OMRF
Oklahoma Medical Research Foundation

PROTOCOL FOR "BIOMARKERS OF LUPUS DISEASE" (the BOLD study)

AN EXPLORATORY, BIOMARKER-LINKED STUDY OF INTRAMUSCULAR METHYLPREDNISOLONE IN PATIENTS WITH LUPUS including PRIMARY IMMUNE PHARMACOLOGY ENDPOINTS ADDITONAL EFFICACY AND SAFETY ANALYSIS LINKED TO BIOMARKERS

Proposed start date: Feb 1, 2009
Estimated completion date: Jan 30 2013

Total patients to be recruited: Up to 290 patients (up to 70 group A, up to 100 group B, up to 50 controls for group C and up to 70 for a new group being added to the protocol (Group E) in order to ensure that we will have 50 independent patients completing the protocol for each of Groups A B and D.

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INTRODUCTION

Drug Development for Lupus

Lupus is a heterogenous autoimmune disorder characterized by sudden flares of organ-threatening inflammation, but with few reliable clinical patterns to predict the timing, nature, or degree of pathology that is likely to strike any individual. The quixotic nature of this disease is underscored by multiple murine models which exemplify clinical features of human lupus, but which bear little resemblance to each other in the underlying genetic defects which lead to that outcome. Human genetics research has confirmed that there are multiple, disparate genes implicated in risk for lupus, and that any two lupus patients have a vanishingly small chance to carry an identical assortment of risk genes. This ongoing genetics work is hampered by the

inability of statistical modeling to organize the variegated data emerging from multiple tests on limited populations, and is now resorting to whole genome scanning. This methodology may identify some of the most prevalent risk factors for lupus but is wholly unable to account for positive and/or negative gene interactions, and will miss some critical causes of the disease.

Although a final common pathway of T Cell defects, B Cell hyper-reactivity, myeloid dysfunctions and impaired apoptosis exist in most lupus patients, patients probably arrive at these shared inflammatory imbalances from many different directions. Thus, any attempts to restore immune balance would be served by understanding that some patients need a push on the left, others a push on the right.

It should be no surprise, then, that since 1994, a slew of Phase II trials for lupus have all failed to meet their primary endpoints. All of these clinical trials involved theoretically promising agents with excellent rationales for their use in lupus, as well as positive results in preclinical studies of murine lupus models. All of these trials used rational clinical endpoints as the primary outcome measure. However none of these trials recruited patients on the basis of demonstrated pathology relevant to the mechanism of action of their agent. None of these trials attempted to optimize dosing, even during Phase I, based on biomarkers relevant to the mechanism of action. Finally, few of these studies attempted to establish primary endpoints that should be relevant to their mechanism of action.

Yet for almost all of these studies, secondary analyses of "failed" trials showed subgroups of patients for whom the treatment, perhaps could have worked, both mechanistically and clinically. Using a carefully controlled, interventional trial of steroids which are known to have global effects on the immune system and elicit predictable response in lupus patients, the current protocol will provide serial blood samples suitable to obtain critical information about the pre-selection of patients by biomarkers relevant to several proposed treatments to be developed by Wyeth, and the degree of suppression in these specific immune pathways that correlates to partial or complete clinical improvement in the patients. More importantly the study incorporates withdrawal of background immune suppression to allow the immediate acquisition of blood samples, just prior to re-treatment, from those patients who flare after the first treatment period ends. This ability to obtain immediate samples from patients on limited background medication who receive copious clinical evaluation using accepted outcome measures will provide an unprecedented resource for the study of lupus biomarkers.

Technology is available to approach lupus drug development more sensibly. And the 20th Century approach to big, fast, drug candidate-wasting, chew them up, spit them out development will not work for lupus. Indeed, as of November 20th 2008, it will be fifty years since a new agent has been approved by the FDA for lupus. The company that succeeds in this mission will be the company that addresses the paradigm shift that is occurring for all complex, multifactorial diseases, towards individualized, 21st Century, biomarker-linked treatments, popularly called "personalized medicine."

By identifying biomarkers that change with steroid improvement, candidate immune pathways can be vetted for more rapid identification of treatment-linked tests to be developed alongside new targeted biologics in the pipeline. Using these tests to guide patient selection and dosing,

ensures the success of clinical trials, and then allows rapid saturation of the market by the diagnostic test once there is treatment approval. The biomarker test will be purchased for all lupus patients in order to select those who will rationally be appropriate to treat with the biomarker-linked agent(s). This then provides an almost instantaneous corrected market share for each new product which would have been difficult to achieve under the old paradigms of drug development and marketing, even after many years of blind competition by trial and error in the clinic. Acquiring this full and appropriate market share more rapidly will be good for business and accompanied by significant improvement in the standards of care for the patients.

CLINICAL PROTOCOL

Inclusion Criteria for Treatment and Non-Treatment SLE Groups: 50 patients will complete the original treatment protocol (Group A) in which background medications are withdrawn, steroids given and patients followed as they improve and then flare again. Up to an additional 100 patients will undergo only the Day 0 procedures without treatment intervention (Group B). We have increased this number because some patients in Group B later become qualified for (and interested in) a prospective study at a later date, therefore we may recruit up to 100 total Group E patients to ensure adequate patients for Group B that do not overlap with the prospectively followed patients. Patients who may have donated samples to Group B and then enter a prospective arm will have the Group B samples utilized as an internal check in consistency of their flare biomarkers. 50 healthy controls will complete Group C (matched to Group A patients).

In addition we are now adding Group E, to include up to 70 entants (50 completing patients) who meet the same entry criteria as Group A and will undergo the same study procedures except that background immune suppressants will not be withdrawn. All three SLE patient groups (A, B and E) must have a minimum of 4 1987 ACR criteria for SLE and have moderate to severe disease activity scores, defined as BILAG B in at least two organ systems, or BILAG A in at least one organ system or SLEDAI score of 6. Patients must also, in the opinion of the investigator, be considered a treatment failure on current medication, those in Group A and E must be appropriate for treatment with steroids.

Those patients who enter the treatment protocol in Group A must be able, on the day of study entry to be reduced to limited background treatment, defined as </= 20 mg prednisone, hydroxychloroquine optional, and no immune suppressants. Although these minimal treatments are allowed, the study will be enriched as much as possible for patients who can be restricted to no background lupus medications and receive only the protocol steroid treatment. Patients who cannot or do not wish to withdraw background treatment have the option of participating in Group B or Group E.

Control Group: n=50healthy individuals who will be matched, one to one with the patients in Group A by being within five years of age, same gender, same ethnicity. Control volunteers will undergo the screening visit protocol without treatment at two visits, at least one month apart. This is to provide a measurement of normal variation in biomarkers. **Exclusion Criteria:**

- Known active infection of any kind at screening
- Known previous HIV, Hepatitis B or C infection

- pregnancy or failure to commit to adequate birth control measures in women of childbearing potential
- cancer (other than basal cell or cervical carcinoma) within five years
- For Group A unwillingness or inability to withdraw background immune suppression
- Any medical condition that, in the opinion of the investigator, would be likely to interfere with completion of the protocol or compromise the patient's safety in the protocol.

Description of Study: Patients who agree to participate in this study will undergo informed consent procedures during which all treatment alternatives to participation in the study will be made available. Patients in Group A will understand that to participate in this protocol, background immune suppressants will be withdrawn and they will be immediately treated with IM depomedrol at a dose (160 mg) which is likely to ameliorate their immediate symptoms. Patients in Group E will receive the same steroid regimen with no alterations in their background treatment. This is a biomarker study, and patients in Groups A and E will have the option to return to the study center up to biweekly (q 3-4 days) for the first two weeks, during which time immediate non-responders will be retreated as necessary with 1-3 consecutive, additional depomedrol shots.

Most patients in Group A or E are expected to only require one depomedrol shot in order to achieve a clinical response (One letter grade decrease in BILAG score as a descriptor of activity in all organ systems that were active at baseline at the day response is determined). However up to four consecutive steroid shots can be given during the first two weeks of the protocol as clinically warranted. Depomedrol shots will stop at the time of response. If improvement, considered significant by the assessor, is not achieved by the time of the four week visit, the patients will be considered non-responders and withdrawn from the protocol (protocol non-completers). Patients who do not complete the Group A or Group E procedures due to failure to improve or to otherwise follow the protocol sufficiently can still have their samples from baseline analyzed (i.e. switched to Group B).

Patients in the BOLD study may come to the treatment center as often as needed during the initial treatment phase and during the follow up phase so that we optimize the chances of monitoring patient safety, achieving improvement and see the patients as soon as their disease flares again. After the four week initial treatment phase, patients who remain in the protocol will have regularly scheduled visits monthly thereafter for up to twelve months total participation. At the time of any symptom flare, they will be asked to immediately inform the clinic and will be scheduled for additional blood drawing, assessment and appropriate treatment within 3 days.

BILAG and SLEDAI disease activity instruments will be performed at baseline and monthly thereafter. Joint counts (tender and swollen 28 joint count) will be performed at every visit, and the CLASI skin scoring system will be used at every visit. At any time that patients are determined to have a BILAG B flare (or greater) or a four point increase in the SLEDAI score which is also considered by the patient or treating physician as necessary to treat, there will be an option for additional steroid shots (optional) or any treatment deemed appropriate.

Patients who complete the baseline, improving and flare visits will be considered study completers. Patients who complete all visits through six months whether they flare or not will

also be considered study completers. Up to 70 patients might be entered into Group A or E to ensure at least fifty completers in each of these Groups. Patients may elect to stay in the study up to 12 months if they have not flared by 6 months.

Safety and Disease Activity Monitoring: In addition to the laboratories needed to complete the BILAG and SLEDAI (CBC with differential, urinalysis, protein/creat ratio, complement studies and anti-dsDNA) blood sugar, liver enzymes and comprehensive blood lipid studies will be performed monthly. Additional biomarker studies are described elsewhere in this proposal. Patients will fill out Oklahoma Lupus Cohort questionnaires and the SF-36 patient reported outcomes measures at every visit. In addition to scoring the SLEDAI, BILAG, CLASI and joint count, clinicians will see the patients at every monthly visit and the flare visit and evaluate their medical condition and well being.

Primary Clinical Outcome Measure: The primary clinical outcome measure will be an assessment of time to flare in patients who enter with moderate vs severe activity and respond to 1-4 steroid shots within one month (moderate disease is defined as 2-3 BILAG Bs, no As, and SLEDAI </= 10) versus severe disease > 3 BILAG B's or at least one BILAG A or SLEDAI > 10 or meets definition for severe flare on SELENA SLEDAI). These data are considered by the lupus community to be very important in designing future clinical trials and will be published along with a BILAG and SLEDAI sensitivity analysis.

Secondary Clinical Outcome Measures:

Comparative BILAG and SLEDAI sensitivity analysis of outcomes using a 3 or 4 point drop or obtaining < 3 points in SLEDAI versus a change in BILAG of one letter grade in one organ, vs one letter grade in all organs or obtaining all Cs or below. The purpose of this analysis will be to test the sensitivity of several constructs of response suggested by consensus panels and used in previous clinical trials in a study of known effective treatment, and to categorize degrees of clinical response for use by the Wyeth and OMRF teams who can use these to evaluate response-predictive biomarker or biomarker changes.

Descriptive safety intent to treat analysis in order to define the expected adverse event rate in this kind of treatment withdrawal protocol

SF-36 Quality of Life Index for patients at entry, at the time of physician determined clinical response, and at the time of subsequent flares

Validation study of the new, revised SLEDAI and BILAG flare indices and the CLASI instrument against a gold standard of study withdrawal and treatment decisions as well as the clinicians simple determination of no flare, mild flare, moderate flare or severe flare (for flare instruments) and additionally no improvement, mild improvement, moderate improvement or major improvement (for the CLASI)

Linkage of Clinical Information to Ancillary and Exploratory Studies:
A searchable database of every individual clinical descriptor on the BILAG, SLEDAI, CLASI and SF-36 assessed at each time point in the study, along with final scores on these instruments,

clinical laboratory results and data fields developed after sorting patients by the above described primary and secondary outcome measures will be provided to the biologic researchers in a manner that can be linked to their laboratory based measurements.

Data Safety and Monitoring:

This is a single site study. An outside adjudicator will be hired in a subcontract to OMRF in order to monitor safety laboratories, flare rates and protocol violations in this study. This adjudicator will prepare a written report summarizing these data for review by the OMRF IRB every two months with additional reports whenever a serious adverse event occurs. A copy of this report will be provided to Pfizer. In the event of an unexpected serious adverse event rate, the adjudicator will be asked to review the reasons for hospitalization and provide a recommendation as to the continuance of the study. As a basis for adjudication of flares rates and infection rates, our data will be compared to safety data and flare rates seen in multi-center clinical trials, which have become available in the past two years. The outside adjudicator will also have the power to end the study for safety concerns.

Monitoring of clinical data entry will be provided by a team of two registered nurses, one from the SLE team in our clinic and one from an outside source without affiliation to the clinic or OMRF, on a regular basis during the data entry process and by a second outside adjudicator when the data has been entered into the database, based on comparing source documents and data entry in the database. This protocol is subject to initial and ongoing approval by the OMRF IRB, which is duly constituted and FDA monitored. The informed consent and HIPAA documents and the protocol are subject to any changes required by this board during initial or ongoing study reviews.

Safety of Treatments and Procedures

Blood drawing will be limited to 40 cc at monthly and flare visits and 30 cc at interim biomarker visits. Please see the breakdown of samples needed for each individual biomarker project.

Steroids are approved by the FDA for SLE and are standard of care treatments for lupus flares. The potential toxicities are well recognized and will be carefully monitored to ensure patients safety and well being. Although background medications will be withdrawn at entry into the study, an argument can be made that patients are not, by definition, responding to these treatments at entry and their withdrawal may help to prevent cumulative toxicities. Furthermore, safety measures are in place to ensure that patients are re-treated if lacking in initial response and will immediately receive different treatments if the repeated steroid treatments are ineffective or if significant flares occur and the patient does not wish retreatment with steroids at that point. Of course, all research participation is voluntary, and patients may withdraw from the study to receive non-protocol treatments (or for any reason) at any time.

Biomarker Testing

To maximize opportunities for biomarker identification and selection based upon this unique study design (e.g. patients flaring with no background immunosuppressive therapy), multiple

biomarker studies at both OMRF and Pfizer are being proposed. These biomarker groups include: (1) standard clinical biomarkers, such as ESR, CRP, complement levels and complement split products, (2) autoantibody evaluation, including screening antibody presence, concentration, epitope specificity and affinity, (3) immune cell populations, including quantitation of ASCs, memory B cells, T cell subsets and human mAb generation, (4) interferon activity by reporter cell assays, (5) inflammatory protein and immune cell signaling/ response profiles, including but not limited to TLDA and other methods of cytokine profiling (6) SNP genotyping of 20 key lupus genetic associations, (7) epigenetics and gene expression profiling with OMRF providing expression before and after immune cell signaling, (8) Epstein-Barr virus immunity, reactivation and gene expression and (9) potential collaborative projects understanding corticosteroid toxicities of hypertension, glucose intolerance, bone effects and lipid profile abnormalities.

(1) Standard clinical biomarkers

Many of the standard biomarkers in current clinical lupus use are incorporated as part of the BILAG and SLEDAI disease activity measures. To capture these reference responses, each patient will have ESR, CRP, and complement levels measures as outlined in the above clinical protocol. In addition, samples from the same blood draws will be tested for complement C4d-bound erythrocytes which are recognized as a measure of SLE disease activity as developed by Drs. Joe Ahearn and Sue Manzi at Pittsburgh (reviewed in Curr Opin Rheum, 2005).

Pfizer requested and funded this study.

(2) Autoantibody evaluation

In addition, select lupus autoantibodies have been historically considered reasonable biomarkers for at least subsets of SLE patients (for example, rising anti-dsDNA levels in combination with increases in complement split products, please see Tseng, et al, Arthritis Rheum, 2006). We will measure panels of standard lupus autoantibodies through traditional immunofluorescence (ANA with titer and pattern, anti-dsDNA by Crithidae), luminex bead based assays (testing for antibodies against 60kD Ro, 52kD Ro, La, Sm/nRNP, nRNP 70K, nRNP A, Sm, chromatin, dsDNA, ribosomal P), and ELISAs (anti-cardiolipin IgG, IgM). In addition, samples will also be tested for panels of 80 additional lupus-associated autoantigens by a slide based proteomic array assays. Positive responses will be tittered out to assess concentrations at different time points for the same individual. For samples positive for antibodies against autoantigens amenable to humoral epitope mapping (spliceosomal proteins, Ro, and ribosomal P), solid phase epitope mapping will be performed to assess epitope specificity changes with improvement of symptoms and clinical flares. Positive antibody responses will also be assessed by BiaCore experiments to assess affinity of antibodies with onset of disease flares. All of these methods are in use in our research laboratories and references are available on our attached biosketches/CVs. Samples for this set of biomarker analyses will be tested at baseline, 2 weeks after response and with any clinical disease flare. Serum will also be stored so that additional evaluations can occur of interim samples as needed to correlate with other biomarkers or disease features.
Pfizer requested and funded the ANA and luminex bead-based assays portion of this study

(3) Immune cell populations

To fully understand the dynamics of the lupus disease process and identify potentially useful and easily tested biomarkers, it is crucial to gather cell surface molecule levels on as many identifiable subsets of cells accessible from whole peripheral blood. The advantage of using whole blood, as opposed to peripheral blood mononuclear cells, is that one is more likely to get a snap-shot of the actual *in vivo* cellular profiles from the patient as near to the time of draw. This minimizes potential instability of particular subsets of cells or surface biomarkers that might lead to a loss of valuable information on these samples.

To maximize the number of cell types examined and the number of cell surface markers measured in particular cell subsets, we propose to use three (3), 9 color flow cytometry panels (T cell, B cell, and myeloid cells). Each of these panels is summarized in the tables below. The total number of visits where profiles would be assessed would 800.

T Cell Profile	**B Cell Profile**	**Monocyte/Macrophage/DC Profile**
Antigen	***Antigen***	***Antigen***
CD3/CD4	CD45	CD45
CD8	CD19	CD209
CD278 (ICOS)	CD27	CD3/CD4
CD275 (ICOSL)	CD38	CD56
CD154 (CD40L)	CD40	CD11b
CD279 (PD-1)	CD194	CD11b active
CD194 (CCR4)	IL-21R	CD279 (PD-1)
IL-21R	CD80	CD194 (CCR4)
CD80	CD86	IL-21R

Pfizer requested and funded this study

(4) Interferon activity assays (OMRF project – samples banked for later)

Over the past several years, increased expression of interferon inducible genes has evolved as a repeated observation in SLE. Indeed, several groups have shown that upregulation of interferon genes is associated with increases in SLE disease activity in cross-sectional studies. Wyeth has plans to test for expression of a panel of such interferon genes. However, serologic evidence of interferon activity through use of reporter cell lines (like the WISH cell line experiments used by Mary Crowe, Timothy Niewold and colleagues) would be useful as this test can be performed on sera and may be more stable than traditional measures of serum cytokine levels directly. This reporter assay tests the ability of serum to upregulate three interferon inducible genes in an interferon-responsive WISH cell line through real-time PCR identification and normalization. We propose to test baseline, 2 week and flare samples by this method for interferon activity. In addition, stored serum would be available for testing of additional interim samples per individual as needed. This information would provide direct evaluation of response of interferon activity to corticosteroids and correlation with disease activity. This assay would also provide a simpler

point of contact assay of interferon activity, as well as allow direct comparison of this method with the proposed gene expression profiling.

(5) Immune cell signaling and response profiling (possible OMRF pilot study).

Another important aspect of understanding lupus is to understand how different cell populations respond to disease relevant biological stimulation. While this is a data intensive proposal, it is important to note that this study offers the highly unique opportunity. Few studies would be amenable to determine such responses in the setting where the patients are on minimal immunomodulatory drug therapy. This proposal enables us to better define responses of individual cell populations to biological modifiers that can be biomarkers and also allows us to gain new insights into mechanisms and potential targets for new therapeutics.

We propose to perform a multiparameter PhosFlow experiment on whole blood cells. Each patient will be assess at two timepoints (minimal disease activity and flare) and approximately 10 controls will be assessed at baseline only. Whole blood cells from each individual will be activated/perturbed with a panel of 20 biologically relevant stimulants. Three stimulation timepoints (5, 7.5 and 15 minutes) will be collected. See table below for list of stimulants to be tested. For every stimulation sample (60 total), a 9 color PhosFlow experiment will be performed. This will allow for 8 or more (T, B, mono/macrophage, DC – activated and unactivated) cell populations to be examined for levels of five (5) phospho proteins (pSTAT1, pSTAT3, pSTAT5, p38 MAP kinase, pERK1/2).

PhospFlow Profile
CD3/CD4
CD8
CD86
CD45
P-Stat3
P-Stat1
P-Stat5
P-p38/MapK
P-ERK1/2

Stimulations (20 conditions, 3 timepoints each)	
Stimulatant	**Stimulant**
PBS	IL-6
PMA + Ionomycin	IFN-alpha
LPS	IFN-gamma
Forskolin	IL-12
FMLP	IL-18
Fibrinogen	IL-12 + IL-18
FMLP + Fibrinogen	IL-23
TNF-alpha	IL-21
anti-CD3	CD40L
anti-IgM F(ab)'2	CD40L + IL-21

(6) SNP genotyping (OMRF project)

Extensive genome wide association studies and candidate gene analyses have provided new information suggesting key genetic pathways which may be dysregulation in SLE. A number of associations have been found and confirmed with SLE; however, the influence of these genetic backgrounds on response to therapy or flare rates have not been studied to date. Although this study is greatly underpowered to find direct genetic associations with clinical responses, we will be able to generate hypothesis regarding potential genetic factors to consider in future trials or

the potential influence of select gentoypes on clinical biomarkers. We proposed to test 20 SNPs from a select number of lupus associated genes. Indeed, today we would select the key markers from IRF5, PTPN22, ITGAM, BLK, and STAT4. To maximize this and other potential analyses an immunochip may be used and exploratory analyses of genes relevant to other biomarker studies may be undertaken as appropriate.

(7) B cell-specific gene expression profiling and functional response assays (50 cases and 10 controls – OMRF Pilot Project)

Pfizer and OMRF plan to do gene expression profiling for a number of interferon and immune response genes. In addition, we propose to collect and bank RNA samples from each patient visit for potential parallel gene expression experiments (baseline and with stimulation) to further dissect immune dysregulation with disease flares, with increased disease activity, and with corticosteroid treatment.

We also propose to assess B cell specific dynamic gene expression responses following B cell activation in low disease activity (3-4 weeks after steroid treatment) patients compared to controls. This would be an opportunity to probe the B cell system for specific pathway components that are dysregulated in a lupus patients and prone to aberrant activation. In phase I of this portion of the study, 4-5 tubes of blood will be collected and used for B cell isolation. Controls will be tested at baseline and patients will be tested at a point of minimal disease activity (2 weeks post-steroid treatment) and at flare. These B cells will be stimulated using various biological stimulations (non-stimulated, EBV infected and anti-IgM + CD40L) and RNA isolated at 1 and 4 hours post-stimulation. All RNAs will be banked for future studies. At two additional times during the protocol, generally on the week following the B cell experiment if possible, three (3) additional tubes will be collected, myeloid cells (monocytes/granulocytes) isolated and frozen for additional experiments on monocyte/neutrophil gene expression or other functional assays. At this time, OMRF only proposes to perform Phase I as listed in the table below. All RNAs will be banked for gene chip analysis at a later time.

(8) Epstein-Barr virus immunity, reactivation and gene expression (OMRF project)

Although genetic predisposition plays a critical role in SLE, based upon the 40% monozygotic twin concordance rate, we know that other environmental factors are crucial in SLE etiology and pathogenesis. OMRF investigators have extensive evidence for molecular mimicry and abnormal humoral immune responses to EBV in SLE patients. They propose to assess EBV serology for viral reactivation, viral loads (in mouthwash and peripheral blood) and EBV gene expression at baseline, 1 week after corticosteroids and with disease flares. This information will help identify the influence of steroids of EBV reactivation and whether SLE patients who flare have reactivation or new EBV gene expression. We will also be able to assess the influence of EBV on interferon activity. These studies would only use extra samples from the study patients and would be supported by investigator funds.

Please see the spreadsheets (attached as excel files) for procedure, sampling and budget breakdowns.

Final Protocol, ~~Amendment #1,~~ 12/14/08

Amendment #2, 6/17/09
Visits .5, 1, 1.5, 3, 5 and 6 are optional for the patients in the study. The patients will be considered completers if these visits are missed.

Amendment #3 11/16/10
The number of patients will be increased to up to 100 patients in each group.

Amendment #4, 4/23/11
This amendment adds one new group of patients (Group D) who will undergo the same procedures as Group A except that their background immune suppressants will <u>not</u> be stopped at the first visit as is being done for Group A. It will also increase the numbers of patients recruited to a total of up to 70 in Groups A and D and 100 in Group B) in order to ensure that 50 different patients can eventually complete the procedures for each group.

Amendment #5, 1/20/12
For Group C healthy control subjects only: A small honorarium will be paid for participation in this protocol in order to complete the study in a timely manner.