5th Annual Meeting of the Lupus Academy
Meeting Report

NH Noordwijk Conference Centre, Leeuwenhorst, Noordwijkerhout, The Netherlands

6–8th May 2016

Introduction

The Lupus Academy is a long-term initiative committed to improving patient outcomes in systemic lupus erythematosus and allied diseases. By providing a highly interactive educational forum, the Lupus Academy is dedicated to sharing best clinical practice through the dissemination and discussion of cutting edge scientific and clinical research.

During the past 5 years the Lupus Academy has built a solid reputation for providing high quality educational meetings, which stimulate discussion, provide clinical practice insight and support improved patient outcomes.

The 5th Annual Meeting of the Lupus Academy was held in Holland in May 2016, with the aim of reviewing and discussing insights in global research and clinical practice in lupus and associated diseases. This two day meeting brought together >115 clinicians and scientists, with a specialist interest in lupus, from around the world. The meeting was CME accredited and was designated for a maximum of 11 European CME credits.

The scientific programme, developed by a Steering Committee of 12 international experts, provided a highly interactive forum through which information and experiences about the management of lupus was exchanged.

This report highlights key content from the main meeting sessions, excluding interactive workshops.

Meeting Objectives

To facilitate improvement in clinical practice and patient outcomes by enabling clinicians to:

- Better diagnose and manage lupus through improved understanding of biomarkers in SLE, early lupus characteristics and fundamentals of the SLE treat-to-target approach
- Have a greater understanding of managing various clinical manifestations of lupus, through new trends in managing lupus nephritis, renal transplantation and early diagnosis/prevention of osteonecrosis and osteoporosis
- Consider the course of and approach to SLE management from conception, through pregnancy and transitioning from childhood onset SLE to adulthood
- Understand cutting edge management of APS and SLE and how this will influence the future management of these diseases
- Reflect on their own clinical cases following participation in interactive workshops designed to bring to life the management of various lupus manifestations seen in clinical practice

Keynote Lectures

The top SLE stories in 2015: Clinical aspects: Richard A. Furie

Professor Furie reviewed impactful publications from the previous year related to the clinical aspects of systemic lupus erythematosus (SLE).

Articles published in the following journals during the 2015 calendar year were reviewed: *Arthritis and Rheumatology, Annals of Rheumatic Disease, JAMA, Lupus, Journal of Rheumatology, Arthritis Care and Research, New England Journal of Medicine, Annals of Internal Medicine* and *Journal of Nephrology*. The results captured included 3550 SLE articles (using the keywords lupus or SLE), 540 publications on antiphospholipid, 5194 articles on rheumatoid arthritis (RA) (using the keyword rheumatoid) and 1004 publications containing the keyword psoriatic.

From these results, Professor Furie identified the following as the 'top' six SLE stories of 2015.

1. The elusive biomarker

A key paper by Dall'Era and colleagues looked at predictors of long-term renal outcome in lupus nephritis trials.[1] Data from the Euro-Lupus Nephritis Trial were analysed to evaluate the performance of proteinuria, serum creatinine (Cr) and urinary red blood cells (RBCs) as predictors of good long-term renal function – defined as a serum Cr value ≤1.0 mg/dL at 7 years. In total, 76 patients with measurements at 3, 6 and 12 months followed-up for a minimum of 7 years were included. Of these, 68% had Cr <1.0 mg/dL at 7 years. Proteinuria less than 0.8 mg/day at 12 months was found to be the single best predictor of good long-term renal function, with a sensitivity of 81% and specificity of 78%. The addition of serum Cr to proteinuria as a composite predictor did not improve the performance of the outcome measure and inclusion of urinary RBCs as a predictor significantly decreased the sensitivity to 47%.

Table: Predictors of long-term renal outcome in lupus nephritis trials

Single Short-term Criterion Predictors of Good Long-term Outcome	Positive Predictive Value	Negative Predictive Value
Proteinuria <0.5 g/d	0.88	0.53
Proteinuria <0.8 g/d	**0.88**	**0.67**
Serum Creat ≤0.8 mg/dL	0.88	0.49
Serum Creat ≤1.0 mg/dL	0.78	0.69

2. A vitamin a day keeps lupus at bay?

Aranow *et al.* carried out a 12-week, randomised, double-blind, placebo-controlled trial investigating the effect of vitamin D3 supplementation on the interferon (IFN) signature in SLE.[2] An IFN signature response was defined as a 50% reduction in the expression of 1 of the 3 genes or a 25% lowering in the expression of 2 of the 3 genes. In total, 57 SLE patients with stable, inactive disease, serum 25-OH vitamin D levels less than 20 ng/mL, dsDNA Ab and an IFN signature were randomised to placebo or vitamin D3 at doses of 2,000 IU or 4,000 IU. In the placebo group, no patient experienced vitamin D repletion, while approximately half of the vitamin D3 group repleted. However, results showed no difference in the percentage of patients with an IFN signature response between those who remained vitamin D deficient and those who demonstrated repletion of vitamin D.

3. From mabs to mibs

On this topic, Professor Furie highlighted interesting research by Alexander and colleagues entitled 'The proteasome inhibitor bortezomib depletes plasma cells and ameliorates clinical manifestations of refractory SLE' which would be discussed further during the course of the meeting.[3]

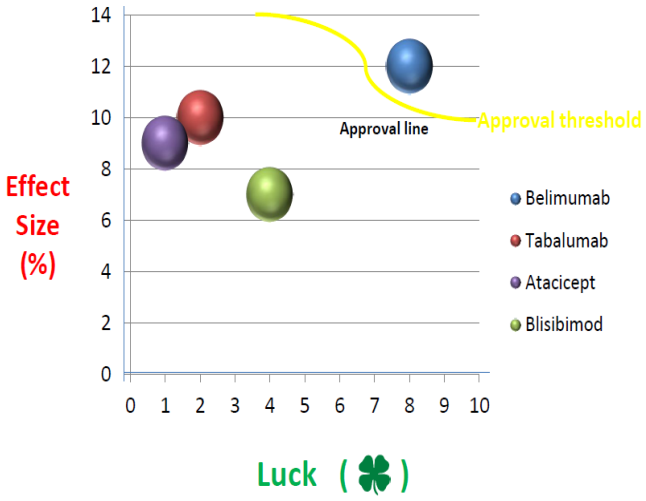
4. A miss with BLys

BLyS is crucial to B-cell maturation, differentiation and survival, providing a solid biological rationale for targeting BLyS in SLE.[4-7] Murine models have shown that transgenic mice develop SLE-like disease and TACI-Ig ameliorates murine lupus activity.[5, 8-11] Furthermore, in human SLE, elevated levels of BLyS are known to be predictive of flare.[12, 13] Several inhibitors of the BLyS/APRIL pathway are under development in SLE but results to date are less than encouraging. Both doses of the BLyS inhibitor tabalumab failed in the Phase 3 ILLUMINATE 1 trial and only the high dose was effective in ILLUMINATE 2.[14, 15]

5. APRIL showers make B cell flowers

The study of atacicept in lupus nephritis has already been terminated. In the extra-renal SLE study (APRIL-SLE) of atacicept for flare prevention, the 150 mg arm was terminated due to two deaths, while the 75 mg dosing arm proved largely ineffective.[16] Of the BLyS pathway inhibitors under clinical development, it is currently only belimumab that possesses an effect size sufficient to cross the approval threshold.

Figure: Outcomes with BLyS pathway inhibitors



6. Damage begets damage

Results from over 1700 patients in the Systemic Lupus International Collaborating Clinics (SLICC) Inception Cohort, published by Bruce *et al.*, have revealed important insights into factors associated with damage accrual in SLE.[17] Patients with no damage at enrolment were significantly less likely to accrue damage than those with damage. Risk factors associated with a transition from an SLICC/ACC damage index (SDI) score of 0 to 1 or more, or an increase in pre-existing damage, were age USA African race/ethnicity, SLEDAI-2K score, steroid use and hypertension. Antimalarial use was associated with lower rates of increases in pre-existing damage. Damage was also linked with patients' future mortality.

In addition to his top six stories of 2015, Professor Furie also drew attention to some noteworthy reviews recently published in the SLE arena, and highlighted some key presentations from ACR 2015.

References

1. Dall'Era M, Cisternas MG, Smilek DE*, et al.* Predictors of long-term renal outcome in lupus nephritis trials: lessons learned from the Euro-Lupus Nephritis cohort. *Arthritis Rheumatol*. 2015;67(5):1305-13.
2. Aranow C, Kamen DL, Dall'Era M*, et al.* Randomized, Double-Blind, Placebo-Controlled Trial of the Effect of Vitamin D3 on the Interferon Signature in Patients With Systemic Lupus Erythematosus. *Arthritis Rheumatol*. 2015;67(7):1848-57.
3. Alexander T, Sarfert R, Klotsche J*, et al.* The proteasome inhibitior bortezomib depletes plasma cells and ameliorates clinical manifestations of refractory systemic lupus erythematosus. *Ann Rheum Dis*. 2015;74(7):1474-8.
4. Batten M, Groom J, Cachero TG*, et al.* BAFF mediates survival of peripheral immature B lymphocytes. *J Exp Med*. 2000;192(10):1453-66.
5. Gross JA, Johnston J, Mudri S*, et al.* TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. *Nature*. 2000;404(6781):995-9.
6. Moore PA, Belvedere O, Orr A*, et al.* BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator. *Science*. 1999;285(5425):260-3.
7. Schneider P, MacKay F, Steiner V*, et al.* BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. *J Exp Med*. 1999;189(11):1747-56.
8. Groom J, Kalled SL, Cutler AH*, et al.* Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjogren's syndrome. *J Clin Invest*. 2002;109(1):59-68.
9. Khare SD, Sarosi I, Xia XZ*, et al.* Severe B cell hyperplasia and autoimmune disease in TALL-1 transgenic mice. *Proc Natl Acad Sci U S A*. 2000;97(7):3370-5.
10. Mackay F, Woodcock SA, Lawton P*, et al.* Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J Exp Med*. 1999;190(11):1697-710.
11. Ramanujam M, Wang X, Huang W*, et al.* Similarities and differences between selective and nonselective BAFF blockade in murine SLE. *J Clin Invest*. 2006;116(3):724-34.
12. Cheema GS, Roschke V, Hilbert DM, Stohl W**.** Elevated serum B lymphocyte stimulator levels in patients with systemic immune-based rheumatic diseases. *Arthritis Rheum*. 2001;44(6):1313-9.
13. Petri M, Stohl W, Chatham W*, et al.* Association of plasma B lymphocyte stimulator levels and disease activity in systemic lupus erythematosus. *Arthritis Rheum*. 2008;58(8):2453-9.
14. Isenberg DA, Petri M, Kalunian K*, et al.* Efficacy and safety of subcutaneous tabalumab in patients with systemic lupus erythematosus: results from ILLUMINATE-1, a 52-week, phase III, multicentre, randomised, double-blind, placebo-controlled study. *Ann Rheum Dis*. 2016;75(2):323-31.
15. Merrill JT, van Vollenhoven RF, Buyon JP*, et al.* Efficacy and safety of subcutaneous tabalumab, a monoclonal antibody to B-cell activating factor, in patients with systemic lupus erythematosus: results from ILLUMINATE-2, a 52-week, phase III, multicentre, randomised, double-blind, placebo-controlled study. *Ann Rheum Dis*. 2016;75(2):332-40.
16. Isenberg D, Gordon C, Licu D*, et al.* Efficacy and safety of atacicept for prevention of flares in patients with moderate-to-severe systemic lupus erythematosus (SLE): 52-week data (APRIL-SLE randomised trial). *Ann Rheum Dis*. 2015;74(11):2006-15.
17. Bruce IN, O'Keeffe AG, Farewell V*, et al.* Factors associated with damage accrual in patients with systemic lupus erythematosus: results from the Systemic Lupus International Collaborating Clinics (SLICC) Inception Cohort. *Ann Rheum Dis*. 2015;74(9):1706-13.

The top SLE stories in 2015: Basic science: Thomas Dörner

Professor Dörner reviewed noteworthy articles related to the basic science of systemic lupus erythematosus (SLE) that were published in 2015.

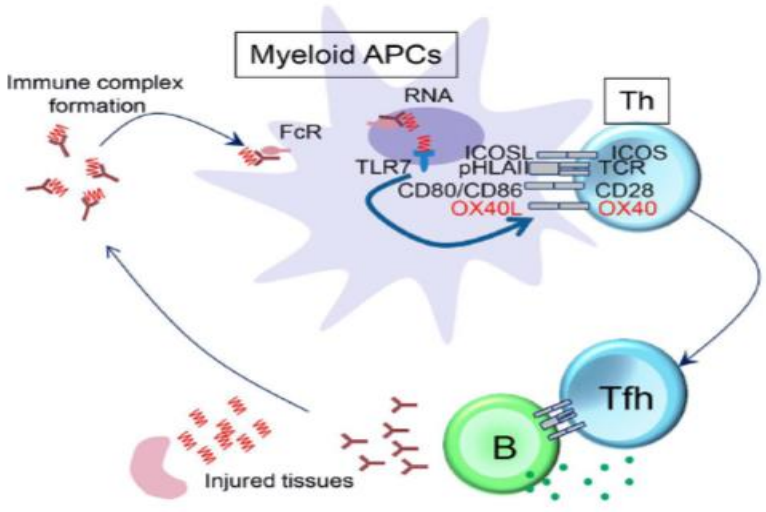
Beginning his presentation with the caveat that SLE, in this instance, may stand for 'subjective, limited, edition,' Professor Dorner highlighted several key discoveries in SLE from the 2015 literature.

- Protein tyrosine phosphatase abnormalities in T and B-cells
- The post-activation/exhaustion status of CD8 B-lymphocytes
- Disturbances of co-stimulation
- Disturbances of naïve B-cells involved in lupus immunopathology.

Protein tyrosine phosphatase non-receptor type 22 (PTPN22 – also known as LYP) associates with autoimmune diseases and remains one of the strongest risk factors for SLE outside the major histocompatibility complex.[1] Research by Holmes *et al*. looked at the role of PEP – the mouse orthologue of PEP – in interferon-alpha (IFN-α) signalling in mice.[2] Results showed PEP plays an inhibitory role in IFN-α and toll like receptor signalling in mice. PEP-/- mice display dysregulated haematopoiesis with anaemia, thrombocytopaenia and neutropaenia. Hence there exists a possible molecular basis for both type I IFN and PTP related to lupus-associated cytopenias.

Professor Dörner also reviewed research from 2015 exploring the role of T follicular helper (Tfh) cells in SLE. Increased activity of Tfh cells is implicated in the pathogenesis of SLE – and the OX40 ligand contributes to this by promoting the Tfh cell response.[2] The OX40 ligand is expressed by myeloid antigen-presenting cells in patients with active SLE. OX40 signals promote the differentiation of human Th cells toward the Tfh lineage. Strong TCR signals promote the expression of Tfh molecules by human Th cells. RNP-Anti-RNP immune complexes also induce monocytes to express OX40L via TLR7 (Figure).

Figure: RNP-Anti-RNP immune complexes induce monocytes to express OX40L via TLR7



Basic research by Yamano *et al.* published in 2015 aimed to uncover whether thymic B-cells also harbour distinct tolerogenic features, similar to the mediation of central tolerance by thymic dendritic cells and medullary epithelial cells.[3] They found that circulating naïve B cells immigrate into the thymus and adopted the licensed phenotype of steady-state thymic B-cells which show enhanced antigen-presenting function. Thymic B-cells are therefore able to present a licensing dependent endogenous self-antigen for negative selection and induction of central T-cell tolerance.

Another basic research question from 2015 related to delineation of the heterogeneity of human bone marrow plasma cells. Distinct plasma cell types in human bone marrow may explain the different serum antibody half-lives that are observed.[4] The human bone marrow is enriched for CD19 plasma cells; these do not change under rituximab therapy and contain anti-dsDNA producing plasma cells in SLE. Tripton *et al*. explored the diversity, cellular origin and autoreactivity of antibody-secreting cell populations in acute SLE.[5] They observed that highly diversified plasma cells are involved in SLE flares and that a substantial fraction of plasma cell clones contain autoantibodies without mutations, a finding consistent with differentiation outside the germinal centres. A substantial plasma cell segment was generated from activated naive cells suggesting selection of SLE autoreactivities occurs during polyclonal activation, with prolonged recruitment of recently activated naive B cells.

SLE is known to be associated with both B-cell hyperactivity and abnormalities of B-cell receptor signalling. Fleischer *et al*. carried out further research in this area to address the linkage between dysregulated BCR signalling and increased B-cell function by looking at immediate phosphorylation events in lupus B-cells.[6] B cells from SLE patients showed diminished Syk phosphorylation and reduced intracellular calcium release after BCR activation as compared to B cells from healthy donors. Phosphorylation of Akt was also significantly increased in SLE B cells. These results indicate that an imbalance between serine and tyrosine phosphatases in SLE contributes to an intrinsically disturbed balance of BCR-initiated signalling pathways, resulting in enhanced survival of lupus B cells and differentiation into plasma cells.

References

1. Burn GL, Svensson L, Sanchez-Blanco C, Saini M, Cope AP**.** Why is PTPN22 a good candidate susceptibility gene for autoimmune disease? *FEBS Lett*. 2011;585(23):3689-98.
2. Jacquemin C, Schmitt N, Contin-Bordes C*, et al.* OX40 Ligand Contributes to Human Lupus Pathogenesis by Promoting T Follicular Helper Response. *Immunity*. 2015;42(6):1159-70.
3. Yamano T, Nedjic J, Hinterberger M*, et al.* Thymic B Cells Are Licensed to Present Self Antigens for Central T Cell Tolerance Induction. *Immunity*. 2015;42(6):1048-61.
4. Mei HE, Wirries I, Frolich D*, et al.* A unique population of IgG-expressing plasma cells lacking CD19 is enriched in human bone marrow. *Blood*. 2015;125(11):1739-48.
5. Tipton CM, Fucile CF, Darce J*, et al.* Diversity, cellular origin and autoreactivity of antibody-secreting cell population expansions in acute systemic lupus erythematosus. *Nat Immunol*. 2015;16(7):755-65.
6. Fleischer SJ, Daridon C, Fleischer V, Lipsky PE, Dorner T**.** Enhanced Tyrosine Phosphatase Activity Underlies Dysregulated B Cell Receptor Signaling and Promotes Survival of Human Lupus B Cells. *Arthritis Rheumatol*. 2016;68(5):1210-21.

Discussion Forum: Issues and Answers

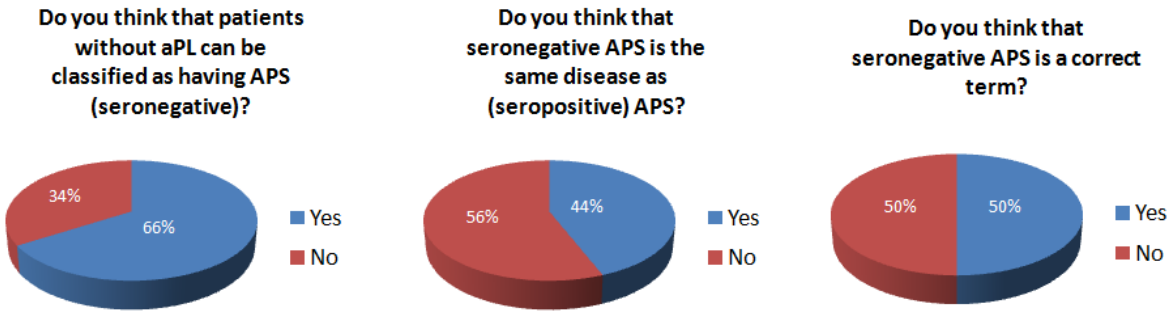
Does seronegative APS exist? Roger A. Levy and Vittorio Pengo

Ricard Cervera introduced the topic and moderated the discussion as Roger Levy (Yes) and Vittorio Pengo (No) debated the existence of seronegative APS.

Diagnosis of antiphospholipid syndrome (APS) is centred on the detection of circulating antiphospholipid (aPL) antibodies. However, in daily clinical practice, patients may present with clinical features suggestive of APS even though they are consistently negative on routine screening tests. The term 'seronegative APS' (SNAPS) has been coined to cover those patients with clinical features of APS who are test negative for aPL. However, there is considerable debate among clinicians about the true existence, and clinical significance, of this so-called seronegative APS.

Professor Cervera set the scene for the ensuing debate by asking the audience to answer three key questions using their keypads, these questions were then asked again at the end of debate following presentation of the case for and against the existence of seronegative APS.

Figure. Questions to the audience: Before the debate



Does seronegative APS exist? Yes: Roger A. Levy (on behalf of Munther A. Khamashta)

Professor Levy began the debate by stating that the major problem with APS lies in the naming of the condition after the antibodies it is associated with, making it hard to distinguish clinical features (such as thrombocytopenia, livedo reticularis etc.) in the absence of detected antibody. He suggested that a preferable name for APS could be autoimmune thrombophilia, APS was first described in the SLE cohort, where 30–40% are aPL positive, but can also exist in a primary, isolated form.[1] There is well-recognised overlap between these two conditions as aPL positive patients with primary APS may progress to overt lupus.

Professor Levy listed additional clinical features which may be associated with APS and/or the thrombosis it produces, and gave a brief overview of the history of APS. β2-glycoprotein 1 (GP1) was the first antigen to be described, however several other proteins involved in the coagulation system can also act as cofactors including prothrombin itself. AntiPS/PT is a more recent test, not part of current criteria, with a striking specificity (97%) and found to be predictive of thrombosis – however it has not yet been fully standardised.[2]

Professor Levy reviewed current criteria for the laboratory diagnosis of APS which is based on assays to detect anticardiolipin antibodies (aCL), lupus anticoagulant (LA) and anti-β2-GP1 antibodies 12 weeks apart.[3,4] Currently, there are no diagnostic criteria for APS hence most clinicians rely heavily on the laboratory classification criteria. In the original St Thomas cohort from 2003, less than 1% of patients had a clinical syndrome suggestive of APS but were aCL and LA negative – these patients were

deemed to have seronegative APS.[5] In current practice, it is now not unusual to find patients with clinical features suggestive for APS who are persistently negative for the routine testing of aCL, anti-β2-GP1 and LA. This could be caused by an inaccurate diagnosis (i.e. not APS), problems in the accuracy of laboratory testing, other phospholipid/cofactor targets (not detected by conventional laboratory testing) or a transitory negative test where the aPL titre has reverted to negative.

Professor Levy pointed to case reports which indicate that even patient with SNAPS can develop catastrophic antiphospholipid syndrome (CAPS).[6] Whether SNAPS accounts for the 20% of patients with Sneddon's syndrome who are negative for aPL also remains unclear.[7] From the lupus Hopkins cohort, ~30% of patients deemed to have APS were negative for the three routine tests.[8]

A proteomic study in 29 patients which aimed to identify new antigenic targets of autoantibodies in APS patients recognised as SNAPS discovered vimentin to be a key endothelial protein cofactor.[9] Antivimentin/ cardiolipin complex antibodies were detected in SNAPS patients but not healthy donors or patients with thrombosis due to another cause. Vimentin therefore represents a promising new target for further study in the SNAPS arena. Research indicates that anti-lysobisphosphatidic acid (LBPA) is another potential candidate antibody target in SNAPS.[10] Professor Levy referred to a recent review which looked at the reasons for negative laboratory results in patients with apparent APS and found seven good reasons to explain this:[11]

1. Weak LA may not be detected if the sample is not centrifuged properly
2. A small proportion of (-) IgG and IgM aCL or anti- β2-GP1 may be positive for IgA
3. Some patients may have antibodies only to other phospholipids not routinely tested
4. Patients may have antibodies to other cofactors like prothrombin
5. In nephrotic patients, there may be urinary loss of IgG APL and/or an increase in catabolism and decrease in synthesis
6. aPL titres may be decreased if a patient is immunosuppressed
7. Transitory disappearance of aPL can occur after a thrombotic event.

Interestingly, the plasma from patients with SNAPS is able to induce expression of tissue factor and increase adhesion molecules like VCAM demonstrating that there is activity in the serum of these patients – even though they remain negative on routine assays.[12] Analysis of clinical manifestations of APS patients with and without aPL antibodies also reveals no clear difference between the groups.[13] Looking at the utility of antiphosphatidylserine/prothrombin and IgA antiphospholipid assays in SLE shows a strong association between history of lupus anticoagulant and antiphospholipid assays for anti-PS/PT, anticardiolipin, anti β2-GP1 and β2-GP1 D1.[14]

Professor Levy reviewed recommendations on the many new panels of tests available and under validation for SNAPs (Table).[15]

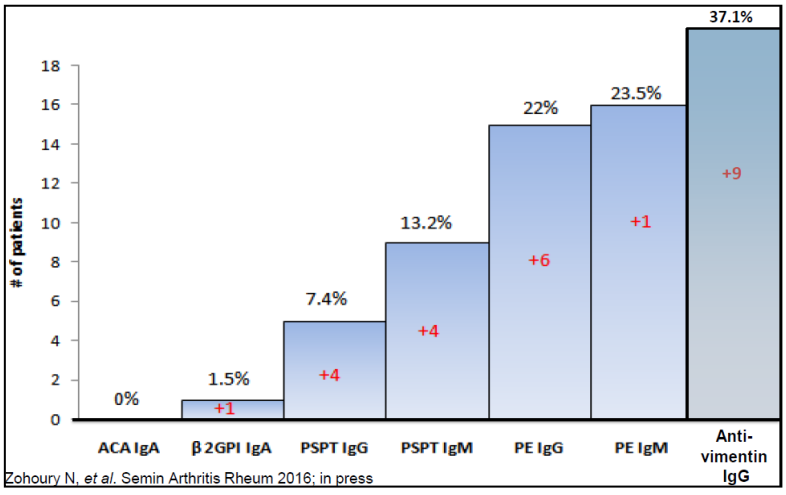
Table: Non-criteria Ab recommendations in SNAPS

'Non-criteria' Ab	Recommendations
Anti-domain I (D1)	Clinical data are encouraging; additional prospective clinical studies and *in vivo* data on the causality of anti-D1 on APS are needed.

Annexin A5 Resistance Assay	Promising use as a mechanistic diagnostic marker; additional data needed before inclusion among the aPL testing panels.
IgA aCL and IgA anti-β2GPI	High clinical suspicion for APS with (-) IgG / IgM aCL
Anti-PE antibodies	Need standardised aPE ELISA and well-designed clinical studies to confirm diagnostic value.
Antibodies to other (-) charged PL: PA, PS & PI	Anti-PS: most promising and relevant in the area of RPL.
Antibodies to Vimentin/CL Complex	**Persistently (+) in almost all APS and many SNAPS patients. Overlaps in SLE and RA, specificity as a diagnostic marker is largely undefined.**
Anti-PT: aPT-A and aPS/PT	**Good specific tests to confirm APS; but standardisation is still needed to be included in the diagnostic criteria.**

Meta-analysis indicates a strong relationship between anti-PS/PT and thrombosis, which has a predictive value for recurring events.[16] Based on this evidence, experts have questioned the need for an updated diagnostic approach to APS which takes into account the detection of 'non-criteria' antiphospholipid antibodies. Work by Professor Khamashta (in press) in 68 SNAPS patients *versus* 107 seropositive controls showed that all SNAPS patients were negative for anti-cardiolipin (aCL), one patient was positive for β2-GP1 IgA, a few were positive for IgG and IgM anti-PE, others were positive for anti-PS/PT IgG and IgM and also anti-vimentin IgG (Figure).[17] Adding these results together reveals that, of the 68 patients included in this study, over 37% could be 'detected' using non-standardised biomarkers. Sensitivity is increased the more non-standardised biomarkers are employed.

Figure: Increasing sensitivity by using non-standardised biomarkers in SNAPS (n=68)



Professor Levy concluded that 'seronegative' as used in the context of APS therefore only really describes those patients who are negative for the standard, classically used tests.

Does seronegative APS exist? No: Vittorio Pengo

Professor Pengo then presented the case for the non-existence of seronegative APS. He began, not by stating that SNAPS does not exist, but by stressing the fact that 'not everyone has figured out which is the real seropositive APS'. Sydney 2005 criteria classify APS patients as category I if more than one laboratory criteria is present and category II if one antibody alone is present.[4] Echoing the point made by Professor Levy, Professor Pengo explained that classification criteria are often mistaken for diagnostic criteria. Thus APS can be diagnosed based on the presence of a single positive test and patients fulfilling these classification criteria are then grouped together in clinical studies – leading to confusion over what these trials are actually looking at.

β2 glycoprotein is the main antigen implicated in APS yet its physiological function is not yet known. Sole aCL positivity (negative for LA and β2-GP1 antibodies) shows no association with thrombosis.[18] In these cases, antibodies are probably directed to cardiolipin binding proteins or directly bind to cardiolipin so are not implicated in APS.[19] Similarly, sole LA positivity (negative for cardiolipin and β2-GP1 antibodies) demonstrates no clear association with the clinical manifestations of APS because there are no antibodies to β2-GP1 which is the main syndrome antigen.[20] Carriers of sole LA positivity followed-up for 10 years showed low levels of thrombosis as evidenced by a low cumulative incidence of thromboembolic events.[21] Equally, Professor Pengo explained that sole anti-β2-GP1 positivity (negative for LA and aCL) also shows no association with clinical manifestations of APS.[22] Anti-domain 4/5 antibodies have been detected in various non-thrombotic conditions and non-domain I anti-β2-GP1 antibodies show no association with thrombosis.[23, 24]

Research carried out by Professor Pengo examined what happened to aPL levels after 12 weeks (the threshold for laboratory antibody testing) in 225 patients initially positive to one or more test – 161 of whom were available for confirmation testing after 3 months.[25] Characteristics at initial testing revealed that triple positivity (LAC+, aCL+, anti-β2-GP1+, same isotype) was strongly associated with female gender and the occurrence of venous or arterial thrombosis. Pregnancy loss was more common in double positive (LAC-, aCL+, anti-β2-GP1, same isotype) patients. After 12 weeks, all initially triple positive patients remained positive as did most of the double positive cohort, however less than half of single positive patients remained positive – suggesting laboratory error or a transient positivity. Professor Pengo stressed that triple positive patients are the 'real' at-risk patients. Follow-up of the cumulative incidence of thromboembolic events over 10 years shows a high risk of events (~40%), despite treatment. Carriers of triple positivity behave in the same way as patients with triple positive APS, developing their first thrombotic events at a similar rate.

Triple positivity is thus unique because, in contrast to single test positivity or no aPL positivity, triple positivity arises from the presence of a single (possible pathogenic) antibody. When the plasma of triple positive patients is passed through a β2-GP1 affinity column and antibodies are eluted and placed into normal plasma, retesting for the presence of aPL shows positivity across all three tests.[26] anti-β2-GP1 domain 1 (Dm1) appears to be the important part of the antigen in terms of syndrome development as all triple positive patients are anti-Dm1 positive and anti-β2-GP1-Dm1 is also associated with thrombosis.[23] In animal models of APS, anti-β2-GP1-Dm1 antibodies contribute to thrombus development after laser injury in wild type mice.

Professor Pengo concluded that APS with triple positivity constitutes the true autoimmune disease for the following key reasons:

- The corresponding antigen is known and the specific epitope is partially known

- An autoimmune reaction is identified in the form of autoantibody
- An analogous response causes a similar disease in experimental animals.

He acknowledged that differential diagnosis is difficult but stressed that cases of non-triple positive APS are likely to be other, distinct, diseases – and should not be considered to be seronegative APS.

Does seronegative APS exist? Roger Levy's Rebuttal (Yes)

Professor Levy agreed with Professor Pengo that triple positives are higher-risk patients, but insisted that other patients with typical syndrome-associated clinical manifestations should also be considered APS. He presented two cases of patients with seronegative APS to highlight this point. The first patient, a 30-year old female, presented in 2005 as only anti-β2-GP1 positive despite prominent livedo reticularis in both legs, severe pre-eclampsia and stillbirth at 34 weeks and evidence of multiple infarcts on brain MRI; in 2011 she was positive for anti-PS/PT. Currently, neither of these are sufficient to diagnose the patient as having APS yet Professor Levy felt this patient should have received treatment right from the outset of her clinical symptoms. The second case was a 38-year old female with a history of premature delivery, pre-eclampsia, cerebellar in fact and mitral and aortic vegetation. This patient was negative for all aPL tests until the most recent which showed positivity for MPL (80 units) and LA. She too would have benefited from earlier treatment intervention in Professor Levy's opinion. Guidelines on the future classification of 'non-criteria' clinical manifestations of APS have recommended the inclusion of seronegative APS because, although the literature evidence is low, SNAPS may lead to recurrent and life-threatening events and is critical for clinical decision-making. Professor Levy concluded with his key messages on seronegative APS:

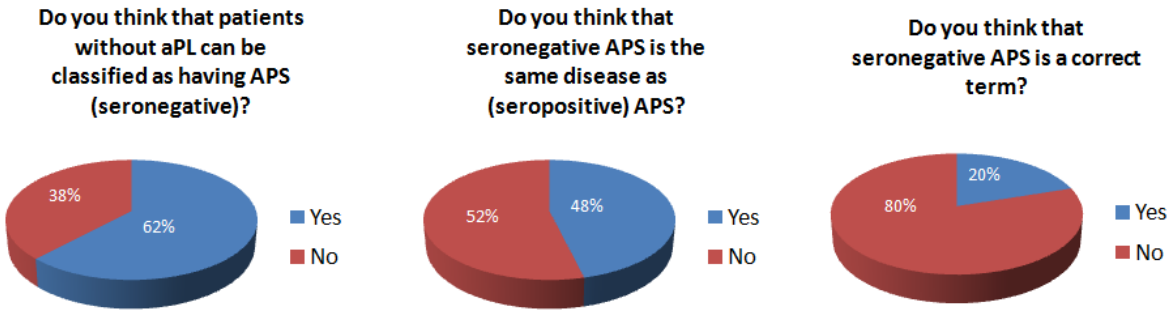
- Trust your clinical judgement and take clinical characteristics into account, offering treatment according to each case
- Consider 'non-criteria' tests for aPL to increase the diagnostic yield
- Standardised and large scale longitudinal studies are needed to confirm the clinical relevance of new non-criteria antibodies
- Follow-up each patient for seroconversion and do not stop treatment if test(s) become negative.

Does seronegative APS exist? Vittorio Pengo's Rebuttal (No)

In his rebuttal, Professor Pengo insisted that adding tests for additional antigens does not add to understanding, or necessarily indicate an underlying disease, and that triple positivity alone is a sufficient criteria to diagnose APS.

A short discussion was then opened with the audience, before the debaters summed up their positions. To conclude, the questions asked at the start of the debate were repeated, to see whether hearing the existence of seronegative APS being debate had altered listeners' opinions.

Figure. Questions to the audience: After the debate



References

1. Hughes GR**.** Thrombosis, abortion, cerebral disease, and the lupus anticoagulant. *Br Med J (Clin Res Ed)*. 1983;287(6399):1088-9.
2. Atsumi T, Ieko M, Bertolaccini ML*, et al.* Association of autoantibodies against the phosphatidylserine-prothrombin complex with manifestations of the antiphospholipid syndrome and with the presence of lupus anticoagulant. *Arthritis Rheum*. 2000;43(9):1982-93.
3. Wilson WA, Gharavi AE, Koike T*, et al.* International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum*. 1999;42(7):1309-11.
4. Miyakis S, Lockshin MD, Atsumi T*, et al.* International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost*. 2006;4(2):295-306.
5. Hughes GR, Khamashta MA**.** Seronegative antiphospholipid syndrome. *Ann Rheum Dis*. 2003;62(12):1127.
6. Lazurova I, Macejova Z, Tomkova Z*, et al.* Severe limb necrosis: primary thrombotic microangiopathy or "seronegative" catastrophic antiphospholipid syndrome? A diagnostic dilemma. *Clin Rheumatol*. 2007;26(10):1737-40.
7. Dutra LA, Braga-Neto P, Pedroso JL, Barsottini OG**.** Sneddon's syndrome: case report and review of its relationship with antiphospholipid syndrome. *Einstein (Sao Paulo)*. 2012;10(2):230-2.
8. Petri M**.** Update on anti-phospholipid antibodies in SLE: the Hopkins' Lupus Cohort. *Lupus*. 2010;19(4):419-23.
9. Ortona E, Capozzi A, Colasanti T*, et al.* Vimentin/cardiolipin complex as a new antigenic target of the antiphospholipid syndrome. *Blood*. 2010;116(16):2960-7.
10. Matonti F, Hoffart L, Trichet E, Conrath J, Ridings B**.** [Central retinal vein occlusion and anti-phosphatidylethanolamine antibodies isolation: a case report]. *Can J Ophthalmol*. 2010;45(3):295-6.
11. Cervera R, Conti F, Doria A, Iaccarino L, Valesini G**.** Does seronegative antiphospholipid syndrome really exist? *Autoimmun Rev*. 2012;11(8):581-4.
12. Conti F, Alessandri C, Sorice M*, et al.* Thin-layer chromatography immunostaining in detecting anti-phospholipid antibodies in seronegative anti-phospholipid syndrome. *Clin Exp Immunol*. 2012;167(3):429-37.
13. Rodriguez-Garcia JL, Bertolaccini ML, Cuadrado MJ*, et al.* Clinical manifestations of antiphospholipid syndrome (APS) with and without antiphospholipid antibodies (the so-called 'seronegative APS'). *Ann Rheum Dis*. 2012;71(2):242-4.
14. Akhter E, Shums Z, Norman GL*, et al.* Utility of antiphosphatidylserine/prothrombin and IgA antiphospholipid assays in systemic lupus erythematosus. *J Rheumatol*. 2013;40(3):282-6.
15. Nayfe R, Uthman I, Aoun J*, et al.* Seronegative antiphospholipid syndrome. *Rheumatology (Oxford)*. 2013;52(8):1358-67.

16. Sciascia S, Sanna G, Murru V*, et al.* Anti-prothrombin (aPT) and anti-phosphatidylserine/prothrombin (aPS/PT) antibodies and the risk of thrombosis in the antiphospholipid syndrome. A systematic review. *Thromb Haemost*. 2014;111(2):354-64.
17. Zohoury N**.** *Semin Arthritis Rheum; in press* 2016.
18. Ruffatti A, Olivieri S, Tonello M*, et al.* Influence of different IgG anticardiolipin antibody cut-off values on antiphospholipid syndrome classification. *J Thromb Haemost*. 2008;6(10):1693-6.
19. Rampazzo P, Biasiolo A, Garin J*, et al.* Some patients with antiphospholipid syndrome express hitherto undescribed antibodies to cardiolipin-binding proteins. *Thromb Haemost*. 2001;85(1):57-62.
20. Pengo V, Biasiolo A, Gresele P*, et al.* A comparison of lupus anticoagulant-positive patients with clinical picture of antiphospholipid syndrome and those without. *Arterioscler Thromb Vasc Biol*. 2007;27(12):e309-10.
21. Pengo V, Testa S, Martinelli I*, et al.* Incidence of a first thromboembolic event in carriers of isolated lupus anticoagulant. *Thromb Res*. 2015;135(1):46-9.
22. Pengo V, Biasiolo A, Pegoraro C*, et al.* Antibody profiles for the diagnosis of antiphospholipid syndrome. *Thromb Haemost*. 2005;93(6):1147-52.
23. Pengo V, Ruffatti A, Tonello M*, et al.* Antibodies to Domain 4/5 (Dm4/5) of beta2-Glycoprotein 1 (beta2GP1) in different antiphospholipid (aPL) antibody profiles. *Thromb Res*. 2015;136(1):161-3.
24. de Laat B, Pengo V, Pabinger I*, et al.* The association between circulating antibodies against domain I of beta2-glycoprotein I and thrombosis: an international multicenter study. *J Thromb Haemost*. 2009;7(11):1767-73.
25. Pengo V, Ruffatti A, Del Ross T*, et al.* Confirmation of initial antiphospholipid antibody positivity depends on the antiphospholipid antibody profile. *J Thromb Haemost*. 2013;11(8):1527-31.
26. Pengo V, Biasiolo A, Rampazzo P, Brocco T**.** dRVVT is more sensitive than KCT or TTI for detecting lupus anticoagulant activity of anti-beta2-glycoprotein I autoantibodies. *Thromb Haemost*. 1999;81(2):256-8.

Plenary I: New Horizons in Basic Science

Inflammatory signals and regulatory B-cells: what goes wrong in lupus patients? Claudia Mauri

Professor Mauri described the role of regulatory B-cells (Bregs) in the maintenance of tolerance and discussed current insights and understanding into the signals that drive Breg differentiation and their potential role in SLE.

Professor Mauri began by explaining that, similar to T-cells, B-cells can be divided into different subsets based on the environment in which they differentiate and, accordingly, the cytokines they produce. Bregs, negative regulators of the immune system, are a distinct subset of B-cells which produce IL-10 that exerts powerful immunosuppressive effects.[1]

In healthy individuals, Bregs make up 10% of the overall B-cell population. These immature, antigen-naive cells have just exited the bone marrow and express high levels of CD24 and CD38.[2] Although any type of B-cell subset can produce IL-10, research shows that when stimulated by anti-CD40 antibodies which mimic the effect of T-cell activation in vivo, the majority of IL-10 production occurs via Bregs.[2] Bregs have been shown to suppress T helper cell differentiation via IL-10 and not TGF-beta, although CD80 and CD86 engagement is key.[2]

But what happens in lupus patients?

Immature B cells isolated from SLE patients are not numerically different from healthy individuals and effector T-cells from SLE patients are not refractory to suppression. Therefore, to answer the question of what goes wrong in lupus patients, Professor Mauri pointed to research from her own centre. When Bregs were depleted from the whole PMBC of a healthy individual there was an increase in TNF-α and interferon production by T-cells, indicating that the Bregs were suppressive. However, no comparable effect was observed in patients with active SLE.[2] Further investigation revealed that Bregs from SLE patients with active disease exhibit impaired IL-10 producing capacity due to an intrinsic defect. This effect is reversible as SLE patients with inactive disease show regeneration of IL-10 producing B cells.

Professor Mauri explained that finding a way to restore the intrinsic defect in Bregs seen in lupus patients may lead to new therapeutic options for the disease – but first it is important to understand the signals that stimulate Breg production. In animals, Breg expansion is directly linked to levels of inflammation.[3] However, in humans, both IL-1beta and IL-6 fail to induce differentiation of Bregs to any significant extent. Subsequent studies have now revealed that IFN-α is the key cytokine in humans that provides the 'danger' stimuli which causes B-cells to differentiate into Bregs

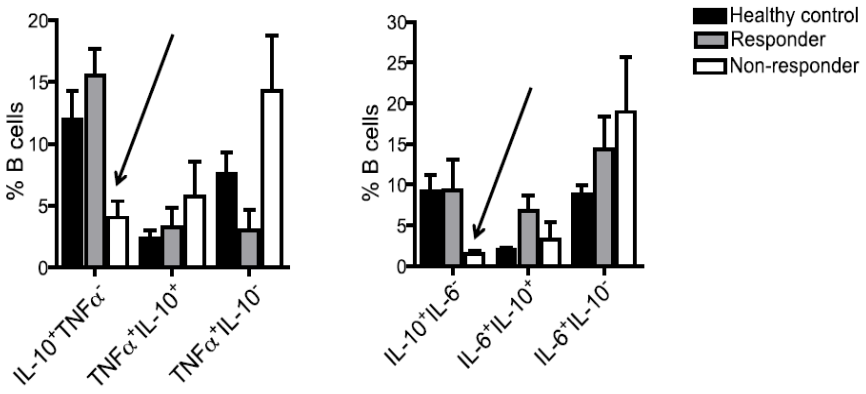
Leading on from this, Professor Mauri outlined important new research showing that plasmacytoid dendritic cells (pDCs) act as the master regulators of human Breg differentiation, driving the differentiation of B-cells into Bregs and plasmablasts in an IFN-α dependent manner. When B-cells that have been in 72-hour contact with pDCs are purified and combined with T-cells, there is suppression of T-cells producing both TNF-α and IFN-γ.[4]

The above research created a conundrum as IFN-α is also known to be a key culprit in SLE disease. To account for this dichotomy, a hypothesis was generated based on the existence of a previously unknown auto-regulatory feedback mechanism between pDCs and Bregs. In testing this hypothesis, characterisation of pDCs from SLE patients showed them to be more active than their healthy counterparts with a clear IFN-α related signature, higher CD86 and CD80 expression and an increased

proliferation index.[4] In patients with SLE, defects in the pDC-Breg cross talk were also found to skew the B-cell response towards plasmablast differentiation rather than Breg induction. Research showed that high exposure to IFN-α actually inhibited Breg differentiation while continuing to promote plasmablast expansion.[4] Overall this shows that IFN-α at low levels has a well-balanced dual effect, however at high concentrations the capacity of B-cells is totally concentrated on becoming plasma cells at the expense of Breg production.

Importantly, the pDC-B-cell interaction has been shown to be normalised in patients with SLE responding to B-cell depletion therapy with rituximab.[4] Could there also be a role for Bregs in patients who manifest as disease activity non-responders after their B-cells have repopulated? Research has shown that the higher the number of Bregs, the lower the activation status of the pDC.[4] Conversely, non-responders to rituximab had a higher level of activated pDCs and a low number of Bregs. Looking at cytokine levels in these patients also shows that rituximab therapy is able to restore Breg function in patients responding to therapy, mimicking what happens in healthy individuals.[4] In non-responders, however, there is an imbalance of Bregs which are no longer primed to make IL-10 but instead are plastic and have been skewed to produce proinflammatory cytokines (Figure).[4]

Figure: Rituximab therapy restores Breg function in patients responding to therapy



Taken together, Professor Mauri concluded that these results indicate that a very fine balance exists between inflammatory signals, induction of Bregs and autoimmunity and SLE.

References

1. Harris DP, Haynes L, Sayles PC*, et al.* Reciprocal regulation of polarized cytokine production by effector B and T cells. *Nat Immunol*. 2000;1(6):475-82.
2. Blair PA, Norena LY, Flores-Borja F*, et al.* CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. *Immunity*. 2010;32(1):129-40.
3. Mizoguchi A, Mizoguchi E, Takedatsu H, Blumberg RS, Bhan AK**.** Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. *Immunity*. 2002;16(2):219-30.
4. Menon M, Blair PA, Isenberg DA, Mauri C**.** A Regulatory Feedback between Plasmacytoid Dendritic Cells and Regulatory B Cells Is Aberrant in Systemic Lupus Erythematosus. *Immunity*. 2016;44(3):683-97.

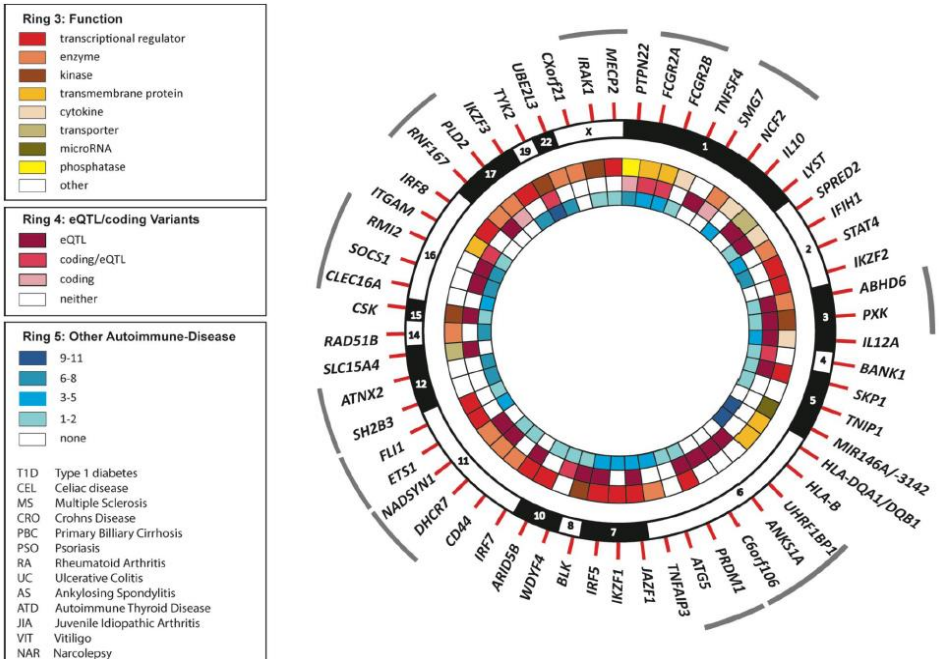
SLE redefined based on molecular pathways: Marta E. Alarcon-Riquelme

Professor Alarcon-Riquelme outlined the impact of genetic studies in SLE and discussed the clinical consequences of key gene expression studies. The role of 'omics' studies in identifying new therapeutic options for SLE was also discussed, along with an overview of the large-scale PRECISESADS project which aims to comprehensively reclassify systemic autoimmune diseases.

Genetics of SLE: Where we are today?

Professor Alarcon-Riquelme began by reviewing current understanding of the genetics of SLE as evidenced by genetic association studies, including both candidate gene studies and genome-wide association studies. Over 50 genes have been identified in SLE, most of which are shared with other autoimmune diseases and across several different ethnicities. The majority of the important polymorphisms identified in SLE are regulatory and control gene expression. This is important because, in the environment-gene interaction, quantitative thresholds exist for immune-cell signalling. The more risk alleles a person possesses, the more likely they are to manifest an excessive immune response to a particular strength stimulus.[1] Unsurprisingly, a large number of the genes implicated in SLE are also involved in the innate and adaptive immune responses.

Figure: SLE genes: Most effects are regulatory and shared with other autoimmune diseases



Adapted from: Bentham DL. *Nat Genet*, 2015;47**:**1457-64.[2]

The possible role of rare mutations in SLE

Rare diseases, particularly interferonopathies, provide useful insights into SLE. Many of these encode genes involved in DNA or nuclei acid metabolism and have been shown to be involved in creating an interferon signature.[3] Professor Alarcon-Riquelme highlighted findings from an analysis of rare variants in 4500 individuals with SLE which revealed enrichment of rare mutations (<1% frequency) involving online Mendelian inheritance in man (OMIM) genes and rare diseases with specific phenotypes such as coagulation, skin manifestations and immunodeficiency's.

From genetics to genomics and multiple–omics: Redefining SLE

New information provided by epigenetic studies suggests that there is significant hypomethylation in interferon-related genes in naïve CD4+ T-cells from lupus patients including BST2 and IFI44L.[4] This indicates the existence of CD4+ naïve T-cells with IFN hypomethylated genes poised for expression, suggesting a potential pathogenic role for abnormal T-cell DNA methylation in lupus. Recently published work by Banchereau *et al.* has further attempted to stratify SLE patients according to genes correlated with disease activity, identifying seven groups of patients overall.[5] Only two of these groups show a true interferon signature where there is evidence of modulation by an underlying IFN-related pathway.

Turning to the question of 'What is autoimmunity?' Professor Alarcon-Riquleme described this as a loss of immune tolerance with a related pathogenesis across several different diseases. The challenge then becomes identifying what makes each autoimmune disease different and at what point in its clinical evolution. A key problem is that systemic autoimmune diseases have partly shared genetics as well as shared biomarkers and signatures. Delayed diagnosis and bad treatment responses are also problems associated with present day clinical classification criteria for autoimmune conditions.

Attempting to address these important issues is the PRECISEADS project, a joint initiative between five pharmaceutical companies, two SMEs and 21 academic institutions. PRECISEADS began in February 2014 and is expected to run for 5 years. It will seek to reclassify systemic autoimmune diseases through the complete characterisation of several 'omics' including genomics, transcriptomics, epigenomics, metabolomics (urine and plasma), proteomics (cytokines) and flow cytometry/mass spectrometry (CyTOF). PRECISESADS will involve cross-sectional study of 2000 cases, 400 of each disease- SLE, rheumatoid arthritis (RA), Sjogren's Syndrome and systemic sclerosis – and controls. The inception cohort will be followed-up from baseline, Month 6 and Month 14, with mouse models also studied in parallel.

Professor Alarcon-Riquelme explained that the ultimate aim of PRECISEADS is to develop a new molecular-based classification for the four diseases in question, however she also acknowledged several key, often overlapping, challenges that the project faces. These include:

- Organising the recruitment and clinical data of patients throughout 18 clinical centres in Europe and managing samples with various requirements including ethical, clinical and laboratory protocols
- Minimising the variation among these different sites
- Ensuring fluorescence compatibility between different flow cytometers, bearing in mind that flow cytometry must be carried out on fresh cells
- Carrying out panel optimisation and ensuring compatibility between dyes and markers
- Integrating different sources of data in a full omic data analysis
- Maintaining a continuous quality control process

Concluding her presentation, Professor Alarcon-Riquelme stressed that it will be the detail that ultimately yields the answers in systemic autoimmune disease – particularly in a heterogeneous condition like SLE.

References

1. Gregersen PK, Diamond B, Plenge RM**.** GWAS implicates a role for quantitative immune traits and threshold effects in risk for human autoimmune disorders. *Curr Opin Immunol*. 2012;24(5):538-43.

2. Bentham J, Morris DL, Cunninghame Graham DS*, et al.* Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus. *Nat Genet*. 2015;47(12):1457-64.
3. Meyts I, Casanova JL**.** A human inborn error connects the alpha's. *Nat Immunol*. 2016;17(5):472-4.
4. Coit P, Jeffries M, Altorok N*, et al.* Genome-wide DNA methylation study suggests epigenetic accessibility and transcriptional poising of interferon-regulated genes in naive CD4+ T cells from lupus patients. *J Autoimmun*. 2013;43:78-84.
5. Banchereau R, Hong S, Cantarel B*, et al.* Personalized Immunomonitoring Uncovers Molecular Networks that Stratify Lupus Patients. *Cell*. 2016;165(3):551-65.

Autoantibodies to neural antigens: Joseph Dalmau

Professor Dalmau gave an overview of the most frequent disorders associated with autoantibodies to neural antigens, focussing on anti-NMDA receptor encephalitis to illustrate the clinical impact of these disorders and their antibody pathogenicity.

Over the last 10 years, attention has focused on a new category of autoimmune disorders of the synapse where autoantibodies are targeted against relevant cell surface proteins and synaptic receptors involved in synaptic signalling and plasticity. Although 16 such disorders have now been characterised, Professor Dalmau explained that the focus of his talk would be on autoantibodies to the NMDA receptor and the associated syndrome of anti-NMDAR encephalitis, which occurs more frequently than any other single viral encephalitis in the US. Antibodies to cell surface and synaptic proteins are easily visualised in optimised tissue samples. However, Professor Dalmau stressed the importance of distinguishing between antibodies against GLUN1 and the NR2 of lupus, which cannot be visualised in live neurones, even though the target is the same receptor.

Patients with paraneoplastic encephalitis are often hospitalised, comatose and display typical, complex movement patterns. In anti-NMDAR encephalitis there is a viral-like prodrome lasting for approximately 1 week followed by psychiatric symptoms including agitation, hallucinations, mania, speech changes and catatonia. Patients then enter a stage where intensive care support is required, suffering seizures, movement disorders, dysautonomia and hypoventilation. Recovery is prolonged, extending over a 12-24 month period, and may be associated with residual deficits.

Professor Dalmau presented an overview of gender and tumour associations from a study of 577 patients with anti-NMDAR encephalitis, showing the two key disease variants – tumour-associated and non-tumour associated. He highlighted that most patients are young, aged <50 years, and that the most common causative tumour in women is an ovarian teratoma containing NMDA-rich neural tissue. 81% of patients with NMDA encephalitis were recovered at 2 years, while 5-7% died during the acute stage of the disease. Relapse rates during the first 24 months were around 12%, but significantly lower in patients with tumour-associated disease. Patients treated with 2nd line therapy – rituximab and/or cyclophosphamide also showed a lower rate of relapse. Around 50% of patients will respond to 1st line treatment with steroids and/or IVIG.

In other disorders of synaptic/cell surface autoimmunity, antibodies target the AMPA receptor, GABAβ receptor or LGI1, leading to classic limbic encephalitis which presents clinically with short-term memory problems. Professor Dalmau also explained that, in contrast to previous thinking on the subject, voltage-gated potassium channel (VGKC) complex antibodies do not exist so laboratory testing should focus instead on LGI1 and CASPR2 as potential autoantibody targets. Antibodies to dipeptidyl-peptidase-like protein-6 (DDPX) can also cause also encephalitis associated with prodromal GI symptoms and weight loss.

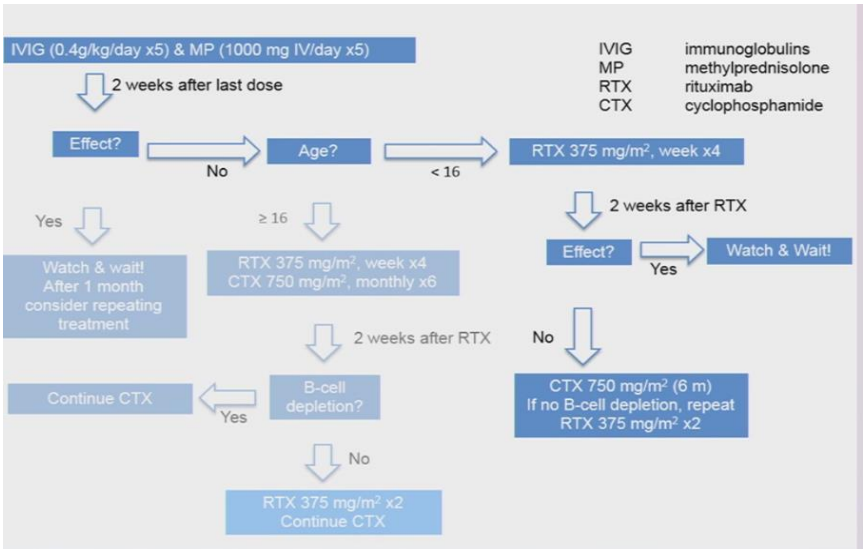
Professor Dalmau outlined how some viral disorders can trigger synaptic autoimmunity, describing the index case of a patient who developed anti-NMDAR encephalitis post herpes simplex encephalitis (HSE). This condition may have been triggered by molecular mimicry or the severe destruction of neurones and inflammation associated with HSE.

GluN1 antibodies from patients with anti-NMDAR encephalitis have well-studied pathogenic effects. For example, patients' antibodies decrease the number of clusters of NMDAR compared to control CSF. If the antibodies are removed and replaced with fresh media, the NMDA receptors return. Across all these disorders, cerebrospinal fluid (CSF) antibody titres correlate better with symptoms and

relapses than serum titres. In an in vivo experiment, mice underwent placement of ventricular catheters connected to osmotic pumps that delivered a continuous infusion of patient or control CSF. Brain tissue showed a progressive increase of brain-bound human antibodies, predominantly in the hippocampus, and a dramatic decrease of synaptic NMDAR. Mice infused with CSF, but not control, also developed progressive memory deficiencies and anhedonic and depressive-like behaviours.

Professor Dalmau concluded his presentation by addressing some of the frequent clinical questions surrounding disorders caused by autoantibodies to neural antigens, including when to suspect these disorders, how to diagnose and treat them and when to stop therapy. These disorders should be suspected when a patient has rapidly progressive encephalopathy of unclear aetiology with multifocal symptoms and inflammatory findings in the CSF. MRI is not that helpful in diagnosing anti-NMDAR encephalitis (remaining normal in ~66% of cases) but may be useful in classic limbic encephalitis associated with anti- LGI1, GABAβ or AMPA antibodies, where FLAIR-T2 changes in the hippocampus may be visible. When diagnosing these disorders, it is important to consider the clinical syndrome in conjunction with patient's age and gender. Serum and CSF samples should be examined for antibodies, with priority given to the CSF results. False results from antibody testing should be considered if the syndrome does not fit with the type of antibody and repeat studies performed in this case, or a research lab consulted. Antibody titres often remain detectable after symptom improvement. Treatment involves checking for, and treating, any underlying tumour, bearing in mind that tumour frequency varies according to the antibody in question and patient age and gender. Immunotherapy consisting of IVIg + steroids or plasma exchange + steroid should be used as first-line treatment, with rituximab and/or cyclophosphamide reserved for second-line therapy. Currently there is no data on the utility of long-term use of steroid-sparing drugs such as azathioprine or MMF.

Figure: Treatment flowchart



Treatment is stopped at the physician's discretion but priority should be given to symptoms over antibody titres, especially given that the recovery process of NMDA receptors is very slow.

Curbside consults

The experts tackle lupus nephritis and lupus arthritis: Richard A. Furie Bevra H. Hahn, Murray B. Urowitz, David A. Isenberg, Roland F. van Vollenhoven, Jamal Al-Saleh

Curbside consults are a common occurrence in everyday clinical practice where a physician may get stopped in the hall of the hospital or paged by another doctor for an unofficial consult and provides input on a case without becoming officially involved in patient care. To replicate this, an expert panel were posed a series of key questions on two specific case studies related to lupus nephritis and lupus arthritis and challenged to give concise, yet clinically comprehensive, short answers.

Case 1: Lupus Nephritis. The Challenge of the Partial Responder

Case 1 addressed the challenge of the partial responder. The panel were provided with the following key clinical information on the patient.

- A 22 year old female was diagnosed with lupus nephritis 1 year ago:
  - Serum creatinine: 1.0
  - DNA 825 (normal < 30 IU/mL); C3: 34; C4: 6
  - Urinary Pr/Cr: 8.2
  - Biopsy: Class IVA-global and V
- Induction therapy consisted of prednisone, hydroxychloroquine 400 mg/d, and MMF (3 g/d)

Date	Baseline	12 months post-induction
Creatinine (<1.2 mg/dL)	1.0	0.9
Urinary Pr/Cr (<0.2)	8.2	2.2
DNA Ab (<30 IU/mL)	825	325
C3/C4 (>90/16 mg/mL)	34/6	94/18

- The good:
  - Creatinine same or a little better
  - >50% improvement in proteinuria
  - >50% improvement in DNA Ab
  - Complements normalized
- The bad:
  - Urinary Pr/Cr 2.2
  - DNA Ab still positive

Question 1: Would you change therapy at 12 months?

Professor Isenberg referred to a paper by Lightstone showing that, in patients treated at the time of LN diagnosis with rituximab followed by MMF, 50% were in complete remission at 12 months, with a 70% complete or partial remission rate. Following this graph out beyond 12 months showed that numbers continued to improve, rising to 70% and 90% after 18 and 24 months, respectively. Hence his advice in this case would be to continue existing MMF therapy as there is a very good chance renal outcomes in the patient will continue to improve.

Professor Urowitz described research showing that response in proteinuria is slow and progressive with standard of care over time, hence he would also opt to continue existing therapy in this patient.

Professor Hahn confirmed that she too would continue existing therapy and seek to reduce prednisolone, but not MMF dose. This is consistent with current ACR guidelines which indicate a patient can be moved to maintenance therapy at 6 months if there is improvement (not defined but based on the clinician's subjective judgement).

Dr Al-Saleh echoed Professor Hahn's position and said he would also add in ACE inhibitors/ARBs and potentially a calcineurin inhibitor.

Professor van Vollenhoven disagreed with the other experts and said he would change therapy for 3 reasons: the patient's levels of proteinuria and protein to creatinine ratio are too high, indicating ongoing kidney damage; the patient is likely require ongoing corticosteroids; and, other good treatment options are available as alternatives to continued MMF. A patient like this could also be considered a candidate for the RING trial.

Question 2: Would you repeat the biopsy at 12 months?

Professor van Vollenhoven noted that his current practice is to repeat biopsy after 6 months of treatment. However, in this case where the patient is not doing well, the biopsy would not be expected to be pristine anyway.

Professor Urowitz felt there was no compelling reason to biopsy at 12 months as the complete response rate is low (~25%) so biopsy would only reveal ongoing active disease.

Professor Hahn agreed that repeating the biopsy at 12 months was unlikely to provide any useful information.

Dr Al-Saleh said he would consider repeating the biopsy.

Question 3: What do you do with your partial responders?

Professor Hahn questioned the patient's race, noting that if the patient was African American or Latino, she would prefer rituximab over cyclophosphamide.

Question 4: We might all try CYC or rituximab, but what about tacrolimus? Where does that fit in?

Asked about the role of calcineurin inhibitors for proliferative disease, Professor Urowitz said he would use these only if the tempo of response was inappropriate.

Professor Hahn referred to a recent Chinese study showing that calcineurin inhibitors combined with low-dose MMF give good induction of improvement. Her experience in practice is that most patients respond to tacrolimus and it is useful for managing a flare, but cannot be used for extended periods of time (>1 year) due to toxicity (higher than that observed in clinical trials) which includes creatinine increases, hypertension and neurological tremor.

Case 2: A 29-year old man with SLE, RA or Rhupus

Case 2 considered the problem of a 29-year old man with SLE, RA or Rhupus.

The panel were provided with the following key clinical information on the patient.

- A 29 year old male was diagnosed with SLE 2 years ago. Manifestations initially included:
  - Malar rash
  - Arthritis (BILAG A level)
  - ANA 1/640 (H)
  - DNA 240 (normal <30 IU/mL)
- Initial therapy consisted of NSAIDs, low dose prednisone, and hydroxychloroquine. However, the debilitating arthritis persisted; methotrexate and MMF also failed to control his arthritis.
- He seeks your opinion as his arthritis is interfering with his ability to function
- Physical Exam:
  - Malar rash
  - Bilateral ulnar deviation
  - Profound polyarticular synovitis (wrists, MCPs, PIPs, knees, MTPs)
- Lab Data:
  - ANA 1/320 (H); DNA 290 (normal <30 IU/mL)
  - RF 125 (normal <13); CCP 250 (normal <18)
- X-rays: erosions at MCPs and ulnar styloids
- MRI: erosions at MCP, ulnar styloids, and wrists; prominent synovitis

Question 1: Is this case SLE with RA serologies, RA with SLE serologies or Rhupus?

Dr Al-Saleh pointed out that this patient fulfils both ACR criteria for SLE and EULAR criteria for RA so his diagnosis would be Rhupus.

Professor van Vollenhoven said he would question the lupus side of the diagnosis in this patient but not the RA.

Professor Urowitz expressed uncertainty on the existence of Rhupus and felt this patient has two separate diseases – SLE and RA – with distinct mechanisms. Overlap of SLE with other autoimmune diseases is well documented.

Question 2: What is the prevalence of CCP Ab in SLE?

Professor Al-Saleh referred to literature indicating a prevalence of 10-15%.

Professor Isenberg referenced his own research from 15 years previous investigating the occurrence of anti-CCP Ab in SLE which arrived at a prevalence figure of ~2%.

Question 3: How common are erosions in SLE?

Professor Hahn felt this patient was a true Rhupus patient as erosions were visible on x-ray (which is true in only 10% of SLE patients with arthritis). In contrast, 50% of SLE patients will have erosions on ultrasound. In her opinion, it is incorrect to categorise SLE as a non-erosive arthritis.

Question 4: Would you use a TNF inhibitor in such a patient?

Professor Urowitz felt that a TNF inhibitor was indicated for this patient's RA. However the concomitant SLE diagnosis requires consideration of the impact this choice of treatment may have on the patient's lupus symptoms. He said he would be 'nervous' about using a TNF inhibitor in this case due to the potential for antiphospholipid antibody development.

Professor Isenberg explained that the concern when TNF inhibitors were first introduced was that they would induce ANAs to DNA. Data from the RA registry in patients followed-up for 15 years indicate that the number of RA patients converting to lupus is 'incredibly small'. However, in lupus patients, he shares the same concerns as Professor Urowitz.

Professor Hahn indicated she would avoid a TNF inhibitor in this patient due to concern about causing a potential lupus flare and the fact that other options are available.

Question 5: What other drugs would you consider?

In preference to TNF inhibitor, Professor Hahn said she would try belimumab as her first choice in this patient (although responses are slow) and may also consider abatacept or tocolimumab. Potential issues with tocolimumab are the fact that, in order to ensure reimbursement, the patient may be required to fail a TNF inhibitor first and also the risk of bowel perforation.

Plenary II: Management of SLE – Compliance, Comorbidities and Drug Toxicities

The importance of assessing medication exposure in SLE: Zahir Amoura

Professor Amoura discussed the importance of assessing medication exposure in SLE, including the need to monitor mycophenolic acid pharmacokinetics and assess blood levels of hydroxychloroquine and azathioprine.

Professor Amoura began his presentation by explaining why it is important to measure medication exposure in SLE – because although clinicians know the dose they are giving to their patients, they don't know what the real exposure to active drug is. The bioavailability of a drug is strongly affected by several factors including absorption, volume of distribution, metabolism and excretion, as well as the pharmacogenomics of the patient and key environmental and genetic factors.

Professor Amoura continued with a detailed look at medication exposure with the key SLE drug mycophenolate mofetil (MMF). Although widely used in SLE treatment, MMF is not approved in this indication. Five generic MMF drugs are now available which increases pharmacokinetic variability further. MMF is an inactive prodrug converted to the active drug mycophenolic acid (MPA) by the action of intestinal, liver and plasma esterase enzymes. MPA is strongly bound to albumin with only a small free fraction. The final target of this free fraction is the enzyme inosine monophosphate dehydrogenase. Blockade of this enzyme strongly inhibits the proliferation of both B and T-cells. MPA is converted into the inactive metabolite MPAG which is partly excreted through the bile into food, thus explaining the digestive side effects of MMF. Biliary excreted MPAG can be converted back to MPA and reabsorbed. This pathway is inhibited by calcineurin inhibitors like cyclosporine A (CsA). Patients who receive both CsA and MMF therefore show higher excretion of MPAG and hence experience more frequent digestive side effects.

MPA blood concentrations after dosing of 1 g of MMF show three distinct peaks, with a Cmax after 0.5-2 hours and an elimination half-life of 11-18 hours. The late third peak occurs due to MPAG hydrolysis back to MPA (not seen in patients on concomitant calcineurin inhibitors). MPA blood concentrations after dosing of 1 g of MMF display high between-patient variability. Tools to measure the actual exposure to MPA in an individual patient include a full PK study, which is the gold standard measure. However, this approach requires 12 blood samples () hence is not practical to perform in routine clinical practice. Another strategy involves using a limited number of samplings and estimating area under the curve (AUC) using a Bayesian estimator (specific to SLE). It requires only 3 hours to perform so can be carried out easily in a day-care unit. The final tool available to clinicians is trough concentrations which show a correlation with MPA AUC, although the strength of this correlation is disputed.[1, 2]

Professor Amoura went on to discuss the relationship between SLE activity and MPA AUC. In a study of 71 consecutive SLE patients, 1 g of MMF was associated with a 1-10 fold variation in MPA AUC.[3] Results showed a strong negative correlation between MPA AUC and SLE disease activity defined by SLEDAI and a positive correlation between MPA AUC and C3 levels.[3] ROC curve analysis revealed the same value using either SLEDAI or BILAG to measure disease activity.[3] This link between SLE activity and MPA PK parameters has been confirmed in many other studies using various tools to assess MPA exposure.[4] However, Professor Amoura acknowledged that currently there is no prospective trial in SLE demonstrating that dosing based on target MPA AUC is better than a fixed dose. The ongoing MYCADO trial in France aims to answer this question. In the interim, Professor Amoura recommended

that adequate MPA exposure is assessed in patients: before MMF dose escalation, before therapy escalation, when there is a low blood albuminaemia levels and in cases of digestive side effects.[5]

Moving on to hydroxychloroquine (HCQ), another drug given at a fixed dose in SLE, Professor Amoura again stressed the strong between-patient variability in blood concentrations. The PLUS study showed that SLE disease activity was higher in patients with low blood HCQ level and that HCQ levels <1000 ng/mL were associated with disease flares.[6] However, this study failed to demonstrate that dosing of HCQ to target could reduce flare.[6] Another drug commonly used in SLE is azathioprine. Azathioprine is a prodrug converted via 6MP to 6-thioguanine (6-TG) nucleotides which are responsible for the drug's cytotoxic and immunosuppressive effects.[7] 6-TG levels are routinely measured in patients with inflammatory bowel disease treated with azathioprine but not in SLE.

Moving on to the final part of this presentation, Professor Amoura highlighted the second key reason why it is important to assess medication exposure in SLE – adherence. Adherence to medication is known to be worse in chronic *versus* acute conditions. Because survival in SLE has improved over the last 5 decades, it has now evolved to become a chronic disease – making it even more important to address issues of adherence.[8] The reported rates of non-adherence in SLE range from 3-76% (depending on definition, methods and drug studied) and non-adherence is associated with a higher risk of flares, morbidity, hospitalisation and poor renal outcomes. HCQ concentration is increasingly used as an objective marker of poor adherence in SLE, with levels <200 ng/mL indicating a non-adherent patient. This tool, validated in clinical studies, can help identify patients who clinicians do not suspect as non-adherent and can also lead to improved adherence via the use of serial monitoring and medical counselling.[9] In SLE flare, HCQ is helpful to distinguish flare due to lack of response from poor adherence. Although no objective markers exist, simple tools are also available for assessing exposure/adherence to other SLE drugs: steroids – increased neutrophil count, decreased basophil/lymphocyte counts or Cushing-like appearance; azathioprine – macrocytosis; oral anticoagulants – international normalised ratio.

Figure: Pharmacokinetic parameters of the main drugs used in SLE

	Active metabolite	Plasmatic half-life	Exposure assessment	Adherence Assessment
Prednisone	Prednisolone	2–4 hours		
Hydroxychloroquine	HCQ	5–40 hours	YES	YES
Methotrexate	Methotrexate	2 hours		
Azathioprine	6-mercaptopurine	30–60 minutes	+/-	
Mycophenolate Mofetil	Mycophenolic Acid	8–16 hours	YES	
Cyclophosphamide	4OH-Cyclophosphamide	4–7 hours		

References

1. van Gelder T, Berden JH, Berger SP**.** To TDM or not to TDM in lupus nephritis patients treated with MMF? *Nephrol Dial Transplant*. 2015;30(4):560-4.
2. Z A**.** , *et al*. Unpublished data.

3. Zahr N, Arnaud L, Marquet P*, et al.* Mycophenolic acid area under the curve correlates with disease activity in lupus patients treated with mycophenolate mofetil. *Arthritis Rheum*. 2010;62(7):2047-54.
4. Croyle L, Morand EF**.** Optimizing the use of existing therapies in lupus. *Int J Rheum Dis*. 2015;18(2):129-37.
5. Arnaud L, Zahr N, Costedoat-Chalumeau N, Amoura Z**.** The importance of assessing medication exposure to the definition of refractory disease in systemic lupus erythematosus. *Autoimmun Rev*. 2011;10(11):674-8.
6. Costedoat-Chalumeau N, Galicier L, Aumaitre O*, et al.* Hydroxychloroquine in systemic lupus erythematosus: results of a French multicentre controlled trial (PLUS Study). *Ann Rheum Dis*. 2013;72(11):1786-92.
7. Askanase AD, Wallace DJ, Weisman MH*, et al.* Use of pharmacogenetics, enzymatic phenotyping, and metabolite monitoring to guide treatment with azathioprine in patients with systemic lupus erythematosus. *J Rheumatol*. 2009;36(1):89-95.
8. Mak A, Cheung MW, Chiew HJ, Liu Y, Ho RC**.** Global trend of survival and damage of systemic lupus erythematosus: meta-analysis and meta-regression of observational studies from the 1950s to 2000s. *Semin Arthritis Rheum*. 2012;41(6):830-9.
9. Costedoat-Chalumeau N, Amoura Z, Hulot JS*, et al.* Very low blood hydroxychloroquine concentration as an objective marker of poor adherence to treatment of systemic lupus erythematosus. *Ann Rheum Dis*. 2007;66(6):821-4.

Managing fatigue in SLE. David D'Cruz

Professor D'Cruz presented an overview of the clinical associations of fatigue in SLE and discussed the assessment of fatigue with objective score tools and available management strategies for use in clinical practice.

Fatigue is defined as an overwhelming sense of extraordinary tiredness or exhaustion that is not relieved by rest or sleep.[1] It can have both physical and mental manifestations. In SLE, fatigue is the single most common and debilitating daily symptom, with a prevalence ranging from 50-92%.[2-5] Over 50% of SLE patients report suffering with severe fatigue.[2-5] Fatigue is associated with work disability and contributes significantly to reduced health-related quality of life (QoL).[6, 7]

Professor D'Cruz explained that, after systematic review of 15 fatigue scales, the Fatigue Severity Scale was selected as the tool of choice for assessing fatigue in SLE, as recommended by the ACR Ad Hoc Committee.[8] On this scale, a score less than 4 equals normal fatigue and the minimal clinically important difference (MCID) is 0.6 points

Professor D'Cruz reviewed some of the key factors associated with fatigue in SLE. The link between disease activity and fatigue is controversial. There is no apparent correlation between fatigue and SLEDAI but good correlation between fatigue and SLAM.[8] Fatigue is also closely correlated with pain, an important issue in SLE given the reported fibromyalgia prevalence of up to 25%.[8] Although fatigue is closely correlated with depression and mood disorders in lupus patients, the link to sleep disorders in SLE is poorly documented and abnormalities of sleep architecture observed in patients with SLE do not explain the severity of fatigue.[4, 8] Fatigue in SLE is also shown to correlate with physical inactivity, deconditioning and obesity.[9]

Moving on to discuss treatment of fatigue, Professor D'Cruz stressed the importance of treating active lupus disease as well as managing underlying comorbid conditions such as anaemia, hypothyroidism, depression and fibromyalgia/pain. Systematic review of the available evidence has identified nine intervention strategies with the potential to alleviate fatigue in SLE: 1. Psychosocial interventions; 2. Exercise; 3. Diet manipulation; 4. Vitamin D supplementation; 5. N-acetylcysteine; 6. Dehydroepiandrosterone (DHEA). 7. Acupuncture. 8. Ultraviolet-A1 (UVA-1) phototherapy; 9. Belimumab.[2] Of these, aerobic exercise and belimumab seem to have the strongest evidence for treatment efficacy.[2] Pooled analysis of clinical data showed no overall benefit of psychosocial interventions in reducing fatigue. However, there is good clinical evidence to show that regular exercise improves fatigue and does not increase the risk of flares.[10] Problems lie in patients' unwillingness to continue exercise and engage with exercise programmes. No major improvement in fatigue has been demonstrated with vitamin D supplementation and there is no clear supportive evidence for DHEA, although N-acetylcysteine and UVA-1 phototherapy may represent potentially promising approaches. Acupuncture is not recommended for patients with multisystem SLE or those on anticoagulation.

The prevalence of obesity is 28-50% in SLE and shows a clear association with fatigue and sleep disorders, plus is exacerbated by corticosteroid use.[11] However, there is currently only one pilot RCT in SLE showing an improvement in fatigue with weight loss.[12] Professor D'Cruz acknowledged that weight loss is very difficult to achieve while on steroids and recommended that patients, instead, be advised to avoid refined sugars and maintain a low GI diet.

Professor D'Cruz went on to show the strong evidence for the anti-BLyS antibody belimumab in reducing SLE-associated fatigue. Belimumab produced significant improvements in FACTIT-Fatigue

score at 52 weeks *versus* placebo in pooled data from the BLISS trials.[13, 14] Greater improvements were seen in fatigue scores in patients who responded to belimumab therapy compared to those who did not.[13, 14]

Professor D'Cruz concluded his presentation by acknowledging that fatigue is a common issue in SLE but difficult to assess and manage. His advice was to treat the active disease, taking care with steroid use, and manage underlying comorbid conditions. Other management recommendations included an aerobic exercise programme, diet/weight loss and sleep hygiene, together with medication.

References

1. Ahn GE, Ramsey-Goldman R**.** Fatigue in systemic lupus erythematosus. *Int J Clin Rheumtol*. 2012;7(2):217-27.
2. Yuen HK, Cunningham MA**.** Optimal management of fatigue in patients with systemic lupus erythematosus: a systematic review. *Ther Clin Risk Manag*. 2014;10:775-86.
3. Zonana-Nacach A, Barr SG, Magder LS, Petri M**.** Damage in systemic lupus erythematosus and its association with corticosteroids. *Arthritis Rheum*. 2000;43(8):1801-8.
4. Tench CM, McCurdie I, White PD, D'Cruz DP**.** The prevalence and associations of fatigue in systemic lupus erythematosus. *Rheumatology (Oxford)*. 2000;39(11):1249-54.
5. Wysenbeek AJ, Leibovici L, Weinberger A, Guedj D**.** Fatigue in systemic lupus erythematosus. Prevalence and relation to disease expression. *Br J Rheumatol*. 1993;32(7):633-5.
6. Baker K, Pope J**.** Employment and work disability in systemic lupus erythematosus: a systematic review. *Rheumatology (Oxford)*. 2009;48(3):281-4.
7. McElhone K, Abbott J, Teh LS**.** A review of health related quality of life in systemic lupus erythematosus. *Lupus*. 2006;15(10):633-43.
8. Ad Hoc Committee on Systemic Lupus Erythematosus Response Criteria for F**.** Measurement of fatigue in systemic lupus erythematosus: a systematic review. *Arthritis Rheum*. 2007;57(8):1348-57.
9. Tench C, Bentley D, Vleck V*, et al.* Aerobic fitness, fatigue, and physical disability in systemic lupus erythematosus. *J Rheumatol*. 2002;29(3):474-81.
10. Tench CM, McCarthy J, McCurdie I, White PD, D'Cruz DP**.** Fatigue in systemic lupus erythematosus: a randomized controlled trial of exercise. *Rheumatology (Oxford)*. 2003;42(9):1050-4.
11. Katz P, Gregorich S, Yazdany J*, et al.* Obesity and its measurement in a community-based sample of women with systemic lupus erythematosus. *Arthritis Care Res (Hoboken)*. 2011;63(2):261-8.
12. Davies RJ, Lomer MC, Yeo SI*, et al.* Weight loss and improvements in fatigue in systemic lupus erythematosus: a controlled trial of a low glycaemic index diet *versus* a calorie restricted diet in patients treated with corticosteroids. *Lupus*. 2012;21(6):649-55.
13. Strand V, Levy RA, Cervera R*, et al.* Improvements in health-related quality of life with belimumab, a B-lymphocyte stimulator-specific inhibitor, in patients with autoantibody-positive systemic lupus erythematosus from the randomised controlled BLISS trials. *Ann Rheum Dis*. 2014;73(5):838-44.
14. Furie R, Petri MA, Strand V*, et al.* Clinical, laboratory and health-related quality of life correlates of Systemic Lupus Erythematosus Responder Index response: a post hoc analysis of the phase 3 belimumab trials. *Lupus Sci Med*. 2014;1(1):e000031.

Using lower doses of glucocorticoids in SLE: less toxicity, same efficacy. Guillermo Ruiz-Irastorza

Professor Ruiz-Irastorza discussed whether glucocorticoids, one of the cornerstones of SLE therapy, can be used at lower doses to minimise toxicity while retaining their anti-inflammatory activity.

Prof Ruiz-Irastorza began by reviewing the mechanism of action of glucocorticoids which involves both genomic and non-genomic pathways, the former being associated with most of the unwanted side effects of treatment.[1] Dose determines which of these pathways is activated. Maximum side effects are elicited at doses over 30 mg/day when the genomic pathway reaches 100% receptor saturation. In contrast, the non-genomic pathway is not activated until doses exceed 100 mg/day.[1]

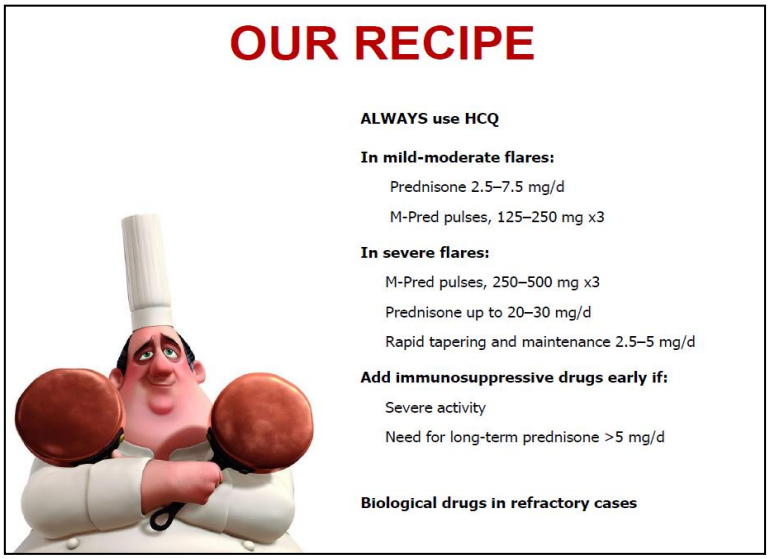
In clinical practice, patients are typically treated for a short period of time with high doses of systemic glucocorticoids – eg, iv pulses of methyl-prednisolone 0.5-1 g/day for 3 days or 1-2 mg/kg/day. However, this preferred starting dose of 1 mg/kg/day appears to be an historical habit rather than evidence-based. Certain damage manifestations of SLE are linked to higher doses of glucocorticoids, as corroborated by the clinical data.[2-4] It has been consistently shown that glucocorticoid-related damage is dose-dependent, with doses below 5–7.5 mg/d appearing largely safe, while doses above 30 mg/d are associated with a substantial increase in the frequency of side effects.[2-4] However, the use of pulses of methyl-prednisolone does not appear contribute to this damage accumulation.[4]

Prof Ruiz-Irastorza pointed out that, although the aim in SLE management is to reduce/taper steroid doses where possible, this does not always happen in clinical practice. In reality, the dose of prednisone given to a patient in the first month appears to be strongly predictive of subsequent dosing practices, and even maintenance doses (eg, 10 mg/day) may still remain in the threshold where damage can occur.[5]

Prof Ruiz-Irastorza then posed the question of whether patients really need to pay the price of steroid side effects to achieve good disease control – or is there another way? Data exists, from observational studies and a small clinical trial, supporting the idea that lower doses of oral prednisone can be as effective as high doses in treating active lupus and renal disease.[6-8] In the clinical trial, 81 lupus nephritis patients were treated with two different glucocorticoid regimens – standard and reduced dose – in combination with MMF. Rates of remission and decrease in disease activity were comparable between the two groups, with the benefit of a reduced infection risk in the lower dose steroid group.[6]

Prof Ruiz-Irastorza explained that he has evaluated lower dose prednisone regimens in his own lupus clinic, in patients with and without nephritis, with favourable results. In fact, the lower dose steroid regimen produced better outcomes in terms of the proportion of patients achieving complete remission, coupled with a lower risk of toxicity.[8, 9] This effect was attributed to the more optimal use of pharmacotherapy, in conjunction with the lower-dose steroid schedules. Prof Ruiz-Irastorza described his optimal 'recipe' for reducing steroid doses based on his own single-centre experience. Combination therapy with hydroxychloroquine, immunosuppressive drugs and, especially, pulses of methylprednisolone (at doses not exceeding 250 mg) can help reduce the initial doses of prednisone to less than 30 mg/d with very rapid tapering, and offer high efficacy while minimising both short- and long-term secondary effects. Long-term follow-up of patients treated with this new steroid 'recipe' showed lower glucocorticoid-related damage, less cardiovascular damage and no more SLE-related damage compared to historical controls.

Figure: Proposed 'recipe' for lowering steroid doses in SLE



References

1. Buttgereit F, Straub RH, Wehling M, Burmester GR**.** Glucocorticoids in the treatment of rheumatic diseases: an update on the mechanisms of action. *Arthritis Rheum*. 2004;50(11):3408-17.
2. Zonana-Nacach A, Barr SG, Magder LS, Petri M**.** Damage in systemic lupus erythematosus and its association with corticosteroids. *Arthritis Rheum*. 2000;43(8):1801-8.
3. Petri M, Purvey S, Fang H, Magder LS**.** Predictors of organ damage in systemic lupus erythematosus: the Hopkins Lupus Cohort. *Arthritis Rheum*. 2012;64(12):4021-8.
4. Ruiz-Arruza I, Ugarte A, Cabezas-Rodriguez I*, et al.* Glucocorticoids and irreversible damage in patients with systemic lupus erythematosus. *Rheumatology (Oxford)*. 2014;53(8):1470-6.
5. Ruiz-Irastorza G**.** *et al*. Lupus Sci Med 2016 (in press).
6. Zeher M, Doria A, Lan J*, et al.* Efficacy and safety of enteric-coated mycophenolate sodium in combination with two glucocorticoid regimens for the treatment of active lupus nephritis. *Lupus*. 2011;20(14):1484-93.
7. Fischer-Betz R, Chehab G, Sander O*, et al.* Renal outcome in patients with lupus nephritis using a steroid-free regimen of monthly intravenous cyclophosphamide: a prospective observational study. *J Rheumatol*. 2012;39(11):2111-7.
8. Ruiz-Irastorza G, Danza A, Perales I*, et al.* Prednisone in lupus nephritis: how much is enough? *Autoimmun Rev*. 2014;13(2):206-14.
9. Ruiz-Arruza I, Barbosa C, Ugarte A, Ruiz-Irastorza G**.** Comparison of high *versus* low-medium prednisone doses for the treatment of systemic lupus erythematosus patients with high activity at diagnosis. *Autoimmun Rev*. 2015;14(10):875-9.

Prevention of accelerated atherosclerosis in SLE: Should everyone be on a statin. Bevra H. Hahn

Professor Hahn described the key features of SLE that can contribute to atherosclerosis development. She went on to review the current status of statin studies in the lupus population and discussed therapeutic options for the prevention of atherosclerosis – including statins and their potential alternatives.

Professor Hahn set the scene for her presentation by highlighting the fact that one third of SLE patients have atherosclerosis as evidenced by surrogate markers such as carotid artery plaque, coronary calcification and myocardial perfusion studies.[1-4] Atherosclerotic deaths also directly account for the worse survival following renal transplant in SLE patients *versus* controls.[5]

Professor Hahn described the multiple inflammatory, oxidative and immune factors that predispose patients to atherosclerosis in SLE. Factors predictive of an individual SLE patient's risk of cardiovascular events (CVE) include traditional Framington parameters – age, male sex, hypertension, diabetes, smoking and hyperlipidaemia.[6, 7] Factors specific to SLE can also predispose to CVE such as sedentary lifestyle, increased waist-to-hip ratio, glucocorticoids, high hsCRP, high disease activity and antibodies to phospholipids and dsDNA.[6, 7]Recent data have shown that a combination of multiple factors – including age ≥48 years, high pro-inflammatory HDL, high leptin, high soluble TWEAK and high homocysteine – are better at predicting carotid plaque formation in SLE patients than just a single biomarker.[7]

Professor Hahn explained that acute inflammation can affect serum lipid levels hence it is important to assess lipids in SLE when the disease is controlled and therapy relative stable. She went on to review therapeutic considerations in the prevention of atherosclerosis in SLE. Antimalarials (eg, hydroxychloroquine) have been shown to reduce thrombotic events, overall damage and lipid and glucose abnormalities, hence are used by 70% of Dutch SLE patients.[8-11] However, potential adverse events must also be considered. The evidence for statins in reducing atherosclerosis risk in SLE is mixed. Several studies have demonstrated no difference in surrogate markers of atherosclerosis in SLE patients treated with stains however post hoc analysis of a trial of pubertal children with high hsCRP did reveal significant benefit.[12-15] Similarly, a recent study from Taiwan indicated that statin use for more than 1 year reduced mortality in SLE patients with hyperlipidaemia.[16] Fluvastatin has also been shown to reduce cardiac events in SLE patients undergoing renal transplantation.[17]

Despite any potential benefits in atherosclerosis risk reduction, Professor Hahn cautioned that statins do not reduce disease activity in SLE and may be associated with adverse events including myopathy and transaminitis – although none of these are increased in SLE patients. The drug interactions between particular statins and CYP3A4 inhibitors/enhancers must also be born in mind in the context of overall SLE management.

In conclusion, Professor Hahn recommended that for atherosclerosis risk reduction in SLE it is important to control disease activity and treat most patients with hydroxychloroquine, maintaining caution for potential retinal damage after 5 years of usage. Lipid levels should be measured when disease activity is as low as possible and therapy is stable. Statins are then indicated if LDL levels are ≥130 or total cholesterol is >200 where SLE is the only risk factor and if LDL is ≥100 or TC >150 where the patient has SLE plus one other risk factor. Statins should also be prescribed for patients with hsCRP above 1.5. In addition to appropriate statin recommendations, it is also important to control patients'

hypertension, diabetes, proteinuria and increased BMI in order to minimise overall atherosclerosis risk.

References

1. Asanuma Y, Oeser A, Shintani AK*, et al.* Premature coronary-artery atherosclerosis in systemic lupus erythematosus. *N Engl J Med*. 2003;349(25):2407-15.
2. Nikpour M, Urowitz MB, Gladman DD**.** Epidemiology of atherosclerosis in systemic lupus erythematosus. *Curr Rheumatol Rep*. 2009;11(4):248-54.
3. Roman MJ, Moeller E, Davis A*, et al.* Preclinical carotid atherosclerosis in patients with rheumatoid arthritis. *Ann Intern Med*. 2006;144(4):249-56.
4. Roman MJ, Shanker BA, Davis A*, et al.* Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. *N Engl J Med*. 2003;349(25):2399-406.
5. Norby GE, Leivestad T, Mjoen G*, et al.* Premature cardiovascular disease in patients with systemic lupus erythematosus influences survival after renal transplantation. *Arthritis Rheum*. 2011;63(3):733-7.
6. Kavanaugh A, Adams-Huet B, Jain R, Denke M, McFarlin J**.** Hydroxychloroquine Effects on Lipoprotein Profiles (the HELP trial): A Double-Blind, Randomized, Placebo-Controlled, Pilot Study In Patients With Systemic Lupus Erythematosus. *J Clin Rheumatol*. 1997;3(1):3-8.
7. McMahon M, Skaggs BJ, Grossman JM*, et al.* A panel of biomarkers is associated with increased risk of the presence and progression of atherosclerosis in women with systemic lupus erythematosus. *Arthritis Rheumatol*. 2014;66(1):130-9.
8. McMahon M, Skaggs B**.** Pathogenesis and treatment of atherosclerosis in lupus. *Rheum Dis Clin North Am*. 2014;40(3):475-95, viii.
9. Tsang ASMW, Bultink IE, Voskuyl AE**.** Long-term evaluation of antimalarials in a Dutch SLE cohort: intolerance and other reasons for non-use. *Clin Exp Rheumatol*. 2014;32(1):95-100.
10. Chen YM, Lin CH, Lan TH*, et al.* Hydroxychloroquine reduces risk of incident diabetes mellitus in lupus patients in a dose-dependent manner: a population-based cohort study. *Rheumatology (Oxford)*. 2015;54(7):1244-9.
11. Durcan L, Clarke WA, Magder LS, Petri M**.** Hydroxychloroquine Blood Levels in Systemic Lupus Erythematosus: Clarifying Dosing Controversies and Improving Adherence. *J Rheumatol*. 2015;42(11):2092-7.
12. Mok CC, Wong CK, To CH, Lai JP, Lam CS**.** Effects of rosuvastatin on vascular biomarkers and carotid atherosclerosis in lupus: a randomized, double-blind, placebo-controlled trial. *Arthritis Care Res (Hoboken)*. 2011;63(6):875-83.
13. Petri MA, Kiani AN, Post W, Christopher-Stine L, Magder LS**.** Lupus Atherosclerosis Prevention Study (LAPS). *Ann Rheum Dis*. 2011;70(5):760-5.
14. Plazak W, Gryga K, Dziedzic H*, et al.* Influence of atorvastatin on coronary calcifications and myocardial perfusion defects in systemic lupus erythematosus patients: a prospective, randomized, double-masked, placebo-controlled study. *Arthritis Res Ther*. 2011;13(4):R117.
15. Schanberg LE, Sandborg C, Barnhart HX*, et al.* Use of atorvastatin in systemic lupus erythematosus in children and adolescents. *Arthritis Rheum*. 2012;64(1):285-96.
16. Yu HH, Chen PC, Yang YH*, et al.* Statin reduces mortality and morbidity in systemic lupus erythematosus patients with hyperlipidemia: A nationwide population-based cohort study. *Atherosclerosis*. 2015;243(1):11-8.
17. Norby GE, Holme I, Fellstrom B*, et al.* Effect of fluvastatin on cardiac outcomes in kidney transplant patients with systemic lupus erythematosus: a randomized placebo-controlled study. *Arthritis Rheum*. 2009;60(4):1060-4.

Plenary III: Management of SLE-Therapies Derived From Other Specialities

Bortezomib in SLE. Reinhard Voll

Professor Voll discussed the therapeutic potential of the proteasome inhibitor bortezomib in SLE based on the role of long-lived plasma cells in refractory disease and their reliance on the ubiquitin-proteasome system for survival.

Professor Voll began by outlining autoantibody-mediated mechanisms in the pathogenesis of SLE. T-cells, B-cells and innate immune cells all contribute to the pathogenesis of lupus and pathogenic antibodies are crucial for many disease manifestations. Long-lived plasma cells provide the body with lifelong protection from re-infection but are not targeted by standard lupus therapies hence can cause refractory disease cases in SLE. These cells survive long-term because the plasma cell niche supplies strong survival signals which induce NF-kappaβ and anti-apoptotic factors.

Professor Voll posed the question of whether long-lived memory plasma cells have an Achilles heel, which can be targeted therapeutically. The answer lies in the ubiquitin proteasome system which is blocked by a class of drugs known as proteasome inhibitors. As a result of their extremely high production of antibodies within the endoplasmic reticulum (ER), plasma cells are highly sensitive to proteasome inhibition, which blocks the degradation of misfolded proteins, thereby inducing ER stress and the terminal unfolded proteins response leading to apoptotic cell death.[1]

The proteasome inhibitor bortezomib, approved in the treatment of multiple myeloma and mantle cell lymphoma, specifically and reversibly inhibits the chymotrypsin-like activity of the 26S proteasome, thereby blocking the degradation of misfolded, defective, supernumerous and regulatory proteins. It is cytotoxic to a variety of cancer cells *in vitro* and *in vivo*. In the New Zealand Black (NZB)/W FI mouse model, bortezomib proved able to deplete both short- and long-lived plasma cells, thereby reducing murine lupus nephritis.[2] The survival of mice treated with preventative bortezomib therapy was dramatically increased compared to control animals. Bortezomib also proved able to ameliorate established disease as evidenced by an improvement in proteinuria in mice with advanced disease treated with the proteasome inhibitor.

Moving on to address whether bortezomib's *in vivo* activity translates into a therapeutic effect in patients with SLE, Professor Voll presented a case series examining the outcomes of 15 patients with SLE who had not sufficiently responded to standard therapy and were treated with bortezomib (1.3 mg/m^2 iv in combination with dexamethasone).[3] SLEDAI disease activity scores and anti-dsDNA antibody titres decreased with bortezomib. In all patients with active lupus nephritis, proteinuria also declined within 6 weeks after start of bortezomib treatment. Total IgG concentrations decreased in most patients by approximately 25%, however largely remained within normal limits. All adverse events were mild or moderate. These results indicate that bortezomib is able to induce a rapid decrease in disease activity with modest adverse events in refractory SLE patients due to preferential elimination of plasma cells, including long-lived plasma cells, through proteasome inhibition. Bortezomib also restores response to regular treatment options for SLE. A placebo-controlled, multicentre clinical trial with the orally bioavailable proteasome inhibitor ixazomib is currently ongoing in refractory lupus nephritis patients.

Professor Voll concluded that proteasome inhibition may represent a new option for induction therapy in antibody-mediated disease like SLE.

References

1. Meister S, Schubert U, Neubert K*, et al.* Extensive immunoglobulin production sensitizes myeloma cells for proteasome inhibition. *Cancer Res*. 2007;67(4):1783-92.
2. Neubert K, Meister S, Moser K*, et al.* The proteasome inhibitor bortezomib depletes plasma cells and protects mice with lupus-like disease from nephritis. *Nat Med*. 2008;14(7):748-55.
3. Alexander T, Sarfert R, Klotsche J*, et al.* The proteasome inhibitior bortezomib depletes plasma cells and ameliorates clinical manifestations of refractory systemic lupus erythematosus. *Ann Rheum Dis*. 2015;74(7):1474-8.

Eculizumab in SLE. Josep M. Campistol

Professor Campistol gave an overview of the potential therapeutic benefits of the humanised monoclonal antibody eculizumab in SLE, focussing on its promising role in managing thrombotic microangiopathy in severe forms of SLE.

Eculizumab is a first-in-class humanised monoclonal antibody (mAb) which targets factor V (C5) of the complement system. The mAb binds with high affinity to C5 thereby blocking terminal complement complex formation. Eculizumab is the first and only approved therapy for patients with paroxysmal nocturnal haemoglobinuria (PNH) and atypical uraemic haemolytic syndrome (aHUS). Compelling evidence from clinical trials supports the long-term clinical benefits that can be achieved with eculizumab in patients with PNH and aHUS. Eculizumab significantly improved survival in PNH patients and demonstrated rapid and sustained improvements in haematological parameters, and continued, on-going improvement in renal function, in adult patients with aHUS.[1, 2]

Professor Campistol discussed the therapeutic potential of targeting the complement system in SLE, based on a sound preclinical rationale for its role in the immunopathogenesis of lupus. Thrombotic microangiopathy (TMA) is a disorder characterised by an acute syndrome of microangiopathic haemolytic anaemia, thrombocytopenia and variable signs of organ injury (particularly affecting the kidney) due to platelet thrombosis in the microcirculation.[3] Professor Campistol reviewed the causes and pathogenesis of TMA, its etiological classification and differential diagnosis. In severe cases of SLE, TMA may develop due to endothelial injury and antibodies capable of activating the complement system develop in severe forms of TMA. Chronic uncontrolled complement activation leads to clinical consequences such as blood clotting, platelet consumption, mechanical haemolysis, vessel occlusion, inflammation and ischaemia, resulting ultimately in systemic organ complications and end-organ damage.[4, 5]

Patients with SLE have a high risk of TMA, which may be associated with auto-antibodies and/or APA and/or severe nephropathy. TMA in lupus nephritis has become a hallmark for the presence of antiphospholipid antibodies. It can occur in any class of lupus nephritis but should not be confused with intracapillary coagula of immunoglobulins. TMA in lupus nephritis is associated with severe renal involvement. Professor Campistol stressed that renal biopsy is always necessary to confirm the differential diagnosis. Preliminary data from case reports have shown promising results with the use of eculizumab to treat TMA in SLE.[6] In these cases, therapy with eculizumab may be helpful in reversing the TMA and avoiding injury or reducing the rate of underlying pathological processes.

TTP can be also present in patients with SLE, although there are clear clinical differences between idiopathic TTP and TTP associated with SLE including the role of the von Willebrand factor multimer protease (ADMATS13).[7] Initial treatment for TTP should always be plasma exchange, but eculizumab may be considered in severe and/or resistant cases.

Professor Campistol concluded that further studies would be needed to analyse the true impact and potential of eculizumab in the management of SLE.

References

1. Hill A, Kelly J, Kulasekararaj A*, et al.* Eculizumab in Paroxysmal Nocturnal Hemoglobinuria (PNH): A Report of All 153 Patients Treated in the UK. 54th ASH Annual Meeting and Exposition. Atlanta, USA; 2012.

2. Legendre CM, Licht C, Muus P*, et al.* Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. *N Engl J Med*. 2013;368(23):2169-81.
3. Benz K, Amann K**.** Thrombotic microangiopathy: new insights. *Curr Opin Nephrol Hypertens*. 2010;19(3):242-7.
4. Licht C, Pluthero FG, Li L*, et al.* Platelet-associated complement factor H in healthy persons and patients with atypical HUS. *Blood*. 2009;114(20):4538-45.
5. Noris M, Remuzzi G**.** Atypical hemolytic-uremic syndrome. *N Engl J Med*. 2009;361(17):1676-87.
6. El-Husseini A, Hannan S, Awad A*, et al.* Thrombotic microangiopathy in systemic lupus erythematosus: efficacy of eculizumab. *Am J Kidney Dis*. 2015;65(1):127-30.
7. Lansigan F, Isufi I, Tagoe CE**.** Microangiopathic haemolytic anaemia resembling thrombotic thrombocytopenic purpura in systemic lupus erythematosus: the role of ADAMTS13. *Rheumatology (Oxford)*. 2011;50(5):824-9.

Romiplostim and eltrombopag for idiopathic thrombocytopenic purpura. Sacha Zeerleder

Dr Zeerleder gave an overview of the pathogenesis of idiopathic thrombocytopenic purpura (ITP), indications to start treatment and current therapeutic options, including the potential role for the thrombopoietin receptor agonists romiplostim and eltrombopag.

ITP is an acquired immune-mediated condition characterised by isolated platelet counts <100 x 10^9/L. It can be primary (i.e. idiopathic) or secondary due to lymphoproliferative disease, autoimmune disease, drugs or hepatitis C infection.[1] ITP is caused by antiplatelet autoantibodies directed against dominant epitopes on platelet antigens which leads to both increased platelet destruction and decreased platelet production. However, Dr Zeerleder stressed that autoantibodies to platelets are not the only 'bad guys' in ITP pathogenesis. Evidence is emerging for the role of specific cytotoxic cells to thrombocytes/megakaryocytes and complement activation via the classical and/or alternative pathway in ITP.[1-3] Up to 59% of ITP patients show evidence of complement activation which results in complement deposition on T cells. Dr Zeerleder explained that current thinking on the pathogenesis of ITP now involves a combination of disturbed cellular immunity, molecular mimicry, inadequate platelet production *and* autoantibodies.

Dr Zeerleder went on to discuss the treatment of ITP, where the goal of therapy should be to increase platelet count to a safe value – not necessarily to achieve normalisation of platelet counts. Steroids are a first-line therapy for ITP, with splenectomy often employed in case of steroid refractoriness. However, clinicians should carefully consider whether there is a true indication for 2nd line therapy and not be impatient to advance treatment beyond first-line steroids. Indications for second-line treatment were listed as:[1]

- Life-threatening bleeding
- Total platelet count <30 x 10^9/
- Platelet count between 30–50 x 109/L and additional risk factors

However, Dr Zeeleder cautioned that less than 15% of patients in the third category will develop a more severe thrombocytopenia and that the risk for major bleeding is low in the absence of aggravating factors. A new category of persistent ITP is also now recognised, distinct from steroid-refractory ITP, which covers the period extending between 3 and 12 months from diagnosis.[1] Although splenectomy provides a high frequency of durable responses for adult patients with ITP, the risk of surgery remains an important consideration.[4] Other options, and alternatives to splenectomy for second-line ITP therapy, include anti-CD20 antibodies such as rituximab and the newer class of agents – thrombopoietin receptor agonists (TPA-RAs).

Dr Zeerleder reviewed the current evidence base for the TPA-RAs, romiplostim and eltrombopag, in second-line ITP. TPO-RAs have demonstrated good efficacy in ITP, producing high response rates (with complete response rates of up to 90%), increased platelet counts and reduced rescue medication requirements.[5-7] TPO-RAs also showed a good short-term safety profile.[7-9] Bone marrow fibrosis was mild-to-moderate and reversible, there has been no evidence of clonal evolutions thus far and the rate of thromboembolic events was only ~5%.[10] The potential disadvantages of TPO-RAs are that the drugs themselves are expensive and continuous treatment is required as stopping therapy results largely in resurgence of ITP. Future generations of TPO-RAs are currently undergoing clinical evaluation in ITP.[11]

References

1. Rodeghiero F, Stasi R, Gernsheimer T*, et al.* Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood*. 2009;113(11):2386-93.
2. Olsson B, Andersson PO, Jernas M*, et al.* T-cell-mediated cytotoxicity toward platelets in chronic idiopathic thrombocytopenic purpura. *Nat Med*. 2003;9(9):1123-4.
3. Najaoui A, Bakchoul T, Stoy J*, et al.* Autoantibody-mediated complement activation on platelets is a common finding in patients with immune thrombocytopenic purpura (ITP). *Eur J Haematol*. 2012;88(2):167-74.
4. Kojouri K, Vesely SK, Terrell DR, George JN**.** Splenectomy for adult patients with idiopathic thrombocytopenic purpura: a systematic review to assess long-term platelet count responses, prediction of response, and surgical complications. *Blood*. 2004;104(9):2623-34.
5. Bussel JB, Cheng G, Saleh MN*, et al.* Eltrombopag for the treatment of chronic idiopathic thrombocytopenic purpura. *N Engl J Med*. 2007;357(22):2237-47.
6. Bussel JB, Provan D, Shamsi T*, et al.* Effect of eltrombopag on platelet counts and bleeding during treatment of chronic idiopathic thrombocytopenic purpura: a randomised, double-blind, placebo-controlled trial. *Lancet*. 2009;373(9664):641-8.
7. Kuter DJ, Bussel JB, Lyons RM*, et al.* Efficacy of romiplostim in patients with chronic immune thrombocytopenic purpura: a double-blind randomised controlled trial. *Lancet*. 2008;371(9610):395-403.
8. Saleh MN, Bussel JB, Cheng G*, et al.* Safety and efficacy of eltrombopag for treatment of chronic immune thrombocytopenia: results of the long-term, open-label EXTEND study. *Blood*. 2013;121(3):537-45.
9. Kuter DJ, Bussel JB, Newland A*, et al.* Long-term treatment with romiplostim in patients with chronic immune thrombocytopenia: safety and efficacy. *Br J Haematol*. 2013;161(3):411-23.
10. Ghanima W, Geyer JT, Lee CS*, et al.* Bone marrow fibrosis in 66 patients with immune thrombocytopenia treated with thrombopoietin-receptor agonists: a single-center, long-term follow-up. *Haematologica*. 2014;99(5):937-44.
11. Bussel JB, Kuter DJ, Aledort LM*, et al.* A randomized trial of avatrombopag, an investigational thrombopoietin-receptor agonist, in persistent and chronic immune thrombocytopenia. *Blood*. 2014;123(25):3887-94.

Plenary IV: Designing Clinical Trials in SLE

Lessons learned from SLE clinical trials. Richard A. Furie

Professor Furie reviewed the key lessons learned from clinical trials in SLE. His presentation covered existing unmet needs relevant to the treatment of lupus patients, issues related to clinical trial design and strategies for drug development in lupus, and a review of clinical studies currently underway in this disease area.

Professor Furie explained the driving forces behind ongoing drug development in lupus which include the need for more effective therapies for lupus nephritis, severe extra-renal lupus, flare prevention and remission induction, as well as the need for safer therapies to replace steroids and cyclophosphamide. He then went to review each of the key challenges associated with SLE clinical trials in turn.

Ensure clinical activity represents active SLE

Professor Furie used the Phase 2 study of belimumab in SLE, where neither co-primary endpoint was reached, to highlight this point.[1] In this trial, 28% of the study population was serologically inactive and post-hoc analysis demonstrated clinical efficacy in serologically-active patients (72% of the original cohort) The rules for entry were therefore changed for Phase 3 and seropositivity was listed as a requirement at the screening stage. To ensure clinical activity represents active and potentially reversible lupus, Professor Furie proposed the following solutions to prevent contamination of a cohort by subjective and/or high-weighted items on a disease activity instrument: 1) require serologic "confirmation" with ANA or DNA, 2) use an adjudication committee and/or photographs, 3) employ joint counts, 4) eliminate certain SS items from the entry criteria such as lupus headache, and 5) demand PD marker like interferon signature. Specifically for lupus nephritis, potential solutions include requirements for proteinuria, serological activity (as evidenced by low C- and/or high DNA Ab), class III/IV lesions biopsied within the last 3-14 months and active urinary sediment.

Design: Sample size, effect size and statistical significance

Professor Furie used a comparison of the belimumab BLISS-76 trial and the LUNAR study of rituximab to illustrate this issue. The former was deemed a success and the latter a failure despite a numerically greater difference in placebo *versus* treatment response in the LUNAR study.[2, 3] To overcome issues with assumptions and sample size determination, Professor Furie suggested reducing the sample size to enrich for patients that can inform (eg, those with higher SLEDAI scores), using innovative trial designs and limiting the number of treatment arms. It is also important to be knowledgeable about potential placebo responses. The Lupus Foundation of America Collective Data Analysis Initiative (LFA CDAI) is collecting, pooling and analysing placebo data from clinical trials in an effort to overcome current trial design challenges in SLE.

Endpoints

Professor Furie used the EXPLORER and abatacept lupus nephritis trials to illustrate the riddle of 'when is a responder not a responder'.[4] He also compared lupus clinical trial endpoints to snowflakes – with no two being truly alike. To overcome these problems, endpoints employed in SLE studies should be

easily measured, valid, reliable, sensitive, clinically relevant, easily understood and able to discriminate treatment from comparator. Standardisation of endpoints is also the ideal – e.g. ACR20 and DAS scores as used in rheumatoid arthritis.

Background therapies work (steroids, immunosuppressives)

The use of background therapies in clinical SLE trials is a key issue as both steroids and immunosuppressive are effective, approved therapies hence can significantly alter trial outcomes. With steroids, it is important to consider maximal dose at entry and during the study, whether doses will be tapered or fixed, and if dose boosts to manage flares are allowed and to what extent. Similar questions exist for immunosuppressives – are they required as part of the trial design and will they be withdrawn as the study continues, tapers or continued? Looking at the design of clinical trials for newer therapies in SLE reveals a marked difference in the use of, and dosing protocols for, background therapies.

Predictors of response

Analysis of change in SRI over 52 weeks in patients with high-disease activity (HDA) *versus* the pooled population in belimumab clinical studies revealed an interesting finding. The placebo group in the HDA group had a lower response rate than that of the pooled group, making the difference between treatment and placebo 19.8% in the HDA group compared to 11.8% in the pooled group.[5-7] In order to maximise response outcomes and placebo *versus* treatment comparisons, current clinical trials of belimumab, blisibimod, epratuzumab and anifrolumab in SLE are therefore all seeking to recruit high disease activity patients. The practical and logistical issue is where all these highly active patients will come from.

Regional safety differences

Differences in regional safety practices at different trial sites can influence the rate of deaths and opportunistic infections seen in SLE studies. For example, in the ALMS and BELONG studies, deaths and opportunistic infections occurred at a substantially higher frequency in Asia as compared to Latin America, North America and Europe.[8, 9]

The tortoise and hare dilemma

Professor Furie considered the relative advantages and disadvantages of short-shot *versus* long-shot drug development strategies. Short-shot involves the traditional clinical development progression from Phase 2 to 3 (eg, as used with belimumab, DV1179 and sirukumab), however, long-shot involves testing a drug (or similar) already approved for another indication (eg, tabalumab) and moving straight to Phase III evaluation. Lessons learned so far from SLE clinical trials indicate that short-shot is more logical and less costly, with options to modify (or terminate) the program at any time. However, the long-shot is more appealing when developing an approved therapy (or one similar to an approved therapy) and when feeling pressure from competition.

More is less or less is more

Professor Furie outlined the lessons learned about dose response in SLE clinical trials. It is important to distinguish an "inverse dose relationship" from a "lack of dose response". An inverse dose relationship is always a worry and may be related to design issues, such as an imbalance in baseline characteristics (improper stratification) or a small sample size. If the biology is real, dosing patients will present issues. Lack of dose response may also be due to a ceiling effect achieved with low dose drug.

Professor Furie concluded his presentation by summarising the key insights gained from trials of new biologic therapies in SLE, noting that – above all – it is important to remain humble, logical and perseverant.

References

1. Wallace DJ, Stohl W, Furie RA*, et al.* A phase II, randomized, double-blind, placebo-controlled, dose-ranging study of belimumab in patients with active systemic lupus erythematosus. *Arthritis Rheum*. 2009;61(9):1168-78.
2. Furie R, Petri M, Zamani O*, et al.* A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. *Arthritis Rheum*. 2011;63(12):3918-30.
3. Rovin BH, Furie R, Latinis K*, et al.* Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the Lupus Nephritis Assessment with Rituximab study. *Arthritis Rheum*. 2012;64(4):1215-26.
4. Merrill JT, Neuwelt CM, Wallace DJ*, et al.* Efficacy and safety of rituximab in moderately-to-severely active systemic lupus erythematosus: the randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. *Arthritis Rheum*. 2010;62(1):222-33.
5. Benlysta® (belimumab) SmPC. GlaxoSmithKline (UK). March 2012.
6. Cervera R,*et al*. ELM 2011; Porto, Portugal. Abstr O12;3.
7. van Vollenhoven RF, Petri MA, Cervera R*, et al.* Belimumab in the treatment of systemic lupus erythematosus: high disease activity predictors of response. *Ann Rheum Dis*. 2012;71(8):1343-9.
8. Appel GB, Contreras G, Dooley MA*, et al.* Mycophenolate mofetil *versus* cyclophosphamide for induction treatment of lupus nephritis. *J Am Soc Nephrol*. 2009;20(5):1103-12.
9. Mysler EF, Spindler AJ, Guzman R*, et al.* Efficacy and safety of ocrelizumab in active proliferative lupus nephritis: results from a randomized, double-blind, phase III study. *Arthritis Rheum*. 2013;65(9):2368-79.

How different is SLE Applying lessons from other diseases to trials in lupus? Ronald F. van Vollenhoven

Professor van Vollenhoven discussed topics such as measuring disease state and understanding the essentials of outcome characteristics in clinical trials, and considered how aspects of clinical trial design in other diseases – in particular RA – could be applied to the development of better clinical studies in SLE.

Professor van Vollenhoven began by distinguishing the four phases of clinical trials – 1, 2, 3 and 4 - in the regulatory sphere and outlining the key differences between industry- *versus* investigator-initiated clinical studies.

He went on to highlight that, while SLE trials are often fraught with failure, clinical trials in rheumatoid arthritis (RA) have been largely successful – so what are we doing wrong and what lessons can be learned? Initial reports of RA patients successfully treated with anti-TNFs were first disclosed in 1993 and, by 1999, two major Phase 3 studies of etanercept and infliximab had been published providing the basis for regulatory approval.[1-4] Professor van Vollenhoven described this 6 years as a 'record setting pace'. A key ingredient in the success of anti-TNF trials was development of the ACR20 composite outcome in the early 1990s which reduced more than 80 possible RA outcomes to a core set of just seven based on consensus and metric data analysis.[5] Further methodological improvements followed with development of another key outcome used in later RA trials (eg, of adalimumab) – the Van der Heijde Modified Sharp score (SHS).[6] This radiological outcome provided a quantifiable and very sensitive measure of structural effects on cartilage and bone damage, separate from the drugs' clinical effects. For a therapy to be classed as a DMARD in RA it must now show both structural and clinical effects.

Around the same time as ACR20 development, measures of disease state and overall disease activity (as opposed to therapeutic response) were also under development, principally the DAS28. This is a single number which effectively captures and conveys all the underlying disease activity in a patient with RA. The DAS28 is important in clinical trials particularly as it allows cut-offs for desirable goals of therapy, such as remission, to be pre-specified.

Comparative medicine is an important tool for comparing the effectiveness of a treatment directly to that of another treatment for the same disease. For example, the Swefot trial compared methotrexate and the anti-TNF infliximab head-to-head with established triple therapy (methotrexate + sulphasalazine + hydroxychloroquine).[7] This trial, and many other comparative, strategy-based RA studies, have used the DAS28 (or DAS-based outcomes) in preference to ACR20.

Professor van Vollenhoven summarised the key reasons underpinning RA trial success. A major factor is that they employ highly sensitive clinical and radiological outcomes such as ACR20 and SHS, even though this may not necessarily be clinically relevant. They also use accurate descriptors of disease state such as DAS29, SDAI and CDAI which *are* clinically relevant. Last, but not least, the therapies being tested in these trials were highly effective drugs. Comparative highly sensitive clinical and damage outcome measures in SLE are the SLDAI, BILAG, SRI and ECLAM, and SLICC damage index, respectively. Accurate descriptors of lupus disease state also exist in the form of LLDAS and remission (as outlined in the DORIS project).

Looking to the future, successful lupus trials may need to consider different outcomes such as organ-specific, flare, steroid-sparing, LLDAs or remission. These will need to be focused on specific patient populations. The organisation of future lupus trials may also need to change. Rather than including

large numbers of patients to demonstrate efficacy, the focus will be on small, tight groups of clinicians who are closely involved and 'invested' in the trial. After all, small trials with big impact were all that was required to establish the effectiveness of current cornerstone treatments for lupus and lupus nephritis such as cyclophosphamide, hydroxychloroquine and MMF.

References

1. Elliott MJ, Maini RN, Feldmann M*, et al.* Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. *Arthritis Rheum*. 1993;36(12):1681-90.
2. Weinblatt ME, Kremer JM, Bankhurst AD*, et al.* A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N Engl J Med*. 1999;340(4):253-9.
3. Furst DE, Breedveld FC, Burmester GR*, et al.* Access to disease modifying treatments for rheumatoid arthritis patients. *Ann Rheum Dis*. 1999;58 Suppl 1:I129-30.
4. Maini R, St Clair EW, Breedveld F*, et al.* Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) *versus* placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. *Lancet*. 1999;354(9194):1932-9.
5. Felson DT, Anderson JJ, Boers M*, et al.* American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. *Arthritis Rheum*. 1995;38(6):727-35.
6. Weinblatt ME, Keystone EC, Furst DE*, et al.* Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. *Arthritis Rheum*. 2003;48(1):35-45.
7. van Vollenhoven RF, Ernestam S, Geborek P*, et al.* Addition of infliximab compared with addition of sulfasalazine and hydroxychloroquine to methotrexate in patients with early rheumatoid arthritis (Swefot trial): 1-year results of a randomised trial. *Lancet*. 2009;374(9688):459-66.