experimental autoimmune encephalomyelitis (EAE) model that replicates aspects of age-dependent MS progression, including leptomeningeal inflammation and subpial hippocampal pathology. Here, we sought to develop an experimental and computational pipeline to identify and therapeutically target neuroimmune pathways that moderate disease progression in EAE mice. To this end, we performed single-cell RNA sequencing (scRNA-Seq) of leptomeninges from young or old mice at EAE initiation, peak, and recovery (for young mice) vs chronic (for old mice) disease phases. In parallel, we developed a novel approach to terminally collect up to 30 μL of pure cerebrospinal fluid (CSF) from individual young vs old mice across disease stages. Using data-independent acquisition LC-MS/MS we analyzed the global proteome of individual mice and resolved >2300 proteins, which varied systematically in abundance between young and old mice throughout EAE. Integrating scRNA-Seq data with complementary CSF proteome and immunofluorescence imaging, we identified production of complement C3 mRNA in the leptomeninges, C3 protein accumulation in the CSF and C3 activation in the hippocampus as a prominent marker of aged EAE disease. Using an adeno-associated viral (AAV) approach to overexpress the C3 inhibitor Crry at sites of C3 activation in the EAE hippocampus, we found that inhibition of C3 activation in old but not young mice resulted in milder disease. These data suggest that C3 activation in EAE is a mechanism driving age-divergent disease worsening in mice. Using imaging mass cytometry and downstream analysis pipeline, C3 activation products were also found on oligodendroglia in the hippocampus of a subset of progressive MS patient brains that showed evidence of leptomeningeal inflammation and hippocampal demyelination. Taken together, our data identifies complement as a driver of age-dependent progression in EAE that is relevant to the human disease.

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Modulation of complement-mediated inflammation in the *mdx* mouse model of duchenne muscular dystrophy by PK007

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Abstract

Background: Duchenne Muscular Dystrophy (DMD) is a progressive neuromuscular disorder marked by chronic inflammation and muscle degeneration. The *mdx* mouse model recapitulates key pathological features of DMD, including elevated complement system activation. In particular, complement component C5 has been implicated in exacerbating muscle inflammation and injury. PK007, an inhibitor of hematopoietic prostaglandin D synthase (HPGDS), has demonstrated anti-inflammatory effects in dystrophic models and may influence complement-related pathways.

Methods: Four-week-old *mdx* mice were treated with PK007 or vehicle control over a 10-day treatment period (postnatal 18 days to postnatal 28 days). Skeletal muscles, including the gastrocnemius (GA), tibialis anterior (TA), and extensor digitorum longus (EDL), were collected for molecular and histological analyses. Bulk RNA sequencing was performed to evaluate transcriptomic changes, with a particular focus on complement-related genes. For protein-level assessment, C5 expression was evaluated by immunofluorescence using a monoclonal anti-C5 antibody. Tissue sections were imaged, and fluorescence intensity was quantified to assess differential expression between treatment groups.

Results: RNA-seq analysis revealed a downregulation of *C5* transcript levels in PK007-treated muscles compared to vehicle controls. Immunofluorescence analysis demonstrated a reduction in C5 protein expression in dystrophic muscle following PK007 treatment.

Conclusion: These findings suggest that PK007 may attenuate complement-mediated inflammation in DMD by downregulating the expression of complement component C5. In combination with its previously observed anti-inflammatory effects, PK007 may contribute to reduced muscle damage in dystrophic muscle, supporting its further investigation as a promising therapeutic candidate in the treatment of muscular dystrophy.

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Targeted treatment with pegcetacoplan for adolescents with C3G or primary (idiopathic) IC-MPGN in the VALIANT phase 3 trial

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Abstract

Background: C3 glomerulopathy (C3G) and primary (idiopathic) immune complex membranoproliferative glomerulonephritis (IC-MPGN) are rare diseases where uncontrolled C3 activation results in excessive glomerular deposition of C3 breakdown products, often diagnosed in adolescence or early adulthood. Current treatments have significant side effects and approximately 20% of children progress to kidney failure within 10 − 15 years of diagnosis. Pegcetacoplan, a C3/C3b inhibitor, targets the central complement pathway components to directly inhibit overactivation of C3 breakdown. In the overall VALIANT (NCT05067127) population, pegcetacoplan led to significant proteinuria reduction and estimated glomerular filtration rate (eGFR) stabilization vs placebo in C3G or primary IC-MPGN patients. Here, we report results for adolescents (12–17 years).

Methods: VALIANT, a randomized, double-blind, placebo-controlled Phase 3 trial, included adolescents (≥12 years) and adults with biopsy-proven C3G or primary IC-MPGN in native or post-transplant kidneys

and proteinuria >1 g/day. In adolescents without a baseline biopsy, eligibility criteria included ≥1 of the following: increased plasma soluble C5b-9, decreased serum C3, presence of hematuria, or presence of C3 nephritic factor. Patients were randomized 1:1 to pegcetacoplan (subcutaneous infusion twice weekly) or placebo for 26 weeks in the RCP. Biopsies were optional for adolescents. The primary endpoint was the log-transformed ratio of urine protein-to-creatinine ratio (UPCR) at week 26 vs baseline. Key secondary endpoints included the proportion of patients achieving a composite renal endpoint criterion (≥50% UPCR reduction and ≤15% eGFR reduction), ≥50% UPCR reduction, and eGFR change from baseline.

Results: In total, 28 adolescents were randomized to pegcetacoplan and 27 to placebo. Pegcetacoplan led to significant and clinically meaningful UPCR reductions at week 26, with a 74.5% relative reduction in proteinuria in the pegcetacoplan vs placebo arms (95% CI 58.5, 84.3; nominal $P < .0001$). A greater proportion of adolescents in the pegcetacoplan vs placebo arm achieved the composite renal endpoint (57.1% vs 3.7%; nominal $P = .0016$) and ≥50% UPCR reduction (71.4% vs 3.7%; nominal $P = .0002$). Pegcetacoplan was associated with clinically meaningful eGFR stabilization.

Conclusions: In VALIANT, pegcetacoplan was well tolerated in adolescents with C3G and primary IC-MPGN and was the first treatment to induce meaningful proteinuria reduction and eGFR stabilization in this population.

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Assessing C3 nephritic factor function using Luminex-based formation and stabilization assays

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Abstract

Background: C3 Nephritic factors (Nefs) are autoantibodies that bind to and stabilize the C3 convertase, thereby enhancing complement activity and driving glomerular diseases such as C3 Glomerulopathy (C3G).[1,2] Recently, a Luminex method was developed to evaluate Nefs.[3] Utilizing this technique, the authors proposed that Nefs influence both convertase *formation* and *stabilization*. In this study, we compare the Luminex-based assay to hemolytic-based and surface plasmon resonance (SPR) assays to assess the formation/stabilization model of Nef activity. Method A test population of polyclonal IgG samples were evaluated for C3Nef activity by hemolysis (n = 21)[1] and for convertase formation and stability by SPR analysis (n = 21).[4] Samples were then applied to the Luminex *Formation* (n = 21) and *Stability* assays[3] (n = 12).

Results: Nef hemolytic scores ranged from 10% (0+) to 100% (4+). Linear regression between hemolysis and Luminex assays was statistically significant: Luminex *Stability*-Hemolysis yielded $R^2 = 0.46$, $F = 8.6$, $p = 0.015$; Luminex *Formation*-Hemolysis yielded $R^2 = 0.29$, $F = 4.3$, $p = 0.013$. ANOVA test with Dunn's multiple comparisons for Luminex data grouped by hemolytic score (0, 1+, or 4+) showed statistically significant difference between 0 and 4+ Nefs by both *Stability* ($p = 0.0003$) and *Formation* ($p = 0.0012$) assays but no significance between 0 and 1+ Nefs by either Luminex assay.

Linear regression between Luminex *Formation* assay and SPR assay analysis showed significance to the SPR "*Formation*" report point ($R^2 = 0.62$, $F = 29.3$, $p = <0.0001$) and SPR-based *stability* analysis ($R^2 = 0.51$, $F = 18.85$, $p = 0.0004$). The Luminex *Stability* assay correlated weakly with the SPR *Stability* analysis but did not reach significance ($p = 0.15$).

Conclusion: Luminex *Formation* correlated better with the SPR *Formation* report point, while Luminex *Stability* showed better correlation to the hemolytic *stability* assay. However, the overall correlations were lower than anticipated, which may point to (1) the small sample size; (2) more nuanced interactions between polyclonal IgG and the C3 convertase; and/or (3) the need for further optimization of the Luminex and SPR methodologies.

References: (1) PMID: 22223606, (2) PMID: 35734939, (3) PMID: 39325562, (4) PMID: 22854646.

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VALIANT: Randomized, multicenter, double-blind, placebo-controlled, phase 3 trial of pegcetacoplan for patients with native or post-transplant recurrent C3G or primary (idiopathic) IC-MPGN

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Abstract

Aims C3 glomerulopathy (C3G) and primary immune complex-membranoproliferative glomerulonephritis (IC-MPGN) are complement-mediated diseases driven by C3 dysregulation with excessive accumulation of C3 breakdown products in the kidney. Pegcetacoplan (PEG) a C3/C3b inhibitor, targets the central components of the complement pathway, directly inhibiting C3 overactivation and preventing further deposition of C3 breakdown products in the glomeruli. VALIANT (NCT05067127) is the first Phase 3 trial investigating PEG in a broad cohort, including adolescents (≥12 yrs) and adults with native or post-transplant recurrent C3G or primary IC-MPGN.