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# Recent developments in C3-targeted complement therapeutics

"The hopes of the educated are better than the wealth of the ignorant"

*Democritus, 4th century BC*

The complement system has long been recognized as an evolutionarily conserved sentinel of innate immunity that responds swiftly to microbial invaders and prevents their spread within the host. However, this simple perception has been abandoned over the last two decades due to a series of findings that point to a broader 'systems-wide' impact of complement on tissue homeostasis and mammalian development [1]. Complement activity appears to orchestrate a broad spectrum of biological processes that reach beyond traditional innate immunology. Indeed, the field has witnessed paradigm shifts that have reshaped our perception of complement biology. These include the following: the evolutionary presence of complement proteins across both invertebrate and vertebrate phylogenesis; the contribution of complement protein diversity to the expanded innate immune recognition of lower vertebrates; the capacity of complement to sculpt and prune the synaptic circuitry of the brain with broad implications in health and disease; the role of complement in promoting tissue regeneration and its intricate involvement in tumorigenesis and cancer immunosuppression, including its potential role as a therapeutic modality for combinatorial cancer immunotherapy [all reviewed in [2–4]]. Growing evidence has revealed the involvement of complement fragments and receptors with pattern recognition signaling pathways, operating both in the intravascular space and within the cytosol of immune or non-immune cells [5]. Complement proteins act as molecular 'rheostats and sense host- or pathogen-derived molecular patterns, thereby driving tissue-restricted and systemic inflammatory responses [2,5]. The magnitude, locale, regulation and duration of these interactions likely determines the outcome of tissue immunosurveillance and ultimately, the maintenance or subversion of tissue homeostasis.

The complement cascade features several key components that can orchestrate effector responses and engage in reciprocal crosstalk with other host defense pathways that mediate immunomodulatory functions in a wide array of cell types [1].

Complement component C3, the most abundant complement protein in the circulation, is positioned at the heart of this highly regulated protein circuitry. C3 serves as the central hub of this system and is also the point at which all pathways converge, regardless of the initiating route or trigger [4]. Indeed, proteolytic cleavage of C3 through the action of multi-protein complexes, termed C3 convertases, sets off structural transformations that expose multiple interaction sites and the release of bioactive fragments endowed with a wide spectrum of opsonizing, immunomodulatory, proinflammatory and cell activating properties. More importantly, C3 activation drives the amplification loop of the alternative pathway (AP) whereby the C3b opsonin is readily deposited on target surfaces, acting as a 'nucleus' for further C3 convertase assembly. Moreover, at high surface densities deposited C3b mediates formation of the C5 convertase, the enzymatic initiator of the terminal complement pathway. Considering that AP amplification can fuel almost 80% of downstream complement effector responses, stringent regulation of this pathway is crucial for maintaining the beneficial homeostatic actions of complement in the host [6].

A growing body of evidence from preclinical and human studies has causally linked complement dysregulation and excessive C3 activity to the pathogenesis of multiple immune-mediated or inflammatory diseases of the hematological, renal, ocular and neurological spectrum [7–9]. The development of high-throughput technologies and disease models, along with focused studies dissecting the contribution of complement signaling to cell trajectories and disease-related phenotypes at single-cell resolution have all contributed to our present understanding of complement's involvement in health and disease. This information has also led to renewed interest of both academic labs and biopharmaceutical companies to develop complement therapeutics that can modulate the activity of multiple proteins and effector pathways within the complement cascade [7].

Despite the broad involvement of C3 signaling in disease pathophysiology across a wide spectrum of pathologies, ranging from acute to chronic diseases and local to systemic indications, selection of C3 as a therapeutic target was met with considerable skepticism by the immunology community [10–12]. The feasibility of prolonged C3 inhibition was downplayed by hypothetical assertions such as the anticipated increased susceptibility of C3-inhibitor treated patients to infections [11,12]. These discussions would be based on observations of rare cases of individuals with primary C3 deficiencies but would fail to recognize the fact that pharmacological C3 inhibition is both tunable and quite distinct from an inborn C3 deficiency (safety concerns linked to therapeutic C3 modulation are extensively reviewed here [13]). In addition, several lines of evidence indicate that the contribution of C3 to pathogen immunosurveillance appears to subside with age, as more compensatory immunological mechanisms develop during our transition to adulthood [14]. Another technical aspect inherent in these discussions was the presumed difficulty in saturating the plasma concentration of C3 with a therapeutic inhibitor [13]. This potential problem would soon be alleviated by the first-in-human studies that showed that small-sized peptidic C3 inhibitors can effectively saturate plasma C3 levels, maintaining prolonged and complete C3 inhibition following systemic delivery [15]. The safety of systemic C3 inhibition, under a prophylactic vaccination regimen, would also be supported by subsequent clinical trials of C3 inhibitors that came to disprove the hypothetical increased risk for infections [16].

Anti-C5 therapy dominated the complement therapeutics field following the clinical approval of eculizumab in 2007. Benefiting from a

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generously rewarding legislation on orphan drug development and incentives like market exclusivity, eculizumab (Soliris, Alexion) soon became a successful, but highly priced therapy in the clinic [17]. The development of this C5-targeting therapeutic antibody was spearheaded in the 1980 s with the identification of the first mouse-specific anti-C5 monoclonal antibody (clone BB5.1) by Frei, Lambris and Stockinger at the Basel Institute for Immunology [18]. While eculizumab's approval for the treatment of paroxysmal nocturnal hemoglobinuria (PNH) was undeniably a milestone in complement drug discovery that bolstered confidence in therapeutic complement modulation, it also perfused the clinical landscape with a misguided perception that C5 blockade was appropriate for all complement-mediated conditions [10]. This notion unavoidably delayed therapeutic development of other complement inhibitors, because corporate drug programs were focused on developing additional anti-C5 agents.

Anti-C5 therapy was successful in the clinical management of PNH patients, controlling intravascular hemolysis and lowering the life-threatening risk for thrombosis. It also garnered approvals in rare autoimmune indications of the neurological space where terminal pathway activity was found to be a pathogenic driver [7]. However, refined preclinical models and new clinical observations soon revealed that C5 targeting could not effectively address multiple pathogenic processes that were driven by complement pathways operating upstream of C5 [19,20]. Indeed, this was first evident in PNH, and was manifested by the persistent C3 opsonization of surviving PNH cells leading to C3-mediated extravascular hemolysis [21]. In addition, there were mixed clinical responses of patients to anti-C5 therapy in heterogenous renal diseases such as C3 glomerulopathy (C3G) [20]. These findings all indicated that anti-C5 agents could not adequately intercept all facets of complement-driven pathology. These unmet medical needs, emerging in the era of anti-C5 therapy, would call for a more disease-focused approach which would exploit discrete therapeutics targeting other components of the cascade.

The clinical approval of Empaveli (APL-2/pegcetacoplan) by the FDA in May 2021 constituted a watershed moment in the history of complement therapeutics and an important validation of therapeutic C3 inhibition in the clinical setting [22]. This approval was ultimately based on laboratory and clinical studies of the action of compstatin-based C3 inhibitors, a distinct class of peptide based C3 therapeutics developed by the Lambris group over 25 years ago at the University of Pennsylvania [23]. In terms of structure, pegcetacoplan comprises two copies of the 4(1MeW)/7 W (Cp05) compstatin analog spaced apart by a PEG linker to enable longer plasma retention. Clinical development of Empaveli was based on structure-guided optimization efforts that yielded compstatin analogs with improved binding affinities and activity profiles through targeted peptide modifications and backbone N-methylation of the original compstatin scaffold [24]. Approval of Empaveli was based on the successful results of the PEGASUS phase III trial in PNH. In this head-to-head comparison with eculizumab in PNH patients remaining anemic under anti-C5 therapy, pegcetacoplan outperformed eculizumab and demonstrated a significantly improved hematological response (i.e., improved hemoglobin levels) compared to anti-C5 therapy [16]. The results of this trial validated the need for alternative complement intervention strategies in diseases in which C3 dysregulation can elicit discrete pathogenic effects, independent of terminal pathway activity and its effectors (C5a, MAC). This was indeed the case with C3-mediated extravascular hemolysis which underpinned the clinical phenotype of PNH patients displaying chronic residual anemia under eculizumab treatment. More importantly, this study provided solid clinical evidence for the broader activity profile of C3 inhibitors in an archetypal complement-mediated disease, thus opening new avenues to explore the clinical feasibility of C3 inhibition in other chronic disorders with prominent C3 dysregulation or excessive C3-driven inflammation (e.g. AP-driven renal disorders and age-related or autoimmune-driven neuroinflammatory and neurodegenerative diseases) [25].

Fifteen years after the clinical approval of eculizumab, the complement field now has numerous opportunities for therapeutic intervention in many clinical indications [7]. In fact, complement drug discovery is now an active area of research based on a long-anticipated expansion of the clinical arsenal of available complement-specific drugs. Following the approval of Empaveli for the treatment of PNH, two subsequent approvals have stirred new expectations in the field: the anti-C1s monoclonal antibody sutimlimab/ BIVV009 (Enjaymo, Sanofi/Bioverativ) was recently approved for the treatment of the rare autoimmune hemolytic condition Cold Agglutinin Disease (CAD), while the orally administered small-molecule C5aR1 antagonist Avacopan/ CCX168 (Tavneos, Chemocentryx) was approved as an adjunctive treatment for patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis [26,27]. This expansion of therapeutic options in the complement drug arena has brought the notion of tailored complement intervention even closer to fruition, offering new opportunities for more personalized patient management. Considering that anti-C5 therapy has already been applied to patients for over 15 years, with only minor adverse events being reported during long-term follow-up, it will be important to similarly monitor the long-term effects of all the newly approved complement therapies, including C3 inhibition.

In launching this special collection our objective was to bring to the forefront the latest developments in the rapidly evolving space of C3-targeted therapeutics. The topics discussed in this issue are indicative of the breadth and scope of transformative work being performed and of the emerging potential for clinical C3 modulation in diseases with inadequate responses to standard therapy or no etiological treatment at hand.

This special issue encompasses a structure-guided perspective on C3 and its multiple interactions with various ligands, receptors and modulators [28]. The wealth of structural data underpinning the dynamic conformational rearrangements of C3 upon activation or interaction with key targets has propelled the molecular development of four generations of compstatin derivatives and may yet be leveraged for the rational design of next-generation C3 inhibitors. Compstatins have not only advanced to the clinic as more efficacious therapeutic agents for PNH but also served as fine molecular tools for dissecting the pathogenic involvement of C3 activation in many disease models. In this regard, our special issue features a perspective on the application of compstatins as tools for interrogating the role of C3 signaling in human whole blood models of microbial-driven inflammation [29]. From a clinical /translational standpoint, the collection features a series of articles discussing the clinical advancement of C3 therapeutics in PNH [32], the sustained and broad anti-inflammatory action of clinical C3 intervention in dysbiosis-driven periodontal inflammation and the prospects of C3 inhibitors in treating peri-implant inflammatory conditions [30]. In addition, the collection features articles on: the emerging potential of C3 therapeutics for indications with prominent involvement of chronic C3 dysregulation or excessive C3 activation in their pathophysiology, such as age-related macular degeneration (AMD); the rare renal disorder C3G; ICU-related pathologies and cancer-driven inflammation and immunosuppression [33–36]. Insights are offered on the clinical feasibility, prospects, and challenges of C3 inhibition in these indications, with emphasis placed on optimal dosing routes, the timing and sites of intervention and identifying the most relevant diagnostic algorithms for stratifying patients into trials. Also, these articles identify meaningful biomarkers and surrogate endpoints that are expected to help clinicians monitor patient responses to therapeutic C3 inhibitors.

Intracellular complement activation has also attracted considerable attention in recent years as another facet of complement biology that may underpin the pathophysiology of autoimmune or chronic inflammatory diseases [5]. While the extent to which therapeutic C3 modulation may impact the complosome's activity remains elusive, the topic is discussed in view of recent evidence that intracellular C3 and C3aR signaling shifts metabolic programs in fibroblasts priming local tissue inflammatory responses [31].

As pointed out above, the decision to launch this special issue was born out of a drive to critically examine the evidence and data emerging from preclinical models, clinical trials and human observations in the context of therapeutic C3 inhibition. It is our strong conviction that the future of complement therapeutics will be bright and full of tangible therapeutic opportunities so long as it remains firmly rooted in science, transparency, and evidence-driven discovery.

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