Review

Biomarkers as tools for improved diagnostic and therapeutic monitoring in systemic lupus erythematosis

Michael F Smith, Jr[1,2], Falk Hiepe[3], Thomas Dörner[3] and Gerd Burmester[3]

[1]Wyeth Research, Discovery Translational Medicine, Collegeville, PA 19426, USA
[2]Present address: Hoffmann-La Roche, Inc., 340 Kingsland Street, Building 721, Nutley, NJ 07110, USA
[3]Universitätsmedizin Charité Berlin, 10098 Berlin, Germany

Corresponding author: Michael F Smith, michael.smith.ms6@roche.com

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Abstract

One of the major challenges in rheumatology is to overcome the classification criteria that previously defined systemic lupus erythematosis, since the heterogeneity of the disease(s) appears to represent a complexity that probably substantially contributed to the failure of a number of recent trials. For those engaged in clinical trials, validated disease activity biomarkers that respond rapidly to treatment and are predictive of clinical response would greatly facilitate early decision-making around futility and dose selection. Likewise, use of validated patient stratification biomarkers possibly in conjunction with autoantibody profiles and disease manifestations will result in the recruitment of more homogeneous patient populations during later stage clinical studies, thereby decreasing size, costs, and risks in pivotal studies.

Challenge of lupus for drug development

Systemic lupus erythematosis (SLE) is perhaps the most clinically and serologically diverse of the autoimmune diseases. The current American College of Rheumatology classification lists 11 criteria for diagnosis of lupus, of which a patient must meet four [1]. The heterogeneity of the patient population results in significant challenges not only in classifying disease activity but also for establishment of therapeutic response to new drug candidates and therapeutic strategies.

Outcome measures used in clinical trials currently rely on one (or more) of several disease activity indices – the Systemic Lupus Erythematosis Disease Activity Index (SLEDAI), the Systemic Lupus Activity Measure, the British Isles Lupus Assessment Group (BILAG), the European Consensus Lupus Activity Measure – and their derivatives. These tools vary in their sensitivities to response, however, dependent upon differential organ involvement and physician assessments [2,3]. Current draft US Food and Drug Administration guidance recommends the use of the BILAG, although the guidance does not rule out the use of other disease activity indices [4]. US Food and Drug Administration guidance on the development of lupus drugs has not yet been formalized, however, despite issuing the draft guidance in 2005. This lack of accepted clinical endpoints makes standardization of study results difficult, and results in significant difficulties for the successful performance of a clinical trial for novel therapeutics for lupus.

In part because of the varied usage of disease activity indices, because of the nature of a flaring disease, and because of associated high placebo response rates, there is considerable interest in the identification and validation of biomarkers for lupus. Physicians, patients, and clinical drug development groups seek biomarkers that more precisely reflect the level of lupus disease activity, are predictive of impending flares, and are associated with or predictive of clinical response to therapeutic intervention. The US Food and Drug Administration has in fact acknowledged the potential utility of validated disease activity biomarkers in its guidance document for lupus development, indicating its willingness to evaluate '… evidence that the proposed surrogate is *reasonably likely to predict clinical benefit*' as part of a registration package for lupus nephritis [4]. Moreover, the use of certain biomarkers may provide diagnostic benefit by defining subsets of a disease that may have a distinct response profile to one or another drug. The inclusion of a definition of the patient's immunological signature as part of the lupus classification criteria could aid in evaluation of novel therapeutics, and ultimately in treatment decision-making.

BAFF = B-cell activating factor of the TNF family; BILAG = British Isles Lupus Assessment Group; CNS = central nervous system; dsDNA = double-stranded DNA; ICOS = inducible costimulator; IFN = interferon; IL = interleukin; sCD25 = soluble IL-2 receptor; Siglec-1 = sialic acid binding immunoglobulin-like lectin 1; SLE = systemic lupus erythematosis; SLEDAI = Systemic Lupus Erythematosis Disease Activity Index; TNF = tumor necrosis factor.

While many cross-sectional studies have identified a plethora of biomarkers that are associated with lupus (specifically or not), there is a significant lack of information from longitudinal and interventional studies that validate the utility of any biomarker for monitoring disease activity or clinical response. This lack of reliable, specific biomarkers for SLE not only hampers precise assessment of disease activity and prompt identification of patients at risk for flares and organ damage, but also impedes the accurate evaluation of responses to treatment [5]. Recent advances in biomarker discovery for lupus, however, are providing new hope that a useful biomarker index can be developed for diagnostic as well as prognostic and response predictors.

Lupus disease activity biomarkers: value for drug development

The pharmaceutical industry realized the unmet medical need for new therapeutics in lupus and has made a considerable investment in bringing new candidates to the clinic. The result of this investment is that there are at least 15 compounds currently in clinical trials [6] with a wide variety of different mechanisms of action. There is therefore considerable incentive to identify biomarkers that will have impact across the broad lupus portfolio, or alternatively define unique SLE subsets that may require and respond to different therapies.

In part because of the challenges around the use of the SLEDAI or the BILAG in clinical trials, pharmaceutical companies have focused phase II proof-of-concept clinical trials on lupus nephritis, where laboratory measurements of proteinuria or the glomerular filtration rate provide objective measurements of renal disease. These designs typically call for 6-month to 12-month clinical endpoint analyses of renal response (if not even longer, as could be concluded from the recent Rituximab clinical trial in lupus nephritis (LUNAR)). Because of increased competition in the lupus field, this patient population will be increasingly difficult to recruit – as a result, the expected length of the proof-of-concept study may be upwards of 2 years or more. Validated disease activity biomarkers that respond rapidly to treatment and are predictive of clinical response at later time points could greatly facilitate early decision-making around futility and dose selection, thereby shortening potentially lengthy proof-of-concept studies. Furthermore, such biomarkers would enhance the development of adaptive trial designs, something not currently possible in lupus nephritis trials, further streamlining the clinical trial process.

An additional advantage to developing a well-validated biomarker toolbox will be the potential to identify patients during early phase studies who are likely to respond to the treatment being tested and who have a higher likelihood to achieve a major response or even remission. Such patient stratification biomarkers can result in the recruitment of a more homogeneous patient population during later stage clinical studies with the promise of decreasing size, costs, and risks in pivotal registration studies.

New approaches to lupus disease activity biomarkers

Complement levels and anti-dsDNA antibodies are classic biomarkers of lupus disease activity that have been shown in some, but not all, studies to be predictors of outcome in lupus nephritis studies [7]. However, to fully capture the multiple clinical manifestations of lupus, developing new strategies that rely on a panel of the most reliable biomarkers will be necessary. Given the heterogeneity of lupus, it is unlikely that a single biomarker will be sufficient for predicting clinical response. The simultaneous collection of multiple biomarkers (cellular, serological and mRNA transcripts) would therefore allow for mathematical modeling in comparison with the SELENA-SLEDAI and the BILAG 2000 disease activity indices leading to generation of a testable composite biomarker score for further studies in lupus. Because of this unmet need, the interest in identifying novel lupus disease activity biomarkers has been remarkable and many potential new indicators of lupus activity have been recently uncovered. Many of these novel biomarkers are only now beginning to be evaluated in clinical settings, however, and none has as yet been qualified as a biomarker that can predict clinical outcomes.

Autoantibody profiles

A number of previous studies reported that SLE patients can be dissected based on their autoantibody profile and associated clinical organ manifestations, but they are also strikingly influenced by the genetic background [8]. In a recent multivariate analysis, Northern European ancestry was significantly associated with photosensitivity (odds ratio = 1.64) and discoid rash (odds ratio = 1.93), while having a protective effect against anticardiolipin autoantibodies (odds ratio = 0.46) and anti-dsDNA autoantibodies (odds ratio = 0.67).

Earlier studies had already suggested that the heterogeneity of SLE patients can be presented more homogeneously [9], with anti-RO (SS-A) production related to the HLA-DQ1/DQ2 heterozygotes, anti-La (SS-B) related to HLA-B8 and HLA-DR3, and anti-nuclear RNP (Sm) related to HLA-DR4. While lymphopenia was associated significantly with anti-Ro (SS-A) and, secondarily, with anti-single-stranded DNA, lupus nephritis was inversely associated with anti-La (SS-B) and associated with anti-dsDNA. It has been repeatedly shown that anti-dsDNA, single anti-Ro antibodies as well as Sm antibodies [10] are associated with lupus nephritis, and in part with central nervous system (CNS) lupus, whereas combined anti-Ro/La antibodies are associated with secondary Sjögren's syndrome and photosensitivity, absence of lupus nephritis and severe CNS involvement. Moreover, anti-ribosomal P antibodies are clearly associated with CNS lupus [11], and anticardiolipin antibodies mark patients with thrombotic events as well as thrombocytopenia [12], serving as

reliable identifiers of these clinical presentations. In a very instructive study, the occurrence of anti-U1RNP/Sm antibodies occurred early on and prior to the disease onset as well as at different times from other autoantibodies [13].

These data indicate that genetic background, including HLA class II, is important for the induction of certain autoantibodies that contribute to the clinical heterogeneity and variation in disease outcomes among SLE patients. The established associations of autoantibody profiles with clinical subtypes may at least require consideration in the design of SLE trials for defining stricter outcome criteria.

The interferon signature

One of the most promising lupus disease activity biomarkers to be identified in recent years is the so-called IFN signature. Increased levels of IFNα were first reported in lupus patients 30 years ago [14]. Twenty years later, seminal work from Lars Rönnblom's group reported the findings that serum from lupus patients contained an IFN-inducing factor and that immune complexes of anti-dsDNA antibodies and DNA could induce IFNα production in normal peripheral blood mononuclear cells [15,16]. Blanco and colleagues expanded this finding by demonstrating that IFNα present in the serum of lupus patients could induce the differentiation of peripheral blood monocytes into dendritic cells [17]. That dysregulation of IFNα production in lupus patients could have a global effect was realized with the advent of transcriptional profiling of peripheral blood mononuclear cells from lupus patients. Gene expression profiling of lupus blood by Baechler and colleagues first demonstrated the upregulation of a group of IFN-stimulated genes in lupus patients [18].

This gene signature is characterized by the highly coordinated upregulation of type I IFN-inducible inflammatory cytokines, chemokines, and other genes whose expression levels are closely correlated with clinical and laboratory measures of SLE disease activity. At least seven separate published studies have now validated the presence of this signature in the peripheral blood of lupus patients [18-24]. Although some variation exists in the gene signatures identified in these different studies, some common markers can be found – including *Mx1*, *IFIT1*, *IFIT4*, *OASL*, *Ly6E*, and *PLSCR1*. Because of patient-to-patient variation in expression levels of individual genes, it has been useful to derive a cumulative IFN-response gene score based upon the sum of the differences in the expression levels of each gene in the patient compared with a healthy donor group. Such a composite score can therefore decrease variability and potentially be more indicative of real changes in disease activity. This approach, however, requires each laboratory to develop its own unique panel of genes and validate the assay with a population of healthy controls and lupus patients. Standardization of an IFN gene signature profile amongst investigators would help significantly for comparing results between studies.

The correlation of the IFN signature with more traditional measures of lupus nephritis activity was evaluated by Bauer and colleagues [25]. Thirty patients were classified as having high or low IFN gene scores (15 patients each) and the association with lupus disease activity indices or other clinical features was determined. High IFN scores were positively associated with increased disease activity as indicated by the SLEDAI and the Systemic Lupus Activity Measure – Revised but not by the BILAG or physician global assessment. Immunologic manifestations and decreased C3 were also associated with high IFN signatures. Similarly, Kirou and colleagues, also found significant correlations between IFN scores, the SLEDAI 2000 immunologic manifestations, and decreased C3 [26]. In addition, this group also identified significant associations between renal involvement and autoantibody profiles.

Similar associations between disease activity scores and IFN scores have been found in all studies that examined them. Importantly, one study also demonstrated that treatment of lupus patients with high-dose steroids rapidly extinguished the IFN gene signature, suggesting that this biomarker may be useful for the evaluation of therapeutic response in clinical studies [19]. The expression of sialic acid binding immunoglobulin-like lectin 1 (Siglec-1) on circulating blood monocytes was very recently shown to be a reflection of the type I IFN signature and a potential biomarker for monitoring disease activity. Siglec-1 cell surface expression correlated with the SLEDAI and the anti-DNA level, and was inversely correlated with complement C3 [27].

Additional longitudinal studies of the response of the IFN gene signature to standard and experimental therapies are essential before this biomarker can be validated as a predictor of clinical response. At least one pharmaceutical company is applying the IFN signature as a rapid indicator of clinical response in early phase I/II trials targeting IFNα by a monoclonal antibody [28]. Notably, the Medimmune group recruited only patients with mild to moderate disease activity and specifically excluded patients with active renal disease. Their study demonstrated that the elevated IFN signature is a characteristic of approximately 60% (37 out of 62) of subjects in this population. Furthermore, the presence of IFN-inducible proteins could be detected in skin biopsies of lesional tissue. If these results can be replicated in larger studies, it would provide strong evidence that IFN-driven disease activity is not confined solely to patients with the most severe disease (that is, lupus nephritis), but is a common characteristic of approximately one-half of lupus patients. This of course raises the important question of which biomarkers may define the other 50% of patients.

Interferon-regulated chemokines

Given the association of the IFN-responsive gene signature described above with lupus nephritis, it is perhaps not surprising that a protein correlate of this activity can be found

in the serum of this patient subset. Bauer and colleagues used a multiplex serum protein analysis to measure the levels of 160 different proteins in the serum of lupus patients and of healthy control subjects [25]. From this original panel, 30 analytes were identified that were dysregulated in lupus nephritis patients – 12 of which were determined by *in vitro* studies to be IFN regulated. Notably, CD25 was also part of this serum signature. As with the IFN gene signature, the composite chemokine protein score positively correlated with the SLEDAI, with the Systemic Lupus Activity Measure – Revised, and with anti-dsDNA antibodies. Lupus patients in this study were also grouped according to the IFN gene signature score (high vs. low), and the cytokine/chemokine profiles between the IFN high and IFN low groups were demonstrated to be remarkably similar and both were very distinct from healthy controls. When correlations between individual chemokines and specific organ manifestations were examined, there appeared to be differences in the serum chemokine profiles between the different groups. In particular, it is interesting to note that there was actually a negative correlation of chemokine levels to the presence of hematological manifestations (predominantly thrombocytopenia). Although sample sizes in this study were very small ($n = 7$ for hematologic samples), this finding is nevertheless very intriguing – and if confirmed in larger studies, may provide additional means for stratifying patients to potential new therapeutic candidates.

A follow-up to this study was reported in an abstract presented at the 2008 American College of Rheumatology meeting [29]. Specific immunoassays were used to quantitate serum levels of IP-10, MCP-1, and MIP-3β in a longitudinal study of 222 patients over 1 year (~1,300 visits). Detailed clinical and laboratory measurements indicated that measurements of serum chemokine levels, in particular IP-10, outperformed standard laboratory tests (anti-dsDNA antibodies and complement levels) as indicators of current disease activity. Furthermore, baseline chemokine levels were also good predictors of future disease course. Further studies on the response of chemokine score to therapeutic intervention will be essential for validating this approach for use in decision-making within clinical studies.

Additional promising biomarkers

The list of serum proteins and leukocyte activation markers that are found elevated in the blood of lupus patients has been rapidly expanding in recent years. Many of these molecules are now emerging as potential therapeutic targets. From a translational medicine perspective, several molecules are also showing promise as biomarkers of lupus disease activity.

Soluble IL-2 receptor

Expression of the IL-2 receptor is upregulated on T cells during activation and the soluble form is released as a result of proteolytic cleavage. Although a precise biological role for soluble IL-2 receptor (sCD25) is unclear, its levels are largely viewed as an indicator of lymphocyte activation, and therefore may not be specific for SLE. Increased levels of sCD25 in serum of SLE patients have been described for 20 years [30-32]. In addition, soluble forms of CD27 and CD40 ligand have also been detected in serum from lupus patients [33,34]. While most studies have demonstrated a positive correlation between levels of sCD25 and disease activity, longitudinal studies have also supported its use as a biomarker that is closely correlated with flare activity and therapeutic responses, particularly in patients with renal involvement [35,36]. sCD25 does not, however, appear to be a highly responsive marker in patients with moderate disease or mild flare.

In total, the results of studies into the association of sCD25 with lupus disease activity strongly support the inclusion of this protein as part of a biomarker panel. As with the measurement of complement activation, however, its utility may be limited to studies of patients with high disease activity.

B-cell activating factor of the TNF family

B-cell activating factor of the TNF family (BAFF) is a cytokine belonging to the TNF superfamily, and is a B-cell activator that controls peripheral B-cell maturation. BAFF (also known as BLys) stimulates B-cell proliferation and is necessary for B-cell survival. Transgenic overexpression of BAFF in mice results in abnormally high B-cell numbers and in the development of a lupus-like disease [37]. This observation suggested a role for BAFF in SLE and was further evaluated in patients. Serum levels of BAFF and BAFF mRNA in peripheral leukocytes are found to be elevated in lupus patients and are positively associated with disease activity indices [38-41]. Of note, BAFF levels tend to fluctuate during the natural course of SLE and appear to be associated with anti-dsDNA antibodies [42]. In addition, BAFF production is apparently influenced/controlled by type I IFNs, as has been suggested by recent studies in $AIRE^{-/-}$ mice [43]. There could therefore be a possibility that enhanced BAFF levels are the result of increased IFN production in SLE and these cytokines are rather interrelated rather than independently enhanced.

Interestingly, one-half of lupus patients show enhanced serum BAFF levels, whereas the remaining patients do not show increased BAFF levels. One study demonstrated a close relationship between mRNA levels of BAFF and disease activity, which however, was not identified for the protein levels of BAFF [44]. Additionally, BAFF levels were also identified to correlate with the severity and therapeutic response in autoimmune thrombocytopenia, suggesting its value as biomarker in this entity [45].

CD40 ligand expression

The expression of costimulatory molecule CD40 ligand, which was previously identified as a biomarker for lupus

disease activity [34], was also demonstrated to be elevated on peripheral T cells from lupus patients and normalized following rituximab treatment [46]. Notably, downregulation of CD40 ligand expression preceded clinical response, as early as 1 month following treatment. These data point towards CD40 ligand being a potential sensitive biomarker of activity and response to treatment.

Inducible costimulator expression

The inducible costimulator (ICOS) is a member of the CD28 family that, like CD28, can enhance T-cell proliferation and cytokine secretion. In the first study in human SLE, Hutloff and colleagues demonstrated overexpression of ICOS on $CD4^+$ and $CD8^+$ T cells and a remarkable reduction of ICOS ligand on $CD27^+$ memory B cells [47]. In addition, lupus nephritis patients had an accumulation of germinal center-like structures in their kidneys. These data suggested that there is continuous crosstalk and activation between T cells and B cells in SLE, leading to the overactivation of the adaptive immunity in lupus.

In confirmation, Yang and colleagues investigated the expression of ICOS on CD4 and CD8 T cells from lupus patients [48] in peripheral blood from moderately and highly active SLE patients. They found elevated numbers of ICOS-positive T cells, both CD4 and CD8, as well as elevated levels of ICOS expression compared with healthy controls or rheumatoid arthritis patients. Furthermore, longitudinal analysis of individual patients indicated that ICOS was significantly elevated during the active phase when compared with clinical remission.

Sialic acid binding immunoglobulin-like lectin 1

Siglec-1 (sialoadhesin, CD169) is a macrophage-restricted receptor that interacts with surface molecules on a number of other cell types, including B cells and T cells [49,50]. Biesen and colleagues recently identified Siglec-1 as a highly upregulated gene expressed by peripheral blood monocytes from lupus and systemic sclerosis patients, but not in rheumatoid arthritis, osteoarthritis, or ankylosing spondylitis [27]. Importantly, Siglec-1 surface expression, as analyzed by fluorescence-activated cell sorting, was positively correlated with the SLEDAI and ant-dsDNA while being negatively correlated with C3 levels – indicating that this expression of this novel marker closely parallels traditional disease activity measures. Furthermore, treatment of four patients with intravenous pulse glucocorticoids resulted in a decrease in Siglec-1-positive monocytes from 77% to 12% within 7 days, suggesting that this may be a useful biomarker for the assessment of clinical response to novel therapeutics.

$CD27^{high}$ plasma cells and peripheral B-cell subset analysis

B cells can be subdivided along the developmental and activation pathway according to the presence or absence of a variety of cell surface molecules. Abnormalities in B-cell subsets in lupus patients have been described in a number of studies. Notably, the expansion of a unique population of $CD19^+/CD27^{high}$ plasma cells was noted under the condition of lupus. Jacobi and colleagues evaluated the correlation of $CD27^{high}$ plasma cells and disease activity in lupus patients [51]. Patients with high disease activity (SLEDAI >8) had significantly increased frequency of $CD19^+/CD27^{high}$ plasma cells and had a predictive value for disease activity that was greater than traditional humoral/clinical measurements (for example, anti-dsDNA, complement, renal manifestations). In addition, there was a statistically significant correlation between these plasma cells with the SLEDAI as well as with the anti-DNA titers. Interestingly, the $CD27^{high}$ cells also correlated with the SLEDAI as measurement of the disease activity in SLE in patients not producing anti-dsDNA autoantibodies. More recent data show that a particular $CD27^-/IgD^-$ B-cell subset is expanded in SLE [52,53]. Only the $CD27^-/IgD^-/CD95^+$ B cells, however, are uniquely expanded in lupus patients, display a memory phenotype, and correlate with lupus activity.

Together, these analyses suggest that monitoring peripheral B cells using CD27 expression may be a reliable biomarker of lupus disease activity and clinical response, but needs to be evaluated in larger trials.

Conclusions

The path to new therapeutics for lupus has been littered with many failed clinical studies, although very recently the phase III trials BLISS-52 and BLISS-76 using belimumab and a phase IIb trial using epratuzumab (both only announced by press releases) could demonstrate effects superior to placebo. The question remains, however, whether the previously failed drug candidates had the wrong target or just a suboptimal study design.

Given the heterogeneity of this disease, we propose that clinical trial design needs to be refocused to take into consideration recruiting patients based upon an *immunological characterization* of the patient with emphasis on relevance to the mechanism of action of the test compound. Such a characterization might include autoantibody profiles, gene signatures, serum proteins, and leukocyte surface markers. For such an approach to be successful, however, well-validated biomarkers are essential. As described above, several novel biomarker strategies have emerged from the literature in recent years, although to date none have been studied in enough detail to be useful in decision-making within clinical trials. In order for a biomarker to be qualified for use in decision-making, epidemiologic or observational studies of the natural history of the disease should establish the relationship between the biomarker, defined clinical cohorts as already widely used for lupus nephritis, and defined clinical endpoints.

To date, the vast majority of biomarker studies in lupus have attempted to link clinical activity to only one, or a few,

potential biomarkers. Given the heterogeneity of the disease – is lupus really one disease? – a multiplexed approach is clearly going to be essential. Biomarker validation is an iterative process requiring multiple studies to identify, characterize, and confirm the validity of the biomarker for the intended purpose. Pharmaceutical companies and clinical trialists need to commit to the evaluation of multiple, exploratory biomarkers within clinical studies and to sharing of the information if we are to improve our understanding of lupus and the response to active therapies. It is a long-term investment but one that the lupus community has recognized and is embracing as the critical path to a successful lupus therapy.

Competing interests

The authors declare that they have no competing interests.

References

1. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ: **The 1982 revised criteria for the classification of systemic lupus erythematosus.** *Arthritis Rheum* 1982, **25:**1271-1277.
2. Merrill JT, Erkan D, Buyon JP: **Challenges in bringing the bench to bedside in drug development for SLE.** *Nat Rev Drug Discov* 2004, **3:**1036-1046.
3. Petri M: **Disease activity assessment in SLE: do we have the right instruments?** *Ann Rheum Dis* 2007, **66(Suppl 3):**iii61-iii64.
4. **Guidance for Industry: Systemic Lupus Erythematosus - Developing Drugs for Treatment** [http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072063.pdf]
5. Liu CC, Manzi S, Ahearn JM: **Biomarkers for systemic lupus erythematosus: a review and perspective.** *Curr Opin Rheumatol* 2005, **17:**543-549.
6. **ClinicalTrials.gov** [www.clinicaltrials.gov]
7. Illei GG, Tackey E, Lapteva L, Lipsky PE: **Biomarkers in systemic lupus erythematosus. I. General overview of biomarkers and their applicability.** *Arthritis Rheum* 2004, **50:**1709-1720.
8. Chung SA, Tian C, Taylor KE, Lee AT, Ortmann WA, Hom G, Graham RR, Nititham J, Kelly JA, Morrisey J, Wu H, Yin H, Alarcon-Riquelme ME, Tsao BP, Harley JB, Gaffney PM, Moser KL, Manzi S, Petri M, Gregersen PK, Langefeld CD, Behrens TW, Seldin MF, Criswell LA: **European population substructure is associated with mucocutaneous manifestations and autoantibody production in systemic lupus erythematosus.** *Arthritis Rheum* 2009, **60:**2448-2456.
9. Harley JB, Sestak AL, Willis LG, Fu SM, Hansen JA, Reichlin M: **A model for disease heterogeneity in systemic lupus erythematosus. Relationships between histocompatibility antigens, autoantibodies, and lymphopenia or renal disease.** *Arthritis Rheum* 1989, **32:**826-836.
10. Riemekasten G, Marell J, Trebeljahr G, Klein R, Hausdorf G, Haupl T, Schneider-Mergener J, Burmester GR, Hiepe F: **A novel epitope on the C-terminus of SmD1 is recognized by the majority of sera from patients with systemic lupus erythematosus.** *J Clin Invest* 1998, **102:**754-763.
11. Shoenfeld N, Agmon-Levin N, Flitman-Katzevman I, Paran D, Katz BS, Kivity S, Langevitz P, Zandman-Goddard G, Shoenfeld Y: **The sense of smell in systemic lupus erythematosus.** *Arthritis Rheum* 2009, **60:**1484-1487.
12. Krause I, Blank M, Fraser A, Lorber M, Stojanovich L, Rovensky J, Shoenfeld Y: **The association of thrombocytopenia with systemic manifestations in the antiphospholipid syndrome.** *Immunobiology* 2005, **210:**749-754.
13. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, Harley JB: **Development of autoantibodies before the clinical onset of systemic lupus erythematosus.** *N Engl J Med* 2003, **349:**1526-1533.
14. Hooks JJ, Moutsopoulos HM, Geis SA, Stahl NI, Decker JL, Notkins AL: **Immune interferon in the circulation of patients with autoimmune disease.** *N Engl J Med* 1979, **301:**5-8.
15. Vallin H, Blomberg S, Alm GV, Cederblad B, Rönnblom L: **Patients with systemic lupus erythematosus (SLE) have a circulating inducer of interferon-alpha (IFN-α) production acting on leucocytes resembling immature dendritic cells.** *Clin Exp Immunol* 1999, **115:**196-202.
16. Vallin H, Perers A, Alm GV, Rönnblom L: **Anti-double-stranded DNA antibodies and immunostimulatory plasmid DNA in combination mimic the endogenous IFN-alpha inducer in systemic lupus erythematosus.** *J Immunol* 1999, **163:**6306-6313.
17. Blanco P, Palucka AK, Gill M, Pascual V, Banchereau J: **Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus.** *Science* 2001, **294:**1540-1543.
18. Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, Shark KB, Grande WJ, Hughes KM, Kapur V, Gregersen PK, Behrens TW: **Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus.** *Proc Natl Acad Sci U S A* 2003, **100:**2610-2615.
19. Bennett L, Palucka AK, Arce E, Cantrell V, Borvak J, Banchereau J, Pascual V: **Interferon and granulopoiesis signatures in systemic lupus erythematosus blood.** *J Exp Med* 2003, **197:**711-723.
20. Crow MK, Kirou KA, Wohlgemuth J: **Microarray analysis of interferon-regulated genes in SLE.** *Autoimmunity* 2003, **36:**481-490.
21. Han GM, Chen SL, Shen N, Ye S, Bao CD, Gu YY: **Analysis of gene expression profiles in human systemic lupus erythematosus using oligonucleotide microarray.** *Genes Immun* 2003, **4:**177-186.
22. Kirou KA, Lee C, George S, Louca K, Papagiannis IG, Peterson MG, Ly N, Woodward RN, Fry KE, Lau AY, Prentice JG, Wohlgemuth JG, Crow MK: **Coordinate overexpression of interferon-alpha-induced genes in systemic lupus erythematosus.** *Arthritis Rheum* 2004, **50:**3958-3967.
23. Nikpour M, Dempsey AA, Urowitz MB, Gladman DD, Barnes DA: **Association of a gene expression profile from whole blood with disease activity in systemic lupus erythematosus.** *Ann Rheum Dis* 2008, **67**:1069-1075.
24. Chaussabel D, Quinn C, Shen J, Patel P, Glaser C, Baldwin N, Stichweh D, Blankenship D, Li L, Munagala I, Bennett L, Allantaz F, Mejias A, Ardura M, Kaizer E, Monnet L, Allman W, Randall H, Johnson D, Lanier A, Punaro M, Wittkowski KM, White P, Fay J, Klintmalm G, Ramilo O, Palucka AK, Banchereau J, Pascual V: **A modular analysis framework for blood genomics studies: application to systemic lupus erythematosus.** *Immunity* 2008, **29:**150-164.
25. Bauer JW, Baechler EC, Petri M, Batliwalla FM, Crawford D, Ortmann WA, Espe KJ, Li W, Patel DD, Gregersen PK, Behrens TW: **Elevated serum levels of interferon-regulated chemokines are biomarkers for active human systemic lupus erythematosus.** *PLoS Med* 2006, **3:**e491.
26. Kirou KA, Lee C, George S, Louca K, Peterson MG, Crow MK: **Activation of the interferon-alpha pathway identifies a subgroup of systemic lupus erythematosus patients with distinct serologic features and active disease.** *Arthritis Rheum* 2005, **52:**1491-1503.
27. Biesen R, Demir C, Barkhudarova F, Grun JR, Steinbrich-Zollner M, Backhaus M, Haupl T, Rudwaleit M, Riemekasten G, Radbruch A, Hiepe F, Burmester GR, Grutzkau A: **Sialic acid-binding Ig-like lectin 1 expression in inflammatory and resident monocytes is a potential biomarker for monitoring disease activity and success of therapy in systemic lupus erythematosus.** *Arthritis Rheum* 2008, **58:**1136-1145.
28. Yao Y, Richman L, Higgs BW, Morehouse CA, de Los Reyes M, Brohawn P, Zhang J, White B, Coyle AJ, Kiener PA, Jallal B: **Neutralization of interferon-alpha/beta-inducible genes and downstream effect in a phase I trial of an anti-interferon-alpha monoclonal antibody in systemic lupus erythematosus.** *Arthritis Rheum* 2009, **60:**1785-1796.
29. Bauer JW, Petri M, Batliwalla F, Wilson JC, Koeuth T, Singh S, Tesfasyone H, Gregersen PK, Behrens TW, Baechler EC: **Baseline IFN-regulated chemokine levels are strong predictors of systemic lupus erythematosus flare.** *Arthritis Rheum* 2008, **58 (9 Suppl):**S805.
30. Campen DH, Horwitz DA, Quismorio FP, Jr, Ehresmann GR, Martin WJ: **Serum levels of interleukin-2 receptor and activity of rheumatic diseases characterized by immune system activation.** *Arthritis Rheum* 1988, **31:**1358-1364.
31. Semenzato G, Bambara LM, Biasi D, Frigo A, Vinante F, Zuppini

B, Trentin L, Feruglio C, Chilosi M, Pizzolo G: **Increased serum levels of soluble interleukin-2 receptor in patients with systemic lupus erythematosus and rheumatoid arthritis.** *J Clin Immunol* 1988, **8:**447-452.
32. Wolf RE, Brelsford WG: **Soluble interleukin-2 receptors in systemic lupus erythematosus.** *Arthritis Rheum* 1988, **31:**729-735.
33. Swaak AJ, Hintzen RQ, Huysen V, van den Brink HG, Smeenk JT: **Serum levels of soluble forms of T cell activation antigens CD27 and CD25 in systemic lupus erythematosus in relation with lymphocytes count and disease course.** *Clin Rheumatol* 1995, **14:**293-300.
34. Kato K, Santana-Sahagun E, Rassenti LZ, Weisman MH, Tamura N, Kobayashi S, Hashimoto H, Kipps TJ: **The soluble CD40 ligand sCD154 in systemic lupus erythematosus.** *J Clin Invest* 1999, **104:**947-955.
35. Davas EM, Tsirogianni A, Kappou I, Karamitsos D, Economidou I, Dantis PC: **Serum IL-6, TNFα, p55 srTNFα, p75srTNFα, srIL-2α levels and disease activity in systemic lupus erythematosus.** *Clin Rheumatol* 1999, **18:**17-22.
36. ter Borg EJ, Horst G, Limburg PC, Kallenberg CG: **Changes in plasma levels of interleukin-2 receptor in relation to disease exacerbations and levels of anti-dsDNA and complement in systemic lupus erythematosus.** *Clin Exp Immunol* 1990, **82:**21-26.
37. Khare SD, Sarosi I, Xia XZ, McCabe S, Miner K, Solovyev I, Hawkins N, Kelley M, Chang D, Van G, Ross L, Delaney J, Wang L, Lacey D, Boyle WJ, Hsu H: **Severe B cell hyperplasia and autoimmune disease in TALL-1 transgenic mice.** *Proc Natl Acad Sci U S A* 2000, **97:**3370-3375.
38. Becker-Merok A, Nikolaisen C, Nossent HC: **B-lymphocyte activating factor in systemic lupus erythematosus and rheumatoid arthritis in relation to autoantibody levels, disease measures and time.** *Lupus* 2006, **15:**570-576.
39. Ju S, Zhang D, Wang Y, Ni H, Kong X, Zhong R: **Correlation of the expression levels of BLyS and its receptors mRNA in patients with systemic lupus erythematosus.** *Clin Biochem* 2006, **39:**1131-1137.
40. Lin Z, Dan-Rong Y, Xiao-Qing T, Hao W, Hong-Lian G, Xian-Tao K, Ren-Qian Z, Jiyu L: **Preliminary clinical measurement of the expression of B-cell activating factor in Chinese systemic lupus erythematosus patients.** *J Clin Lab Anal* 2007, **21:**183-187.
41. Morimoto S, Nakano S, Watanabe T, Tamayama Y, Mitsuo A, Nakiri Y, Suzuki J, Nozawa K, Amano H, Tokano Y, Kobata T, Takasaki Y: **Expression of B-cell activating factor of the tumour necrosis factor family (BAFF) in T cells in active systemic lupus erythematosus: the role of BAFF in T cell-dependent B cell pathogenic autoantibody production.** *Rheumatology (Oxford)* 2007, **46:**1083-1086.
42. Stohl W: **SLE – systemic lupus erythematosus: a BLySful, yet BAFFling, disorder.** *Arthritis Res Ther* 2003, **5:**136-138.
43. Lindh E, Lind SM, Lindmark E, Hassler S, Perheentupa J, Peltonen L, Winqvist O, Karlsson MC: **AIRE regulates T-cell-independent B-cell responses through BAFF.** *Proc Natl Acad Sci U S A* 2008, **105:**18466-18471.
44. Collins CE, Gavin AL, Migone TS, Hilbert DM, Nemazee D, Stohl W: **B lymphocyte stimulator (BLyS) isoforms in systemic lupus erythematosus: disease activity correlates better with blood leukocyte BLyS mRNA levels than with plasma BLyS protein levels.** *Arthritis Res Ther* 2006, **8:**R6.
45. Emmerich F, Bal G, Barakat A, Milz J, Muhle C, Martinez-Gamboa L, Dorner T, Salama A: **High-level serum B-cell activating factor and promoter polymorphisms in patients with idiopathic thrombocytopenic purpura.** *Br J Haematol* 2007, **136:**309-314.
46. Sfikakis PP, Boletis JN, Lionaki S, Vigklis V, Fragiadaki KG, Iniotaki A, Moutsopoulos HM: **Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the T cell costimulatory molecule CD40 ligand: an open-label trial.** *Arthritis Rheum* 2005, **52:**501-513.
47. Hutloff A, Buchner K, Reiter K, Baelde HJ, Odendahl M, Jacobi A, Dorner T, Kroczek RA: **Involvement of inducible costimulator in the exaggerated memory B cell and plasma cell generation in systemic lupus erythematosus.** *Arthritis Rheum* 2004, **50:**3211-3220.
48. Yang JH, Zhang J, Cai Q, Zhao DB, Wang J, Guo PE, Liu L, Han XH, Shen Q: **Expression and function of inducible costimulator on peripheral blood T cells in patients with systemic lupus erythematosus.** *Rheumatology (Oxford)* 2005, **44:**1245-1254.
49. Hartnell A, Steel J, Turley H, Jones M, Jackson DG, Crocker PR: **Characterization of human sialoadhesin, a sialic acid binding receptor expressed by resident and inflammatory macrophage populations.** *Blood* 2001, **97:**288-296.
50. van den Berg TK, Nath D, Ziltener HJ, Vestweber D, Fukuda M, van Die I, Crocker PR: **Cutting edge: CD43 functions as a T cell counterreceptor for the macrophage adhesion receptor sialoadhesin (Siglec-1).** *J Immunol* 2001, **166:**3637-3640.
51. Jacobi AM, Odendahl M, Reiter K, Bruns A, Burmester GR, Radbruch A, Valet G, Lipsky PE, Dorner T: **Correlation between circulating CD27^{high} plasma cells and disease activity in patients with systemic lupus erythematosus.** *Arthritis Rheum* 2003, **48:**1332-1342.
52. Jacobi AM, Reiter K, Mackay M, Aranow C, Hiepe F, Radbruch A, Hansen A, Burmester GR, Diamond B, Lipsky PE, Dorner T: **Activated memory B cell subsets correlate with disease activity in systemic lupus erythematosus: delineation by expression of CD27, IgD, and CD95.** *Arthritis Rheum* 2008, **58:**1762-1773.
53. Wei C, Anolik J, Cappione A, Zheng B, Pugh-Bernard A, Brooks J, Lee EH, Milner EC, Sanz I: **A new population of cells lacking expression of CD27 represents a notable component of the B cell memory compartment in systemic lupus erythematosus.** *J Immunol* 2007, **178:**6624-6633.