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TRANSLATIONAL SCIENCE

Molecular characterisation of lupus low disease activity state (LLDAS) and DORIS remission by whole-blood transcriptome-based pathways in a pan-European systemic lupus erythematosus cohort

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ABSTRACT

Objectives To unveil biological milieus underlying low disease activity (LDA) and remission versus active systemic lupus erythematosus (SLE).

Methods We determined differentially expressed pathways (DEPs) in SLE patients from the PRECISESADS project (NTC02890121) stratified into patients fulfilling and not fulfilling the criteria of (1) Lupus LDA State (LLDAS), (2) Definitions of Remission in SLE remission, and (3) LLDAS exclusive of remission.

Results We analysed data from 321 patients; 40.8% were in LLDAS, and 17.4% in DORIS remission. After exclusion of patients in remission, 28.3% were in LLDAS. Overall, 604 pathways differed significantly in LLDAS versus non-LLDAS patients with an false-discovery rate-corrected p (q)<0.05 and a robust effect size (dr)≥0.36. Accordingly, 288 pathways differed significantly between DORIS remitters and non-remitters (q<0.05 and dr≥0.36). DEPs yielded distinct molecular clusters characterised by differential serological, musculoskeletal, and renal activity. Analysis of partially overlapping samples showed no DEPs between LLDAS and DORIS remission. Drug repurposing potentiality for treating SLE was unveiled, as were important pathways underlying active SLE whose modulation could aid attainment of LLDAS/remission, including toll-like receptor (TLR) cascades, Bruton tyrosine kinase (BTK) activity, the cytotoxic T lymphocyte antigen 4 (CTLA-4)-related inhibitory signalling, and the nucleotide-binding oligomerization domain leucine-rich repeat-containing protein 3 (NLRP3) inflammasome pathway.

Conclusions We demonstrated for the first time molecular signalling pathways distinguishing LLDAS/remission from active SLE. LLDAS/remission was associated with reversal of biological processes related to SLE pathogenesis and specific clinical manifestations. DEP clustering by remission better grouped patients compared with LLDAS, substantiating remission as the ultimate treatment goal in SLE; however, the lack of substantial pathway differentiation between the two states justifies LLDAS as an acceptable goal from a biological perspective.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Remission and low disease activity (LDA) constitute goals of treatment in systemic lupus erythematosus (SLE), but differential biological milieus underlying these states have yet to be explored.

WHAT THIS STUDY ADDS

⇒ For the first time, we demonstrate molecular signalling pathways distinguishing Lupus LDA State (LLDAS) and Definitions of Remission in SLE remission from active SLE.

⇒ We found LLDAS and DORIS remission to be linked with reversal of biological processes related to SLE pathogenesis and specific clinical manifestations.

⇒ Through cluster analysis of differentially expressed molecular pathways, we demonstrated that remission better grouped patients compared with LLDAS, but there was no substantial pathway differentiation between the two states.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study substantiates remission as the ultimate treatment goal in SLE and justifies LLDAS as an acceptable goal when remission is not achievable, also from a biological perspective.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease that is characterised by heterogeneity of immunological aberrancies and clinical manifestations.[1] The disease exhibits relapsing-remitting patterns, with acute inflammatory tissue injury that needs prompt and effective therapy, aiming for remission which is characterised by quiescence in terms of clinical features. Treating to remission, or to low disease activity when

remission is not achievable, emerged as a conceptual framework for the management of SLE in 2014,[2] which was later endorsed in the 2019 and the 2023 European Alliance for Associations for Rheumatology (EULAR) updates of the recommendations for the management of SLE.[3 4]

Several definitions of remission have been proposed, yet the prevailing definition by the Definitions of Remission in SLE (DORIS) taskforce[2] is the one most frequently used in studies.[5] Likewise, several criteria have been proposed to define low disease activity, with the lupus low disease activity state (LLDAS)[6 7] being the most frequently used. Attainment of DORIS remission and LLDAS has been coupled with prevention of organ damage[8 9] and favourable experience of health-related quality of life,[10] which substantiates their relevance as treatment goals, but the biological milieus underlying these states and how these differ from lupus biology during active disease have yet to be explored.

RNA from whole-blood samples collected with RNA stabilisers allows the study of large populations in multicentre studies, mitigating technical and source variability that limits the reproducibility of results and introduces methodological bias,[11] and has therefore been proven useful in the context of autoimmune diseases.[12–14] RNAseq data are commonly interpreted on analysis of differentially expressed (DE) genes at a single-gene level, which substantially limits statistical power as well as interpretability due to redundant results not accounting for similarity across genes. Gene-set analysis has been proposed as an attempt to overcome this issue,[15] through grouping similar transcripts belonging to specific pathways, thus allowing the interpretation of results in the context of biologically related groups of genes (ie, DE pathways (DEPs)). Among several existing methods, the Functional Analysis of Individual Microarray Expression (FAIME) algorithm has proven particularly powerful,[16] as shown in our previous results from a multicohort study.[12] Along with this analysis, the Reactome Knowledgebase[17] that systematically links protein-coding genes to their molecular functions can be used to annotate pathways of interest and to discover functional biological relationships.

The above formed the scope of the present investigation, where we aimed at determining DEPs in LLDAS versus non-LLDAS as well as in remission versus non-remission states in European patients with SLE.

METHODS

Patients and controls

Patients with a diagnosis of SLE according to the revised 1997 American College of Rheumatology (ACR) classification criteria[18] and/or the 2012 Systemic Lupus International Collaborating Clinics (SLICC) criteria,[19] who participated in the PRECISESADS project[20] (NTC02890121) were included in the present study. Clinical data were extracted from the PRECISESADS SLE substudy case report forms and included Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) scores,[21] Physician Global Assessment (PGA; scale 0–3), and detailed information about therapies, which allowed stratification of the study population into patients fulfilling and not fulfilling the criteria of (1) LLDAS,[6] (2) DORIS remission,[2] and (3) LLDAS after exclusion of patients in remission. As per study protocol, none of the patients had been treated with cyclophosphamide within 6 months and/or depletive therapies within 12 months prior to sampling.

The study protocol was reviewed and approved by local ethics committees at all recruiting centres. All patients provided written informed consent for participation in the PRECISESADS project. The present research has been reviewed and approved by the Swedish Ethical Review Authority (registration number: 2022-03907-01).

Table 1 Demographics and clinical characteristics of patients at the time of sampling

Feature	n=321
Female sex, n (%)	299 (93.1)
Age (years), mean±SD	46.7±13.6
Disease duration (years), mean±SD	14.7±10.0
Low complement, n (%)	157 (48.9)
Anti-dsDNA positivity, n (%)	126 (39.3)
Musculoskeletal involvement, n (%)	48 (14.9)
Renal involvement, n (%)	66 (20.6)
Mucocutaneous involvement, n (%)	161 (50.2)
CNS involvement, n (%)	26 (8.1)
Serositis, n (%)	7 (2.3)
Leucopenia, n (%)	54 (16.8)
Thrombocytopenia, n (%)	21 (6.5)
PGA (0–3), mean±SD	0.5±0.5
SLEDAI-2K, mean±SD	6.2±5.6
cSLEDAI, mean±SD	4.6±5.4
DORIS remission, n (%)	56 (17.4)
LLDAS, n (%)	131 (40.8)
Prednisone use, n (%)	146 (45.5)
HCQ use, n (%)	231 (72.0)
Immunosuppressants, n (%)	106 (33.1)
MMF, n (%)	28 (8.7)
MTX, n (%)	19 (5.9)
AZA, n (%)	30 (9.3)
CNIs, n (%)	2 (0.1)
Not specified, n (%)	27 (29.0)

AZA, azathioprine; CNIs, calcineurin inhibitors; CNS, central nervous system; cSLEDAI, clinical SLEDAI-2K (excluding the serological descriptors); DORIS, definitions of remission in systemic lupus erythematosus; HCQ, hydroxychloroquine; LLDAS, lupus low disease activity state; MMF, mycophenolate mophetil; MTX, methotrexate; PGA, Physician Global Assessment; SLEDAI-2K, systemic lupus erythematosus disease activity index 2000.

Procedures

A detailed description of the methodology followed for the RNAseq, DEP, and druggability analyses, including statistical procedures, can be found in the online supplemental material, pages 3–10.

RESULTS

Clinical characteristics

Complete clinical data were available for 321 patients (n=310 for LLDAS; n=321 for DORIS remission). Baseline characteristics and demographics are summarised in table 1. A total of 131 patients (40.8%) were in LLDAS, while 56 (17.4%) were in DORIS remission; 75 of 265 non-remission patients (28.3%) were in LLDAS. Patients in LLDAS (n=23/131 (17.6%) vs n=79/179 (44.1%); p=0.013) as well as patients in DORIS remission (n=10/56 (17.9%) vs n=96/265 (36.2%); p=0.013) were less likely to be currently treated with immunosuppressants compared with patients not fulfilling the criteria of LLDAS or DORIS remission, respectively.

No differences regarding the use of hydroxychloroquine (HCQ), anti-dsDNA positivity, or complement consumption

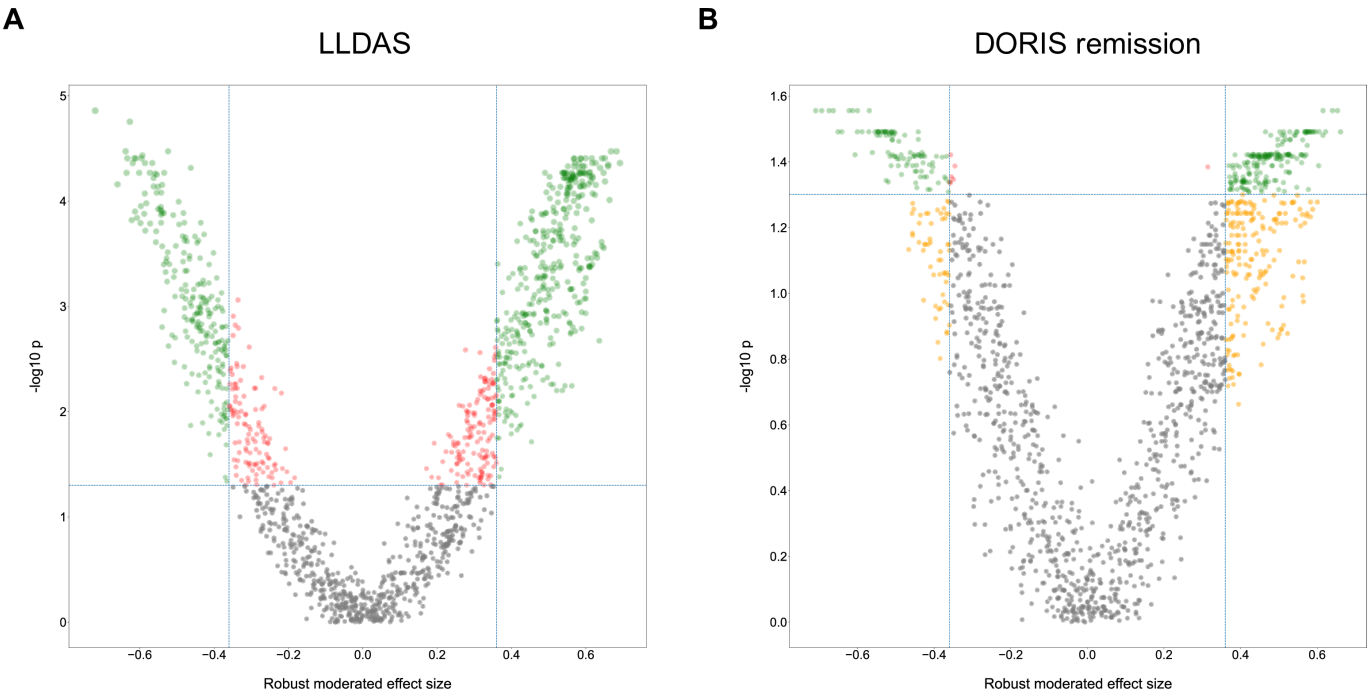

Figure 1 Volcano plots in SLE patients fulfilling the LLDAS and DORIS remission criteria. Volcano plot of Reactome pathways in (A) patients in LLDAS or (B) patients in DORIS remission. The horizontal dashed line indicates the log-transformed false-discovery rate (FDR)-corrected probability threshold (q=0.05) for the moderated t-test statistic; the vertical dashed lines indicate the moderated robust effect size threshold (|dr|=0.36). Pathways not differing significantly yet with a sufficient effect size are highlighted in yellow, whereas significantly differing pathways with an insufficient effect size for both conditions are highlighted in red. Significantly differing pathways with a sufficient effect size for both conditions are highlighted in green. DORIS, definitions of remission in systemic lupus erythematosus; LLDAS, lupus low disease activity state; SLE, systemic lupus erythematosus.

were observed between patients in DORIS remission and non-remission individuals. By contrast, LLDAS patients were less frequently hypocomplementemic (n=50/131 (38.2%) vs n=107/179 (59.8%); p<0.001) and positive for anti-dsDNA (n=27/131 (20.6%) vs n=94/179 (52.5%); p<0.001) compared with non-LLDAS subjects, but no difference was noted regarding the proportion of patients on HCQ between the two groups. Patients fulfilling the LLDAS criteria had a mean dose of prednisone equivalents of 1.54 (±2.32) mg/day, patients in DORIS remission had a mean dose of 0.71 (±1.56) mg/day, and patients in LLDAS exclusive of remission had a mean dose of 2.08 (±2.58) mg/day. Patients not in LLDAS had a mean dose of prednisone equivalents of 4.59 (±5.14) mg/day, and patients not in DORIS remission had a mean dose of 3.83 (±4.68) mg/day.

Reactome pathway analysis in LLDAS versus non-LLDAS

Overall, 604 pathways differed significantly between patients who fulfilled the LLDAS criteria and patients who did not with a false-discovery rate (FDR)-corrected p (q)<0.05 and a robust effect size (dr)≥0.36 (online supplemental material, sheet 1; figure 1A); of those, 226 pathways were downregulated and 378 were upregulated in patients in LLDAS compared with the non-LLDAS population; 218 and 366 pathways, respectively, fulfilled the statistical selection criteria for root comparison (count of Reactome roots>5; online supplemental material, sheet 2). The proportion of pathways among those with a positive correlation with LLDAS exceeded the proportion of pathways among those with a negative correlation within three roots: DNA repair (n=34/366 (9.3%) vs n=1/218 (0.3%); $q=4.1\times10^{-6}$), metabolism of RNA (n=31/366 (8.5%) vs n=0/218 (0.0%); $q=1.8\times10^{-6}$), and cell cycle-related pathways (n=41/366 (11.2%) vs n=2/218 (0.9%); $q=1.8\times10^{-6}$). Conversely, higher proportions among pathways with negative correlations with LLDAS were observed within roots for the immune system (n=44/218 (20.2%) vs n=24/366 (6.6%); $q=3.5\times10^{-5}$), extracellular matrix organisation (n=6/218 (2.8%) vs n=0/366 (0%); q=0.007), and metabolism-related pathways (n=52/218 (23.9%) vs n=21/366 (5.7%); $q=4.9\times10^{-8}$).

After clustering analysis, three distinct groups of patients could be identified, as illustrated in figure 2A (separation of classes among clusters, $\chi^2=25.3$; p<0.001). Clusters 1 and 2 comprised the highest proportions of patients in LLDAS (n=49/91 (53.8%) and n=70/148 (47.3%), respectively), while cluster 3 had the lowest LLDAS prevalence (n=12/71 (16.9%)). Clusters 1 and 3 were distinctly different in terms of enriched pathways, while cluster 2 was characterised by a mixed pattern.

The prevalence of clinical features in the musculoskeletal, mucocutaneous, and renal domains differed across the three biological clusters, as did the use of immunosuppressants, the latter being less prevalent in the LLDAS-enriched clusters 1 and 2 (table 2). Cluster 3 was characterised by an increased prevalence of hypocomplementemia and anti-dsDNA positivity compared with the LLDAS-enriched clusters 1 and 2.

Reactome pathway analysis in DORIS remission versus non-remission

A total of 1465 unique Reactome pathways were selected for analysis. Overall, 288 pathways differed significantly (q<0.05 and dr≥0.36) between DORIS remitters and non-remitters with a FDR-corrected p (q)<0.05 and a robust effect size (dr)≥0.36 (online supplemental material, sheet 3; figure 1B); of those, 97 were found to be downregulated and 191 upregulated in DORIS remitters compared with the non-remission population;

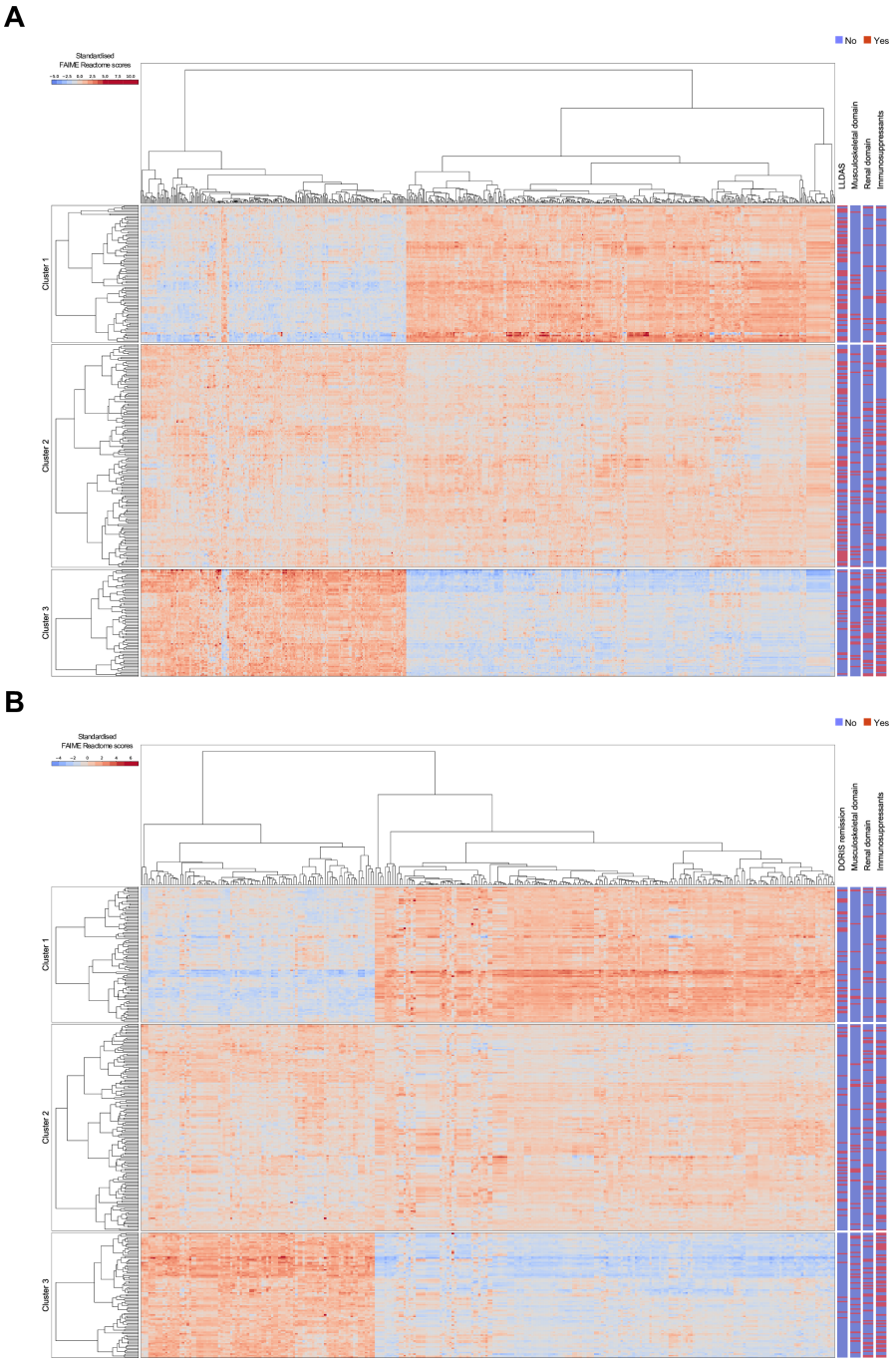

Figure 2 Clusters of individualised Reactome pathways. Individualised Reactome pathways after clustering of selected features associated with (A) LLDAS or (B) DORIS remission. The bars to the right illustrate the distribution of relevant clinical features across clusters. Cluster 1, LLDAS/DORIS remission-enriched cluster; cluster 2, mixed cluster; cluster 3, non-LLDAS/DORIS remission cluster; DORIS, definitions of remission in systemic lupus erythematosus; LLDAS, lupus low disease activity state.

82 and 172 pathways, respectively, qualified for statistical analysis, that is, they had Reactome roots with >5 entries (online supplemental material, sheet 4). The proportion of pathways among those with a positive correlation with DORIS remission exceeded the proportion of pathways among those with a negative correlation within four roots: DNA repair (n=21/172 (12.2%) vs n=0/82 (0.0%); $q=6.7\times10^{-4}$), metabolism of RNA (n=20/172 (11.6%) vs n=0/82 (0.0%); $q=6.7\times10^{-4}$), signal transduction (n=32/172 (18.6%) vs n=5/82 (6.1%); q=0.015), and gene expression (n=9/172 (11%) vs n=1/82 (1.2%); q=0.005). Conversely, higher proportions of negative correlations with DORIS remission were observed for roots within the immune system (n=39/82 (47.6%) vs n=16/172 (9.3%); $q=2.2\times10^{-11}$) and metabolism-related pathways (n=22/82 (26.8%) vs n=7/172 (4.1%); $q=6\times10^{-7}$).

After clustering analysis of significant pathways, three distinct groups of patients could be identified, as illustrated in figure 2B (separation of classes among clusters, $\chi^2=12.3$; p=0.002). Cluster 1 encompassed the highest proportion of patients in DORIS remission (n=25/93; 26.9%), while cluster 3 encompassed the lowest proportion of DORIS remitters (n=6/86; 7%); these two clusters showed distinctly different biological phenotypes and separation of over-expressed and under-expressed Reactome pathways. Cluster 2 had an intermediate number of

Table 2 Summary of clinical features in Reactome pathway clusters

	Clusters by LLDAS (n=310)				Clusters by DORIS remission (n=321)			
Feature, n (%)	**Cluster 1 (n=91)**	**Cluster 2 (n=148)**	**Cluster 3 (n=71)**	**P value**	**Cluster 1 (n=93)**	**Cluster 2 (n=142)**	**Cluster 3 (n=86)**	**P value**
DORIS remission	21 (23.1%)	31 (20.9%)	4 (5.6%)	**<0.010**	25 (29.9%)	25 (17.6%)	6 (7.0%)	**0.001**
LLDAS	49 (53.8%)	70 (47.3%)	12 (16.9%)	**<0.001**	52 (55.9%)	63 (44.4%)	16 (18.6%)	**<0.001**
CNS	8 (8.8%)	13 (8.8%)	5 (7.0%)	NS	8 (8.6%)	11 (7.7%)	7 (8.1%)	NS
Musculoskeletal	8 (8.8%)	21 (14.2%)	19 (26.8%)	**<0.010**	9 (8.6%)	20 (14.1%)	20 (23.3%)	**<0.050**
Renal	11 (12.1%)	30 (20.3%)	24 (33.8%)	**<0.005**	11 (11.8%)	29 (20.4%)	26 (30.2%)	**0.010**
Mucocutaneous	38 (41.8%)	73 (49.3%)	44 (62%)	**<0.050**	39 (41.9%)	71 (50.0%)	51 (59.3%)	NS
Serositis	1 (1.1%)	3 (2.0%)	3 (4.2%)	NS	1 (1.1%)	3 (2.1%)	3 (3.5%)	NS
Positive serology	59 (64.8%)	86 (58.1%)	52 (73.2%)	NS	62 (66.7%)	78 (54.9%)	62 (72.1%)	**<0.050**
Low C3/C4 levels	42 (46.1%)	70 (47.3%)	45 (63.4%)	0.050	46 (49.5%)	59 (41.5%)	52 (60.5%)	**<0.050**
Anti-dsDNA (+)	33 (36.3%)	51 (34.5%)	37 (52.1%)	**<0.050**	36 (38.7%)	47 (33.1%)	43 (50%)	**<0.050**
Altered CBC	18 (19.8%)	28 (18.9%)	17 (23.9%)	NS	17 (18.3%)	29 (20.4%)	21 (24.4%)	NS
Leucopenia	16 (17.6%)	21 (14.2%)	14 (19.7%)	NS	15 (16.1%)	22 (15.5%)	17 (19.8%)	NS
Thrombocytopenia	3 (3.3%)	11 (7.4%)	6 (8.4%)	NS	4 (4.3%)	9 (6.3%)	8 (9.3%)	NS
Hydroxychloroquine	68 (74.7%)	102 (69.0%)	53 (74.6%)	NS	70 (75.3%)	95 (66.9%)	66 (76.7%)	NS
Immunosuppressants	18 (19.8%)	44 (29.7%)	40 (56.3%)	**<0.001**	18 (19.4%)	42 (29.6%)	46 (53.5%)	**<0.001**

Prevalence of clinical and laboratory features in clusters from significantly differential Reactome pathways.
P values are derived from Pearson's χ squared (χ^2) or Fisher's exact tests.
Significant results are in bold.
(+),increased binding.
C3, complement protein 3; C4, complement protein 4; CBC, complete blood count; CNS, central nervous system involvement; DORIS, definitions of remission in systemic lupus erythematosus; LLDAS, lupus low disease activity state; NS, non-significant p-value.

DORIS remitters (n=25/142; 17.6%) and a biological mixture of pathways without a clear-cut distinct biotype.

The three clusters were characterised by a different prevalence of musculoskeletal and renal activity, as well as use of immunosuppressants, with an apparent trend across clusters from the lowest prevalence in DORIS remission-enriched cluster 1, to intermediate prevalence in the mixed cluster 2, and the highest prevalence in cluster 3 that comprised the lowest proportion of DORIS remitters, as summarised in table 2. Differences were observed concerning positive serology, hypocomplementemia, and anti-dsDNA positivity, yet with no clear trend (lower prevalence in cluster 2). No other differences were observed.

Reactome pathway analysis in DORIS remission versus LLDAS exclusive of remission versus non-LLDAS

Analysis of adjacent levels of disease activity using forward difference coding in linear regression models showed no DEPs between patients in DORIS remission compared with patients in LLDAS after suppression of patients in DORIS remission. This comparison was deemed sufficient for testing the null hypothesis of equivalence of the means between the partially overlapping samples of patients in DORIS remission and patients in LLDAS.[22] By contrast, 662 DEPs were documented between patients in LLDAS after suppression of DORIS remitters and the non-LLDAS patient population (online supplemental material, sheet 5).

Analysis of Reactome pathways in relation to renal involvement and other clinical manifestations

The expression of Reactome pathways within the renal domain showed that 223 pathways were differentially expressed with $q<0.05$ and $dr\geq0.36$ (online supplemental material, sheet 6); of these, 141 were upregulated and 82 were downregulated in patients with current renal activity versus patients no renal activity, 123 and 77, respectively, qualifying for association analysis (online supplemental material, sheet 7). Renal involvement was positively associated with enriched metabolic (renal involvement: n=39/123 (31.7%) vs no renal involvement: n=1/77 (1.3%); $q=4.8\times10^{-8}$) and signal transduction pathways (renal involvement: n=37/123 (30.1%) vs no renal involvement: n=3/66 (3.9%); $q=4.8\times10^{-6}$) and negatively associated with cell cycle processes (renal involvement: n=0/123 (0.0%) vs no renal involvement: n=10/77 (13.0%); $q=1.1\times10^{-4}$), DNA repair (renal involvement: n=0/123 (0.0%) vs no renal involvement: n=18/77 (23.4%); $q=4.8\times10^{-8}$), and metabolism of RNA (renal involvement: n=37/123 (30.1%) vs no renal involvement: n=3/77 (3.9%); $q=1.1\times10^{-4}$). Only 19 pathways were found to be associated with haematological manifestations, which limited us from conducting association tests. Exploratively, it was found that four immune system pathways related to IFN functions were positively correlated with haematological manifestations, including IFN-α/β signalling (q=0.046; dr=0.417) and IFN-γ signalling (q=0.0488; dr=0.414). No other associations were found between Reactome pathways and musculoskeletal, mucocutaneous, pulmonary, neurological, and serological features of disease.

Explorative analysis showed that several pathways were correlated with SLEDAI-2K scores (online supplemental material, sheet 8), although with a small effect size (Cohen's $f^2\geq0.02$); of these, 121 had a negative correlation and 92a positive correlation, 114 and 80, respectively, qualifying for association analysis (online supplemental material, sheet 9). Consistent with LLDAS and DORIS remission, which incorporate SLEDAI-2K, increasing scores were negatively associated with DNA repair mechanisms (positive correlation: n=0/80 (0.0%) vs negative correlation: n=22/114 (19.3%); $q=3.9\times10^{-15}$) but positively associated with immunological functions (positive correlation: n=20/80 (25.0%) vs negative correlation: n=5/114 (4.4%); $q=5.4\times10^{-4}$). The overlap of DEPs by LLDAS, DORIS remission, and SLEDAI-2K scores is depicted in the online supplemental figure S1. Comparing the frequencies of pathways that were positively or negatively associated with the different

outcomes (online supplemental material, sheet 10), DORIS remission was more strongly associated with a negative regulation of the immune system than LLDAS or low SLEDAI-2K scores. Heatmaps depicting immune system pathways in relation to LLDAS- and DORIS remission-enriched clusters are illustrated in figure 3, where inhibitory pathways are shown to be

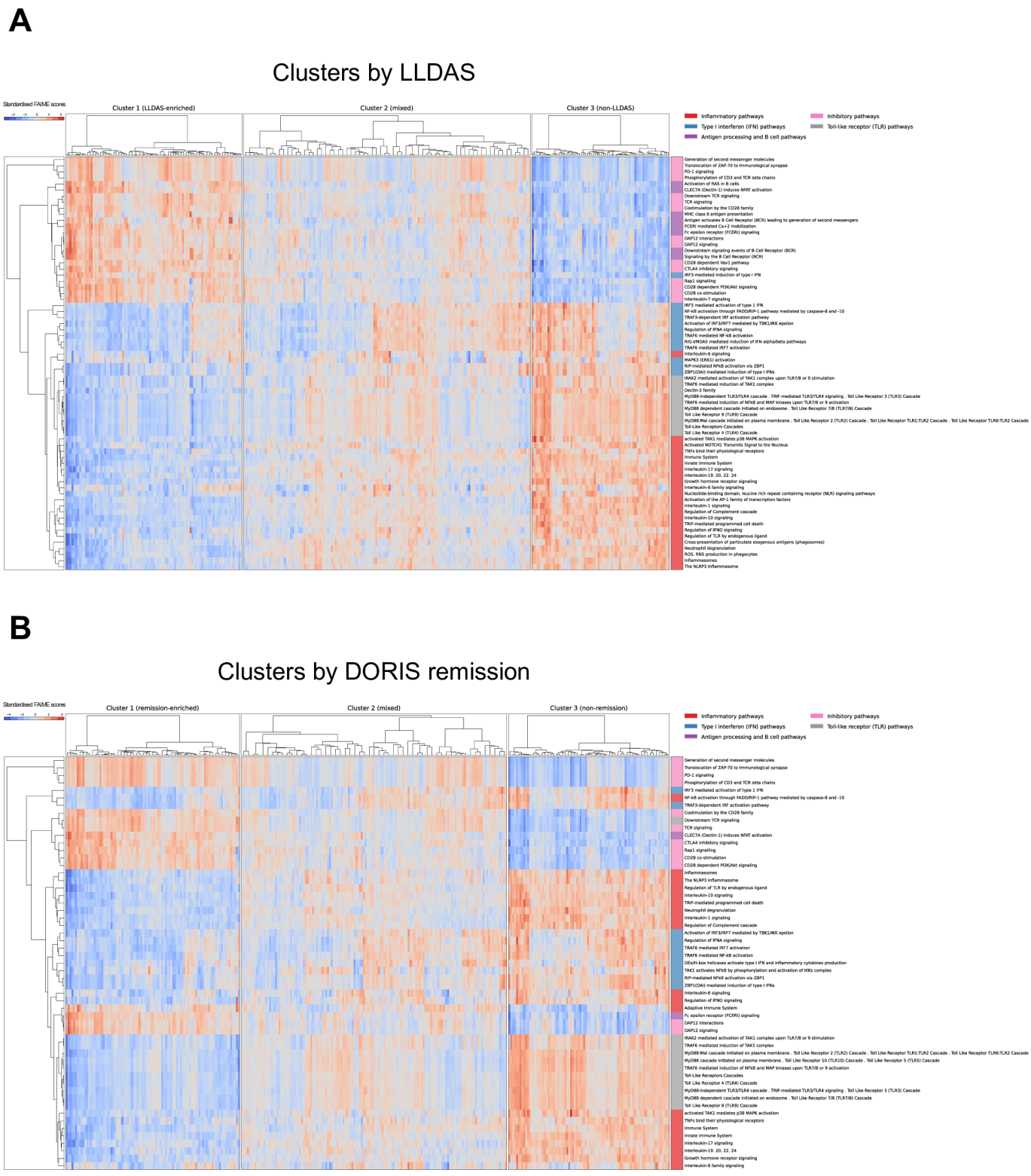

Figure 3 Immune system Reactome pathways according to biological clusters and main functions. Distribution of individualised immune system Reactome pathways in clusters (A) by LLDAS and (B) by DORIS remission. The coloured bars represent manual annotation according to the main functions of each pathway cluster, with pink denoting pathways with inhibitory functions on the immune system, red denoting inflammasome/inflammatory pathways enriched in cytokines, grey denoting toll-like receptor (TLR) and related functions, blue denoting type I interferon (IFN) pathways, and purple denoting antigen processing and B-cell pathways. DORIS, definitions of remission in systemic lupus erythematosus; LLDAS, lupus low disease activity state.

Table 3 Summary of relevant mechanisms and supporting evidence

Mechanism	Finding	Supporting literature
DNA repair	Increased in LLDAS/DORIS remission; downregulated in active SLE.	Defective in SLE;[23 24] defective DNA repair predisposes to RA;[29] ameliorates with symptom improvement in RA.[30]
	Reduced GG-NER and TC-NER in active renal SLE.	Defective DNA-repair in lupus nephritis.[25]
	Reduced POLB-dependent long patch base excision repair pathway in active renal SLE.	Mutations in POLB associated with SLE;[26] defective POLB caused nephritis[27] and correlated with severe glomerulonephritis in murine lupus.[28]
RNA metabolism	tRNA processing and post-transcriptional modification of mRNA metabolism of non-coding mRNA associated with LLDAS/DORIS remission.	Altered RNA metabolism in inflammation and autoimmune diseases;[31 32] mutations in NMD elicit type I IFN responses;[33] inhibition of NMD increases p53,[34] linked to SLE activity[35] via promoting apoptosis;[36] inhibition of p53-dependent apoptosis reverses alveolar haemorrhage in murine lupus.[37]
Gene expression	Reduced caspase-related apoptosis in active renal SLE.	Reduced apoptosis in kidney biopsy samples from patients with lupus nephritis.[38]
	Increased in LLDAS/DORIS remission.	Transcription of death genes impaired in remittent patients.[39]
Immune system *Type I IFN*	Reduced type I IFN responses in LLDAS/DORIS remission.	Type I IFN pathways induce inflammation,[40] correlating with SLE activity.[41 42]
	No association with active neurological, respiratory, musculoskeletal, mucocutaneous, or renal SLE.	No correlation with musculoskeletal, mucocutaneous, or renal manifestations in phase III trials of anifrolumab.[43]
	Associations with haematological activity.	Subgroup analysis of phase III anifrolumab trial demonstrated an effect of anti-IFN therapy on leucopenia and thrombocytopenia.[44]
Immune system *TLR pathways*	TLRs downregulated in LLDAS/DORIS remission.	TLR7/8 and TLR9 elicit B-cell proliferation and function[45] and associate with disease activity.[46]
	Association between SLE activity and TLR1:TLR2 and TLR6:TLR2 heterodimers.	TLR1:TLR2 and TLR6:TLR2 heterodimers promote type I IFN production.[47]
	TLR2 associated with active renal SLE.	TLR2 promotes ischemia/reperfusion injury in the kidneys.[48]
	Activation of TLR3, TLR4, and TLR5 cascades in patients with active disease.	TLR3 and TLR4 promote inflammation and production of type I IFN;[49 50] TLR5 associated with IL-17 and IL-22 production in tissues.[51]
Immune system *Interleukins*	IL-1, IL-4, IL-13, IL-6, IL-7, IL-10, IL-17, and IL-20 family associated with active SLE.	Interleukins associated with SLE activity and pathogenesis.[52–54]
	IL-2, IL-3, IL-5, and GM-CSF signalling associated with LLDAS/DORIS remission.	Inverse association with SLE activity thoroughly described.[55–57]
Immune system *Inflammasome*	Inflammasome and related pathways associated with active SLE.	Inflammasome implicated in SLE pathogenesis;[58] potential therapeutic target.[60]
	NLRP3 showed a trend toward an association with active renal SLE.	NLRP3 pathway activated in tissue samples from lupus nephritis patients.[59]
Immune system *CTLA-4*	CTLA-4 pathway upregulated in LLDAS/DORIS remission.	Polymorphisms reducing CTLA-4 function increase type I IFN.[61]
Immune system *DAP-12*	DAP-12-related pathways upregulated in LLDAS/DORIS remission.	DAP-12 exerts inhibitory signals on natural killer cells and regulates natural immunity.[63]
Immune system *PD-1*	PD-1 pathway function increased in LLDAS/DORIS remission.	PD-1 negatively regulates B-cell and T-cell function.[64]
Metabolism	Acetylation increased in LLDAS/DORIS remission.	Epigenetic mechanisms linked to acetylation control autoimmunity;[71] defective histone acetylation in murine models promotes dsDNA production and tissue injury.[72]
	Eicosanoid reduction linked to the absence of renal involvement.	Eicosanoids suggested as potential targets in renal disease.[73]
	Reduction of eicosanoids and leukotrienes linked to LLDAS/DORIS remission.	Eicosanoids suggested as potential targets for SLE.[74]

The table lists the main mechanisms that were found to be associated with systemic lupus erythematosus (SLE) or specific clinical manifestations and LLDAS or DORIS remission based on differential Reactome pathway analysis.
CTLA-4, cytotoxic T lymphocyte antigen 4; DAP-12, 12kDa transmembrane protein; DORIS, definitions of remission in SLE; dsDNA, double-strand DNA; GG-NER, global genome NER; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; LLDAS, lupus low disease activity state; mRNA, messenger RNA; NER, nucleotide excision repair; NLRP3, nucleotide-binding oligomerisation domain leucine-rich repeat-containing protein 3; NMD, nonsense-mediated mRNA decay; PD-1, programmed death-1; POLB, DNA polymerase beta; RA, rheumatoid arthritis; SLEDAI, systemic lupus erythematosus disease activity index; TC-NER, transcription-coupled NER; TLR, toll-like receptor; tRNA, transfer RNA; UV, ultraviolet.

upregulated in patients in LLDAS and DORIS remission, while IFN, inflammatory, and toll-like receptor (TLR) signalling pathways are shown to be upregulated in the non-LLDAS and non-remission groups.

Associations of interest between LLDAS or DORIS remission states, or disease manifestations, and immune mechanisms are summarised in table 3, along with literature relating to the findings.

Illustrative examples of druggable Reactome pathways

As LLDAS and DORIS remission were positively associated with several TLR cascades (online supplemental material, sheets 1 and 3), we explored known drug–target interactions within these pathways (https://idg.reactome.org/PathwayBrowser/#/R-HSA-168898). TLR7/8 and TLR9 cascades interact with two drugs: HCQ, an antagonist of both TLR7 and TLR9, and imiquimod, an agonist of TLR7 (figure 4A–C; detailed in online supplemental figure S2).

The TLR2 cascade as well as TLR1:TLR2 and TLR6:TLR2 heterodimer cascades do not have direct drug interactions, neither do TLR5 and TLR10, yet all these pathways converge to the MyD88:MAL(TIRAP) cascade that is initiated on the plasma membrane, which was also associated with renal involvement (dr=0.361; q=0.033) (https://reactome.org/PathwayBrowser/#/R-HSA-166058). Bruton tyrosine kinase (BTK) is a key player in these pathways; its activity is affected by at least three inhibitors, that is, acalabrutinib, ibrutinib, and zanubrutinib (figure 4D,E; detailed in online supplemental figure S2).

DISCUSSION

In the present study, several Reactome pathways were able to distinguish between LLDAS and non-LLDAS, as well as between DORIS remission and non-remission states, lending support to the clinical meaningfulness of LLDAS and DORIS remission as treatment goals. We herein coupled these states of disease with underlying biology for the first time. DORIS remission yielded

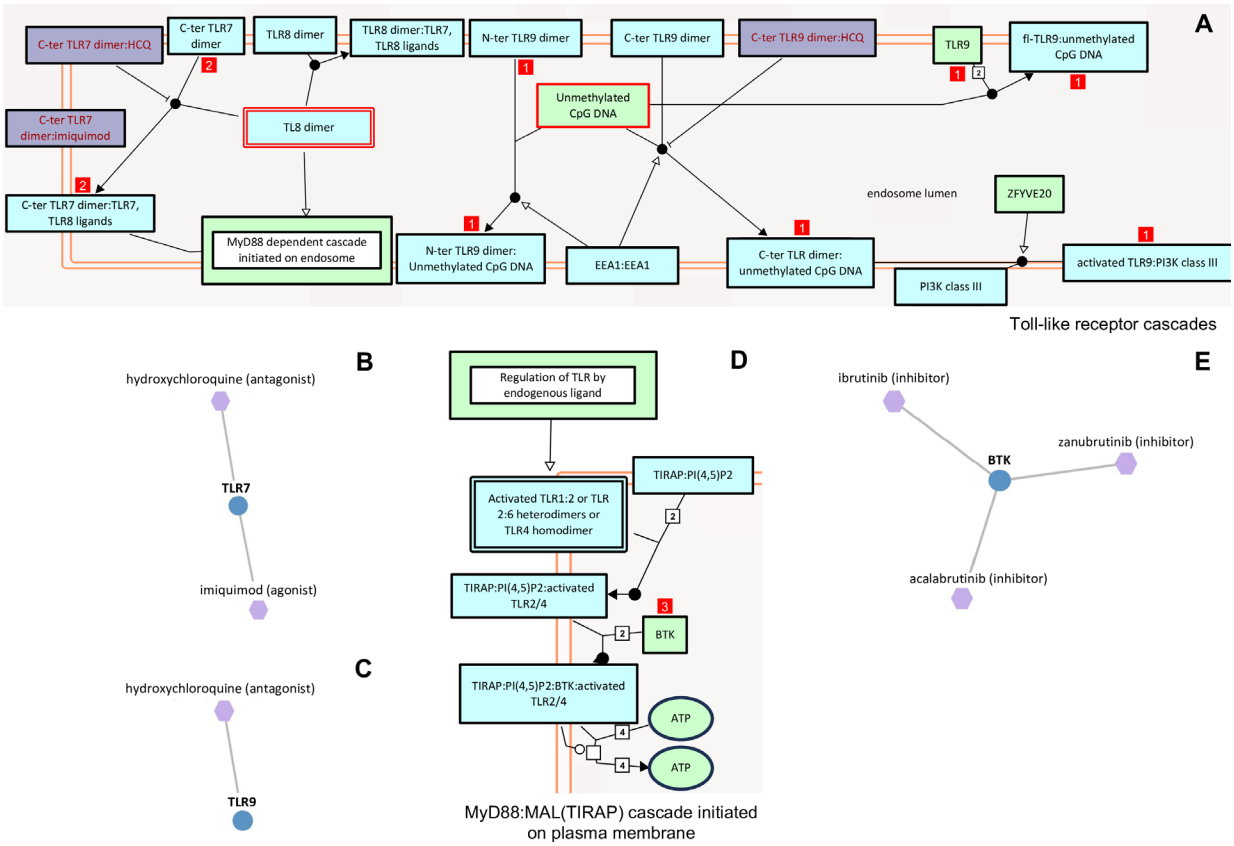

Figure 4 Druggable toll-like receptor (TLR) cascades. Drug–pathway interactions within TLR cascades associated with definitions of remission in systemic lupus erythematosus (DORIS) remission (from: https://idg.reactome.org, with modifications); only selected parts of pathways are shown, and irrelevant pathways or parts of pathways are omitted. The complete pathways are detailed in online supplemental figure S2. Panel A depicts TLR 7/8 and TLR9 pathways; pathway–drug interactions with TLR7 and TLR9 are highlighted with red squares (the number of related drugs is indicated) and demonstrated in panels B and C. Parts of the MyD88:MAL(TIRAP) cascade initiated on the plasma membrane belonging to the druggable TLR cascades are detailed in panel D; this cascade constitutes the terminal effector of TLR2, TLR5, and TLR10 pathways. Within this pathway, Bruton tyrosine kinase (BTK) is a key druggable component, whose inhibitors are shown in panel E.

more distinct separations across differentially enriched pathways and biological clusters compared with LLDAS, presumably owing to the greater degree of stringency of remission, corroborating the concentric distribution of LLDAS and DORIS remission, also at a biological level. However, a non-substantial pathway differentiation was demonstrated between DORIS remission and LLDAS exclusive of remission, suggesting a minimal added modulation at a biological level between these two states. Collectively, these observations suggest that although management of SLE should aim for DORIS remission, LLDAS is an acceptable goal in biological terms when remission is not achievable.

We documented associations between LLDAS or DORIS remission and several immune mechanisms. Evidence indicates that DNA repair is defective in patients with SLE, with implications in SLE pathogenesis.[23 24] Interestingly, defective DNA repair mechanisms were here found to be associated with renal activity, corroborating previous literature.[25] Mutations in the POLB genes have been associated with SLE and replicated in independent genome-wide association studies,[26] while POLB defective function has been shown to cause lupus in murine models,[27] also correlating with severe glomerulonephritis.[28] In line with these previous findings, the POLB-Dependent Long Patch Base Excision Repair Reactome pathway was found in our sample to be defective in patients with renal involvement. Defective DNA repair has also been linked to loss of T cell tolerance, predisposing individuals to rheumatoid arthritis.[29] Notably, this deficiency has been found to be reversible following treatment, with a tendency to decrease alongside symptom improvement.[30] This aligns with our findings of hampered DNA damage mechanisms in patients fulfilling the LLDAS or DORIS remission criteria.

Another mechanism that was enriched in patients in LLDAS or DORIS remission was RNA metabolism. Different processes involved in RNA transcription products were found to be associated with LLDAS and DORIS remission, including transfer RNA (tRNA) processing, post-transcriptional modification of messenger RNA (mRNA), as well as pathways involved in the metabolism of non-coding mRNA. Dysregulation of RNA metabolism has been linked to inflammation and autoimmunity.[31 32] Mutations in NMD affecting the quality control of aberrant mRNA mutations may predispose to an autoimmune phenotype via type I IFN signalling.[33] Inhibition of NMD upregulates the tumour suppressor protein p53,[34] which has been correlated with SLE activity,[35] likely through induction of apoptosis,[36] and targeting p53-dependent apoptotic mechanisms was shown effective in treating murine diffuse alveolar haemorrhage.[37] Our finding of reduced NMD pathways in patients not fulfilling the LLDAS or DORIS remission criteria is well in line with the aforementioned evidence, further corroborating the relevance of these targets at the biological level.

LLDAS and DORIS remission were also associated with increased gene-expression processes, and apoptotic processes

related to caspases and caspase activators were downregulated in patients with renal involvement compared with SLE patients quiescent in the renal domain. This accords with reports of reduced apoptosis in kidney tissue from patients with lupus nephritis.[38] Interestingly, remission has been linked with impaired transcription of death genes, but preserved transcription and regulation of DNA repair.[39]

Not unexpectedly, LLDAS and DORIS remission were associated with reduced activity of immune system processes. Induction of type I IFN pathways and elicited inflammatory responses, a mechanism of known importance in SLE pathogenesis,[40] which also correlates with the degree of SLE activity,[41 42] was here found to be upregulated in SLE patients not fulfilling the LLDAS or DORIS remission criteria. This is the first report describing a relative to counter states downregulation of the IFN signature in SLE patients in LLDAS and DORIS remission. Interestingly, renal activity correlated poorly with IFN-related pathways, as also observed in a post-hoc analysis of the phase III trials of anifrolumab, a human monoclonal antibody targeting the type I IFN receptor, approved for the treatment of active SLE.[43] On the contrary, haematological alterations were found to correlate with INF-related pathways, also in accordance with findings from the same study, where anifrolumab was found to improve leucopenia and thrombocytopenia.[44]

Furthermore, a wide range of TLR cascades were downregulated in remittent patients. This pertains to the known role of TLR7/8 and TLR9 in B-cell functions and proliferation,[45] as well as to a previous report on their association with SLEDAI scores in a small population of untreated SLE subjects.[46] We herein describe for the first time associations between TLR2 or TLR1:TLR2 and TLR6:TLR2 heterodimers, which promote inflammation and type I IFN production,[47] and active SLE disease state. Importantly, TLR2 activity was here also associated with renal activity. Previous studies have shown that TLR2 mediates ischaemia/reperfusion injury in kidney tissue,[48] and our data indicate that haemostatic functions, including platelet glycoprotein Ib (GPIb)-mediated adhesion and platelet activation, signalling, aggregation, and degranulation with thromboxane release are prominent in patients with active renal SLE. Moreover, we provide for the first time evidence of activation of TLR3, TLR4, and TLR5 cascades in patients not fulfilling the LLDAS or DORIS remission criteria. TLR3 and TLR4 have been shown to promote inflammation and production of type I IFN,[49 50] while TLR5 has been linked to IL-17 and IL-22 production in tissues.[51] Inflammatory and regulatory cytokine signalling pathways were herein found to be associated with active SLE, including several that previously have been coupled with SLE pathogenesis or activity.[52–54] In contrast, IL-2, IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor signalling was associated with LLDAS and DORIS remission, also corroborating previous literature.[55–57]

The inflammasome and inflammasome-related pathways were here associated with active SLE. Importantly, the inflammasome pathway has been advocated to be relevant in SLE pathogenesis and disease progression,[58 59] with therapeutic implications.[60] We found that the inhibitory signalling related to cytotoxic T lymphocyte antigen 4 (CTLA-4) was upregulated in SLE patients in LLDAS or DORIS remission. Defective CTLA-4 function coupled with a genetic polymorphism has been linked to increased type I IFN activity.[61] Abatacept, a CTLA-4/IgG fusion protein with inhibitory functions on T cell proliferation and cytokine production, failed to demonstrate efficacy in lupus nephritis, but was effective in reducing the occurrence of articular flares.[62] Associated with LLDAS and DORIS remission were also inhibitory signals such as the innate-immunity inhibitor DAP12, which primary acts on natural killer (NK) cells, and the programmed cell death 1 signalling pathway, which attenuates signalling downstream of the T cell receptor (TCR) and the costimulatory receptor CD28, suppressing cellular functions such as activation, proliferation, metabolic regulation, cytotoxicity, and cytokine production.[63 64]

Finally, our data provide implications of targeting certain pathways to ameliorate disease. We demonstrated examples of druggable Reactome pathways pointing to inhibitors of TLR cascades as putative drug candidates. These results are independent of HCQ use, a well-known modifier of TLR7 and TLR9 functions,[65] and further stress the need to globally tackle immune system activation to promote remission. Nevertheless, the use of HCQ in our population may explain the lack of association between TLR pathway signalling and renal involvement, as this drug is known to be beneficial in the treatment of lupus nephritis in conjunction with mycophenolate mofetil[66] and is recommended for all SLE patients unless contraindicated, especially those with kidney involvement.[4 67] In our analysis, BTK emerged as a potential target of downstream mediators of TLR pathway signalling. The use of BTK inhibitors has been advocated in lupus,[68] with evidence from animal models indicating that they may ameliorate renal lupus.[69 70] It is worth mentioning that a phase II RCT of the dual TLR7/8 inhibitor enpatoran is currently ongoing (NCT05162586).

Our study has strengths but is not exempt from drawbacks. The lack of replication in independent cohorts or in vitro experiments, the cross-sectional study design, and the overall limited numbers of SLE patients with high disease activity constitute main limitations. The lack of longitudinal data precluded investigation of restoration or reversal of biological aberrancies while shifting from active SLE to disease quiescence defined by LLDAS or DORIS remission, which would be of interest to study in a future work. Another important limitation was the lack of data on the duration of LLDAS and DORIS remission prior to sampling, which may be expected to affect the extent and depth of transcriptome alterations that are associated with these states. Strengths include the large number of patients and a multitude of results corroborating previous research, which provides reassurance while contributing to the robustness of current evidence. Importantly, this was the first thorough analysis of pathways in SLE patients in association with low disease activity and remission, providing biological relevance to current treatment goals in SLE management. We demonstrated how pathways can be explored to gain insights into SLE pathogenesis, substantiate the distinction between quiescent and active disease, and reveal potential therapeutic targets.

In summary, we demonstrated for the first time molecular signalling pathways distinguishing LLDAS and DORIS remission from active SLE. Compared with active disease, LLDAS and remission were associated with downregulated biological processes related to SLE pathogenesis and biological processes linked to specific disease manifestations. While DEP clustering by DORIS remission better grouped patients than clustering by LLDAS, substantiating the conceptual testimonial of remission being the ultimate treatment goal in SLE, the lack of substantial pathway differentiation between the two states justifies LLDAS as an acceptable goal from a biological perspective when remission is not achievable. Notably, DORIS remission was associated with a higher proportion of pathways with negative regulatory effects on the immune system compared with LLDAS or low SLEDAI-2K, underlying the importance of reversing immune system activation to attain adequate clinical control of the

disease. The study revealed the potentiality of existing drugs that could be repurposed to treat SLE and important pathways underlying active SLE whose modulation could aid attainment of disease quiescence. Among those, TLR cascades, BTK activity, CTLA-4-related inhibitory signalling, and the NLRP3 inflammasome pathway were of particular interest.

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