RESEARCH PROTOCOL

Title of project: Dipyridamole Assessment for Flare REduction in SLE: The DARE study

Principal Investigator: Aikaterini Thanou, MD

Co-Investigator: Joan Merrill, MD

Co-Investigator: Judith James, MD, PhD

Co-Investigator: Eliza Chakravarty, MD

Co-Investigator: Samera Vaseer, MD

Co-Investigator: Teresa Aberle, PA-C

Consultant: Stavros Stavrakis, M.D.

Abstract

T cells in systemic lupus erythematosus (SLE) express an abnormal phenotype characterized by increased effector functions and deficient regulatory responses. Dipyridamole, a phosphodiesterase inhibitor extensively used in combination with low dose aspirin in secondary stroke prevention, has been proposed as a specific T cell directed treatment for SLE. Dipyridamole inhibits the calcium/calcineurin/NF-AT pathway in SLE T cells *in vitro* and abrogates expression of cytokines and costimulatory molecules, eventually also affecting B cell responses. Dipyridamole delays the emergence of lupus related pathology in lupus prone mice, but has not yet been studied in humans with SLE. We aim to investigate the efficacy of dipyridamole in the prevention of flares in SLE patients after withdrawal of background immunosuppressive medications. We will additionally evaluate the safety and tolerability of dipyridamole and its impact on quality of life measures in this population. Furthermore, the effect of dipyridamole on T and B cell biomarkers will be examined.

A. Specific aims

Primary objective: To examine the efficacy of dipyridamole/aspirin (Aggrenox, Boehringer Ingelheim Pharmaceuticals, Inc.) for flare prevention in patients with SLE as measured by the BICLA endpoint (improvement in disease activity compared to baseline without new flare or additional treatments).

Secondary objectives: To examine the effect of dipyridamole on:

1. Flare rate and time to first flare

2. Systemic response index (SRI) 4/5 at week 24, BICLA and SRI 4/5 at each month, SRI component analyses, CLASI and DIAL responses

3. Patient reported outcomes, including: health related quality of life (measured by the Lupus QoL, LupusPRO and the Short Form 36v2), fatigue (measured by the FACIT-fatigue score), sleep quality (measured by the Pittsburgh Sleep Quality Index (PSQI)) and depression (measured by the Center for Epidemiologic Studies Depression (CES-D) Scale)

4. Tolerability and safety

5. T and B cell biomarkers, including: serum immunoglobulin levels, evidence of B cell hyperactivity measured by cell bound complement activation products (Exagen Diagnostics, Inc.), levels of T and B cell cytokines, serum INFα activity, immunophenotypes of T and B cell subsets, T cell biomarkers and cytokine production after in vitro T cell stimulation, immunoglobulin production after in vitro stimulation of peripheral blood mononuclear cells (PBMCs), T and B cell gene expression profiles,

6. Urine biomarkers of lupus disease activity (cells, cytokines, proteomics, mRNA)

7. Heart rate variability in an ancillary study (see attached protocol).

B. Background and significance

T cells in SLE express an abnormal phenotype characterized by increased effector functions and deficient regulatory responses (1). SLE T cells provide aberrant costimulation to B cells and can also directly invade host tissues, where they produce proinflammatory cytokines. Upon engagement of the TCR, SLE T cells display a particularly robust calcium response and mobilize NF-AT into the nucleus (2). T cells from MRL/lpr mice can aberrantly help normal mouse B cells in a calcineurin dependent manner, linking the SLE T cell hyperactivity to T cell helper functions.

The traditional calcineurin inhibitor tacrolimus effectively blocks NF-AT activation in SLE T cells, but has an unfavorable side effect profile in patients with SLE. Dipyridamole, a phosphodiesterase inhibitor that increases intracellular cAMP, has been recently recognized as a specific inhibitor of calcineurin/NF-AT interactions (3). Dipyridamole has been extensively use as an anti-platelet agent and vasodilator for various indications, including stroke (4) and certain types for nephritis (5). Dipyridamole in combination with low dose aspirin is superior to aspirin alone in secondary prophylaxis of ischemic stroke and other vascular events, with a good tolerance and safety profile (4). Dipyridamole has been additionally recognized to selectively amplify the anti-inflammatory effects of corticosteroids (6).

The enhanced calcium/calcineurin/NF-AT pathway in human and murine SLE T cells can be suppressed in presence of dipyridamole (7). Dipyridamole abrogates expression of costimulatory molecules and production of cytokines by SLE T cells and inhibits T cell directed B cell antibody production. Administration of dipyridamole to MRL/*lpr* mice inhibits production of IL6 and delays the emergence of lupus related pathology, including nephritis and skin disease. Taken together, these observations justify a clinical study of dipyridamole in patients with SLE.

C. Preliminary studies

There is an ongoing need for new treatments in SLE that will target specific immune pathways and provide immunomodulation with limited immunosuppression and acceptable tolerance. Recent clinical trials of new medications for treatment of SLE have however been hampered by disease heterogeneity and confounded by background medications, as well as the use of various, inadequately validated measures of disease activity (8).

Recent genetic and mechanistic studies in SLE provide valuable insight into disease heterogeneity, and biomarkers of disease activity (9). A gene expression array of genes contributing to aberrant T cell function was recently found useful in SLE diagnosis and classification (10). Similar arrays may be also useful for monitoring of T cell targeted therapies.

Only certain SLE subgroups or organ systems may respond to a particular agent, an important consideration for early phase trials. Concurrent use of immunosuppressive medications, like mycophenolate mofetil, azathioprine, methotrexate and corticosteroids, creates further biologic variability in an already heterogenous disease and confounds the interpretation of data in human studies, since these medications might interfere with or confound the mechanism of the agent(s) studied. This practice also increases the response in the comparator arms, limiting the possible differences between treatment and placebo in SLE clinical trials, necessitating the prolonged study of larger patient numbers for adequate power, and further compromising the clinical interpretability of results. Withdrawal of background immune suppression has been considered as an option to limit study time and patient exposures to untested therapies, but the community has been concerned about its safety. The BOLD (Biomarkers of Lupus Disease) trial, in which a brief course of parenteral steroids is utilized to induce a state of remission or low disease activity while background medications are withdrawn, has shown evidence this approach is safe and effective (11). Time to first flare averaged 72 days (CI 43–91), with 100% of the patients flaring by 24 weeks.

In the BLISS trials of belimumab in SLE the systemic response index (SRI), a composite measure of disease activity, was used as the primary endpoint. SRI is a landmark index defined as the composite of ≥4 point reduction in total SLEDAI score, with no new BILAG A or no more than one new BILAG B domain score and no more than 10% worsening in physician's global assessment (PGA), compared to baseline. Another composite measure, the SRI-BILAG (BICLA) has been used in the EMBLEM phase II trial of epratuzumab in SLE. BICLA requires the achievement of BILAG-2004 improvement (BILAG A to B/C/D, BILAG B to C/D, and no BILAG worsening), along with no deterioration in SLEDAI total score and no more than 10% worsening in PGA. In the BOLD study, BICLA was found to be more sensitive to change than SRI, when anchored to the simpler BOLD improvement criteria (drop of ≥ one BILAG grade or ≥4 SLEDAI points) anchored to the clinician's determination of significant clinical improvement (11).

The dipyridamole dose administered in mice (50mg/kg) by Kyttaris VC *et al* (7) is considered equivalent to 4mg/kg in humans (12), the dose routinely use for thromboprophylaxis in clinical practice (oral administration). Pharmacodymanic studies for the anti-inflammatory and T cell inhibitory effects of dipyridamole have however not been conducted in humans, not excluding the possibility of lower doses also being effective. Immediate release dipyridamole and an extended release formulation of dipyridamole (200mg) in combination with low dose aspirin (25mg) (Aggrenox, Boehringer Ingelheim Pharmaceuticals, Inc.) are available in the US market.

Dipyridamole at standard daily dosing (400mg daily) is often poorly tolerated, mainly due to dizziness, headache and abdominal distress. In the large ESPRIT study of the combination of aspirin and dipyridamole, rates of treatment discontinuation due to intolerance were substantial (34% in the dipyridamole plus aspirin group versus 13% in the aspirin alone group) (13), and similar rates were observed in the ESPS2 trial (14). In clinical practice, a titration scheme of dipyridamole at initiation could be used to try to resolve the problem of drug-induced headache (15).

In ESPRIT, patients allocated to aspirin and dipyridamole had fewer major bleeding complications than patients allocated to aspirin alone, though this finding was not significant (13). Moreover, an equal rate of minor bleeding complications between groups was found in both ESPRIT and ESPS2, and few major bleeding complications were reported in either group. Similarly, the ESPS2 showed no difference in the frequency of severe or fatal bleeding complications between the two groups (14). Thus, treatment with dipyridamole and aspirin is overall considered at least as safe as low dose aspirin.

Heart rate variability (HRV) is an easily obtainable electrocardiographic measure of vagus nerve activity in humans and has been inversely correlated with inflammatory markers (C-reactive protein and IL-6) in the general population (16) (please see attached protocol). Decreases in HRV have been demonstrated in a broad array of pathologic conditions, including lupus (17, 18). The relation of HRV with disease activity in lupus patients has not been conclusively examined. Similarly, no previous studies have addressed the effect of dipyridamole on HRV.

D. Research design and Methods

This is a prospective placebo-controlled randomized study. Patients will enter with active disease. After informed consent and screening procedures are completed, enrolled patients may be optionally treated with intramuscular methylprednisolone acetate and background immune-suppressive medications will be withdrawn. Injection of methylprednisolone acetate may be repeated in week 2 at the discretion of the investigator, to induce a state of remission/low disease activity. Patients will be assigned to begin the study medication upon enrollment (baseline, week 0). Patients will be randomized 1:1 to Aggrenox or half of an 81mg aspirin tablet twice daily for 24 weeks. Aggrenox will be initiated at a single bedtime dose for a week, followed by twice daily administration for the rest of the study, to minimize the risk of side effects (e.g. dizziness, headache). Aspirin will be titrated similarly to preserve blinding. Gelatin capsules will be used to cover the pills in order to preserve the blinding.

Participants will be evaluated at baseline (week 0), week 2, week 4 and every 4 weeks until the completion of the study at week 24. An additional study visit may be scheduled at the time of a clinically significant flare (flare visit). Evaluation of general health status, adverse events and lupus disease activity (SLEDAI, BILAG, PGA, CLASI, DIAL, SF-36v2, Lupus QoL, FACIT-fatigue, PSQI, CES-D) will be performed at baseline, week 4 and every subsequent visit. Time to first flare and severity of first flare will be examined. At the time of a clinically significant flare

(defined as at least one BILAG letter grade increase or an increase of ≥3 points in SLEDAI AND clinician determination of clinically significant deterioration) patients may elect further treatment with intramuscular methylprednisolone acetate or, if necessary DMARD therapy, but will continue the study as permanent nonresponders.

Patients will have CBC with differential, metabolic profile, urinalysis, anti-dsDNA, and complement studies performed at baseline, each of the subsequent monthly visits through week 24 and the flare visit. If urine dipstick is ≥ 2+, a protein/creatinine ratio will be performed at the following visit (or sooner at the discretion of the investigator). Pharmacodynamic and exploratory blood and urine biomarker samples will be drawn prior to first dose at baseline, at weeks 8 or 12, week 24 and at the flare visit. Some of the stimulated cell gene expression and signaling pathway work will be performed on deidentified samples in collaboration with Drs. George Tsokos and Vasileios Kyttaris at Beth Israel Medical Center in Boston Massachusetts. We will have a material transfer agreement with that institution. In the context of our ancillary study of heart rate variability in lupus, a 5-minute ECG will be performed at each monthly visit and at the flare visit (please see attached protocol).

Summary of Medications and Medication Changes

At screening, participants may be receiving daily prednisone at a dose of 20mg or less, and/or one or more of the following DMARDs: hydroxychloroquine, methotrexate, azathioprine and mycophenolate mofetil, and must be agreeable to discontinue their background immune suppressants upon enrollment. Patients taking hydroxychloroquine upon enrollment will however continue the medication throughout the study.

All participants may be administered optional intramuscular methylprednisolone acetate upon enrollment (usually 160mg, but dose may adjusted based on clinical judgment). Intramuscular steroid treatment may be repeated at weeks 2 at the discretion of the investigator, to induce a state of remission/low disease activity while background medications are withdrawn. Patients on oral steroids will be given an optional rapid taper over 3-4 weeks.

Duration of participation

Participants will receive treatment for a total of 24 weeks.

Patient enrollment

A maximum of 50 patients meeting the inclusion criteria and not meeting the exclusion criteria will be enrolled in the study. The enrollment period is expected to last for at least 18 months but could last as long as four years.

Data collection and analysis

Data will be collected by study personnel who will be kept blinded to treatment assignments. A trained study coordinator will provide data monitoring and quality assurance checks on the database. The final data will be pooled and analyzed by the investigators.

E. Statistical methods

The primary analysis will be an intention-to-treat two by two table.

Primary endpoint: A two by two table analysis of rate of response by the BICLA response index at week 24 compared to baseline (week 0) in patients on aspirin versus Aggrenox regimens, with non-response attributed to patients who receive defined non-responder treatments and/or withdraw early. Please see below for power calculations based on expected response rates. Independent predictors of BICLA response, including baseline biomarkers, biomarker responses to Aggrenox treatment, demographics and amount of steroids administered, will be further investigated by exploratory logistic regression modeling.

Secondary endpoints: Flare rates at week 24 compared between completers among treatment groups. Time to flare (log rank test). SRI 4/5 at week 24, BICLA and SRI 4/5 at each month. HRQOL comparing treatment groups versus baseline at week 24 and at each month. Although our primary endpoint is based on the BICLA response, an accepted outcome for lupus clinical trials which serves as a pilot response indicator for future trials, it is likely that some secondary endpoints, such as time to flare will be more sensitive, and more likely to achieve significance in a pilot study.

Exploratory endpoints: SRI component analyses and CLASI responses. Refinement in BICLA and SRI analysis using a propensity score derived by multiple potential confounders identified in exploratory single variate analysis. Relation of clinical and biologic endpoints with heart rate variability measures by multivariate analysis.

Biologic Endpoints: Changes in B cell and T cell subsets, relevant gene expression patterns, B-T cytokine profiles and cell-bound complement split products and urinary biomarkers at baseline versus week 24 in each treatment group. Evaluation of B Cell/T cell expression patterns in patients with higher versus lower IL6, IL17 and IL23 expression detected at baseline. These endpoints will be evaluated both as changes with Aggrenox versus aspirin treatment (non parametric analysis) and as components of multivariate modeling and propensity score analysis as described above. An exploratory responder analysis will also be performed (changes in these biomarkers with response versus non-response in patients receiving Aggrenox). The latter analysis may need to be observational only, as it is likely to be underpowered and diluted by patients who may not have begun the trial with the characteristic abnormalities in T cell subset activity.

Power/Sample size calculation

We base this calculation on preliminary data from the BOLD study (11) which is an exploration of underlying biology in patients with SLE who receive steroids and are withdrawn from background treatments, in other words identical to the aspirin group in the current proposal. To date we have analyzed data from 31 patients who completed the BOLD protocol and these outcomes suggest that no more than 20% of the aspirin group will achieve a BICLA response at week 16 (range 1-20%) and no more than 5% achieves a BICLA response at week 24 (range 0-10%). Assuming a 35 to 50% response rate in the Aggrenox group at week 24, a sample size of 50 patients will provide a power around 80% at a significance level of 0.05 at week 24. By these settings and assumptions, the following power estimates are obtained.

Power calculations for a 50 patient study, if alpha=0.05 with response rates:

	Rate 1	Rate 2	Rate 3	Rate 4	Rate 5	Rate 6	Rate 7
Aggrenox n=25	60%	55%	50%	45%	40%	35%	35%
Aspirin n=25	20%	15%	10%	10%	5%	5%	3%
power	0.84	0.86	0.89	0.80	0.86	0.77	0.84

An absolute difference of ≥12 to 15% in the response rate is however considered clinically significant in other lupus trials. Since this is a pilot study, a clinically significant (12% or greater absolute difference), but not statistically significant difference, might be considered justification for a larger study. Even if this pilot study does not reach statistical significance, the proposed exploratory biomarker studies evaluating T and B cell phenotypes, T/B cell functional studies, gene expression or serum cytokine/chemokine profiles might be used to refine the entry criteria and biologic treatment goals for a further study and/or underscore the rationale for an expansion of this study for more power of discrimination. Finally, the BICLA and other flare analyses will be performed at each monthly visit, increasing the likelihood of finding differences, should they exist, when including these secondary analyses.

An interim analysis will be performed after 20 patients will have completed the study, and will be used to assess the quality of the data collected and the treatment effects. Consideration of sample size changes can be discussed at that time.

F. Gender/Minority/Pediatric Inclusion for Research

Participants will be age 18 or older. Race, minority status and gender will not affect enrollment.

G. Human participants

The study population will include male/female patients with SLE, of age 18 or older and of all races.

Inclusion Criteria:

1. Patients with SLE meeting the ACR Classification Criteria (19)
2. Males or females, age 18-70
3. Evidence of positive ANA or anti-dsDNA within one year of screening
4. SLEDAI ≥4 or ≥1 BILAG B or A at screening, despite standard of care

Exclusion Criteria

1. WBC <2.000/mm^3, lymphocytes < 300/mm^3 (last known labs)
2. AST or ALT >3 times above normal cut off values (last known labs)
3. Acute lupus nephritis defined as class II,III, IV or V nephritis diagnosed within 6 months or prot/creat > 1.5 gm/gm due to active lupus or in process of receiving induction therapy for nephritis
4. Active CNS lupus affecting mental status
5. Pregnancy or breast feeding
6. Current requirement for anticoagulation
7. Contraindication to aspirin or dipyridamole, including history of recent or severe GI bleeding, hemoglobin <9 mg/dL, platelet count of <30,000 /mm^3 or unstable platelet count (last known labs)
8. Any other medical condition, whether or not related to lupus which, in the opinion of the investigator would render the patient inappropriate or too unstable to complete the study protocol
9. Inability or unwillingness to understand and/or sign informed consent

2. Identify sources of research material in the form of specimens, records or data.

The research information will consist of clinical assessment performed by the physicians, health assessment questionnaires filled by the patients, and blood and urine specimens collected at each visit.

3. Describe plans for recruitment and consent procedures to be followed.

a. Describe the location where consent is most likely to take place

Patients will be recruited at the lupus clinic at the Oklahoma Medical Research Foundation. Candidate subjects will have the purpose of the study explained to them, including the benefits, risks and options, will be provided with the consent form, and after questions have been answered, will be invited to participate. No study specific procedures will be performed until a participant completes the informed consent process and signs the IFC form.

b. Describe provisions for recruiting non-English speaking participants.
Only English-speaking persons will be recruited.

c. Describe measures to decrease coercion of participants
Participants will be allowed adequate time to review the consent and encouraged to discuss the study with other caregivers or family. An important element of the process will be the understanding by the patient that their decision to participate or not will not affect their access to medical care in the clinic. Employees and clinic staff will be excluded from participation.

4. Describe risks and assess likelihood and seriousness.

The study medication, extended release dipyridamole in combination with low dose aspirin (Aggrenox, Boehringer Ingelheim Pharmaceuticals, Inc.), is known to be generally well tolerated and has been tested in several large randomized controlled trials (4). Aggrenox was approved in 1999 for prevention of stroke and has been used safely and effectively in high risk populations for many years. The main adverse events described are headache (39%), but tolerance usually develops. Other common adverse events are abdominal pain (18%), dyspepsia (18%), nausea (16%) and diarrhea (13%).

5. Describe procedures for protecting against or minimizing potential risks.

Patients will be told about potential side effects during the informed consent process and will be provided with contact information for the clinic and the investigators. Patients will be questioned about adverse events at every visit. Patients with severe episodes of GI bleeding will be excluded. To decrease to risk of GI irritation concurrent use of a proton pump inhibitor (PPI) may be recommended for some patients as clinically warranted.

6. Describe potential benefits and importance to the participants and others.

Immune modulating therapies in current use for SLE are either extremely expensive (belimumab) or have significant side effects (prednisone and other immune suppressants). There is a definite unmet need for safer treatments for use alone or in combination to control this difficult illness as well as for the insights into pathophysiology of the disease that can be obtained by studying treatments with known effects on immunity.

7. Discuss why risks are reasonable in relation to benefits

The risks are minimal and acceptable compared to alternative treatments for SLE. The potential benefits in knowledge and determining whether there may be potential usefulness of this treatment therefore justify these risks.

H. Data and Safety Monitoring Plan

Information on adverse events will be collected throughout the study and reported to the IRB

annually. Serious adverse events will be reported to the IRB within 24 hours of the site becoming aware of them. Adverse events will also be adjudicated quarterly by an independent data safety monitoring board (DSMB) consisting of four physicians with expertise in lupus who will submit a report to the OMRF IRB every annually and at any additional time deemed appropriate. The DSMB will consist of Dr. Diane Kamen, Assistant Professor of Medicine at the Division of Rheumatology at the Medical University of South Carolina in Charleston, Dr. Maria Dall'Era, Associate Professor of Medicine and Director of the Lupus Clinic and Rheumatology Clinical Research Center at the University of California in San Francisco, Dr. Amr Sawalha, Associate Professor of Medicine at the Division of Rheumatology at the University of Michigan in Ann Arbor and Dr. John Harley, Director at the Division of Rheumatology at the Cincinnati Children's Hospital Medical Center in Cincinnati. Data monitoring will be performed by a trained study coordinator or study monitor who is not assigned to this project, but may be an employee of OMRF.

I. Literature sited

1. Crispin JC, Liossis SN, Kis-Toth K, Lieberman LA, Kyttaris VC, Juang YT, et al. Pathogenesis of human systemic lupus erythematosus: recent advances. Trends Mol Med. 2010;16(2):47-57.
2. Kyttaris VC, Zhang Z, Kampagianni O, Tsokos GC. Calcium signaling in systemic lupus erythematosus T cells: a treatment target. Arthritis Rheum. 2011;63(7):2058-66.
3. Mulero MC, Aubareda A, Orzaez M, Messeguer J, Serrano-Candelas E, Martinez-Hoyer S, et al. Inhibiting the calcineurin-NFAT (nuclear factor of activated T cells) signaling pathway with a regulator of calcineurin-derived peptide without affecting general calcineurin phosphatase activity. J Biol Chem. 2009;284(14):9394-401.
4. Leonardi-Bee J, Bath PM, Bousser MG, Davalos A, Diener HC, Guiraud-Chaumeil B, et al. Dipyridamole for preventing recurrent ischemic stroke and other vascular events: a meta-analysis of individual patient data from randomized controlled trials. Stroke. 2005;36(1):162-8.
5. Kamei K, Nakanishi K, Ito S, Saito M, Sako M, Ishikura K, et al. Long-term results of a randomized controlled trial in childhood IgA nephropathy. Clin J Am Soc Nephrol;6(6):1301-7.
6. Zimmermann GR, Avery W, Finelli AL, Farwell M, Fraser CC, Borisy AA. Selective amplification of glucocorticoid anti-inflammatory activity through synergistic multi-target action of a combination drug. Arthritis Res Ther. 2009;11(1):R12.
7. Kyttaris VC, Zhang Z, Kampagianni O, Tsokos GC. Calcium signaling in systemic lupus erythematosus T cells: a treatment target. Arthritis Rheum;63(7):2058-66.
8. Bruce IN, Gordon C, Merrill JT, Isenberg D. Clinical trials in lupus: what have we learned so far? Rheumatology (Oxford);49(6):1025-7.
9. Perl A, Fernandez DR, Telarico T, Doherty E, Francis L, Phillips PE. T-cell and B-cell signaling biomarkers and treatment targets in lupus. Curr Opin Rheumatol. 2009;21(5):454-64.

10. Juang YT, Peoples C, Kafri R, Kyttaris VC, Sunahori K, Kis-Toth K, et al. A systemic lupus erythematosus gene expression array in disease diagnosis and classification: a preliminary report. Lupus;20(3):243-9.
11. Sudhakar T ST, Zhou T, Immermann F, Lehmann M, Masferrer JL, Honczarenko M, Rawdon JC, James JA, Merrill JT. Low Placebo Responses and Clinical Components of the Biomarkers of Lupus Disease (BOLD) Study May Provide Useful Insights for Systemic Lupus Erythematosus Clinical Trial Design. Arthritis Rheum. 2011;63(10).
12. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J. 2008;22(3):659-61.
13. Halkes PH, van Gijn J, Kappelle LJ, Koudstaal PJ, Algra A. Aspirin plus dipyridamole versus aspirin alone after cerebral ischaemia of arterial origin (ESPRIT): randomised controlled trial. Lancet. 2006;367(9523):1665-73.
14. Diener HC, Cunha L, Forbes C, Sivenius J, Smets P, Lowenthal A. European Stroke Prevention Study. 2. Dipyridamole and acetylsalicylic acid in the secondary prevention of stroke. J Neurol Sci. 1996;143(1-2):1-13.
15. Lindgren A, Husted S, Staaf G, Ziegler B. Dipyridamole and headache--a pilot study of initial dose titration. J Neurol Sci. 2004;223(2):179-84.
16. Sloan RP, McCreath H, Tracey KJ, Sidney S, Liu K, Seeman T. RR interval variability is inversely related to inflammatory markers: the CARDIA study. Mol Med. 2007;13(3-4):178-84.
17. Stojanovich L, Milovanovich B, de Luka SR, Popovich-Kuzmanovich D, Bisenich V, Djukanovich B, et al. Cardiovascular autonomic dysfunction in systemic lupus, rheumatoid arthritis, primary Sjogren syndrome and other autoimmune diseases. Lupus. 2007;16(3):181-5.
18. Laversuch CJ, Seo H, Modarres H, Collins DA, McKenna W, Bourke BE. Reduction in heart rate variability in patients with systemic lupus erythematosus. J Rheumatol. 1997;24(8):1540-4.
19. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40(9):1725.

RESEARCH PROTOCOL AMENDMENT, January 13, 2015

We will modify the DARE protocol as following: The randomized controlled part of the study (group A) will be temporarily interrupted in order to focus our work on the pharmacology of Aggrenox. Patients enrolled will be assigned to open label Aggrenox for 3 months (group B) using the same protocol as in the randomized controlled study. There will be no control arm in this modified phase. We plan to enroll in group B 10 more patients with mild to moderate disease, using the same inclusion and exclusion criteria as in the parent study (group A). However, only patients will a Th17 signature, defined by serum cytokine and gene expression profiling, will be invited to participate in group B. Patients will be instructed to hold their background medications as in the parent study, and will not be allowed to receive any additional steroids. Patients who flare during this study or who have persistently active disease, may elect to be treated with steroids and other immune suppressants as needed, but will be nonresponders for the primary endpoint at 3 months.