the second month, and then monthly (+/- 2 weeks) starting in month 3
- Reticulocyte count (at 1 and 3 months only, Home MDs only if available)
- ECHO (at 3 months only +/- 2 weeks)
- Serum troponin-I (at 3 months only +/- 2 weeks)
- Thyroid function tests (at 3 months only +/- 2 weeks)
- Flow cytometry of the peripheral blood for GPI-cells (at 3 months only +/- 2 weeks)

## 6.4 Post Treatment Monitoring 4 Months to 6 Months

After the 3 month visit, subjects will be followed by their home physician or at the Clinical Center and have blood work done as detailed below. Progress notes and laboratory results from home physicians will be faxed to the Research nurse, Barbara Weinstein, 301 402-3088. Subjects must be evaluated at the Clinical Center at the 6 month (+7 days) time point

- Physical examination
- Complete blood counts with differential (every other week +/- 3 days)
- DAT (direct antiglobulin test) (6 months +/- 1 month)
- Type and screen (at 6 months only +/- 2 weeks)
- Acute care, mineral, hepatic and other panel (every other week +/- 3 days) (Home MDs: electrolytes, transaminases, urea nitrogen (BUN), serum creatinine, total bilirubin)
- EBV and CMV PCR in the blood monthly (+/- 2 weeks) to 6 months.
- Reticulocyte count (at 6 months only, Home MDs only if available)
- Thyroid function tests (at 6 months only +/- 1 month)

- Bone marrow biopsy and aspiration with cytogenetics (6 months (+/- 1 month only)
- Flow cytometry of the peripheral blood for GPI-cells (6 months (+/- 1 month only)
- If Cyclosporine (CsA) is added: CsA levels will be monitored every week (+/- 3 days) for the first month and then every other week (+/- 3 days) for the remainder of the treatment period. Less frequent monitoring may occur if CsA drug level is therapeutic and steady. Dosing will be adjusted to maintain blood level of 200 – 400 ng/ml. More frequent drug serum levels may be obtained as needed to achieve constant therapeutic levels.

## 6.5 Long Term Follow Up After 6 Months

After the 6 month visit, subjects must be evaluated at the Clinical Center (12 months (+/- 2 month) and then yearly (+/- 2 month) thereafter for 5 years and have blood work done as detailed below. Subjects will be seen by their home physician as clinically indicated and the Clinical Center and home physician will continue to communicate.

- Physical examination
- Complete blood counts with differential (annually +/- 1 month)
- Acute care, mineral, hepatic and other panel(annually +/- 1 month)
- Reticulocyte counts (annually +/- 1 month)
- DAT (direct antibody titer) (annually +/- 1 month)
- Flow cytometry of the peripheral blood for GPI-cells (annually +/- 1 month )
- EBV and CMV PCR in the blood (at 12 months only +/- 1 month )
- Thyroid function tests (annually +/- 1 month)
- Bone marrow biopsy and aspiration with cytogenetics (at 12 months +/- 1 month and annually thereafter, as clinically indicated)
- Type and screen (annually +/- 1 month or if clinically indicated)
- Lymphocyte phenotyping (12 months (+/- 1 month) and as clinical indicated)
- If cyclosporine (CsA) is added: CsA levels will be monitored every week (+/- 3 days) for the first month and then every other week (+/- 3 days) for the remainder of the treatment period. Less frequent monitoring may occur if CsA drug level is therapeutic and steady. Dosing will be adjusted to maintain blood level of 200 – 400 ng/ml. More frequent drug serum levels may be obtained as needed to achieve constant therapeutic levels

## 7.0 LABORATORY RESEARCH STUDIES

**Intended use:** During the course of participating on this study, an additional 105 cc of blood (at baseline) , 5 cc (Day 5), 10 cc (Day 10), or 50 cc of blood (at 1 month post treatment), 45 cc of blood (3months, 6 months and 12 months post treatment), 20 cc of blood (annually thereafter) and 5 cc of bone marrow aspirate (baseline, 6 months, 12 months, and annually thereafter as clinically indicated) may be collected for exploratory ancillary laboratory research studies which have been approved by the IRB and are listed in Appendix A. These studies will not be used to assess the primary endpoint, may or may not be done, and if done, may be correlated with the presence or absence of response. These specimens will not be read by a pathologist or used for diagnostic purposes. Campath research studies may include:

- Anti-alemtuzumab (Campath®) antibody may be measured.
- Drug sensitivity testing assessment may be collected. In order to assess the mechanisms of infusion related toxicities (fever, hypotension, wheezing, rash, etc.) following alemtuzumab (Campath®). Blood sample will be collected at baseline and 2-4 hours after alemtuzumab (Campath®) infusion and correlated with clinical adverse events:

- Tryptase (2.5 ml blood)
- Inflammatory cytokines (e.g., TNF-a, IL-1ra, IL-6) and IL-10

**Storage:** Research samples will be stored with identifiers (subject initials, data time point, and date of sample collection) in the secure laboratory of the principal investigator.

**Tracking:** Samples will be ordered and tracked through the CRIS Research Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Specimens will be entered in the NHLBI Biospecimen Inventory System (BSI). Samples will not be sent outside NIH without IRB notification and an executed MTA.

**End of study procedures:** Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

**Loss or destruction of samples:** Should we become aware that a major breach in our plan for tracking and storage of samples has occurred, the IRB will be notified.

## 8.0 BIOSTATISTICAL CONSIDERATIONS

### 8.1 Primary Endpoint

The primary endpoint is hematologic response measured at 3 months after first dose alemtuzumab (Campath®) sustained on at least two serial measurements at least one week apart at landmark time points (3, 6 and 12 months) will be defined using standard hematologic and cytogenetic response criteria for MDS (See Appendix B for detailed description)<sup>77</sup>.

**Complete response:** Normalization of all affected cell lines (Hemoglobin, Reticulocyte count, Platelets, ANC)

**Partial response:** At least one minor response

#### 1. Hemoglobin Response

**Major response:** For subjects with pretreatment hemoglobin less than 11 g/dL, greater than 2 g/dL increase in hemoglobin; for RBC transfusion-dependent subjects, transfusion independence or an absolute reticulocyte count to  $\geq 60,000/\mu\text{L}$  of blood in transfusion-dependent patients.

**Minor response:** For subjects with pretreatment hemoglobin less than 11 g/dL, 1 to 2 g/dL increase in hemoglobin; for RBC transfusion-dependent patients, 50% decrease in transfusion requirements.

#### 2. Platelet response

**Major response:** For subjects with a pretreatment platelet count less than  $100,000/\text{mm}^3$ , an absolute increase of  $30,000/\text{mm}^3$  or more; for platelet transfusion-dependent subjects, stabilization of platelet counts and platelet transfusion independence.

Minor response: For subjects with a pretreatment platelet count less than 100,000/mm<sup>3</sup>, a 50% or more increase in platelet count with a net increase greater than 10,000/mm<sup>3</sup> but less than 30,000/mm<sup>3</sup>.

### 3. ANC response

Major response: For absolute neutrophil count (ANC) less than 1500/mm<sup>3</sup> before therapy, at least a 100% increase, or an absolute increase of more than 500/mm<sup>3</sup> whichever is greater.

Minor response: For ANC less than 1500/mm<sup>3</sup> before therapy, ANC increase of at least 100%, but absolute increase less than 500/mm<sup>3</sup>.

## 8.2 Secondary Endpoints

**8.2.1 Transfusion-independence** for red blood cells and/or platelets: ability to maintain a hemoglobin >8gm/dl and a platelet count > 20 000/ $\mu$ L without the need for transfusions for a period of 6 weeks regardless of subsequent events.

**8.2.2 Overall survival** which is defined as the time elapsed between time of treatment and death and will be compared between responders and non-responders. Any such comparison may be biased and needs to be interpreted cautiously.

**8.2.3 Life threatening toxicity.** Subjects who do not complete the treatment course because of toxicity or voluntary withdrawal will be included in analyses.

**8.2.4 Overall survival and transformation-free survival**, which is defined as the time elapsed (reported at 3 month intervals for the first four years, then yearly thereafter) between time of treatment, and bone marrow morphologic evidence for progression (as determined by FAB criteria), development of acute leukemia, or death will be compared between responders and non-responders. Any such comparison may be biased and needs to be interpreted cautiously.

**8.2.5 Response duration** will be determined as the interval between time of onset of response as outlined above, and the time of relapse or loss of response characteristic (reported at 3 month intervals for the first four years, then yearly thereafter).

**8.2.6 Affects of the addition of cyclosporine.** Subjects who relapse after responding to Campath will be given CsA to determine if CsA will be beneficial under these circumstances. For this protocol, CsA is investigational, subjects will be followed, data will be collected and analyzed as secondary endpoints. The number of subjects who will receive CsA cannot be predicted since it will depend on the initial response to Campath which is unknown.

Response proportion, response duration, overall survival, and transformation free survival will be determined and compared within the FAB sub-groups and International Prognostic Scoring System categories of MDS and with the results of previous studies at the NIH using h-ATG alone or CsA alone to treat the cytopenia of MDS.

## 8.3 Sample Size

Because the efficacy of this treatment is almost completely unknown, we would like to reject the treatment as quickly as possible with a small number of subjects if the treatment is not effective.

A response probability of 30% or less would warrant terminating the treatment on this subject population, and we hypothesize that the actual response probability using this treatment would reach 50% or more.

Let  $p$  be the probability of complete or partial response at 3 months. Our sample size is determined by testing the null hypothesis  $H_0: p \leq 30\%$  versus the alternative  $H_1: p \geq 50\%$  at 0.05 significance level and 0.80 of the power. We determine the sample size using the Two-Stage Minimax Design outlined in Table 1 of Simon (1989)<sup>75</sup> This design is selected because it requires a smaller total number of subjects ( $n=39$ ) than the Simon's Two-Stage Optimal Design ( $n=46$ ). At the first stage, 19 subjects will be accrued and the null hypothesis will be accepted (i.e., the treatment will be terminated) if 6 or less subjects respond to the treatment at 3 months. If 7 or more subjects respond to the treatment at 3 months in the first stage, then an additional 20 subjects will be accrued, bringing the total number of subjects to  $n=39$ . The null hypothesis of  $p \leq 30\%$  will be accepted if the total number of complete and partial responders at 3 months is 16 or less.

An additional 39 subjects will be enrolled to allow for statistically valid subgroup comparisons of secondary endpoints such as response to cyclosporine in subjects who relapse within six months of treatment.

#### **8.4 Statistical Methods**

The planned analyses will include descriptive statistics on the proportions of responses (i.e. % subjects with partial or complete response) and the time to response. The response probabilities will be estimated using the sample proportions and their inferences including confidence intervals and hypotheses testing will be evaluated using Binomial distributions. The time to responses will be analyzed using appropriate tools in survival analysis such as Kaplan-Meier estimates and Cox regression taking consideration of random censoring. In addition methods based on survival analysis, cumulative incidence rates for response and other competing risk models will be used to evaluate the treatment effects. Graphical tools will be used to display the appropriate estimates (i.e. estimated proportions and Kaplan-Meier curves) and their corresponding 95% confidence intervals. Methods based on multiple regression, analysis of variance and logistic regression will also be employed if deemed appropriate.

#### **8.5 Stopping Rules**

The following two types of treatment related severe adverse events will be monitored and considered for early stopping for all or part of the study:

- Death considered to be definitely related to alemtuzumab (Campath-1H®)
- Any grade IV toxicity considered to be definitely related to alemtuzumab (Campath-1H®), i.e. opportunistic infection such as tissue-invasive CMV or *Pneumocystis carinii*, with exception of temporary cytopenias.

We will monitor the numbers of subjects who have developed one or more of the above treatment related severe adverse events (TRSAE). The study will be seriously considered for early stopping if the corresponding number of subjects who have developed one or more of the above TRSAE is over a pre-specified threshold value.

From our experience using this agent in other clinical settings, we anticipate the rate of developing at least one of the above TRSAE to be 10% or less. Following Geller et al. (2003, "Design of Early

Trials in Stem Cell Transplantation: A Hybrid Frequentist-Bayesian Approach”), our stopping rule is determined by a Bayesian approach.<sup>76</sup> The stopping boundary is reached if the Bayesian posterior probability that the true probability of developing one or more of the above TRSAE exceeds this benchmark rate of 10% is at least 90%. We take our prior distribution to be a beta distribution with the sum of the two beta parameters to be 6, i.e. the parameters of the beta prior distribution are 1.0 and 5.0. Since we have seen in the past that the first few subjects to be accrued are possibly sicker than the rest of the subjects in the sample, we will start safety monitoring when 3 or more subjects have developed TRSAE. The following table summarizes the threshold numbers for stopping an experiment:

Number of Subjects in the experiment	Stop if the number of subjects who have developed any of the TRSAE reaches:
≤ 9	3
≤ 16	4
≤ 23	5
≤ 31	6
≤ 38	7
≤ 46	8
≤ 54	9
≤ 62	10
≤ 70	11
≤ 78	12

We investigated the performance of the above stopping rule by a simulation study. In each simulation run, we generated a study with 78 independent Bernoulli trials, each had a probability  $p$  for having the above TRSAE and  $q=1-p$  for not having such TRSAE and compared the TRSAE outcomes with the above stopping boundary to determine whether the study was stopped. We repeated the simulation 100,000 times and computed the proportion of stopped studies (i.e. “number of stopped studies”/100,000) which were stopped using the above stopping rule. The following table summarizes the proportions of stopped studies under a number of scenarios for  $p$ :

Prob of TRSAE = $p$	2%	5%	10%	15%	20%	25%
Proportion of Stopped Studies	0.7%	1.7%	21.8%	65.4%	92.8%	99.6%
Average Number of Subjects	77.95	76.93	67.32	46.97	28.46	18.17
Average Numbers of Subjects Suffering TRSAE	1.55	3.86	6.73	7.03	5.70	4.55

These results suggest that our stopping rule has a low probability of stopping a study when the proportion of the above TRSAE is below the benchmark value of 10%, and the probability of stopping a study is high when the true proportion of the above TRSAE exceeds this benchmark value. Based on these results, we believe that our Bayesian stopping rule has satisfactory statistical properties.

## 8.6 Off Study Criteria

- *Per Subject choice:* Subjects may be removed from study at their request. The risks of

withdrawing will be discussed, as will alternative treatment options. Those subjects who choose to withdraw while taking alemtuzumab (Campath®) will continue to have labs monitored through the 6 month off drug time point.

- *Per principal investigator decision:* Alemtuzumab (Campath®) administration will be discontinued, the subject will be followed until the event has resolved and labs have been monitored through the 6 month off drug time point if the subject develops:
  - Intolerance of alemtuzumab (Campath®) infusion as manifested by hypotension, fever, chills and/or rash that is refractory to pre-medication with anti-histamines and acetaminophen.
  - Life threatening acute hypersensitivity reactions
  - Lymphoproliferative disease or malignancy.
  - Worsening of MDS with increased numbers of blasts fulfilling a diagnosis of RAEBt or leukemia
  - Pregnancy or unwillingness to refrain from pregnancy
  - Initiation of additional immunosuppressive therapy (except steroids or CsA)
  - Initiation of the herbal supplement *Echinacea purpurea* or *Usnea barbata* (*Old Man's Beard*)
  - Failure to respond to Campath-1H therapy
  - Failure to improve with CsA after relapse.

Once off study, subjects will be referred back to their referring physician or consented to the Hematology Branch evaluation and treatment protocol (94-H-0010) for consideration for standard therapy or evaluation for eligibility for another branch protocol, depending on what is considered to be in the best interest of the subject.

## 8.7 Data Management

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system 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 human subjects personally identifiable information (PII) as defined in accordance to the Health Insurance Portability and Accountability Act, eligibility and consent verification will be recorded in DIR's Clinical Data System (CDS) or the Laboratory of Cardiac Energetics (LCE) database. Primary 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, e.g., study-specific identifying number (SSPIN) generated by CDS or other unique code or minimum PII required for subject identification.

**End of study procedures:** Data will be stored in locked cabinets and in a password protected database until it is no longer of scientific value.

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

No data will be sent outside NIH without prior IRB approval and an executed MTA or CTA.

**Publication Policy:** Given the research mandate of the NIH, patient data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate human subject protection

institutional approval such as prospective NIH IRB review and approval or an exemption from the NIH Office of Human Subjects Research (OHSR).

## **9.0 DATA AND SAFETY MONITORING**

### **9.1 Safety Monitoring**

*Principal investigator:* Accrual, efficacy and safety data will be monitored by the PI.

*NHLBI IRB:* Accrual and safety data will be reviewed annually by the Institutional Review Board (IRB). Prior to implementation of this study, the protocol and the proposed patient consent and assent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to the Title 45 CFR 46. This committee must approve all amendments to the protocol or informed consent, review all SAEs, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects.

*NHLBI DSMB:* The NHLBI Data safety and Monitoring Board will review the protocol at 6 to 12 month intervals. A progress report will be forwarded to the DSMB at these times and their recommendations will be expeditiously implemented. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

### **9.2 Event Characterization and Reporting**

Events include adverse events (AE), serious adverse events (SAE), protocol deviations (PD), unanticipated problems (UP), and non-compliance.

The principal investigator will review all events (AEs, protocol deviations, UPs, SAEs) to determine the seriousness, expectedness, and reportability of the event. As required and/or needed, the principal investigator will review the events with the Sponsor to make the final determination of seriousness and reportability.

#### **9.2.1 Definitions**

*Adverse Event (AE):* Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

*Serious Adverse Event (SAE):* A serious adverse event that:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in in-patient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant incapacity;
- results in a congenital anomaly/birth defect; or
- 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.

*Suspected adverse reaction:* Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

*Serious event:* An event is serious if it meets the definition of a serious adverse event (above) or if it requires immediate corrective action by a PI and/or IRB to protect the safety, welfare or rights of subjects.

*Unexpected adverse reaction:* An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. “Unexpected”, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

*Unanticipated Problem (UP):* Any incident, experience, or outcome that meets all of the following criteria:

1. 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
2. related or possibly related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

*Unanticipated Problem that is not an Adverse Event:* An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involves risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

*Protocol Deviation (PD):* Any change, divergence, or departure from the IRB approved research protocol.

*Non-compliance:* The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human research. Noncompliance may be further characterized as:

1. **Serious non-compliance:** Non-compliance that:

- a. Increases risks, or causes harm, to participants.
- b. Decreases potential benefits to participants.
- c. Compromises the integrity of the NIH HRPP.
- d. Invalidates the study data.

2. **Continuing non-compliance:** Non-compliance that is recurring. An example may be a pattern of non-compliance that suggests a likelihood that, absent an intervention, non-compliance will continue. Continuing noncompliance could also include a failure to respond to IRB requests to resolve previous allegations of non-compliance.

3. **Minor (non-serious) non-compliance:** Non-compliance that, is neither serious nor continuing.

### 9.2.2 Adverse Events Management:

. All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded. The AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease and graded by severity utilizing CTC version 2.0. A copy of the criteria can be down-loaded from the CTEP home page at <http://ctep.cancer.gov/reporting/ctc.html>.

**Abnormal laboratory findings** used to evaluate the safety of this protocol regimen will be collected to include any change from laboratory assessments done prior to first dose of study medication that results in a progression to a grade 3 or 4 laboratory toxicity and/or are 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.

The laboratory toxicities will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease and graded by severity utilizing CTC version 2.0.

**Length of Adverse Event reporting:** Adverse event collection will start at the baseline and continue for 6 months after the last dose of Campath and when applicable cyclosporine, after which the events will be recorded in the medical record but not abstracted to toxicity tables.

### 9.2.3 Serious Adverse Events Management

Serious adverse events will be attributed as definitely (clearly related to the research), probably (likely related to the research), possibly (may be related to the research), unlikely (doubtfully related to the research) and unrelated (clear not related to the research).

**Treatment related SAEs (TRSAEs)** are those attributed as definitely, probably, or possibly. As detailed in section 8.5 stopping rules, Death and any grade IV toxicity considered to be definitely related to alemtuzumab (Campath-1H®) will be monitored and considered for early stopping the study according to statistically determined criteria. John Tisdale, MD, NHLBI will serve as the independent monitor who reviews the attribution of TRSAEs

Hospitalization (overnight admission) for routine supportive care (platelet or RBC transfusions) or admission from the NIH inpatient unit to the NIH ICU for routine monitoring will not be reported as a serious adverse event.

**Duration of Serious Adverse Event collecting and reporting:** The collection of SAEs will begin on the first day of initiation of the study drug and will continue along as the subject is on study. SAE reporting will continue as long as the subject remains on study.

### 9.2.4 Reporting of Events

*PI:* All events will be reported to the Principal Investigator of this study

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## **Reporting Timeframes to IRB Chair, Clinical Director, and/or NHLBI IRB**

### **Serious Events**

*Reports to the IRB and CD:* The PI must report Serious UPs, and Serious PDs to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event via PTMS using the NIH Problem Report Form.

*Reports to the IRB Chair and CD:* The PI must report all SAEs that do not meet the definition of UP to the IRB chair and CD not more than 14 days after the PI first learns of the event via PTMS, using the SAE submission form.

### **Non-serious Events**

*Reports to the IRB and CD:* The PI must report all UPs that are not Serious to the IRB and CD, and PDs that are not Serious to the IRB, not more than 14 days after the PI first learns of the event via PTMS using the NIH Problem Report Form.

### **Deaths**

The PI must report all deaths (that are not UPs) to the CD as soon as possible, but not more than 7 days after the PI first learns of the event.

### **At continuing review, the PI will provide to the IRB a summary of:**

- All UPs
- All PDs
- All AEs (except for those granted a waiver of reporting)
- If, while preparing the continuing review, the PI identifies a greater frequency or level of severity of expected adverse events than was previously identified in the protocol or investigational brochure (IB), these should be reported separately as a UP. If such an observation occurs before the time of continuing IRB review, it should be reported to the IRB and CD as a UP in the time frames noted above, and summarized at the time of continuing review.

### **Exclusions to data reporting:**

The following Adverse Events will be captured only in the source documents and will not be reported to the IRB at the time of continuing review.

- Laboratory values that do not meet the definition of a laboratory AE listed in Section 9.2.2.
- All grade 1 events listed as expected in the investigator's brochure, package insert, and/or anticipated events.

### ***NHLBI DSMB Reporting:***

Reports of serious adverse events that are unexpected and thought to be related to the experimental drug will also be forwarded no later than seven (7) days in the case of death or life-threatening

serious adverse events or within fifteen (15) days after the occurrence of all other forms of serious adverse events to the Data and Safety Monitoring Board (DSMB). A summary of events will be included in DSMB reports for review by the DSMB.

If the serious adverse event is thought to be due to the experimental component of the protocol, accession to the protocol will be stopped until a full discussion with the IRB has been held.

## **10.0 HUMAN SUBJECTS PROTECTION**

### **10.1 Rationale for Subject Selection**

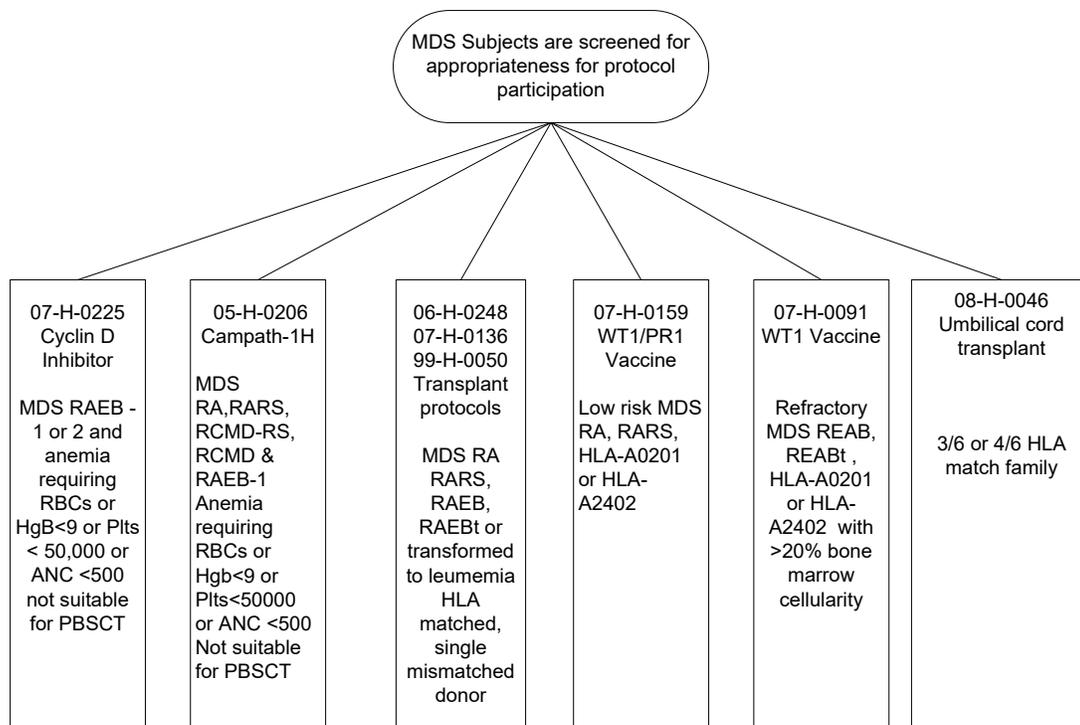
MDS is not known to show a racial or gender preference in incidence, as such, there will be no restrictions based on ethnicity or gender in patient selection. One of the difficulties clinicians face is distinguishing between hypoplastic MDS and aplastic anemia when faced with a hypoplastic marrow. For the purposes of this protocol, patients with hypocellular marrows (<30%) will be classified as MDS. A cytogenetic abnormality in the setting of a hypoplastic marrow makes a diagnosis of MDS. When there are no cytogenetic abnormalities the distinction between MDS and aplastic anemia needs to be made on morphologic grounds. However, dyserythropoiesis, which is a feature of both AA and MDS will not be used as a sole diagnostic criterion.

The Hematology Branch has a robust source of patients with bone marrow failure, and we do not anticipate recruitment to be problematic. We have screened over 950 patients with MDS since 1997. Because the inclusion/exclusion reflects a prognostic index that incorporates age, we anticipate the study population will be slightly younger.

**Reimbursement:** Reimbursement for protocol participation, travel, food, and lodging will be consistent with NIH guidelines. In determining reimbursement, the following factors are considered applicable to this protocol: the patients are diagnosed with a rare disease; the patient population is sick; the protocol offers the potential for direct benefit; the protocol regimen is demanding; and in order to complete accrual in a reasonable timeframe a geographically dispersed participant population is required.

**Recruitment efforts:** The study will be listed on [clinicaltrials.gov](http://clinicaltrials.gov), Clinical Center research studies, the NHLBI patient recruitment website, and the Aplastic Anemia and MDS Foundation websites. If recruitment goals are not met, a recruitment plan will be developed by the Office of Patient Recruitment.

**Competition among other Branch protocols:**



## 10.2 Participation of Children.

Pediatric MDS patients less than 18 yrs old are excluded from protocol participation. Less than 3% of patients with the diagnosis of MDS are under the age of 30. MDS may also be biologically a different disease in children. The treatment of choice in an MDS patient under age twenty with MDS is a bone marrow transplant, even from a matched unrelated donor. Furthermore, at this time, we do not believe treatment with *Campath*® will be curative which should be the goal of therapy in a young patient.

## 10.3 Risks and Discomforts

### 10.3.1 Related to alemtuzumab (*Campath*®)

#### WARNING: CYTOPENIAS, INFUSION REACTIONS, and INFECTIONS

Cytopenias: Serious, including fatal, pancytopenia/marrow hypoplasia, autoimmune idiopathic thrombocytopenia, and autoimmune hemolytic anemia can occur in patients receiving Campath. Single doses of Campath greater than 30 mg or cumulative doses greater than 90 mg per week increase the incidence of pancytopenia.

Infusion Reactions: Campath administration can result in serious, including fatal, infusion reactions. Carefully monitor patients during infusions and withhold Campath for Grade 3 or 4 infusion reactions. Gradually escalate Campath to the recommended dose at the initiation of therapy and after interruption of therapy for 7 or more days.

Infections: Serious, including fatal, bacterial, viral, fungal, and protozoan infections can occur in patients receiving Campath.

Administer prophylaxis against *Pneumocystis jiroveci* pneumonia (PCP) and herpes virus infections.

**The most commonly reported infusion related adverse events were** rigors (86%), drug-related fever (85%), nausea (54%), vomiting (41%), and hypotension (32%). Hematologic toxicities included pancytopenia/marrow hypoplasia (6%), anemia (80%), thrombocytopenia (72%), neutropenia (85%), and profound lymphopenia, and should be monitored. Infections reported included sepsis (15%), pneumonia (16%), and opportunistic infections such as CMV (8% - Study 1), Candidiasis (5% - Study 1), Aspergillosis (2% -Study 1), and Mucormycosis (2% - Study 1).

**Infusion-related symptoms and signs:** Alemtuzumab (Campath®) can result in serious infusion reactions which include nausea, vomiting, fever, chills, rigors, hypotension, rash, fatigue, headache, diarrhea, pruritus, urticaria, bronchospasm and dyspnea. An attempt to prevent these reactions is made by using premedication. Patients will be carefully monitored during infusions. In case of mild reaction, the patient will be treated according to symptoms (antiemetics, IV fluid hydration, acetaminophen, antihistamines, inhaled bronchodilators). In case of moderate or severe reaction, the infusion will be discontinued and restarted at a lower rate once symptoms have subsided. When alemtuzumab is administered subcutaneously, rare and usually mild or moderate injection site reactions can result in pain, erythema, itching, or edema at the injection site. These reactions are usually Grade 1 or 2 and can be minimized by applying ice before and after the injection and with medications like Tylenol and Benadryl.

**Infections:** Serious, sometimes fatal bacterial, viral, fungal and protozoan infections have been reported in patients receiving alemtuzumab (Campath®). Patients will be at risk for opportunistic infections. Therefore patients will receive prophylaxis for Herpes virus and *Pneumocystis carinii*.

**Cardiac toxicities:** The following were reported in at least one patient treated on studies where alemtuzumab (Campath®) was used as a single agent: cardiac failure, cyanosis, atrial fibrillation, cardiac arrest, ventricular arrhythmia, ventricular tachycardia, angina pectoris, coronary artery disorder, myocardial infarction, pericarditis. Some of these cardiac abnormalities may be irreversible. For this reason, we will monitor subjects with an echocardiogram and a 24 hour holter monitor before treatment begins and after the last dose of alemtuzumab (Campath®). The ECHO will also be repeated at the 3 month follow up visit. We will closely monitor subjects for cardiac symptomology and ask them to immediately report any cardiac symptoms (palpitations, irregular pulse, difficulty in breathing, dizziness, swelling in the ankles, chest discomfort or pain).

**Hematologic toxicities:** Myelosuppression involves all 3 cell lineages. Serious and in rare instances fatal pancytopenia/marrow hypoplasia, autoimmune idiopathic thrombocytopenia, and autoimmune hemolytic anemia have occurred in patients receiving alemtuzumab (Campath®).

**Other side effects** (< 5% in CLL studies) include headache, anorexia, asthenia, skeletal pain, myalgias, peripheral edema, dyesthesias, dizziness, tremor, stomatitis, mucositis, dyspnea, cough, pneumonitis, rhinitis, abdominal pain, back pain, dyspepsia, constipation, insomnia, depression, somnolence, diarrhea, and EBV related lymphoproliferative disease.

**Associated with pregnancy and breast feeding:** Animal reproductive studies have not been conducted. It is not known whether alemtuzumab (Campath®) can affect reproductive capacity or cause fetal harm. Human IgG is known to pass the placental barrier and thus may potentially cause fetal B-cell depletion; therefore alemtuzumab (Campath®) should only be given to pregnant women only if clearly needed. It is not know whether alemtuzumab (Campath®) is excreted in human milk. Because human IgG is excreted in human milk and the potential for absorption and immunosuppression in the infant is unknown, women should be advised to discontinue nursing for at least 3 months following last dose of alemtuzumab (Campath®).

### 10.3.2 Related to Cyclosporine:

#### Potential serious side effects include

*Infection related:* Because of low white blood cell counts, patients with MDS are susceptible to infections. By further blocking the immune system, CsA further increases this risk.

*Cancer related:* When used at high doses in transplant patients, CsA may be associated with an increased risk of cancer, especially lymphoma (4 of every 10,000 patients who receive the medication). Transplant patients receive higher doses than you will be given and are treated for longer periods than the duration of this study. However, because of the way that CsA acts on the body, there is a chance that it may cause effects that may not occur until years after the medicine is used.

*Blindness:* In very rare instances (less than .01%), CsA has been reported to cause blindness.

#### Potential side effects:

Although it is metabolized primarily in the liver, CsA major toxicity is renal. CsA causes a decrease in creatinine clearance, which almost always returns to normal range on cessation of the drug or lowering of the dose. Rare development of a hemolytic-uremic syndrome has been reported in patients with CsA after allogeneic bone marrow transplant. In our patients with SAA, frequent creatinine measurements have allowed prompt adjustment of dose and serious renal complications are infrequent.

Evidence of hepatotoxicity is common, usually as transient increases in bilirubin and transaminases. These levels often normalize with continued administration of the drug; reduction of the dose is uniformly associated with a return to normal levels.

Additional complications include hypertrichosis, gingival hypertrophy (possibly related to pre-existing poor dental hygiene), hyperesthesia, hirsutism, tremors, headaches, nausea and nonspecific gastrointestinal complaints. Hypertension may occur, and be high enough to require treatment.

Neurologic complications include insomnia, dizziness, anxiety, confusion, and vertigo. We have observed seizures in patients receiving CsA, when drug levels were within the therapeutic range. Posterior Reversible Encephalopathy Syndrome (PRES) is an increasingly recognized neurologic disorder seen in 1% of patients on cyclosporine following solid organ transplantation which manifest with acute to subacute hypertension and/or seizures.<sup>84</sup> In the event of hypertension, subjects will be prescribed 1 or more medications to control blood pressure in an effort to decrease the risk of this complication.

Hypomagnesemia and hyperkalemia may occur but are asymptomatic. Increases in uric acid may occur and attacks of gout have been rarely reported. Cyclosporine therapy may be associated with a modest increase of serum triglycerides or cholesterol.

#### Less frequent adverse events include:

*Autonomic Nervous System:* dry mouth, increased sweating

*Systemic:* allergy, asthenia, hot flushes, malaise, weight decrease, weight increase  
*Cardiovascular:* abnormal heart sounds, cardiac failure, myocardial infarction, peripheral ischemia  
*Central and Peripheral Nervous System:* hypoesthesia, neuropathy, vertigo  
*Endocrine:* goiter  
*Gastrointestinal:* constipation, dysphagia, enanthema, eructation, esophagitis, gastric ulcer, gastritis, gastroenteritis, gingival bleeding, glossitis, peptic ulcer, salivary gland enlargement, tongue disorder, tooth disorder  
*Infection:* abscess, bacterial infection, cellulitis, folliculitis, fungal infection, herpes simplex, herpes zoster, renal abscess, moniliasis, tonsillitis, viral infection  
*Hematologic:* anemia, epistaxis, leukopenia, lymphadenopathy  
*Liver and Biliary System:* bilirubinemia  
*Metabolic and Nutritional:* diabetes mellitus, hyperkalemia, hyperuricemia, hypoglycemia  
*Musculoskeletal System:* arthralgia, bone fracture, bursitis, joint dislocation, myalgia, stiffness, synovial cyst, tendon disorder  
*Neoplasms:* breast fibroadenosis, carcinoma  
*Psychiatric:* anxiety, confusion, decreased libido, emotional lability, impaired concentration, increased libido, nervousness, paroniria, somnolence  
*Reproductive (Female):* breast pain, uterine hemorrhage  
*Respiratory System:* bronchospasm  
*Skin and Appendages:* abnormal pigmentation, angioedema, dermatitis, dry skin, eczema, nail disorder, pruritus, skin disorder, urticaria  
*Special Senses:* abnormal vision, cataract, conjunctivitis, deafness, eye pain, taste perversion, tinnitus, vestibular disorder, blindness  
*Urinary System:* abnormal urine, hematuria, increased BUN, micturition urgency, nocturia, polyuria, pyelonephritis, urinary incontinence

### ***10.3.3 Related to bone marrow aspirate and biopsy***

No major risks are involved with bone marrow aspirate and biopsy. However, a small risk of infections, pain, bleeding, and hematoma formation at the site of the aspiration exists with the procedure.

***10.3.4 Related to blood draws:*** No major risks are involved with blood draws. Minor complications including bleeding, pain, and hematoma formation at the site of blood draws or infections may rarely occur.

***10.3.5 Related to central line placement:*** A catheter may be placed in a large vein of the neck, chest, or arm using local anesthetic. Patients will sign a separate consent for the placement procedure. Intravenous line placement carries a small risk of bleeding, bruising or pain and a very low risk of accidental injury to the adjacent artery and nerve. Some patients may experience a vasovagal reaction (lightheadedness, or, rarely, fainting due to temporary lowering of blood pressure). Only trained experienced staff will place the line in order to minimize these procedure related risks.

### ***10.3.6 Related to cardiac monitoring***

- **EKG:** An electrocardiogram (EKG) is a test that measures the electrical activity of the heartbeat. With each beat, an electrical impulse (or “wave”) travels through the heart. This wave causes the muscle to squeeze and pump blood from the heart. A technician will put

patches (electrodes) on the chest, arms and legs. The electrodes are soft and don't cause any discomfort when they're put on or taken off by the technician. The machine only records the EKG. It doesn't send electricity into the body. There's no pain or risk associated with having an electrocardiogram.

- **ECHO:** The ECHO uses sound waves to visualize and evaluate the function of the heart. There are no associated risks.
- **Holter monitor:** The Holter involves wearing a monitor for 24 hours during which time the electrical activity of the heart is recorded. There are no associated risks other than the inconvenience of wearing the apparatus.

### ***10.3.7 Related to infection prophylaxis medications***

- ***Cirpofloxacin*** (Cipro, Cipro XR, Proquin XR) The most frequent side effects include nausea, vomiting, diarrhea, abdominal pain, rash, headache, and restlessness. Rare allergic reactions have been described, such as hives and anaphylaxis (shock). Rare instances of seizures, other neurologic sequella and tendon rupture have been reported. Drug interactions with other medications have been reported and these will be reviewed before antibiotic start.

Fluoroquinolones, including CIPRO®, are associated with an increased risk of tendinitis and tendon rupture in all ages. This risk is further increased in older patients usually over 60 years of age, in patients taking corticosteroid drugs, and in patients with kidney, heart or lung transplants. Fluoroquinolones, including CIPRO, may exacerbate muscle weakness in persons with myasthenia gravis. Avoid CIPRO in patients with known history of myasthenia gravis."

- ***Pentamidine:*** cough (31-47%), bronchospasm (10-23%), decreased appetite (53-72%), fatigue, metallic taste, shortness of breath, decreased appetite, dizziness, rash, nausea, pharyngitis, chest pain/congestion, night sweats, chills, vomiting.
- ***Valacyclovir:*** Nausea and/or vomiting, headache, dizziness, abdominal pain, dysmenorrhea, arthralgia, acute hypersensitivity reactions, elevations in liver enzyme laboratory values (e.g. AST). Renal failure and CNS symptoms have been reported in patients with renal impairment who received valacyclovir at greater than the recommended dose.

## **10.4 Risks in Relation to Benefits**

*For adult subjects:*

The potential benefits to the subject include reduction or even abolition of transfusion requirements and/or improvement of cytopenia, resulting in improved quality of life and also decreased morbidity and mortality from transfusion-associated viral agents, iron overload, and/or a susceptibility to infections. For patients with MDS who have failed initial immunosuppression therapy and do not have a histocompatible matched sibling donor, therapeutic options are limited. Although unrelated donor stem cell transplantation has been reported with success, the morbidity

and mortality are still considerably higher than a transplant with a matched sibling donor. Potentially, treatment with other more toxic therapies could also be avoided or postponed.

Therefore, the research involves greater than minimal risk to subjects with the prospect of direct benefit (45 CFR 46.102)

**As of January 27, 2017, this study will no longer be in subject follow-up and will continue in data analysis only; the level of risk is now minimal.**

## **10.5 Consent Processes and Procedures**

The investigational nature and research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and benefits, and potential alternative therapies will be carefully explained to the patient during the initial clinic evaluation. A copy of the consent form will be presented to the patient, with instructions to note down questions or underline areas of the consent form they do not properly comprehend. The principal investigator or an associate investigator on this protocol from the Hematology Branch will lead this discussion and obtain the informed consent. Investigators who may obtain consent are listed on page 1 of the protocol. The consent form will be signed in the presence of the investigator and a witness prior to commencement of the treatment plan. The treatment plan and risks will be discussed again and in detail during their hospital visit for treatment.

If at any time during participation in the protocol, new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to each enrolled or prospective patient. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

We anticipate enrollment of non-English speaking participants into this study. The IRB approved consent will be translated into the subject's language in accordance with Clinical MAS Policy M77-2. If there is an unexpected enrollment of a research participant for which there is no translated 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, 45 CFR 46.117 (b) (2), 21 CFR 50.27 (b)(a). The summary that will be used is the English version of the IRB approved consent document. We request prospective IRB approval of the use of the short form for up to a maximum of 5 participants in a given language and will notify the IRB at the time of continuing review of the frequency of the use of the short form. Should we reach the threshold of 5, we will notify the IRB of the need for an additional use of the short form and that we will have the consent document translated into the given inherent language.

## **10.6 Conflict of Interest**

The Principal Investigator assured that each associate investigator listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. Investigators added subsequent to the initial circulation were provided a copy of the document when they were added. Copies of the Conflict of Interest Statement were forwarded to the Clinical Director. No initial or subsequent members of the research team reported a potential conflict of interest.

This protocol has no associated patents, CRADAs or CTAs. No transfer of material will be accomplished until a Material Transfer Agreement is fully executed through the NHLBI Office of Technology Transfer and Development (OTTAD).

### **FWA Coverage Agreement**

Dr. Catherine Lai has recently undertaken the role of Director of Leukemia at MedStar Georgetown University Hospital's Lombardi Comprehensive Cancer Center, Washington DC. She still maintain her work at NIH as an associate investigator and will continue to analyze identifiable data for the purpose of publishing a manuscript based on subject outcomes. Dr. Lai's role in the research will be limited to data analysis. An FWA coverage agreement to cover this activity will be executed by Dr. Lai and Dr. Hourigan, once this amendment is approved.

## **11.0 PHARMACEUTICALS**

### **11.1 ALEMTUZUMAB (CAMPATH®)**

*Generic:* Alemtuzumab

*Classification:* Monoclonal antibody

*Action:* Monoclonal antibody directed against CD52 antigen, a surface glycoprotein expressed by lymphocytes

*Availability:* Commercial, Genzyme Corporation

*Product Description:* Alemtuzumab injection is available in single-use, clear glass vials containing 30 mg of alemtuzumab in 1 ml of solution

*Storage:* Stored at 2 to 8 degrees Celsius (36 to 46 degrees Fahrenheit) and protected from direct sunlight. Protect from freezing; discard if frozen.

*Stability:* Stability data is not available for undiluted alemtuzumab drawn into a syringe. Once the prescribed dose is drawn into a syringe the syringe, the dose should be administered within 4 hours of syringe preparation (i.e. 4 hour expiration) whether at room temperature or refrigerated. Protect from light.

*Preparation for Administration:* Parenteral drug products should be inspected for visible particulate matter and discoloration prior to administration. If particulate matter is present or the solution is discolored, the vial should not be used. DO NOT SHAKE VIAL PRIOR TO USE. As with all parenteral drug products, aseptic technique should be used during the preparation and administration of alemtuzumab (Campath®).

Withdraw the protocol prescribed amount of alemtuzumab from the vial (solution concentrated to 30mg/ml) into a 1 ml syringe calibrated in increments of 0.1 ml.

### **11.2 CYCLOSPORINE (Gengraf, Sandimmune, Neoral)**

*Supply:* Cyclosporine will be obtained by the NIH Clinical Center Pharmacy Department from commercial sources and is available in capsules (25 mg and 100 mg), USP [MODIFIED], oral solution (100 mg/ml), USP [MODIFIED], and as a parenteral concentrate for injection (50 mg/ml). When oral capsules are prescribed for this protocol, the cyclosporine capsules, USP [NON-MODIFIED] should NOT be used.

*Preparation:* For parenteral doses, each milliliter of concentrate (50mg/ml) should be diluted in 20 to 100ml of dextrose 5% in water or sodium chloride 0.9%. Parenteral doses of cyclosporine will be prepared in non-PVC containers and infused with non-PVC administration sets/tubing. Oral cyclosporine solution may be mixed in orange juice or other beverages, but not milk.

*Storage and Stability:* Capsules, oral solution, and ampules of parenteral concentrate bear expiration dates and are stored at room temperature and protected from light. Cyclosporine concentrate for

injection that has been diluted to a final concentration of approximately 2mg/ml is stable for 24 hours in 5% dextrose or 0.9% sodium chloride injection in glass, PVC or non-PVC plastic containers. To minimize the potential for sorption to PVC plastic bags and tubing as well the leaching of phthalate plasticizer (DEHP) into the solution, only non-PVC plastic bags and intravenous administration sets should be utilized.

*Administration:* Cyclosporine may be given intravenously or orally.

*Adverse Reactions:* see section 10.3.2, Risks and discomforts.

### 11.3 CIPROFLOXACIN (CIPRO)

*Drug Class and Mechanism:* Many common infections in humans are caused by single cell organisms, called bacteria. Bacteria can grow and multiply, infecting different parts of the body. Medicines that control and eradicate these bacteria are called antibiotics. Ciprofloxacin is an antibiotic that stops multiplication of bacteria by inhibiting the reproduction and repair of their genetic material (DNA).

*Supply:* Commercially available

*Drug interactions:* Ciprofloxacin administered together with **theophylline** can lead to elevated blood levels of theophylline. Theophylline is used to open airways in the treatment of **asthma**. Toxic levels of theophylline can lead to seizures, and disturbances in heart rhythm. If concurrent use of ciprofloxacin and theophylline cannot be avoided, frequent blood tests to monitor theophylline blood levels are performed. Ciprofloxacin should be used with caution in patients with central nervous system diseases such as seizures, because rare seizures have been reported in patients receiving this medication. Ciprofloxacin should be avoided in children and adolescents under 18 years old, as safe use in these patients have not been established.

Many antibiotics, including ciprofloxacin, can alter the normal bacteria in the colon and encourage overgrowth of bacteria responsible for the development of inflammation of the colon (pseudomembranous colitis). Pseudomembranous colitis can cause fever, **abdominal pain**, **diarrhea**, and sometimes even shock. Patients taking ciprofloxacin can develop sensitivity of the skin to direct sunlight. Ciprofloxacin can enhance the action of the anticoagulant **warfarin** (Coumadin), and increase the risk of bleeding.

*Storage and Stability:* Ciprofloxacin should be stored at below 86 degrees F.

*Administration:* Ciprofloxacin may be taken with or without food. Ciprofloxacin is partially metabolized by the liver and excreted by the kidney. Dosages require adjustment in patients with severely abnormal liver or kidney function. Antacids block the absorption of ciprofloxacin and they should not be taken together.

*Adverse reactions:* see section 10.3.8, Risks and discomforts

### 11.3 PENTAMIDINE (NebuPent®)

*Supply:* Commercially available (NebuPent®, American Pharmaceutical Partners, Inc.)

*Product description:* Pentamidine isethionate is available as a 300 mg single dose vial containing 300 mg of lyophilized powder in a 15 mL capacity vial. The contents of one vial must be dissolved in 6 mL of sterile water for injection, USP. It is important to use only sterile water, saline solution will cause the drug to precipitate.

*Storage and stability:* Store dry product at controlled room temperature 15-30°C (59-86°F).

*Route of administration:* Inhalation; Once reconstituted, the entire contents of a vial should be placed into the Respigard® II nebulizer (Marquest) reservoir for administration by inhalation Do not mix the pentamidine solution with any other drugs.

*Adverse Reactions:* see section 10.3.8, Risks and discomforts.

### 11.4 VALACYCLOVIR (VALTREX)

*Generic name:* valacyclovir

*Brand Name:* VALTREX

*Supply:* Commercially available.

*Pharmacology:* Valacyclovir is the hydrochloride salt of L-valyl ester of the antiviral drug acyclovir. After oral administration, valacyclovir is rapidly absorbed from the GI tract and nearly completely converted to acyclovir and L-valine by first-pass intestinal or hepatic metabolism.

*Product description:* Valacyclovir is available in 500mg tablets and 1gm tablets. Dose adjustment is necessary in patients with significant renal impairment (refer to the manufacturer's labeling for dose adjustment guidelines).

*Storage and Stability:* Oral tablets should be stored at 15° to 25°C (59° to 77°F).

*Route of administration:* Oral

*Adverse Reactions:* see section 10.3.8, Risks and discomforts.

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**APPENDIX A NHLBI HEMATOLOGY BRANCH LABORATORY RESEARCH STUDIES  
-2/5/2013**

	DESCRIPTION OF LABORATORY STUDY BY BRANCH SECTION	Does this test pose a greater than minimal risk to pediatric subjects per 45 CFR 46.404?	Does this test pose a greater than minimal risk to healthy pediatric donors per 45 CFR 46.404?
<b>A</b>	<b>Stem Cell Allograft Transplantation Section (Dr. A. John Barrett)</b>		
<b>A.1</b>	Measurement of lymphocyte function and immune responses directed toward allogeneic tissues, malignant cells, and infectious agents. Assay of a variety of antigens, including standard proliferation, cytotoxicity, and intracellular cytokine detection including GVHD predictive markers. Measurement of antigen-specific responses including employment of tetramers, ELISPOT technique, gene amplification-based assays, and flow cytometry. Selection of cells using immunomagnetic beads or flow cytometry. Culture, expansion, and selection of cells. Surface marker analysis of PB MC using flow cytometry. Cytokine/chemokine analysis of plasma/serum samples using ELISA and/or Luminex techniques.	No	No
<b>A.2</b>	Generation of cell lines for the study of immune cell interactions with other cells. Transformation of B-lymphocytes using Epstein-Barr virus. Derivation of malignant cell lines from patient leukemic or solid tumor samples.	No	No
<b>A.3</b>	Infection of cells and cell lines with recombinant genes to ascertain the effects of expressed molecules on immune responses and on growth and development. Transfection of cell lines with specific molecules to study antigen-specific responses.	No	No
<b>A.4</b>	Assays of peripheral blood and bone marrow progenitor cells including primitive and late erythroid progenitor-derived colonies, myelomonocytic colonies, and primitive multi-potential progenitor-derived colonies.	No	No
<b>A.5</b>	Injection of human cells into experimental animals to study the immune system and the growth of normal and malignant cells under varying conditions.	No	No
<b>A.6</b>	Testing of selection methods, cell isolation, and cell expansion leading to the development of new cell-based therapies requiring scale-up for clinical application.	No	No
<b>A.7</b>	Identification of individual T cell clones by their T cell receptor sequence.	No	No
<b>A.8</b>	Measurement of tumor and tissue specific antigens in cells of subjects and donors by mRNA, protein, or peptide expression in cells or fluids.	No	No
<b>A.9</b>	Laser capture micro dissection of cells from biopsies for GVHD to determine clonotypes.	No	No
<b>A.10</b>	DNA and RNA typing of genes that control immune responses in lymphocytes.	No	No
<b>A.11</b>	Microassay studies utilizing cellular DNA, cDNA, and RNA for neoplasia and host-tumor interactions.	No	No
<b>B</b>	<b>Molecular Hematopoiesis Section (Dr. Cynthia Dunbar)</b>		
<b>B.1</b>	Flow cytometric analysis of cell surface and cytoplasmic proteins, including cell adhesion molecules, putative retroviral receptors, and markers of differentiation, using bone marrow and mobilized peripheral blood cells.	No	No
<b>B.2</b>	Hematopoietic progenitor-derived colony ascertainment in vitro (as described above), and engraftment of immunodeficient mice for detection of human stem cell number and function.	No	No

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<b>B.3</b>	Testing ability of hematopoietic progenitor cells to be transduced with retroviral, lentiviral, and novel gene transfer vectors in vitro.	No	No
<b>B.4</b>	Reprogramming of adult mature cells, including skin fibroblasts and blood cells, into induced pluripotent stem cells in vitro.	No	No
<b>C</b>	<b>Cell Biology Section (Dr. Neal Young)</b>		
<b>C.1</b>	Studies of blood and bone marrow hematopoietic progenitor numbers, including early and late erythroid progenitors, myelomonocytic progenitors, and multi-potential progenitor cells. In addition, bone marrow may be placed in long-term bone marrow culture to assess the function of stroma and stem cells and to assay more primitive progenitors, as well as organelle culture. Whole or selected bone marrow populations are cultured short-term for CD34 cell expansion.	No	No
<b>C.2</b>	Assays of apoptosis in hematopoietic cells and their progeny, using flow cytometric methods such as annexin and caspase-3 staining, propidium iodide uptake, and mitochondrial permeability tests.	No	No
<b>C.3</b>	Separation and functional study of cell populations characteristic of paroxysmal nocturnal hemoglobinuria, identified by absence of glycosylphosphatidylinositol anchored proteins.	No	No
<b>C.4</b>	Studies of mutation rates in hematopoietic cells and in buccal mucosa cells, using conventional hypoxanthine phosphoribosyltransferase activity functional assays, sequencing of mitochondrial DNA after specific gene amplification, and measurement of GPI-anchored deficient cells in blood and bone marrow.	No	No
<b>C.5</b>	Assays of immune function of T-cells, including intracellular cytokine staining, ELISPOT, semiquantitative gene amplification for gamma-interferon, tumor necrosis factor, interleukin-2, and other cytokines, and functional assessment in co-culture using specific neutralizing monoclonal antibodies. In addition, peripheral blood lymphocytes are subjected to spectratyping for CDR3 size distribution as well as nucleotide sequence of CDR3 peaks obtained.	No	No
<b>C.6</b>	Studies of engraftment of human normal and diseased bone marrow and peripheral blood in immunodeficient mice in order to determine the presence of hematopoietic repopulating stem cells as well as functional differences among selected populations.	No	No
<b>C.7</b>	Flow cytometric analysis of blood and bone marrow for lymphocyte phenotype, especially for evidence of activation of lymphocytes, for markers of apoptosis, and for antigens associated with primitive and mature hematopoietic cell populations.	No	No
<b>C.8</b>	Flow cytometric analysis of blood and bone marrow for hematopoietic stem cell progenitors and CD34 positive cells.	No	No
<b>C.9</b>	Studies of chromosomal instability in myelodysplastic syndromes including BM cell and CD34 cell response to PAS crosslinking and examination of the cytotoxic effect of lymphocytes to the abnormal clone of cells.	No	No
<b>C.10</b>	Surface Enhanced Laser/Desorption Ionization (SELDI) time-of-flight mass spectrometry (Ciphergen) (proteomics methodology).	No	No
<b>C.11</b>	Mitochondrial DNA (mtDNA) sequence heterogeneity.	No	No
<b>C.12</b>	Measurement of EBV viral load.	No	No
<b>C.13</b>	Measurement of EBV LMP-1 via RT-PCR for LMP-1 RNA or flow cytometry for LMP-1.	No	No
<b>C.14</b>	Outgrowth assay of EBV transformed B cells.	No	No
<b>C.15</b>	Quantification of serum chemokines and cytokines (e.g. SDF-1, IL-10, IL-6, CXCR4, CXCL12).	No	No
<b>C.16</b>	Quantification of EBV cytotoxic T cells (tetramer staining).	No	No
<b>C.17</b>	Telomere length measurement by Southern blot, Q-PCR, flow-fish, in situ hybridization and STELA	No	No

C.18	Telomere repair complex gene mutations by nucleotide sequencing of some or all of the following: <i>DKC1</i> , <i>TERC</i> , <i>TERT</i> , <i>SBDS</i> , <i>NOP10</i> , <i>NHP2</i> .	No	No
C.19	Analysis of inflammatory markers and/or bacterial, viral, fungal or protozoal elements in plasma or serum using molecular, colorimetric, enzymatic, flow cytometric or other assays in subjects receiving immunosuppressive therapy, chemotherapy and/or bone marrow transplantation.	No	No
C.20	Confocal microscopic imaging of bone marrow.	No	No
C.21	Characterization of intracellular signaling proteins by cell permeabilization and flow cytometry, and quantitative immunoblots.	No	No
C.22	Assays for chromosomal aneuploidy by fluorescence in situ hybridization (FISH) and other molecular techniques.	No	No
C.23	Conversion of human dermal fibroblasts into hematopoietic progenitors using Oct4 transfection.	No	No
<b>D</b>	<b>Virus Discovery Section (Dr. Neal Young) THESE ASSAYS WILL NOT BE PERFORMED ON SAMPLES FROM HEALTHY PEDIATRIC DONORS</b>		
D.1	Assays of serum, blood cells, and bone marrow cells for B19 parvovirus and possible B19 variants using gene amplification, cell culture, and hematopoietic colony inhibition assays.	No	N/A
D.2	Assays of blood, bone marrow, liver, and other tissues for potentially novel viruses, using a variety of techniques including RNA and DNA assays, differential display, gene amplification with conserved and random primers, cell culture assays, immunohistochemical methods, and inoculation of mice, rabbits, and monkeys, as well as antibody measurements.	No	N/A
D.3	Assays of blood, bone marrow, and liver for known viruses, including herpesviruses such as cytomegalovirus, human herpesviruses 6, 7, and 8, enteric viruses such as A-6, circoviruses, and parvoviruses, using assays as in (2).	No	N/A
D.4	Spectra-typing of blood cells to determine response to known or putative viral infections.	No	N/A
D.5	HLA typing or subtyping to determine risk factors/determinants for hepatitis-AA studies.	No	N/A
D.6	Cytotoxic lymphocyte assays with intracellular cytokine measurement for determining anti-viral response and lymphocyte cloning to obtain clones with specific antiviral activity.	No	N/A
<b>E</b>	<b>Solid Tumor Section (Dr. Richard Childs)</b>		
E.1	Cr51 cytotoxicity assay to evaluating killing of patient tumor cells by patient NK cell clones and T-cells.	No	No
E.2	ELISA for IL-12 maturity of DC's made from subjects monocytes.	No	No
E.3	ELISA for IFN $\alpha$ to evaluate specificity of CTL clones.	No	No
E.4	H thymidine uptake to evaluate proliferation potential of antigen specific T-cells.	No	No
E.5	PCR of STR to assess chimerism status of cellular subsets grown in-vitro or retrieved from subjects post-transplant.	No	No
E.6	Flow sorting of PBL and/or tissue samples to evaluate chimerism of different subsets.	No	No
E.7	Surface marker analysis of peripheral blood mononuclear cells using flow cytometry.	No	No
E.8	cDNA expression arrays to evaluate T-cells expression/gene patterns in subjects with GVHD and a GVT effect.	No	No
E.9	Geno typing of tumor or tissue samples by high density cDNA arrays.	No	No
E.10	VHL mutation analysis on kidney cancer tissue.	No	No
E.11	Transduction of dendritic and tissue cells with tumor antigens using plasmids, viral vectors and hybrid fusions.	No	No

E.12	Lasar capture microdissection of cells from tumor biopsies and tissue samples to determine origin (donor vs patient).	No	No
E.13	Quantification of polyoma virus BK exposure by serology and PCR in stem cell transplant donors and recipients from blood and urine samples.	No	No
E.14	Quantification of polyoma virus BK specific T cells in stem cell transplant donors and recipients from peripheral blood samples.	No	No
E.15	Determination of origin of neovasculature endothelial cells in tumor and tissue samples obtained from subjects post transplant.	No	No
E.16	Quantification of lymphocyte subsets CD34 progenitors and endovascular progenitors in G-CSF mobilized peripheral cell allografts.	No	No
E.17	Testing for polyoma virus BK latency in CD34 progenitors, B cells and T cells in the G-CSF mobilized peripheral cell allografts.	No	No
E.18	Determination of etiology of membranous nephropathy using serum from subjects.	No	No
E.19	Serum Proteomic patterns analysis to diagnose complications related to allogeneic transplantation.	No	No
E.20	Determine cell origin (donor vs patient) of tissue samples using IHC, IF, sorting, and FISH.	No	No
<b>F</b>	<b>Lymphoid Malignancies Section (Dr. Adrian Wiestner)</b>		
F.1	Culture of cells from research subjects to investigate molecular disease mechanisms, model host tumor interactions, and to test effect of drugs on cell survival and cellular functions.	No	No
F.2	Generation of stable cell lines for the study of hematologic malignancies.	No	No
F.3	Modifications of cells using standard expression systems or biologic molecules, e.g. interfering RNA, to investigate the effects of candidate genes on cellular functions.		
F.4	Identification and monitoring of B or T cell populations as identified by flow cytometry and by their B cell or T cell receptor expression.	No	No
F.5	Measurement of gene expression in cells or tissues. Techniques frequently used include gene expression profiling on microarrays, quantitative RT-PCR, Western blotting, flow cytometry and ELISA assays.	No	No
F.6	Analysis of chromosomal abnormalities or mutations in malignant cells and non-malignant cells including FISH technology and DNA sequencing.	No	No
F.7	Assays of immune function of B-cells and T-cells, including intracellular cytokine staining, ELISPOT, quantitative RT-PCR for cytokines or other immune regulatory genes.	No	No
F.8	Analysis of antibody specificities in serum and antigen specificity of the B-cell receptor on cells. Techniques may include expression of antibodies in phage display systems, generation of antibodies in cell culture systems and use of such antibodies to screen for cognate antigens.	No	No
F.9	Transplantation of human cells into mice (xenograft model) to study disease biology and to investigate the effect of experimental therapy.	No	No
F.10	Measurements of drug concentrations, biologic molecules and disease markers in blood, serum, and plasma.	No	No

## APPENDIX B :INTERNATIONAL WORKING GROUP PROPOSED STANDARDIZED CRITERIA FOR RESPONSE IN MDS<sup>77</sup>

### I. Altering Disease Natural History

#### Ia. Complete remission (CR)

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*Peripheral blood evaluation* (absolute values must last at least 2 months)†:

- Hemoglobin greater than 11 g/dL (untransfused, patient not on erythropoietin)
- Neutrophils 1500/mm<sup>3</sup> or more (not on a myeloid growth factor)
- Platelets 100 000/mm<sup>3</sup> or more (not on a thrombopoietic agent)
- Blasts, 0%
- No dysplasia\*

*Bone marrow evaluation:* Repeat bone marrow showing less than 5% myeloblasts with normal maturation of all cell lines, with no evidence for dysplasia.\* When erythroid precursors constitute less than 50% of bone marrow nucleated cells, the percentage of blasts is based on all nucleated cells; when there are 50% or more erythroid cells, the percentage blasts should be based on the nonerythroid cells.

### **Ib. Partial remission (PR)**

All of the CR criteria (if abnormal before treatment) (absolute values must last at least 2 months); except:  
*Bone marrow evaluation:* Blasts decreased by 50% or more over pretreatment, or a less advanced MDS FAB classification than pretreatment. Cellularity and morphology are not relevant.

**Ic. Stable disease:** Failure to achieve at least a PR, but with no evidence of progression for at least 2 months.

**Id. Failure:** Death during treatment or disease progression characterized by worsening of cytopenias, increase in the percentage bone marrow blasts, or progression to an MDS FAB subtype more advanced than pretreatment.

**Ie. Relapse after CR or PR:** one or more of the following:

- a) Return to pretreatment bone marrow blast percentage.
- b) Decrement of 50% or greater from maximum remission/response levels in granulocytes or platelets.
- c) Reduction in hemoglobin concentration by at least 2 g/dL or transfusion dependence.§
6. Disease progression
  - a) For patients with less than 5% blasts: a 50% or more increase in blasts to more than 5% blasts.
  - b) For patients with 5% to 10% blasts: a 50% or more increase to more than 10% blasts.
  - c) For patients with 10% to 20% blasts: a 50% or more increase to more than 20% blasts.
  - d) For patients with 20% to 30% blasts: a 50% or more increase to more than 30% blasts.
  - e) One or more of the following: 50% or greater decrement from maximum remission/response levels in granulocytes or platelets, reduction in hemoglobin concentration by at least 2 g/dL, or transfusion dependence.§

**If. Disease transformation:** Transformation to AML (30% or more blasts).

**Ig. Survival and progression-free survival**

## **II. Cytogenetic response**

**IIa Major:** No detectable cytogenetic abnormality, if preexisting abnormality was present.

**IIb Minor:** 50% or more reduction in abnormal metaphases.

Requires 20 analyzable metaphases using conventional cytogenetic techniques. Fluorescent in situ hybridization may be used as a supplement to follow a specifically defined cytogenetic abnormality.

## **III. Hematologic Improvement (HI)**

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Improvements must last at least 2 months in the absence of ongoing cytotoxic therapy.† Hematologic improvement should be described by the number of individual, positively affected cell lines (eg, HI-E; HI-E 1 HI-N; HI-E 1 HI-P 1 HI-N).

### **IIIa. Erythroid response (HI-E)**

- *Major response:* For patients with pretreatment hemoglobin less than 11 g/dL, greater than 2 g/dL increase in hemoglobin; for RBC transfusion-dependent patients transfusion independence.
- *Minor response:* For patients with pretreatment hemoglobin less than 11 g/dL, 1 to 2 g/dL increase in hemoglobin; for RBC transfusion-dependent patients, 50% decrease in transfusion requirements.

### **IIIb. Platelet response (HI-P)**

- *Major response:* For patients with a pretreatment platelet count less than 100 000/mm<sup>3</sup>, an absolute increase of 30 000/mm<sup>3</sup> or more; for platelet transfusion-dependent patients, stabilization of platelet counts and platelet transfusion independence.
- *Minor response:* For patients with a pretreatment platelet count less than 100 000/mm<sup>3</sup>, a 50% or more increase in platelet count with a net increase greater than 10,000/mm<sup>3</sup> but less than 30,000/mm<sup>3</sup>.

### **IIIc. Neutrophil response (HI-N)**

- *Major response:* For absolute neutrophil count (ANC) less than 1500/mm<sup>3</sup> before therapy, at least a 100% increase, or an absolute increase of more than 500/mm<sup>3</sup>, whichever is greater.
- *Minor response:* For ANC less than 1500/mm<sup>3</sup> before therapy, ANC increase of at least 100%, but absolute increase less than 500/mm<sup>3</sup>.

### **IIId. Progression/relapse after HI:**

One or more of the following: a 50% or greater decrement from maximum response levels in granulocytes or platelets, a reduction in hemoglobin concentration by at least 2 g/dL, or transfusion dependence.§

For a designated response (CR, PR, HI), all relevant response criteria must be noted on at least 2 successive determinations at least 1 week apart after an appropriate period following therapy (eg, 1 month or longer).

\* The presence of mild megaloblastoid changes may be permitted if they are thought to be consistent with treatment effect. However, persistence of pretreatment abnormalities (eg, pseudo-Pelger-Huët cells, ringed sideroblasts, dysplastic megakaryocytes) are not consistent with CR.

† In some circumstances, protocol therapy may require the initiation of further treatment (eg, consolidation, maintenance) before the 2-month period. Such patients can be included in the response category into which they fit at the time the therapy is started.

§ In the absence of another explanation such as acute infection, gastrointestinal bleeding, hemolysis, and so on.