

6th Annual Meeting of the Lupus Academy Meeting Report

Lisbon, Portugal

5–7th May 2017

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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 6 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 6th Annual Meeting of the Lupus Academy was held in Portugal in May 2017, 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 >100 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:

- Discuss known associations between SLE, DNA damage and cancer, and appreciate the effects of DNA-damaging autoantibodies on SLE pathophysiology and cancer risk.
- Better diagnose and manage lupus through improved understanding of biomarkers in individual SLE manifestations.
- Improve their clinical practice by better identifying and more effectively managing individual SLE manifestations, including cutaneous lupus, haematological challenges such as clotting, cardiovascular lupus and musculoskeletal manifestations of lupus.
- Develop their understanding of the immunopathology of SLE.
- Understand the place of oral anticoagulants in the management of SLE.
- Improve their management of SLE through improved understanding of drug induced lupus, lupus and pregnancy and a greater understanding of the clinical balance of vaccination and lupus management.
- Consider the latest therapeutic advances in SLE, and how these may improve clinical outcomes, including up to date understanding of B cell activating factors and interferon α blockade.

Keynote Lecture

Lupus and malignancy: The risk and the reasons: Ann Clarke

Professor Clarke reviewed the risk of malignancy in adult and paediatric patients with systemic lupus erythematosus (SLE), evaluating the risk factors mediating risk and providing recommendations for screening and management.

Professor Clarke began her presentation by highlighting the risk of malignancy in adults with SLE as shown by data from the largest clinical cohort examining cancer in SLE. This study compared the observed number of cancers with those expected by linking the clinical cohort to regional tumour registries; *in situ* cancers were excluded. Expected cancers were calculated by multiplying the person-years in the cohort by the geographically matched age, sex, calendar year-specific cancer rates.^{1, 2} The study included 30 centres following a largely female (90%) cohort of 16,409 patients between 1958 and 2009 and found that patients with SLE were 14% more likely to develop a malignancy than the general population. Looking at specific cancers, it was noted that the incidence of all types of haematologic cancers were increased in patients with SLE, particularly non-Hodgkin's lymphoma (NHL) (standardised incidence ratio [SIR]: 4.39); moreover, the highest rate of disease was evident early in the disease, as opposed to later in the disease when patients have been exposed to a higher cumulative dose of immunosuppressants. Other, non-hematologic cancers, with higher standardised incidence ratios in patients with lupus included thyroid cancer (SIR: 1.76), lung cancer (SIR: 1.30) and hepatobiliary cancer (SIR: 1.87). Interestingly, patients with lupus are less likely to develop breast (SIR: 0.73), uterine (SIR: 0.44) and ovarian (SIR: 0.64) malignancies than the general population, yet they are more likely to develop vulvar (SIR: 3.78) malignancies. Professor Clarke then contextualised the work of her centre alongside the work of others to draw a broad consensus on the risk of cancers in patients with SLE (below).

The risk of cancer overall

Overall cancer risk in patients with SLE appears to be similar across US, Taiwanese, Swedish and Danish cohorts with SIRs ranging from 1.14 (US) to 1.76 (Taiwanese).²⁻⁶ A meta-analysis of nine studies by Cao et al in 2015 showed a pooled SIR of 1.28 for overall cancer risk in SLE patients.⁷

The risk of non-Hodgkin's lymphoma (NHL)

Other large population studies of US, Taiwanese and Swedish patients with SLE show elevated SIRs (range 3–7) for NHL risk in these cohorts.^{2-6, 8} Likewise, a meta-analysis by Cao et al in 2015 showed a pooled SIR of 5.4, which is consistent with the large population studies in showing the increased risk of NHL in patients with SLE.⁷

The risk of solid tumours

The risk of lung cancer in a meta-analysis by Ni et al (2014)⁹ showed an SIR of 1.68 in patients with SLE, which again is comparable with the results by Bernatsky et al (2013).² Similarly, liver cancer results from the meta-analysis showed an SIR of 2.44 compared with 1.87 by Bernatsky et al (2013). Reproductive cancers (breast, ovarian and uterine), albeit not supported by meta-analysis, had SIRs predominantly <1, indicating a reduced risk of these types of cancers in patients with SLE; whereas vulvar-vaginal cancers appear more likely to occur in patients with SLE than the general population (SIR: 2.7–5.7).²⁻⁶ The risk of high-grade squamous cervical intraepithelial lesions is also increased in SLE (SIR: 8.66).¹⁰ Given the data for this meta-analysis were taken primarily from patients who live in

countries without a nationwide screening programme for cervical cancer, it could be that patients with lupus undergo more scrutiny or surveillance than the general populations, thus artificially increasing the SIR or the absence of a screening programme could mean that lesions go unnoticed until they become high grade. It should be noted, that in countries with screening programmes, the SIR is much lower for patients with high-grade squamous intraepithelial lesions. In males with SLE, the risk of prostate cancer is reduced, with and SIR of 0.77 according to a meta-analysis by Huang et al (2014).¹¹

The risk of paediatric malignancy

In the paediatric SLE population (<18 years) the risk of malignancy is higher than in the general paediatric population. A study conducted over 12 centres, in 1,168 patients between 1974 and 2009 (8,839 patient years) showed that the SIR for all malignancies in this population was 4.13 and, notably, in NHL the SIR was 18.6; it should be noted that the confidence interval was wide and attributable to the small sample size.^{12, 13} The highest SIRs in children occur early in the SLE disease course (<1 year) and then again over a decade later 10–19 years.

The reasons for increased risk of malignancy: Lymphomas and cervical dysplasia

NHL has the highest SIR of all cancers in SLE and the risk of development is greater earlier in the SLE disease course, thus suggesting that NHL is associated with disease activity and immune dysregulation rather than immunosuppressive drugs, the cumulative dose of which is higher later in the disease course. It is, therefore, postulated that chronic immune stimulation leads to upregulated lymphocyte proliferation, which predisposes to a translocation and juxtaposition of an oncogene beside a gene regulating immune function with malignant transformation.¹⁴ In addition, among the lymphomas, the ones that are particularly increased include the diffuse large B-cell lymphomas (DLBCL), particularly from activated B-cells/non-germinal center, which are derived from activated lymphocytes, again supporting a role for chronic immune stimulation.¹⁵ Other studies have shown there is increased expression of APRIL and BAFF in SLE patients with DLBCL.¹⁶ In addition there is a resistance to apoptosis and prolonged survival of B-cells, as well as a potential role for IL-6, IL-10, IFN.¹⁷ Lymphomas are also associated with primary Sjogren's syndrome, although these tend to be marginal zone lymphomas as opposed to DLBCL. Given this, Bernatsky et al conducted a study to determine if SLE disease activity and/or treatment increases risk of lymphoma.^{18, 19} The study found that in the fully adjusted regression model, males and older patients were more likely to get lymphomas, however, there was no significant relationship between SLE medications or disease activity and lymphoma. However, in the partially adjusted regression model, patients exposed to cyclophosphamide, systemic steroids (>3.5g) or those with secondary Sjogren's syndrome were more likely to develop lymphoma.

Other recent studies have assessed the risk of cancer in SLE patients taking immunosuppressive drugs. Hsu et al (2017) found a higher risk of malignancy with higher cyclophosphamide exposure and, interestingly, a lower risk of malignancy with higher hydroxychloroquine exposure.²⁰ A Swedish study by Wadstrom et al (2017) looked at the association between immunosuppressive drugs and cervical neoplasia and found that SLE patients generally had a higher risk of cervical cancer than the general population (Hazard Ratio: 2.12), moreover, those SLE patients taking immunosuppressants *versus* antimalarials had a higher risk of cervical dysplasia than those taking antimalarials alone (Hazard Ratio : 1.83).²¹ Similar results were seen in patients with cervical intraepithelial neoplasia, whereas, there was no increased risk of invasive cervical cancer.

In summary, cyclophosphamide is associated with an increased risk of lymphoma and overall malignancy, immunosuppressive drugs appear to be associated with cervical dysplasia, but there is no association between disease activity and risk of malignancy.

Lupus-related autoantibodies and the risk of malignancy

The reason SLE patients have a decreased risk of breast, ovarian and prostate cancers may be explained by the role of lupus-related autoantibodies. Noble et al described antibodies that penetrate the cell nucleus, causing breaks in dsDNA, resulting in cell death in those cells unable to repair the DNA.²² Other antibodies, including those to the centromere protein and RNA polymerase in patients with SLE and cancer, are also being studied.

Other factors modifying the risk of cancer in SLE

Patients with SLE have an increased risk of lung cancer and are more likely to smoke than the general population (Hazard ratio for smoking: 6.4). Breast and endometrial cancer risk in patients with SLE is lower than the general population, possibly because of decreased oestrogen exposure, likewise, male patients are less likely to get prostate cancer, possible because of decreased androgen exposure. In addition, viral infections are likely to influence cancer in SLE patients, with SLE patients being more likely to be infected with Human Papilloma Virus (HPV) and the risk of cervical cancer in countries with nationwide screening programs being approximately 2-fold higher than the general population. Likewise, an increased risk of vulvar/vaginal cancer (SIR: >3), anus cancer (SIR: 26.9) and head and neck cancer (SIR: 2.1) is evident in patients with SLE and is potentially related to HPV.^{23,10} Hepatic cancer may potentially be associated with Hepatitis B Virus/Hepatitis C Virus and NHL with Epstein Barr Virus, although there is currently no evidence in SLE to support this.

Professor Clarke concluded her presentation by reviewing the evidence for a shared genetic susceptibility to SLE and cancer. Bernatsky et al (2012), using data from a breast cancer genome wide association study (GWAS), compared the frequency of SLE-related single nucleotide polymorphisms (SNPs) in breast cancer cases versus the general population. They showed that there was very little difference in the frequency of SNPs between those with and without breast cancer.²⁴ However, Bernatsky et al (2017), using data from a DLBCL GWAS, showed that two SLE-related SNPs occurred more frequently in patients with DLBCL than in the general population.²⁵ Likewise, Ekström Smedby et al (2008) showed that SLE was a risk factor for lymphoma, independent of shared environmental risk factors.²⁶

Table: Summary of risks, reasons and practice implications.

Risks

Malignancy type/Cause	Risk
Overall cancer	Minimally increased – 1.28 (95% CI 1.17, 1.41)
Hematological malignancies	Highly increased NHL – SIR 5.40 (95% CI 3.75, 7.77)
Virally-associated cancers	High grade cervical dysplasia – SIR 8.66 (95% CI 3.75, 20.00)
Breast, endometrial, ovarian, prostate	Decreased Breast – SIR 0.76 (95% CI 0.67, 0.86)

	Endometrial – SIR 0.60 (95% CI 0.40, 0.87) Ovarian – SIR 0.82 (95% CI 0.54, 1.20) Prostate – SIR 0.69 (95% CI 0.50, 0.93)
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Reasons

Cause	Example
Immune dysregulation	SLE disease activity – no association Lupus-related autoantibodies – protective?
Immunosuppressive exposure	Cyclophosphamide – potential association Antimalarials – protective?
Environmental exposures	Smoking & lung cancer; HPV & cervical cancer
Genetic	Shared genetic susceptibility to SLE and lymphoma

Practice Implications²⁷⁻²⁹

Consideration	Action Taken
No history of malignancy	Use ISDs as clinically indicated Duration determined by usual risk:benefit ratio
Remote history of malignancy	Use ISDs as clinically indicated Duration determined by usual risk:benefit ratio
Recent history of malignancy	Avoid ISD if possible & limit duration if possible Consider rituximab and belimumab
Malignancy screening	Follow general population recommendations EXCEPT: Annual screening for cervical dysplasia Particular vigilance if cyclophosphamide exposure
Other considerations	HPV vaccination – safe and effective Hydroxychloroquine

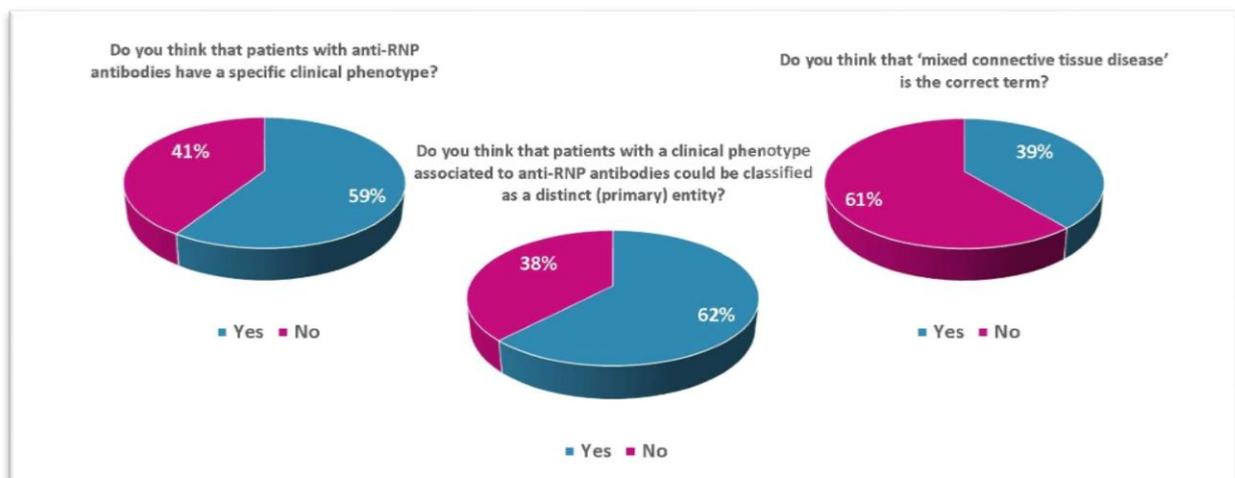
The Great Debate

This house believes the term 'mixed connective tissue disease' is archaic and should be replaced? David Isenberg (For) and Andrea Doria (Against)

In the 1970s Sharp et al claimed to have described an apparently distinct rheumatic disease syndrome with clinical characteristics including a combination of features similar to those in systemic lupus erythematosus, scleroderma, and polymyositis. The term mixed connective tissue disease (MCTD) was born. Since then, there has been criticism of the term, with some suggesting the MCTD is nothing more than a diagnostic uncertainty that soon develops into one of the more definitive CTDs (ie. SLE, scleroderma, polymyositis).

Ricard Cervera introduced the topic, presenting the history of the debated term MCTD, and moderated the discussion as David Isenberg (For) and Andrea Doria (Against) debated the term 'mixed connective tissue disease' (MCTD). Professor Cervera set the scene for the ensuing debate by asking the audience to answer three key questions using their keypads (**Figure**), these questions were then asked again at the end of debate following presentation of the case for and against the existence of (MCTD).

Figure. Questions to the audience: Before the debate



This house believes the term 'mixed connective tissue disease' is archaic and should be replaced?

For: David Isenberg

Professor Isenberg began the debate by presenting the natural history, including clinical descriptions, serological connections, and classification, of the autoimmune rheumatic disease (ARD), systemic lupus erythematosus (SLE), Sjögren's syndrome and antiphospholipid syndrome (APS); highlighting, that all have a well-documented progression from clinical description, through serology to classification. Professor Isenberg continued to review the 'unnatural history of MCTD, beginning with Sharp et al's (1972) discovery of patients (from laboratory notebooks) with high levels of antibodies to RNP and the 25 case notes that formed the basis (clinical and serological features: **Table**) of their definition of MCTD.³⁰

Table: Clinical features and serological abnormalities of MCTD³⁰

Clinical Features	Serological Abnormalities
Raynaud's	Anti-RNP antibodies
Arthralgia/mild arthritis	Hypergammaglobulinaemia
Puffy hands	Anaemia
Abnormal oesophageal mobility	Leukopaenia
Lymphadenopathy	
Myositis	

In addition, Sharp et al noted that Lupus' features (e.g. photosensitivity, alopecia, rashes, serositis) were rare as were pulmonary, renal and neurological involvement, along with no report of vasculitis, low steroid requirement and ultimately benign/low mortality. This basis for MCTD implying that it is a bad news/good news story with favourable outcomes for patients. However, Professor Isenberg highlighted, within just 8 years of Sharp's definition of MCTD, others began to question its existence. Nimelstein et al (1980) reviewed 22 of the 25 original patients (three were not traced) and noted that clinical evolution to scleroderma had occurred in many, there was a relatively high rate of mortality (36.4%), not all of the patients had anti-RNP antibodies, some even had high-RNP antibodies without displaying clear features of overlap syndrome and some patients did require high-dose steroids.³¹ In conclusion Nimelstein et al said the results indicated that certain features of the patients that had originally been thought to make them clinically distinct had not held true over time. Conversely, changes in the criteria for lupus over time (1971–1982) are much less pronounced than those found in MCTD. Notably, only Raynaud's and alopecia were removed from the clinical criteria between 1971 and 1982, likewise the serological criteria only changed slightly with the grouping of LE cells and false positive syphilis being grouped and proteinuria (>3.5 g/day) and urine cellular casts being grouped under renal disorders, with just the addition on lymphopenia being the only real change in serological criteria. These minor alterations were not reflected in MCTD, which are dramatic by comparison (**Table**).

Table: Changes in clinical and serological criteria in MCTD (1972–1984)

	1972 (%)	1984 (%)
Lymphadenopathy	68	50
Serositis	24	35
Renal	0	26
Pulmonary (intrinsic)	0	85
Alopecia	0	41
Cardiac	0	26
Malar rash	0	29
Anaemia	48	24
Leucopaenia	52	21

Hypergammaglobulinaemia	80	32
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Professor Isenberg questioned why the MCTD story did not end, given this evidence. He noted that it may have been because in spite of the dubious nature of the original claims, exposed by Nimelstein et al, there really is a group of patients with mild overlapping features who do not change over time. Moreover, physicians like to give patients good news. In the 1970s the outlook for many patients with ARD was still relatively poor, so that any notion of a milder 'variety' was warmly welcomed and quickly established in the collective consciousness – and has proved hard to eradicate. In addition, there are now four sets of criteria for MCTD developed between 1987 and 1991, each having varying degrees of sensitivity. In 1995, Gendi et al reported that of 39 patients with MCTD, as diagnosed using Sharp's criteria, 11 went on to be diagnosed with scleroderma, 10 with SLE, 2 with RA and 2 with overlap syndromes, only 14 remained unchanged within 10 years of diagnosis.³² Similar results were reported by van den Hoogen et al in 1994, highlighting that again over half of patients (n=18) originally diagnosed with MCTD, with high levels of antibodies to RNP, went on to develop scleroderma, SLE, RA or a combination of these.³³ Conversely, Frandsen et al (1996) found that of 151 patients 26% were originally diagnosed with MCTD, yet after 7 years of follow up 64% were diagnosed with MCTD; however, MCTD criteria were not established in this study and on a third of patients with high RNP and a 15 year disease duration maintained a diagnosis of MCTD.³⁴

Professor Isenberg then highlighted some of the subtleties of measuring anti-RNP antibodies, noting that they are directed against a complex variety of protein structures (A,C, 70k and occ. UI-RNA) and that 40% of SLE patients too have antibodies to RNP (ie. RNP antibodies are not disease specific). Greidinger et al (2001) studies >3000 patients over 10 years and found that the portion of the target molecule that anti-RNP molecules were binding to changed over time, with 70k and B/B' proteins being 'early immunogens' - A and C proteins were recognised later.³⁵ Conversely, Lundberg et al (1992) found that 29 anti-RNP positive patients had consistent antibody specificity even though clinical features were accruing.³⁶ In summary,

Professor Isenberg concluded that physicians try to distinguish individual diseases as a matter of convenience so we can more easily talk to each other and inform patients in broad terms about treatment, prognosis etc, and for 'straightforward' conditions (e.g. measles, mumps etc) this approach works well. However, for patients with ARD it is much harder. The definitions have been much more difficult to agree and there is a significant degree of overlap and concordance. He went on to note that the definitions of RA/SLE/Sjögren's have all been 'refined' over the past 30 years; but in essence have changed little, however virtually all of the claims made for MCTD when it was first 'announced' in 1971/2 are demonstrably false. In addition, the four sets of classification criteria used to define MCTD are excessive and that Sharp et al's original criteria have little basis in fact. Professor Isenberg, did however note that there may be a modest number of patients with overlapping features of well-established ARDs – among whom many (but not all) have anti-RNP antibodies – whose disease can 'hold true' over time (though it often doesn't!). In 1972 MCTD stood for 'Mixed Connective Tissue Disease'. In 2017 MCTD should stand for 'Muddled Concept To be Discarded!'

This house believes the term ‘mixed connective tissue disease’ is archaic and should be replaced?

Against: Andrea Doria

Professor Andrea Doria began his side of the debate highlighting the risk alleles shared by CTDS, noting that there are common pathogenetic pathways across these diseases, and the common features of CTDs noting that some of these are early features of CTDs and must therefore be considered as ‘red flags’ for CTDs (**Table**).³⁷⁻³⁹

Table: Common features of CTDs

Features of CTDs	Early Features of CTDs
Raynaud’s phenomenon	Raynaud’s phenomenon
Non erosive arthritis	Non erosive arthritis
Hand oedema	Hand oedema
Pulmonary fibrosis	
Serositis	
Oesophageal hypomotility	
Subcutaneous calcification	
ANA	ANA

Professor Andrea Doria noted that the existence of common genetic and clinical findings supports the existence of undifferentiated connective tissue diseases and connective tissue disease overlap syndromes. Citing work by Mosca et al, Professor Doria presented preliminary classification criteria for undifferentiated connective tissues disease, noting that there should be the presence of antinuclear antibodies determined on two different occasions and there should be signs and symptoms suggestive of a CTD, but not fulfilling the criteria for any of the defined CTDs for at least 3 years.⁴⁰ He noted that overlap CTDs are more complicated and recent work has suggested the classification of patient with overlap CTDs into two groups: (1) those associated with a specific antibody profile, and (2) those without a specific antibody profile.⁴¹ Professor Doria highlighted that MCTD lies within overlap syndromes and is different from undifferentiated connective tissue disease (UCTD), noting that the confusion between MCTD and UCTD was probably derived from the first description of UCTD (Puffy Hand syndrome) Hanby LeRoy et al (1980), who described this as an possible early state of MCTD.⁴² Before addressing each of the three original questions posed at the beginning of the debate, Professor Doria highlighted that in order to make a diagnosis of MCTD, patients must have positive anti-RNP antibodies and at least two clinical manifestations of CTDs including SLE, scleroderma and polymyositis as detailed in the Japanese diagnostic criteria for MCTD.⁴³

Question 1: Have patients with anti-RNP antibodies a specific clinical phenotype?

Professor Doria noted that anti-RNP is present in 100% of patients with MCTD, usually at high titre and in the absence of anti-SM antibody, whereas it is found in 20-40% of patients with SLE, usually in association with anti-SM antibody.⁴⁴⁻⁴⁶ 2–14% of patients with systemic sclerosis⁴⁷⁻⁴⁹ and 6–9% of those with idiopathic inflammatory myopathies^{50, 51} have anti-RNP antibodies. Professor Doria also highlighted the anti-RNP associations in patients with CTDs, linking this directly with clinical phenotype (**Table**).

Table: Anti-RNP associations in patients with CTDs

Disease	Clinical Phenotype
Systemic lupus erythematosus	Raynaud's phenomenon ^{52, 53} Polyarthritis ^{54, 55} Leucopaenia ⁵³ Pleuritis ⁵⁶ Myositis ⁵⁴ Interstitial lung disease ^{55, 57} PAH ⁵⁸
Systemic sclerosis	Myositis ⁵⁹ PAH ⁶⁰
Idiopathic inflammatory myopathies	Pulmonary fibrosis ⁶¹

Question 2: Could patients with a clinical phenotype associated with anti-RNP antibodies be classified as a distinct (primary) entity?

Professor Doria presented the major criticisms to MCTD as a distinct clinical entity, dealing with each in turn:

(1) Many evolve to another CTD over time

In each CTD clinical features appear over clinical course. Indeed, in classification criteria it is specified: " ...items have to be present at a single time point or over the disease course". In overlap syndromes different from MCTD, the single CTD components rarely occur at the same time. Professor Doria, then reviewed the evolution of MCTD, noting that although historically a high percentage (34–71%) of MCTDs have evolved to another CTD,^{31-33, 62, 63} more recently the percentage of MCTDs evolving to another CTD has dropped (<15%).^{64, 65} Moreover, research has shown that there are specific factors associated with the development of a new CTD component in MCTD (Table).

Table: Factors associated with the development of a new CTD component in MCTD^{34, 63}

Development of another CTD	<ul style="list-style-type: none"> • Disease duration >5 years • Low anti-U1RNP titre
Systemic lupus erythematosus	<ul style="list-style-type: none"> • Anti-dsDNA
Systemic sclerosis	<ul style="list-style-type: none"> • Sclerodactily • Oesophageal hypomotility
Myositis	-

(2) The clinical picture is similar to that of other CTDs

Comparing the clinical prevalence of MCTD and SLE, Professor Doria highlighted the significant differences between the prominent features of these diseases, as is the case in patients with scleroderma, myositis⁶⁶ and systemic sclerosis.⁶⁷

(3) That there is a lack of pathogenic clues.

Professor Doria presented data supporting the pathogenicity of anti-RNP antibodies in MCTD. A large cohort study by Flám et al (2015) showed that the HLA profiles of MCTD were distinctly different from the profiles of clinical related CTDs, reinforcing the notion that MCTD is a separate disease entity.⁶⁸ An *in vivo* study by Greidiner et al (2006) also provides evidence for the pathogenicity of anti-RNP antibodies in MCTD, which highlights that the induction of immune response by the same antigens can lead to different diseases (eg. MCTD and SLE).⁶⁹

Question 3: Do you think that 'mixed connective tissue disease' is the correct term?

Professor Doria presented the rationale for changing names of rheumatic diseases, highlighting those that have come into question in recent years (**Table**).

Table: Rheumatic diseases that have changed their names (or ought to)

Disease	Reason for changing	Effects
Wegener's granulomatosis	Wegener supported Nazism	The new name GPA better defines the disease but is quite similar to EGPA
Reiter's syndrome	Reiter was a Nazi supporter	The name 'reactive arthritis' better defines disease
Churg-Strauss syndrome	Need for eponym abolishment	EGPA may be confused with GPA
Horton disease	Need for uniformity between Europe and the rest of the world	The new name GCA gives some hints to pathophysiology
Rheumatic fever	Obsolete and not indicative of the disease process	Still waiting
Rheumatoid arthritis	Confusion with osteoarthritis	Still waiting
Spondyloarthritis	Extra-spinal manifestations	Still waiting

Even RA is misleading and faces an identity crisis, with the lay person having little appreciation of the differences between RA and osteoarthritis. Moreover, patients with spondyloarthropathies are often diagnosed, without spinal manifestations of the disease, just because they have peripheral arthritis. Professor Doria asked if we should change the names to "all rheumatic diseases", before suggesting that MCTD includes lots of factors that should be considered carefully including the combination of different disease traits, the systemic nature of the disease, the role of anti-RNP and the underlying

common genetics. Professor Doria explored alternative names for MCTD, before excluding these as inappropriate (**Table**). Professor Doria concluded that although MCTD is probably not the best term, it does already exist, it is comprehensive enough, physicians have become confident with it and the best is the enemy of the good.

Table: Reasons not to rename MCTD

New name	Excluded because...
Undifferentiated autoimmune rheumatic disease?	It's very unspecific; it generates confusion with UCTD
Anti-U1RNP syndrome?	Anti-U1RNP is not exclusive to MCTD
Overlap' SLE-PMDM-SSc?	It's not really any better
Mixed connective tissue syndrome?	It looks like a syndrome, but has common underlying pathogenic pathways like a disease

This house believes the term 'mixed connective tissue disease' is archaic and should be replaced?

For: David Isenberg (Rebuttal)

Professor Isenberg revisited the term anti-RNP, reminding the audience that these are not specific to any one CTD. Looking at the structure of anti-RNP, an epitope mapping study looked at antibodies to 15 peptides tested in SLE (n=68), MCTD (n=29), and healthy (n=20) subjects. The study concluded 'Although nearly all of the antibodies to the peptides were strong predictors of autoimmune illness none were successful at distinguishing between SLE and MCTD'. In a more detailed study by Mesa et al (2013), no differences in IgG anti-U1 RNP response were found between the SLE and MCTD groups, however, IgM anti-U1 RNP responses did however distinguish SLE and MCTD.⁷⁰ The same study showed that 31 of 41 laboratory tests and 24 of 40 clinical features were not distinguished between SLE and MCTD. Professor Isenberg highlighted that antibodies to RNP keep changing over time, unlike anti-Ro antibodies, which remain constant overtime.⁷¹ Moreover, long-term follow up studies have shown MCTD to be somewhat of a 'chameleon' of a disease, one in which the core clinical features develop overtime.⁶² The mortality rate in patients with MCTD is similar to that in SLE, with approximately 15% of patients dying over a period of 6-15 years. Professor Isenberg concluded by highlighting Nimmelstein (1980) saying 'MCTD is not a benign condition and that patients with MCTD can lack antibodies to RNP'.³¹ Moreover, Lázaro (1989) reported that 'antibodies to nRNP do not identify a particular subgroup within the overlap syndromes and that MCTD does not appear to be a distinct entity',⁷² and Lundberg (2005) reported that 'one third of patients with 'MCTD' had a benign course, one third had an aggressive course and one third improved with immunosuppressive therapy that was required for several years'.⁷³ Professor Isenberg, summarise his 'riposte' with a portrayal of the 'San Miguel Solution' (see video presentation on Lupus Academy Website), where healthy patients develop mild, undifferentiated autoimmune disease, some of them go on to develop myositis, SLE or scleroderma, yet few remain in the undifferentiated autoimmune disease category, ie, the term MCTD should be abolished.

This house believes the term ‘mixed connective tissue disease’ is archaic and should be replaced?

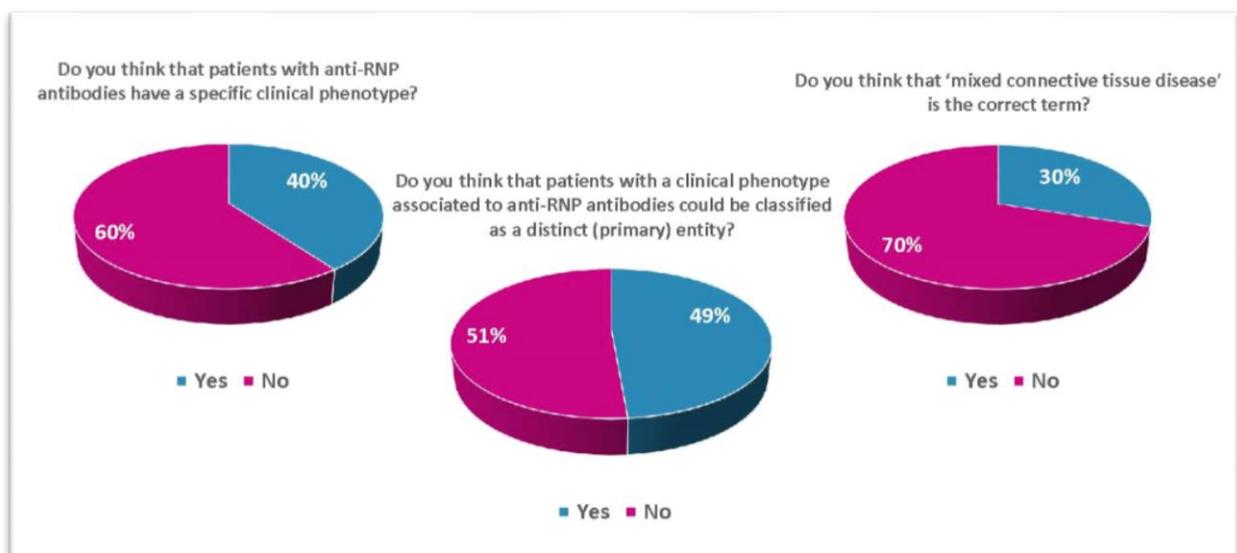
Against: Andrea Doria (Rebuttal)

Professor Doria opened his rebuttal by distinguishing diagnostic and classification criteria, highlighting that these are useful for defining a group of patients, but not useful in identifying single patients (ie. diagnosis is largely down to the clinical experience of the Rheumatologist).⁷⁴ Responding to Professor Isenberg’s reference to the high number of classification criteria for MCTD, Professor Doria noted that MCTD actually has a low number of criteria (4) compared to other CTDs. Refereeing to Professor Isenberg’s publication (1992) claiming MCTD does not exist,⁷⁵ Professor Doria noted that this had little impact on subsequent publications or interest in MCTD, moreover, MCTD continued to be diagnosed in clinical practice, and included in the classification of rare diseases at Padova University.

Ricard Cervera concluded the debate by asking the audience to answer three key questions using their keypads (**Figure**), these questions relating to the case for and against the existence of (MCTD). The audience concluded that the debate was in favour of abolishing the term MCTD, with a majority of people agreeing that:

1. Patients with anti-RNP antibodies do not have a specific clinical phenotype
2. Patients a clinical phenotype associated to anti-RNP should not be classified as a primary entity
3. Mixed connective tissue disease is not the correct term for these (aforementioned) patients.

Figure. Questions to the audience: After the debate



Plenary I: Biomarkers in SLE Update

dsDNA antibodies: New insights: David Pisetsky

Professor Pisetsky reviewed the role of dsDNA antibodies as the quintessential diagnostic and prognostic biomarkers in lupus.

Professor Pisetsky opened his presentation by noting the importance of understanding DNA antibodies (anti-DNA) in order to fully understand their significance as a diagnostic and prognostic biomarker in patients with lupus. Professor Pisetsky highlighted work from Schur (1968)⁷⁶ and Tan (1966)⁷⁷ showing that levels (unlike anti-SM) of both antibody (anti-DNA) and antigen (DNA) can vary over time, and because an important mechanism of anti-DNA is the formation of immune complexes one must understand both where both antibody and antigen are.

Professor Pisetsky highlighted that while anti-DNA is highly associated with SLE, it is not a biomarker for everything; it depends on the disease manifestation, and is expressed most prominently in lupus nephritis. Increasingly, anti-positivity is becoming an entry criterion for clinical trials in lupus, as well as biomarkers such as ANA, anti-SM whereby anti-DNA is being assessed as a companion biomarker (or “theranostic”) in determining SLE therapy. Anti-DNA can be viewed in several contexts as a marker for SLE, including pathological (ie. abnormal production), pathogenic (ie. causing disease) and nephritogenic (ie. causing nephritis). What is not known is how many are nephritogenic nor what the nephritogenic species are. Aside the complexity of pathogenicity of anti-DNA, it is the formation of immune complexes that is important. DNA is a highly charged large molecule with an unusual polymeric structure, whose job is to bind proteins in the nucleus of the cell and also bind proteins (antibodies) outside of the cell to form immune complexes in the blood or the kidney (*in situ*) where cytokine production, in the form of an immune complex, is initiated.

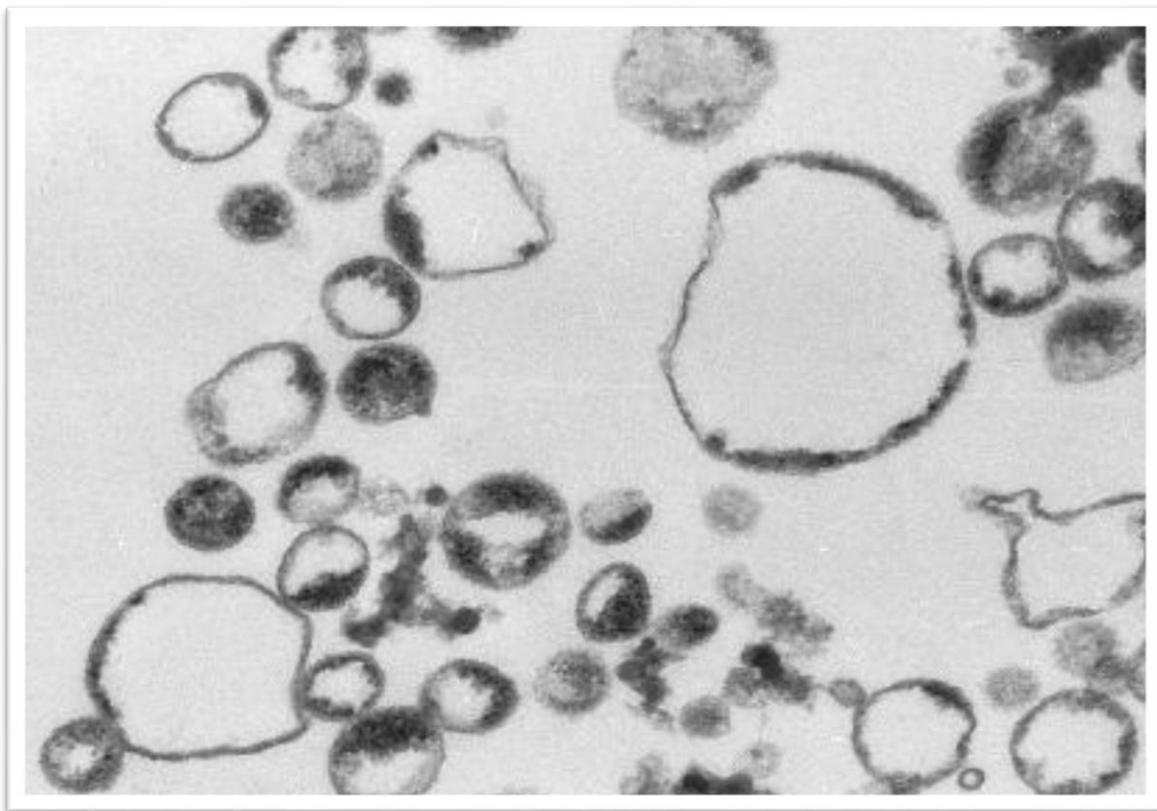
Focusing on the specificity of SLE anti-DNA, Professor Pisetsky asked how antibodies bind DNA and what is the target antigen? The most common presentation of DNA in the cell is nucleosome (DNA wrapped around histone), therefore DNA can be viewed as a subset of antibodies that bind to the nucleosome; other antibodies include histones and DNA-histone complexes. Therefore, by just assaying antibodies to DNA, one may be missing important components of an overall anti-nucleosome response. There has been a lot of discussion about differences between single and double stranded DNA, however most anti-DNA antibodies bind both single and double stranded DNA; another way in which antibodies to DNA bind is by monogamous bivalent interaction. Anti-DNA is not just found in lupus patients, but also in normal individuals, however the specificity of anti-DNA in lupus patients is distinct from anti-DNA found in sera of normal individuals; the healthy immune system recognizes bacterial DNA in a conventional way, whereby it finds area of sequence that are distinct, in contrast lupus antibodies see the backbone.⁷⁸ Therefore, most anti-DNA bind to both single and double stranded DNA, it is difficult to prepare dsDNA without ssDNA regions and vice versa, DNA can undergo conformational rearrangement to expose ssDNA regions and assays with ssDNA are technically easier and frequently more sensitive in patients with lupus.

Professor Pisetsky highlighted the large number of anti-DNA assays that have been carried out in lupus patients, including immunoprecipitation, Farr (ammonium sulfate or PEG), Filter binding, crithidia and ELISA, noting that comparison among assays difficult because of differences in detection systems as well as DNA sources; the most appropriate assay will depend on the individual patient and whether they are used for diagnosis or follow-up. Anti DNA is typically recognised where classic immunofluorescence shows it forming an immune complex in the kidney (lupus nephritis); however, another function of anti-DNA is to induce interferon production, for which an immune complex is essential.^{79, 80} One origin of the interferon signature is immune complexes of DNA and anti-DNA or anti-DNA and components of apoptotic cells.⁸⁰ The process by which this occurs includes

DNA on the inside of the cell, when cells die or become inactivated DNA moves to the outside of the cell, an immune complex is formed, which takes the DNA back into the cell, triggering toll-like or non-toll-like receptors, resulting in the output of interferon; antibodies to RNA binding proteins do exactly the same. Professor Pisetsky emphasized that when DNA leaves the cells it does so in the format of large material such as a nucleus or fragment of nucleus (ie it is not leaving the cell as pure DNA).

Explaining the formation and clearance of apoptotic cells through the formation of microparticles, Professor Pisetsky noted that DNA is present in these particles, as are viruses, bacteria and platelets; hence information transfer takes place in particles rather than through the presence of 'free' nucleic acids in the blood, these particles are being released from cells all the time and dramatically increase during apoptosis (**Figure**).⁸¹

Figure. Microparticles expelled from apoptotic cells.

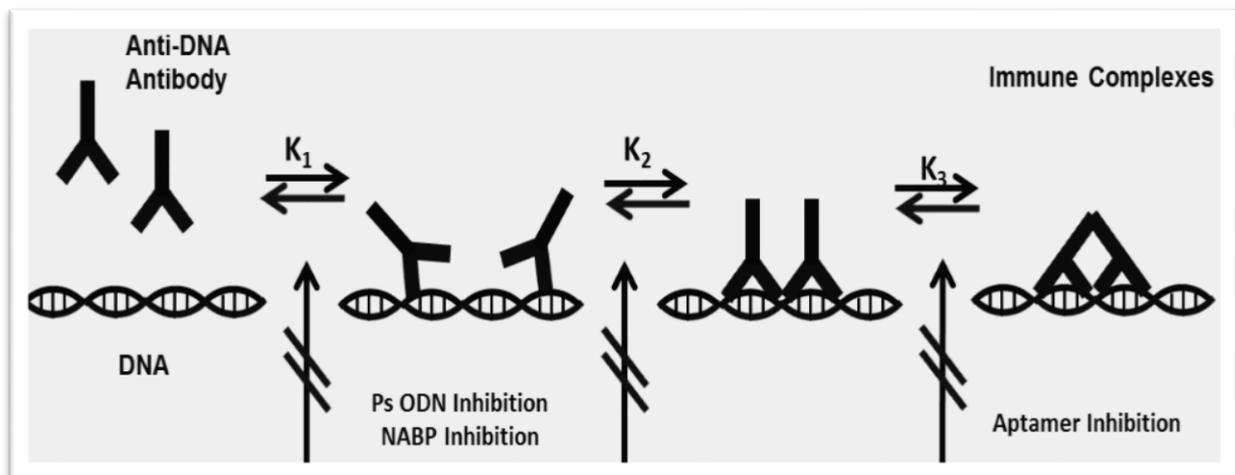


In patients with lupus, the number of particles (eg. lactadherin +/-) in the blood can be significantly increase, compared with normal subjects.⁸² These particles may comprise DNA, but also T-cells, B-cells, platelets and endothelium. Studies have shown that ANA binds to microparticles that display DNA and nucleosomal molecules in an antigenic form and represent a source of immune complexes in SLE.⁸³ Binding of SLE plasma to microparticles generated in vitro have shown that in patients with SLE have increased levels of IgG microparticles, with a clear relationship between these and anti-DNA levels.⁸³ In addition to IgG, the microparticles also contain bound complement (C4d) in patients with SLE.⁸³

Professor Pisetsky revisited anti-DNA, asking the question how anti-DNA bind to DNA? In patients with lupus antibodies bind to DNA by charge; DNA is a large and highly (negatively) charged polymer and the antibodies are positively charged. The polymeric structure can have a huge impact on avidity of antibody interactions by facilitating cross-linking by Fab sites. One piece of DNA can bind multiple

antibodies, resulting in high avidity. This was shown by Papalian et al (1980), who proposed the mechanism of monogamous bivalent binding of DNA by anti-DNA, where a piece of DNA spanned two Fab sites.⁸⁴ Professor Pisetsky's team investigated how big a piece of DNA would be needed to bind these sites, using ELISA and DNA of varying sizes. They found that piece of DNA, even hundreds or thousands of bases long, were not good antigens, even though the actual span of the Fab sites was only about 50 bases.⁸⁵ The study concluded that "the antigenicity of DNA fragments is dramatically altered by solid-phase binding and suggest that constraints on topological or conformational rearrangements of DNA in the solid phase limit antibody interaction". An additional study looked at Fab fragments prepared from IgG. The study showed that Fab fragments, which can only bind monovalently, had negligible activity, likewise bivalent fragments also showed poor anti-DNA activity, thus demonstrating anti-DNA avidity depends on monogamous bivalency, with the antibody Fc portion also influencing DNA binding, in a mechanism, which can be termed Fc-dependent monogamous bivalency.⁸⁶ Professor Pisetsky questioned why Fc fragment is necessary for anti-DNA binding, noting that Fc can alter the conformation of Fab, it may Contact with DNA to act as a third combining site or Fc:Fc interaction promotes multivalent binding. Professor Pietsky concluded by proposing that anti-DNA antibodies bind by one Fab site binding the anti-DNA and, because or proximity and it is a polymer the second binding site binds the anti-DNA, resulting in monogamous bivalency (a stable interaction) and, thirdly Fc:Fc interaction which is high affinity (Figure).

Figure. Novel strategies to inhibit DNA-anti-DNA immune complex formation.



Summarising, Professor Pietsky highlighted that the relevant circulating form of DNA may be particulate and not soluble. As such, complexes may be very large and differ from classical model immune complexes. Anti-DNA antibodies bind by a non-conventional mechanism called Fc-dependent monogamous bivalent interaction. Finally, elucidating DNA-anti-DNA complexes may entail fundamentally different assays that utilize particles and less "pure" forms of DNA to allow detection of interactions that occur *in vivo*.

Plenary I: Biomarkers in SLE Update

Atherosclerosis biomarkers: Anisur Rahman

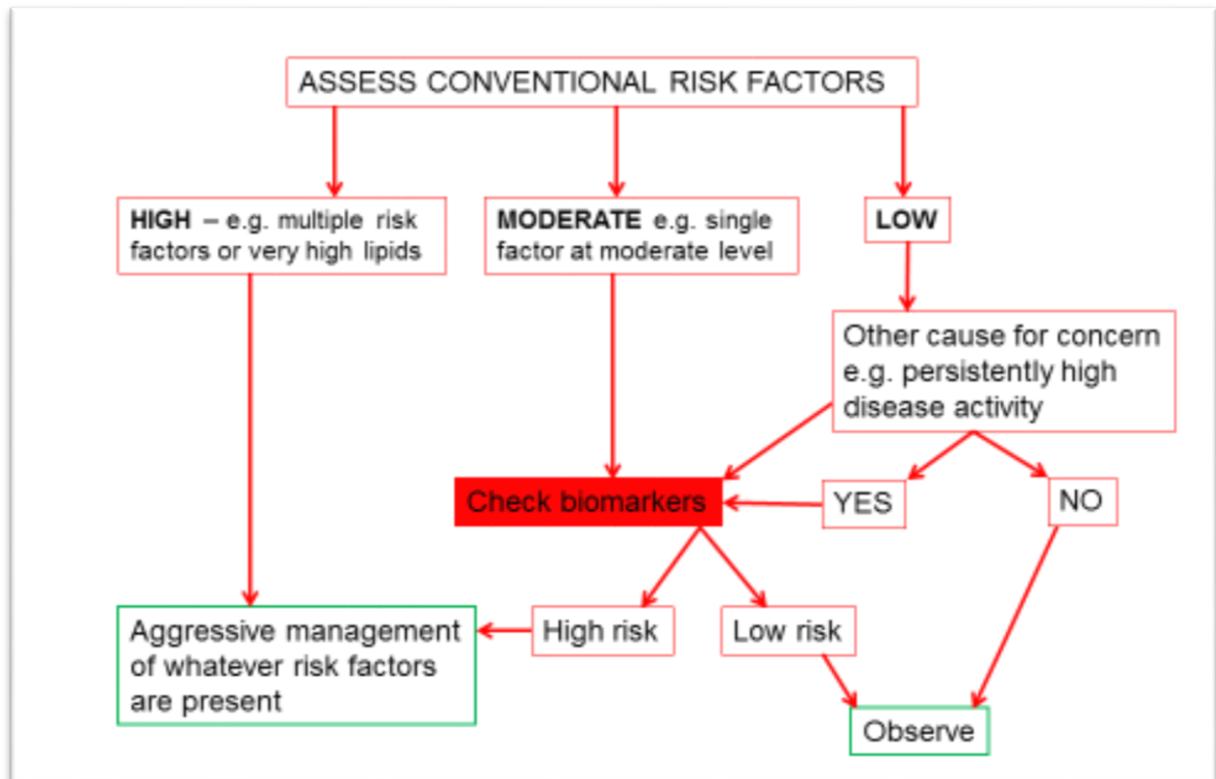
Professor Rahman reviewed the increased risk of atherosclerosis and CVD in patients with systemic lupus erythematosus (SLE) and why establishing biomarkers for atherosclerosis in SLE could help in managing this risk.

Professor Rahman highlighted the importance of identifying atherosclerosis biomarkers, noting that a surprising number of patients with SLE have cardiovascular problems, which can develop at a young age and be very serious. It is important to know who is likely to be affected, why this is and how it can be prevented.

SLE is an autoimmune rheumatic disease that affects primarily young to middle aged women (Female:Male = at least 10:1). Generally the risk of CVD in women is low, but in women with SLE it is high, with a large multicentre mortality study showing that 25% of deaths were due to CVD.⁸⁷ An earlier study by Manzi et al (1997) comparing the age-specific incidence of myocardial infarction/angina in 498 patients with SLE, revealing a 52.4 fold increased risk in women aged 35-44 years.⁸⁸ Similar results have been found in studies across the USA, Canada and Europe, highlighting an relative risk of 7 to 10 fold. Such incidence may be explained by the effect of conventional risk factors (eg. diabetes, smoking, lipids, hypertension), disease activity resulting in chronic inflammation, or other risk factors such as aPL.

Professor Rahman continued to focus on about what can be done to improve CV outcomes in these patients, such as improving the management of conventional risk factors (eg. statins, antihypertensives and smoking cessation) and improved management of disease activity in patients where inflammation drives CVD (eg. increased steroid and immunosuppressants). However, it is difficult to do so, as many of the patients are young women in whom you would not normally use statins. Moreover, the Framingham risk score underestimates the 'true risk' of CVD in patients with SLE, as these programmes are biased towards age and sex. For example, a study by O'Neil et al found that only 35/308 patients had a 10-year risk of >7.5%.⁸⁹ The equations are not accurate for SLE. In addition, Increasing immunosuppressive treatment specifically to reduce CVD risk is hard to justify. Professor Rahman continued to explain an alternative approach.

A study of conventional risk factors in a UCLH cohort (2015) of 309 patients (mean age 47 years; 94% female; 55% white, 25% Afro-Caribbean, 14% Asian) found 10% of them smoked, which is typical of this type of cohort, only six had under-treated hypertension and 64 had raised lipids, yet only 13 were treated. Professor Rahman questioned if we could do better by identifying lupus patients at high risk of CVD and then targeting the management of conventional risk factors and make better decisions about controlling disease activity. To do this there is a need for biomarkers of high CVD risk. Professor Rahman highlighted the pathways to assess risk in lupus patient with CVD (Figure), noting that biomarkers are central to the management of CVD risk in patients with lupus.

Figure. CVD risk assessment in patients with SLE.

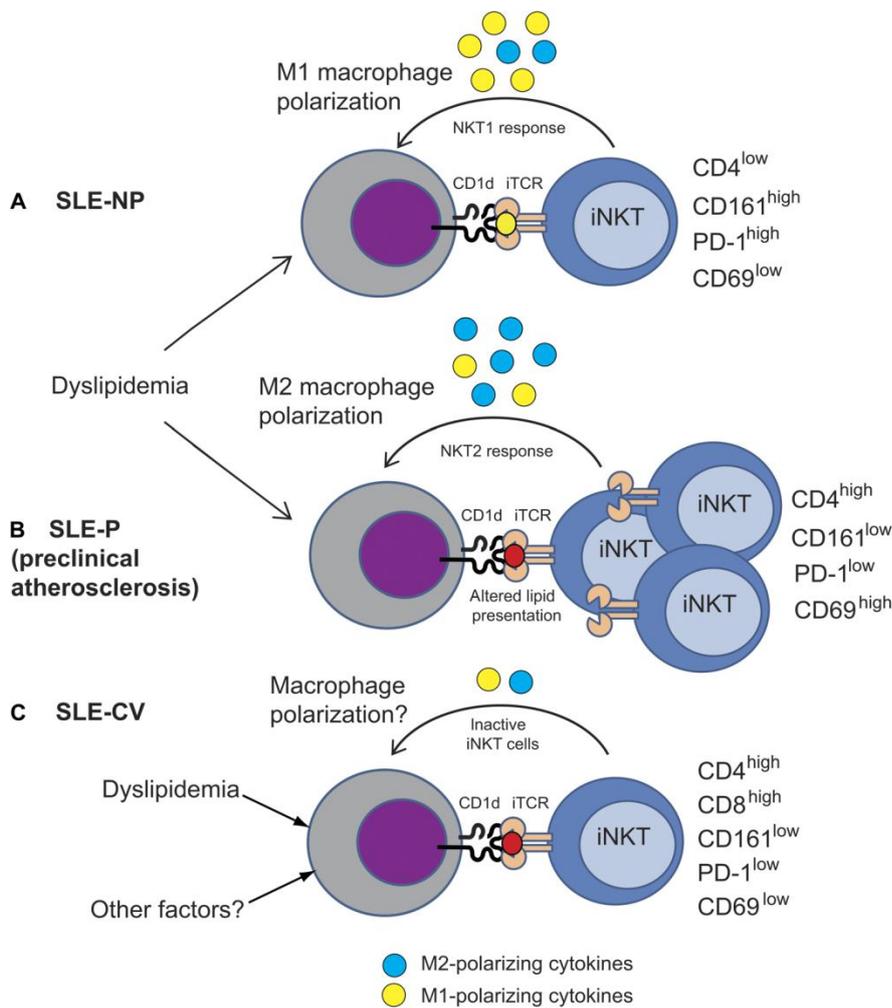
Biomarkers we could use fall into two groups, imaging and serological biomarkers. Imaging biomarkers give direct information about the blood vessels and are proven to predict CVD outcomes; however, this is a difficult process and outcomes are operator dependent. Conversely, serological biomarkers are easy to measure and they are directly associated with disease activity, however there is no good evidence that they predict CVD outcome. Therefore a combination of both groups would be best.

Professor Rahman highlighted that imaging biomarkers include vascular ultrasound, coronary CT, electron beam tomography, cardiac MRI and, most promising, ultrasound. Ultrasound is non-invasive, there is no radiation, it's fast and easy, results from multiple groups show that 30-40% of SLE patients with no symptomatic CVD have carotid plaque, and multiple outcomes such as thickness, area, and echolency can be measured, all of which are attractive. The best evidence comes from a cohort of 394 American women with SLE who were followed for 8 years. Baseline intramedial thickness and place were measured and followed up, demonstrating a 0.05 mm increase in baseline in baseline intima-media thickness increases risk of CVD by 1.4 fold and the presence of plaque at baseline gives 4.26-fold increase in risk of CVD.⁹⁰

Professor Rahman noted that serological risk factors include anti-phospholipid antibodies, anti-apolipoprotein A1 antibodies, endothelial microparticles and invariant natural killer T cells. Anti-phospholipid antibodies are present in 25-30% of patients with SLE, and they increase the risk of CVD (stroke more than MI) and can have direct effects on the endothelium. However, Farzaneh-Far *et al* (2006) found no increase in carotid artery plaque in aPL-positive compared to aPL-negative patients with SLE.⁹¹ Moreover, aPL levels do not vary with disease activity. Conversely, anti-

apoprotein A1, part of HDL, may be more promising as it is protective against CVD. IgG anti-apoA1 has been found in 21% of patients with acute coronary disease. In RA patients, elevated IgG anti-apoA1 levels have been associated with increased risk of developing CVD,⁹² as they have in SLE. However, it has been found that IgG anti-apoA1 are present in the serum of 27% of patients within the first year of diagnosis of SLE, but levels vary with disease activity and were higher in patients on high dose corticosteroids but lower in those on HCQ, yet there was no association with CVD or mortality.⁹³ Professor Rahman, continued to describe the role of endothelial microparticles (EMP) (1 μm), which are released from activated endothelial cells and have biological effects on clotting and the vascular wall. The presence of these cells is raised in patients with diabetes, hypertension, SLE and APS as measured by FACS analysis. Parker *et al* (2014) studied 27 patients with active SLE before treatment then 4-5 months following treatment, measuring vascular function with flow-mediated dilation (FMD).⁹⁴ The study found that EMPs were higher in patients than in control, yet FMD was lower in patients than in controls, implying dysfunction of vascular health. However, after treatment (24 steroids, 14 immunosuppression, 13 rituximab) activity fell, EMP fell and FMD rose. Finally, Liz Jury's group studied iNKT cells, a rare cell comprising less than 1% of peripheral blood mononuclear cells, which are the only T cells that are stimulated by lipids via CD1d and have proven to be important in autoimmunity and atherosclerosis in mouse models.⁹⁵ A study of 100 patients with SLE and no history of CVD compared iNKT cells from 30 patients with SLE and plaque, 35 patients with SLE and no plaque and 50 controls.⁹⁶ The iNKT from SLE-P were more numerous and with anti-inflammatory phenotype compared to SLE-NP. It was also found that iNKT differentiated in the presence of SLE-P serum can polarize monocytes towards protective M2 phenotype. It was found, paradoxically, more iNKT cells protect against plaque. Professor Rahman explained the paradox, noting that there are some, but not many, iNKT cells in patients with no plaque and the cytokines they produce are making macrophages migrate to the M1 phenotype, then you get asymptomatic plaque that switches on the immune system to reverse the process (eg. like a fire brigade coming to put out a fire before it takes hold), ie. if there is no problem the 'fire brigade' does not respond. Those patients who progress to full blown plaque have been failed by the 'fire brigade', ie. the fire became too big to extinguish, full-blown CVD is established and the iNKT cells give up. (Figure)

Figure. iNKT paradox.



Professor Rahman concluded by stating that there is no established way of using biomarkers of CVD risk in SLE. However, in future, biomarkers could help us target patients for management of conventional risk factors and disease activity and both imaging and serological biomarkers could be used to achieve this.

Plenary I: Biomarkers in SLE Update

Renal biomarkers: Rich Furie

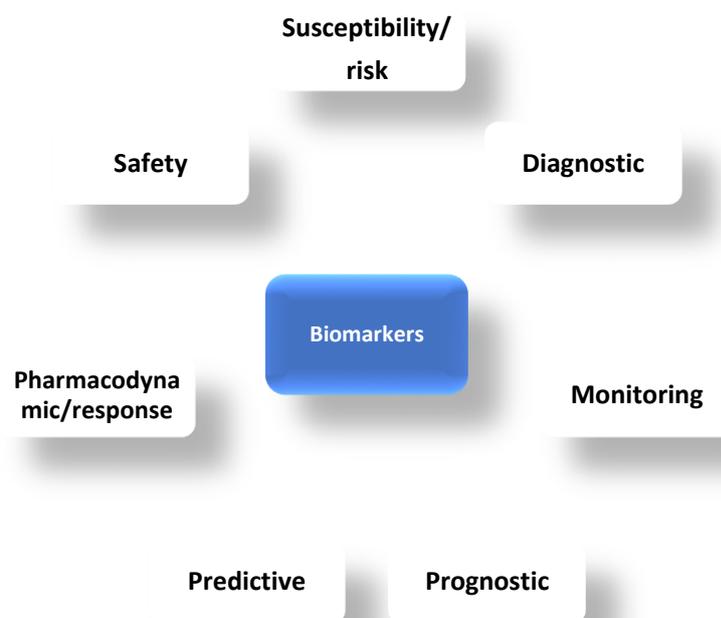
Professor Furie highlighted the need for biomarkers in the treatment of lupus nephritis before presentation potential biomarkers that could prove to be of clinical value in patients with lupus nephritis.

Professor Furie began by highlighting the challenge of identifying renal biomarkers and the definition of a biomarker per se, as well as current needs for biomarkers that predict disease, facilitate targeted treatment, predict response to induction treatment, correlate well with disease activity and predict long-term outcomes.

Since 2008 there have been several discussions between the FDA and NIH, culminating in BEST “Biomarkers, EndpointS, and other Tools”, and a definition of biomarkers and biomarker types, which Professor Furie adopted the structure for his presentation (Table 1)

“A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions. Molecular, histologic, radiographic, or physiologic characteristics are types of biomarkers. A biomarker is not an assessment of how an individual feels, functions, or survives”

Figure 1. Biomarker types



Susceptibility/risk biomarkers

Susceptibility/risk biomarkers are defined as a biomarker that indicates the potential for developing a disease or medical condition in an individual who does not currently have clinically apparent disease or the medical condition. Several risk factors exist for lupus nephritis, including demographics (age and race),⁹⁷ genetic polymorphisms (4q11-q13; 16p12; 6p 22),⁹⁸ epigenetic modifications (HIF3A, IFI44, PRR4)⁹⁹ and, importantly, autoantibody profile (anti-DNA, anti-C1q, anti-Sm).

Diagnostic biomarkers

Diagnostic biomarkers are defined as biomarkers used to detect or confirm presence of a disease or condition of interest or to identify individuals with a subtype of the disease. For example, urinalysis is a simple test that is widely used. The SLICC group studied autoantibody profiles in 308 SLE patients with anti-C1q in combination with anti-dsDNA and found low complement strongly correlated with lupus nephritis (OR 14.9;95% CI 5.8–38.4;p<0.01).¹⁰⁰

Monitoring biomarkers

Proteinuria

Monitoring biomarkers are measured serially for assessing status of a disease or medical condition or for evidence of exposure to (or effect of) a medical product or an environmental agent. The main goal in lupus nephritis patients is to reduce proteinuria with complete response being <0.5. Professor Furie continued to describe a ‘sobering’ publication by Malvar et al (2015), which correlated histologic and clinical responses in 69 patients with Class III (29%) and IV (71%) lupus nephritis managed with IV CYC or MMF, steroids, ACE/ARB and antimalarials.¹⁰¹ Taking two biopsies 6 months apart, they found that 41% achieved complete remission (Pr/Cr <0.5). However, approximately half of those achieving complete remission still had disease activity according to the NIH activity index and one fifth of patients in histologic remission still had proteinuria. This study demonstrates a clear discordance between what is deemed response to treatment and histologic activity.

Anti-DNA antibody

Professor Furie then highlighted the LUNAR study by Rovin et al (2012) demonstrating the association between anti-dsDNA antibody response and good Pr/Cr ratio.¹⁰² Another study from the mid-1990s, launched a clinical trial revolution in lupus nephritis when looking at prevention of renal flares in patients with reductions in anti-dsDNA antibodies.¹⁰³ The studies showed a fivefold reduction in flares in patient with anti-dsDNA antibodies compared with those who did not.

Complement

In the USA, there is an assay used to detect cell-bound complement activation products (CB-CAPS) for diagnostic purposes. C4d has been shown to bind to different cell types including erythrocytes, platelets, B cells and T cells. A correlation between CB-CAPs and complement activation has been shown as well as CB-CAPs being present at higher levels in SLE patients. A study across four centres was conducted to assess this.¹⁰⁴ The study found a strong correlation between erythrocyte C4d and UPCR as well as correlations between C3, C4 and anti-c1q, but not anti-dsDNA. This test is now available for SLE patients in the US.

Urinary biomarkers

There is a lot of literature and a lot of candidate biomarkers (eg. TIMP-1, PAI-1, PF4, vWF, IL-15, adiponectin, sVCAM-1, IL-6¹⁰⁵, MCP-1¹⁰⁶, NGAL¹⁰⁷ and TWEAK.¹⁰⁸

TWEAK is linked to inflammation and fibrosis in patients with lupus nephritis. It acts through its receptor, fn14, which is upregulated in the kidney, but not expressed on T or B cells. A study of a TWEAK-inhibitor (BIIB023) in 188 patients with lupus nephritis showed no

correction between clinical and biomarker response, despite a reduction in urinary TWEAK.¹⁰⁹

Prognostic biomarkers

Prognostic biomarkers are used to identify likelihood of a clinical event, disease recurrence, or progression in patients who have the disease or medical condition of interest. The ALMs study demonstrated that normalising C3 or C4 and proteinuria by Week 8 was a predictor of renal response at 24 weeks.^{110, 111} Kidney biopsy shows predictors of renal failure to be age, male, BP, proteinuria, or proliferative nephritis.¹¹² Importantly, kidney biopsy highlights severe activity and chronicity; Class IV nephritis; electron dense deposits, cellular crescents and interstitial fibrosis as key to future progression of nephritis.¹¹³⁻¹¹⁵ Likewise, combinatorial urine biomarkers LFABP (Liver-type Fatty Acid Binding Protein), MCP-1, albumin, and transferrin also help predict long-term outcomes, but more work is needed in this area.¹¹⁶

A study by Dall'EraM et al looked at data from the Euro-Lupus Nephritis Trial to evaluate the performance of proteinuria, Cr and urinary red blood cells as predictors of good long-term renal outcome.¹¹⁰ Data were analysed at 3, 6, and 12 months post-randomisation to predict good long-term renal outcome (defined as a serum Cr value <1.0 mg/dL at 7 years) and showed:

- 52/76 subjects (68%) had Cr <1.0 mg/dL at 7 years
- **Proteinuria <0.8 g/day at 12 months is the single best predictor of good long-term renal function (sensitivity 81% and specificity 78%)**
- Addition of serum Cr to proteinuria as a composite predictor did not improve the performance of the outcome measure
- Addition of urinary RBCs as a predictor significantly decreased the sensitivity to 47%

Predictive biomarkers

Predictive biomarkers are used to identify individuals who are more likely than similar individuals without the biomarker to experience a favorable or unfavorable effect from exposure to a medical product or an environmental agent. Predictive biomarkers are supported by the outcomes of several studies. The abatacept lupus nephritis trial showed that nephrotic patients had greater response rates¹¹⁷, LUNAR found a suggestion of greater effects in African American and Hispanic patients,¹⁰² and BLISS-52 and 76 found greater effect size in patients with high disease activity.¹¹⁸ Professor Furie also presented Phase 2 data from the anifrolumab study, which showed interferon high may be a biomarker for predicting response in patients with lupus nephritis.¹¹⁹

Pharmacodynamics response biomarkers

Pharmacodynamic/response biomarkers are used to show that a biological response has occurred in an individual who has been exposed to a medical product or an environmental agent. Despite many successes in clinical trials there have been few clinical responses. For example, abetimus sodium's 40% reduction in anti-DNA Ab did not translate into a clinical response.¹²⁰ Rituximab treatment has resulted in a depletion of CD20 B cells and 70% reduction in anti-DNA Ab, yet the EXPLORER and LUNAR trials did not meet their endpoints.¹⁰² Likewise, TWEAK, anti-CD40L, Phase I studies of eculizumab and belimumab have all shown biological activity, but not all translate in to clinical improvement.

Safety biomarkers

Safety biomarkers are measured before or after an exposure to a medical product or an environmental agent to indicate the likelihood, presence, or extent of toxicity as an adverse effect. However, there are none for SLE. Yet SLE trials do have safety issues, including deaths and opportunistic infections.¹²¹⁻¹²³

Professor Furie concluded by saying biomarkers are more than science, it's also about changing the behaviours of physicians, a lesson which can be learned from oncology. Science aside, there is an art to taking care of patients with lupus.

Plenary I: Biomarkers in SLE Update

CNS biomarkers: Raquel Faria

Dr Faria reviewed the need for CNS biomarkers, highlighting the heterogeneity of the disease, risk factors for neuropsychiatric SLE (NPSLE) and the why many antibodies and cytokines have been studied for NPSLE, only few are validated for use in clinical practice.

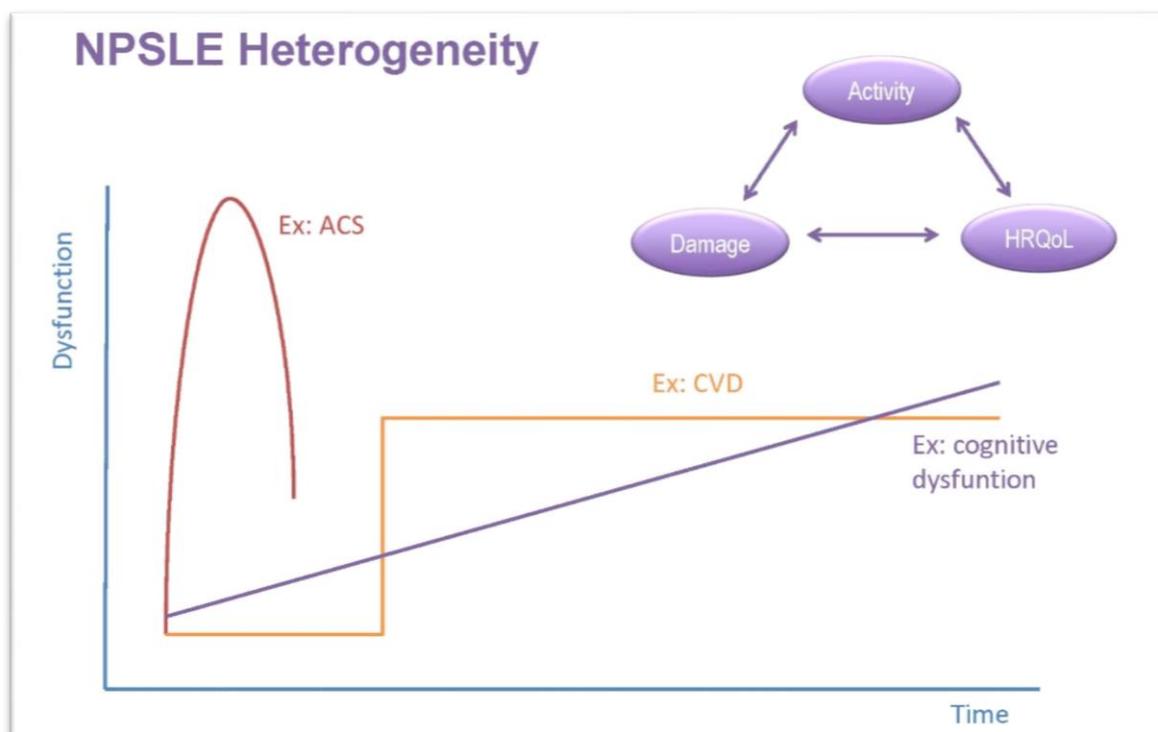
Dr Faria questioned why there are so few CNS lupus biomarkers, compared with lupus nephritis biomarkers. Despite its first description by Kaposi in 1872, NPSLE remains the most clinically challenging visceral involvement of lupus, with a lack of standardised markers. Moreover, NPSLE affects approximately 60% of all SLE patients and is manifest in several heterogeneous syndromes, including neurological and psychiatric manifestations.

NPSLE events may precede, coincide, or follow the diagnosis of SLE. 50–60% of events happen within a year of diagnosis, in the presence of generalised disease activity this is 40–50% and when restrict models of attribution are applied 8-13% of events are due to SLE itself. Risk of an NPSLE event is five time greater with generalised SLE, if there is a history of NPSLE (cognitive or seizures) or aPL (CVD, seizures, chorea).¹²⁴⁻¹²⁷

NPSLE syndromes

The ACR Classification Criteria for SLE list 19 syndromes across CNS and PNS. Looking the cause of any one of these syndromes is challenging, disease activity, damage and HRQoL of the patient are all assessed.¹²⁶ Dr Faria, highlighted the different ways in which NPSLE symptoms manifest themselves. For example, acute confusion syndrome manifests as a reduction in all mental capacity such as cognition, memory, thought, but if the generalised activity is treated the dysfunction quickly reduces. Conversely, cerebral vascular disease, results in a increase in dysfunction which is sustained over time prolonged, Finally, some cognitive function, increases overtime and the patient only become symptomatic after a couple of years (Figure).

Figure. NPSLE heterogeneity



NPSLE pathophysiology

Dr Faria focused on the seven major the CNS syndromes including aseptic meningitis, cerebrovascular disease, movement disorder (chorea), seizure disorders, acute confusional state and major cognitive dysfunction, psychosis; excluding myelopathy, headache, anxiety disorder, cognitive function and mood disorder. Presenting the work of Hanley et al (2014) Dr Faria focused on the pathophysiology of NPSLE.¹²⁸ Dividing the symptoms into focal and diffuse disease (or a mix of both), Dr Faria looked at the contribution of SLE to these states including primary SLE, secondary SLE (eg. SLE therapy, infection) and concurrent non-SLE neuropsychiatric disease (eg. diabetes). It is not possible to separate the symptoms by clinical exclusion to identify primary SLE as the cause of the symptoms, therefore there is a need for biomarkers.

Dr Faria highlighted the biomarkers in NPSLE (ie. antibodies, cytokines, genes—in serum, CSF) and the tests that complement these (ie. psychometrics and imaging). Focal NPSLE has been shown to be related to aPL, with mainly vascular syndromes, and also immunocomplexes and leukostasis, with vasculopathy and thrombosis being the main issues. Diffuse SLE is driven by systemic production of antibodies, such as aPL, antiendothelial cells and complement, which result in dysfunction of the blood-brain barrier. However, other factors, such as infection, fever, and hypertension also disrupt the blood-brain barrier and result in anti-bodies passing to the CSF, resulting in pro-inflammatory feedback.

NPSLE pathophysiology (antibodies)

aPL, antiendothelial and anti-MAP2 have been shown to disrupt the blood-brain barrier as well as playing a role in diffuse NPSLE.¹²⁹ For focal NPSLE, aPL and anti-endothelial antibodies play a role,¹³⁰ but also in seizures aCL and anti-ribosomal P have been found to be present in blood and CSF and anti-NR2 in serum and could be related to seizures in the first year of diagnosis.^{131, 132} Likewise, aCL, aPL and ANA are present in CVD and aCL (blood) and anti-ribosomal P (CSF) are present in chorea. For diffuse NPSLE, anti-NMDA (NR2) is one of the more promising antibodies, with some studies showing that anti-dsDNA could cross-reactive with NR2, mimicking NR2 activity.¹³³⁻¹³⁵ Cross-reaction has also been observed with anti-ribosomal P and anti-Sm, resulting in apoptotic neuronal death.¹³⁶ Anti-SSA subtypes (anti-TROVE2) are also related to NPSLE pathophysiology.¹³⁷ Studies have also shown that psychosis is related to the presence of anti-ribosomal P (serum) and anti-alfaGDI (serum), acute confusional state related to aCL (serum) and anti-ribosomal P (CSF) and the association of major cognitive dysfunction with aPL, aCL, LA, anti-NMDA and anti-NR2 present in serum.^{131, 138}

NPSLE pathophysiology (cytokines)

Cytokines have been shown to have a major role in the dysfunction of the blood-brain barrier. There are some studies that show the role of interferon 1 signature in disease pathogenesis,^{139, 140} TWEAK has also been associated with cognitive impairment,^{141, 142} neuron specific enolase has been shown to be lower in patients with SLE and elevated ubiquitin, which plays a role in regulation of apoptosis, has been linked to neural degeneration.

NPSLE pathophysiology (genetics)

Genes associated with the risk of NPSLE include: TREX1, an interferon regulator gene exhibiting several polymorphisms associated with high NPSLE risk;¹⁴³ TRPC6, which is involved in the regulation of NMDA and may be protective against NPSLE;¹⁴⁴ TCF4 is associated with reduction in spontaneous germinal centre reaction¹⁴¹ and VEGF polymorphisms have been found to be high in patients with NPSLE.¹⁴⁵

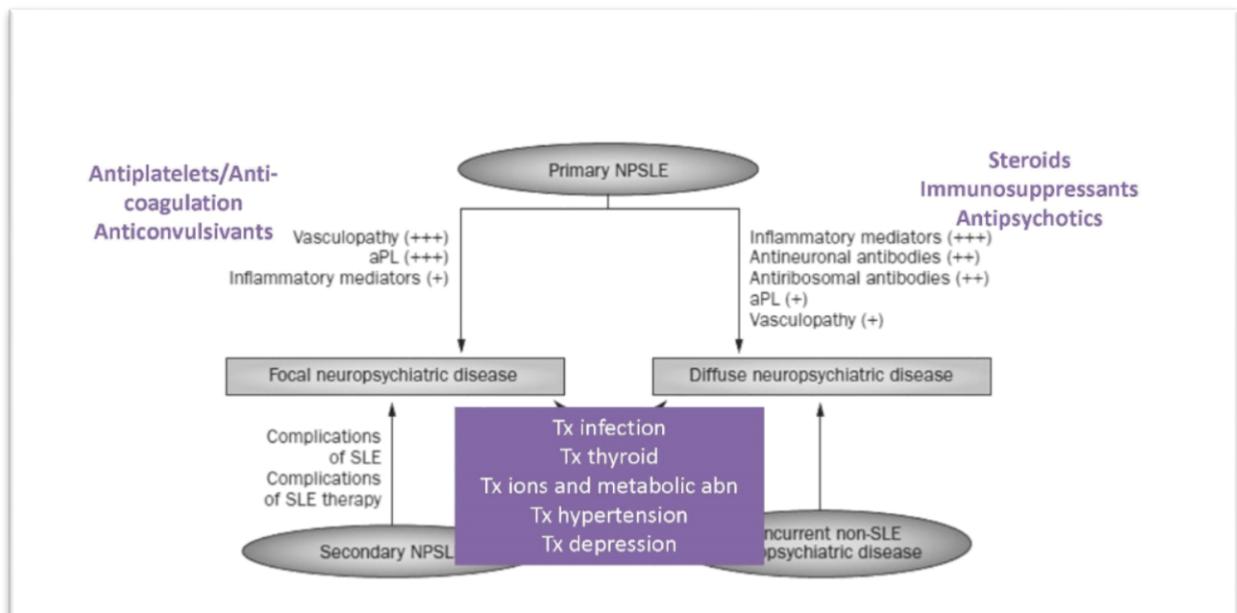
NPSLE: “Minor” psychiatric events

Mood disorders such as HRQoL-related anxiety, depression and suicidal ideation are all raised in patients the NPSLE.¹⁴⁶⁻¹⁴⁹ Coping mechanisms appear to be weaker in patients with NPSLE, who are more prone to insomnia and perceived stress.^{150, 151} Studies are ongoing to identify a personality trait associated with NPSLE. Cognitive impairment, with brain atrophy (mainly anti-NMDA-related and in the hippocampus and amygdala) and attention disorders are evident in NPSLE.¹⁵²

Biomarkers for NPSLE “minor” psychiatric events include anti-ribosomal P (serum) and antiβ2GPI for mood disorders (not anxiety) and APL, aCL, LA and anti-NMDA (serum) for headache, which is also associated with diminished grey matter.

Dr Faria summarised by noting that CNS biomarkers are indicators of an association between neuropsychiatric events and SLE rather than predictors. There is a need for more data. Allthesame, patients with NPSLE need effective treatment; Dr Faira concluded by presenting an overview of the recommended EULAR management guidelines for NPSLE (Figure).¹²⁷

Figure. Recommended management of NPSLE (EULAR 2010)



Plenary II: Management of SLE

Drug-induced lupus: Rober Rubin

Professor Rubin reviewed the similarities and differences between drug-induced lupus and SLE, highlighting the complexities of differentiating the two, while explaining the oxidative metabolism of lupus-inducing drugs and drug metabolites that disrupt immune tolerance ‘machinery.’

Professor Rubin began his presentation by defining drug-induced lupus as the idiosyncratic side effect of various medications in which symptoms and signs overlap with those of SLE and is fully reversible by discontinuation of the medication. The significance of drug-induced lupus is that it exposes the autoimmune propensity of the adaptive immune system.

Guidelines for the diagnosis of drug-induced lupus recommend the continuous treatment with a known lupus-inducing drug for at least 1 month and usually much longer, which complicates implicating the role of the drug in the disease process.¹⁵³ Presenting symptoms and the laboratory profile of drug-induced lupus are highlighted in the following Table.

Table. Presenting symptoms and laboratory profile for drug-induced lupus

Presenting symptoms	
Common	arthralgias, myalgias, malaise, fever, serositis, polyarthritis
Rare	vasculitic rash or other dermatologic problems, glomerulonephritis
Laboratory Profile	
Common	ANA which are due to anti-histone or -nucleohistone antibodies especially IgG anti-[(H2A-H2B)-DNA], anti-ssDNA, increased ESR, pANCA
Uncommon	leukopaenia, thrombocytopaenia, mild anaemia
Rare-absent	antibodies to native DNA*, Sm, RNP, SS-A/Ro, SS-B/La, hypocomplementaemia

Improvement and resolution of symptoms within days or weeks after discontinuation of therapy; serologic abnormalities, especially autoantibody levels, often require months to resolve

Similarities and differences

Professor Rubin continued to highlight similarities and differences between drug-induced lupus and SLE, noting cutaneous, CNS, nephrotic and haematological manifestations being lower in patients with drug induced lupus as compared with SLE. Likewise, some serological markers (eg. anti-chromatin, antihistone) are similar in drug-induced lupus whereas others (eg. anti-sm, anti-dsDNA, hypocomplementaemia) are lower in drug-induced lupus. Given this it is difficult to distinguish drug-induced lupus from idiopathic SLE. Features suggesting SLE, rather than drug-induced lupus include symptoms like malar or discoid rash, photosensitivity, oral ulcers, alopecia, renal disease, neurological disorders, or laboratory parameters such as hypocomplementaemia, autoantibodies to native DNA, Sm, RNP, SS-A/Ro, SS-B/La, and a history of rheumatologic disorders.

Drug involvement

Drugs associated with the induction of lupus-like disease are numerous and include some antiarrhythmics, antihypertensives, anti-inflammatories, anticonvulsants, diuretics and

antipsychotics, statins, anti-thyroidals and others. Symptoms and signs of drug-induced lupus include pleuropericarditis resulting from procainamide or statins and polyarthrititis resulting from quinidine and minocycline use. Other abnormalities occurring in the presence or absence of frank drug-induced lupus are outlined in the following table.

Signs	Drugs
Cytopenias	hydralazine, isoniazid, statins
Glomerulonephritis	hydralazine
Haemolytic anaemia	methyldopa
Hepatitis	minocycline, atorvastatin, sulfasalazine
pANCA	hydralazine, propylthiouracil, minocycline
Anti-histone and anti-ssDNA antibodies	Many drugs

Differences between drug-induced lupus and drug hypersensitivity

Lupus patients tend to have a predisposition to reacting to many medications, resulting in: exacerbation of SLE, drug-induced haemolytic anaemia; autoimmunity associated with small molecule immune-modulating therapeutics or macromolecular immune-modulating biologics; drug-induced autoimmune hepatitis; and drug hypersensitivity.

There are some important differences between drug-induced lupus and drug hypersensitivity.

1. Drug hypersensitivity includes disease mediated by drug-altered B-cell epitope, T-cell epitope, or MHC, whereas in drug induced lupus autoantibody binding unaffected by drug; drug-specific T cells not associated with autoantibodies.
2. With drug hypersensitivity there is a rapid recurrence of symptoms upon challenges, whereas with drug-induced lupus once autoimmunity has waned, drug re-introduction restarts the induction period.
3. Symptoms are triggered by low or transient drug exposure, when a patient is hypersensitive, whereas with drug induced lupus there is a strong correlation between the development of autoimmunity and accumulative dose

Epidemiology and genetic factors in drug-induced lupus

Retrospective studies have reported that 10–12% of patients initially diagnosed with SLE actually have drug induced lupus. Drug induced lupus is 2–4x more likely in women (procainamide, hydralazine) and there is higher propensity in those of Caucasian “race”. Association between those with HLA-DR2 and -DR4 (minocycline, hydralazine, penicillamine, atorvastatin) has been found, as with the C4A or C4B null allele and, importantly, there is an association with slow drug acetylator phenotype.

Professor Rubin, noted that it is unlikely that drug-induced lupus is directly mediated by the ingested medication itself, for the following reasons:

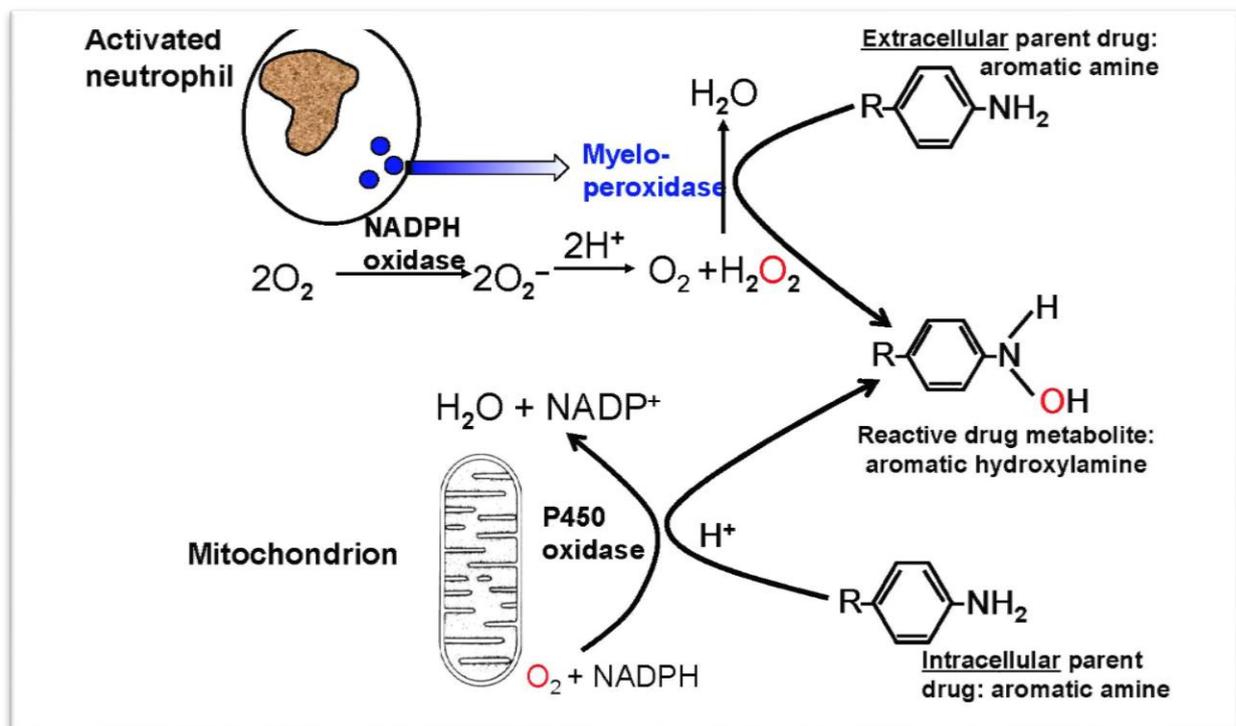
- Lupus-inducing drugs are highly diverse in chemical structure and pharmacological action, yet the laboratory and clinical features of lupus induced by most drugs are essentially the same

- Except for their pharmacological action, lupus-inducing drugs are largely inert at normal doses. Drug-induced lupus is an idiosyncratic drug reaction not predicted by any known property of the implicated drugs
- Drugs reach steady-state concentrations within a few hours, but drug-induced autoimmunity and lupus require many months or years of continuous treatment for manifestation

Oxidative metabolism of lupus-inducing drugs

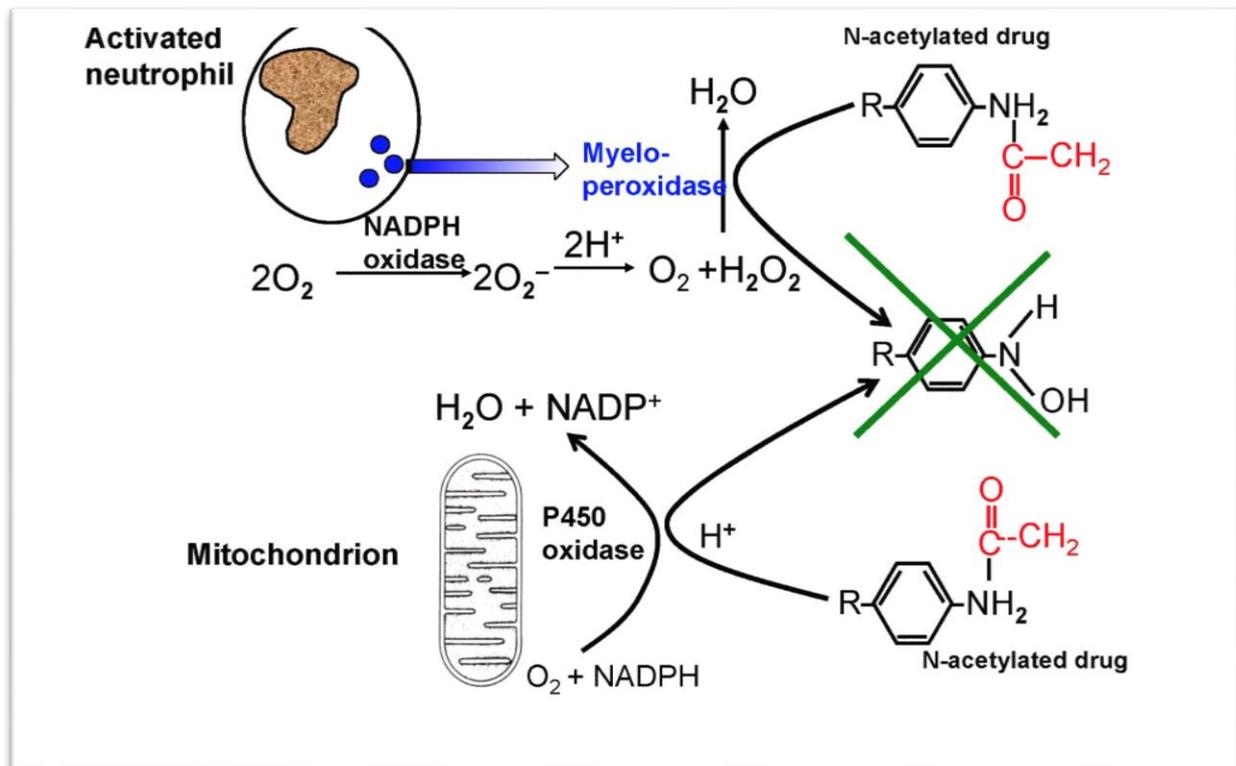
Professor Rubin summarised the oxidative metabolism of lupus-inducing drugs (Figure).

Figure. Oxidative metabolism of lupus-inducing drugs



Upon activation, neutrophils produce superoxide anion (O_2^-) in the extracellular environment through the ectoenzyme NADPH oxidase. O_2^- spontaneously or enzymatically dismutates to hydrogen peroxide (H_2O_2). Neutrophil degranulation often follows, releasing myeloperoxidase (MPO) from azurophil granules. If a drug with an appropriate functional group is present, it will participate in electron transfer with the H_2O_2 -MPO intermediate, resulting in incorporation of an oxygen atom. Another mechanism leading to the same end-product occurs when hepatic NADPH-dependent cytochrome P-450 mixed-function oxidases catalyze the reduction of molecular oxygen (O_2), resulting in the incorporation of an oxygen atom into the functional group of the drug. In this example a hydroxylamine-reactive group is created (Figure).

Figure. Acetylation by N-acetyl transferases blocks N-oxidation



Neutrophil-dependent drug cytotoxicity measures the capacity of the indicated drug in the presence of activated neutrophils to affect the viability of a target cell line and is expressed as the percent of cells killed by the drug metabolite. Inhibition of MPO activity measures the capacity of the drug to act as a competitive inhibitor of the enzymatic activity of MPO. Both *in vitro* assays were measured at a drug concentration of 10 μM . Drugs are grouped as pairs with the incidence of drug-induced lupus indicated for the upper drug, while the lower member being the an inactive analogue or metabolite.

Possible mechanisms in drug induced lupus

Professor Rubin, continued to note the possible mechanisms in drug induced lupus, including:

1. Immune response to drug-modified self-molecule initiates autoimmunity
2. Drug metabolite-specific T-cell responses spread to autoantigens
3. Cytotoxic drug metabolites release autoantigens that induce autoimmunity
4. Non-antigen-specific activation of lymphocytes by drug leads to autoimmunity
5. Drug metabolites disrupt immune tolerance machinery

Highlighting drug metabolites that disrupt immune tolerance machinery (point 5), Professor Rubin explained that all T-cells undergo a selection process, in which pre-T-cells must engage with their T-cell receptor self-MHC. Noting that all T cells are potentially autoreactive as a result of T-cell development in the thymus, Professor Rubin explained the hypothesis that drug metabolites disrupt acquisition of tolerance during positive selection, highlighting a study, which showed the appearance of autoreactive T cells after exposure of thymuses to procainamide-hydroxylamine *ex vivo*, and autoimmune response after injection of procainamide-hydroxylamine into the thymus of B6D2 mice.^{154, 155}

Summarising, Professor Rubin explained that in contrast to a “normal” situation, in which positive selection results in the appearance of T cells tolerant to the selecting self-antigen, in presence of procainamide-hydroxylamine this process fails so tolerance to the selecting self-antigen is not established, allowing the cells to react with the cognate antigen, proliferate and induce autoantibodies.

Plenary II: Management of SLE

Optimising lupus management in pregnancy: Catherine Nelson-Piercy

Professor Nelson-Piercy provided guidance on managing lupus during pregnancy and breastfeeding, with focus on the importance of pre-pregnancy counselling, understanding the risk factors for adverse pregnancy outcomes in patients with SLE and how best to manage/treat SLE during pregnancy.

Professor Nelson-Piercy began her presentation by stressing the importance of pre-pregnancy counselling and controlling disease prior to beginning pregnancy or fertility treatment for pregnancy, as active lupus affects fertility and also increased the risk of miscarriage and preterm delivery.^{156, 157} Importantly, more harm comes to mother and fetus from uncontrolled disease (preterm delivery, pre-eclampsia, growth restriction) than from the medication used to treat/control lupus.¹⁵⁸ Highlighting a study in patients with rheumatoid arthritis, Professor Nelson-Piercy showed that age, nulliparity, DAS score, NSAID and steroid use were all factors associated with prolonged time to pregnancy, with a dose-dependent effect.¹⁵⁷

Women with lupus need informed, evidence-based, women-centered counselling addressing likely complications, safety and advisability of medications during pregnancy and breast feeding and the importance of disease control. Importantly, clinicians advising pre pregnancy and caring during pregnancy should have expertise in management of the particular condition in pregnancy. Main concerns for the patient and the doctor include: the effect of SLE on pregnancy outcome, the effect of pregnancy on SLE and drugs, both in pregnancy and while breastfeeding.

Pregnancy outcomes in SLE

Several factors affect outcomes in SLE, including disease activity, lupus nephritis (hypertension, renal impairment), anti Ro/La, antiphospholipid antibodies and cardiac and lung involvement.^{159, 160}

Disease activity at conception or first presentation of SLE in pregnancy increased the risk of pre-eclampsia, fetal-growth reduction and pre-term delivery. Pregnancy also increased the risk of disease flares, which are more difficult to diagnose during pregnancy because all of the symptoms of flare are the same as the symptoms of pregnancy. The PROMISE study looked at the predictors of pregnancy outcome in SLE. The study found that the overall adverse pregnancy outcomes was 19%, including fetal death (4%), neonatal death (1%), preterm delivery (9%) and very low birth weight (10%).¹⁶¹ The study also showed non-Hispanic white race was protective (OR 0.45; 95% CI 0.24 to 0.84) and maternal flares, higher disease activity, and smaller increases in C3 level later in pregnancy also predicted adverse pregnancy outcomes. Likewise, patients who were LAC-positive/LAC-negative nonwhite/Hispanic and using antihypertensives, had a 58.0% chance of adverse pregnancy outcomes and fetal or neonatal mortality was 22.0%.

Professor Nelson-Piercy noted the lack of awareness and the importance of the association between neonatal cutaneous lupus and anti-Ro/La antibodies, with 30% of women with lupus being Ro/La positive. These women, need counselling about the risk of complete heart block (2% incidence) and cutaneous lupus (5% incidence), both occurring independent of each other, rarely together. A systematic review found that 86% of women (n=1416) with babies with acute complete heart block were anti-Ro positive, with about half of them being asymptomatic. Professor Nelson-Piercy looked at the use of hydroxychloroquine and the risk reduction of cardiac neonatal lupus. Analysis of three databases (US, UK and France) revealed a reduced recurrence of CHB by 77% with patients taking hydroxychloroquine. Anti-Ro positive patients should be given hydroxychloroquine during pregnancy.¹⁶²

Lupus nephritis also results in complications in pregnancy, such as the increased risk of pre-eclampsia/growth restriction/preterm delivery. Even quiescent lupus nephritis increases risk of fetal loss, especially if hypertensive or proteinuric. The risk of deterioration also is higher with higher serum creatinine and the chance of successful outcome is lower with higher serum creatinine. SLE nephropathy may manifest or be diagnosed for the first time in pregnancy. It is advised to delay pregnancy for 6 months after renal flare. A study illustrates the 30% increased risk of pre-eclampsia in patients with renal involvement during pregnancy.¹⁶³ Likewise, in a study of Italian women with lupus nephritis in complete (49%) or partial remission (27%) at conception, one third of patients had preterm delivery, one third a low-birth weight baby, and one third had a renal flare. These outcomes can be predicted with hypocomplementaemia at conception (RR 19.02; 90% CI 4.58–78.96) or low dose aspirin during pregnancy (RR 0.11; 90% CI 0.03–0.38).¹⁶⁴ Therefore low-dose aspirin is recommended for pregnant patients with lupus. In addition, a systematic review of pregnancy outcomes in 37 studies of patients (n=1842) with SLE and lupus nephritis also showed high rates of unsuccessful pregnancy (23.4%), premature birth (39.4%) and low birth weight (12.7%) as well as high flare rates (25.6%), hypertension (16.3%), nephritis (16.1%) and pre-eclampsia (7.6%) in the mother.¹⁶⁵

Professor Nelson-Piercy highlighted the patient type, who should be advised not to get pregnancy (ie. those with pulmonary hypertension, CKD Stage 4/5, active lupus nephritis and those with severe restrictive lung disease,¹⁶⁶ as well as the drugs which are contraindicated during pregnancy (ie. mycophenolate mofetil [MMF], methotrexate, cyclophosphamide [except 2nd/3rd trimester] and warfarin/new oral anticoagulants).^{167, 168}

Pregnancy management

For pregnancy management, it is important to ensure disease remission is maintained, flares are treated with prednisolone, pregnancy compatible drugs are continued, growth scans are conducted if there is active disease, prescribe low-dose aspirin for APS, APL and lupus nephritis patients, ensure there are no indications for premature/early/Caesarean delivery unless superimposed pre-eclampsia/growth restriction/obstetric indication, and counsel about drugs in pregnancy and breastfeeding (Table).

Table. Drugs in lupus pregnancy.^{167, 168}

Yes (safe)	No (dangerous)
<ul style="list-style-type: none"> • Steroids • Azathioprine • Cyclosporin/tacrolimus • Hydroxychloroquine • Sulfasalazine • Anti TNFα agents • IVIG 	<ul style="list-style-type: none"> • NSAIDs (3rd trim) • Cyclophosphamide (1st trim) • Methotrexate, thalidomide • Chlorambucil • Gold, D-penicillamine • Mycophenolate mofetil • Leflunamide • Rituximab/abatacept

Professor Nelson-Piercy briefly explained the value of the updated British Society for Rheumatology guidelines on prescribing during pregnancy as a reference for all physicians counselling pregnant patients with autoimmune diseases.¹⁶⁹ In addition, Professor Nelson-Piercy presented a study by Fischer-Betz et al (2013) about the management of lupus nephritis patients taking MMF. It is advised that patients switch to azathioprine as it is as good as MMF in maintaining remission in lupus nephritis in women who are pregnant. Risk for adverse pregnancy outcome increased with every mg of prednisone dosage (OR 2.03) and every single unit of SLEDAI score (OR 3.92).¹⁷⁰

The EULAR guidelines provide some key overarching principles for the management of lupus in pregnancy.¹⁷¹

- A. Family planning should be addressed in each patient of reproductive age and adjustment of therapy considered before a planned pregnancy.
- B. Treatment of patients with rheumatic disease before/during pregnancy and lactation should aim to prevent or suppress disease activity in the mother and expose the fetus/child to no harm.
- C. The risk of drug therapy for the child should be weighed against the risk that untreated maternal disease represents for the patient and the fetus or child.
- D. The decision on drug therapy during pregnancy and lactation should be based on agreement between the internist/rheumatologist, gynaecologist/obstetrician and the patient, and including other healthcare providers when appropriate.

Professor Nelson-Piercy highlighted some important common clinical scenarios that challenge the physician treating lupus during pregnancy (Table).

Table. Common challenging clinical scenarios/symptoms.

Nephritic Flare <i>versus</i> Pre-eclampsia	
Symptomatic of Both	Symptomatic of SLE Only
<ul style="list-style-type: none"> • Hypertension • Proteinuria • Thrombocytopaenia • Renal impairment 	<ul style="list-style-type: none"> • Rising anti-DNA titre • RCs or cellular casts in urine • Fall in C3/C4 • No increase in uric acid • No abnormal LFTs • Other features of active SLE
Infection <i>versus</i> Inflammation	
Symptomatic of Infection	Symptomatic of Inflammation
<ul style="list-style-type: none"> • Raised CRP • Raised/reduced WBC • Fever • Response to antibiotics • Raised procalcitonin (sensitivity 70%) 	<ul style="list-style-type: none"> • Normal CRP (↑in pericarditis/lupus pneumonitis/arthritis) • Reduced WC • Fever • Response to immunosuppression

There are also few data on which drugs to use in breastfeeding, although azathioprine is effective and safe, with metabolites not seen in the baby, only the milk.¹⁷² Likewise, tacrolimus is also not absorbed in breast milk.¹⁷³ Importantly, women who have a pregnancy complicated by low birth

weight, preterm baby or pre-eclampsia are at increased risk of subsequent cardiovascular disease, therefore it is important to ask women about pregnancy history to enable assessment of subsequent cardiovascular risk.¹⁷⁴

Professor Nelson-Piercy summarised by highlighting the importance of pre-pregnancy counselling to explain risks and stratify these, noting that the outcome is dependent on disease activity and systemic involvement, that high-risk pregnancies require multidisciplinary care and that many drugs have an acceptable benefit-risk profile for use in pregnancy.¹⁷⁵⁻¹⁷⁷

Plenary II: Management of SLE

Immunisation of patients with SLE: Bevra Hahn

Professor Hahn presented an overview of infection risk in patients with SLE, explaining the key treatment strategies and prophylactic immunization approaches for effectively managing infection risk in patients with SLE.

Professor Hahn opened her presentation with the fact that one-third of patients' die of infection, regardless of duration of disease.¹⁷⁸ She highlighted that infection risk, requiring hospitalisation, is increased by 10-20% in patients with lupus, compared with those who don't have lupus, with pneumonia being markedly higher in patients with SLE. Also, sepsis is now more common in all populations than it was in 1995, a similar observation has been made with skin infections. Conversely, mortality is steadily falling in infection patients with and without lupus.

Several factors increase the risk of infection in SLE including age (>51 years), male gender, black/African American race, lupus nephritis, medication (hydroxychloroquine, immunosuppressives, glucocorticoids, rituximab).¹⁷⁹ Professor Hahn continued to present a study of patients with rheumatoid arthritis requiring hospitalisation for infection whilst being treated with biologics.¹⁸⁰ The study showed that rituximab-treated patients were more likely to be hospitalised for infection, followed by those taking an anti-TNF and those taking abatacept.

Prevention of infection in SLE is clearly important and requires the physician to be vigilant as infections requiring therapy occur in at least 2/3 of immunosuppressed SLE patients. Secondly, consider prophylactic therapies for high-risk patients, and finally immunize or vaccinate these patients.

There are many opportunistic infections that are of particular concern in lupus, including herpes zoster (which could be treated with a vaccine), tuberculosis (which should be screened with a skin test), candida albicans (which is often overlooked and should be treated early), pneumocystis jirovecii (for which prophylactic treatment should be considered) and progressive multifocal encephalopathy (which is common with immunosuppressive treatment, notably rituximab).

Prophylaxis for infections in SLE is possible. For pneumocystis there are no strong efficacy data in SLE, however, there are good data for transplant and HIV, such as trimethoprim-sulfamethoxazole twice a week. However, the problem is increased allergy in SLE to sulfa drugs (and penicillins) and 11% of SLE patients have adverse events. Other drugs include atovaquone 1500 mg/d and can be used in patients with lung disease, asthma etc. Dapsone once a week is also effective. For herpes simplex/vaginal acyclovir 400 mg/d or 800 mg/d works well. Human papilloma virus (HPV), however, is a little more problematic, notably there is increased cervical dysplasia in SLE, possible due to azathioprine, MMF, prednisolone or cyclophosphamide. It is therefore important for SLE patients to have regular PAP smears and use the quadrivalent HPV vaccine in women ≤27 years old, especially with >2 sexual partners: this has shown to be protective in 74% *versus* 93% controls at 7 months.¹⁸¹⁻¹⁸⁴

Professor Hahn continued to present details of what to do to detect latent tuberculosis in SLE patients, highlighting the high specificity of interferon release *versus* tuberculin tests. Should the test be positive and chest X-ray normal, then treatment of latent tuberculosis is required for one month before immunosuppression. Such treatment should include INH plus rifampin once a week for 3 months.

Professor Hahn raised the question 'Is it safe to vaccinate/immunize in SLE?' noting that flares in vaccinated patients are no higher than those in patients receiving placebo (ie. 10-12%).¹⁸⁵⁻¹⁹⁰ There

is, however, some controversy as recombinant HPV4 is associated with a variable risk for induction of 1.0-7.6.¹⁹¹

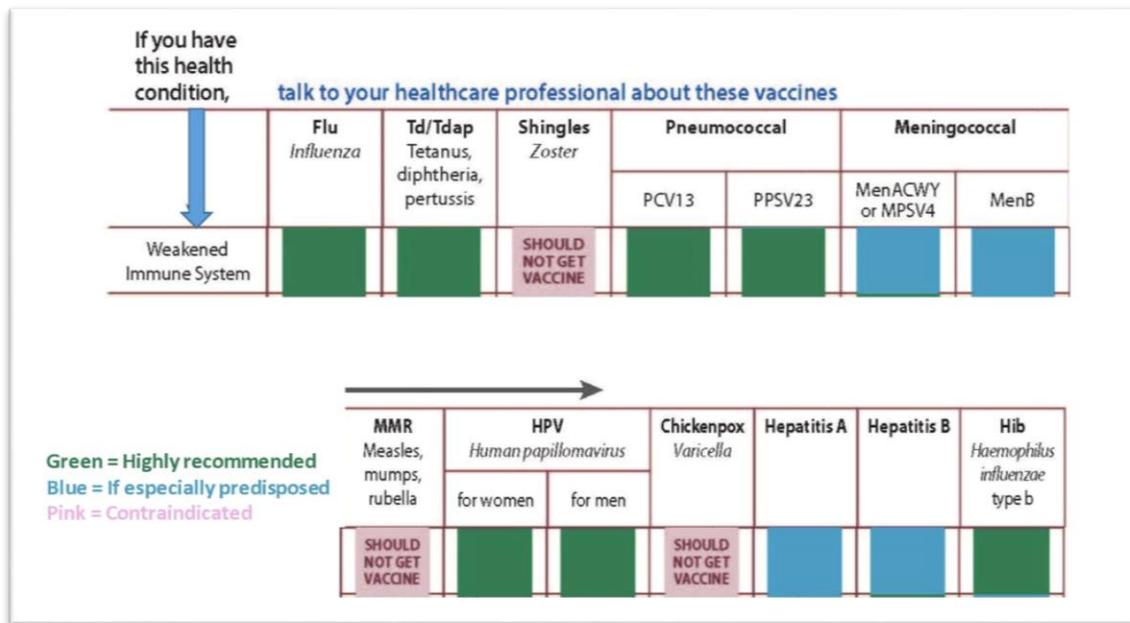
Allthesame, vaccines and immunisations are effective in SLE, especially those who are immunosuppressed. The majority of patients develop antibodies and/or T-cell responses, but they are not as good as in people who don't have lupus; this is primarily attributable to the dose of glucocorticoids plus use of other immunosuppressives, including azathioprine, mycophenolate mofetil and cyclophosphamide. Hydroxychloroquine is NOT associated with lower responses and Rituximab is the biologic most highly associated with lower than normal responses to vaccinations.

Professor Hahn highlighted the recommended vaccinations for patients in SLE.

- Take immunisation history during first visit
- Ideally immunise when disease is clinically stable
- Avoid live attenuated vaccines, especially if prednisolone >20 mg qd or other immunosuppressive
- **Influenza vaccine should be considered for everyone!**
- **PPV23 plus PPV13 at least 8 weeks apart; should be considered for everyone!**
- Tetanus toxoid when due normally: if wound after B cell depletion give tetanus Ig
- **Hyposplenic? Give Infl, PPV, HInflB, meningococcal C – before surgery if possible**
- B cell depletion (rituximab,? belimumab) suppresses response for 6 months
- Consider herpes zoster vaccine (see later discussion) to high risk: negative for HZ antibodies
- Consider HPV in selected patients: women <27 yo with multiple sex partners
- Give Hepatitis A and/or B if pts plan travel to high endemic areas or live there and seronegative
- Travel vaccines as recommended (not live organisms as in yellow fever, BCG, oral polio, oral typhoid)
- BCG is not recommended

There are several vaccines that contain attenuated live organisms and should be avoided in those patients who are immunosuppressed, these include nasal influenza, measles/mumps/rubella, BCG, yellow fever, varicella/herpes zoster, brucella and anthrax. Professor Hahn continued by highlighting the recommended immunisations for adults by health condition (Figure), and also the importance of knowing the geographic distribution of infections.

Figure. Recommended immunisations for adults: By health condition.



Professor Hahn presented the prevalence of herpes zoster in lupus patients, 10-15% in total¹⁹² before explaining the development of a promising new vaccine, containing recombinant varicella-zoster virus glycoprotein E and the AS01_B adjuvant system, which has been shown to reduce the herpes zoster virus by 97.2% compared with placebo.¹⁹²

Professor Hahn summarized her presentation, with a reminder that infections are the cause of death in 1/3 of SLE patients, therefore it is important for physicians to be alert to them. Testing for tuberculosis before starting immunosuppression is important as is consideration of prophylaxis for pneumocystis and herpes simplex. Immunization is important but attenuated live virus vaccines should be avoided.

Plenary III: New Insights in SLE

Where do new oral anticoagulants fit in SLE?: Hannah Cohen

Professor Cohen presented the potential benefits of direct oral anticoagulants (DOACs) compared with VKAs before describing the available data on the use of DOACs in APS patients with SLE and considerations for the appropriate use of DOACs in patients with SLE.

Professor Cohen opened her talk by introducing direct oral anticoagulants (DOACs) as an alternative to warfarin in haemorrhagic a number of indications, but raised the question as to whether they are ok for patients with SLE. Current licensed DOACs include apixaban , edoxaban and rivaroxaban and, the direct thrombin IIa inhibitor, dabigatran.¹⁹³

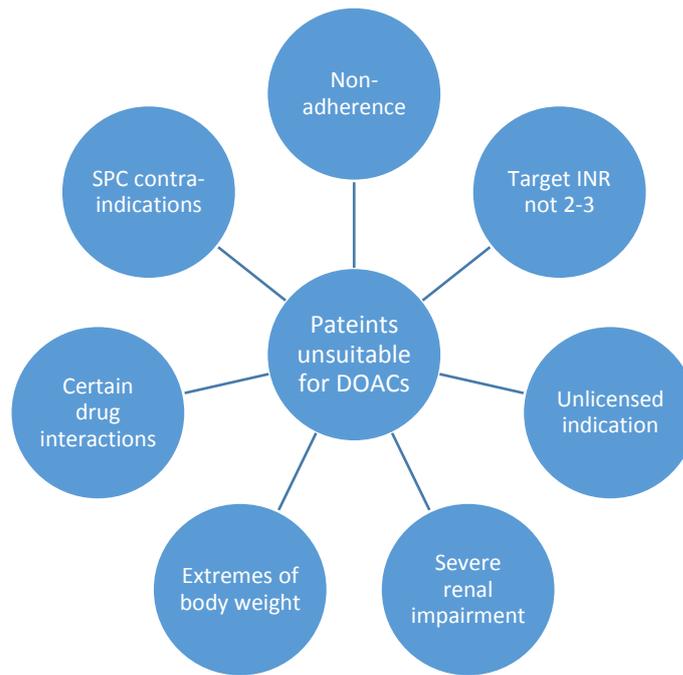
DOACs: Efficacy and bleeding risk

Data from a meta-analysis of >70,000 patients with atrial fibrillation gathered from the pivotal DOAC studies in AF, show that overall the composite outcome of patients taking DOAC was a lower incidence of stroke or systemic embolic events, this was largely driven by a substantial reduction in haemorrhagic stroke.¹⁹⁴ There was also a significant reduction in all-cause mortality, whilst exhibiting a favourable safety profile, with decrease in intracranial haemorrhage, but an increase in gastrointestinal bleeding *versus* warfarin. In another meta-analysis of patients with recurrent venous thromboembolism (VTE), including VTE-related death, DOACs were shown to have similar efficacy to vitamin K agonists, whilst significantly reducing the risk of bleeding.¹⁹⁵ Acknowledging the successes of short-term DOAC usage, Professor Cohen went on to review the use of long-term anticoagulation. Extended use of DOAC for VTE has been studied with apixaban (2.5 mg/bd and 5 mg/bd) and rivaroxaban (10 mg/d and 20 mg/d), with both showing the lower dose DOAC was effective and safe as compared with placebo and aspirin, respectively.^{196, 197}

Patients unsuitable for DOACs

Professor Cohen highlighted the patients who should not be given DOACs (Figure). Highlighting that guidelines state that DOACs should not be used in pregnancy or breast feeding, Professor Cohen explained a study showing a 2.2% incidence of fetal abnormality in patients using DOACs compared with 0.6% in patients with warfarin.^{198, 199}

Figure. Patients who should avoid DOACs.



Reversal of anticoagulant effect

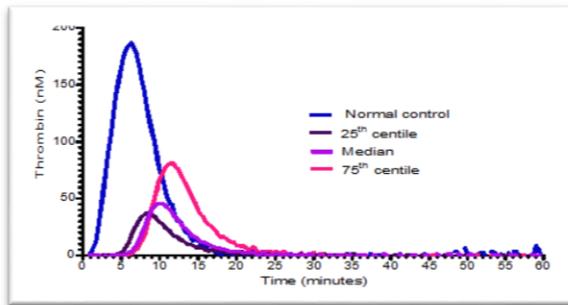
Reversal of the anticoagulant effect of dabigatran with the monoclonal antibody, idaricizumab, has been shown to reverse the anticoagulant effect and provide effective haemostasis in 39/51 patients with serious bleeding.^{200, 201} Anti-Xa inhibitors are also used to reverse the anticoagulant effect. Andexanet alfa is a 'decoy' molecule, modified recombinant human factor Xa, which binds and sequesters factor Xa inhibitors. A study has shown andexanet alfa to provide effective haemostasis in 79% of patients with acute major bleeding.^{202, 203}

Studies in thrombotic APS

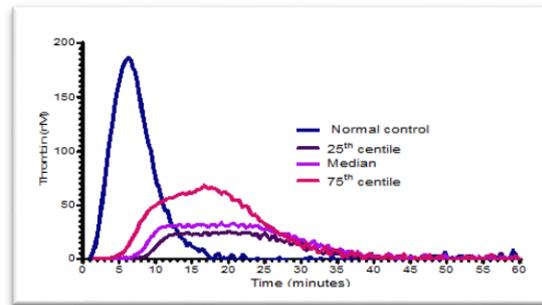
Rivaroxaban in APS, with or without SLE (RAPS) trial was conducted as there was some uncertainty as to whether DOAC use should be extended to APS: aPL is estimated to occur in 10% of VTE patients, but aPL status not systematically documented in DOAC trials, furthermore, it was questioned if aPL could interfere with the anticoagulant action of DOACs. The RAPS trial included patients who required standard intensity coagulation for VTE, and aimed to demonstrate that the intensity of anticoagulation achieved with rivaroxaban (n=57) was not inferior to that of warfarin (n=59) using thrombin generation.²⁰⁴ The primary outcome measure was the percentage change in endogenous thrombin potential (ETP) from randomisation to Day 42. Approximately 20% of patients had SLE, with 25-32% of patients being triple positive for aPL. At Day 42, ETP was found to be higher in the rivaroxaban group than the warfarin group, however, all results were within or below the normal range with the study not reaching the non-inferiority threshold. Conversely, peak thrombin generation was lower with rivaroxaban than with warfarin, and all results were within or below the normal range. RAPS thrombin generation curves revealed a more protracted thrombin generation curve compared to warfarin, suggesting inhibition of the positive feedback of factor Xa on factor 7a generation, because factor Xa is being inhibited by rivaroxaban (Figure).

Figure. RAPS thrombin generation curves.

Warfarin



Rivaroxaban

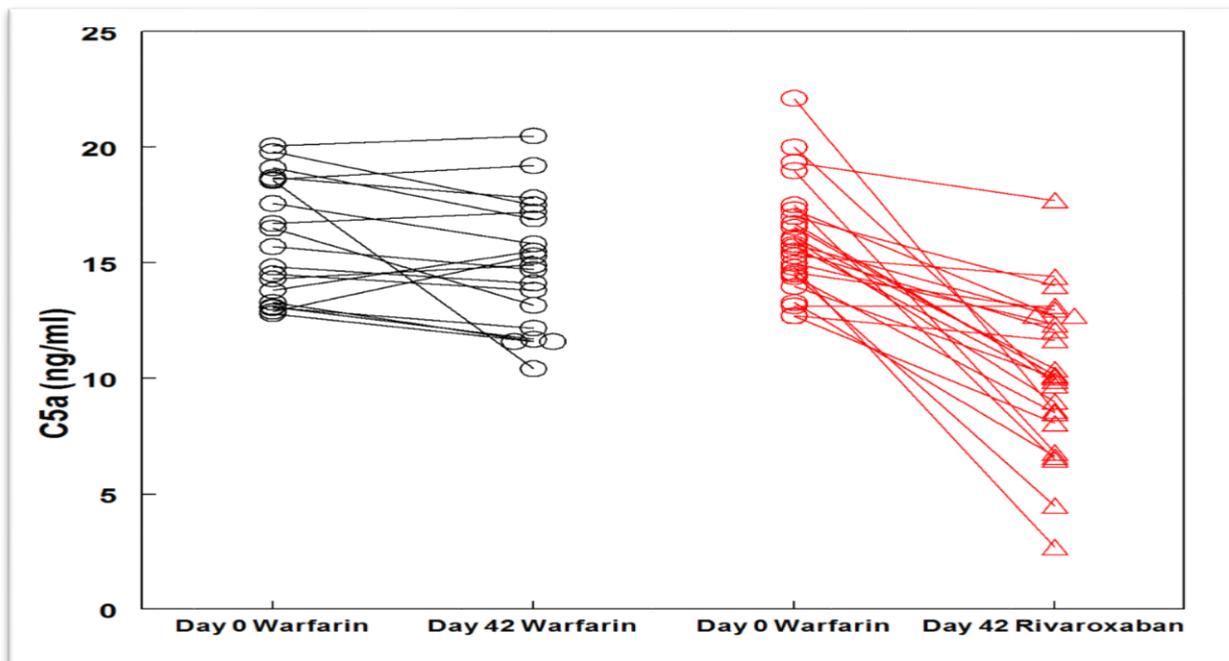


At follow up there were no new thrombosis, no major bleeds and no differences in clinically-relevant or minor bleeding, with better quality of life in the rivaroxaban groups.

High-intensity rivoroxiban (15mg/bd) versus high-intensity warfarin is being assessed in stroke patients with APS (RISAPS) with or without SLE. The primary outcome is rate of change in brain white matter hyperintensity volume on MRI.

Complement levels in APS patients were also been assessed in the RAPS trial, with complement C5a levels significantly decreasing at Day 42 in rivaroxaban-treated patients (Figure).

Figure. Samples with elevated C5a at baseline *versus* Day 42.



Case studies of patients with VTE requiring standard intensity anticoagulation have no recurrent thrombosis on DOACs, yet patients with arterial thrombosis or microvascular thrombosis and those with triple positive APS do not do well. ²⁰⁵

Studies in thrombotic SLE

There is not much guidance/evidence in patients with SLE. However, patients with aPS/aPL at VTE requiring standard intensity anticoagulation should be treated with rivaroxaban 20 mg/d. DOACs should be avoided in patients with recurrent VTE requiring high intensity anticoagulation or arterial or microvascular thrombosis. Those SLE patients with obstetric APS/ asymptomatic aPL should be given rivaroxaban 20 mg/d; however, every patient should be assessed on individual merit and it is important to ensure there is no arterial thrombosis. DOAC use in SLE patients who test negative for antiphospholipid antibodies is reasonable in line with the SPC.

Plenary III: New Insights in SLE

Immunopathology in SLE: The pathogenic engines: Thomas Dörner

Professor Dörner presented insights into the pathogenesis of SLE, describing the altered function of B cells, T cells and plasma cells and how these could be driving the development of SLE.

Professor Dörner began by presenting the immunopathogenic concept in SLE, looking at three sub-phenotypes in lupus including genetic risk factors and questioning how genetic predisposition, linked to epigenetic modulation, leads to modification of the innate immune system, ie. pDc and dendritic cells, and how this translates into the modification of adaptive immunity, resulting in the production of autoantibodies and organ damage.²⁰⁶ There are many genetic risk factors and key immune pathways involved in SLE susceptibility.²⁰⁷ Some risk alleles show an association with clinical sub-phenotypes of SLE, but importantly all risk genes are linked to immune activation pathways. Professor Dörner highlighted an important paper by Zharkova et al (2017), in which the interaction of the immunological pathways leading to SLE is described.²⁰⁸ Dendritic cells lay at the centre of the immune pathway and give T cells instruction to enter a pro- or anti-inflammatory pathway, they also instruct plasmacytoid dendritic cells and B cells (directly), resulting in the production of autoantibodies. There is also a B-cell centric view, of the immunopathology of SLE, on which several traditional and emerging therapies as based, but the timing of certain immune pathways is important too.²⁰⁹

Lessons from recent interventions in SLE

Professor Dörner noted that a lot of lessons have been learned from rheumatic diseases and taking a 'bed to bench' or 'reverse translation' view of these a 'heat map' of targeted therapies for autoimmune diseases can be created (Figure). Much has been learned about key immune pathways for given diseases. However, for lupus there is only one 'successful' pathway for approved targeted therapy.²¹⁰

Figure. "Heat Map" of targeted therapies of autoimmune diseases: Dissecting immune pathways. Blue = successful (approved); Red = failed.



Professor Dörner reiterated that in SLE is a heterogeneous disease and targeting one cell system may not be the answer, given there is more than one component to target (eg. B cell, dendritic cell etc). The two main pathogenic engines in SLE may include (1) B-cells, with their immune amplification functions, and (2) plasma cells, which contribute with autoantibody production and immune complex formation.²¹⁰ None of the existing studies have investigated targeting both B and plasma cells simultaneously.

Arbuckle et al (2003) showed that autoantibody production begins 8-10 years before the time of diagnosis or appearance of the first clinical manifestation of SLE.²¹¹ Moreover, this accumulation of autoantibodies peaks just prior to diagnosis, indicating that early in the disease there is immune tolerance. More recently, it was shown that in the same sample interferon γ was present early in disease, before classification and, thus, accumulation of autoantibodies, whereas interferon α only increased close to SLE diagnosis (within 2 years).²¹² Therefore, it is apparent that breach of tolerance and subsequent appearance of autoantibodies, appearance of type II interferon and then appearance of type I interferon occur at different times prior to SLE manifestation.²¹³

Altered T-cell function in SLE

Given this, Professor Dörner questioned if patients with SLE have an overly activated immune system or impaired immune regulation. Altered T-cell function in SLE has been studied with Tsokos et al (2011) postulating that the composition of the T-cell receptor in patients with lupus is abnormal.²¹⁴ They showed that amplification of B-and T-cell signalling events involved downstream signalling via Syk instead of ZAP-70; that there was an overexpression of PP2Ac, which modulates calcium levels and suppresses IL-2 production; that SLE patients were also deficient in the production of IL-2, which decreases CD8+ T-cell cytotoxicity and increases T-cell longevity in SLE; and CREM- α transcription factor, which suppresses IL-2 production and drives IL-17 expression. Abnormalities of cytokine

production by differentiated subsets of CD4 T cells from patients with SLE and controls, have revealed decreased IL-2 production in naïve CD4 T cells from patients with SLE.²¹⁵ Furthermore, the same study revealed a correlation between low IL-2 production and high SLEDAI score. The key question is whether this relates to the CREM- α transcription factor. Another study showed regulatory T cells deficiency, as shown by reduced CD25 expression, correlated with IL-2 deficiency in SLE.²¹⁶

Altered T-cell function in SLE: Therapeutic immune rebalancing?

An *in vivo* proof-of-concept study by Humrich et al (2015) examined the effect of low-dose IL-2 in SLE patients and found that following four courses of IL-2 markedly reduced SLE disease activity (SLEDAI).²¹⁷ Creatinine, anti-dsDNA and steroid dose were also reduced. Lupus skin manifestations also improved, even on standard of care in follow up. Another study of low-dose recombinant human IL-2 selectively modulated number of regulatory T (Treg) cells, follicular helper T (TFH) cells and IL-17-producing helper T (TH17) cells and was accompanied by marked reductions of disease activity, as shown by SRI-4, SLENA-SLEDAI, as well as reducing the need for steroids.²¹⁸

Altered B-cell and plasma cell function in SLE

Professor Dörner reiterated that there may be two possible engines of autoimmunity—auto-antibody production and overactive B-cells. Siber et al (2014) demonstrated that, in SLE patients, the capacity of B cells to produce cytokines, following TLR engagement, diminishes with increased disease activity, therefore suggesting that B cells become exhausted or tolerant to TLR stimulation with worsening disease.²¹⁹ This raises the question that may be the B cells are overactive because they need to drive B cell receptor activation.²²⁰ Patients with lupus has been shown to have diminished B-cell receptor response to SyK due to poor SyK phosphorylation; conversely, active phosphorylation is observed for Akt.²²¹

Professor Dörner concluded by outlining the remaining challenges to understanding the immunopathology of SLE, including the clinical heterogeneity of the disease, the apparent overactive immune system and the dysregulations of naïve CD4 T and memory B cells (either intrinsic or of secondary nature) and impaired Treg numbers and function. It is clear, however, that inhibiting key nodes of immune activation as well as improving immune regulation in SLE represent attractive therapeutic approaches.

Plenary IV: Therapy Updates in SLE

Where are we know with blocking B-cell activating factors? Ronald van Vollenhoven

Professor van Vollenhoven explained the role of biologics targeting B cells in patients with SLE and described the results of several clinical trials of biologics targeting these B cells, whilst reviewing the implications of these result for clinical trials' design and relevance to clinical practice.

Professor van Vollenhoven began his presentation by giving an overview of the pathogenesis of SLE, noting that there are many cell types that have a role in lupus. Lupus is characterised by multiple autoantibodies which originate from B cells that differentiate into autoreactive plasma cells.²²² The central role for B cells in SLE was identified in a genome-wide study by Hu et al (2008), which identified multiple cells in rheumatoid arthritis, a small role for B cells in Crohn disease, yet with SLE there was predominant transcription and dysregulation of B cells, highlighting their likely role in the disease pathogenesis, hence supporting the rationale for targeting B cells in SLE patients.²²³ Furthermore, there is evidence to suggest that SLE patients can be grouped by blood transcriptome into three subsets: (1) IFN signature negative; (2) IFN signature positive plus B-cell signature; and (3) IFN signature positive plus neutrophil signature, highlighting that also SLE may have different pathophysiologies, B cells are predominant.^{224, 225}

B cells as a target for SLE therapy

Professor van Vollenhoven switched focus to targeting B cells in patients with SLE, noting that one strategy that has been used for many years, but remains unproven is B-cell depletion with anti-CD20 monoclonal antibodies (eg. rituximab, ocrelizumab, ofatumumab), B-cell modulation through CD22 or B-cell antagonism, through cytokine inhibition (eg. tabalumab, blisibimod, atacicept, belimumab). Professor van Vollenhoven continued to present drugs developed to target B cells in SLE patients.

Tabalumab (Phase 3)

Tabalumab is similar to belimumab and was studied in two large simultaneous Phase 3 trials (ILLUMINATE I and II).^{226, 227} Some outcomes were positive, but others were not. The conclusion was that there was not a clear clinical benefit so the development of tabalumab was stopped. Professor van Vollenhoven, noted that the molecule was probably too similar to belimumab to offer new hope.

Blisibimod (Phase 2)

Like belimumab, Blisibimod targets BLISS, but is built up from modular structures. Phase 2 results were promising, with improvements in SRI response,²²⁸ however, in Phase 3 it did not meet its primary endpoint.

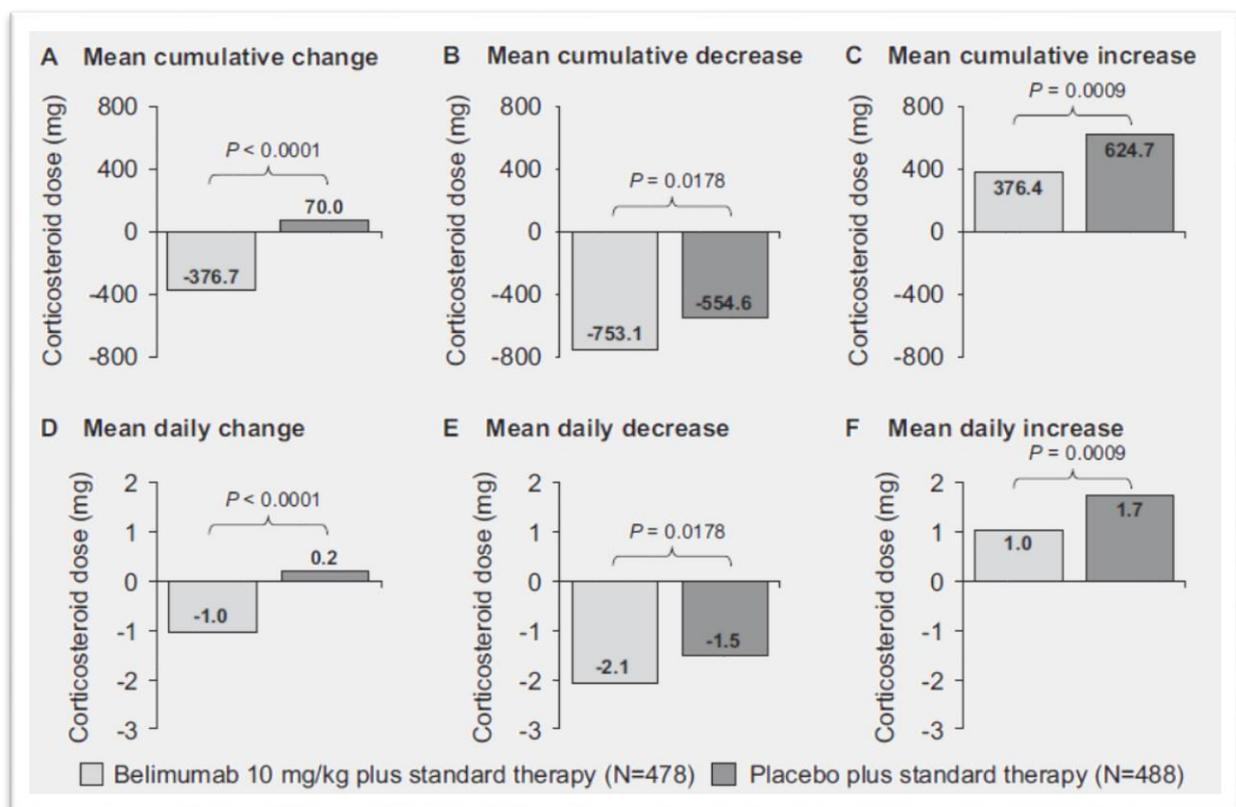
Blisibimod (Phase 3)

Belimumab is the only approved biologic for SLE. Two large Phase 3 trials, BLISS-52 and 76, supported belimumab's approval by the EMA and FDA.^{229, 230} These trials allowed identification of an activated B-cell compartment, with patients exhibiting anti-DNA and low complement (C3/C4) having the highest likelihood of response to belimumab.

Recommendations for targeted treatment of SLE

Professor van Vollenhoven noted that corticosteroids tend to be effective today, but at the expense of the patient's health tomorrow. Therefore, it is recommended that maintenance treatment for SLE should aim for the lowest glucocorticoid dosage needed to control disease and, if possible, glucocorticoids should be withdrawn completely, which begs the question: Can belimumab help achieve this? The BLISS-52 and 76 trials^{229, 230} included patients with active SLE despite background therapy and approximately 75% of these were taking glucocorticoids at baseline. During the trial, patients were allowed to adjust their glucocorticosteroid dose depending on how they were responding to therapy.^{118, 229} The mean cumulative corticosteroid dose over the trial duration (one year) increased significantly ($p < 0.0001$), which was an extraordinary result.¹⁵⁵ This was due to the fact that there were small reductions in steroid dose when titrating down compared to large increases in steroid dose when titrating up. Looking at the subgroups further, it was clear that belimumab had a glucocorticoid sparing effect (Figure).

Figure: Change in corticosteroid dose (all routes of administration) for patients treated with belimumab 10 mg/kg and patients treated with placebo.



The change in corticosteroid use did not however translate into improvements in weight, blood pressure or glucose. This may have been because the difference between groups was too small or the trial duration was too short. Professor van Vollenhoven also highlighted the SRI response scale that was used in the BLISS trials, noting that if a patient has an SRI response then a lot of other parameters are good, including improved PGA, risk of flare and corticosteroid dose.

Professor van Vollenhoven concluded by asking how we can do better to manage lupus, including better medications and using existing medications in a better way, highlighting the importance of improving treatment strategies based on our understanding of lupus in clinical practice as well as clinical trials. Instead of only focusing on approval wording, based on a clinical trial design that may

not be consistent with clinical practice, it important to consider better strategies, including early or intermitted use, induction and maintenance and even immunophenotype, but overall it is important to be proactive and speak to patients before deciding on the optimal use of belimumab.

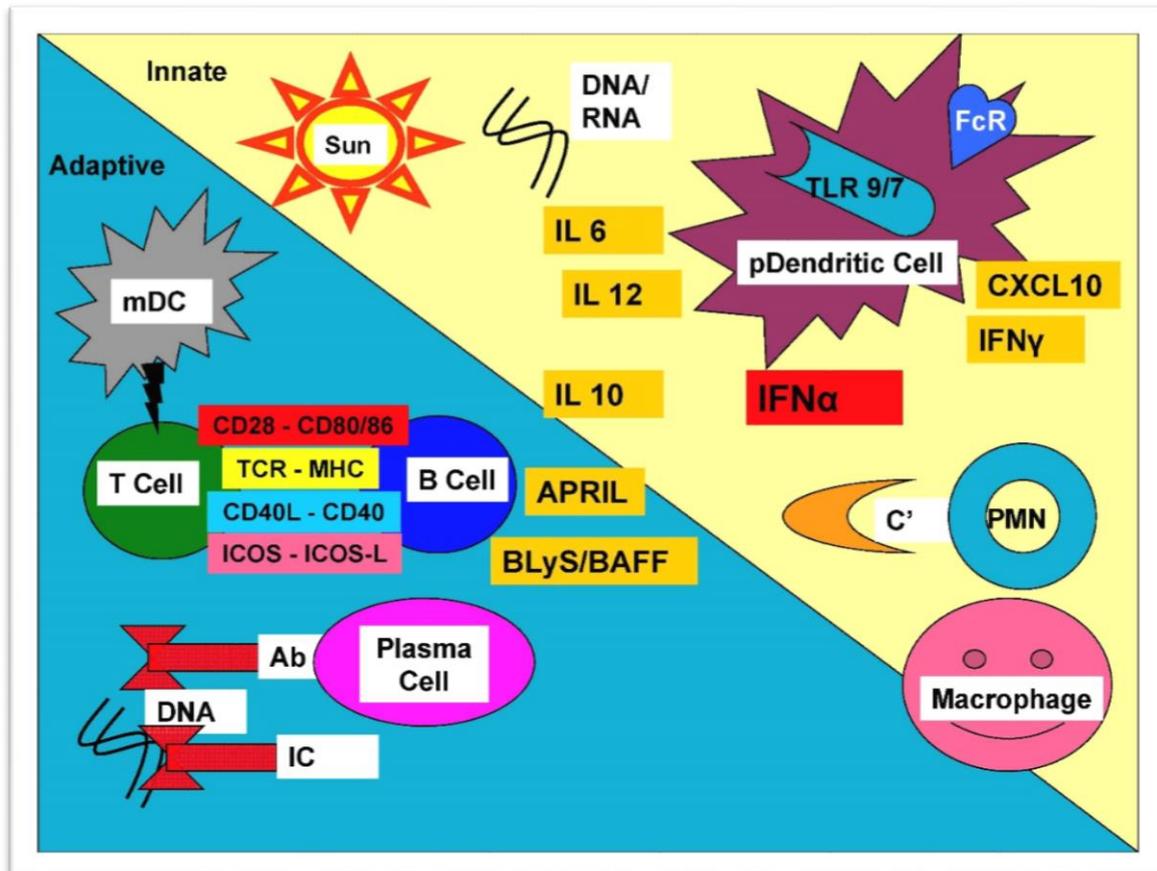
Plenary IV: Therapy Updates in SLE

Targeting the interferon pathway in SLE? Richard Furie

Professor Furie explained the role of type 1 interferons in SLE, describing the strategic approaches to inhibiting the interferon pathway and the results of clinical trials with experimental therapies targeting the interferon pathway in patients with SLE.

Professor Furie opened his presentation with a brief recap of the innate and adaptive immune systems. The innate immune system being evolutionarily ancient, common across all plant and animal life, provides non-specific responses and immediate defense rather than sustained defense and comprising complement, neutrophils, macrophages and toll-like receptors (TLR). The innate immune system is an activator of the adaptive immune system. The adaptive immune system is evolutionarily advanced, found only in vertebrates, provides antigen specific responses, has memory to enable to mount stronger attacks (somatic mutations) and comprises B lymphocytes, antibodies and T lymphocytes. There are several key players in the immunopathogenesis of lupus. In the genetically susceptible host there is an environmental trigger. For example the sun, which induces apoptosis of the skin cells, releasing RNA and DNA, the DNA enters the plasmacytoid dendritic cell, signalling TLR 9, resulting in the elaboration of many cytokines including interferon α . Crossing over to the adaptive immune system, myeloid dendritic cells are activated by many of the cytokines, which in turn triggers an interaction between T cell and B cell via the T cell receptor and major histocompatibility complex, as well as other important costimulatory molecules. B cells are dependent upon some key cytokines, they can differentiate into plasma cells, which release antibodies (ie. DNA antibodies) that form immune complexes, activate complement, attract neutrophils and activate macrophages (Figure). Professor Furie noted that all of these have been or are potential drug targets in lupus, but highlighted that the focus of his talk would be on what is being done to target the early pathways (ie. the innate immune system).

Figure. Key players in the immunopathogenesis of lupus.



Professor Furie briefly explained the interferon α gene signature and why this is important in lupus.²³¹ Patients with SLE have elevated interferon α levels, SLE sera induce interferon gene signatures, >60% of lupus patients have interferon gene signatures and the more disease activity there is the greater the interferon gene expression, which has also proven to decrease with anti-interferon α antibodies; thus, raising the question--can interferon inhibitors reduce SLE activity?

Targets for drug development

There are many strategies/studies linking SLE pathogenesis to drug development targets, Professor Furie presented each in turn.

rhDNase

In the late 1990s, rhDNase was targeted in a study of 17 patients with lupus nephritis. The rationale for targeting rhDNase was to dispose of the debris so the plasmacytoid dendritic cells could not be activated. However, there was no change in serology or immune complexes and no change in the skin biopsy, so the rhDNase strategy was abandoned.²³²

RNA

Conversely, a cross-sectional analysis of 228 SLE patients measured U1 & Y1 RNA complexed with antibodies and immune complexes. It was found that RNA "burden" correlated with SLEDIA and the

expression of interferon gene signature far more than auto antibodies. Subsequently, RSLV-132 (RNase-Fc fusion protein) was tested in a Phase 1b in 32 patients and demonstrated RNase catalytic activity. A Phase 2 study is underway.

Immune complexes

Immune complexes have been targeted for a long time. RBC transfusions have been given to reduce circulating immune complexes, because RBCs have complement receptor 1 on their surface and that receptor binds circulating immune complexes. Column therapy with C1q has failed²³³ as has the use of plasmapheresis in removing circulating immune complexes. However, a trial of the FcR: Recombinant human FcγIIb receptor (SM101) in 51 SLE patients, with SLEDAI >6; DNA antibody or low C3, produced some interesting results with SRI scores at 24 weeks in the active treatment arms improving twice as much as placebo.

Toll-like receptors

The TLR 7/9 inhibitor DV1179 failed to meet its pharmacodynamics endpoint, in a Phase 1 study of 52 patients with an interferon gene signature. A second TLR 7,8,9 inhibitor (IMO-8400) is under investigation (Psoriasis, diabetes mellitus, Waldenström's), but not for SLE.

Interferons

There are three types of interferon: Type I interferon has five subtypes (α , β , ω , ϵ , κ) and, importantly, all bind to the same receptor; Type II interferon (γ) and Type III interferon (λ).

There are different target strategies for targeting Type I interferons.

Interferon α -Kinoïd: vaccine (inactive interferon- α -KLH)

This vaccine comprises inactive interferon α conjugate to KLH, allowing the patient to make their own antibodies to interferon α . This is being studied in a Phase 1/2 dose-escalation study of 28 women with SLE (SLEDIA 4-10), resulting in anti-interferon α antibodies peaking at 56-128 days, and in those who were interferon gene-signature positive interferon gene-signature was reduced. Professor Furie headed caution with this approach as even if the patient wanted to stop the drug the response would continue, stating that it would be logical to give antibodies to the various cytokine or receptor.

Antibodies to interferon α

This said, a Phase 2 extra renal study of rontalizumab failed.²³⁴ A phase 2 study of sifalimumab was more successful, with 60% of patients in the high-dose group having an SRI response versus 45% in the placebo group.²³⁵ Thirdly, anifrolumab, which is different to the aforementioned antibodies, because it binds to the Type I receptor, inhibiting all type I interferon signalling. A Japanese study demonstrated a 50% greater interferon gene signature suppression with anifrolumab *verses* sifalimumab and a has shown promise in a Phase II, three arm study of >300 patients.¹¹⁹ Professor Furie presented an overview of the anifrolumab Phase 2 study, noting that anifrolumab met its primary endpoint with a greater effect size in patients with a high interferon signature at baseline. SRI was greater as was BICLA response, as well as remarkable responses for skin manifestations, demonstrated by improvement in CLASI scores. Joint scores were also improved in anifrolumab-

treated patients *versus* placebo. Professor Furie explained why the low dose results outperformed the high dose results, noting that there were more dropouts in the high dose group and that the biologic effect was similar in both groups, indicating a ceiling effect.

Professor Furie noted that although there are differences in gene signature inhibition between the three antibodies to interferon α there are mysterious similarities in SRI, which indicates a need to look more carefully at the interferon high and low patients (Table).

Table. Interferon mysteries

	Rontalizumab ¹ 750 mg IV/300 SC		Sifalimumab ^{2a,b} 1200 mg		Anifrolumab ³ 300 mg	
	N=79	N=159	N=108	N=107	N=102	N=99
	<u>PLC</u>	<u>RON</u>	<u>PLC</u>	<u>SIF</u>	<u>PLC</u>	<u>ANF</u>
Baseline						
SLEDAI (baseline)	9.5	10.0	11.1	11.3	11.1	10.7
IFN High (baseline)	69.6%	78.6%	81.5%	81.5%	74.5%	75.8%
Results						
IFN GS Inhibition	~<10%	~30%	~<6%	~25%	0%	~90%
SRI (4)	45.6%	50.6%	45.4%	59.8%	40.2%	62.6%
SRI (4) IFN high	47.3%	44.7%	42.0%	57.5%	35.5%	60.0%
SRI (4) IFN low	41.7%	72.7%	60.0%	70.0%	53.8%	70.8%
Zoster			0.9%	8.4%	2.0%	5.1%

Type 2 interferons

AMG 811 an antibody to IFN γ has been studied in 26 patients with a mean SLEDAI <4, which demonstrate dose-dependent modulation of the interferon γ gene signature as well as a reduction in serum levels of CXCL 10 (IP-10: interferon γ inducible protein 10).²³⁶

Plasmacytoid dendritic cells

BDCA2 is uniquely expressed on pDCs and when ligating BDCAR pDC function is suppressed, without depleting the cell itself. Anti-BDCA2 (BIIB059) inhibits pDC activation *in vitro* via dual mechanisms:²³⁷

- Fc-independent: inhibits production of pDC-derived cytokines and chemokines
- Fc-dependent: CD32a (Fc γ RIIa) down-modulation; blocks pDC activation by immune complexes

It has been shown to reduce *in vitro* production of all type I and III interferons as well as IL-6, TNF and chemokine production and is currently being studied in a Phase I trial.

In addition, there is an anti-IL-3 in (CD123), but in SLE *in vitro* studies the compound depletes approximately 90% of pDCs. Another study has shown that Bcl-2 antagonists deplete pDC in NZB/NZW mice,²³⁸ and venetoclax (approved for CLL with 17p deletion) has been studied in SLE with a > 80% B-cell depletion.

JAK/STAT

Several studies have looked at targeting the JAK/STAT pathway in lupus, with varying levels of success:

- GSK2586184: JAK1 inhibitor; program terminated
- R333: topical JAK/SYK inhibitor; failed study in DLE
- Tofacitinib: JAK1/JAK3 inhibitor
- Baricitinib: JAK1/JAK2 inhibitor; Phase II completed

Hydroxychloroquine

Professor Furie concluded with a reminder of our new understanding of hydroxychloroquine, noting that it is recommended for all patients with lupus because it:

- Improves rash and arthritis
- Increases survival: LUMINA cohort²³⁹
- Reduces lipid levels (TC: -8%; LDL: -14%)²⁴⁰
- Has anti-thrombotic effects²⁴¹
- Reduces risk of early cumulative damage²⁴²
- Prevents flares²⁴³

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