# Classical Complement Activation in Lupus Nephritis Correlates with Disease Biomarkers: Results from Two Observational Studies

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ANNEXON biosciences

## INTRODUCTION

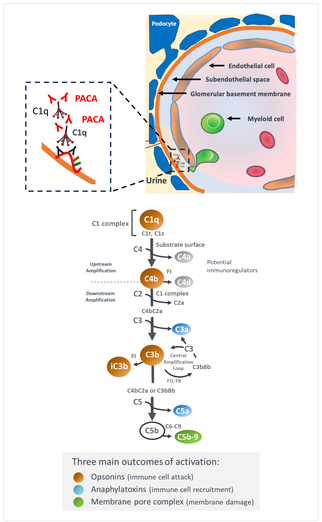
### Systemic Lupus Erythematosus (SLE) is Driven by Autoantibodies Against Numerous Antigens Including DNA and Even C1q Itself

- Circulating immune complexes (ICs) trapped by the glomerular basement membrane (GBM) within subendothelial space
- ICs activate C1q and the classical complement cascade (with immune cell recruitment / attack and direct membrane damage)
- Pathogenic anti-C1q autoantibodies (PACAs) accumulate on ICs, recruiting and activating more C1q

### C1q Accumulation Drives C4 Cleavage, the Pivot Point of Classical Complement Activation

- Accumulation of C1q-activating substrates (eg, autoantibody complexes) can cause chronic complement activation that overwhelms complement regulatory molecules
- C4 cleavage is the first step in the "upstream" cascade that results in deposition of a covalent opsonin, beyond which "downstream" process take hold
- Tissue damage can arise from:
  - Surface attack by phagocytic cells through the major opsonins C4b, C3b, and C1q
  - Immune cell recruitment and activation by soluble anaphylatoxins C3a and C5a
  - Direct membrane damage/cell lysis by the C5b-9 membrane attack complex pore
- Cutting off upstream amplification of the classical cascade before C4 cleavage has the maximum impact by inhibiting all factors that cause tissue damage
  - Prevents deposition / forward pressure of first covalent opsonin (C4b)
  - Slows cascade advancement and leverages backpressure of pathway inhibitors
  - Leaves lectin and alternative pathway initiation and amplification in place for normal immune function

### Figure 1. A Classical Complement Focused View[1-3]




FD/FB, Factor D/Factor B; PACA, pathogenic anti-C1q autoantibody.

## OBJECTIVES

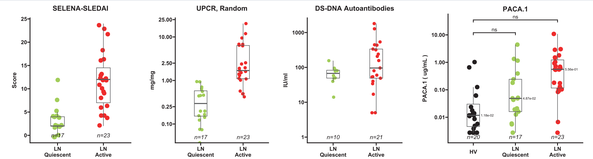
- Plasma samples taken from population-based registries of patients with lupus nephritis (LN) were used to describe the relationship of C4d/C4 ratio with pathogenic anti-C1q antibodies (PACAs) and urine protein-creatinine ratio (UPCR)

## METHODS

- **UCSF Study:** A cohort of 40 patients, 23 with active disease ("Active LN") and 17 without active disease ("Quiescent LN"), were selected, along with 20 healthy volunteers from the California Lupus Epidemiology Study (CLUES) and other population-based registries. Diagnosis of lupus nephritis and disease activity were made by physicians on study.
- **Sanguine Study:** Annexon contracted with Sanguine Biosciences to recruit 24 LN patients and 10 healthy volunteers and to profile serological samples collected over 4-6 weeks. Study inclusion was based on past LN diagnosis and self-reporting of Class III or IV with/out Class V with proteinuria between 0.5-3 g/g. LN patients must also be on active management for LN.
- **Internal Assays:** Complement proteins were profiled using ELISA immunoassays targeting substrate proteins and cleavage products. Plotted values are from visit time at study enrollment.
- **External Assays:** C4d, C4 and UPCR profiling were done using validated assays at ICON Clinical Laboratory Services

## RESULTS

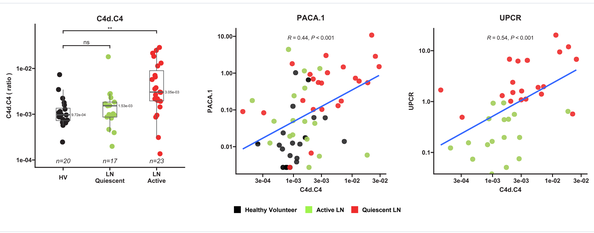
### Figure 2. SLEDAI, UPCR, dsDNA Autoantibodies and PACAs Are Elevated in Active LN




dsDNA, double-stranded DNA; HV, healthy volunteer; LN, lupus nephritis; PACA, pathogenic anti-C1q antibody; SELENA-SLEDAI, safety of estrogens in lupus erythematosus national assessment disease activity index - systemic lupus erythematosus disease activity index; UPCR, urine protein-creatinine ratio.

- Clinical parameters of systemic lupus erythematosus are elevated in patients with active disease

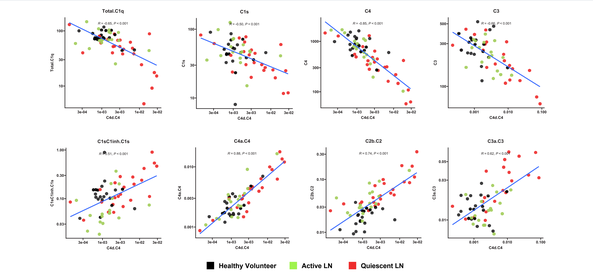
### Figure 3. Classical Complement Activation and Consumption Are Driven by PACAs and Correlate with Disease in LN




HV, healthy volunteer; LN, lupus nephritis; PACA, pathogenic anti-C1q antibodies; UPCR, urine protein-creatinine ratio.

- C4d/C4 correlates with PACAs and proteinuria, suggesting value in focusing on patients with high complement activation for study of anti-C1q therapy

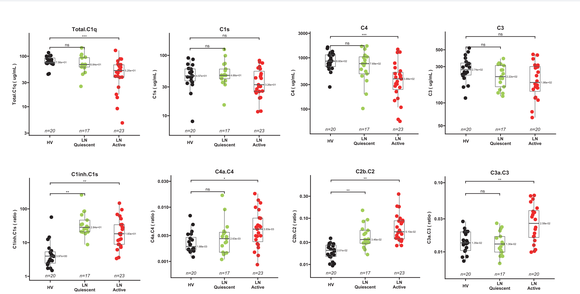
### Figure 4. Classical Complement Consumption Correlates with C4d/C4 Activation Ratio




HV, healthy volunteer; LN, lupus nephritis.

- Active LN marked by greater consumption of classical complement factors C1q, C4, and C2

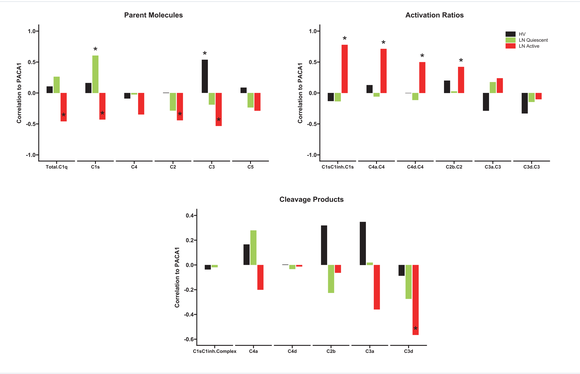
### Figure 5. Classical Complement Activity Highest in Patients with Active LN




HV, healthy volunteer; LN, lupus nephritis.

- C1 inhibitor binds to active site of C1r/C1s and inactivates C1 complex
- Formation of C3 convertase (C4bC2a) is accompanied by release of non-protease fragments (C2b, C4a) and subsequent C3a generation

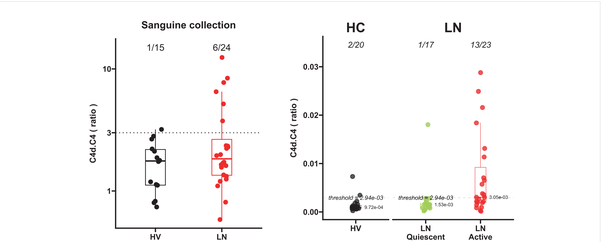
### Figure 6. PACA1 Levels Correlate with Classical Complement Activity




*Statistical significance (p<0.05).
HV, healthy volunteer; LN, lupus nephritis; PACA, pathogenic anti-C1q antibody.

- Pearson correlation coefficients with PACA IgG1 levels were calculated for complement substrate molecules, cleavage products, and the respective activation ratios

### Figure 7. LN Patients from an Independent Sanguine Cohort Are Also Enriched for High C4d/C4




Thresholds set at 90th percentile of HV range.
HV, healthy volunteer; LN, lupus nephritis; PACA, pathogenic anti-C1q antibodies; UCSF CLUES, University of California San Francisco California Lupus Epidemiology Study.

- C4d/C4 activation ratios were calculated for the UCSF cohort (healthy volunteers, n=20; LN, n=40) and independent LN cohort collected by Sanguine Bioscience (healthy volunteers, n=15; LN, n=24)

## CONCLUSIONS

- **The classical complement pathway is a key driver of LN pathology, potentiated by PACAs**
  - **PACAs are associated with elevated classical complement activity and with disease activity**
  - **PACAs are primarily IgG1 and IgG3 (data not shown) subclasses, which are more efficient at activating the classical complement pathway**
  - **PACAs potentiate complement-driven local inflammation and increase likelihood of renal flares**
- **LN patients with classical complement-mediated disease are identifiable by high C4d/C4 ratio**
- **C4d/C4 positively correlates with PACAs, linking pathway mechanism with disease pathology**
- **Classical complement activation and consumption are hallmarks of active LN**
- **Targeting C1 can potentially lead to more durable remission**
- **LN-01 (NCT05780515) is a phase 1b study in patients with LN receiving an anti-C1q therapeutic (ANX009) to test the hypothesis that blockade of upstream classical complement activation and amplification will prevent activation of downstream components**

### REFERENCES

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2. Nowling TK, Gilkeson GS. *Arthritis Res Ther*. 2011;13(6):250.
3. Lintner KE, et al. *Front Immunol*. 2016;7:36.

### DISCLOSURES

**EC:** Employment with and equity ownership in Annexon Biosciences; stock holdings in Gilead Sciences.
**JL, NY, QC, HAK, YAZ, TY, AM:** Employment with and equity ownership in Annexon Biosciences.
**MB:** Prior employment with and equity ownership in Annexon Biosciences, Inc.; prior employment with Roche/Genentech; prior equity ownership in Genentech.
**JO:** Employment with and equity ownership in Annexon Biosciences; prior employment with FibroGen.
**DRA:** Employment with and equity ownership in Annexon Biosciences; stock ownership in Bristol-Myers Squibb, Johnson & Johnson, and Merck.
**MCD:** Consultancy/advisory role with Annexon Biosciences, AstraZeneca, Aurinia, Biogen, GlaxoSmithKline, and Pfizer; research funding from Annexon Biosciences and GlaxoSmithKline.

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