Abbreviated Title: Autologous transplantation for SLE
CC# 04-C-0095
Amendment G
Version: July 26, 2012

PROTOCOL TITLE: A Pilot Study of Intensified Lymphodepletion Followed by Autologous Hematopoietic Stem Cell Transplantation in Severe Systemic Lupus Erythematosus

Principal Investigator: Steven Z. Pavletic, M.D.[1] Telephone: 301-402-4899

Scientific Chairpersons: Frances Hakim, Ph.D.[1] Telephone: 301-402-3627 Ronald Gress, M.D.[1] Telephone: 301-496-1791

Associated Investigators:

Juan Gea-Banacloche M.D.[1] Claude Sportes, M.D.[1] Daniel Fowler, M.D.[1] Seth Steinberg, Ph.D.[4] Susan Leitman, M.D.[5] Diane Arthur, M.D.[6] Ann Berger, M.D.[13] Howard Austin, M.D.[8] James Balow, M.D.[8] Barbara Mittleman, M.D.[2] Gabor Illei, M.D.[10] Sarfaraz Hasni, M.D.[2] Bradford Wood, M.D.[9] James Shelhamer, M.D.[11] Thomas Hughes, Pharm.D.[12] Alicia Armstrong, M.D.[7] Nikolay Nikolov, M.D.[3]

[1] Experimental Transplantation and Immunology Branch, National Cancer Institute
[2] Office of the Clinical Director, National Institute for Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
[3] National Institute of Dental and Craniofacial Research
[4] Biostatistics and Data Management Section, Center for Cancer Research, National Cancer Institute
[5] Department of Transfusion Medicine, Clinical Center, National Institutes of Health
[6] National Cancer Institute, Center for Clinical Research, Laboratory of Pathology
[7] National Institute of Child Health and Human Development
[8] National Institute for Diabetes, Digestive and Kidney Diseases
[9] Department of Radiology, Clinical Center, National Institutes of Health
[10] Human Genome Sciences, 14200 Shady Grove Road, Rockville, Maryland
[11] Department of Critical Care, Clinical Center, National Institutes of Health
[12] Pharmacy Department, Clinical Center, National Institutes of Health
[13] Department of Pain and Palliative Care, Clinical Center, National Institutes of Health

PRECIS
Background:

- Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that can involve almost any organ and can range in severity from mild to life-threatening. In spite of significant improvements in survival of SLE patients over last 20 years, a small but significant portion of patients still develop progressive therapy-refractory disease that impairs organ function and overall survival.
- Since 1996, more than 500 patients have been treated worldwide in pilot trials of autologous hematopoietic stem cell transplantation (autoHSCT) for autoimmune diseases, including about 80 patients with SLE.
- The rationale for autoHSCT in autoimmune disease is to ablate autoreactive immune effectors and allow reconstitution of a new self-tolerant immune system from the hematopoietic stem cell. Studies have demonstrated acceptable safety and promising short-term efficacy of high-dose cyclophosphamide-based (200 mg/kg) autoHSCT for about 60% of patients with advanced refractory SLE and reacquisition of sensitivity to conventional drugs have been demonstrated in many cases. However, these trials were designed to address the primary endpoint of safety and were inadequate for assessing the disease-response.
- Numerous questions about the true efficacy of autoHSCT, optimal transplant regimen, patient selection and mechanisms of action remain unaddressed.

Objectives:

- The primary objective is to assess the rate of continuous relapse-free complete clinical responses at 24 months post-transplant, with statistical power of 84% to detect, if greater than 70% of patients meet the primary endpoint.
- The long-term goal of this research is to develop a basis for future transplant protocols that would incorporate new cellular or other immunotherapeutic interventions to further improve results of transplants with the ultimate goal to cure SLE.

Eligibility:

- Subjects age 15-40 years who fulfill at least 4 of the 11 criteria for SLE as defined by the American College of Rheumatology
- Have severe and active lupus, refractory to immunosuppressive therapy. Included are subjects with nephritis, CNS lupus, pulmonary lupus or hematologic disease

Design:

- Fourteen patients with active and standard dose cyclophosphamide-resistant SLE will be enrolled on this phase II pilot study.
- Study design is intended to improve the efficacy of autoHSCT. A lymphoablative conditioning regimen (rituximab, fludarabine and cyclophosphamide) is explored for the first time in autoimmune disease.
- The treatment schedule consists of two parts; the priming regimen prior to stem cell mobilization and collection, and the conditioning regimen with transplant.
- In contrast to other studies, this study has precisely defined eligibility and disease response criteria with strict schema of tapering immunosuppression that should allow accurate

interpretation of the treatment results.

- The study includes a carefully chosen battery of laboratory research studies designed to investigate SLE biology and mechanisms of post-transplant responses.

Study Schema

Inclusion criteria

Age 15-40 years
≥ 4/11 ACR criteria for SLE
Active and refractory lupus:
-nephritis
-CNS lupus
-pulmonary lupus
-hematologic disease
Organ function acceptable for transplantation

1. Priming regimen

Dose of administration	Days
Methylprednisolone 1000 mg i.v.	day 1
Rituximab 375 mg/m^2 i.v.	days 1 & 4
Cyclophosphamide 2000 mg/m^2 i.v.	day 2
G-CSF 10 µg/kg/day s.c. starting on until the end of the leukapheresis	day 6

Leukapheresis and cell processing

Leukapheresis starts at WBC > 5.0/µL

CD34 positive selection by Isolex 300i, v 2.5

Target CD34+ cell infusion dose 3 x 10^6/kg and at minimum 1.5 x 10^6/kg

Cryopreserve CD34+ fraction for transplant

Cryopreserve CD34- fraction for back-up

Research samples from both fractions

2. Conditioning regimen and transplant

Rituximab 750 mg/m^2 i.v.	day -7
Fludarabine 30 mg/m^2 i.v.	days -6, -5, -4, -3
Mesna 150 mg/m^2 i.v.	day -6 only
Cyclophosphamide 1200 mg/m^2 i.v.	days -6, -5, -4, -3
Mesna 1200 mg/m^2 c.i.v.	days -6 to -3
Stem cell infusion	day 0
G-CSF 5 µg/kg/day s.c. starting	

Study design
Phase II pilot study
Accrual goal 14 patients

Primary efficacy endpoint

Continuous relapse-free complete clinical response at 24 mo post-

transplant for at least 3 mo

Study evaluations

- Clinical response and blood immunology studies at: baseline, after priming, day 0 prior to stem cell infusion, at 1, 3, 6, 9, 12, 18 & 24 mo
-Marrow biopsy and aspirate at baseline, 6, 12 and 24 months lymph node needle aspirates or tissue biopsies for disease evaluation: at baseline, 6, 12 and 24 months.

day 1 until ANC> 500/μL for one day

Steroid tapering schedule

Tapering starts no later than day +28 post-transplant
Prednisone at least ≤ 10 mg/day at 6 months
Prednisone at least ≤ 5 mg/day at 12 months
Indeterminate or minor flare: repeat evaluation in 1-2 weeks and may increase prednisone up to
0.5 mg/kg/day for 2 weeks and taper to baseline over 4 weeks (allowed only once)
Major flare = treatment failure

TABLE OF CONTENTS

1 INTRODUCTION

1.1 Study Objectives

1.1.1 Primary objective:

To evaluate the rate of durable relapse-free complete clinical responses after using a lymphodepleting non-myeloablative regimen for autologous hematopoietic stem cell transplantation (autoHSCT) in patients with severe systemic lupus erythematosus (SLE).

1.1.2 Secondary objectives:

a) To evaluate toxicity of a lymphodepleting autoHSCT regimen in the setting of severe SLE.
b) To evaluate biological markers of disease after autoHSCT for severe SLE.
c) To investigate immunological efficacy and mechanisms of responses after lymphodepleting autoHSCT for SLE.

1.2 Background and rationale

1.2.1 Pathogenesis

Systemic lupus erythematosus (SLE) is the prototypic systemic autoimmune disease that can involve almost any organ and can range in severity from mild to life-threatening. The pathogenesis of SLE is complex involving a spectrum of disease promoting mechanisms. A general model of pathogenesis is that in a genetically susceptible individual an initial breakdown in tolerance creates primary autoreactive effectors, which then propagate the autoimmune response by a variety of mechanisms that include positive-feedback amplification loops [1, 3]. Autoantibody production, complement activation, immune complex deposition, and leukocyte infiltration of target organs are key immunopathogenic events. There is a 24% concordance of disease between monozygotic twins, compared to only 2% between dizygotic twins [4] suggesting an important effect of the genetic background but at the same time implying an important role for environmental or stochastic factors. Although single gene defects can lead to lupus-like conditions in animal models, the genetic influence in humans is clearly polygenic and may be different from one patient to another. Elegant studies in mice that spontaneously develop lupus have demonstrated that different genetic loci are responsible for the initial loss of

tolerance, the amplification of the autoimmune process and the severity and specific organ involvement [5]. Most autoantibodies in human lupus are targeted against conserved nuclear antigens, such as DNA, nuclear proteins and nucleosomes [6]. The original inciting event leading to loss of tolerance is not known, but in both animal models and humans, central tolerance seems to remain intact [1]. In mice immunologic abnormalities can be detected well before the onset of clinical symptoms suggesting that the underlying pathogenic mechanisms are different at various stages of the disease. At the time of clinical presentation, the immune system of patients with systemic lupus is characterized by a myriad of abnormalities. These include abnormalities of T-cells, B-cells, antigen presenting cells (APC) and the complement and cytokine network. At this stage it is difficult to determine what the primary abnormality was, but it is evident that there are mechanisms that form positive feedback loops that maintain the auto-inflammatory process and may prevent complete remission or cure with the currently used treatments. The T cell/B cell interaction is key in the amplification process, therefore targeting these cells is a reasonable approach of therapy. Presentation of autoantigens is also abnormal in SLE and modifying the way these antigens are presented to the regenerating immune system may lead to tolerance. At this point we cannot directly influence antigen presentation, but creating a non-inflammatory environment and deleting the potentially “autoreactive” APCs, such as B-cells, may achieve this goal.

1.2.2 B Lymphocytes in the pathogenesis of systemic lupus erythematosus

Although the number of B cells is low or normal in SLE, they have an increased rate of proliferation and increased secretion of immunoglobulins, especially pathogenic IgG autoantibodies [7]. The increased B cell activity is T-cell dependent, as demonstrated by isotype switching and affinity maturation of B-cells [8] [9] [10] [11]. Additional factors contributing to the increased activity of B-cells may involve decreased Fc receptor mediated inhibition or an enhanced activity of the mutational machinery [12] [13]. This may either reflect an intrinsic defect in the mutational machinery or, more likely, an altered state of B cell activation that effects the mutational process and may be associated with an escape of self-reactive B cells from the elimination process in the germinal center [13, 14]. Circumstantial evidence based primarily on clinical observations suggests that there may be at least two different types of autoantibody

producing B-cell populations in SLE patients. The first is exemplified by anti-dsDNA antibody producing cells. The anti-dsDNA titer exhibits a correlation with disease activity, and responds to immunosuppression. It rises before and during disease flares and decreases after immunosuppressive therapies. This implies that this population of autoantibody producing cells may be proliferating lymphoblasts or early plasmablasts that are continuously replenished in a rapid and transient manner that is sensitive to antiproliferative agents. The second type of antibody producing cells includes B-cells secreting antinuclear antibody and autoantibodies to Ro, La, Sm/RNP and cardiolipin. Titers of these antibodies do not usually correlate with disease activity or therapy. These observations suggest that B-cells secreting antinuclear antibodies or autoantibodies to the nucleus, Ro, La, Sm/RNP may have fully differentiated to non-proliferating, long-lived plasma cells that secrete immunoglobulins but do not proliferate and thus are not susceptible to antiproliferative agents. The conventional view of the role of B-cells in the pathogenesis of lupus has been that B-cells function solely as producers of autoantibodies. Emerging data have challenged this theory and indicate that B-cells may also play a role in the early events of the pathogenesis of SLE. B-cells are efficient antigen presenting cells for a variety of autoantigens [15] and may promote the breakdown of peripheral T-cell tolerance [16] [17] possibly by activating populations of T cells with low affinity toward autoantigens [16]. B-cells from patients with SLE produce higher levels of IL-10 [18] providing a positive autocrine feedback loop on B-cells for the production of autoantibodies. B cells may also produce other cytokines that could contribute to T-cell activation. The central role of B cells in the pathogenesis of lupus was demonstrated in a series of experiments in the MRL/lpr mouse model of SLE, that spontaneously develops a lupus-like autoimmune disease in an age-dependent manner [19]. First it was shown that B-cell deficient MRL/lpr mice do not develop glomerulonephritis (GN) [20] and more surprisingly, had a marked reduction in the number of activated memory T cells compared to B cell-intact controls [21]. The significant drop in activated memory T cell numbers correlated with the absence of cellular infiltrates in the kidney and skin suggesting a connection between T cell amplification and disease. Spontaneous T-cell activation was restored in mice reconstituted with B-cells that expressed membrane bound IgM but did not secrete soluble immunoglobulins [22]. These mice developed interstitial nephritis and vasculitis; histologically renal lesions were similar in appearance to the MRL/lpr animals. The presence of end-organ pathology in the absence of circulating antibodies supports the notion that

the role of B cells in promoting autoimmunity goes beyond the production of autoantibodies. This was further corroborated by studies in which B-cell deficient mice were infused with pooled serum from diseased MRL/lpr mice. Despite marked glomerular immunoglobulin deposition, no renal disease was observed in B-cell deficient mice. Taken together, these data provided evidence that the physical presence of B-cells is essential for the clinical manifestations of lupus nephritis and T-cells are essential for tissue damage.

1.2.3 T-cells in SLE

The pathogenic autoantibodies in SLE show features associated with T cell dependent responses, including isotype-switching and somatic mutation. In fact, T-cell clones that can help autologous B cells to produce anti-dsDNA antibodies have been isolated from lupus patients. The majority of these cells were CD4+, whereas a small fraction were αβ TCR+CD4-CD8- and γδ TCR+ [23] [24] [15]. Similarly, autoreactive T-cell clones that respond to nucleosomes have been isolated from lupus patients [25]. Spectratyping of TCR β-chains revealed restricted CDR3 length polymorphism and oligoclonal CD4+ T cell activation in peripheral blood from SLE patients. These effects were more pronounced in active patients compared patients in remission [26].

1.2.4 Hypothesis for the central role of T-cell-B-cell interaction in SLE

Although the primary event in the initiation of autoimmunity is unknown, by the time SLE is clinically evident there is an ongoing activation of autoreactive B- and T-cells in the lymphoid organs in a self-perpetuating process (**Figure 1**) [3] [1, 7].

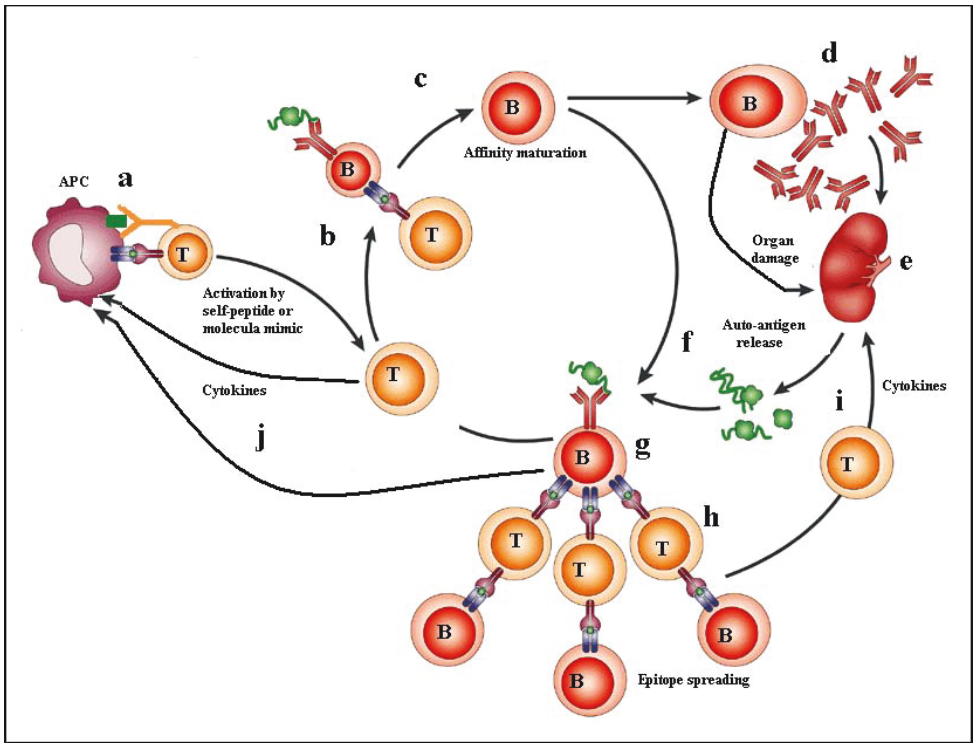

FIGURE 1. Central role of T-B cell interaction in SLE. Autoantigens are presented to T cells in an inflammatory milieu by antigen presenting cells (a) and B cells. Once activated, they provide stimulation to hyper-responsive B-cells (b). These autoantigenstimulated B cells undergo somatic hypermutation and affinity maturation (c) and produce autoantibodies (d). Pathogenic autoantibodies (such as anti-dsDNA ab) deposit in the target organs and elicit an inflammatory response (e), leading to organ damage and release of self antigens (f) which are taken up by antigen presenting B cells (g) that leads to further T cell activation resulting in a positive amplification loop (h). Activated T cells will also migrate to the target organ (i) where they contribute to organ damage. Both activated B cells and T cells produce inflammatory cytokines that contribute to organ damage and provide an inflammatory milieu for abnormal presentation of auto-antigen by APCs (j). Modified from [1, 2].

Once activated, B cells can form memory cells; differentiate into auto-antibody forming plasma cells or plasmablasts; and present auto-antigen to T-cells, inducing proliferation of more activated T cells leading to amplification of this process. Deleting B-cells when the disease is established may lead to a decrease in autoantigen presentation in the lymphoid organs that may in turn decrease the number of autoreactive T-cells and decrease both autoantibody production and activation of other effector cells. Based on the animal data presented above, B-cell depletion may also reduce leukocyte infiltration at the sites of inflammation, and ameliorate tissue damage. Using agents that delete premature and mature B-cells, but not plasma cells, may decrease the

levels of pathogenic anti-dsDNA antibody levels, without reducing immunoglobulin levels globally, which could increase susceptibility to infections. Deleting activated T cells at the same time may be necessary to prevent activation of remaining precursors of autoreactive B cells and to prevent T cell help to naturally occurring low affinity autoreactive cells that may develop during the regeneration of the immune system.

1.2.5 Epidemiology

Systemic lupus erythematosus is an autoimmune disorder affecting 24 out of 100,000 individuals in the United States [27]. The majority (90%) of patients is female and it is more common in minorities, particularly among African-Americans. Clinically, lupus is a heterogeneous disease ranging from a relatively mild condition to severe life-threatening disease involving major organs, such as the kidney, brain, lung or the hematopoetic system. Lupus nephritis is the most common major organ manifestation; about 80 % of patients have microscopic evidence of kidney involvement and about half of these develop clinically significant nephritis. Severe, potentially life-threatening major organ manifestations occur much less frequently.

1.2.6 Factors associated with survival

Overall 10-year survival rates range from 64-93% in North-American series published since 1990. The standardized mortality rate (SMR), which compares mortality to the general population, was found to be four-fold increased in two different lupus cohorts [28], the increased mortality risk was higher in younger patients. Regarding the clinical and laboratory features of SLE, major organ involvement and persistent overall disease activity are by far the most important predictors of poor outcome [reviewed in [29] [30].

The presence of renal disease was associated with increased risk of mortality in all studies [31] [32] [33] [34] [35] [36] [37] [38]. Central nervous system involvement has also been associated with decreased survival in several studies [32] [39] [40] [41]. Lung involvement including pleuritis, lupus pneumonitis, pulmonary hemorrhage and pulmonary hypertension was found to be a predictive factor of mortality [34]. Anemia was an independent predictor of poor survival in one study even after adjustment for the presence of kidney disease [32] whereas thrombocytopenia was an independent predictor of poor survival in several studies [33] [34] [35] [42]. Overall disease activity has been shown to be a risk factor for mortality in a number of studies [36] [43] [44] [45] [46]. Greater overall disease activity, as measured by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) at the time of first visit to a lupus clinic and a high weighted SLEDAI score, depicting persistent disease activity were both independent predictors of worse survival in a Canadian cohort [34], whereas a high weighted SLEDAI score was associated with decreased survival in another study [47].

1.2.7 Causes of Death

The majority of fatalities among patients with SLE are attributable to active disease with major organ involvement, followed by infections [30]. The infections most commonly occur in the

setting of active SLE and in patients receiving high dose corticosteroids and/or immunosuppressive therapy. Comorbid conditions and other complications of therapy account for the remainder of deaths. During the past decade, increasing attention has been directed toward a potentially preventable cause of mortality: corticosteroidassociated coronary artery disease. In one study the incidence of myocardial infarction was nine-fold higher in SLE patients than in controls [48], while another study found that women with lupus in the 35-44 year age group were over 50 times more likely to have a myocardial infarction than women of similar age in the Framingham Offspring Study [30] [2]. Several studies identified corticosteroid therapy as risk factor for cardiovascular disease in SLE patients [29]. Thus, in addition to controlling traditional risk factors of atherosclerosis, efforts should be made to minimize the life-long cumulative dose of corticosteroids.

1.2.8 Treatment of major organ manifestations of SLE

Lupus nephritis is the only major organ manifestation for which effective treatment was established in controlled clinical trials, most of which were done at the NIH [49] [50] [51].

The treatment of other potentially life threatening major organ manifestations, such as cerebritis, transverse myelitis, pneumonitis and pulmonary hemorrhage, is based on the experience gained in the treatment of severe lupus nephritis combined with organ-specific supportive therapy. Immune mediated hemolytic anemia and thrombocytopenia in SLE are treated as their idiopathic counterparts, AIHA and ITP [52]. The treatment of severe proliferative lupus nephritis will be summarized below. As mentioned above, the same principles apply to the treatment of most other major organ manifestations.

There have been no controlled clinical trials proving the benefit of corticosteroids over supportive therapy in lupus nephritis. Similarly, there have been no studies directly comparing conventional prednisone with methylprednisolone pulse therapy. However, long clinical experience amply demonstrates the value of steroids and they remain the cornerstone of the management of patients with SLE and lupus nephritis.

The current standard of treatment for focal and diffuse proliferative lupus nephritis has been established in a series of controlled studies of different regimens of cyclophosphamide and corticosteroids. The first of a series of controlled trials at the National Institutes of Health has shown that cyclophosphamide is superior to corticosteroids and azathioprine in preventing end-stage renal disease (ESRD) [51]. The next study proved that a 6 month course of monthly bolus cyclophosphamide is effective in inducing renal response but prolonged courses (up to over 2 years) of quarterly pulse cyclophosphamide are needed to maintain response [50]. The most recent study showed that renal remission was achieved somewhat more rapidly with the combination pulse methylprednisolone and pulse cyclophosphamide therapy [49]. Extended follow-up (median 11 years) of this study cohort, demonstrated persistent benefit of cyclophosphamide containing regimens compared to methylprednisolone alone. Indeed, the majority of the patients who initially received methylprednisolone eventually required

cyclophosphamide for the control of renal disease. At the end of the extended follow-up, eleven of the 82 patients died, 20 patients had doubled their serum creatinine and 15 of these progressed to end-stage renal disease [53].

Approximately one-third to one-half of patients has a relapse of nephritis after achieving partial or complete remission of proliferative lupus nephritis. Since each major exacerbation is expected to leave residual and cumulative irreversible damage aggressive treatment is recommended for patients with moderate or severe nephritic flares. A kidney biopsy is strongly recommended in ambiguous situations [54].

Alternatives to pulse cyclophosphamide induction therapy continue to be used at various centers around the world. Azathioprine and mycophenolate mofetil are among the most popular. Regarding azathioprine, most studies indicate that this drug adds marginally to the efficacy of prednisone alone. Thus, at the present time, azathioprine is used as primary therapy mainly in milder forms of lupus nephritis, in patients strongly opposed to use of cyclophosphamide and as maintenance therapy after induction of remission with cyclophosphamide. Mycophenolate mofetil (MMF) has been claimed to be equivalent to daily oral cyclophosphamide in a small study of 42 patients with diffuse proliferative glomerulonephritis. All patients had significant improvement and there were no differences in response or early relapse rates between the two arms [55]. However, the flare rate in the mycophenolate group was significantly higher at the 2 and 3 years follow-ups. The comparative efficacy of MMF is being further evaluated in several randomized controlled studies.

1.2.9 Historical background for HSCT in SLE

Since 1996 a number of investigators worldwide have carried out phase I-II studies of high-dose immunosuppression and autoHSCT in approximately 500 patients with autoimmune disease [56]. The majority of these transplants have been performed in patients with high-risk therapy-refractory multiple sclerosis, systemic sclerosis, systemic lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis and idiopathic thrombocytopenic purpura. The stage for these early studies has been set by demonstrated increase in efficacy and safety of allogeneic and autologous HSCT for hematologic malignancies in humans as well as by experimental animal models showing efficacy of syngeneic and autologous transplantation in antigen-induced autoimmunity [57]. Additional impetus to the field was given by the number of case reports of patients with hematologic malignancy or aplastic anemia who had concomitant autoimmune disease and were rendered free of both processes after autologous or allogeneic HSCT [58], [59] [60]. For the reasons of safety and clarity of initial trial results, international consensus guidelines in 1996 recommended that initial studies of HSCT in autoimmune disease should use autologous hematopoietic stem cell sources rather than allogeneic [61]. There are several theoretical mechanisms for how autoHSCT for autoimmune disease should work. First is the high-dose intensity effect by analogy with the treatment of malignant lymphoma; dose-intensified immunosuppression is supposed to overcome therapeutic resistance and result in

prolonged anti-inflammatory effect and decrease and delay in target-tissue injury. In that case hematopoietic stem cells source serves solely as a vehicle for hematopoietic rescue. The second presumed mechanism envisions restoration of self-tolerance by eliminating the bulk of autoreactive cells and restoration of the naïve immune system by the process of de-novo immune reconstitution from the hematopoietic stem cell. This favorable reconstitution would occur either in the absence of the initiating autoantigen or the tolerance would be acquired by the de novo process of thymic education in spite of the autoantigen presence. Finally, high-dose therapy could be effective in eliminating cell populations which mediate complex and dysregulated immune circuits which are associated with the disease (concept of "resetting" the autoimmunity). Although there is indirect clinical evidence that at least some of these mechanisms are operational in the clinical autoHSCT, their relative contributions are unclear and may be different from disease to disease. Studies in monozygotic twins showed only a 15-30% rate of concordance for autoimmune disease, indicating that in spite of strong genetic component, environmental factors play a significant role in the development of autoimmunity. In fact, around 70% of patients with autoimmune disease initially respond well to autoHSCT. About 2/3 demonstrate durable remissions/stabilizations. Those patients who relapse frequently respond to salvage agents that were previously ineffective. Reasons for the recurrent autoimmune disease in patients after autoHSCT may include insufficient eradication of autoreactive lymphocytes during the transplant process or strong genetic component that could not be overcome by the immunoablative therapy.

1.2.10 Summary of preclinical and clinical experience

a. Animal models of hematopoietic stem cell transplantation Experiments in animal models of autoimmune disease provided the rationale and stimulus for clinical studies of autoHSCT in the treatment of a variety of severe, refractory autoimmune diseases. There are two types of animal models (**Table 1**): 1. Autoimmune-prone animals that have genetic tendency to spontaneously develop either generalized autoimmunity such as the classic model of SLE in NZBxNZW F1 mice, or organ-specific autoimmunity, such as the model of diabetes mellitus in NOD mice; and 2. antigen-induced models of autoimmune diseases such as adjuvant arthritis (AA) and experimental allergic encephalomyelitis (EAE). In cases of spontaneous autoimmune diseases, such as murine models of SLE, syngeneic bone marrow transplantation early in life has not prevented autoimmunity; however, allogeneic transplantation using autoimmune resistant strain has prevented, or in some instances when given early after the disease onset, ameliorated the autoimmune disease. In contrast, studies of antigen-induced autoimmune diseases in EAE and AA by van Bekkum and his colleagues have shown both therapeutic and preventive efficacy from syngeneic or pseudoautologous transplantation [62] [57]. Application of findings from murine lupus-like models to clinical SLE must be done with caution since these mice originate from highly inbred strains raised in controlled environments. In outbred human populations, environmental exposure and regulatory cells may have a more significant role in modulating disease-associated processes. Therefore, antigen-induced models of autoimmunity may be a

much more realistic tool for translational research. One of the most important messages that emerged from the antigen-induced disease models such as adjuvant arthritis and EAE is that, in both models, the best results for syngeneic or autologous transplantation have been obtained with the strongest lymphomyeloablative regimens [57]. By drawing the analogy to a human situation, the rate of cure could be increased by intensifying conditioning regimens but with the cost of more toxicity - unless the target cell specificity can be improved. Since most likely target cell candidates in overt autoimmune disease are T and B lymphocytes, a high priority is to search for agents or regimens that would increase the clinically effective window between lymphotoxicity and myelotoxicity. It is reasonable to expect that reinfusing T and B lymphocytes in the inoculum would add substantially to the residual population and should be avoided.

TABLE 1. Animal models of autoimmunity and effect of marrow transplantation [63]

Disease	**Transplantation effect**
Spontaneous autoimmune disease	
-Models of SLE (NZBxNZW) F1 mice BXSB mice (NZWxBXSB) F1 mice MRL/lpr mice	Prevented and treated by allogeneic transplantation
-Model of diabetes mellitus NOD mice	Prevented by allogeneic transplantation
Antigen-induced autoimmune disease	
-Adjuvant arthritis Buffalo rat	Treated by syngeneic and allogeneic transplantation
Experimental allergic encephalomyelitis Buffalo rat SJL mice Lewis rat	Treated by syngeneic and allogeneic transplantation

b. Phase I/II studies in SLE

Since 1996 more than 80 patients have been enrolled in early studies of autoHSCT for severe systemic lupus erythematosus worldwide. This experience can be grouped in four ways: a) Published literature cases, b) European Bone Marrow Transplant group (EBMT) registry data, c)

International Bone Marrow Transplant Registry (IBMTR) data, and d) Northwestern University experience. Marmont reported in 1997 a first case of autoHSCT where SLE was the only indication for transplant (non concomitant disease) in a female patient with recurrent lupus nephritis; her SLE relapsed after a three-year steroid-free clinical remission [64]. Since that time a total of 29 autoHSCT cases with severe SLE have been reported in the literature by 10 transplant centers, most patients had severe renal, CNS, pulmonary or hematologic lupus manifestations after failing standard treatments (**Table 2**).

TABLE 2. Published results on autologous HSCT for non concomitant SLE

Author	N	Conditioning regimen	Outcome
Marmont 1997 [64]	1	thiotepa 15 mg/kg cyclophosphamide 100 mg/kg	Relapse after 3 yrs
Fouillard 1999 [65]	1	BEAM	Relapse at 9 mo
Trysberg 2000 [66]	1	cyclophosphamide 4940 mg/m2 TBI 1000 cGy	CNS lupus still absent at 18 mo but SLE relapsed at 6 mo, new AIHA
Rosen 2000 [67]	3	cyclophosphamide 200 mg/kg rabbit ATG 270 mg/kg methylprednisolone 3000 mg	In remission at 10, 16 and 19 mo, on 4-5 mg/d PDN
Marmont 2001 [68]	1	thiotepa 10 mg/kg cyclophosphamide 100 mg/kg	Relapse at 6 months
Wulffraat 2001 [69]	2	cyclophosphamide 200 mg/kg rabbit ATG 20 mg/kg TBI 400 cGy	In remission at 12 and 18 months, off immunosuppression
Musso 2001 [70]	3	cyclophosphamide 100 mg/kg rabbit ATG 30 mg/kg methylprednisolone 3000 mg	2 in remission at 4 and 30 mo, 1 on PDN 10 mg/d at 17 mo
Shaughnessy [71] 2001	1	cyclophosphamide 200 mg/kg horse ATG 90 mg/kg methylprednisolone 3 mg/kg	On hemodialysis at transplant, died at 2 mo, non-engraftment
Brunner 2001 [72]	1	cyclophosphamide 200 mg/kg ATG 90 mg/kg	Pneumonitis resolved normal lung function, in remission at 21 mo
Traynor 2002 [73]	15	cyclophosphamide 200 mg/kg horse ATG 90 mg/kg methylprednisolone 3000 mg	12 in remission 12-66 months, 2 relapsed, 8 completely off immunosuppression
TOTAL	29	7 (24%) IN REMISSION ON MINIMAL STEROIDS 13 (45%) IN REMISSION NO IMMUNOSUPPRESSION (only 2 remission patients with less than 12 mo follow-up)	

The majority of 29 published SLE cases (87%) responded to high-dose cyclophosphamide-based regimens and 20/29 (69%) maintained clinical remissions, 18/20 remissions were durable at 12-66 months post-transplant (**Table 2**). In spite of selection of very poor-risk cases for these early trials, only one (3%) transplant-related death was reported in a hemodialysis dependent lupus-nephritis patient irreversible post-transplant marrow aplasia [71]. Publications of small case

series are always prone to a possible reporting bias in favor of success. This may be overcome by looking at transplant registries. The EBMT international registry seated in Basel is the first systematic database to record activities related to BMT in autoimmune diseases and recently conducted a preliminary analysis of the reports in transplantation for SLE (David Jayne, personal communication). Fifty-tree out of 55 registered patients who received autoHSCT for SLE were analyzed. Cases were reported by 23 teams from 12 countries including Peoples Republic of China. Median patient age was 28 years (9-52). In 42/53 cases the stem cell source was peripheral blood rather than bone marrow and the most common method of stem cell mobilization was a combination of cyclophosphamide and G-CSF. Conditioning regimens were typically non-myeloablative, high-dose cyclophosphamide (n=32) or BEAM (BCNU, etoposide, cytarabine, melphalan) (n=4), with or without anti-thymocyte globulin. Eleven patients received TBI-based regimens and one a combination of busulfan and cyclophosphamide. Remission (defined as SLEDAI score <3 and prednisone < 10 mg/d) on an intention to treat basis occurred in 31 (58%) cases. At the median follow-up of two years, projected survival without evidence of disease activity was 59 +/- 17%. Eight out of 31 patients (26%) who achieved remission have relapsed and three have died of progressive SLE. There were seven (12%) transplant-related deaths in this multi-center series, three from septicemia, one from TTP, one from bleeding, one from secondary AML, and one from unknown causes. At the time of last follow-up steroids were successfully tapered only in 8 (15%) patients. Fourteen patients were still on steroids for disease control, and in additional individuals on post-transplant immunosuppression for disease control [(mycophenolate mofetil (n=12), cyclosporine (n=2) or cyclophosphamide (n=1)]. In summary, this EBMT experience demonstrated the feasibility of dose-intensification of immunosuppresion in patients with severe recurrent SLE many of whom had failed prior cyclophosphamide (70%) or other standard therapies. Although some patients are able to stay in remission off steroids, durable immunosuppresion-free disease control remains a problem in most and the curative potential of this strategy remains uncertain. Transplant-related mortality remains difficult to interpret due to the insufficient details about patient clinical status at the time of transplant and due to the unknown center-effect typical in such multi-center registry analyses. The International Bone Marrow Transplant registry (IBMTR) started tracking activities in the transplants for autoimmune diseases more recently, in 2001, and no formal data analysis has been yet performed. By November 2002, 27 transplant patients with nonconcomitant SLE have been registered with the IBMTR from 8 teams in the United Sates (n=25) and one team from Europe (n=2). One patient received an HLA-identical allogeneic sibling transplant and all others received autologous transplants. According to the unofficial registry information, survival data are available for 16 cases, of which 13 are alive at 12 (3-30) months post-transplant. All deaths were within 100 days post-transplant, two due to unspecified infections (one of them after allotransplant) and one of idiopathic pneumonia. The only significant single center series of autoHSCT for severe SLE is by the Northwestern University group in Chicago. Traynor et al published recently a five-year summary analysis of their data [73]. Fifteen patients with persistent and severe SLE, 7 of whom were critically ill at the time of transplant, underwent autologous HSCT. Stem cells were mobilized with cyclophosphamide 2.0 gm/m^2 and G-CSF 5

μg/kg/day. Lymphocytes were depleted from the graft by selection of CD34 positive cells. The conditioning regimen used was cyclophosphamide 200 mg/kg, horse antithymocyte globulin 90 mg/kg, and methylprednisolone 3 gm. Outcome was evaluated by the SLEDAI activity index, serum complement levels, serologic features, function of diseased organs and immunosuppressive medication requirements. Median follow-up is 36 months (12-66 mo) and no deaths occurred post-transplant. All patients demonstrated marked improvement and the SLEDAI score declined to < 5 in 12 patients. Only two patients demonstrated recurrent lupus, one of whom responded to a course of standard doses of cyclophosphamide that were previously ineffective and is currently off immunosuppression. At the time of the last follow-up, 8 patients are completely off immunosuppresion, one is receiving i.v. cyclophosphamide and six are on tapering prednisone doses of 5-15 mg/day. (After this follow-up was published one patient, who is now followed at the NIH and achieved complete clinical remission post-transplant, experienced a major lupus flare with pericarditis, rapidly progressing glomerulonephritis, pneumonitis and severe hematologic manifestations.) An additional two patients were enrolled in to the Northwestern study, and underwent stem cell harvest but died before receiving the conditioning regimen. One died of *Mucor* mycosis two weeks following chemomobilization and the other died of lupus cerebritis. Most common toxicities post-transplant included culture negative neutropenic fever (n=12), fluid retention necessitating dialysis (n=4), plantar dysesthesia or foot drop (n=4) and bacterial infection (n= 4), five patients required transient transfer to the intensive care unit. Patients at highest risk for more serious toxicities were those who had active glomerulonephritis with concurrent pulmonary or brain manifestations.

1.2.11 Summary of the current status in transplantation for SLE

Although dose intensification by high-dose cyclophosphamide-based autoHSCT regimens demonstrates major clinical benefit in about 60% of standard-therapy refractory lupus patients, there are limitations associated with this procedure at its current stage. The first and major limitation is, assessment of the true magnitude of the benefit is obscured due to lack of standardization of study entry criteria, clinical response criteria, post-transplant immunosuppression schedule, and therapy of relapses. All these early studies were designed primarily as toxicity and feasibility trials, with disease response assessments being secondary endpoints. Patients enrolled in such studies were frequently too ill and were treated in numerous transplant centers, many of which had little experience in applying this treatment to SLE. The second major deficiency of these early studies is the profound lack of the biological studies, so the immunological mechanisms that accompany disease responses in patients after autoHSCT for SLE are not clear. For example, relative contributions of the dose-intensification versus *de-novo* establishment of immune tolerance post-transplant remain to be determined. Current clinical research strategies in HSCT for SLE are going in two general directions: One is to test high-dose cyclophosphamide-based regimens in the randomized phase III clinical trials as compared to the conventional NIH regimen of serial doses of intravenous cyclophosphamide [74]. If successful, this strategy may result in the establishment of autoHSCT as a new therapy standard for selected

patients with severe SLE. However, based on the current clinical experience in animal models and humans, high-dose cyclophosphamide alone may not be sufficiently immunodepleting to induce cures in the autologous transplant setting [75] [76]. The second clinical research strategy implies that the long term goals in autoimmune disease should be achievement of the immunological tolerance and cure rather than the delay of disease progression or attenuation of symptoms. To achieve these goals, it is critical to explore new approaches to autoHSCT. One proposed direction is based on the data from the animal experiments that suggest the use of more intense myeloablative preparative regimens to accomplish more effective depletion of the memory cells from the host [57]. Although this hypothesis still needs its formal testing in humans, the currently available data demonstrate that myeloablative regimens are the major risk factor for transplant-related mortality after autologous transplantation in autoimmune disease, and this hypothetical benefit may not be without serious increase in risks [57]. An alternative strategy to be explored includes the development of more specific immunodepleting regimens which spare patient from myeloablation. The main potential disadvantages of autoHSCT are its perceived toxicities and costs. On the other hand, in a genetically complex multifactorial disease such as SLE, HSCT procedure offers a unique potential of ablation of the disease process that could be theoretically followed by the spontaneous or guided reconstruction of a functional immune system. Current conventional and novel biological therapies for autoimmune disease typically do not eliminate the disease activity but only suppress it with a need for life-long pharamacological maintenance. Toxicities of long-term therapies and increasing costs with new biologicals are surpassing one-time costs associated with autoHSCT procedure. The situation may become even more complicated since biological therapies (to be effective) may need to combine multiple agents with additional unpredictable compounding financial and toxic effects. On the other hand developing immunotherapeutics could become very valuable tools in future transplant regimens if their fundamental efficacy and mechanisms could be established. Developing a technology that has a one-time curative potential is a highly attractive therapeutic goal for patients with severe autoimmune disease. Study rationale

1.2.12 Selection of the study population

A. Disease criteria The general goal is to select patients who have therapy resistant, active severe lupus with the potential of long-term response. The specific disease eligibility criteria are defined by the following guiding principles:

- Eligible patients must have severe major organ manifestations associated with increased mortality or severe disability due to uncontrolled disease activity and/or anticipated toxicity associated with conventional immunosuppressive therapy.
- Subjects must have active disease manifestations that are known to respond to immunosuppressive therapy. Active disease must be differentiated from permanent damage (eg: proteinuria would be only regarded as active if the activity is supported by active sediment or a biopsy).
- Treatment-refractory disease is defined as worsening of disease despite adequate

immunosuppressive therapy or the inability to taper prednisone.

- The minimum length of prior immunosuppressive therapy is defined specifically for each organ involved.
- If a patient fulfills criteria on the basis of more than one major organ involvement, the primary target organ will be defined based on the degree of assessed risk for morbidity or mortality.

B. Age criteria Patients eligible for this protocol will be 15-40 years old. The guiding criteria that determined the eligibility age range are the following:

- This age range captures most SLE patients who are potential candidates for this protocol.
- SLE in patients younger than 15 years of age has a different clinical course and there is no experience with cyclophosphamide and fludarabine in this age group.
- The upper age limit is based also on the studies from our laboratory on the recovery of thymic function after autologous-transplant, showing the lack of the TRECs and $CD4^+$ cell number recovery in most patients older than 40 years of age. Since certain mechanisms of the establishment of the post-transplant immune competence and autoantigen tolerance are dependent on functional thymus, patients older than 40 years of age will not be enrolled in this pilot study.
- Age above 40 years was the main predictive factor for treatment failure in a large registry study of patients transplanted for autoimmune disease [77]. This supports the notion that age groups older than 40 should be excluded at least from the pilot studies in this setting.

1.2.13 Selection of response criteria

The ultimate goal of the study is to evaluate the sustained remission-inducing potential of intensive immunoablation followed by autoHSCT. The response criteria are designed to reflect major favorable responses of the underlying autoimmune process in the target organ with a concomitant improvement of the general disease activity. An important aspect of evaluating response is the differentiation between disease activity and irreversible damage that is already present at the time of autoHSCT. Since concomitant corticosteroid therapy is a significant confounder of outcome, the protocol includes a standard regimen of steroid taper and successful tapering of corticosteroids a necessary element of response. Different grades of responses (complete, partial or no response) are defined for every target organ. All definitions will incorporate three aspects of response:

1. Response in the primary target organ.
2. Improvement in general disease activity.
3. Ability to taper prednisone according to a predetermined schedule. Response will be evaluated at various time-points. Flares will be defined for all target organs and will be classified as severe and mild. At the end of the study patients will be classified as complete responders, partial responders or non-responders based on the degree and duration of response as defined for the primary target organ. The occurrence or absence of flares will be incorporated in the final classification of patients.

1.2.14 Fludarabine and cyclophosphamide

It is now evident, that even if given in highest possible doses (200 mg/kg), cyclophosphamide does not sufficiently eliminate T-cells from the host and the recurrences of autoimmunity in the autologous setting or graft-rejections in the allogeneic setting are frequent problems. One strategy to address the insufficient lymphodepleting potential of cyclophosphamide is to use it in conjunction with total-body irradiation or high-doses of busulfan at myeloablative doses. Unfortunately, high-intensity myeloablative regimens correlate with a significant increase in the regimen-related mortality. An alternative strategy is to search for new regimens with better lymphotoxicity versus myelotoxicity ratio. In a separate line of investigation in B-cell chronic lymphocytic leukemia, *in vitro* studies demonstrated a synergistic antitumor effects of fludarabine and cyclophosphamide and were first to support the use of this combination in clinical trials [78]. These studies in BCLL showed that patients resistant to either drug respond to the combination of the two with good tolerance, most common side-effects being immunosuppression and increased incidence of herpes simplex and varicella-zoster infections. In studies at the NCI in a murine F1-into-parent marrow transplant rejection model, a preparative regimen using the combination of daily fludarabine and cyclophosphamide showed much better selectivity in lymphodepletion and better efficacy in preventing the graft rejection than lethal total-body irradiation [79]. We took these experimental results into the clinic and incorporated this regimen in two NCI transplant protocols, 99-C-0143 (immunoablative allogeneic transplantation for hematologic malignancies) and CC 00-C0119 (immunoablative allogeneic transplantation and graft T-cell depletion for metastatic breast cancer). Sixty patients have been treated on these studies and pertinent findings demonstrated profound lymphodepletion after fludarabine/cyclophosophamide, excellent full engraftment rate and only 3% preparative regimen-related mortality (one sepsis and one cardiac arrhythmia) in a very high-risk group of allogeneic transplant patients, many of whom had advanced age or decreased organ function. This daily concurrent fludarabine plus cyclophosphamide regimen has been adopted as a current standard preparative regimen for allogeneic stem cell transplantation at the NCI for its excellent immunodepleting properties and good clinical tolerance [80]. The same will be used in the current autoHSCT protocol for SLE. Various other schedules and combinations of fludarabine and cyclophosphamide are the mainstay of preparative regimens for nonmyeloablative allogeneic stem cell transplantation worldwide; however, no study to date addressed its role as lymphodepletion in transplantation for autoimmune disease.

1.2.15 Phase I/II lupus nephritis study of cyclophosphamide plus fludarabine at NIAMS

For their immunosuppressive effects nucleoside analogs fludarabine and cladribine have been explored and shown activity in systemic lupus, rheumatoid arthritis, dermatomyositis and polymyositis [81] [82] [83] [84, 85]. Based on these experiences, we conducted a Phase I/II study to evaluate a short course of low-dose cyclophosphamide combined with fludarabine in patients with lupus nephritis (LN) (Illei et al, manuscript in preparation). Patients with active proliferative LN were treated with monthly oral boluses of low-dose cyclophosphamide (0.5

gm/m^2 on day 1) and subcutaneous fludarabine (30 mg/m^2 on days 1-3). Prednisone was aggressively tapered to a low-dose, alternate day schedule. Complete response (CR) was defined as stable creatinine, proteinuria < 1 gm/day and inactive urine sediment (<10 RBC/hpf and no cellular casts). Partial response was defined as > 50% reduction in 24 h proteinuria, if baseline > 2 g/day, with persistently active urine sediment; or inactive urine sediment (if active at baseline) with proteinuria < 2 g/day if nephrotic at baseline or < 50% of baseline if non-nephrotic. The primary and secondary outcomes were determined at 12 months. Patients were regularly followed for at least 24 months to establish long-term effects of therapy. Thirteen patients were enrolled in the study; 11 received three or more cycles. The study was terminated early because of the higher than expected rate of bone marrow suppression. One patient had severe aplastic reaction and died from a subsequent transfusion-associated graft versus host disease (TA-GVHD); two other patients had self-limited grade 4 neutropenia, not associated with any clinical sequelae. Three patients had mild leukopenia. In terms of efficacy, 8/10 patients with at least 24 months post-treatment follow-up had sustained significant response: 5 achieved and maintained CR, 3 had significant response but continued to have persistent stable mild hematuria (n=2) or low grade proteinuria (n=1). Additional treatment was started in the 2 patients who had no significant response and in 1 patient with partial response who had a kidney biopsy at 24 months showing mild focal proliferative nephritis – much improved from her baseline. Prolonged CD4 lymphocytopenia was noted in most patients (mean CD4: 98/uL at 7 months and 251/uL at 12 months); this was not associated with an increased rate of infection.

1.2.16 Rationale for Rituximab

Rituximab is a chimeric IgG1 human/mouse anti-CD20 monoclonal antibody with human constant regions and mouse variable regions [86]. It is the first monoclonal antibody approved for clinical use in cancer and has become part of the standard therapy for patients with B-cell non-Hodgkin's lymphoma. To date more than 300,000 patients have been treated with rituximab worldwide where this antibody has been used alone or in combinations with cytotoxic agents or hematopoietic stem cell transplantation. The monoclonal antibody is generally well tolerated up to single-infusion doses of 2,250 mg/m^2 [87]. Adverse events are typically infusion-associated, mostly occur with the first dose and most commonly include chills, fever and rigor related to the release of cytokines. Rituximab treatment in lymphoma patients results in impaired secondary immune responses to recall antigens but does not result in decrease in immunoglobulin levels or increase in the number of infections [88]. Rituximab binds avidly to the CD20 antigen which is expressed on 95% of B-cell lymphoma cells and on normal B-cells and in much lower levels or not at all on plasma cells. Importantly, CD20 is not present on precursor B-cells or hematopoietic stem cells. The CD20 antigen is an appealing target for a selective immunotherapy for several reasons: CD20 does not circulate in the plasma, it is not shed from the surface of $CD20^+$ cells after antibody binding, and it is not internalized or down regulated [86]. Although the function of CD20 is not fully understood, factors such as complement-mediated lysis, effector cell-mediated

lysis, induction of apoptosis and interference with calcium influx into cell contribute to the cytotoxic activity. Infusion of rituximab to lymphoma patients results in selective depletion of $CD20^+$ lymphocytes lasting 6-12 months. Terminal half-life of rituximab in lymphoma patients is variable and ranges between 76.3 h after the first infusion to 205.8 h after the fourth infusion [89]. Many autoimmune disorders and especially SLE are mediated in large part by B-cells and the elimination of autoreactive B-cell clones provides a rationale for the increasing use of rituximab in these diseases. Currently, the most promising information about the efficacy of rituximab is available from the treatment of idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, rheumatoid arthritis, dermatomyositis, and type II mixed cryoglobulinemia, as well as in systemic lupus erythematosus [90]. For example, in rheumatoid arthritis a short induction dose of rituximab was well tolerated and resulted in durable responses in majority of patients as well as a significant additive effect if used in combination with a short course of relatively low-doses of cyclophosphamide or methotrexate [91]. In an ongoing study in the United States, Anolik et al reported on 18 patients with active but non-organ threatening SLE who were treated with three dose levels of rituximab, a single infusion of 100 mg/m2 (n=6), a single infusion of 375 mg/m2 (n=6) and a full course of four weekly doses of 375 mg/m2.

Rituximab was well tolerated and it induced a dose-dependent depletion of CD19+ lymphocytes. Furthermore, the efficacy of B-cell depletion was influenced by the polymorphism of the Fc receptor genotype on immune effector cells but this effect seemed to be abrogated by the higher levels of rituximab in the higher-dose cohorts. The degree of B-cell depletion correlated with the clinical improvement in the symptoms of SLE, 7/12 patients who had good depletion of B-lymphocytes (<1% CD19+ lymphocytes) had a statistically significant decrease in disease activity (SLAM) scores at 2-3 months post-rituximab. In contrast, those patients who did not achieve a good CD19+ cell depletion experienced a continuous increase in their SLAM scores during the same period of time [92]. Overall, the early data indicate promising safety and efficacy of rituximab in the treatment of patients with SLE warranting future larger trials. Another report from England by Leandro et al described six patients with active SLE resistant to standard immunosuppressive therapy who were treated on an open-label basis. During the two-week period each patient received two 500 mg infusions of rituximab, two 750 mg infusions of cyclophosphamide, and high-doses of oral corticosteroids. No significant side-effects were observed during the follow-up. Five out of six patients had improved as evidenced by British Isles Lupus Assessment Group global scores from a median of 14 (range 9-27) at baseline to a median of 6 (range 3-8) at 6 months. Manifestations of SLE such as fatigue, arthralgia/arthritis, and serositis responded particularly well to this protocol. Disease parameters improved after rituximab in most patients as assessed by the increase in hemoglobin, decrease in the erythrocyte sedimentation rate, increase in complement level, and in two cases by the improvement in renal function and proteinuria. At the follow-up of 6-18 months, two patients are still well enough without additional immunosuppression despite fluctuating evidence of disease activity, two patients relapsed at 7 and 8 months [93]. In another report from Europe, Perrotta et al reported a resolution of SLE related autoimmune hemolytic anemia after two doses of 375 mg/m2 of

rituximab that remained durable at 7 months after therapy [94]. These preliminary data on efficacy and safety of rituximab in SLE and its potent B-cell depleting activity provide the rationale for its use in combination with other cytotoxic and immunodepleting drugs for autologous stem cell transplantation. Immunodepleting regimens with high-dose cyclophosphamide or combinations of cyclophosphamide and fludarabine are more biased towards T-cell depletion than B-cell depletion and their use in combination with a potent anti-B-cell agent is rational given the role of both cell populations in the pathogenesis of the disease. Rituximab sensitizes to and significantly enhances the efficacy of cytotoxic drugs on neoplastic lymphocytes in vitro [95]. Combinations of rituximab with fludarabine and cyclophosphamide have been shown in clinical trials to be very effective and safe in non-transplant therapy of B-CLL, resulting in a dramatic increase of profound molecular remissions and clinical responses providing the rationale for further use of this combination for lymphodepletion. Khouri used with success a combination of rituximab, cyclophosphamide, and fludarabine in a conditioning regimen for non-myeloablative allogeneic stem cell transplantation in low-grade lymphoma [96]. The combination was extremely well tolerated with promising clinical anti-lymphoma efficacy and low-incidence of acute graft-versus-host disease, suggesting a potential clinical benefit of added depletion of malignant and non-malignant B-lymphocytes. Finally, a number of trials used rituximab in conjunction with autologous stem cell transplantation for patients with lowgrade non-Hodgkin's lymphoma, showing promising safety and efficacy in this setting [86].

1.2.17 Rational for priming regimen and stem cell support

The priming regimen to be used in this study has several purposes: a) mobilization of hematopoietic stem cells, b) control of disease activity, c) in vivo graft-purging, and d) control of infusional side-effects. The addition of cyclophosphamide to G-CSF for hematopoietic stem cell mobilization in autoimmune disease results in better CD34+ cell yields, better control of the autoimmune disease and the elimination of G-CSF associated disease flares [97, 98]. The 2 gm/m2 cyclophosphamide dose has been shown to be as effective in obtaining good CD34+ cell doses but is less toxic than 4 gm/m2 cyclophosphamide. The one-time bolus dose of high-dose methylprednisolone at the beginning of mobilization has been commonly used in autoimmune disease protocols to accomplish a rapid disease control, attenuate antibody-induced infusional side effects, and prevent flares. Several studies of autologous stem cell transplantation for B-CLL and mantle cell lymphoma showed the efficacy and safety of rituximab when used for in vivo graft purging of malignant cells from the stem cell product [99-103]. Administration of rituximab resulted in dramatic reduction of B-cells from the graft, no interference with the engraftment and high-rate of long-term molecular remissions post-transplant in patients with advanced low-grade B-cell malignancies. In vivo administration of rituximab for B-cell depletion does not interfere with the procedures for the CD34 cell selection [100, 104]. There is an additional possibility that administration of G-CSF may enhance the effector cell function and improve killing of the target cells by the anti-CD20 monoclonal antibody [105-107]. To minimize the reinfusion of memory T-cells, hematopoietic stem cell product will be further

depleted ex vivo of T-cells by using CD34 cell positive selection. It is expected that such manipulation will provide about 4 log T-cell depletion from the graft with >90% CD34 cell purity. The hematopoietic stem cell support in the current protocol is given to minimize the myelotoxicity of the regimen and to build a foundation for the future graft engineering protocols.

1.2.18 Rationale for the proposed therapy

The goal of the proposed treatment is to delete the majority of auto-reactive immune cells and create a non-inflammatory environment for the regenerating immune system in which the potential for the development of tolerance against nuclear antigens is maximal. Cyclophosphamide is standard therapy for major organ manifestations in lupus and the combination of low dose cyclophosphamide and fludarabine was shown to be effective in lupus nephritis in a pilot study. The inclusion of rituximab will ensure profound depletion of B cells. This regimen is expected to lead to profound and durable responses in most patients. We doubled the single dose of rituximab in the conditioning regimen. The rationale for this higher rituximab dosing is for the sake of simplicity and also to be consistent with the intention of dose-intensification during conditioning [108]. This is based also on the evidence from the dose escalation trial conducted by O'Brien et al where rituximab was given safely up to the six times higher single doses than prescribed by the drug label [87]. Since toxicities of rituximab are most common during the first infusion, we still decided to adhere to the standard dose during the initial administration in the priming regimen. Available information supports also a clear dose-response effect of rituximab. The ultimate goal of this study is to evaluate if reeducation of the immune system and cure can be achieved with intensive immunoablative therapy approaches.

1.3 SUMMARY

This proposal is a phase II pilot study of intensive lymphoablative preparative regimen using the combination of fludarabine, cyclophosphamide and rituximab followed by autoHSCT for patients with severe SLE. The primary goal is to assess the rate of durable complete clinical responses. It is expected that such transplant regimen with attenuated doses of cyclophosphamide would result in improved efficacy/toxicity ratio in treating patients with severe SLE. The study also incorporates tightly defined patient selection and clinical response criteria and precise definitions for the use of post-transplant immunosuppression and therapy of flares. Such strictly defined criteria should improve the interpretation of the results of the study. Major components of this research are the in-depth studies of SLE biology and transplantation immunology. The long-term goal is to develop a basis for future transplant protocols that would incorporate new cellular or other immunotherapeutic interventions to further improve results of transplants with the ultimate goal of cure for SLE.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 Eligibility Criteria

2.1.1 Inclusion criteria

1. Age 15-40 years
2. Must fulfill at least 4 of the following 11 criteria for SLE as defined by the American College of Rheumatology [109, 110]:

• *Malar rash.* Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds.

• *Discoid rash.* Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions.

• *Photosensitivity.* Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation.

• *Oral ulcers.* Oral or nasopharyngeal ulcerations, usually painless, observed by a physician.

• *Arthritis.* Nonerosive arthritis involving two or more peripheral joints, characterized by tenderness, swelling, or effusion.

• *Serositis.* a.) Pleuritis - convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion OR b.) Pericarditis - documented by ECG or rub or evidence of pericardial effusion

• *Renal disorder.* a.) Persistent proteinuria greater than 0.5 grams per day or greater than 3+ if quantitation not performed OR b.) Cellular casts – may be red cell, hemoglobin, granular, tubular, or mixed.

• • *Neurologic disorder.* a.) Seizures – in the absence of offending drugs or known metabolic derangements; eg, uremia, ketoacidosis, or electrolyte imbalance

• OR b.) Psychosis - in the absence of offending drugs or known metabolic derangements; eg, uremia, ketoacidosis, or electrolyte imbalance

• *Hematologic disorder.* a.) Hemolytic anemia – with reticulocytosis OR b.) Leukopenia – less than 4000/µL total on two or more occasions OR c.) Lymphopenia – less than 1500/µL on two or more occasions OR d.) Thrombocytopenia – less than 100,000/µL in the absence of offending drugs

• *Immunologic disorder.* a.) Anti-DNA: antibody to native DNA in abnormal titer OR b.) Anti-SM: presence of antibody to SM nuclear antigen OR c.) Positive finding of antiphospholipid antibodies based on (1) an abnormal serum level of IgG or IgM anti-cardiolipin antibodies, (2) a positive test result for lupus anticoagulant using a standard method, or (3) false positive serologic test for syphilis known to be positive for at least 6 months and confirmed by Treponema Pallidum immobilization or fluorescent treponemal antibody absorption test

• Antinuclear antibodies. An abnormal titer of ANAs by immunofluorescence or an equivalent assay at any point in time in the absence of drugs known to be associated with “drug-induced lupus” syndrome.

3. Have severe and active lupus, refractory to immunosuppressive therapy, defined as one of the following (a-d):

a. Nephritis: Biopsy proven Diffuse Proliferative Glomerulonephritis (WHO Class IV) with or without superimposed membranous changes

i. Active disease:

1. A kidney biopsy within three months of enrollment showing active WHO Class IV disease. Activity will be determined based on the presence of endocapillary cellular proliferation compromising the capillary loops or cellular crescents or necrosis on light microscopy or subendothelial deposits on electron microscopy.
2. If a biopsy is contraindicated patients can be enrolled if they had a previous biopsy showing Diffuse Proliferative Glomerulonephritis (WHO Class IV) and at the time of enrollment have all of the following:

a. Proteinuria > 1gm/day

b. Active urine sediment defined as hematuria (> 10 RBC/hpf on a nephrology urinalysis of a 50 mL urine sample) with dysmorphic RBC and/or cellular casts on a nephrology urinalysis of a 50 mL urine sample

c. Low C3 (<69 mg/dL) and/or elevated dsDNA antibodies (>25EU) 3. Need for prednisone >20 mg/day due to increased renal activity after at least 6 months of cyclophosphamide.

ii. Treatment resistant: Patients with active disease after at least 6 months of mycophenolate mofetil or intravenous pulse cyclophosphamide ± iv methylprednisolone and daily oral prednisone, or

Early flare: those who have reactivation of their nephritis during or within 6 months of completing cyclophosphamide therapy

Recalcitrant disease: two or more recurrences of lupus nephritis within five years of enrollment. All flares must have received adequate therapy and at least one of the episodes must have been treated with minimum 6 months of intravenous pulse cyclophosphamide ± iv methylprednisolone and maintenance oral prednisone.

b. **CNS lupus:** Lupus CNS manifestations indicative of encephalitis or myelitis or vasculitis. Concomitant CNS diseases should be excluded. (e.g. infections, multiple sclerosis; patients fulfilling MS and SLE criteria will be excluded). Clinical signs and symptoms must be supported by objective findings of CNS inflammation.

i. Active disease:

Signs/symptoms that are accepted for disease activity:

- Clinical signs and symptoms compatible with focal CNS damage
- Severe global neurocognitive/psychiatric impairment (eg: psychosis, organic brain syndrome, severe depression)
- Intractable seizures

Clinical findings must be supported by at least one of the following:

a) MRI findings consistent with transverse myelitis or CNS vasculitis

-Signs of inflammation on MRI are either the presence of Gadolinium (Gd)-enhancing lesions, or the increase of the number and/or volume of T2-weighted lesions (or lesions showing up on FLAIR imaging). We will use the standard MS protocol sequences, which are routinely used in the Clinical Center to evaluate inflammatory CNS lesions.

b) If patient has seizures/psychiatric signs and symptoms in the absence of clear signs of vasculitis or cerebritis by MRI, the CSF should show protein elevation above normal levels and abnormal number of WBCs or intrathecal IgG synthesis/or oligoclonal bands.

c) Need for prednisone >20 mg/day due to increased CNS activity (see above) after at least three monhs of cyclophosphamide therapy.

ii. Treatment resistant:

a) Active disease after a minimum of three months of oral or intravenous cyclophosphamide, or

b) Early flare: reactivation of CNS lupus within 6 months of completing cyclophosphamide therapy

c) Recalcitrant disease: two or more recurrences of CNS lupus within five years of enrollment. All flares must have received adequate therapy and at least one of the episodes must have been treated with minimum 3 months of oral or intravenous cyclophosphamide

c. Pulmonary lupus

Recalcitrant disease: two or more recurrences of pulmonary as described above within five years of enrollment. All flares must have received adequate therapy and at least one of the episodes must have been treated with minimum 3 months of oral or intravenous cyclophosphamide

d) Hematologic disease

i) Active disease:

a) Severe immune-mediated thrombocytopenia (platelet count < 20,000/mm^3 or < 50,000/mm^3 with history of bleeding), or b) Severe immune-mediated anemia (requiring transfusions to maintain Hb> 8.0 g/dL or to treat symptoms of anemia) c) Need for prednisone >20 mg/day due to increased hematologic lupus activity after therapy as described in section ii.a).

ii) Treatment resistant:

i. Active disease:

a) Lung biopsy showing active pneumonitis, alveolitis or pulmonary vasculitis after the minimally required therapy within one month of enrollment or

b) If a biopsy is contraindicated within one month of enrollment, patients may be included if they had a biopsy at the start or during cyclophosphamide treatment showing active pneumonitis, alveolitis or pulmonary vasculitis (as above) and have abnormal or worsening pulmonary function tests with a chest CT consistent with active pneumonitis, alveolitis or vasculitis within 2 weeks of enrollment and at the time of enrollment have a CT consistent with active disease.

c) Need for prednisone >20 mg/day due to increased pulmonary lupus activity after minimum of three months of cyclophosphamide. Treatment resistant:

d) Ongoing or recurrent active pulmonary lupus after a minimum of three months of oral or intravenous cylophosphamide, or Early flare: reactivation of pulmonary lupus (as defined above) within 6 months of completing cyclophosphamide therapy.

a) Active disease as defined above after a minimum of three months of high dose oral or pulse corticosteroids ± IVIg (or WinRho) and splenectomy, or

b) Early flare: reactivation of hematologic lupus (as defined above) within 6 months of completing above therapy.

c) Recalcitrant disease: two or more recurrences of immune-mediated thrombocytopenia or anemia, as described above, within five years of enrollment. All flares must have received adequate therapy and at least one of the episodes must have been treated by splenectomy.

2.1.2 Exclusion Criteria

1. Inability to provide written informed consent prior to entry in the protocol
2. Pregnant or lactating women. Women of childbearing potential are required to have a negative pregnancy test at screening
3. Women of childbearing potential who are not practicing or who are unwilling to practice birth control during the entire study
4. Men who are unwilling to practice birth control for the first 6 months after the transplant
5. Evidence of infection with hepatitis B, hepatitis C, or HIV
6. History of malignancy other than basal cell carcinoma of the skin
7. DLCO corrected <45%
8. LVEF <45%, determined by ECHO cardiogram or MUGA
9. SGOT or SGPT >2x upper limit of normal (unless active myopathy is proven by elevation of serum aldolase levels and the patient has no obvious hepatic disease) and/or bilirubin >2.0 (unless due to isolated hemolysis).

10.Calculated glomerular filtration rate < 30 ml/min using the MDRD equation estimate [111] :

$GFR\ (ml/min/173\ m^2) = 186.3 \times (Pcr)^{-1.154} \times (age)^{-0.203} \times 1.212\ (\text{if black}) \times 0.742\ (\text{if female})$

Late flare (patients who have a single episode of target organ flare, that is not within the time frame defined as early flare), will not be considered as treatment failures until they receive the minimally required therapy for this flare episode and fail to respond to it

Abnormal bone marrow cytogenetics

Significant concurrent medical condition or any significant circumstance that could affect the patient's ability to tolerate or complete the study

Live vaccines within 4 weeks of starting the priming regimen

2.1.3 Concurrent therapy

It is anticipated that all subjects prior to enrollment in this protocol will be on immunosuppressive therapy and moderate-to high doses of corticosteroids. It is also anticipated that the priming regimen will provide short-term control of lupus. Therefore, all immunosuppressive and disease modifying treatments, with the exception of steroids, will be discontinued before the beginning of the priming regimen. Analgesics (except NSAIDs) including narcotics will be allowed if needed. Flares that occur before HSCT will be treated with corticosteroids. Prednisone will be tapered according to a predetermined schedule based on the dose at the time of transplant. The treatment of flares after HSCT is specified later in the protocol (section 5.2.5). The following treatments must be discontinued before mobilization;

a. Pulse cyclophosphamide at least 3 weeks before mobilization

b. Antimalarials at least 2 weeks before mobilization

c. Methotrexate, mycophenolate mofetil, azathioprine, oral daily cyclophosphamide, leflunomide, cyclosporine or any other medication for lupus at least 1 week before mobilization

2.2 Research Eligibility Evaluation:

2.2.1 To confirm the eligibility status the following clinical, laboratory and radiologic screening assessments must be performed at the time of the evaluation at NIAMS:

a) Complete medical history and physical examination

b) CBC with differential, PT, PTT, ESR

c) Chem 20 panel

d) ANA, ENA, dsDNA, RF, C3, C4, lupus anticoagulant, anticardiolipin antibody, direct and indirect Coombs test

e) ABO typing, and infection disease markers: Anti-HIV-1/2, Anti-HTLV-I/II, Anti-HCV, Anti-HBc, HBSAg, Anti-HA, Anti-CMV, NAT (nucleic acid testing) for HIV, HCV and WNW, EBV, adenovirus, HSV and BK virus serology, RPR, Toxo-titers

f) Nephrology and routine urine analysis

g) Urinalysis

h) 24 hrs urine collection x2 for renal SLE pts only

i) TSH, free T3, free T4

j) Pregnancy test

k) Lumbar puncture if CNS SLE (unless contraindicated)

l) Kidney biopsy for renal SLE (unless contraindicated) and lung biopsy for pulmonary SLE (unless contraindicated). The Laboratory of Pathology will review all biopsy specimens of SLE involved organs. CCR; kidney biopsies will be sent to AFIP as per routine CC policy

m) Bronhoalveolar lavage to exclude active infection if pulmonary SLE

n) Chest CT for pulmonary SLE

o) MRI scans for CNS SLE

p) Neurocognitive testing, if necessary for evaluation for NPSLE

q) Other diagnostic studies as clinically indicated to evaluate SLE disease activity

r) STATCLOT

Additional testing to be performed as part of the eligibility screening at the NCI:

s) History and physical

t) Female reproductive endocrinology consultation

u) Chest x-ray

v) Social work consultation

w) Cardiac ECHO or MUGA for ejection fraction, ECG

x) Pulmonary function tests with DLCO measurements

y) Unilateral bone marrow aspirate and biopsy (CBC/Diff + Lym Pheno TBNK panel at the same day with bone marrow biopsy) for flow cytometry, conventional and FISH cytogenetics and research studies

z) Other specific serum or urine diagnostic studies clinically appropriate for the health status evaluation

2.2.2 Following studies should be completed at the NCI prior to starting the priming regimen (duplicate testing will not be performed, unless medically indicated, if the previous test was done within two weeks):

a) History and physical
b) CBC, differential, acute care panel, hepatic panel, mineral panel
c) ANA, ENA, dsDNA, RF, C3, C4, lupus anticoagulant, anticardiolipin antibody
d) Nephrology and routine urine analyses
e) Urinalysis
f) Pneumococcal and tetanus serum titers
g) FSH, LH, Estradiol (female) and Testosterone (male), Inhibin A and B
h) Nutritional assessments consult
i) Social work consults
j) Dental consult
k) HLA-A, -B, and –DR typing
l) PPD
m) Placement of central venous line
n) Lymph node ultrasound survey and fine needle aspirates for conventional cytology and research studies
o) FDG-PET scan focused on the lymphoid organs
p) Leukapheresis for the peripheral blood mononuclear cells for baseline research studies
q) Storage of mononuclear cells, RNA and sera for future research
r) Quality of life assessments
s) Neurocognitive testing: every patient able to cooperate will undergo neurocognitive testing with ANAM, a computerized neurocognitive test that is not language dependent. Cooperative patients with good English will also have a set of traditional neurocognitive tests. We will apply a panel that is used in a study evaluating neurocognitive function in SLE
t) SLEDAI, SLAM, SLICC-ACR damage index.

2.3 Patient Registration

2.3.1 The patient's entry date on protocol is considered to be the day that treatment consent forms have been signed by the patient. The treatment start date is considered to be the day the recipient begins her/his priming regimen.

2.3.2 Authorized staff must register an eligible candidate with Central Registration no later than 24 hours after patient has signed consent forms. A registration checklist must be completed confirming eligibility and faxed to the NCI Central Registration Office at 301-480-0757. After confirmation of eligibility at the NCI Central Registration Office, the NCI Central Registration Office staff will call Pharmacy to advise them of the acceptance of the patient on the protocol. For registration, authorized staff should call 301-402-1732 between the hours of 8:30 a.m. and 5:00 p.m., Monday through Friday. A recorder is available during non-working hours.

2.3.3 Principal investigator will hold a formal meeting of the coinvestigators that will summarize and document the eligibility criteria for each patient prior to entry onto study. Meeting participants will include at minimum: principal investigator, clinical chairperson, one study physician with marrow transplant expertise, one study physician with SLE expertise, one physician with primary lupus-affected organ expertise; study research nurse, and an external physician with SLE expertise who is not a co-investigator on this study (Sandy Via, MD, of University of Maryland agreed to fulfil this function for the purpose of this protocol as a primary external SLE expert). In case the primary external expert is not available or in an unusual situation where the external expert is at the same time a referring physician, an alternate independent SLE expert will be designated by the PI or the clinical chairperson. The study eligibility will be properly documented and signed by all meeting participants. If necessary, the meeting can also include communication over the phone in the form of a conference call – as long as all necessary documentation was provided to the participating investigators and experts.

3 STUDY IMPLEMENTATION

3.1 Study Design

This is a phase II pilot study of autologous hematopoietic stem cell transplantation with the goal to enroll 14 patients. The treatment schedule consists of two parts, the priming regimen and the conditioning regimen with transplant.

3.2 Drug Administration

3.2.1 Priming regimen:

Drug	**Dose**	**Days**
Methylprednisolone*	1000 mg i.v. over 30 minutes	on day 1
Rituximab	375 mg/m^2 i.v	on days 1 and 4
Cyclophosphamide	2000 mg/m2 i.v. infusion/2 hours	on day 2
Mesna	600 mg/m2 i.v. immediately prior to cyclophosphamide and repeat at 4 and 7 hours after the first dose	on day 2
Filgrastim (G-CSF)	10 µg/kg/day s.c.	starting day 6**

*An alternative parenteral glucocorticoid may be used at equivalent doses depending on the availability. **G-CSF is given daily approximately at 7.00 AM until the last day of leukapheresis, G-CSF can be stopped earlier if total WBC exceeds 100,000/µL.

3.2.2 Hydration with 0.9% NaCl i.v. will begin at 500 cc/hr and run for one hour before starting cyclophosphamide, then will be continued at 200 cc/hr during cyclophosphamide and then at 250 cc/hr for additional 5 hours after finishing cyclophosphamide infusion. Mesna will be given by i.v. route over 15-30 minutes, 600 mg/m^2 immediately prior cyclophosphamide (time 0), and then 4 and 7 hours after the start of the cyclophosphamide.

- Chem 20 including serum potassium level will be checked before and 8 hours in to hydration. Serum potassium will be supplemented as clinically necessary. During hydration, 20 mg of furosemide can be administered once by i.v. route to maintain diuresis, with additional doses of furosemide to be given as needed for weight gain due to fluid retention. In general, furosemide doses should be separated by at least a four-hour observation interval. Routine hematologic monitoring, cardiac monitoring, and urinalysis will be performed as deemed clinically necessary.

3.2.3 Patients will visit the DTM Dowling Apheresis Clinic during the week prior to apheresis for a venous assessment. Prior to starting the priming regimen all patients will be scheduled for placement of an apheresis compatible central venous catheter by the Vascular Access Device (VAD) service of the Critical Care Medicine Dept (10D).

3.2.4 Apheresis will start on the day the WBC first exceeds >5,000/μL. 15-25 liters will be processed daily. At least one hour will elapse between G-CSF administration and the beginning of leukapheresis. Apheresis and filgrastim administration will continue daily until a target cell dose of at least 3×10^6 CD34+ cells/kg-recipient weight is obtained after completion of cell processing and allocation of research samples, or until 3 consecutive procedures are performed, whichever comes first. Starting at latest on day 10 after the priming dose of cyclophosphamide patients will be started on early morning monitoring of CBC and CD34 counts so that samples arrive to the laboratory by 6.30-7.00 AM and the apheresis can start by 7.30-8.00 AM. Unless the WBC is rising very rapidly from the prior day, leukapheresis for stem cell collection will not start unless the circulating CD34 cell count is $\geq$8/μL. Maximal total number of peripheral blood stem cell collection procedures will be 3.

3.2.5 T-cell depletion will be performed in the cell processing laboratory of the Department of Transfusion Medicine using the Baxter Isolex 300i Stem Cell Collection System version 2.5, for CD34 positive selection. The infused progenitor cell target dose will be $\geq 3 \times 10^6$ CD34+cells/kg, it will be attempted not to infuse $>8 \times 10^6$ CD34+cells/kg.

Transplantable minimal progenitor cell dose to be infused will be 1.5×10^6 CD34+ cells/kg, patients who do not collect minimal number of stem cells will not be evaluable for the primary endpoint and will go off study. Samples of the stem cell product will be obtained for extended immunophenotyping including CD3, CD4, CD8, CD19, CD20, CD56, CD14, CD33, and CD34 surface markers and for the FISH cytogenetics. Cells will be cryopreserved and stored according to DTM policy and procedure until needed for infusion.

3.2.6 **Conditioning Regimen and Transplant:** Three weeks after administration date of cyclophosphamide patients will become eligible to start the transplant conditioning regimen. Any non-hematologic toxicities of mobilization must resolve to at least < grade 2 in order to proceed. If more than 14 days of additional recovery time is required for resolution of toxicity or other medical complications (for example documented infection), PI approval is required prior to initiation.

Transplant Conditioning Regimen

Drug	**Dose and administration**	**Days***

Rituximab	750 mg/m2 i.v. infusion	Transplant Day -7
Fludarabine	30 mg/m2 per day i.v. infusion over 30 minutes, daily for 4 days	Transplant Days -6, -5, -4, -3
Cyclophosphamide	1200 mg/m2 per day i.v. infusion over 2 hours, daily for 4 days	Transplant Days –6, -5, -4, -3
Mesna	1200 mg/m2 per day continuous 24 hr i.v. infusion, daily for 4 days, start concurrently with the start of cyclophosphamide	Transplant Days –6, -5, -4, -3
Stem cell infusion	Product will be infused rapidly i.v. after premedication with diphenhydramine 25-50 mg orally or i.v.	Day 0
Filgrastim (G-CSF)	5 µg/kg/d s.c.	Day +1 until ANC >500/µL

3.2.7 Hydration during the conditioning regimen:

a) Hydration will be initiated 12 hours prior to cyclophosphamide infusion (on day –7 of the transplant), consisting of 0.9% sodium chloride supplemented with 10 mEq/liter potassium chloride (KCl) at an initial rate of 100 ml/hour. For patients with poor oral intake, the rate of hydration may be increased as clinically indicated to meet fluid requirements. Hydration will continue until 24 hours after the last cyclophosphamide dose has been completed.

b) During hydration, serum potassium level will be monitored every 12 hours. If serum potassium is > 4.5 mEq/l, KCl will be removed from the saline infusion. If serum potassium is < 3.0, KCl concentration in the saline will be increased to 20 mEq/L.

c) During hydration, 20 mg of furosemide will be administered daily by IV route to maintain diuresis, with additional doses of furosemide to be given as needed for weight gain due to fluid retention. In general, furosemide doses should be separated by at least a four-hour observation interval.

d) During hydration, if fluid intake exceeds urine output by greater than 500 ml during an 8-hour period, an additional 20 mg of furosemide will be administered.

e) Routine hematologic monitoring, cardiac monitoring, and urinalysis will be performed as deemed clinically necessary.

3.3 Treatment modifications

Patients with GFR 30-70 ml/min/1.73m^2 will have fludarabine dose reduction of 20%.

3.4 Protocol Evaluation/Monitoring

3.4.1 The following studies will be obtained in the patient during hospitalization for transplantation:

3.4.1.1 CBC and differential count twice daily.

3.4.1.2 Chem 20 twice daily during the conditioning, then daily.

3.5 Concurrent therapies

3.5.1 Corticosteroids:

3.5.1.1 Pre-HSCT: all immunosuppressive and disease modifying drugs will be discontinued before mobilization. Flares in disease activity before HSCT will be controlled with corticosteroids as clinically indicated. The daily dose of prednisone will be maximized at 1mg/kg with 80 mg/day being the maximal dose; if a patient requires higher doses, they will be treated with pulse methylprednisolone (1 gm methylprednisolone i.v. for 1-3 days).

3.5.1.2 Post-HSCT: corticosteroids will be tapered according **to Appendix C**. Tapering can be started as early as clinically feasible as but not later than Day 28 post transplant. The dose of prednisone will be decreased every two weeks by the equivalent of 10 mg/day for doses > 35mg/day and by the equivalent of 5mg/day for doses ≤35 mg/day. After reaching 20 mg/day a taper to alternate day dosing will be attempted. If patients show no clinical sign/symptoms of lupus activity, a more rapid taper can be attempted; if this results in reappearance of disease activity the dose can be increased to the dose shown **in Appendix C** Table for that time point. This will not be considered a flare. The taper can be suspended in case of signs and symptoms of adrenal insufficiency or severe steroid withdrawal. Stress doses of steroids can be used for intercurrent infections or other stressful situations, when clinically indicated. The goal is to discontinue prednisone unless signs and symptoms of adrenal insufficiency or severe steroid withdrawal develop.

3.6 Off Study Criteria

3.6.1 Patients will be removed from protocol for any of the following reasons:

a) Inadequate stem cell collection (Section 3.2.4).

b) Irreversible non-hematologic toxicity (> grade 3) during the priming.

c) The patient refuses to continue therapy or withdraws consent.

d) No response to therapy (see 5.2.6). Patient can still be followed every 6 months up to five years for survival and the SLE treatment history data.

e) In addition, the patient may at any time be removed from protocol at the principal investigator's discretion, if the PI deems the patient to be at unacceptable risk to remain on study. Reasons for this action may include (but are not limited to) disease progression with declining organ function/performance status before transplantation; inadequate family/caregiver support; noncompliance.

3.6.2 Authorized physicians must notify Central Registration office when a patient is taken off study.

3.7 Post SCT Evaluation

3.7.1 After completion of therapy the patient will be followed for potential complications related to HSCT. After discharge from the transplant inpatient service, the patient will be followed at least twice weekly in the NCI outpatient setting until Day +28 and then at least weekly until discharge home. The patient will be seen thereafter in follow-up at the Clinical Center to evaluate disease status, immunological status and late problems related to HSCT, at minimum monthly for the first six months, and then at 9, 12, 18, and 24 months post-transplant, then yearly up to five years post-transplant, or at the time of the disease flare or progression. Evaluation of immunological status will include assessments of peripheral blood lymphocyte and mononuclear cell subpopulations by flow cytometry to determine the efficacy of lymphocyte depletion and the time course of lymphocyte reconstitution. Enclosed is the flow-chart summarizing the standard pre and post-transplant evaluations:

3.7.2 Study Calendar

Study Evaluations	Screening NIAMS NCI	Baseline *	After Priming	Conditioning Day -7	Conditioning Day -1	Scheduled Follow-up**
History and physical	x x	x		x	x	x
CBC and differential	x x	x		x	x	x
ESR	x	x		x	x	x
PT/PTT	x					
Chem 20 panel	x	x		x	x	x
ANA, ENA, dsDNA, RF, C3,	x	x		x	x	x
ABO typing, TTV viral screen	x					
Lym Pheno TBNK panel	x					At 4, 5, 7, 8 mo
Lupus anticoagulant	x	x		x		x
Anticardiolipin antibody	x	x		x		x
STATCLOT	x					
Direct and indirect Coombs test	x					x
Urinalysis	x	x		x	x	x
24 Hrs URINE if renal SLE	x (Twice)					At 3, 6, 9, 12, 18, 24 mo
TSH, free T3, free T4	x					
Pneumococcal and tetanus Abs		x				At 12 mo & before boost
Fertility consult	x					
Pregnancy test	x	x				
FSH, LH, Estradiol (f), Testosterone (m) (l)		x		x		x
Inhibin A&B		x				At 24 months
Chest x-ray	x					Q month 1st 3 mo
Nutritional assessment		x				x

Social work consult	x	x				x
Dental consult		x				
HLA typing		x				
PPD test		x				
Cardiac ECHO/MUGA & ECG	x					
Pulmonary function & DLCO	x					x if lung SLE
Lumbar puncture for CNS SLE	x					At 1, 3, 6, 9, 12, 18 and 24 mo
Marrow biopsy	x				x	At 6,12, 24 mo
Marrow cytogenetics	x					At 6,12, 24 mo
Lymph node FNA/US guided		x				At 6, 12, 24 mo
PET scans		x			x	At 1, 6, 12 and 24 mo
Kidney biopsy if renal SLE	x					At 12 mo
Lung biopsy if lung SLE	x					
BA lavage if lung SLE	x					At 12 mo
Chest CT for lung SLE	x					At 1, 3, 6, 9, 12, 18 and 24 mo
MRI scans for CNS SLE	x			x		At 1, 3, 6, 9, 12, 18 and 24 mo
Store serum		x		x	x	"
Store PBMNC		x		x	x	"
Blood research studies	x	x	x	x	x	" (please see footnote#)
Stem Cell Collection			x			
Quality of life questionnaires		x				"
SLEDAI and SLAM		x		x	x	At 1, 3, 6, 9,12,18 and 24 mo
SLICC-ACR Damage Index		x				At 6, 12, 18, and 24 mo
HAQ, SF-36, KFFS		x		x	x	At 3, 6, 9, 12, 18 and 24 mo
Neurocognitive testing ANAM		x				At 12 and 24 mo
Leukapheresis for research***		x				At 1, 3, 6, 9, 12, 18, 24 mo and relapse
*Duplicate testing will not be performed unless medically indicated if the previous test was done within two weeks. Please note: None of the optional invasive tests done solely for research indication will be offered to patients younger than 18 years of age						

(day-1 marow biopsy, PET scans, lymph node aspirates, 12 mo kidney biopsy and 12 mo BAL)

**Post-transplant follow-up evaluations are monthly during first six months, then at 9, 12, 18, 24 months then yearly for five years, or at the time of documented flare or progression.

*** For leukapheresis to collect cells for *in vitro* research five liters will be processed by continuous leukapheresis (two needle, two arm device) in patients with adequate bi-lateral peripheral venous access. In patients who do not have adequate peripheral venous access, two liters of blood will be processed by discontinuous flow apheresis (single-needle device). The goal is to obtain approximately 2×10^9 MNCs per procedure[#]. An additional peripheral blood draw for research will take place at 2 weeks, 7, 8 months post-transplant. Also, if at monthly evaluations during first nine months absolute B-cell count in peripheral blood gets for the first time >25/μL, an extra research blood draw will be repeated for studies of early B-cell recovery.

3.7.3 Patient flow coordination between two institutes Candidates for this study will be identified through the NIAMS Lupus clinic. The eligibility will be assessed by the primary rheumatology team in consultation with relevant subspecialists and thereafter by the NCI transplant team. After confirmation of eligibility informed consent signature will be obtained and patients' care will be transferred to the NCI primary team until the discharge from the transplant inpatient and day-hospital service, which is anticipated to occur by the day 100 post-transplant. While patient is under the care of the NCI primary team, a rheumatology team member will attend transplant inpatient rounds at least 2x/weeks on the inpatient service and at least 1x/week on the outpatient service. All SLE-specific clinical response evaluations will be conducted by the NIAMS team. At the points when patients return to the NIH for the routine post-transplant long-term follow-up (beyond 100 days), evaluations will be scheduled in both NIAMS and NCI facilities. The NCI research team will hold the ultimate responsibility that all protocol-related data are collected, recorded on case report forms and stored as planned.

3.8 Research studies of SLE and transplantation

Research studies will focus on the degree and nature of the immune cell depletion and the regeneration of the immune system following immunoablation and autoHSCT. Special emphasis will be put on the evaluating B- and T-lymphocyte and dendritic cell (DC) subsets and their functional status. Clinical correlations with lupus activity will be made. We will attempt to obtain a detailed and comprehensive picture of the degree and nature of the immune cell depletion and immune reconstitution following HSCT by analyzing not only the changes in peripheral blood but also the bone marrow and secondary lymphoid organs, such as lymph nodes and thymus either directly or indirectly. To achieve these goals, we will collect routine CBC at each time point TBNK assay and phenotyping is done in addition to the other research blood samples throughout the protocol and collect PBMCs for research by apheresis at pre-specified time-points. Bone marrow biopsy will be done at baseline and at 6,12 and 24 months. To evaluate the role of lymph nodes, we will perform ultrasound surveillance and fine needle aspiration at baseline and at 6, 12 and 24 months. In addition, FDG-PET studies using GE Advance Scanners will be done to evaluate the metabolic and immunologic activity of peripheral lymph nodes and the thymus before priming, before conditioning and 1, 6, 12 and 24 months post transplant. This is based on the results of a previous study that suggested that activity in the lymph nodes is increased in all lupus patients but only patients with active disease have increased FDG uptake in the thymus. PBMCs, bone marrow samples and lymph node aspirates will be characterized by multiparameter FACS analysis, functional studies and mRNA microarrays. Cytogenetic studies using FISH will be done at baseline, 6, 12 and 24 months after the transplant to detect small clones of abnormal myeloid progenitor cells.

Specific research studies:

1 T- and B-lymphocyte, NK and DC subsets and their activation status will be determined

by multiparameter FACS analysis using a panel of B- and T-cell and myeloid-cell specific antibodies from PBMCS, bone marrow and fine needle aspirates of lymph nodes.

2 Detection of TRECs from PBMCs to assess the number of recent thymic emmigrants

3 Assays of T- cell, B-cell and NK function.

4 B-cells: ELISPOT assays to detect anti-dsDNA producing plasma cells.

5 RNA isolation: mRNA from whole blood and B- and T-cell subsets will be isolated and tested with mRNA micro-arrays for change in expression of inflammatory molecules.

6 Serum cytokine levels using multiplex cytokine assays.

7 Urine cytokine levels.

Serum, urine, CSF , cells and RNA will be stored and may be used for future studies evaluating markers of lupus activity and/or imunnoregeneration. A more detailed description of research studies is given in **Appendix A**. Please refer to **Appendix M** for a description of the processes used in this study for the tracking and storage of human specimens. The PI will report any loss or destruction of samples to the IRB.

4 SUPPORTIVE CARE

4.1 Infection Prophylaxis

See **Appendix B** for detailed description. All patients will receive:

4.1.1 Prophylaxis against Pneumocystis carinii pneumonia, prior to priming and continuing until transplantation, and resuming at the time of neutrophil and platelet recovery. PCP prophylaxis will continue until 12 months, steroids are tapered and CD4 counts >200/μL, whichever comes later. Trimethoprim/sulfamethoxazole is the preferred regimen, but alternative regimens are available for patients with a history of adverse reactions to Bactrim. See Appendix B, Section 9.1.1 for details.

4.1.2 Caspofungin during the period of neutropenia post-transplant. See Appendix B, Section 9.1.2 for details.

4.1.3 Valacyclovir for prophylaxis against herpes simplex virus and varicella zoster virus infection/reactivation. This therapy will start at the time of chemomoblization, continuing through transplantation until 12 months, steroids are tapered, and CD4>200/μL. See Appendix B, Section 9.1.3.1 for details.

4.1.4 If pre-transplant serology for Cytomegalovirus (CMV) is positive, monitoring for CMV reactivation by weekly testing for CMV antigenemia and clinical evaluation will be done. Positive antigenemia will be treated according to the schema in the Appendix B, Section

9.1.3.2. Weekly monitoring will continue through day +100 and until CD4 count >200/μL – see Appendix B).

4.1.5 Monitoring for Epstein-Barr virus reactivation by polymerase-chain reaction testing, and treated preemptively with a single dose of rituximab as outlined in the appendix B, section 9.1.4.

4.1.6 Intravenous antibiotics while neutropenic post-transplant. Ceftazidime is preferred as described in Appendix B, Section 9.1.5.

4.1.7 PPD testing prior to starting the chemomobilization and if positive will be treated with INH and pyridoxine as outlined in the Appendix B, section 9.1.6.

4.1.8 Standard vaccinations beginning 12 months post-transplant. See Appendix B, Section 9.1.7 for details.

4.2 Menses Suppression and Contraception

Menstruating pre-menopausal women who have not undergone hysterectomy will be placed on adequate suppression regimen as recommended by the gynecology consultants. Female transplant recipients will be advised to use contraception for at least 2 years after transplantation and to additionally have their male partners use condoms. Male transplant recipients will be advised to use contraception, preferably condoms, for 6 months after transplantation.

4.3 Blood Product Support

Patients will receive packed red blood cells and platelets as needed to maintain Hb > 8.0 gm/dl, and plts > 10,000/mm^3 (or higher, if clinically indicated). All blood products with the exception of the stem cell product will be irradiated.

4.3.1 Leukocyte-reduction filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused leukocytes and decrease the risk of CMV infection.

4.4 Nutritional Support

When mucositis or other patient condition prevents adequate PO intake, parenteral hyperalimentation will be instituted and discontinued under the direction of the dietary service. Oral intake will resume when clinically appropriate under the supervision of the dietary service of the Clinical Center.

4.5 Anti-emetics

Anti-emetic usage will follow Clinical Center Guidelines as well as recommendations from the Pharmacy service.

4.6 Hematopoietic Growth Factor Support

All patients will start filgrastim (GCSF) 5μg/kg/day on day +1. The dose will be given subcutaneously (SC). G-CSF will be continued until the absolute neutrophil count (ANC) is > 500/μL, it can be resumed if ANC drops or stays below 1000/μL per discretion of the attending physician if clinically indicated.

5 DATA COLLECTION AND EVALUATION

5.1 Data Collection

Data will be prospectively collected and entered into the Center for Clinical Research database. All laboratory adverse events will be collected from grade ≥2. Protocol-specific case report forms will be developed and filled out by the NIAMS or NCI teams depending on the phase of the protocol and the type of evaluations (all lupus-specific evaluations such as SLEDAI will be performed exclusively by the rheumatologists). Working relationships between the teams are described in section 3.7.2. NCI research team is ultimately responsible for the appropriate data collection and storage as defined by the protocol.

5.2 Response Criteria

5.2.1 **Primary efficacy endpoint:** Continuous relapse-free complete clinical response at 24 months post-transplant. Patients will be considered to achieve this endpoint if they fulfill the criteria for complete clinical response at anytime and maintain it for at least three months.

5.2.2 Definitions:

I. Complete clinical response:

- Complete response in the target organ and no clinical signs of active lupus as determined by a SLEDAI score of ≤ 3
- Prednisone ≤10mg/day at 6 months and ≤5mg/day at 12 months or later

II. Partial response:

- At least partial response in the target organ (defined on an organ basis)
- At least 50 % improvement in general disease activity as measured by SLEDAI
- At least 50% decrease in prednisone dose at 6 months and ≤10mg/day at 12 months or

later

III. No response:

- No response in target organ
- Inability to taper prednisone due to increased disease activity
- Need for additional immunosuppressive therapy

5.2.3 Secondary efficacy endpoints:

- Time to flare-free complete response
- Time to complete clinical response (in months)
- Time to partial response (in months)
- Time to relapse (in months)
- Improvement in general disease activity measured by SLAM and SLEDAI
- Time to normalization of dsDNA and complement levels
- Pain scale, quality of life
- Functional assessment by HAQ score
- Neurocognitive testing and depression scale

5.2.4 Organ-specific efficacy definitions:

Renal:

Complete response

a. Stable or improved renal function: <30% increase in serum creatinine or <20% decrease in GFR and

b. Inactive urine sediment (< 10 RBC/hpf and no cellular casts) and

c. Improvement in proteinuria

   i. If nephrotic range proteinuria at baseline, decrease in proteinuria to < 1.0 g/day or

   ii. If non-nephrotic range proteinuria at baseline: proteinuria < 0.5 gm/day

Partial Response

a. Stable or improved renal function, as above and

b. Improvement in proteinuria (as above) with a persistently active urine sediment

or

Inactive urine sediment and non-nephrotic proteinuria and >50% decrease from baseline

No response

a. Not fulfilling complete or partial response

Relapse

a. Minor: increase in hematuria and/or > 50% increase in proteinuria and stable renal function (as defined above)

b. Major: reappearance of sustained (repeatedly present at least 1 month apart) nephritic sediment with biopsy proven proliferative nephritis or for patients where a biopsy is contraindicated: reappearance of sustained (repeatedly present at least 1month apart) nephritic sediment with sustained > 30% increase in serum creatinine or > 20% decrease in GFR

5.2.5 CNS Lupus

Complete response:

Stable or improved clinical exam defined by the Scripps Neurological Ration Scale (NRS), (**Appendix H**) and no objective signs of active CNS inflammation.

a. Stable or improved clinical exam: no more than a 5 point worsening on the NRS required on two consecutive exams (3 months apart). The presence and frequency of seizures before HSCT will be documented. If the seizures disappear, this will be considered a response. If seizures persist, but do not increase in frequency, it will be assumed that this is a response to treatment assuming that other objectives measure of CNS inflammation, such as MRI and/or CSF normalize. If the seizure frequency and/or severity increase, we will consider this a treatment failure. De novo occurrence of seizures will similarly be considered a failure.

b. No objective signs of CNS inflammation: No signs of inflammation on MRI and/or CSF

(Normal CSF protein or cell count within normal range with the protein/IgG ratio equal or improved compared to pretreatment).

Partial response: Not meeting the criteria for either complete or no response

No response/failure:

a) Persistence or re-appearance of any of the signs/symptoms that

define active CNS lupus b) NRS worsening of >15 points c) Any requirement for specific treatment of CNS lupus-associated signs/symptoms, e.g. seizures Relapse

a. Minor: Anything less than major relapse

b. Major: Longer than 24 hours appearance/re-appearance of signs/symptoms compatible with CNS lupus. These findings should not be otherwise attributable, i.e. to interfering infections, compromise of pulmonary, renal function, or other Pulmonary:

Complete response:

♦ Improvement in DLCO by >50% or normal DLCO and no evidence of alveolitis on high resolution CT Partial response: ♦ stable DLCO (<150% and > 85% of baseline) and no evidence of alveolitis on high resolution CT

No response:

♦ ≥15% worsening in DLCO or evidence of alveolitis on high resolution CTRelapse a. Minor: any of the following: new onset or worsening dyspnea on exertion, <20% decrease in DLCO, new infiltrate of unknown significance on chest CT

b. Major: any of the following: pulmonary hemorrhage, >20% decline in DLCO, dyspnea at rest, need for oxygen, respiratory failure, new or worsening alveolitis on high-resolution CT

For both minor and major relapse other causes of pulmonary disease such as pulmonary emboli and infections have to be excluded Hematologic:

Complete response:

♦ Platelet count > 100,000/mm^3 and normalization of Hb with no signs of hemolysis and independent of transfusions or any other therapy for at least three months

Partial response:

♦ self-sustained (platelet counts >50,000/mm^3 without signs of bleeding and Hb > 10g/dL No response:

♦ not meeting criteria for complete or partial response Relapse:

a. Minor: platelets >20,000-100,000 without signs of bleeding or Hb > 8 g/dL – normal

b. Major: platelets <20,000 or <100,000 with bleeding or requiring transfusions to maintain Hb > 8.0g/dL or to treat symptoms of anemia

5.2.6 Treatment of relapses (flares)

1. Indeterminate or minor:

a. Repeat evaluation in 1-2 weeks

b. Increase prednisone up to 0.5 mg/kg/day for 2 weeks, taper prednisone to baseline over 4 weeks

2. Major: treatment failure Treat as clinically indicated

5.2.7 Assessment of response

Response to therapy will be assessed every 3 months following transplant. At each time-point patients will be classified as

- Complete clinical responders or
- Partial responders or
- Non-responders

based on the criteria defined previously. The final outcome of the patients will be determined at 24 months. They will be classified as

- Responders, if they fulfill criteria for relapse-free complete clinical response
- Partial responders: if they fulfill criteria for partial response and have maintained it for at least 3 months or they had one (1) episode of indeterminate/minor flare and maintained partial or complete clinical response for at least 3 months (as above)
- Non-responders: if they never achieved partial or complete response criteria or they had a major flare or they had more than one indeterminate/minor flare that required increase in their prednisone dose.

5.3 Toxicity Criteria

This study will use the CTC version 3.0 for toxicity and adverse event reporting. A copy of the CTC version 3.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov). Known toxicities for each agent used in this protocol are listed in section 8.0 of this protocol.

5.4 Statistical Section

The primary objective of this study is to determine if the strategy identified in section 3.1 is associated, in a pilot fashion, with a sufficiently high success-rate to warrant further study in subsequent trials. The primary efficacy endpoint will be continuous relapse-free complete clinical response at 24 months post transplant, which will be evaluated as a dichotomous variable. Using an exact binomial test from nQuery Advisor, if 14 subjects are enrolled, there is 84% power to be able to detect the difference between a null proportion of 70% with success and a more desirable alternative as high as 95%, using a one-sided 0.05 alpha level test. In other words, if we conducted this study repeatedly, and if the true major response rate were 95%, then with 14 patients, 84% of the time, we would obtain the result that the lower bound of a one-sided 95% confidence interval exceeded 70%. Since we are also concerned about the possible toxicity associated with the proposed therapy, this will also be closely monitored and used to determine the suitability of continuing to enroll patients onto the trial. It would be expected that there is no more than a 5% probability of any individual patient experiencing an early non-lupus related death on the study. As such, the probability of observing 0 such deaths in 14 patients would be 49% and 1 such death in 14 would be 36%. Thus, there is approximately 85% probability that no more than one such death would be observed in 14 patients if the true probability of this happening to any individual patient were 5%. If one such death occurs prior to accrual of the 14 patients, then this would result in a re-evaluation of protocol procedures, but not termination of accrual. If there are two early non-lupus related deaths on the study, the trial will cease enrollment. This more likely indicates that the true probability of these events is perhaps 20% rather than 5%, since the probability of 2 or more events in 14 is 80% if the true probability were 20%.

The evaluation for continuous relapse-free complete clinical response at 24 months post transplant will be based on 14 patients who are able to mobilize sufficient numbers of stem cells. An occasional patient who is not able to mobilize sufficient stem cells to infuse at least 1.5×10^6 selected CD34+ cells/kg will be taken off study and considered inevaluable for response. However, the trial will terminate early due to toxicity if there are two early non-lupus related deaths in any enrolled patients, regardless of whether they are evaluable for response or not. In order to assure 14 evaluable patients, a total of up to 16 patients may be enrolled.

All patients will be monitored closely by the principal investigator and other clinical personnel associated with the trial. Should any severe toxic events arise other than that specifically referred to above, the investigators will carefully decide how to proceed with the treatment of any subsequent patients on the protocol in order to protect patient safety.

All secondary evaluations of biologic markers and other similar laboratory determinations will be done with exploratory intent. In view of the limited number of subjects to be included in this pilot trial, non-parametric evaluation of changes from baseline will be performed, as well as descriptive analyses. In any cases in which p-values are reported from these analyses, they will be reported as being unadjusted for multiple comparisons, and will be carefully noted to be the

result of hypothesis generating studies, requiring replication in larger populations in order to confirm any effects noted.

As noted in section 5.2.3, a number of secondary efficacy endpoints have been proposed. All endpoints relating to time to an event will be analyzed via the Kaplan-Meier method. Other measures which involve baseline and then follow-up evaluations will be done by subtracting the baseline evaluation from each follow-up evaluation (see section 3.7.1 for details of the follow-up points), and using paired non-parametric tests to compare the changes. Since these evaluations will be considered secondary, the results will not be adjusted for multiple comparisons, and will be carefully stated as being the result of exploratory evaluations.

It is expected that approximately one such patient per month may be enrolled onto this trial; thus accrual should be completed in 1.5 years, depending on the referral of such patients to the NCI.

5.4.1 Data and Safety Monitoring Plan

5.4.2 In accordance with NCI IRB policies, this pilot study does not require independent monitors or review by a Data Safety and Monitoring Board. However, an independent 3-person review committee has been established to review all deaths to determine if those are related to the underlying disease or to the experimental treatment. The committee consists of 2 lupus experts (Mark Gourley NIEHS, Peggy Crow, Hospital for Special Surgery, NY) and 1 bone marrow transplant expert (Harry Malech, NIAID). In exceptional special circumstances (for example but not limited to - inability to serve on the panel, or in an unusual situation where the SLE expert is at the same time a referring physician), PI in consultation with the clinical chairperson or one of the scientific chairpersons can designate a substitute for a member of this committee and inform immediately the IRB about this change. Also it is anticipated that deliberations of this committe will be done by a conference call under provision that all necessary and requested documentation on the respective patient is provided in advance. The committee will provide its assessment to the PI who will forward it to the IRB and FDA, as appropriate. If the death is considered to be at least probably related to the experimental therapy the protocol procedures will be re-evaluated but enrollment will not be terminated. If there are two early non-lupus related deaths on the study, the trial will cease enrollment.

5.4.3 The PI and Clinical Chairperson will provide continuous, close monitoring, with prompt reporting of unexpected or serious adverse events to the IRB and FDA (Section 7.1). On a weekly basis, the PI, Clinical Chairperson, Protocol Research Nurse, and Transplant Coordinator will review clinical and laboratory data from each of the transplant recipients. A complete summary of patient outcomes will be provided annually to the IRB and FDA. Protocol Support Office of the Medical Oncology Clinical Research Unit, CCR, NCI will assist with annual internal auditing of subject case records. After accrual

of 3 subjects, the Protocol Support Office will also perform a protocol activation audit to review adherence to the informed consent procedures, eligibility documentation, and initial treatment.

5.4.4 The expected heterogeneity of study participants with respect to risk factors for toxic events renders these parameters impractical to evaluate in a statistical manner; accordingly, formal stopping rules will not incorporate toxic events but will instead be monitored as described in statistical section.

6 HUMAN SUBJECT PROTECTIONS

6.1 Rationale for subject selection

Subjects with severe treatment refractory SLE will be enrolled. The gender distribution (F:M) for lupus is 9:1 and the peak age of onset is in the third and fourth decade. SLE is ~3 times more common in African-Americans therefore women and minorities are expected to be well represented in this study. If the referral base cannot provide a population that is representative of the population of the US, then alternative methods for recruitment will be considered such as contacting our referring physicians and request the appropriate study subjects and/or contacting health professionals in urban tertiary referral centers for African-, Hispanic-, or Asian-American subject recruitment.

6.1.1 Participation of children

This is a study that involves greater than a minor increment over minimal risk with the prospect of direct benefit to subjects. Minors over the age of 15 will be enrolled in the study. The clinical course of patients older than 15 years is similar to adult patients and we have experience with this age group. SLE in patients younger than 15 years of age has a different course and there is no experience with fludarabine and cyclophosphamide or rituximab in lupus patients in this age group, therefore patients under 15 years of age will not be included in the study. If the safety of this treatment is established, younger patients may be considered for future studies.

6.2 Evaluation of Benefits and Risks/Discomforts

The patient population eligible may benefit from this treatment by achieving the remission of their disease or prolonged disease control. It is expected that this benefit may occur in at least half of the cases, in this population of patients who failed all available standard therapies. Achievement of durable responses is expected to result in improved survival, less disability, and better quality of life. This is however a more than standard risk procedure with estimated procedure-related mortality risk of up to 5%, there is also a theoretical but probably small risk of irreversible procedure-related injury to vital organs due to transplant-related complications such as unusual drug-induced side effects or infections or, in theory, a risk for bone marrow injury that would result in secondary myelodysplastic syndromes. Other more common risks would be

reversible side-effects of chemotherapy, antibodies, growth factors, mobilization, apheresis, or central-line placement. Transient negative psychological impacts of the transplant process on the patient and caregiver are possible. Adverse legal, social or financial impacts are not expected but all attempts will be made to anticipate them, prevent them and attenuate them from the very beginning and through the transplant process. Alternative treatments for these patients exist but none of them are likely to result in disease remissions, including continuation of pulse-cyclophosphamide with or without additional other immunosuppression. Another alternative are other investigational therapies as available. Confidentiality of the subjects will be protected by safe-record keeping and the avoidance of the usage of patient identifiers. In case of the adverse effects of the study related therapy all medical intervention will be provided at the site by the study team.

6.3 Risks/Benefits Analysis

The anticipated risks of this therapy are considered justifiable given the fact that for this patient population, no effective standard therapies exist. These patients are at a much higher risk for mortality and disability from their disease or its conventional treatments than of this therapy if the therapy provides the anticipated results. To ensure the anticipated risk/benefit ratio, the protocol has implemented careful monitoring of toxicities, stopping rules, as well as a data safety monitoring plan. In addition to the potential benefit to the patients enrolled in this study, there is a strong potential for a broader benefit to future patients by acquiring a better knowledge of the systemic lupus disease process and ways to cure it. Some patients younger than 18 years of age (15-18 years) are anticipated to be enrolled in this study. Although the overall risk is considered to be greater than minimal, there is a strong potential for a therapeutic benefit and intent built in this protocol that is in accordance with federal guidelines for approvable research in children. In fact, due to the better prospects for complete immune reconstitution in younger individuals, children are those who are the most likely to have successful outcome with transplant therapy.

6.4 Consent and Assent Process and Documentation

The procedures and treatments involved in this protocol, with their attendant risks and discomforts, potential benefits, and potential alternative therapies will be carefully explained to the patient by the study team and the principal investigator personally. Particular emphasis will be placed on the subject's understanding that this treatment is investigational, that it may not result in the direct benefit, and that there is a short term risk form the procedure-related mortality of 5%. An informed consent document, and assent for individuals under age 18, will be obtained and signed from the patients by the PI (or one of his clinical associates), in the presence of a NIAMS and an NCI team representative and an independent monitor who is familiar with the ethical and regulatory requirements for informed consent. The independent monitor will ascertain that the subject understood that this is an experimental approach, which may not lead to direct benefit and may be associated with significant risks, including death. The independent monitors who agreed to observe and document the process of obtaining the consent are Ms

Mildred Wilson, RN and Beverly Barham RN. Both are research nurses at NIAMS with all necessary training and both are currently doing the observed consent for the neurocognitive lupus study at NIAMS. Thereafter, the Principal Investigator (or his designee) and the subject (and/or his/her legal representative) will sign the consent document in the presence of an independent witness. The independent monitor will also assist the investigators to identify subjects for whom additional assessment of decisional capacity would be appropriate. Such participants will then be evaluated for their decisional capacity to participate in this protocol by the consultation psychiatry liaison service. The original signed consent and assent documents will be kept with the patient's chart. The Data Management Section will also retain a copy of the informed consent and assent documents. A copy of the informed consent and assent documents will also be given to the patient.

7 DATA REPORTING

7.1 Adverse Event Reporting

This protocol is registered with the FDA under IND number 66,867 (Holder: Dr Steven Pavletic). The IND was withdrawn from the FDA in May 2012. Reports of adverse events will be made using the Common Terminology Criteria for Adverse Events version 3.0 for reference according to the guidelines published by the DCTD, NCI on June 10, 2003. A copy of the CTCAE v3.0 can be obtained from the CTEP home page (http://ctep.cancer.gov). Guidelines for AE reporting are summarized as follows:

7.1.1 The protocol PI will report to the NCI-IRB:

All serious adverse events (SAEs) that are **not** in the consent form, but are possibly, probably or definitely related to the research. An SAE is defined as an untoward medical occurrence that:resulted in a death;

was life-threatening; required or prolonged hospitalization; caused persistent or significant disability/incapacity; resulted in congenital anomalies or birth defects; or required intervention to prevent permanent impairment or death. All other deaths not included in the SAE category above.

All grade 3 and 4 (CTCAE) events that are not in the consent and that are possibly, probably or definitely related to the research, but not included in the SAE category above. All life-threatening or disabling events (i.e., grade 4, except for myelosuppression), which may be due to administration of the study drug(s). Since hospitalization or re-hospitalization are more a rule rather than exception in HSCT trials, hospitalization by itself will not automatically qualify as an adverse event [112]. Failure to recover neutrophil count >500/μL by day +21 post-transplant. Cases of secondary AML/MDS Reports must be received by the NCI-IRB within 7 days of notification of the event. Severe adverse events due to FDG-PET will be also reported to Dr. Peter Herscovitch, Building 10, Room 1 C 495 (301-451-4248). As of 2012, this protocol was removed from the FDG IND.

7.1.2 FDA reporting requirements include:

(1) Reporting any unexpected fatal or life-threatening adverse events associated with the use of the drugs by telephone (301-827-2090) or fax (301-827-2531) no later than 7 calendar days after initial receipt of the information (21 CFR 312.32(c)(2);

(2) Reporting any adverse experience associated with use of drugs that is both serious and unexpected in writing no later than 15 calendar days after initial receipt of information (21 CFR 312.32(c)(1); and

(3) Submitting annual progress reports (21 CFR 312.33).

7.1.3 The principal investigator will review each report and determine whether an adverse event is expected or unexpected and its possible relationship to the research study based on the information available in the protocol consent document, drug label, status of the underlying SLE disease and preexisting conditions.

7.1.4 All fatal events will be reported to an independent review committee within 10 days. The committee will determine if these events are related to the experimental treatment.

7.1.5 Information on adverse events that do not qualify for immediate reporting will be collected at 3-month intervals from the case report forms, C3D database and medical records. The following information, specifically, will be collected and reported:

- All Grade 2 events that are not in the consent form, but are possibly, probably or definitely related to the research;
- All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
- All Grade 5 events regardless of attribution;
- All Serious Events regardless of attribution.

The study personal will compile this information for the PI who will notify IRB if action is warranted. Beginning at the one year post transplant follow up, only those adverse events that meet the reporting criteria above will be recorded.

7.1.6 Annual progress reports will be submitted in parallel to the IRB and the FDA. Such annual reports will also include the summary of any adverse events on this protocol.

7.2 Record Keeping

7.2.1 All patients must have signed an Informed Consent, and an on-study confirmation of eligibility form will be filled out before entering on the study. Study data will be reported as case-report forms and study summaries.

8 PHARMACEUTICAL AND INVESTIGATIONAL DEVICE INFORMATION

8.1 CYCLOPHOSPHAMIDE (CTX, Cytoxan, NSC-26271)

8.1.1 Supply – Cyclophosphamide will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a lyophilized powder in various vial sizes.

8.1.2 Preparation - will be reconstituted with sterile water for injection to yield a final concentration of 20 mg/ml as described in the package insert.

8.1.3 Storage and Stability - The vials are stored at room temperature. Following reconstitution as directed, solutions of cyclophosphamide are stable for 24 hours at room temperature, or 6 days when refrigerated at 2-8° C.

8.1.4 Route of Administration - The cyclophosphamide used in this regimen will be mixed in 100 ml normal saline and given as an IVPB over 2 hours.

8.1.5 Toxicities:

a) Nausea and vomiting - variable; symptomatically improved with standard anti-emetics and/or benzodiazepines [e.g., lorazepam].

b) Water retention – cyclophosphamide may rarely provoke the syndrome of inappropriate antidiuretic hormone secretion and resultant hyponatremia, usually manifested 12-48 hrs after IV administration, necessitating frequent accurate assessment [q 1-2 hrs] of intake, urine output and urine specific gravity. This effect can be counteracted by furosemide. Fluid restriction is not feasible during administration of high dose cyclophosphamide.

c) Cardiomyopathy -cyclophosphamide may cause severe, sometimes lethal, hemorrhagic myocardial necrosis or congestive cardiomyopathy. Patients may present with congestive cardiomyopathy as late as 2 weeks after the last dose of cyclophosphamide. The clinical syndrome has been observed in patients receiving the dose of cyclophosphamide in this regimen

in allogeneic transplantation patients. In an attempt to minimize this complication, patients with significant cardiac dysfunction are excluded from this protocol [see patient eligibility]. Congestive failure is managed according to standard medical therapeutics.

d) Hemorrhagic cystitis – this is a serious, potentially life-threatening complication related to injury of the bladder epithelium by cyclophosphamide metabolites. Although subclinical hematuria is not uncommon at this dose level, clinically significant hematuria or serious hemorrhage can usually be avoided by maintaining a high urine volume and frequent voiding and the administration of Mesna. Diuresis is maintained for 24 hrs after completion of last dose by parenteral infusions of 0.9% Sodium Chloride with potassium chloride, as specified in section 3.2. Careful monitoring of serum and urine electrolytes is mandated. Furosemide may be required to ensure this diuresis. Continuous bladder irrigation may be used for control of significant hematuria.

e) Sterility after cyclophosphamide is a possibility and more commonly occurs in female patients above age 26-30 years.

f) Less common but serious complications include pulmonary fibrosis and secondary malignancies. Less common but reversible toxicities include alopecia and skin rash.

8.2 MESNA (Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex, NSC113891)

8.2.1 Supply – Mesna will be will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a 100mg/ml solution.

8.2.2 Preparation

Priming regimen: The Mesna should be prepared in 100 ml of either 5% Dextrose in Water, USP or 0.9% sodium chloride injection, USP and infused over 15-30 minutes.

Conditioning regimen: Dilute up to 20 mg Mesna/ml fluid in D5W or 0.9% Sodium Chloride. Mesna should be started concurrently with the the start of cyclophosphamide at 1200 mg/m^2 in 500 ml by continuous IV infusion over 24 hour infusion for four doses (days -6, 5, -4, and -3).

8.2.3 Storage and Stability – Intact ampules are stored at room temperature. Diluted solutions (1 to 20 mg/dl) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48-72 hours in D5W, 48-72 hour in D5W/0.45% sodium chloride, or 24 hours in 0.9% sodium chloride.

8.2.4 Administration - To be administered intravenously as continuous infusion.

8.2.5 Toxicities - Nausea, vomiting, diarrhea.

8.3 FILGRASTIM (G-CSF, Neupogen®)

8.3.1 Supply – Commercially available as filgrastim injection in a concentration of 300µg/ml in 1ml (300µg) and 1.6ml (480µg) vials.

8.3.2 Preparation – For subcutaneous administration, the appropriate prescribed dose is drawn up from the vial with no further dilution prior to administration. For intravenous administration, the commercial solution for injection should be diluted prior to administration. It is recommended that the prescribed dose be diluted with dextrose 5% in water to a concentration greater than 5µg/ml. Dilution of filgrastim to a final concentration of less than 5µg/ml is not recommended at any time. Do not dilute with saline at any time; product may precipitate. Filgrastim diluted to concentrations between 5 and 15µg/ml should be protected from absorption to plastic materials by the addition of Albumin (Human) to a final concentration of 2mg/ml. When diluted in 5% dextrose or 5% dextrose plus Albumin (Human), filgrastim is compatible with glass bottles, PVC and polyolefin IV bags, and polypropylene syringes. The dose may be "rounded down" to within 10% of patient's calculated dose to use the drug cost-effectively.

8.3.3 Storage and Stability – Filgrastim for injection should be stored in the refrigerator at 2° to 8° C (36° to 46° F). Avoid shaking.

8.3.4 Administration – Subcutaneous injection is preferred. If clinically indicated, filgrastim may be administered as an intravenous infusion over 4 or 24 hours.

8.3.5 Toxicities – Medullary bone or skeletal pain is the most commonly reported toxicity. In addition, reversible elevations in uric acid, lactate dehydrogenase, and alkaline phosphatase are common laboratory abnormalities. Four cases of splenic rupture have been reported in healthy donors when given filgrastim or other myeloid growth factors for peripheral blood stem cell mobilization; 1 of these cases resulted in fatality. Five additional cases of splenic rupture have been reported in cancer patients undergoing chemotherapy or peripheral blood stem cell mobilization; splenic rupture may have contributed to deaths in 2 of these cases. One additional death due to splenic rupture after filgrastim therapy was reported to the manufacturer without additional information. According to the manufacturer, the reporting rate for splenic rupture with filgrastim is less than 1 in 486,000.

8.4 FLUDARABINE (Fludara, Berlex Laboratories)

8.4.1 Supply - Fludarabine monophosphate will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a white, lyophilized

powder. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH. Fludara is stored at room temperature.

8.4.2 8.4.2 Preparation - FLUDARA IV should be prepared for parenteral use by aseptically adding Sterile Water for Injection, USP. When reconstituted with 2 ml of Sterile Water for Injection, each ml of the resulting solution will contain 25 mg of Fludarabine Phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7–8.5. Fludarabine will be diluted in 100 to 125ml of either 5% dextrose in water or 0.9% sodium chloride, and infused IV over 30 minutes.

8.4.3 Storage and Stability - Reconstituted FLUDARA IV is chemically and physically stable for 24 hours at room temperature or for 48 hours if refrigerated. Because reconstituted FLUDARA IV contains no antimicrobial preservative, care must be taken to assure the sterility of the prepared solution; for this reason, reconstituted FLUDARA IV should be used or discarded within 8 hours.

8.4.4 8.4.4 Administration - Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

8.4.5 Toxicities – Fludarabine toxicities include myelosuppression (dose limiting toxicity), fever, nausea, vomiting, stomatitis, diarrhea, gastrointestinal bleeding, anorexia, edema, skin rashes, myalgia, headache, agitation, hearing loss, transient episodes of somnolence and fatigue, auto-immune hemolytic anemia, auto-immune thrombocytopenia, paresthesias, peripheral neuropathy, renal, and pulmonary toxicity (intersititial pneumonitis). Severe fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status were encountered almost exclusively after very high doses of fludarabine monophosphate. Such toxicity has only rarely been demonstrated at the 25-30 mg/m^2/day dosage of fludarabine. Very rarely described complications include transfusion-associated graft-versus-host disease, thrombotic thrombocytopenic purpura, and liver failure. Tumor lysis syndrome following fludarabine administration has been observed, especially in patients with advanced bulky malignant disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed postfludarabine, especially in heavily pre-treated individuals, and in individuals receiving fludarabine combined with other agents.

8.5 PREDNISONE

8.5.1 Supply – Prednisone will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources. Prednisone is commercially available as tablets, in strengths of 1, 2.5, 5, 20, and 50 mg.

8.5.2 Storage and Stability - Prednisone tablets should be stored in the container provided away from heat. The product labeling bears the manufacturer's expiration dating for stability.

8.5.3 Administration - Prednisone will be administered at a maximum dose of 1 mg/kg/day or at a maximum total daily dose of 80 mg orally and according the enclosed post-transplant tapering guidelines (appendix). In patients unable to tolerate oral medication, methylprednisolone can be substituted at an equivalent dosage, diluted in a small volume (e.g. 25-50ml) of 5% dextrose in water or 0.9% sodium chloride and infused over 15 minutes. To reduce gastrointestinal side effects, prednisone should be taken with food.

8.5.4 Toxicities -Prednisone frequently causes gastritis, immunosuppression, muscle wasting, fluid retention, and hyperglycemia.

8.6 RITUXIMAB (Rituxan)

8.6.1 Supply – Rituximab will be obtained commercially and is supplied as a 10 mg/ml sterile, preservative-free solution for injection, in vials of 100 mg and 500 mg.

8.6.2 Preparation – The desired dose will be diluted to a final concentration of 1 to 4 mg/mL in either 0.9% Sodium Chloride or D5W.

8.6.3 Storage and Stability – Rituximab vials should be stored at 2–8°C (36–46°F) and should be protected from direct sunlight.

8.6.4 Administration – First Infusion: The rituximab solution for infusion should be administered intravenously at an initial rate of 50 mg/hr. Rituximab should not be mixed or diluted with other drugs. If hypersensitivity or infusion reactions do not occur, escalate the infusion rate in 50 mg/hr increments every 30 minutes, to a maximum of 400 mg/hr. If hypersensitivity (non-IgE-mediated) or an infusion reaction develops, the infusion should be temporarily slowed or interrupted. The infusion can continue at one-half the previous rate upon improvement of patient symptoms. If the first infusion is tolerated well, subsequent infusions can begin at a rate of 100 mg/hr and will be increased by 100 mg/hr increments at 30-minute intervals. However, if the first infusion is not tolerated well, then the guidelines for the initial infusion should be followed for subsequent administration. Premedication will routinely be administered 30 to 60 minutes prior to the beginning of each rituximab infusion, consisting of acetaminophen 650 mg PO and diphenhydramine 50 mg IV.

8.6.5 Toxicities – The most serious adverse reactions caused by rituximab include infusion reactions, tumor lysis syndrome, mucocutaneous reactions, hypersensitivity reactions, cardiac arrhythmias and angina, and renal failure.

a) Fatal and Severe Infusion Reactions: Deaths within 24 hours of rituximab infusion have been reported. Approximately 80% of fatal infusion reactions occurred in association with the first infusion. Severe reactions typically occurred during the first infusion with time to onset of 30 to 120 minutes. Signs and symptoms of severe infusion reactions may include hypotension, angioedema, hypoxia or bronchospasm, and may require interruption of rituximab administration. The most severe manifestations and sequelae include pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, and cardiogenic shock. In the reported cases, the following factors were more frequently associated with fatal outcomes: female gender, pulmonary infiltrates, and chronic lymphocytic leukemia or mantle cell lymphoma. Management of severe infusion reactions: The rituximab infusion should be interrupted for severe reactions and supportive care measures instituted as medically indicated (e.g., intravenous fluids, vasopressors, oxygen, bronchodilators, diphenhydramine, and acetaminophen). In most cases, the infusion can be resumed at a 50% reduction in rate (e.g., from 100 mg/hr to 50 mg/hr) when symptoms have completely resolved. Patients requiring close monitoring during first and all subsequent infusions include those with pre-existing cardiac and pulmonary conditions, those with prior clinically significant cardiopulmonary adverse events and those with high numbers of circulating malignant cells (= 25,000/mm^3) with or without evidence of high tumor burden.

b) Severe Mucocutaneous Reactions: Mucocutaneous reactions, some with fatal outcome, have been reported in patients treated with rituximab. These reports include paraneoplastic pemphigus (an uncommon disorder which is a manifestation of the patient's underlying malignancy), Stevens-Johnson syndrome, lichenoid dermatitis, vesiculobullous dermatitis, and toxic epidermal necrolysis. The onset of the reaction in the reported cases has varied from 1 to 13 weeks following rituximab exposure. Patients experiencing a severe mucocutaneous reaction should not receive any further infusions and seek prompt medical evaluation. Skin biopsy may help to distinguish among different mucocutaneous reactions and guide subsequent treatment. The safety of readministration of RITUXAN to patients with any of these mucocutaneous reactions has not been determined.

c) Infusion reactions and lymphopenia are the most commonly occurring adverse reactions. Mild to moderate infusion reactions consisting of fever and chills/rigors occur in the majority of patients during the first rituximab infusion. Other frequent infusion reaction symptoms included nausea, pruritus, angioedema, asthenia, hypotension, headache, bronchospasm, throat irritation, rhinitis, urticaria, rash, vomiting, myalgia, dizziness, and hypertension. These reactions generally occur within 30 to 120 minutes of beginning the first infusion, and resolved with slowing or interruption of the rituximab infusion and with supportive care (diphenhydramine, acetaminophen, IV saline, and vasopressors). In an analysis of data from 356 patients with relapsed or refractory, low-grade NHL who received 4 (N = 319) or 8 (N = 37) weekly infusions of rituximab, the incidence of infusion reactions was highest during the first infusion (77%) and

decreased with each subsequent infusion (30% with fourth infusion and 14% with eighth infusion).

8.7 DIPHENHYDRAMINE

8.7.1 Supply – Commercially available. Diphenhydramine HCl injection is available in an injectable solution at a 50mg/ml concentration in single dose ampules, syringes and vials as well as multi-dose vials from multiple manufacturers.

8.7.2 Preparation – Diphenhydramine HCl may be given by direct intravenous injection without additional dilution. Alternatively the prescribed dose may be diluted in a small volume (e.g. 25-50ml) of 5% dextrose in water (D5W) or 0.9% sodium chloride (NS) and infused over 10-15 minutes.

8.7.3 Storage and Stability – Store commercially available injectable product at controlled room temperature.

8.7.4 Administration – Diphenhydramine HCl injection may be administered by direct IV injection (IV push) at a rate generally not exceeding 25mg/min. Alternatively, diphenhydramine HCl injection may be diluted and given over 10-15 minutes (see Preparation).

8.7.5 Toxicities – Sedation, sleepiness, dizziness, disturbed coordination, epigastric distress, thickening of bronchial secretions. Diphenhydramine can provide additive effects with alcohol or other CNS depressants. Diphenhydramine can cause anticholinergic side effects (e.g. dry mouth, fixed or dilated pupils, flushing, urinary retention). Diphenhydramine should be used with caution in patients with a history of bronchial asthma, increased intraocular pressure, hyperthyroidism, cardiovascular disease or hypertension.

8.8 ACETAMINOPHEN

8.8.1 Supply – Commercially available as 325 mg or 500 mg tablets for oral administration from multiple manufacturers.

8.8.2 Storage – Store at controlled room temperature.

8.8.3 Administration – Oral. For analgesia and antipyresis, the usual dose is 650 to 1000 milligrams every 4 to 6 hours, to a maximum of 4 grams/day.

8.8.4 Toxicities – No toxicities are anticipated to result from single doses of acetaminophen administered as premedication for rituximab infusions.

8.9 VALACYCLOVIR (Valtrex®)

8.9.1 Supply – Commercially available as 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.)

8.9.2 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.

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

8.9.4 Administration – Oral.

8.9.5 Toxicities – 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 or acyclovir at greater than the recommended dose. Dose reduction is recommended in this patient population (refer to the manufacturer's labeling for dose adjustment guidelines).

8.10 CASPOFUNGIN (Cancidas®)

8.10.1 Supply: Commercially available.

8.10.2 Product description: Caspofungin is available in 70mg and 50mg single-use lyophilized vials that require reconstitution with appropriate diluent and final dilution prior to administration.

8.10.3 Preparation: For reconstitution, aseptically add 10.5ml of an appropriate diluent (see manufacturer's package insert for suggested diluents) to either the 70mg or 50mg lyophilized vial. Then aseptically transfer 10ml of the reconstituted caspofungin to an IV bag (or bottle) containing 250ml of 0.9% sodium chloride injection or another suitable parenteral solution (see manufacturer's package insert for suggested solutions). NOTE: DO NOT USE DILUENTS CONTAINING DEXTROSE AS CASPOFUNGIN IS NOT STABLE IN DILUENTS CONTAINING DEXTROSE. In fluid restricted patients receiving the 50mg dose, a volume of 100ml (instead of 250ml) of an appropriate parenteral solution can be utilized.

8.10.4 Storage and Stability: The lyophilized vials should be stored refrigerated at 2° to 8°C (36° to 46°F). Reconstituted caspofungin may be stored at ≤ 25°C (≤ 77°F) for one hour

prior to the preparation of the patient infusion solution. The diluted product (final patient infusion solution) in the IV bag or bottle should be stored at ≤ 25° C (≤ 77° F) for 24 hours or at 2° to 8° C (36° to 46° F) for 48 hours.

8.10.5 Route of administration: Parenteral. Parenteral doses should be administered by slow IV infusion over approximately one hour.

8.10.6 Toxicities:

Fever, nausea, vomiting, flushing, infused-vein complications (e.g. phlebitis), and headache have generally been reported in clinical trials at an incidence ≥ 2%. Possible histamine-mediated symptoms have been reported including isolated reports of rash, facial swelling, pruritis, sensation of warmth, or bronchospasm. Anaphylaxis has been reported during administration of caspofungin. In post marketing experience, rare cases of clinically significant hepatic dysfunction has been reported. Elevations in liver transaminases (AST, ALT), total serum bilirubin, and serum alkaline phosphatase have been reported in clinical trials at an incidence usually ≥ 2%. Concomitant use of caspofungin with cyclosporine has been associated with a higher risk of liver tranasaminase elevations in healthy subject volunteers and concomitant use is not recommended by the manufacturer.

8.11 TRIMETHOPRIM/SULFAMETHOXAZOLE (TMP/SMX, Cotrimoxazole, Bactrim, Septra)

8.11.1 Supply – Commercially available as a single strength tablets containing trimethoprim 80mg and sulfamethoxazole 400mg and a double strength (DS) tablet containing trimethoprim 160mg and sulfamethoxaole 800mg. It is also available in a oral suspension at a concentration of 40mg of trimethoprim and 200mg sulfamethoxazole per 5ml. Parenteral TMP/SMX is available in a solution for injection at a concentration of 80mg of trimethoprim and 400mg of sulfamethoxazole per 5ml.

8.11.2 Preparation – For parenteral administration, the commercial solution for injection must be diluted prior to administration. It is recommended that each 5ml of the solution for injection be diluted with 100-125 ml or, if fluid restriction is required, in 75ml of dextrose 5% in water. Normal saline (0.9% sodium chloride) may be substituted as a diluent but the resulting solutions have reduced stability. Consult with pharmacy for questions regarding diluent, volume, and expiration.

8.11.3 Storage and Stability – Oral tablets and oral suspension should be stored at 15° to 30° C (59° to 86° F) in a dry place and protected from light. TMP/SMX for injection should be stored at room temperature between 15° to 30° C (59° to 86° F) and should not be

refrigerated. Stability of intravenous doses after final dilution is dependent on concentration and diluent. Consult with pharmacy for questions regarding stability and expiration dating.

8.11.4 . Administration – Oral and parenteral. Parenteral doses should be administered by an intravenous infusion over 60 to 90 minutes.

8.11.5 Toxicities – The most common adverse effects from TMP/SMX are gastrointestinal disturbances (nausea, vomiting, anorexia) and allergic skin reactions (such as rash and urticaria). Fatalities associated with the administration of sulfonamides, although rare, have occurred due to severe reactions, including Stevens-Johnson syndrome, toxic epidermal necrolysis, fulminant hepatic necrosis, agranulocytosis, aplastic anemia and other blood dyscrasias. For TMP/SMX injection, local reaction, pain and slight irritationon IV administration are infrequent. Thrombophlebitis has rarely been observed.

8.12 BAXTER ISOLEX 300i Stem Cell Collection System, version 2.5

8.12.1 Availability - The Isolex 300i Stem Cell Separation System, version 2.5, is an automated cell separation system based on immunomagnetic methods to purify CD34+ hematopoietic progenitor cells. It is manufactured by Baxter Healthcare Corporation and has been approved by the FDA; however, use of this system in this protocol is considered an "off label" use. CD34+ cell selection is performed according to the manufacturer's instructions and the DTM standard operating procedure based on those instructions.

8.12.2 Preparation and Administration – A leukapheresis concentrate of mobilized peripheral blood stem cells is incubated with human IVIgG, filtered through a standard blood recipient filter, and attached to the disposable set of the Isolex system. Using the Isolex closed system, the cells are washed to remove platelets and plasma, and concentrated for incubation with a murine anti-human CD34 monoclonal antibody. After washing, the sensitized cells are rosetted with paramagnetic microspheres coated with sheep anti-murine antibody. The rosetted cells are separated from the remaining cells by exposure to the primary magnet. A release agent is added to release the CD34+ cells from the paramagnetic microspheres coated with sheep anti-murine antibody. The released CD34+ cells are washed, concentrated, and transferred to a plastic bag for infusion or cryopreservation.

8.12.3 Toxicities – There are no significant toxicities associated with the infusion of autologous CD34+ cells that have been purified by the Isolex method. The cells will be cryopreserved and theoretically there may be adverse effects associated with the use of dimethylsulfoxide (DMSO) as a cryoprotectant such as hives, flushing, chest tightness, or cardiovascular instability. However, these effects are unlikely to occur or would be

relatively minor because the dose of DMSO is very low with elected CD34+ cells. The stem cell product will be additionally infused after premedication with diphenhydramine (Benadryl) 25-50 mg p.o. or i.v.

8.13 ^{18}FDG-PET

8.13.1 Supply

Fluorine-18-fluorodeoxyglucose (FDG) is an investigational new drug approved by the FDA for use in a limited number of patients. The current holder of this IND#23195 is Dr. Peter Herscovitch, Chief, PET Department. The FDG is produced as outlined in the IND. FDG is an analog of glucose labeled with F-18 (half-life is109 minutes). The FDG is taken up by the same mechanism as glucose and then is phosphorylated. Once intracellular and phosphorylated, it is not subject to the breakdown by glucose phosphatase and therefore accumulates intracellularly.

8.13.2 Preparation

The FDG in this protocol will be provided by the PET Department, CC, NIH or from a commercially available source in compliance with the FDG IND. The formulations will be prepared on the day of use and injected within 9 hours of formulation. The FDG is typically prepared at a specific activity of > 1 Ci/mmol. Radionuclide purity is analyzed periodically and to date no radionuclide impurities have been detected. Preparation

The FDG for human use is in sterile isotonic phosphate buffer and is passed over terminal millipore filter (0.22μ). It has high specific activity and is isomerically pure. A sample of each FDG preparation will be tested for pyrogenicity by the limulus amebocyte lysate assay; sterility and radiochemical purity by thin layer chromatography by the Quality Control Group of the PET department. Quality control will be performed as outlined in the IND. Alternatively the FDG will be purchased from a commercial vendor who provides FDG for clinical use.

8.13.3 Administration

The product is in liquid form and will be administered intravenously.

8.13.4 Dosimetry

FDG dosimetry has been calculated using methodology of the Medical Internal Radiation Dose (MIRD) Committee of the Society of Nuclear Medicine. The cumulative radiation dosimetries with bladder voiding 1.5 hours after injection (for a total of 15 mCi) are summarized in the RSC form 88-23(A). All radioactive drugs will be administered under the supervision of an authorized user. The dosimetry for FDG and transmission scans performed under this protocol is given in the NIH 88-23 form.

8.13.5 Toxicity

FDG has been used extensively at the NIH since 1983 and at many other centers throughout the world and it is well tolerated. Because we use microgram amounts there are no clinically significant side-effects.

9 APPENDICES

9.1 Appendix A

. Summary of laboratory research studies

A-1Examination of B cell precursor and mature B cells in SLE before and after auto-HSCT (By the laboratories of Amrie Grammer and Peter Lipsky)

SLE-prone individuals may have an abnormality in the generation and/or maintenance of their functional repertoire of B cells expressing specific Ig genes and may have been subjected to conditions that bias specific immunoglobulin variable region usage, thereby predisposing to the development of autoAb [113]. Intrinsic B cell over-reactivity may provide the drive for pathogenic autoAb formation by overwhelming the normal mechanisms that protect against autoimmunity and thereby permit the escape of pathogenic autoantibody producing progeny. AutoHSCT of SLE patients will determine whether deleting a large percentage of memory B-cells and effector plasma cells and replacing them with autologous stem cell-derived naïve cells in a non-inflamed environment will lead to long lasting remission in systemic autoimmune disease. The goal of this research is to examine the extent of depletion of memory B-cells and effector plasma cells as a result of the conditioning regimen and the nature of the surviving cells and the cells reconstituted from the autologous stem cells and to relate these parameters to the extent of clinical response and the length and quality of remission.

The human B cell repertoire is generated following differentiation of pluripotent hematopoietic stem cells in the bone marrow to immature B cells that migrate through the blood to finish their differentiation to mature B cells in the spleen. The techniques that can be used to monitor the nature and completeness of B cell depletion before transplantation as well as reconstitution of B cell subsets include multiparameter flow cytometry, single cell PCR of sorted B cell populations for Ig rearrangement, repertoire and mutational analysis, as well as microarray and the proteomic technique immunoaffinity capillary electrophoresis (ICE) for analysis of genes and proteins that are not able to be detected by multiparameter flow cytometery.

A-2. Reconstitution of regulatory and effector T cell populations

following autologous HSCT in systemic lupus (By the laboratory of Fran

Hakim and Ronald Gress)

Systemic lupus patients display multiple defects in T cell function, resulting in problems distinguishing which defects may be primary and which secondary effects of the disorder or of therapies applied. Immunoablation provides a unique opportunity to attempt to dissect primary and secondary defects in T lymphocytes of lupus patients and to correlate them with disease activity. Durable remissions in SLE following cyclophosphamide treatment and autologous transplant have been associated with a normalization of T cell populations, repertoire and function in a few patients studied [114]. Certainly the depletion of activated T cells associated with the disease process is a key element in remission. Recovery of naïve T cell populations through a renewal of thymopoiesis post transplant would also contribute to the recovery of a broad T cell receptor repertoire. T cell regeneration will be evaluated by studying the peripheral T cell receptor repertoire and subsets of T cells by multicolor FACS analysis. The role of thymus will be evaluated by studying the presence of recent thymic emigrants determined by the presence of TRECs.

Special emphasis will be placed on studies of regulatory T lymphocytes (some of these studies will be done in Dr. Lipsky's laboratory). Little is known of the role of TR in the pathogenesis of systemic autoimmune disorders in man. The complete lack of TR in the *scurfy* mouse results in an aggressive lethal autoimmune disorder that can be corrected by early transplant of CD4+CD25+ TR cells [115]. On the other hand, depletion of TR by neonatal thymectomy in lupus-prone (SWR x NZB)F1 mice increased levels of autoantibodies and organ specific autoimmunity, but also shifted the immunoglobulin isotype of the predominant autoantibodies and reduced glomerulonephritis [116]. Some of the immunological abnormalities present could be consistent with a reduction in TR, such as the common finding of an inability to make TGFβ, a key cytokine in generation and function of TR. We have recently shown that levels of CD4+CD25+ TR cells are abnormal in active lupus patients in contrast to those with inactive lupus [117]. Furthermore, the TR cells in lupus patients lack suppressive activity in vitro.

One potential explanation for durable remission with auto-HSCT is the recovery of regulatory T cell populations. Immune reconstitution studies post transplant have focused on expansion of residual memory cells and generation of new naïve T cells. The ability of the adult thymus to reconstitute TR populations following transplant has never been examined. The severe reduction in lymphocyte populations produced by the fludarabine and cyclophosphamide regimen and the CD34 selected graft should provide optimal conditions for assessing the recovery of TR cells.

We will assess the levels of Tr cells in at the pretreatment time point and establish a time course of recovery of TR cells in the peripheral blood by the use of multiparameter flow cytometry, functional analysis and quantitative RT-PCR.

A-3. Feasibility of Th2-cell type differentiation (By the laboratory of Daniel Fowler)

As previously referenced, SLE is an autoimmune disease with a complex disease pathogenesis. With respect to the role of T cells in SLE, there is considerable debate whether the Th1/Th2 paradigm of T cell cytokine biology applies to this disease. As such, we have generated the

hypotheses that, like most inflammatory disorders, Th1-type immunity plays a role in SLE pathogenesis, and that Th2-type immunity present in SLE patients may represent an unsuccessful attempt at immune auto-regulation. Significantly, recent murine data would lend support to this hypothesis [118]. This biology would be analogous to that of GVHD, which is initiated by Th1-type cells, yet after disease establishment, both Th1- and Th2-type patterns of T cells can contribute to disease pathogenesis. However, in the allogeneic transplant setting, if Th2-type cells are predominant at initiation of the transplant, the initial Th1-type response is abrogated, and acute GVHD is prevented. By extension of these principles, one would predict that in the setting of immune ablation and autologous transplantation for the therapy of SLE, that re-establishment of an anti-inflammatory immune response that is devoid of Th1-type elements and enriched for Th2-type elements might prevent the re-development of an SLE autoimmune predisposition. Given this possibility, using established methods of T cell expansion we have developed, we will evaluate the feasibility of whether $CD4^+$ T cells from SLE patients (at the time of study entry) can be induced into a state of Th2-type differentiation. In the event that this is possible, such an autologous Th2-type product might be utilized in future studies to promote the reconstituting immune system to a Th2 phenotype after transplantation for SLE. 40 ml of peripheral blood will be harvested from SLE patients at the time of study entry. $CD4^+$ T cells will be enriched by negative selection, and an initial CD4 cell cytokine phenotype will be determined by ELISA assay and Miltenyi cytokine secretion assay. Such CD4 cells will then be expanded under in vitro culture conditions in an attempt to polarize towards Th1- or Th2-phenotypes. In addition to this Th2 cell therapy feasibility evaluation, post-autoHSCT time points will also be evaluated by the same methods. These evaluations will be aimed at determining whether autologous transplantation resulted in a shift in the capacity of patient CD4 cells to develop towards Th1 or Th2 phenotypes.

B . Cytogenetics

(By the laboratory of Diane Arthur)

A relatively rare but serious potential complication of autoHSCT is the development of secondary, therapy-related myelodysplastic syndrome (t-MDS) or acute myelogenous leukemia (t-AML). Although documented most extensively inpatients transplanted for non-Hodgkin's lymphomas, t-MDS and t-AML have alsobeen reported following autoHSCT for non-hematologic solid tumors and nonmalignant diseases [119] (D. Jayne personal communication). Both pretransplantation and transplant-related factors likely play a role in the occurrence of secondary malignancy, but the relative contributions of these various factors remain a matter of some controversy. Factors that have been implicated include dose, type, and duration of prior cytotoxic therapy; total body irradiation; number and source of hematopoietic stem cells infused; chemotherapy priming of peripheral blood stem cells; and older age. The preparative regimen for patients entered on this study has been carefully chosen to decrease myelotoxicity and optimize immunodepletion. Nonetheless, we anticipate these patients will be at some risk for developing

t-MDS or t-AML due, in part, to previous cytotoxic chemotherapy, particularly the alkylating agent cyclophosphamide [120], and perhaps to cyclophosphamide and fludarabine in the priming and preparative regimens. Recent studies have shown that small clones of abnormal myeloid progenitor cells, not evident by routine G-banding, can be detected prior to transplant using interphase fluorescence in-situ hybridization (FISH) techniques in patients who go on to develop t-MDS or t-AML after transplant [121, 122]. The investigators suggest that pre-transplant FISH studies can potentially identify patients at increased risk of developing secondary malignancy and, therefore, permit more informed clinical management.To test the hypothesis that prospective interphase FISH analyses can detect small clones of abnormal myeloid progenitor cells prior to transplant, we propose to perform interphase FISH analyses using a carefully chosen panel of probes on bone marrow collected at baseline and 6, 12, and 24 months after transplant, as well as purified CD34+ stem cells collected for re-infusion on Day 0. Based upon prior studies done in our laboratory and others, we anticipate the most common chromosomal abnormalities following alkylating agent chemotherapy will be loss or deletion of the long arms of chromosomes 5 and 7 (-5/5q-, -7/7q-). Other common abnormalities include deletions of band 13q14, the short arm of chromosome 17, and the long arm of chromosome 20; and trisomy of chromosome 8. We will therefore employ a panel of six probes to detect these abnormalities.

Routine G-banded metaphase chromosome analysis will be performed on all bone marrow aspirates in the CAP-accredited NCI/CCR/LP Clinical Cytogenetics Laboratory under the direction of Dr. Diane Arthur. Processing and analysis will be performed according to standardized laboratory protocols. Final reports will be issued to the patients' medical records, with copies to Dr. Pavletic. Interphase FISH analyses will be done on all bone marrow aspirates, and also on purified CD34+ stem cells. These analyses will also be performed in Dr. Arthur's laboratory according to procedures established and quality-controlled for each probe in her laboratory. A panel of six probes purchased from Vysis, Inc. will be applied to each sample including LSI EGR1/D5S23, D5S721 Dual Color Probe, LSI D7S486/CEP 7 Dual Color Probe, LSI 13 (RB1) 13q14 SpectrumOrange Probe, LSI p53 (17p13.1) SpectrumOrange Probe, LSI D20S108 (20q12) SpectrumOrange Probe, and CEP 8 DNA Probe Kit. The FISH tests will be performed on a research basis, with results to Dr. Pavletic only.

C . F-18 FDG Positron Emission Tomography to Assess the Distribution of Activated Lymphocytes in Systemic Lupus Erythematosus

(By Peter Herscovitch, M.D. Participating investigator: M. Whatley).

Positron emission tomography (PET) is a physiology-based method of imaging based on the uptake and metabolism of radiopharmaceutical by the tissues. PET with FDG is a functional imaging technique that provides information about glucose metabolism. FDG has been used successfully to image a variety of tumors [123]. FDG accumulation in tissue is by no means tumor specific and accumulation has been reported in a variety of infectious and inflammatory processes [124, 125]. Uptake in sarcoidosis has been reported and patients with AIDS often have hypermetabolic nodes. Similar findings have been found in our study of patients with AIDS and

high viral loads at the clinical center (protocol 00-I-0109). Furthermore patients with autoimmune lymphoproliferative disorder (ALPS) who have a defect in apoptosis and who typically exhibit enlarged lymph nodes will often have increased uptake of FDG in their peripheral nodes (based on our experience here at NIH (02-I-0308).

Patients who are receiving G-CSF typically will change their FDG biodistribution exhibiting a marked increase in bone marrow uptake and increase in splenic uptake.

In patients with active SLE both T and B-lymphocytes express higher levels of activation markers compared to inactive patients and effective treatment leads to a decrease in activated lymphocytes [84]. The anatomic site of lymphocyte activation is unknown in humans.

In a recently completed pilot study, we compared the pattern of PET images in lymphoid organs between patients with active and inactive disease. There were two important findings of this study. First, both active (n=10) and inactive (n=9) lupus patients had increased FDG uptake in lymph nodes when compared to healthy volunteers, with no statistical difference between the two groups. This suggests that the metabolic and probably immunologic activity of lymphoid organs is enhanced, not only in active, but also in clinically quiescent SLE patients. It would be of interest to see whether achieving clinical remission after the autoHSCT results in a more normal PET pattern. Second, thymic uptake, was demonstrated in 5/10 active patients compared to 0/9 of inactive patients. This suggests a qualitative difference in the immune response between different stages of clinical activity and points toward an important role for the thymus during active disease. Age might be a confounding factor but thymic uptake was seen in active patients even in their fourth decade, when the thymus is usually involuted. In fact, 3/5 patients with thymic uptake were 29 years or older. Thymic uptake was seen in only 1/10 healthy controls of similar age. Imaging the thymus will be of interest for several reasons. The correlation between thymic activity and regeneration of the T-cell repertoire following immunoablation is of general interest, whereas the correlation between thymic uptake, changes in T lymphocyte subsets and disease activity will provide important information about the pathogenesis of lupus.

Eligibility Assessment and Enrollment for PET studies Exclusion Criteria:

i) Patients who weigh ≥ 136 kg (weight limit for the PET scanner table).
ii) Patients with uncontrolled glucose due to diabetes may be studied, but will be evaluated separately.
iii) If logistical difficulties arise patients may not be scanned and this will not be considered a protocol violation.
iv) Pregnancy test for women of childbearing potential prior to imaging within 24 h of imaging.
v) Patients under the age of 18.

FDG-PET scanning

FDG-PET scans of the whole body will be performed at baseline. On day -1 before transplant and at 1, 6, 12, and 24 months post-transplant. The patients will be fasting for at least 6 hours except for water. They will be encouraged to drink fluids prior to the FDG injection. An intravenous line with a 0.9% saline solution will be inserted into the subject's arm for isotope injection. Lines should be inserted prior to arrival for their PET scan. The patient will be positioned on the FDG-PET scanning bed. A transmission scan using Ga-68/Ge-68 rotating pin source of positron emitting radioactivity will be performed to measure the attenuation. These measurements will be used to correct the emission data. Typically, each area imaged will require a 3 minute transmission scan (total transmission scan < 8 min per study). This will be followed by an emission scan of the same area. Transmission scans will be used for attenuation correction. In total there will be a 1 h uptake period and less than 90 min imaging time

One blood sample for glucose and insulin determination will be obtained during the scanning sequence. The patients will be instructed to void frequently for the next 6 hours. Patients will be allowed to void after their emission scan. These scans will be reconstructed and images will be reviewed visually. The PET scan images will be read by a physician experienced in the interpretation of whole body PET imaging. The region of interest will be performed over areas in the baseline and follow up scans that will include the liver, spleen, bone marrow and any lymph node regions or areas that are felt to warrant evaluation by the nuclear physican. We will use quantitative or semi-quantitative parameters to assess lesion activity.

For FDG-PET scans the following data will be collected at baseline and follow up:

i. SUV max and mean

ii. Metabolic volume

iii. Comparison with CT (if available)

iv. Comparison with fine needle aspirates (if available)

-As many patients as possible that are being accrued into the protocol will be imaged with FDG. Patient's baseline images will compare to the follow up images. Human Subject Protections

-FDG-PET is an approved procedure for the evaluation of patients with metastatic cancer. In this study the main purpose of the FDG scan is to try evaluate if we can see metabolic changes related to lymphocyte homing and trafficking, thus no direct benefit is expected from serial FDG-PET imaging.

D . Ultrasound surveillance and fine needle aspiration of lymph nodes

(By Bradford J. Wood, Ziv Neeman and Andrea Abati) For the lymph node specimen research sampling patients will undergo a limited superficial ultrasound survey at regular intervals during the protocol, including pre-transplantation (not to exceed 6 weeks prior to transplantation), and 6,

12, and 24 months following transplantation. This limited ultrasound will include a survey of bilateral axillae, groins (inguinal and femoral nodes), and the neck (cervical chains). Grossly abnormal nodes will be measured and recorded on the imaging datasheet (**Appendix K**). The largest node in each region (6 regions: left and right neck, groin, and axilla) will be measured in 3 dimensions, and the exact location will be recorded in relation to nearby anatomy and vessels for future localization. Depth below skin surface will be recorded. Note will be made if no nodes are visible on ultrasound in a region.

Fine needle aspiration: Fine needle aspirations will be performed with 23 or 22 gauge needles (like Chiba cutting needle) using standard image guided technique with ultrasound. Clinical Center guidelines will be adhered to for all stages of the procedure following the special procedures policies. If conscious sedation is required, Clinical Center sedation guidelines will be adhered to. Standard laboratory studies will be performed within 7 days of the procedures to include platelets and PT/PTT. History and physical will be on chart within 30 days of procedure. Research specimen paperwork will be used. Cytology staff will be available at the time of the aspirations for rapid bedside interpretation to estimate the quantity and quality of lymphoid material sampled.

Patients will be prepped and draped in the usual sterile fashion, and the subcutaneous tissues will be anesthetized with 1 – 2 % lidocaine along the chosen pathway. This should render the remainder of the procedure relatively pain-free following the mild burning sensation induced by lidocaine anesthetic. 22 or 23 gauge needles will be inserted into the nodal target with real-time ultrasound visualization to ensure accurate sampling. The first pass will be a “capillary” pass, with no suction applied, to minimize red blood cell contaminant in the specimen. Subsequent passes will have suction applied, usually with an automatic syringe. All specimens will be split between slide and test tube with normal saline (or fixative such as RPMI).

Up to three nodes may be sampled in a given patient, at a given time period. Fine needle aspirates may be performed at baseline (pre-transplantation), as well as at 6, 12 and 24 months following transplantation.

Ultrasound guided fine needle aspiration of superficial nodes is a very low-risk procedure. The location of the nodes superficially makes bleeding less likely since pressure can be held to control any theoretical bleeding. Such small needles have a much lower risk of causing bleeding or other complications that larger needles typically used for image-guided biopsy procedures. Measurements will be made of nodes before and after aspiration, and the size pre-aspiration may be used for the timeline measurement.

9.2 Appendix B: Guidelines for prophylaxis of infectious complications in lymphodepleting autologous stem cell transplantation for SLE *Note:*

The practice guidelines included in this Appendix are based upon the best available clinical and circumstantial evidence and may change as additional data become available. Although the current protocol is in the autologous transplantation setting, this patient population of lupus patients is considered to be at an infection risk that is potentially higher than in a non-lymphodepleting autologous transplantation protocol, therefore the whole infection prevention philosophy here is to err on the side of safety and at minimum matches the allogeneic stem cell transplantation protocol prophylaxis standards.

Pneumocystis carinii pneumonia

PCP prophylaxis will start prior to priming. After finishing leukapheresis and prior to starting the conditioning regimen, all patients, except those with lupus nephritis, will complete (as latest by day -1) a 7 day course of trimethoprim 160 mg/sulfamethoxazole 800 mg (TMP/SMX 160/800), one tablet PO BID. Lupus nephritis patients will continut the usual 3x/week prophyliactic schedule until day -1. The treatment will resume post transplant when ANC is >500/μL and unsupported platelets >50,000/mm^3 at one tablet a day dosing three days per week (e.g., on each Monday, Wednesday and Friday). This will continue for 12 months post transplant and until CD4 count >200/μL and prednisone dose ≤ 5 mg/day, whichever comes later.

9.2.1.1 The alternative regimens for PCP prophylaxis are less effective than TMP/SMX and include:

-Pentamidine inhalation 300 mg every 4 weeks

-Dapsone PO 100 mg/day

-Atovaquone PO 1500 mg/day

9.2.2 Fungal Infections (including Aspergillus)

Since majority of patients will be on long-term high doses of steroids, caspofungin usually at 50 mg i.v./day will be used starting on day 2 until post-transplant ANC is >1000/μL.

9.2.3 Viral pathogens

9.2.3.1 Herpes Simplex Virus (HSV) and Varicella Zoster Virus (VZV)

a) All patients will receive valacyclovir 500 mg PO QD for suppression of HSV and VZV infection/reactivation. This prophylaxis will begin when patients start the priming regimen and

continue throughout transplantation. Valacyclovir will continue for 12 months after transplantation, until CD4 count >200/μL and prednisone ≤5mg/day, whichever comes later. If a patient cannot take oral medications, acyclovir 250 mg/m^2 IV q12h will be substituted for valacyclovir.

9.2.3.2 Cytomegalovirus (CMV) *Preemptive Therapy:* Patients with positive pre-transplant serologies for CMV will undergo weekly monitoring of CMV antigenemia. Antigenemia monitoring will begin at the time of starting priming regimen and continue through day +100 or until CD4 count is > 200/μL. Antigenemia will be assessed if clinically indicated independently of the pre-transplant CMV serology status, for example if fever of unknown origin, decreasing counts or pulmonary symptoms occur. If a patient has an ANC < 1000/μL or the specimen is obtained from a long distance CMV monitoring will be performed via polymerase chain reaction (PCR) instead of antigenemia. If antigenemia becomes positive in two consecutive samples (expressed as the number of positive cells per 400,000 white blood cells) before day +100, then patients will be treated with oral valganciclovir, IV ganciclovir, or IV foscarnet according to the following schema:

# Of (+) cells	Rx: VALGANCICLOVIR	Rx: IV GANCICLOVIR	Monitoring	Reinduction
Any	Valganciclovir induction: 900 mg PO q12h for 7 days, then maintenance: 900 mg/24h until antigenemia is negative x 2 weeks*	Ganciclovir induction: 5 mg/kg IV q12h for 7 days, then maintenance: 5 mg/kg/24h 5 days/week until antigenemia is negative x 2 weeks*	Weekly antigenemia	1. If antigenemia becomes positive after being negative 2. For doubling of antigenemia

*I.e., on two consecutive weekly antigenemia tests.

NOTES:

- Valganciclovir and ganciclovir may cause bone marrow suppression. If ANC < 1000: Start filgrastim (G-CSF) 5-10 μg/kg/d

- Weekly IVIG infusion has no proven benefit in the setting of CMV reactivation without established disease. Therefore, its use will be limited to patients with CMV pneumonitis (or other target organ infection, at the investigators' discretion).

Indications for IV ganciclovir:

- clinical suspicion of malabsorption

Indications for foscarnet:

- ANC < 500/µl despite G-CSF
- Platelets < 20,000/mm^3
- Resistance to ganciclovir: rising antigenemia after 3 weeks with no response to ganciclovir reinduction

Dose Adjustments for Renal Insufficiency

Valganciclovir		
Calculated CrCl	Induction	Maintenance
>60	900 mg q12h	900 mg q24h
40-59	450 mg q 12h	450 mg q 24h
25-39	450 mg q 24h	450 mg q 48h
10-24	450 mg q 48h	450 mg 2x/week

Ganciclovir		
Calculated CrCl	Induction	Maintenance
>70	5 mg/kg q12h	5 mg/kg q24h
>50-69	2.5 mg/kg q12h	2.5 mg/kg q24h
25-49	2.5 mg/kg/24h	1.25 mg/kg/24h
10-24	1.25 mg/kg/24h	0.625 mg/kg/24h
<10	1.25 mg/kg 3x/wk	0.625 mg/kg 3x/wk

9.2.4 Epstein-Barr virus

All patients will be monitored following the completion of the conditioning regimen at weekly intervals for EBV-DNA by a quantitative assay at least until the day 100 post-transplant and until CD4 count is >200/µL, whichever comes later. Patients experiencing an EBV reactivation more than or equal to 1000 geg/mL will be candidates for preemptive therapy using a single 375

mg/m^2 infusion of rituximab [126]. Prior to such preemptive rituximab infusion patients will be carefully examined for signs and symptoms to exclude EBV post-transplant lymphoproliferative disorder (PTLD), including if needed, CT scans and bone marrow biopsy. EBV load will be monitored daily during the first 72 hours then twice weekly until 2 negative (<50 geg/mL) tests were obtained and thereafter at the each outpatient visit.EBV-PTLD is extremely rare after autologous stem cell transplantation but is more commonly described in transplants for both malignant and/or autoimmune disease in situations of profound T-cell depletion by graft manipulations or anti-T cell antibody administration.

9.2.5 Bacterial Pathogens

Since most of these patients will be on steroids, ceftazidime at doses of 2 g IV Q 8 hrs will be given prophylactically during the period of post-transplant neutropenia (defined as ANC <500/μL).

9.2.6 Tuberculosis

All patients will undergo PPD testing prior to starting chemomobilization, those with positive skin tests would receive prophylactic INH 300 mg QD with pyridoxine 50 mg QD.

9.2.7 Vaccination

All patients will undergo standard HSCT immunizations that include: 1. seasonal flu vaccine that may start at 6 months post-transplant, 2. Tetanus-Diphteria toxoid, Haemophilus influenza type B conjugate, inactivated Polio at 12, 18, and 24 months. 3. Pneumococcal 7-valent conjugate will be given at 12 months only

9.3 Appendix C: Prednisone taper schema

Week	**Maximally allowed daily dose of prednisone mg/day (or equivalent)#**												
0 – 2	**80**	**75**	**70**	**65**	**60**	**55**	**50**	**45**	**40**	**35**	**30**	**25**	**20**
3 – 4	80	75	70	65	60	55	50	45	40	35	30	25	20
5 – 6	70	65	60	55	50	45	40	35	30	30	25	20	15
7- 8	60	55	50	45	40	35	30	30	25	25	20	15	10
9 – 10	50	45	40	35	30	30	25	25	20	20	15	10	10
11 –12	40	35	30	30	25	25	20	20	15	15	10	10	*
13 – 14	30	30	25	25	20	20	15	15	10	10	10	*	*
15 –16	25	25	20	20	15	15	10	10	10	10	*	*	*
17 – 18	20	20	15	15	10	10	10	10	*	*	*	*	*
19 – 20	15	15	10	10	10	10	*	*	*	*	*	*	*
21 – 22	10	10	10	10	*	*	*	*	*	*	*	*	*
23 -24	10	10	*	*	*	*	*	*	*	*	*	*	*
25 - 26	*	*	*	*	*	*	*	*	*	*	*	*	*
48	<5mg/day												

* A taper from the equivalent of 10 mg/day can start. Decrease dose every 4 weeks by 2.5 mg/day. Taper to 0 mg/day, if tolerated.

#: Taper to alternate day regimen, if tolerated, for doses <20 mg/day

9.4 Appendix D: Modified SELENA-SLEDAI

Descriptor	Definition	Wt	Present
Seizure	Recent onset (last 10 days). Exclude metabolic, infections or drug cause, or seizure due to past irreversible	8	
Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized, or catatonic behavior. Exclude uremia and drug causes.	8	
Organic Brain Syndrome	Altered mental function with impaired orientation, memory or other intellectual function, with rapid onset and Syndrome fluctuating clinical features. Include clouding of consciousness with reduced capacity to focus, and inability to sustain attention to environment, plus at least two of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious or drug causes.	8	
Visual Disturbance	Retinal and eye changes of SLE. Include cytoid bodies, retinal hemorrhages, serous exudate or hemorrhages in the choroid, optic neuritis, scleritis or episcleritis. Exclude hypertension, infection, or drug causes.	8	
Cranial Nerve Disorder	New onset of sensory or motor neuropathy involving cranial nerves. Include vertigo due to lupus.	8	
Lupus Headache	Severe persistent headache: may be migrainous, but must be non-responsive to narcotic analgesia.	8	
CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis or hypertensive causes	8	
Vasculitis	Ulceration, gangrene, tender finger nodules, periungual infarction, splinter hemorrhages, or biopsy or an angiogram proof of vasculitis.	8	
Arthritis	More than 2 joints with pain and signs of inflammation (i.e., tenderness, swelling, or effusion).	4	
Myositis	Proximal muscle aching weakness, associated with elevated creatine phosphokinase/adolase or electromyogram changes or a biopsy showing myositis.	4	
Urinary Casts	Heme-granular or red blood cell casts.	4	
Hematuria	>10 red blood cells/high power field. Exclude stone, infection or other cause.	4	
Proteinuria	New onset or recent increase of more than 0.5 g/24 hours.	4	
Pyuria	>5 white blood cells/high power field. Exclude infection.	4	
Rash	Ongoing inflammatory lupus rash.	2	
Alopecia	Ongoing abnormal, patchy or diffuse loss of hair due to active lupus.	2	
Mucosal Ulcers	Ongoing oral or nasal ulcerations due to active lupus.	2	
Pleurisy	Classic and severe pleuritic chest pain or pleural rub or effusion or new pleural thickening due to lupus.	2	
Pericarditis	Classic and severe pericardial pain or rub or effusion, or electrocardiogram confirmation.	2	
Low Complement	(Will not be rated in this study.)	N/A	N/A
Increased DNA Binding	>25% binding by Farr assay or above normal range for testing laboratory.	2	
Fever	>38 C. Excluding infectious causes.	1	
Thrombocytope	<100,000-platelets/ mm3.	1	

nia			
Leukopenia	<3,000 white blood cells/mm3. Excluding drug causes.	1	
TOTAL SCORE (Sum of weights for conditions present)			

MD Global Rating of SLE Activity
(over last month, use most active rating during the month)
(assume that "NONE" and "MOST ACTIVE" refer to what patient has experienced)

0 (NONE) Mild Moderate Severe

Patient Global Rating of Their Disease Activity

0 (NONE) 10 (MOST)

9.5 APPENDIX E: SLAM (Systemic Lupus Activity Measure)

Date last seen at NIH (mm/dd/yyyy): ____/____/_____

Today's date (mm/dd/yyyy): ____/____/_____

Rater:_____

Constitutional	Absent or normal	Mild	Moderate	Severe	Not recorded
Weight Loss	***Physical Examination,*** *VITALS, Weight*				
	0	1 < 10% body weight		3 > 10%	0
Fatigue	***Source Document for Physician****, Review of systems*				
	0	1		3	
Fever	***Source Document for Physician,*** *Review of systems* ***Physical Examination****, VITALS, Temperature*				
	0	1 37.5 – 38.5ºC	2	3 > 38.5ºC	

Integument	Absent or normal	Mild	Moderate	Severe	Not recorded
Oral/nasal ulcers, or Periungal erythema, or					

Malar rash, or
Photosensitive rash, or
Nail fold infarct

Physical Examination, *Skin*

0	1			0
	Present			

Alopecia

Physical Examination, *Skin*

0	1	2		

Integument (Continued)

Absent or normal	Mild	Moderate	Severe	Not recorded

Erythematous, maculopapular rash, or
Discoid lupus, or
Lupus profundus, or
Bullous lesions

Physical Examination, *Skin*

0	1	2	3	
	Present			

Vasculitis
Leukocytoclastic vasculitis, or
Urticaria, or
Palpable purpura, or
Livedo reticularis, or
Ulcer, or
Panniculitis

Physical Examination, *Skin*

0	1	2	3	
	< 20% TBA	20-50% TBA	> 50% TBA or necrosis	

Eye

Cytoid bodies

Physical Examination, *HEENT*

0	1	3	
	Present	Visual acuity < 20/200	

Hemorrhages (retinal or choroidal), or

Episcleritis

Physical Examination, *HEENT*

0	1	3	
	Present	Visual acuity < 20/200	

Papillitis, or

Pseudotumor cerebri

Physical Examination, *HEENT*

0	1	3	
	Present	Visual acuity < 20/200 or field cut	

Reticuloendothelial	Absent or normal	Mild	Moderate	Severe	Not recorded

Diffuse lymphadenopathy (cervical, axillary, epitrochlear)

Physical Examination*, Lymph nodes*

0	1	2	
	Shotty	> 1cm × 1.5cm	

Hepato- or splenomegaly

Physical Examination*, Abdomen*

0	1	2	3	
	Palpable only with inspiration	Palpable only without inspiration		

Pulmonary	Absent or normal	Mild	Moderate	Severe	Not recorded

Pleural effusion

Pleurisy

Source Document for Physician*, Review of systems*

0	1	2	3	
	Shortness of breath or pain only with	Shortness of breath or pain only with	Shortness of breath or pain at rest, decreased	

	prompting. Exam normal or nearnormal	exercise decreased breath sounds and dull lower lobe (s)	breath sounds and dull middle and lower lobes		

Pneumonitis

***Source Document for Physician**, Review of systems*

0	1	2	3	
	X –ray infiltrate only	Shortness of breath with exercise at rest	Shortness of breath	

Cardiovascular

Absent or normal	Mild	Moderate	Severe	Not recorded

Raynaud's

***Source Document for Physician**, Review of systems*

0	1	
	Present	

Hypertension

***Physical Examination**, VITALS, BP*

0	1	2	3	
	Diast. 90-105		Diast. 105-115	Diast. > 115

Carditis

***Source Document for Physician**, Review of systems*

0	1	2	3	
	Pericarditis by EKG&/or RUB &/oreffusion byEcho; no symptom	Chest pain or arrhythmia	Myocarditis withhemodynamic compromise&/or arrhythmia	

Gastrointestinal

Absent or normal	Mild	Moderate	Severe	Not recorded

Abdominal pain (Serositis, pancreatitis, ischemic bowel, etc)

***Source Document for Physician**, Review of systems*

0	1	2	3	
	Complaint Limiting pain		Peritoneal signs/ascites	

Neuromotor

Stroke syndrome Mononeuritis multiplex,	0	1	2	3	
		Single TIA			

Transient ischemic attack (TIA), Reversible ischemic neurologic deficit (RIND), Cerebrovascular accident (CVA), Retinal vascular thrombosis

Multiple TIA/RIND, or mononeuritis multiplex, or Cranial neuropathy or Chorea

CVA/myelitis, retinalvascular occlusion

Seizure

***Source Document for Physician**, Review of systems*

0	1	2	3	
	1-2/month > 2/month		Status epilepticus	

Cortical dysfunction

***Source Document for Physician**, Review of systems*

0	1	2	3	
	Mild depression/personality disorder or cognitive deficit	Change in sensorium or severe depression orlimiting cognitive impairment	Psychosis or dementia or coma	

Headache

(including migraine equivalents)

***Source Document for Physician**, Review of systems*

0	1	2	3	
	Symptoms or transient neuro deficit with normal activities	Interferes somewhat meningitis	Incapacitating/aseptic	

Myalgia/myositis

***Source Document for Physician**, Review of systems*

0	1	2	3	
	Complaint Limits some activity		Incapacitating	

Joints

Absent or normal	Mild	Moderate	Severe	Not recorded

Joint pain from synovitis, and/or tenosynovitis

***Physical Examination**, Musculoskeletal*

0	1	2	3	
	Arthralgia only	Objective inflammation	Limited function	

Other

Absent or normal	Mild	Moderate	Severe	Not recorded

(Write rules for ascertainment and ad hoc scale)

0	1	2	3	

Laboratory

	0	1	2	3	
Hematocrit	> 35	30-35	25-29.9	< 25	
WBC	> 3500	3500-2000	2000-1000	< 1000	
Lymphocyte count	1500-4000	1499-1000	999-500	< 499	
Platelet count	>150T	100-150T	99-50T	< 50T	
ESR (Westergren)	>25	25-50	51-75	>75	
Serum creatinine, or creatinine clearance	0.5-1.3 mg/dLor 80-100% CrCl	1.4-2 mg/dL or 79-60% CrCl	2.1-4 mg/dL or 30-60% CrCl	> 4 mg/dL or < 30% CrCl	
Urine sediment	0	1 > 5 RBC &/or WBC/hpf or 0 to 1-3 granular &/or cellular casts/hpf or 1-2+ proteinuria or < 500 mg/L 24° urine protein	2 > 10 RBC &/or WBC/hpf or > 3 granular &/or cellular casts/hpf or 3 or 4+ proteinuria or 500-3500 mg/L 24° urine protein	3 > 25 RBC or WBC/hpf or red cell cast or > 4+ proteinuria or > 3500 mg/L 24° urine protein	

9.6 Appendix F

SLICC/ACR DAMAGE INDEX FOR SYSTEMIC LUPUS ERYTHEMATOSUS

Overview:

The Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index for Systemic Lupus Erythematosus (SLE) records damage occurring in patients with SLE irregardless of causation. This can be used to monitor patients over time especially for comparing periods of disease activity and inactivity.

Glossary of Terms

Term	Definition
Damage	Nonreversible change not related to active inflammation occurring since diagnosis of lupus ascertained by clinical assessment and present for at least 6 months unless otherwise stated. **Repeat episodes must occur at least 6 months apart to score 2.** The same lesion cannot be scored twice.
Cataract	A lens opacity (cataract) in either eye ever whether primary or secondary to steroic therapy documented by ophthalmoscopy
Retinal damage	Documented by ophthalmoscopic examination may result in field defect legal blindness
Optic atrophy	Documented by ophthalmoscopic examination.
Cognitive impairment	Memory deficit difficulty with calculation poor concentration difficulty in spoken or written language impaired performance level documented on clinical examination or by formal neurocognitive testing.
Major psychosis	Altered ability to function in normal activity due to psychiatric reasons. Severe disturbance in the perception of reality characterized by the following features: delusions hallucinations (auditory visual) incoherence marked loose associations impoverished thought content marked illogical thinking bizarre disorganized or catatonic behavior.
Seizures	Paroxysmal electrical discharge occurring in the brain and producing characteristic physical changes including tonic and clonic movements and certain behavioral disorders. Only seizures requiring therapy for 6 months are counted as damage.
Cerebrovascular accident (CVA)	Cerebrovascular accident resulting in focal findings such as paresis weakness etc. or surgical resection for causes other than malignancy.
Neuropathy	Damage to either cranial or peripheral nerve excluding optic nerve resulting in either motor or sensory dysfunction.
Transverse myelitis	Lower extremity weakness or sensory loss with loss of rectal and urinary bladder sphincter control
Rena	Estimated or measured glomerular filtration rate <50% proteinuria ≥ 3.5 gm per 24 hours or end-stage renal disease (regardless of dialysis or transplantation)
Pulmonary	Pulmonary hypertension (right ventricular prominence or loud P2); pulmonary fibrosis (physical or radiograph); shrinking lung (radiograph); pleural fibrosis (radiograph); pulmonary infarction (radiograph); resection for cause other than malignancy)
Cardiovascular	Angina or coronary artery bypass; myocardial infarction (documented by electrocardiograph and enzyme studies) ever; cardiomyopathy (ventricular dysfunction documented clinically), valvular disease (diastolic murmur or systolic murmur >3/6);

Term	Definition
Peripheral vascular	Claudication persistent for 6 months by history; minor tissue loss such as pulp space ever; significant tissue loss such as loss of digit or limb or resection ever; venous thrombosis with swelling ulceration or clinical evidence of venous stasis
Gastrointestinal	Infarction or resection of bowel below duodenum by history resection of liver spleen or gallbladder ever for whatever cause; mesentric insufficiency with diffuse abdominal pain on clinical examination; chronic peritonitis with persistent abdominal pain and peritoneal irritations on clinical examination; esophageal stricture on endoscopy upper gastrointestinal tract surgery such as correction of stricture ulcer surgery etc. ever by history; pancreatic insufficiency requiring enzyme replacement or with a pseudocyst
Musculoskeletal	Muscle atrophy or weakness demonstrated on clinical examination; deforming or erosive arthritis including reducible deformities (excluding avascular necrosis) on clinical examination; osteoporosis with fracture or vertebral collapse (excluding avascular necrosis) demonstrated radiographically; avascular necrosis demonstrated by any imaging technique osteomyelitis documented clinically and supported by culture evidence; tendon ruptures
Skin	Scarring chronic alopecia documented clinically; extensive scarring or panniculum other than scalp and pulp space documented clinically; skin ulceration (excluding thromboss) for more than 6 months
Premature gonadal failure	Secondary amenorrhea prior to age of 40
Diabetes	Diabetes requiring therapy but regardless of treatment.
Malignancy	Documented by pathologic examination excluding dysplasia

Scoring

Organ	Item	Points
Ocular (either eye by clinical assessment)	Any cataract ever	1
	Retinal damage or optic atrophy	1
Neuropsychiatric	Cognitive impairment (e.g. memory deficit difficulty with calculation poor concentration difficulty in spoken or written language impaired performance level) or major psychosis	1
	Seizures requiring therapy for 6 months	1
	Cerebrovascular accident ever (score 2 if more than 1) or surgical resection for causes other than malignancy	1 or 2
	Cranial or peripheral neuropathy (excluding optic)	1
	Transverse myelitis	1
Renal	Estimated or measured glomerular filtration rate <50% **or**	1
	Proteinuria ≥ 3.5 g per 24 hours **or**	1
	End-stage renal disease (regardless of dialysis or transplantation)	3

SLICC/ACR Damage Index for Systemic Lupus Erythematosus continued.

9.7 Appendix G: ANAM

The ANAM test will be administered by trained NIAMS investigators in a room both quiet and confidential. The ANAM test is on a CD-ROM, available on a computer in the clinic. Each participant's results will be stored on the NIAMS's hard drive and will be labeled only with his/her unique identification number that will be assigned at his/her entrance to the study and will be kept in the participant's research chart. Coded data will be exported to a CDROM and will be shipped to Dr Joseph Bleiberg at the National Rehabilitation Hospital for interpretation. The following ANAM subtests will be conducted;

- Simple reaction time (SRT)
- Sternberg memory (STN)
- Mathematical processing (MTH)
- Matching to sample (MSP)
- Spatial processing (SPD)
- Code substitution (CDD)

Conventional neurocognitive tests

The whole test will take approximately 25 minutes. The following battery of neuropsychological tests will be administered to each participant by trained NIAMS investigators in the same order using standardized instructions and pre-recorded audio tapes in a quiet room.

- California Verbal Learning Test (memory)
- Rey-Osterrieth Complex Figure Test Copy (Visual-Spatial Processing)
- Stroop Color-Word Test (interference) (Complex Attention/Executive Functions)
- Wechsler Adult Intelligence Scale (WAIS-III) Digit Symbol Substitution Test (Psychomotor Speed)
- Trail Making Test (Part B) (Complex Attention/Executive Functions)
- Controlled Oral Word Association Test (Language)
- Rey-Osterrieth Complex Figure Test Recall (Spatial Memory)
- Grooved Pegboard (Motor function)

The whole procedure will take approximately 1.5 hours. Participants will be allowed to drink water and to take a break as necessary.

Self-report instrument for depression

Depression will be evaluated by Beck Depression Inventory – Second Edition (BDI-II), which will be administered to each participant by trained NIAMS investigators using standardized instructions. The BDI-II constitutes the latest revision of the original Beck Depression Inventory (BDI), an instrument that was extensively used over a long period of time for measuring the

severity of depression. The BDI-II consists of 21 self-administered items that measure affective, cognitive, and somatic symptoms of depression. The BDI-II requires approximately 15 minutes to complete. Each item evaluates a category according to a scale of four possible responses of increasing severity, and the total score ranges from 0 to 63. In analyzing data, we will follow the cut score guidelines suggested for patients diagnosed with major depression. Thus, participants will be categorized into four levels based on the total scores: minimal (0-13); mild (14-19); moderate (20-28); severe (29-63).

9.8 Appendix H: Neurological Rating Scale

System Examined		Maximum Points	Normal	Degree of Impairment Mild	Mod.	Severe
Mentation and Mood		10	10	7	4	0
Cranial Nerves:	Visual Acuity	21	5	3	1	0
	Fields, Discs, Pupils		6	4	2	0
	Eye Movements		5	3	1	0
	Nystagmus		5	3	1	0
Lower Cranial Nerves		5	5	3	1	0
Motor:	RU	20	5	3	1	0
	LU		5	3	1	0
	RL		5	3	1	0
	LL		5	3	1	0
DTRS:	UE	8	4	3	1	0
	LE		4	3	1	0
Babinski: R; L (2 ea)		4	4	—	—	0
Sensory:	RU	12	3	2	1	0
	LU		3	2	1	0
	RL		3	2	1	0
	LL		3	2	1	0
Cerebellar:	UE	10	5	3	1	0
	LE		5	3	1	0
Gait, Trunk and Balance		10	10	7	4	0
Special Category: Bladder/Bowel/Sexual Dysfunction		0	0	−3	−7	−10
Totals		100				
Neurological Rating Scale Score						

* Points assigned for each component of the neurologic examination are subtotaled, and points for autonomic dysfunction are subtracted, leaving the final (NRS) score.

From Sipe et al, Neurology (Cleveland) 34:1368-1372, 1984

9.9 Appendix I: Graceley Scales

9.9.1 Intensity


20
19
18
EXTREMELY INTENSE
17
VERY INTENSE
16
INTENSE
15
STRONG
14
13
SLIGHTLY INTENSE
12
BARELY STRONG
11
MODERATE
10
9
8
MILD
7
VERY MILD
6
5
WEAK
4
VERY WEAK
3
2
1
FAINT
0
NO PAIN SENSATION

9.9.2 Unpleasantness

9.10 Appendix J

FACIT-Sp (version 4)

Below is a list of statements that other people with your illness have said are important. **By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.**

	PYSICAL WELL-BEING	Not at all	A little bit	Some	Quite a bit	Very much
GP1	**I have a lack of energy**	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain.	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	**I am forced to spend time in bed**	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	Not at all	A little bit	Some	Quite a bit	Very much
GP1	**I have a lack of energy 0**	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain.	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	**I am forced to spend time in bed**	0	1	2	3	4

Sp1

Sp2

9.11 Appendix K: Ultrasound lymph node surveillance data sheet

Name sticker

Date

Circle one:
0, 6, 12, 24 months

Region / location/ depth / size / aspirated? / notes

L groin

R groin

L axilla

R axilla

L neck

R neck

9.12 APPENDIX L: Research Specimen Handling

TIME POINTS	PRODUCTS	SEND TO	2° recipient
PRE TREATMENT- Baseline			
PBL/HAKIM	1GTT/1CPTred/green	Fran Hakim 5A07	(10ml hep/10ml red&green heparized cell prep.tub
PBL/FOWLER	4GTT	Dan Fowler 12C210	(10ml hep tube)
SERUM	1RTT (10 ml red top, serum tub	Fran Hakim 5A07	(10 ml red top, serum tube)
LEUKAPHERESIS (1 - 2 x109)	entire bag	Fran Hakim 5A07	200 E6 Amrie Grammer
MARROW	3-5ml, heparized,	Diane Arthur, cytogenetics	
MARROW	Remainder of 10ML ASPIRATE	Amrie Grammer 6D50	
PBL/GRAMMER(the same day w/B	1CPT red/green	Amrie Grammer 6D50	
HAKIM	TBNK (Lym Pheno TBNK panel	Clinical Immunology	
KIDNEY	BIOPSY	Clinical lab; 3rd pass to AG	AG & FH request laser scanning cytometer
PBL/Illei	1CPT red/green	Gabor Illei 9S210	8ml CPT
RNA/ILLEI	1 Special red top tube	Gabor Illei 9S210	2.5 ml
Plasma/ILLEI	6ml, purple tube	Gabor Illei 9S210	6ml
LYMPH NODE	FNA (B.Wood, Z.Neeman)	Andrea Abati	AG 6D50 (Fran will collaborate in T cell data)
URINE	FIRST IN MORNING	Gabor Illei 9S228	
PBSC APHERESIS			
PBL/HAKIM	1GTT, before apheresis	Fran Hakim 5A07	
BAG FACS	1x107	Fran Hakim 5A07	
CD34	1x107	Diane Arthur, cytogenetics	
CD34	1x107	Amrie Grammer 6D50	(Fran will collaborate in T cell data)
CD34	any yield ? 8 x106/kg	Amrie Grammer 6D50	Sarah for mice
non-CD34 ELUATE	200 x106	Amrie Grammer 6D50	(Fran will collaborate in T cell data)
CONDITIONING (DAY -7)			
FACS/HAKIM	1GTT	Fran Hakim 5A07	
PBL/Illei	1CPT red/green	Gabor Illei 9S210	8ml CPT
RNA/ILLEI	1 Special red top tube	Gabor Illei 9S210	2.5 ml
Plasma/ILLEI	6ml, purple tube	Gabor Illei 9S210	6ml

SERUM/HAKIM	1RTT	Fran Hakim 5A07	
CONDITIONING (DAY -1)			
PBL/HAKIM	1GTT	Fran Hakim 5A07	
SERUM/HAKIM	1RTT	Fran Hakim 5A07	
URINE	FIRST IN MORNING	Gabor Illei 9S228	
MARROW	10ML ASPIRATE	Amrie Grammer 6D50	Fran will collaborate
PBL/GRAMMER(the same day w/B	1CPT red/green	Amrie Grammer 6D50	
2 WEEKS			
PBL/HAKIM	1GTT/6CPTred/green	Fran Hakim 5A07	
SERUM/HAKIM	1RTT	Fran Hakim 5A07	
1 MONTH			
PBL/HAKIM	1GTT/6CPTred/green	Fran Hakim 5A07	
Plasma/ILLEI	6ml, purple tube	Gabor Illei 9S210	6ml
SERUM/HAKIM	1RTT	Fran Hakim 5A07	
URINE			
2 MONTHS			
Plasma/ILLEI			
URINE			
3 MONTHS			
PBL/HAKIM	1GTT/1CPTred/green	Fran Hakim 5A07	
PBL/FOWLER	4GTT	Dan Fowler 12C210	
PBL/Illei	1CPT red/green	Gabor Illei 9S210	8ml CPT
RNA/ILLEI	1 Special red top tube	Gabor Illei 9S210	2.5 ml
Plasma/ILLEI	6ml, purple tube	Gabor Illei 9S210	6ml
SERUM	1RTT	Fran Hakim 5A07	
LEUKAPHERESIS (2-3 x108)	entire bag	Fran Hakim 5A07	Sort/phenotype with Randy
URINE	FIRST IN MORNING	Gabor Illei 9S228	
4 MONTHS			
Hakim	6 CPT	Fran Hakim 5A07	
Hakim	TBNK (Lym Pheno TBNK panel)	Clinical Immunology	

Plasma/ILLEI	6ml, purple tube	Gabor Illei 9S210	6ml
URINE	FIRST IN MORNING	Gabor Illei 9S228	
5 MONTHS			
Hakim	6 CPT	Fran Hakim 5A07	
Hakim	TBNK (Lym Pheno TBNK panel)	Clinical Immunology	
Plasma/ILLEI	6ml, purple tube	Gabor Illei 9S228	6ml
URINE	FIRST IN MORNING	Gabor Illei 9S210	
TIME POINTS	PRODUCTS	SEND TO	2° recipient
6 MONTHS			
PBL/HAKIM	1GTT/1CPTred/green	Fran Hakim 5A07	
SERUM	1RTT	Fran Hakim 5A07	
PBL/Illei	1CPT red/green	Gabor Illei 9S210	8ml CPT
RNA/ILLEI	1 Special red top tube	Gabor Illei 9S210	2.5 ml
Plasma/ILLEI	6ml, purple tube	Gabor Illei 9S210	6ml
PBL/GRAMMER(the same day w/B	1CPT red/green	Amrie Grammer 6D50	
LEUKAPHERESIS (3-4 x 108)	entire bag	Fran Hakim 5A07	Sort/phenotype with Randy
LYMPH NODE	FNA (B.Wood, Z.Neeman)	Andrea Abati	AG 6D50 (Fran will collaborate in T cell data)
URINE	FIRST IN MORNING	Gabor Illei 9S228	
MARROW	3-5ml, heparized,	Diane Arthur, cytogenetics	
MARROW	Remainder of 10ML ASPIRATE	Amrie Grammer 6D50	Fran will collaborate
7 MONTHS			
Hakim	6 CPT	Fran Hakim 5A07	
Hakim	TBNK (Lym Pheno TBNK panel)	Clinical Immunology	
8 MONTHS			
Hakim	6 CPT	Fran Hakim 5A07	
Hakim	TBNK (Lym Pheno TBNK panel)	Clinical Immunology	
9 MONTHS			
PBL/HAKIM	1GTT/1CPTred/green	Fran Hakim 5A07	
PBL/Illei	1CPT red/green	Gabor Illei 9S210	8ml CPT

RNA/ILLEI	1 Special red top tube	Gabor Illei 9S210	2.5 ml
Plasma/ILLEI	6ml, purple tube	Gabor Illei 9S210	6ml
SERUM	1RTT	Fran Hakim 5A07	
LEUKAPHERESIS (3-4 x 108)	entire bag	Fran Hakim 5A07	Sort/phenotype with Randy
URINE	FIRST IN MORNING	Gabor Illei 9S228	
12 MONTHS			
PBL/HAKIM	1GTT/1CPTred/green	Fran Hakim 5A07	
PBL/FOWLER	4GTT	Dan Fowler 12C210	
PBL/Illei	1CPT red/green	Gabor Illei 9S210	8ml CPT
RNA/ILLEI	1 Special red top tube	Gabor Illei 9S210	2.5 ml
Plasma/ILLEI	6ml, purple tube	Gabor Illei 9S210	6ml
SERUM	1RTT	Fran Hakim 5A07	
PBL/GRAMMER(the same day w/B	1CPT red/green	Amrie Grammer 6D50	
LEUKAPHERESIS (1 x 10^9)	Entire bag	Fran Hakim 5A07	Sort/ phenotype with Randy
MARROW	3 – 5 mL, heparinized	Diane Arthur, cytogenetics	
MARROW	Remainder of 10 mL aspirate	Amrie Grammer 6D50	
KIDNEY	Biopsy	Clinical lab; 3rd pass to A	AG & FH request laser scanning cytometer
LYMPH NODE	FNA (B. Wood, Z. Neeman)	Andrea Abati	AG 6D50 (Fran will collaborate in T cell data)
URINE	FIRST IN MORNING	Gabor Illei 9S228	
18 MONTHS			
PBL/HAKIM	1GTT/1CPTred/green	Fran Hakim 5A07	
PBL/Illei	1CPT red/green	Gabor Illei 9S210	8ml CPT
RNA/ILLEI	1 Special red top tube	Gabor Illei 9S210	2.5 ml
Plasma/ILLEI	6ml, purple tube	Gabor Illei 9S210	6ml
SERUM	1RTT	Fran Hakim 5A07	
LEUKAPHERESIS (1 x109)	entire bag	Fran Hakim 5A07	Sort/phenotype with Randy
URINE	FIRST IN MORNING	Gabor Illei 9S228	
24 MONTHS			
PBL/HAKIM	1GTT/1CPTred/green	Fran Hakim 5A07	
PBL/FOWLER	4GTT	Dan Fowler	

		12C210	
PBL/Illei	1CPT red/green	Gabor Illei 9S210	8ml CPT
RNA/ILLEI	1 Special red top tube	Gabor Illei 9S210	2.5 ml
Plasma/ILLEI	6ml, purple tube	Gabor Illei 9S210	6ml
SERUM	1RTT	Fran Hakim 5A07	
PBL/GRAMMER(the same day w/BM	1CPT red/green	Amrie Grammer 6D50	
LEUKAPHERESIS (1 - 2 x109)	entire bag	Fran Hakim 5A07	Sort/phenotype with Randy
MARROW	3-5ml, heparized,	Diane Arthur, cytogenetics	
MARROW	Remainder of 10ML ASPIRAT	Amrie Grammer 6D50	
LYMPH NODE	FNA (B.Wood, Z.Neeman)	Andrea Abati	AG 6D50 (Fran will collaborate in T cell data)
URINE	FIRST IN MORNING	Gabor Illei 9S228	
RELAPSE			
PBL/HAKIM	1GTT/1CPT; if no leukapheresis, get 10	Fran Hakim 5A07	
PBL/Illei	1CPT red/green	Gabor Illei 9S210	8ml CPT
RNA/ILLEI	1 Special red top tube	Gabor Illei 9S210	2.5 ml
Plasma/ILLEI	6ml, purple tube	Gabor Illei 9S210	6ml
SERUM	1RTT	Fran Hakim 5A07	
PBL/GRAMMER	1CPT red/green	Amrie Grammer 6D50	
LEUKAPHERESIS (1 - 2 x109)	entire bag	Fran Hakim 5A07	Sort/phenotype with Randy
MARROW	3-5ml, heparized,	Diane Arthur, cytogenetics	
MARROW	Remainder of 10ML ASPIRAT	Amrie Grammer 6D50	
KIDNEY	BIOPSY	Clinical lab; 3rd pass to A	AG & FH request laser scanning cytometer
LYMPH NODE	FNA (B.Wood, Z.Neeman)	Andrea Abati	AG 6D50 (Fran will collaborate in T cell data)
URINE	FIRST IN MORNING	Gabor Illei 9S228	

9.13 APPENDIX M: Experimental Transplantation and Immunology Branch Preclinical Service Policy for Sample Handling

9.13.1 Storage/Tracking

Normal donor and patient blood and tissue samples, collected for the purpose of research under IRB approved protocols of the Experimental Transplantation and Immunology Branch, may be archived by the ETIB Preclinical Service. All data associated with archived clinical research samples is entered into the ETIB Preclinical Service's Microsoft Excel databases on frozen cells and plasma. These databases are stored on the NCI group drive in the ETIB Preclinical Service folder. Access to this folder is limited to ETIB clinical staff, requiring individual login and password. All staff in the Preclinical Service laboratory have received annually updated NIH/CIT training and maintain standards of computer security.

The data recorded for each sample includes the patient ID, name, trial name/protocol number, date drawn, treatment cycle/post transplant time point, cell source (e. g. peripheral blood, lymphapheresis, mobilized peripheral blood stem cells, marrow, pleural fluid) as well as box and freezer location. Patient demographics that correlate treatment outcomes and therapies with the samples can be obtained only through the NCI/ETIB clinical records. As of January 2007, all newly received samples will receive a unique bar code number, which will be added to the sample Preclinical Service database. Only this bar code will be recorded on the sample vial and the vials will not be traceable back to patients without authorized access to the Preclinical Service database. All non-coded samples previously archived will be stripped of identifiers prior to distribution for any use other than as a primary objective of the protocol under which they were collected.

Samples are stored in locked freezers at -85°C (sera and plasma) or under liquid nitrogen (cells), according to stability requirements. These freezers are located onsite at the Preclinical Service laboratory (12C216) (-85° freezer) or in ETIB common equipment space (CRC/3-3273). Access to samples from a protocol for research purposes will be by permission of the Principal Investigator of that protocol or through his/her submission and IRB approval of the NCI IRB Authorization Form (appended) stipulating whether IRB review is not necessary or IRB approval is granted for the pursuit of this new research activity. All researchers are required to sign a form (attached) stating that the samples are only to be used for research purposes associated with objectives of the original protocol for which the samples were collected, or (using only unlinked or coded samples) for an IRB approved protocol as stipulated on the IRB Authorization Form, and that any unused samples must be returned to the Preclinical Service laboratory.

9.13.2 Protocol Completion/Sample Destruction

Once primary research objectives for the protocol are achieved, researchers can request access to remaining samples, providing they have both approval of the Principal Investigator of the original protocol under which the samples or data were collected and either an IRB approved protocol and patient consent or the IRB Authorization Form stipulating that the activity is exempt from IRB review (see attached authorization form from the NCI IRB).

Samples, and associated data, will be stored permanently unless the patient withdraws consent. If researchers have samples remaining once they have completed all studies associated with the protocol, they must be returned to the Preclinical Service laboratory.

The Preclinical Service staff will report to the Principal Investigators any destroyed samples, if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container), lost in transit between facilities or misplaced by a researcher. The Principal Investigators will annually report this information to the IRB.

9.13.3 ETIB Preclinical Service Form for Requesting Samples

Please fill in the relevant information and initial and date the form below. Note that all unused samples or portions of samples are to be returned to the ETIB Preclinical Service laboratory.

I am requesting the following samples from transplant protocols of the Experimental Transplantation and Immunology Branch for analysis as a primary objective of the protocol under which the samples were obtained. I am a principle/associate investigator under Protocol _____.

Investigator Name _____ Initials____ Date _____

I am requesting coded samples of cells/plasma/sera of patients obtained from transplant protocols of the Experimental Transplantation and Immunology Branch for analysis under IRB Protocol _____. Relevant demographic data may be made available, but patient identifiers have been stripped from these samples.

Investigator Name _____ Initials____ Date _____

Attached is the IRB authorization Form indicating that no IRB review is necessary for the stipulated research activity.

Investigator Name _____ Initials____ Date _____
****
******

Protocol Number Cell Type/Plasma Transplant Stage

Request to Conduct Research for Same Use of Stored Human Samples, Specimens,or Data Collected in a Terminated NCI-IRB Protocol Protocol Number of Terminated Protocol: Please Check One: [ ] Exempt from IRB Review:

If the NIH investigator CANNOT identify the subjects, then the research activity may be exempt from IRB review and approval (Under 45CFR46.101(b). Use form I on this OHSR Website **http://ohsr.od.nih.gov/info/info.html** .

[ ] Same Use as Described in the Terminated Protocol

For this submission, complete the 1195 for as with any other initial protocol, respond to the required new protocol information, and provide a copy of the Consent Form/s from the Terminated Protocol:

I. Brief Description of Data/Specimens (how many, types, storage):

II. Research/Analysis being Conducted:

III. Timeframe for Analysis of Data/Specimens: (Please note – if longer than one year the

requirement for Continuing Review of the Research applies.)

IV. Indicate how the Rights and Welfare of Human Subjects will be protected and Risks to Human Subjects will be minimized: (This section must include a description of the processes for maintaining confidentiality of identifiable data/specimens/samples and what will happen to identifiable data/specimens/samples at the end or completion of the protocol. Where human samples or specimens are involved - describe how they will be stored; how they will be tracked; and under what circumstances would the PI report to the IRB loss or destruction of the samples/specimens.

9.13.4 NIAMS Laboratory Policy for Research Use, Storage and Disposition of Human Subject's Samples and Data.

Research records will be stored in locked files in our offices and in password-protected databases on a secure server. Clinical data will be stored indefinitely in the Medical Records department of the NIH Clinical Center where they will be secured and confidentiality will be protected under the standard procedures of these departments.

Serum, plasma, and DNA samples will be stored in locked freezers in Building 10. All stored biological samples and specimens will be coded with a study identification number only. Only NIH investigators associated with this study will have access to the secure database linking the study identification number with individual patients. Research data and samples may be shared with additional investigators in the future. Any data or samples sent to these investigators will be coded and will not include any patient identifying information. Codes need to be retained to allow the possibility of adding new patient-derived information in the future.

We do not plan to destroy research-related data or specimens at the completion of the protocol, as these data may be useful in the future care of subjects. The IRB will be notified at each annual review of any data or biological specimens lost or destroyed.

10 REFERENCES

1. Shlomchik, M.J., J.E. Craft, and M.J. Mamula, *From T to B and back again: positive feedback in systemic autoimmune disease.* Nat Rev Immunol, 2001. **1**(2): p. 147-153.
2. Manzi, S., et al., *Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study.* Am.J.Epidemiol, 1997. **145**(5): p. 408-415.
3. Lipsky, P.E., *Systemic lupus erythematosus: an autoimmune disease of B cell hyperactivity.* Nat Immunol, 2001. **2**(9): p. 764-766.
4. Deapen, D., et al., *A revised estimate of twin concordance in systemic lupus erythematosus.* Arthritis Rheum, 1992. **35**(3): p. 311-318.
5. Wakeland, E.K., et al., *Delineating the genetic basis of systemic lupus erythematosus.* Immunity, 2001. **15**(3): p. 397-408.
6. Plotz, P.H., *The autoantibody repertoire: searching for order.* Nat.Rev.Immunol, 2003. **3**(1): p. 73-78.
7. Datta, S.K., *Production of pathogenic antibodies: cognate interactions between autoimmune T and B cells.* Lupus, 1998. **7**(9): p. 591-596.
8. Demaison, C., et al., *Somatic diversification in the heavy chain variable region genes expressed by human autoantibodies bearing a lupus-associated nephritogenic anti-DNA idiotype.* Proc Natl Acad Sci USA, 1994. **91**(2): p. 514-518.
9. Winkler, T.H., H. Fehr, and J.R. Kalden, *Analysis of immunoglobulin variable region genes from human IgG anti-DNA hybridomas.* Eur J Immunol, 1992. **22**(7): p. 1719-1728.
10. Livneh, A., et al., *Lupus anti-DNA antibodies bearing the 8.12 idiotype appear to be somatically mutated.* J Clin Immunol, 1992. **12**(1): p. 11-16.
11. van Es, J.H., et al., *Somatic mutations in the variable regions of a human IgG anti-double-stranded DNA autoantibody suggest a role for antigen in the induction of systemic lupus erythematosus.* J Exp Med, 1991. **173**(2):
p. 461-470.
12. Dorner, T., et al., *Enhanced mutational activity of Vkappa gene rearrangements in systemic lupus erythematosus.* Clin Immunol, 1999. **92**(2): p. 188-196.
13. Munakata, Y., et al., *Somatic mutation in autoantibody-associated VH genes of circulating IgM+IgD+ B cells.* Eur J Immunol, 1998. **28**(5): p. 1435-1444.
14. Manheimer-Lory, A.J., et al., *Lupus-specific antibodies reveal an altered pattern of somatic mutation.* J Clin Invest, 1997. **100**(10): p. 2538-2546.
15. Rajagopalan, S., et al., *Pathogenic anti-DNA autoantibody-inducing T helper cell lines from patients with active lupus nephritis: isolation of CD48- T helper cell lines that express the gamma delta T-cell antigen receptor.* Proc Natl Acad Sci USA, 1990. **87**(18): p. 7020-7024.
16. de Vos, A.F., et al., *Breakdown of tolerance to a neo-self antigen in double transgenic mice in which B cells present the antigen.* J Immunol, 2000. **164**(9): p. 4594-4600.
17. Roth, R., R.J. Gee, and M.J. Mamula, *B lymphocytes as autoantigenpresenting cells in the amplification of autoimmunity.* Ann NY Acad Sci, 1997. **815**: p. 88-104.
18. Llorente, L., et al., *Spontaneous production of interleukin-10 by B lymphocytes and monocytes in systemic lupus erythematosus.* Eur Cytokine Netw, 1993. **4**(6): p. 421-427.
19. Andrews, B.S., et al., *Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains.* J Exp Med, 1978. **148**(5): p. 1198-1215.
20. Shlomchik, M.J., et al., *The role of B cells in lpr/lpr-induced autoimmunity.* J Exp Med, 1994. **180**(4): p. 1295-1306.
21. Chan, O. and M.J. Shlomchik, *A new role for B cells in systemic autoimmunity: B cells*

promote spontaneous T cell activation in MRL-lpr/lpr mice. J Immunol, 1998. **160**(1): p. 51-59.
22. Chan, O.T., et al., *A novel mouse with B cells but lacking serum antibody reveals an antibody-independent role for B cells in murine lupus.* J Exp Med, 1999. **189**(10): p. 1639-1648.
23. Shivakumar, S., G.C. Tsokos, and S.K. Datta, *T cell receptor alpha/beta expressing double-negative (CD4-/CD8-) and CD4+ T helper cells in humans augment the production of pathogenic anti-DNA autoantibodies associated with lupus nephritis.* J Immunol, 1989. **143**(1): p. 103-112.
24. Rajagopalan, S., C. Mao, and S.K. Datta, *Pathogenic autoantibodyinducing gamma/delta T helper cells from patients with lupus nephritis express unusual T cell receptors.* Clin Immunol Immunopathol, 1992. **62**(3): p. 344-350.
25. Datta, S.K. and A. Kaliyaperumal, *Nucleosome-driven autoimmune response in lupus. Pathogenic T helper cell epitopes and costimulatory signals.* Ann NY Acad Sci, 1997. **815**: p. 155-170.
26. Kolowos, W., et al., *CD4 positive peripheral T cells from patients with systemic lupus erythematosus (SLE) are clonally expanded.* Lupus, 2001. **10**(5): p. 321-331.
27. Jacobson, D.L., et al., *Epidemiology and estimated population burden of selected autoimmune diseases in the United States.* Clin Immunol Immunopathol, 1997. **84**(3): p. 223-243.
28. Urowitz, M.B., et al., *Mortality studies in systemic lupus erythematosus. Results from a single center. III. Improved survival over 24 years.* J Rheumatol, 1997. **24**(6): p. 1061-1065.
29. Rus, V. and Hochberg.MC., *The epidemiology of systemic lupus erythematosus*, in *Dubois' Lupus Erythematosus*, D.J. Wallace and B.H. Hahn, Editors. 2002, Lippincott Williams & Wilkins: Philadelphia. p. 65-86.
30. Gladman, D.D. and M.C. Hochberg, *Epidemiology of systemic lupus erythematosus*, in *Systemic Lupus Eythematosus*, R.G. Lahita, Editor. 1999, Academic Press: San Diego. p. 537-550.
31. Wallace, D.J., et al., *Systemic lupus erythematosus--survival patterns. Experience with 609 patients.* JAMA, 1981. **245**(9): p. 934-938.
32. Ginzler, E.M., et al., *A multicenter study of outcome in systemic lupus erythematosus. I. Entry variables as predictors of prognosis.* Arthritis and Rheumatism, 1982. **25**(6): p. 601-611.
33. Reveille, J.D., A. Bartolucci, and G.S. Alarcon, *Prognosis in systemic lupus erythematosus. Negative impact of increasing age at onset, black race, and thrombocytopenia, as well as causes of death.* Arthritis and Rheumatism, 1990. **33**(1): p. 37-48.
34. Abu-Shakra, M., et al., *Mortality studies in systemic lupus erythematosus. Results from a single center. I. Causes of death.* J Rheumatol, 1995. **22**(7): p. 1259-1264.
35. Massardo, L., et al., *Survival of Chilean patients with systemic lupus erythematosus.* Semin Arthritis Rheum, 1994. **24**(1): p. 1-11.
36. McLaughlin, J.R., et al., *Kidney biopsy in systemic lupus erythematosus.*

III. Survival analysis controlling for clinical and laboratory variables. Arthritis and Rheumatism, 1994. **37**(4): p. 559-567.
37. McLaughlin, J., et al., *Kidney biopsy in systemic lupus erythematosus. II. Survival analyses according to biopsy results.* Arthritis and Rheumatism, 1991. **34**(10): p. 1268-1273.
38. Ward, M.M., E. Pyun, and S. Studenski, *Mortality risks associated with specific clinical manifestations of systemic lupus erythematosus.* Arch Intern Med, 1996. **156**(12): p. 1337-1344.
39. Ward, M.M., E. Pyun, and S. Studenski, *Long-term survival in systemic lupus erythematosus. Patient characteristics associated with poorer outcomes.* Arthritis and Rheumatism, 1995. **38**(2): p. 274-283.
40. Estes, D. and C.L. Christian, *The natural history of systemic lupus erythematosus by prospective analysis.* Medicine (Baltimore), 1971. **50**(2): p. 85-95.
41. Swaak, A.J., et al., *Systemic lupus erythematosus. I. Outcome and survival: Dutch experience with 110 patients studied prospectively.* Ann Rheum Dis, 1989. **48**(6): p. 447-454.
42. Nossent, J.C. and A.J. Swaak, *Prevalence and significance of haematological abnormalities in patients with systemic lupus erythematosus.* Q J Med, 1991. **80**(291): p. 605-612.
43. Esdaile, J.M., et al., *The time-dependence of long-term prediction in lupus nephritis.* Arthritis Rheum, 1994. **37**(3): p. 359-368.
44. Goulet, J.R., et al., *The longterm prognosis of lupus nephritis: the impact of disease activity.* J Rheumatol, 1993. **20**(1): p. 59-65.
45. Cohen, M.G. and E.K. Li, *Mortality in systemic lupus erythematosus: active disease is the most important factor.* Aust NZJ Med, 1992. **22**(1): p. 5-8.
46. Iliopoulos, A.G. and G.C. Tsokos, *Immunopathogenesis and spectrum of infections in systemic lupus erythematosus.* Semin Arthritis Rheum, 1996. **25**(5): p. 318-336.
47. Gladman, D.D., et al., *The nature and outcome of infection in systemic lupus erythematosus.* Lupus, 2002. **11**(4): p. 234-239.
48. Jonsson, H., O. Nived, and G. Sturfelt, *Outcome in systemic lupus erythematosus: a prospective study of patients from a defined population.* Medicine (Baltimore), 1989. **68**(3): p. 141-150.
49. Gourley, M.F., et al., *Methylprednisolone and cyclophosphamide, alone or in combination, in patients with lupus nephritis. A randomized, controlled trial.* Ann Intern Med, 1996. **125**(7): p. 549-557.
50. Boumpas, D.T., et al., *Controlled trial of pulse methylprednisolone versus two regimens of pulse cyclophosphamide in severe lupus nephritis.* Lancet, 1992. **340**(8822): p. 741-745.
51. Austin, H.A., III, et al., *Therapy of lupus nephritis. Controlled trial of prednisone and cytotoxic drugs.* N Engl J Med, 1986. **314**(10): p. 614-619.
52. Boumpas, D.T., et al., *Systemic lupus erythematosus: emerging concepts. Part 1: Renal, neuropsychiatric, cardiovascular, pulmonary, and hematologic disease.* Ann Intern Med, 1995. **122**(12): p. 940-950.
53. Illei, G.G., et al., *Combination therapy with pulse cyclophosphamide plus pulse methylprednisolone improves long-term renal outcome without adding toxicity in patients with lupus nephritis.* Ann Intern Med, 2001. **135**(4): p. 248-257.
54. Illei, G.G., et al., *Renal flares are common in patients with severe proliferative lupus nephritis treated with pulse immunosuppressive therapy: long-term followup of a cohort of 145 patients participating in randomized controlled studies.* Arthritis and Rheumatism, 2002. **46**(4): p. 995-1002.
55. Chan, T.M., et al., *Efficacy of mycophenolate mofetil in patients with diffuse proliferative*

lupus nephritis. Hong Kong-Guangzhou Nephrology Study Group. N Engl J Med, 2000. **343**(16): p. 1156-1162.
56. Tyndall, A. and T. Koike, *High-dose immunoablative therapy with hematopoietic stem cell support in the treatment of severe autoimmune disease: current status and future direction.* Intern Med, 2002. **41**(8): p. 608-12.
57. Van Bekkum, D.W., *Experimental basis for the treatment of autoimmune diseases with autologous hematopoietic stem cell transplantation.* Bone Marrow Transplant, 2003. **32 Suppl 1**: p. S37-9.
58. Lopez-Cubero, S.O., K.M. Sullivan, and G.B. McDonald, *Course of Crohn's disease after allogeneic marrow transplantation.* Gastroenterology, 1998. **114**(3): p. 433-40.
59. McSweeney, P., *Nonmyeloablative allogeneic hematopoietic cell transplants: any role for rheumatoid arthritis?* J Rheumatol Suppl, 2001. **64**: p. 49-54.
60. Hinterberger, W., M. Hinterberger-Fischer, and A. Marmont, *Clinically demonstrable anti-autoimmunity mediated by allogeneic immune cells favorably affects outcome after stem cell transplantation in human autoimmune diseases.* Bone Marrow Transplant, 2002. **30**(11): p. 753-9.
61. Tyndall, A. and A. Gratwohl, *Blood and marrow stem cell transplants in auto-immune disease: a consensus report written on behalf of the European League against Rheumatism (EULAR) and the European Group for Blood and Marrow Transplantation (EBMT).* Bone Marrow Transplant, 1997. **19**(7): p. 643-5.
62. Burt, R.K., et al., *Induction of tolerance in autoimmune diseases by hematopoietic stem cell transplantation: getting closer to a cure?* Blood, 2002. **99**(3): p. 768-84.
63. Openshaw, H., R.A. Nash, and P.A. McSweeney, *High-dose immunosuppression and hematopoietic stem cell transplantation in autoimmune disease: clinical review.* Biol Blood Marrow Transplant, 2002. **8**(5): p. 233-48.
64. Marmont, A.M., et al., *Autologous marrow stem cell transplantation for severe systemic lupus erythematosus of long duration.* Lupus, 1997. **6**(6):
p. 545-8.
65. Fouillard, L., et al., *Control of severe systemic lupus erythematosus after high-dose immunusuppressive therapy and transplantation of CD34+ purified autologous stem cells from peripheral blood.* Lupus, 1999. **8**(4): p. 320-3.
66. Trysberg, E., I. Lindgren, and A. Tarkowski, *Autologous stem cell transplantation in a case of treatment resistant central nervous system lupus.* Ann Rheum Dis, 2000. **59**(3): p. 236-8.
67. Rosen, O., et al., *Autologous stem-cell transplantation in refractory autoimmune diseases after in vivo immunoablation and ex vivo depletion of mononuclear cells.* Arthritis Res, 2000. **2**(4): p. 327-36.
68. Marmont, A.M., *Lupus: tinkering with haematopoietic stem cells.* Lupus, 2001. **10**(11): p. 769-74.
69. Wulffraat, N.M., et al., *Prolonged remission without treatment after autologous stem cell transplantation for refractory childhood systemic lupus erythematosus.* Arthritis Rheum, 2001. **44**(3): p. 728-31.
70. Musso, M., et al., *Autologous peripheral blood stem and progenitor (CD34+) cell transplantation for systemic lupus erythematosus complicated by Evans syndrome.* Lupus, 1998. **7**(7): p. 492-4.
71. Shaughnessy, P.J., et al., *Graft failure in a patient with systemic lupus erythematosus (SLE) treated with high-dose immunosuppression and autologous stem cell rescue.* Bone Marrow Transplant, 2001. **27**(2): p. 221-4.

72. Brunner, M., et al., *Autologous blood stem cell transplantation in refractory systemic lupus erythematosus with severe pulmonary impairment: a case report.* Arthritis Rheum, 2002. **46**(6): p. 1580-4.
73. Traynor, A.E., et al., *Hematopoietic stem cell transplantation for severe and refractory lupus. Analysis after five years and fifteen patients.* Arthritis Rheum, 2002. **46**(11): p. 2917-23.
74. Burt, R.K., et al., *Development of a phase III trial of hematopoietic stem cell transplantation for systemic lupus erythematosus.* Bone Marrow Transplant, 2003. **32 Suppl 1**: p. S49-51.
75. Moore, J.J., et al., *Hematopoietic stem cell transplantation for severe rheumatoid arthritis.* Bone Marrow Transplant, 2003. **32 Suppl 1**: p. S536.
76. Brodsky, R.A., *High-dose cyclophosphamide for aplastic anemia and autoimmunity.* Curr Opin Oncol, 2002. **14**(2): p. 143-6.
77. Fassas, A., et al., *Hematopoietic stem cell transplantation for multiple sclerosis. A retrospective multicenter study.* J Neurol, 2002. **249**(8): p. 1088-97.
78. O'Brien, S.M., et al., *Results of the fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia.* 2001. **19**(5): p. 1414-1420.
79. Petrus, M.J., et al., *An immunoablative regimen of fludarabine and cyclophosphamide prevents fully MHC-mismatched murine marrow graft rejection independent of GVHD.* Biol Blood Marrow Transplant, 2000. **6**(2A): p. 182-9.
80. Bishop, M.R., et al., *Establishment of early donor engraftment after reduced-intensity allogeneic hematopoietic stem cell transplantation to potentiate the graft-versus-lymphoma effect against refractory lymphomas.* Biol Blood Marrow Transplant, 2003. **9**(3): p. 162-9.
81. Kuo, G.M., et al., *Fludarabine pharmacokinetics after subcutaneous and intravenous administration in patients with lupus nephritis.* 2001. **21**(5): p. 528-533.
82. Boumpas, D.T., et al., *A pilot study of low-dose fludarabine in membranous nephropathy refractory to therapy.* 1999. **52**(2): p. 67-75.
83. Davis, J.C., Jr., et al., *High dose versus low dose fludarabine in the treatment of patients with severe refractory rheumatoid arthritis.* J Rheumatol, 1998. **25**(9): p. 1694-704.
84. Davis, J.C., Jr., et al., *A pilot study of 2-chloro-2'-deoxyadenosine in the treatment of systemic lupus erythematosus-associated glomerulonephritis.* Arthritis Rheum, 1998. **41**(2): p. 335-43.
85. Adams, E.M., et al., *A pilot study: use of fludarabine for refractory dermatomyositis and polymyositis, and examination of endpoint measures.* 1999. **26**(2): p. 352-360.
86. Boye, J., T. Elter, and A. Engert, *An overview of the current clinical use of the anti-CD20 monoclonal antibody rituximab.* Ann Oncol, 2003. **14**(4): p. 520-35.
87. O'Brien, S.M., et al., *Rituximab dose-escalation trial in chronic lymphocytic leukemia.* J Clin Oncol, 2001. **19**(8): p. 2165-70.
88. van der Kolk, L.E., et al., *Rituximab treatment results in impaired secondary humoral immune responsiveness.* Blood, 2002. **100**(6): p. 2257-9.
89. McLaughlin, P., et al., *Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program.* J Clin Oncol, 1998. **16**(8): p. 2825-33.
90. Silverman, G.J. and S. Weisman, *Rituximab therapy and autoimmune disorders: prospects for anti-B cell therapy.* Arthritis Rheum, 2003. **48**(6):
p. 1484-92.
91. Edwards, J.C. and G. Cambridge, *Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes.* Rheumatology (Oxford), 2001. **40**(2): p. 205-11.
92. Anolik, J.H., et al., *The relationship of FcgammaRIIIa genotype to degree of B cell depletion*

by rituximab in the treatment of systemic lupus erythematosus. Arthritis Rheum, 2003. **48**(2): p. 455-9.
93. Leandro, M.J., et al., *An open study of B lymphocyte depletion in systemic lupus erythematosus.* Arthritis Rheum, 2002. **46**(10): p. 2673-7.
94. Perrotta, S., et al., *Anti-CD20 monoclonal antibody (Rituximab) for life-threatening autoimmune haemolytic anaemia in a patient with systemic lupus erythematosus.* Br J Haematol, 2002. **116**(2): p. 465-7.
95. Chow, K.U., et al., *Anti-CD20 antibody (IDEC-C2B8, rituximab) enhances efficacy of cytotoxic drugs on neoplastic lymphocytes in vitro: role of cytokines, complement, and caspases.* Haematologica, 2002. **87**(1): p. 3343.
96. Khouri, I.F., et al., *Nonablative allogeneic hematopoietic transplantation as adoptive immunotherapy for indolent lymphoma: low incidence of toxicity, acute graft-versus-host disease, and treatment-related mortality.* Blood, 2001. **98**(13): p. 3595-9.
97. Burt, R.K., et al., *Collection of hematopoietic stem cells from patients with autoimmune diseases.* Bone Marrow Transplant, 2001. **28**(1): p. 1-12.
98. Pavletic, S.Z., et al., *Intensive immunoablation and autologous blood stem cell transplantation in patients with refractory rheumatoid arthritis: the University of Nebraska experience.* J Rheumatol Suppl, 2001. **64**: p. 1320.
99. Mangel, J., et al., *Pharmacokinetic study of patients with follicular or mantle cell lymphoma treated with rituximab as 'in vivo purge' and consolidative immunotherapy following autologous stem cell transplantation.* Ann Oncol, 2003. **14**(5): p. 758-65.
100. Voso, M.T., et al., *In vivo depletion of B cells using a combination of high-dose cytosine arabinoside/mitoxantrone and rituximab for autografting in patients with non-Hodgkin's lymphoma.* Br J Haematol, 2000. **109**(4): p. 729-35.
101. Magni, M., et al., *Successful in vivo purging of CD34-containing peripheral blood harvests in mantle cell and indolent lymphoma: evidence for a role of both chemotherapy and rituximab infusion.* Blood, 2000. **96**(3): p. 864-9.
102. Buckstein, R., et al., *Stem cell function and engraftment is not affected by "in vivo purging" with rituximab for autologous stem cell treatment for patients with low-grade non-Hodgkin's lymphoma.* Semin Oncol, 1999. **26**(5 Suppl 14): p. 115-22.
103. Berkahn, L., et al., *In vivo purging with rituximab prior to collection of stem cells for autologous transplantation in chronic lymphocytic leukemia.* J Hematother Stem Cell Res, 2002. **11**(2): p. 315-20.
104. Flohr, T., et al., *Rituximab in vivo purging is safe and effective in combination with CD34-positive selected autologous stem cell transplantation for salvage therapy in B-NHL.* Bone Marrow Transplant, 2002. **29**(9): p. 769-75.
105. Stockmeyer, B., et al., *Enhanced killing of B lymphoma cells by granulocyte colony-stimulating factor-primed effector cells and Hu1D10--a humanized human leucocyte antigen DR antibody.* Br J Haematol, 2002. **118**(4): p. 959-67.
106. Kimby, E., *Beyond immunochemotherapy: combinations of rituximab with cytokines interferon-alpha2a and granulocyte colony stimulating factor [corrected].* Semin Oncol, 2002. **29**(2 Suppl 6): p. 7-10.
107. van der Kolk, L.E., et al., *Analysis of CD20-dependent cellular cytotoxicity by G-CSF-stimulated neutrophils.* Leukemia, 2002. **16**(4): p. 693-9.
108. Berinstein, N.L., et al., *Association of serum Rituximab (IDEC-C2B8) concentration and anti-tumor response in the treatment of recurrent low-grade or follicular non-Hodgkin's lymphoma.* Ann Oncol, 1998. **9**(9): p. 995-1001.

109. Tan, E.M., et al., *The 1982 revised criteria for the classification of systemic lupus erythematosus.* Arthritis Rheum, 1982. **25**(11): p. 1271-7.
110. Hochberg, M.C., *Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus.* Arthritis Rheum, 1997. **40**(9): p. 1725.
111. Manjunath, G., M.J. Sarnak, and A.S. Levey, *Prediction equations to estimate glomerular filtration rate: an update.* Curr Opin Nephrol Hypertens, 2001. **10**(6): p. 785-92.
112. Martin, P.J., et al., *Reporting of adverse event data in hematopoietic stem cell transplantation clinical trials involving investigational new drugs or devices: a report from the William Guy Forbeck Foundation 2001 focus meeting on clinical trials in hematopoietic stem cell transplantation.* Biol Blood Marrow Transplant, 2002. **8**(6): p. 295-302.
113. Grammer, A.C., T. Dorner, and P.E. Lipsky, *Abnormalities in B cell activity and the immunoglobulin repertoire in human systemic lupus erythematosus.* Molecular Pathology of Autoimmune Diseases, 2001. **2**: p. 282-318.
114. Traynor, A.E., et al., *Treatment of severe systemic lupus erythematosus with high-dose chemotherapy and haemopoietic stem-cell transplantation: a phase I study.* Lancet, 2000. **356**(9231): p. 701-7.
115. Smyk-Pearson, S.K., et al., *Rescue of the autoimmune scurfy mouse by partial bone marrow transplantation or by injection with T-enriched splenocytes.* Clin Exp Immunol, 2003. **133**(2): p. 193-9.
116. Bagavant, H., et al., *Differential effect of neonatal thymectomy on systemic and organ-specific autoimmune disease.* Int Immunol, 2002. **14**(12): p. 1397-406.
117. Valencia, X., L. He, and P.E. Lipsky, *CD4+CD25+ T regulatory cells in systemic lupus erythematosus.* Arthritis and Rheumatism, 2002. **SY09**.
118. Theofilopoulos, A.N., et al., *The role of IFN-gamma in systemic lupus erythematosus: a challenge to the Th1/Th2 paradigm in autoimmunity.* Arthritis Res, 2001. **3**(3): p. 136-41.
119. Nichols, G., et al., *Therapy-related myelodysplastic syndrome after autologous stem cell transplantation for breast cancer.* Leukemia, 2002. **16**(9): p. 1673-9.
120. Rosenthal, N.S. and D.C. Farhi, *Myelodysplastic syndromes and acute myeloid leukemia in connective tissue disease after single-agent chemotherapy.* Am J Clin Pathol, 1996. **106**(5): p. 676-9.
121. Abruzzese, E., et al., *Detection of abnormal pretransplant clones in progenitor cells of patients who developed myelodysplasia after autologous transplantation.* Blood, 1999. **94**(5): p. 1814-9.
122. Lillington, D.M., et al., *Detection of chromosome abnormalities pre-highdose treatment in patients developing therapy-related myelodysplasia and secondary acute myelogenous leukemia after treatment for non-Hodgkin's lymphoma.* J Clin Oncol, 2001. **19**(9): p. 2472-81.
123. Gambhir, S.S., et al., *A tabulated summary of the FDG PET literature.* J Nucl Med, 2001. **42**(5 Suppl): p. 1S-93S.
124. Alavi, A., et al., *Positron emission tomography imaging in nonmalignant thoracic disorders.* Semin Nucl Med, 2002. **32**(4): p. 293-321.
125. Zhuang, H. and A. Alavi, *18-fluorodeoxyglucose positron emission tomographic imaging in the detection and monitoring of infection and inflammation.* Semin Nucl Med, 2002. **32**(1): p. 47-59.
126. van Esser, J.W., et al., *Prevention of Epstein-Barr virus lymphoproliferative disease by molecular monitoring and preemptive rituximab in high-risk patients after allogeneic stem cell transplantation.* Blood, 2002. **99**(12): p. 4364-9.