Compstatin: a C3-targeted complement inhibitor reaching its prime for bedside intervention

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ABSTRACT

There is a growing awareness that complement plays an integral role in human physiology and disease, transcending its traditional perception as an accessory system for pathogen clearance and opsonic cell killing. As the list of pathologies linked to dysregulated complement activation grows longer, it has become clear that targeted modulation of this innate immune system opens new windows of therapeutic opportunity for anti-inflammatory drug design. Indeed, the introduction of the first complement-targeting drugs has reignited a vibrant interest in the clinical translation of complement-based inhibitors. Compstatin was discovered as a cyclic peptide that inhibits complement activation by binding C3 and interfering with convertase formation and C3 cleavage. As the convergence point of all activation pathways and a molecular hub for crosstalk with multiple pathogenic pathways, C3 represents an attractive target for therapeutic modulation of the complement cascade. A multidisciplinary drug optimization effort encompassing rational 'wet' and *in silico* synthetic approaches and an array of biophysical, structural and analytical tools has culminated in an impressive structure-function refinement of compstatin, yielding a series of analogues that show promise for a wide spectrum of clinical applications. These new derivatives have improved inhibitory potency and pharmacokinetic profiles and show efficacy in clinically relevant primate models of disease. This review provides an up-to-date survey of the drug design effort placed on the compstatin family of C3 inhibitors, highlighting the most promising drug candidates. It also discusses translational challenges in complement drug discovery and peptide drug development and reviews concerns related to systemic C3 interception.

Keywords clinical translation, complement-based drug design, compstatin, Cp40, nonhuman primate models, peptidic C3 inhibitors.

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Introduction

In the wake of a new era in biopharmaceutical research that has strongly embraced the driving forces of scientific cross-disciplinarity, high-throughput data integration and system-wide bioevaluation, complement-targeted drug discovery has taken a new turn down fascinating avenues of opportunity for novel anti-inflammatory therapeutics that will be amenable to clinical use [1–3]. Complement research has traditionally been dominated by the prevalent, albeit quite misleading, concept that this innate immune system is merely a first-line sentinel for microbial invaders and only plays an accessory role in the backdrop of inflammatory processes (i.e. in antibody-triggered effector pathways). Nevertheless, the advent of complement gene 'knockout' models, together with the development of high-resolution and dynamic-range analytical and structural tools, has provided the essential underpinnings for better understanding the molecular basis of complement's involvement in human pathophysiology [4–6]. Complement is now widely recognized as an important modulator of tissue homeostasis and a multitasking protein network that contributes to tissue immunosurveillance through the fine tuning of innate and adaptive immune responses [4,7,8]. Underscoring the crucial role of complement in human homeostasis and

Figure 1 Overview of the complement cascade illustrating its multiple interconnections to human physiology and disease, with an emphasis on key aspects of complement-targeted therapeutic design. (a) Complement activation on diseased host surfaces involves diverse recognition molecules and initiation mechanisms that underlie three distinct activation pathways operating either in parallel or independently of each other. Briefly, binding of C1 to antigen–antibody complexes (e.g. in autoimmune pathologies) triggers the classical pathway (CP), while binding of MBL/ficolin or properdin to target surfaces, via recognition of distinct molecular patterns, initiates the lectin pathway (LP) and alternative pathway (AP), respectively. Irrespective of their initiating trigger, all pathways converge at the proteolytic activation of C3, the central component of the cascade. Assembly of C3 convertases via any of the three aforementioned activation pathways promotes surface opsonization by C3 fragment deposition mainly through an AP-mediated amplification loop and also leads to effector generation (e.g. C3a anaphylatoxin release). C3 activation is the prerequisite for the downstream activation of the lytic pathway that begins with the cleavage of C5 and culminates in the assembly of the cell-perforating MAC. Both fluid-phase and surface-bound complement regulators (e.g. factor H, CR1, MCP) promote the factor I-mediated degradation of C3b into bioactive fragments that mediate a plethora of immunomodulatory functions in health and disease (including inflammatory signalling, phagocytic activation and modulation of adaptive immune responses). Cleavage of the central complement components C3 and C5 by their respective convertases results in the generation of the anaphylatoxins C3a and C5a that, through interaction with their cognate receptors (C3aR, C5aR1), trigger potent inflammatory signalling and chemotactic responses in a variety of myeloid and nonmyeloid cell types. (b) Therapeutic modulation of complement using the peptidic C3-targeted inhibitor compstatin: Compstatin acts as a highly selective protein–protein interaction inhibitor. Binding of compstatin to native C3 and also to C3b-containing convertases results in potent and broad complement inhibition through abrogation of C3 activation and AP-mediated amplification of C3 cleavage. The lack of C3b deposition also prevents C5 convertase assembly and downstream effector generation (e.g. C5a, MAC). The complement inhibitory action of compstatin is exerted regardless of the upstream trigger or complement pathway (CP, LP, AP) involved. C. Therapeutic intervention at different steps of the complement cascade affords distinct benefits and limitations to each targeting approach. Intervention at the early stages of complement initiation, such as inhibition of C1s and MASPs by C1-INH, affords effective blockade of the CP and LP, while letting the alternative pathway operate and preserve its homeostatic and immune surveillance functions. However, upstream inhibition may also still allow amplification of potentially harmful activation on diseased tissue. C3 interception using peptidic inhibitors of the compstatin family results in abrogation of C3 activation by the convertases, inhibition of AP-mediated amplification and downstream effector generation. However, compstatin does not affect initial activation of the AP through the 'C3 tick-over' mechanism and also allows for C4b generation through upstream activation of the CP and LP. Therefore, compstatin treatment may still allow for residual opsonic activity and complement-mediated immune surveillance. Finally, C5 blockade selectively targets the lytic pathway of complement abrogating C5 activation and generation of detrimental downstream effectors (i.e. C5a, MAC), while leaving intact upstream activation pathways including C3 activation and the AP-mediated amplification loop. *Abbreviations*: BCR, B-cell receptor; CR1 complement receptor type 1; CR2, complement receptor type 2; CP, classical pathway, AP, alternative pathway; LP, lectin pathway; MAC, Membrane Attack Complex; MBL, mannose-binding lectin; MASP, MBL-associated serine protease; MCP, membrane cofactor protein; DAF, decay-accelerating factor; CR's, complement receptors.

disease, an increasingly long list of clinical disorders and pathological manifestations has been associated with dysregulation or impaired function of distinct complement components across the entire spectrum of activation pathways (see [5] for an updated review).

Given the multifaceted role of complement in human pathophysiology, it becomes apparent that targeted inhibition and fine spatiotemporal modulation of its activity can provide unprecedented opportunities for therapeutic drug design and effective clinical intervention [2,9,10]. The clinical introduction of the first complement-specific drug in 2007, a therapeutic antibody that blocks the terminal complement component C5 (Eculizumab; Soliris®; Alexion Pharmaceuticals, Cheshire, CT, USA), further contributed to a renewed interest in complement as a target system. However, approved treatment options have remained scarce, and currently, they include only eculizumab and a serine protease inhibitor with broad target specificity within and outside the complement cascade (C1 inhibitor; available from various manufacturers). Hence, the distinct

clinical landscape of each disease, the emerging importance of therapy-modifying complement haplotype variations and the context-specific contribution of complement to disease progression all point to the need for comprehensive therapeutic strategies that will exploit broader complement intervention, affording coverage of multiple pathways.

Notably, the complement field is currently witnessing a surge of creative approaches aiming to block C3 activation through diverse means, tools and conceptual frameworks [9]. It remains to be seen which of these elegant drug design strategies will safely navigate through the Symplegades (i.e. 'clashing rocks') of preclinical development, eventually entering the clinic as potent anticomplement therapeutics.

Target selection: interception at the level of C3

With some 50 plasma-based and cell surface-bound proteins contributing to complement function [4], this intricate innate immune pathway offers numerous points of pharmacological intervention. Understandably, targeting a single complement
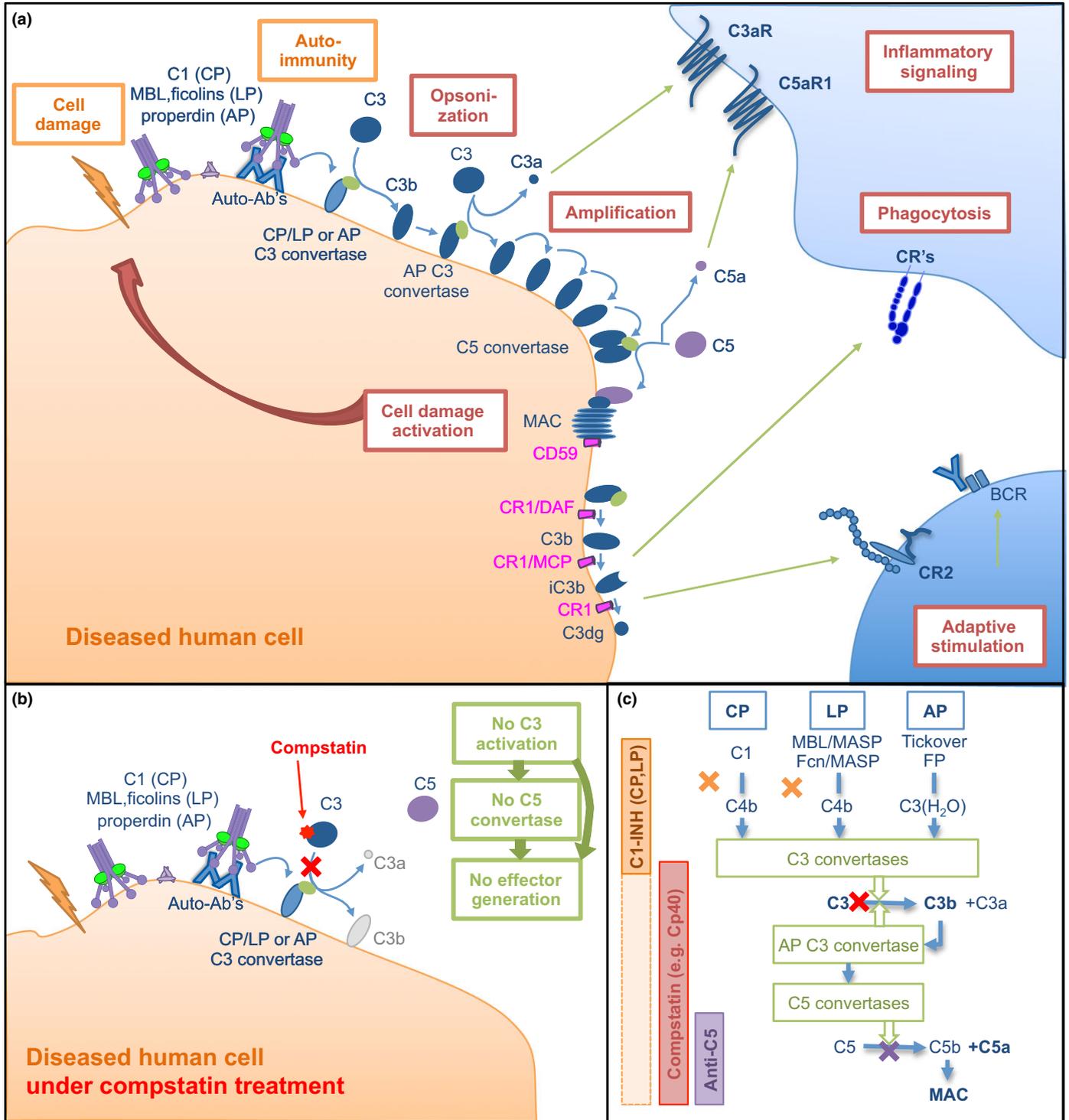
component in all pathologic states and clinical disorders is not a panacea. Therapeutic strategies aiming for upstream, central or downstream (terminal) complement inhibition each have their own merits and limitations (extensively reviewed in [2]). Being the convergence point of all activation pathways and a hub forging molecular interconnections with multiple pathogenic pathways, C3 serves as a particularly attractive target for therapeutic modulation of the complement cascade [2] (Fig. 1a).

Indeed, several lines of evidence indicate that C3 inhibition would qualify as a more comprehensive approach than would targeting downstream effector pathways (e.g. via C5 blockade or C5aR1 antagonism) in a variety of clinical conditions [2,9,11]. Notably, derivatives of the compstatin family (e.g. AMY-101, Amyndas Pharmaceuticals) are currently the only low molecular weight inhibitors of C3 that have advanced through clinical development for the treatment of several indications. Other antibody or complement regulator-based approaches, targeting complement at the level of C3 or the C3 convertase, have also entered preclinical development (e.g. mAbs S77 and 3E7 and the engineered regulators TT30 and mini-FH; for a review see [9]).

Because of its central role within the complement cascade, intercepting C3 naturally sparks questions concerning safety, particularly in the case of long-term, systemic intervention. Thus far, however, these discussions have remained mostly hypothetical because of our limited experience with C3-targeted inhibitors in the clinic. The often-raised concern that complete shutdown of C3 activity will generally increase susceptibility to infections, as the result of a loss of C3's opsonic capacity, has been based primarily on observations in C3-deficient patients. Indeed, individuals with primary C3 deficiencies show an increased risk for episodic infection by a limited set of pyogenic pathogens, primarily in the early stages of life when immunity is not yet fully developed [12,13]. Despite these clinical observations in the rare cases of homozygous C3 deficiency, it should be noted that long-term pharmacological C3 inhibition might not necessarily recapitulate the same phenotype. This may hold true particularly in view of a putative role of C3 in the early stages of development of the immune system and its multifaceted impact on various lymphoid and non-lymphoid compartments that may modulate the immune response to infections. In contrast to genetic C3 deficiency, targeted C3 interception using inhibitors such as compstatin would not be expected to interfere with such developmental pathways. Furthermore, in some animal disease models, C3 deficiency or inhibition even beneficially modulates the course of infection [14–16], underscoring the context-specific contribution of C3 in host–pathogen interactions. Notably, anti-C3 treatment with small-sized inhibitors (such as compstatin) can be easily interrupted in a clinical protocol, allowing for swift recovery of C3 activity during an active infection. In fact, even residual amounts of C3 can effectively contribute to host defence, and inhibition at the level of C3 still allows for C4b opsonization via the classical and lectin pathways [2].

Taken together, it remains unclear how much and how directly the observations in C3-deficient patients would translate to a C3-inhibitory therapy. At least until more clinical experience is gained, the risk for infection and its prophylactic management need to be carefully considered.

Patients subjected to chronic C3-targeted intervention [e.g. in paroxysmal nocturnal hemoglobinuria (PNH)] should likely be vaccinated against encapsulated bacteria, including meningococci, pneumococci and *Haemophilus influenza,* to eliminate the potential risk for infections during treatment. Similar prophylaxis, in particular against meningococcal infection, has successfully been applied in C5-targeted therapy for many years now. Apart from vaccination, long-term prophylactic use of antibiotics may also be considered as an option for further diminishing the risk for infection in cases of chronic C3-targeted intervention. Conversely, acute treatment with C3 inhibitors (i.e. in haemodialysis settings) is not expected to increase the risk of infection and would likely not require prophylactic measures for the duration of therapy. Moreover, transient C3 inhibition in transplantation settings should not evoke unwanted infectious complications, as clinical protocols already include antimicrobial prophylaxis to counterbalance this risk [17]. A recent study evaluating the efficacy of a soluble form of CR1 in a patient with C3GN-DDD has provided proof of concept for the safety and tolerability of C3 interception in acute clinical protocols involving over 2 weeks of C3-targeted intervention [18]. It is noteworthy, however, that potential safety issues do not apply to the local administration of C3 inhibitors, which in fact may have indirect antimicrobial effects, as in periodontitis [14]. Provided that certain safety precautions are taken into consideration, as in the case of anti-C5 therapy, it is expected that C3 interception protocols may afford therapeutic benefit with low or controllable adverse consequences.

Another concern that often sparks debate regarding C3-targeted therapies is the purported risk of autoimmune reactions that might be triggered by prolonged C3 inhibition. Complement component and receptor deficiencies have long been considered predisposing factors for autoimmune pathologies (e.g. SLE) [7,19]. Importantly, however, while deficiencies of the early components of the classical pathway (C1q, C2 and C4) render patients prone to autoimmune manifestations (e.g. SLE), C3 deficiency has only rarely been associated with a similar risk [19]. Recent studies have provided mechanistic insight into this seemingly paradoxical role of C3 in autoimmunity, by showing that the absence of C3 from dendritic cells downregulates antigen presentation and blunts downstream T-cell responses to aberrantly expressed self-antigens (e.g. apoptotic cells), thereby attenuating the risk for autoimmune reactions [20,21]. Notably, the absence of spontaneous autoimmunity in C3-deficient mice, as opposed to C1q-deficient mice, also corroborates these findings [19]. Altogether, these lines of evidence suggest that systemic C3 interception in a clinical setting would not run the risk of 'fuelling' autoimmune responses, and they further underscore the necessity of weighing conceptual extrapolations about prolonged C3 inhibition and autoimmunity against actual clinical data.

Finally, an often-raised concern in discussions over the safety of long-term C3 intervention is the impaired clearance of immune complexes (ICs) and the potential exacerbation of IC-mediated inflammatory responses. Indeed, alternative pathway activation and increased binding of C3 fragments appear to be important for solubilizing immune precipitates, and IC disorders have occasionally been reported in C3-deficient patients. Still, when compared to the susceptibility to episodic infections discussed above, the risk for developing IC-mediated diseases appears to be lower and not as well defined [22], suggesting that other mechanisms may override the requirement for C3 in these processes. Of note, even in the absence of C3, upstream components of the classical or lectin pathways (MBL, C1q, C2, C4) can handle several aspects pertinent to IC clearance [22]. For example, binding of C1 or C4b to immune complexes may interfere with Fc–Fc interactions, thus reducing rapid IC aggregation and precipitation [22]. Furthermore, C3 inhibition and the abrogation of downstream effector generation (e.g. C5a) may even beneficially modulate the inflammatory response triggered by IC-Fc gamma receptor interactions in certain cases of IC-driven pathology [23]. Although a direct correlation between long-term C3 inhibition and development of IC disorders remains to be established, it will be important to monitor IC levels during future clinical studies.

As compstatin derivatives and other C3-targeted inhibitors make their way into clinical evaluation, it will be important to replace these hitherto largely hypothetical discussions about safety with real clinical experience.

Molecular development of compstatin

Discovery, initial characterization, and proof-of-concept of its therapeutic potential

The drug design effort that culminated in the discovery of compstatin was bolstered by the concept that small-molecule complement inhibitors targeting all three pathways at their focal point of convergence (i.e. the C3 activation step) might have a more promising profile for clinical application in pathological disorders that entail deregulated complement activation. In line with this concept, compstatin was discovered by phage-displayed peptide library screening as a 13-residue, cyclic peptide (I[CVVQDWGHHRC]T-NH$_2$) [24] that selectively binds to native C3 and its bioactive fragments C3b and C3c [24,25]. Initial functional characterization revealed that compstatin controls the activation of C3 by the convertases but acts by sterically hindering the binding of C3 to the convertases, unlike the natural regulators (i.e. FH, CD35, CD46 and CD55), which destabilize the C3 convertase or accelerate degradation of the enzyme complex [24–26]. Importantly, a series of structure-guided activity studies have revealed that specific type I β-turn residues, hydrophobic interactions and the cyclic

core structure itself are critical not only for the conformational stability of the peptide but also for binding to C3 and the inhibitory action of the peptide (for detailed reviews see [11,27,28]). The therapeutic efficacies of the original compstatin and its acetylated derivative have been evaluated in a spectrum of clinically relevant *in vitro* and *ex vivo* models ranging from biomaterial-induced complement activation by extracorporeal circuits to xenotransplantation (for a more detailed review see [11,28]).

Species specificity of compstatin and exclusive binding to primate C3

The inhibitory potency of compstatin against C3 from various species quickly emerged as a critical question, particularly in terms of validating its therapeutic potential in appropriate animal models of disease. A series of complement assays in different species (human, various monkeys, pig, rabbit, guinea pig, rat and mouse) suggested that this inhibitor exhibits a narrow species selectivity profile, binding exclusively to human and nonhuman primate (NHP) C3 [29]. Subsequent interaction analysis confirmed that compstatin binds NHP C3 (baboon) and human C3 with similar affinity, but it does not bind to mouse or rat C3 [25,29]. Importantly, and despite an improvement in the C3-binding affinity by several orders of magnitude, this narrow species specificity is still found in the most recent analogues of compstatin. Recent studies, employing an ELISA-based assay to quantify the inhibitory effect of the current lead analogue Cp40 on classical pathway-mediated complement activation, showed exclusive inhibition in human and NHP plasma, but not in mouse, rat, rabbit, swine and canine complement assays (J.D. Lambris unpublished observations). On the other hand, the highly comparable binding of compstatin to human and NHP C3 was recently confirmed by surface plasmon resonance (SPR)-based studies, in which the binding profiles of Cp40 and related analogues were almost identical for C3 from humans, baboons and cynomolgus and rhesus monkeys [30]. In view of this consistently observed primate specificity of compstatin analogues, recent studies reporting the effects of compstatin-mediated C3 inhibition in rodent models of disease [31,32] and using a commercial, nonlicensed compstatin product (sold by Tocris) can only be explained as experimental artifacts. Moreover, the product tested in these studies corresponds to the original compstatin, which is up to 6000-fold less active than the derivatives currently in clinical development.

This exquisite specificity of compstatin for primate C3 was originally considered surprising, given the high degree of phylogenetic conservation of C3 from various mammalian species, with a pronounced homology in the primary structure that varies from 85% to 94%. Over the past decade, an array of biochemical, functional and structure-based investigations have

corroborated the original premise that compstatin binds its target protein (C3) according to distinct species-specific 'constraints'. In particular, the release of a cocrystal structure of C3c with a 45-fold more potent analogue of the parental peptide (analogue 4W9A) has largely explained the high selectivity of compstatin for human and NHP C3 [33]. Whereas an earlier study had already identified a 40-kDa C-terminal fragment of the β-chain of C3 as the binding region of compstatin [34], the crystal structure revealed a shallow binding pocket formed by macroglobulin (MG) domains 4 and 5 of C3 [33]. Several critical residues involved in the interaction between C3 and compstatin, that is, Gly-345, His-392, Pro-393, Leu-454 and Arg-459, were found to be highly conserved in human and primate C3, whereas all of them differed in other mammalian species [33]. The presence of different residues in these positions may lead to steric restrictions, loss of specific contacts within the binding site or conformational differences in the local structure. Overall, these structural aspects largely explain the species specificity of compstatin and provide a structural basis for its exclusive binding to human and primate C3.

Collectively, these studies attest to the high target selectivity of compstatin. Moreover, the comparable binding profiles towards human C3 and C3 from different monkey species strongly support the concept that preclinical NHP models of therapeutic C3 intervention have clear potential for successfully translating the therapeutic efficacy and safety profile of the most promising compstatin analogues into the human setting.

## Unravelling the binding and unique inhibitory modes of compstatin

The resolution of the crystal structure of native C3 and its C3b and C3c fragments not only provided critical insight into the conformational dynamics of C3 but also catalysed efforts to unravel the structural basis of compstatin's mode of action [35–37]. One particular milestone in this context was the resolution of the C3c-compstatin cocrystal structure that led to the mapping of the binding site in a shallow pocket between the MG4 and MG5 domains of the β-chain of C3 (Fig. 2a) [33]. Strikingly, the cocrystal structure revealed that compstatin undergoes substantial conformational changes upon complex formation, whereas C3 itself appears largely unchanged [33]. Once the peptide switches from an open, U-shaped conformation in solution to a twisted O-shaped form upon binding, the peptide snugly fits the shallow binding site, with some 40% of its surface being buried in C3c [33]. The importance of this conformational switch was later extended by NMR and binding studies and has had important implications for the structural optimization of compstatin that has led to more potent analogues with impressively improved binding affinities ([30], see also below). Details concerning the binding mode of compstatin have been discussed in previous publications [28,33].

It soon became evident that compstatin, unlike physiological regulators, does not act on the C3 convertases, but rather protects native C3 from convertase-mediated cleavage. Localization of the binding site in the β-chain of C3 essentially excluded the possibility that compstatin directly interfered with the release of the C3a fragment that is located in the α-chain of C3. At the same time, the cocrystal structure suggested that the peptide occupies a potential dimerization site of C3/C3b, and this hypothesis was later supported by the analysis of the structure of the C3 convertase [33,38]. Based on these observations, the prevalent model of compstatin's action indicates that this peptide acts as a protein–protein interaction inhibitor by sterically hindering the binding of native C3 to the convertases [33] (Fig. 2b), thereby preventing the cleavage of C3 by both the classical/lectin and the alternative pathway C3 convertases and blocking the generation of effector fragments. Despite the structural homology that exists between the complement components C3, C4 and C5, compstatin is specific for C3 and does not prevent the activation of C4. Also, it does not block the hydrolysis of C3 to the active $C3(H_2O)$ form (i.e. tick-over activation), nor does it prevent convertase-independent cleavage of C3 by proteases such as thrombin that may occur under certain disease conditions. As such, compstatin and its derivatives efficiently control the major routes of complement activation and block the often-devastating amplification of the response, while leaving residual (upstream) complement activity intact.

Towards a new generation of more potent compstatin analogues

The resolution of the crystal structure of the C3c–compstatin complex has helped to streamline the refinement effort on the parental peptide, paving the way for the generation of more potent analogues that are now in clinical trials for diseases such as age-related macular degeneration (AMD) and also show promise for clinical intervention in PNH, haemodialysis-associated thromboinflammation, periodontal disease, acute graft rejection and sepsis [9] (an overview of the milestones achieved throughout the preclinical development of compstatin is provided in Fig. 3).

Even in the absence of structural information from the cocrystal, a range of complementary approaches guided by biochemical, and biophysical, analyses led to a remarkable improvement in compstatin's activity (reviewed in [28]) that culminated in the discovery of analogue 4(1MeW) [39], which formed the basis for the clinical development of POT-4 by Potentia (now Apellis Pharmaceuticals). It was in the past few years, however, that the molecular improvement of the compstatin family reached several milestones, with the most recent analogues showing more than a 6000-fold improvement in target binding affinity when compared to the parent peptide
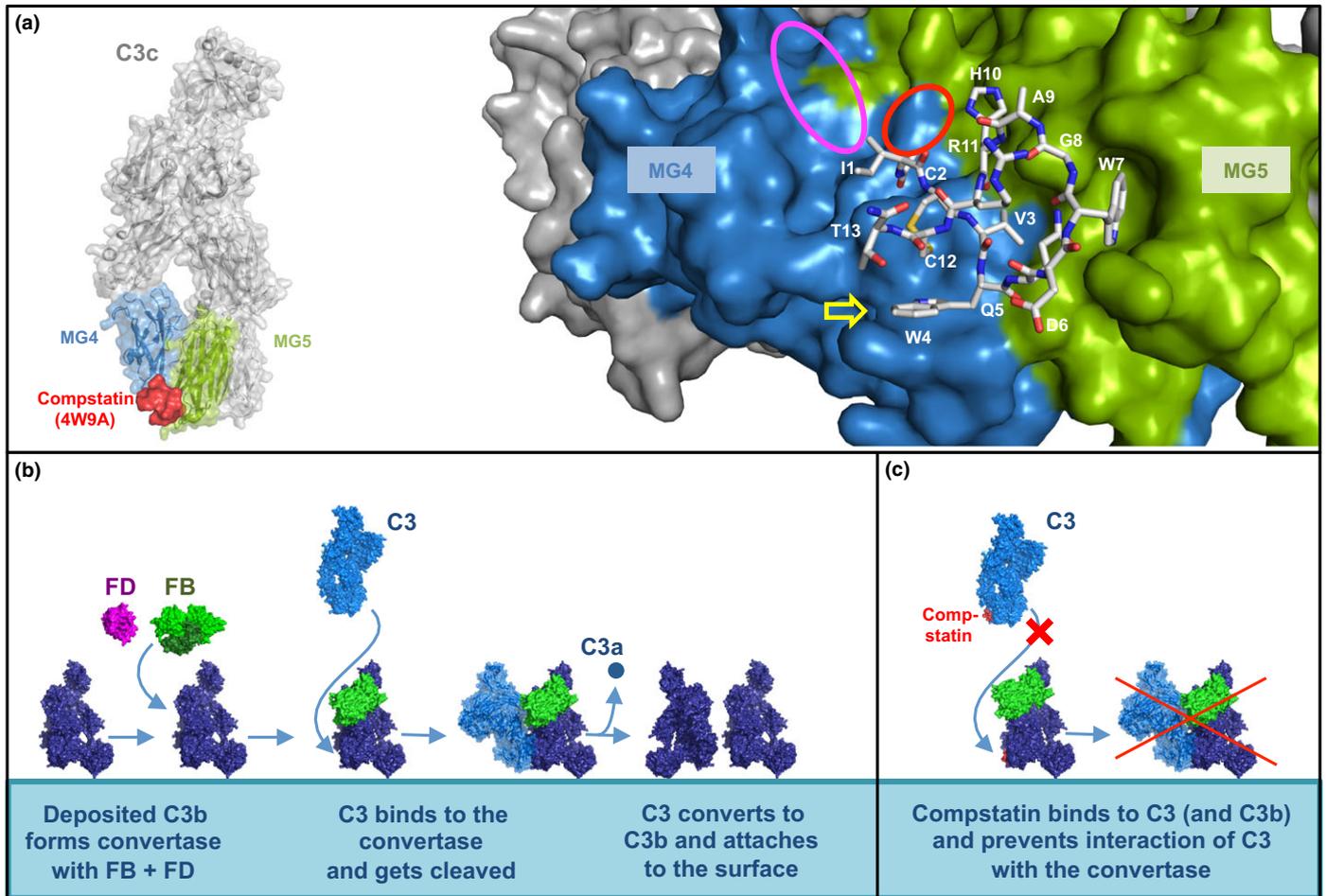
Figure 2 The unique binding and inhibitory modes of compstatin on human C3. (a) The resolution of the crystal structure of the C3c–compstatin complex revealed that the binding of compstatin to C3 occurs at the macroglobulin (MG) ring of the β-chain, in a shallow pocket formed between the MG4 and MG5 domains of the β-chain (coloured blue and green respectively). Open circles depict additional contact sites on C3 that have been explored through targeted modifications of compstatin, i.e. via N-terminus extension in analogue Cp40 (red) or by adding albumin-binding tags (magenta). (b) Schematic illustration of the key protein interactions leading to the formation of the alternative pathway (AP) C3 convertase on a target surface, displaying the subsequent binding of native C3 to the nascent convertase and its cleavage into C3a and C3b. (c) Compstatin acts as a protein–protein interaction inhibitor. It binds both native C3 and C3b and sterically inhibits the binding and cleavage of native C3 by C3 convertases.

(Fig. 3). This enormous progress has been facilitated by the resolution of the C3c cocrystal structure and has involved state-of-the-art techniques such as backbone methylation and the exploration of an extended binding site. Backbone N-methylation is regarded as an effective approach to lowering the binding entropy of peptides by imposing constraints in the peptide backbone that, in turn, affect its secondary structure and side-chain orientation [40,41]. N-methylation has also been shown to increase the binding selectivity of small peptides for their targets, improve stability and solubility (by reducing intramolecular hydrogen bonds and electron-inducing effects) and extend the half-life of modified peptides in the plasma

[41,42]. It was therefore hypothesized that backbone N-methylation of compstatin would influence the solution structure and contact network, while at the same time, it would have positive effects on the plasma stability and pharmacokinetic (PK) profile. A systematic N-methylation scan of a compstatin template was therefore employed to identify suitable sites of methylation; while most of the modifications left the activity unaffected or reduced, backbone methylation at Gly-8 and Thr-13 led to a significantly improved inhibitory potency [43]. In fact, one of the generated analogues (Ac-I[CV(MeW)QDW-Sar-AHRC](NMe)I-NH$_2$, with Sar standing for sarcosine or N-methyl-glycine), termed Cp20, displayed a profound
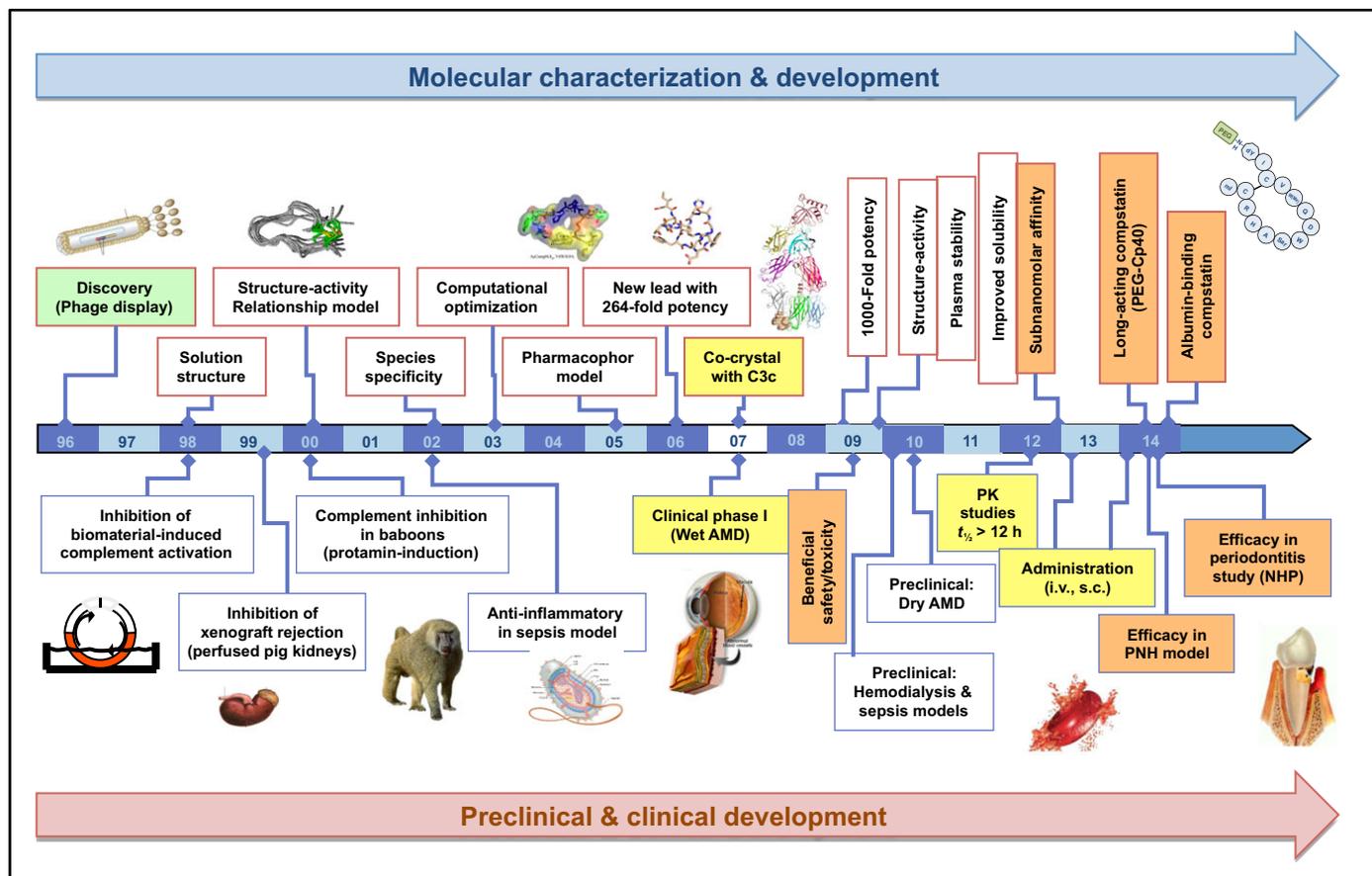
Figure 3 Milestones of the preclinical and clinical development of compstatin and its most potent derivatives. A comprehensive timeline of the molecular characterization, structure-guided optimization preclinical and clinical evaluation of the most promising compstatin derivatives, including milestones that have illuminated key structural elements of the interaction with C3 and have propelled the drug design effort placed on this family of peptidic C3 inhibitors (see text for more details).

increase in potency (IC_{50} = 62 nM) and C3b binding affinity (K_D = 2·3 nM) over the first-generation analogues [43]. It was later shown that N-methylation of the N-terminus, by replacing the terminal acetyl group with a sarcosine residue, further improves the target affinity and positively affects peptide solubility; the resulting analogue, Cp30, featured a K_D of 1·6 nM [30]. As an interesting side note, these cyclic, N-methylated compstatin analogues increasingly resemble the clinically successful peptide drug cyclosporine in terms of both structure and physicochemical properties; the target specificities and clinical profiles of the two peptides are, however, highly distinct.

Biophysical and structural analyses have provided molecular explanations for the observed improvements achieved through backbone methylation. As mentioned above, compstatin undergoes rigorous conformational rearrangement upon binding to C3 when compared to the free (unbound) molecule [33].

It was therefore hypothesized that backbone N-methylation would afford a more constrained conformation that would more closely resemble the bound form of the peptide in complex with C3. Indeed, kinetic analysis confirmed that analogues with sarcosine at position 8 consistently show an enhancement of the association rate (as a measure of complex formation) when compared to the nonmethylated glycine derivatives [43]. This hypothesis was further corroborated by NMR analysis of a Sar-8 analogue and by computational studies, which revealed a significant change in the solution structure to a more compact, twisted form resembling bound compstatin, when compared to the 'relaxed' form of an early analogue [30]. This conformational shift appears to be induced by the ability of Sar-8 to stabilize a β-turn encompassing residues 8–11 rather than residues 5–8, as observed in the nonmethylated analogues. From a structure-guided perspective, methylation at position 8 therefore appears to induce a 'bound-like' structure of compstatin in

solution by shifting the conformation of the peptide [30]. In addition, N-methylation may also induce a better orientation of the hydrophobic side chains in key residues of compstatin and increase the hydrophobic interactions with the hydrophobic binding pocket in C3 [30,43]. This increased binding to the hydrophobic pocket in C3 may also hold true for the effect of changes at the C-terminal residue 13, because the replacement of Thr-13 with the smaller, hydrophobic Ile-13 conferred improved affinity on the peptide. Imposing further structural constraints through backbone N-methylation of the flanking residue at position 13 has resulted in analogues with significantly enhanced inhibitory potency and binding affinity, indicating that the nature of the residue at position 13 can exert a modifying effect on the binding of compstatin to C3 [43].

The initial characterization of compstatin and its cocrystal structure with C3c have both indicated that the N-terminus plays a comparatively minor role in C3 binding when compared to the cyclic scaffold [24,33]. However, the improved activity of an analogue with a sarcosine residue replacing the N-terminal acetyl group (Cp30) has indicated that an extension of the N-terminus may confer beneficial effects [30]. Indeed, the addition of a panel of distinct chemical groups, natural and nonproteinogenic amino acids, at the N-terminus of Cp20 significantly influenced activity. One of the resulting analogues featuring an N-terminal D-tyrosine, termed Cp40 (yI[CV(MeW)QDW-Sar-AHRC](NMe)I-NH$_2$), has shown markedly improved inhibitory activity and a K_D of 0·5 nM for C3b, making it the first compstatin derivative with subnanomolar target affinity [30]. Computational analysis of the Cp40–C3c complex using docking approaches has revealed that an additional hydrophobic patch on the surface of C3c might be available for further contact with N-terminally extended versions of the compstatin scaffold [30] (Fig. 2a). Notably, some of the latest N-terminally modified analogues seem to exhibit a higher solubility than does Cp20, a feature that may facilitate systemic dosing. Overall, these optimization efforts have not only produced highly potent compstatin analogues with high suitability for therapeutic administration but have also revealed promising avenues for further improvement of this peptide family. As detailed in the section below, PK optimization of compstatin has already led to the discovery of a secondary binding site that may be tapped to increase the potency of future analogues [44].

## Clinical development of compstatin derivatives

Pharmacokinetic profile of compstatin and optimization strategies

Peptide drug development has traditionally been hampered by factors such as limited administration options, poor cell penetration, low metabolic stability and rapid plasma elimination

[45]. Whereas cell penetration is of limited importance in the case of compstatin, because the target molecules are primarily found in the circulation, the other aspects have been investigated and optimized during preclinical development of compstatin analogues. Early on, it was recognized that the cyclic nature of the peptide is an important contributor to its high plasma stability, with the acetyl group of the initial analogues further contributing to protecting the N-terminus from proteolytic degradation (the C-terminus has always been capped by amidation) [25]. In newer analogues, nonproteinogenic amino acids such as sarcosine (used in the compstatin analogue Cp30) or D-tyrosine (used in the compstatin analogue Cp40) have replaced the acetyl group [30]. One potential point of lability is the disulphide bridge, which is susceptible to reduction. Although changes in the cyclic conformation of the peptide are typically not well tolerated [25,33], the disulphide bridge may be replaced with a thioether bond without profound loss of potency [46]. As expected, the resulting thioether derivatives of compstatin showed largely improved stability against reduction. In plasma, however, even the disulphide-bridged analogues have shown extraordinary stability; thus, it appears advisable to reserve the thioether approach for specific conditions in which reductive stress may be prevalent [46].

The rapid elimination profiles of the first-generation compstatin analogues in human and NHP plasma have posed a major hurdle in the development of therapeutic compstatin analogues for systemic drug administration. The clinical candidate POT-4 (Potentia), for example, has been evaluated for local treatment of AMD via intravitreal injection, thereby largely bypassing these limitations. Recent PK studies of second-generation analogues (Cp20, Cp30, Cp40) in cynomolgus monkeys have surprisingly revealed apparent terminal plasma half-life values of up to ~12 h after a single intravenous injection [30]. Notably, these plasma elimination profiles have followed a biphasic mode that can be correlated with the plasma C3 concentration, with a rapid elimination of peptide exceeding the C3 level, followed by a much slower phase of elimination (Fig. 4a). This pattern suggests a 'target-driven' model in which the tight binding of compstatin to C3 prevents renal elimination of the peptide. Indeed, the half-life values determined for the three tested analogues were found to be directly related to their binding affinities for C3, with Cp40 showing the longest plasma residence [30]. Future optimization of their binding properties and efficacy may therefore simultaneously affect the plasma elimination of compstatin analogues.

Although the beneficial PK profiles of the second-generation compstatin analogues make them directly amenable for systemic use, an additional extension of the time that the drugs are resident in the plasma may confer an advantage for indications that rely on long-term administration. Two recent studies have evaluated distinct strategies for retaining compstatin analogues
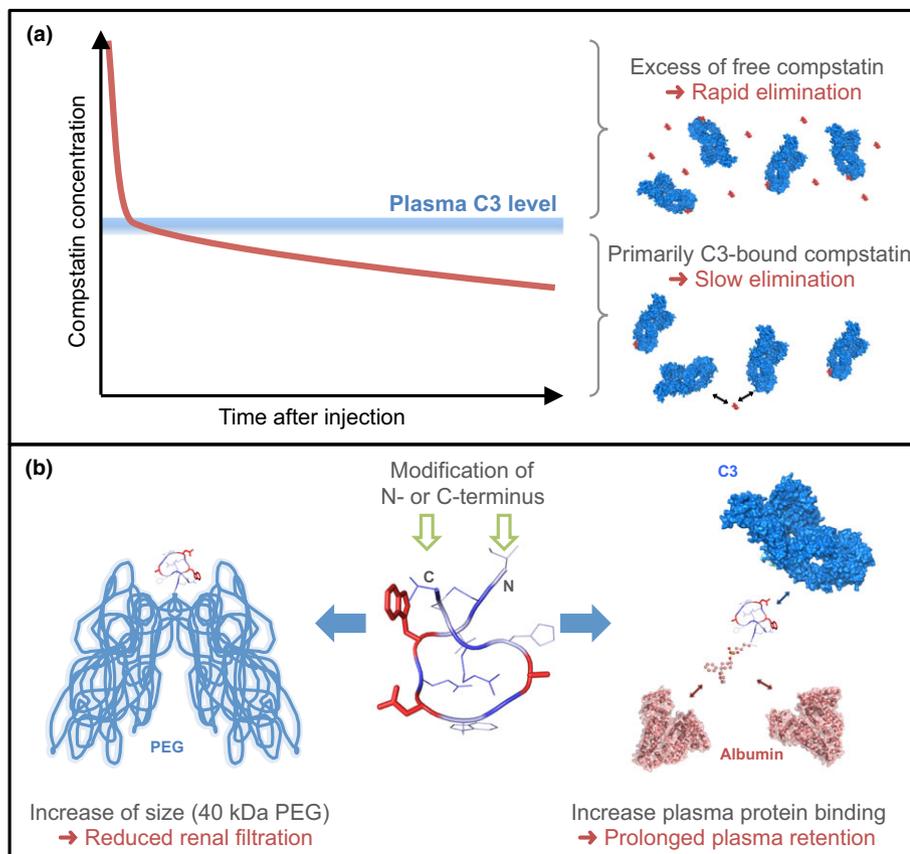
Figure 4 The pharmacokinetic profile of compstatin and structure-guided modifications aimed at improving its plasma residence. (a) A 'target-driven' model of compstatin's elimination from plasma: A first rapid phase of clearance of the excess of free (unbound) compstatin is followed by a second, slower phase of plasma elimination that is driven by the tight binding of compstatin to its target protein, C3. (b) Schematic overview of N- or C-terminal modifications for the development of compstatin derivatives with increased plasma residence. These compstatin derivatives are designed to display longer plasma retention via binding to carrier proteins (i.e. albumin) or via reducing renal filtration by conjugation to high molecular weight-PEG moieties. More details on the various peptide modification strategies employed to improve the pharmacokinetic profile and plasma residence of compstatin can be found in the text.

in the circulation in concentrations that exceed the C3 concentration. The option of attaching modifying groups to either the N- or C-terminus of the compstatin analogues without profoundly affecting their inhibitory activity has greatly facilitated these strategies (Fig. 4b). Following a traditional approach to the development of biologics [47], polyethylene glycol (PEG) moieties were attached to the compstatin analogue Cp40. The size of the branched PEG group (40 kDa) was expected to reduce renal filtration and prolong the half-life in a target-independent manner. Indeed, when injected into cynomolgus monkeys, the PEGylated Cp40 showed a remarkably enhanced half-life of more than 5 days [48]. Despite its impressive extension of the half-life by a factor of 10, the PEGylation approach poses several questions that still need to be addressed: first, an observed increase in the C3 plasma concentration during treatment with PEG-Cp40 suggested that the binding of a large PEGylated compound might interfere with the metabolism of C3 [48]. However, this observation needs to be further corroborated, and there is no evidence as yet as to whether this increase in C3 affects dosing schemes. Second, the long half-life of PEG-Cp40 makes it more difficult to adjust the drug levels or even phase patients off treatment in cases of potential therapy-modifying events (e.g. infections). Third, it is

not clear whether the PEG moiety itself induces some level of toxicity, particularly at the comparatively high concentrations required to inhibit C3. Indeed, complications such as vacuolization have been described for PEG modifiers [47,49]. Nevertheless, PEGylation of compstatin may prove useful in certain indications, and PEGylated derivatives are currently been developed and evaluated by Potentia and Apellis.

Another approach for extending the half-life of drugs relies on exploiting the transporter/depot function of abundant serum proteins such as albumin. For example, conjugation of peptide drugs to an albumin-binding peptide (ABP) has previously been shown to increase their half-life in circulation [50]. This strategy was successfully adopted with a first-generation compstatin analogue by adding an ABP to the terminus via a mini-PEG linker; the resulting ABP-fusion peptides retained their inhibitory activity and displayed a prolonged plasma residence (i.e. still detectable 24-h postinjection) after injection in mice; in contrast to compstatin, the APB moiety binds to albumin of both human and mouse [51]. Although the results are encouraging, the synthesis and linkage of two disulphide-bridged peptides are challenging and potentially costly. A corresponding approach involving a low molecular weight albumin-binding molecule (ABM), which is used in the clinic to

increase the half-life of a contrast agent [52], has recently been implemented to improve the PK properties of compstatin analogue Cp20 (Fig. 4b). N-terminal conjugation of different ABM entities to Cp20 resulted in derivatives with markedly increased plasma protein binding [44]. Most notably, one of the resulting derivatives (ABM2-Cp20) displayed a 20-fold higher binding affinity for C3 (K_D = 150 pM) over the parental peptide, making it the most potent compstatin analogue to date. Kinetic and structural analysis indicated that the ABM2 moiety occupies a secondary site on C3b, thus affording additional contacts that improve the dissociation rate of the complex [44]. Going forward, this intriguing strategy may allow for extending the plasma residence of free compstatin exceeding the C3 level (via albumin binding) while at the same time reducing clearance by prolonging the bound state via increased C3 affinity. Undoubtedly, these promising PK profiles will pave the way for further clinical development of lead compounds. Along the way, open questions concerning biodistribution, route of excretion, immunogenicity and the potential generation of metabolites still need to be answered.

Probing the therapeutic efficacy of compstatin analogues in preclinical and clinical studies

Since its discovery, compstatin has been tested as an attractive drug candidate in a plethora of disease models associated with dysregulated or excessive complement activation [2,5,28]. Previous reviews have provided a thorough survey of the initial therapeutic evaluation of compstatin and its first-generation analogues [11,28]. In this section, we provide an updated account of its therapeutic evaluation, encompassing the most recent developments, promising indications and suitable treatment strategies.

Age-related macular degeneration. Age-related macular degeneration is the most prevalent eye disease in the industrialized world [53]. This debilitating disorder gradually leads to irreversible blindness through the progressive development of geographic atrophy (GA; dry AMD) and/or choroidal neovascularization (CNV; wet AMD) of the patient's retina [54,55]. Chronic inflammation has been recognized as a potential driving factor in the disease, and a particularly prominent role for complement was first demonstrated by genome-wide association studies (GWAS) identifying single nucleotide polymorphisms within the factor H locus as the most strongly predisposing genetic factor for the development of AMD [56–59]. Meanwhile, other complement polymorphisms have been associated with AMD, underscoring the intricate involvement of complement-driven inflammation in disease progression [60].

Given this strong association of complement dysregulation with AMD, compstatin has long been considered an attractive therapeutic option for the treatment of this disease. A first-generation compstatin analogue has been evaluated in a unique NHP model of early-onset AMD in cynomolgus monkeys. These animals develop drusen, lipoprotein deposits in the retinal tissue that are considered an early hallmark of dry AMD, at a much earlier age than do unaffected monkeys or humans. This model therefore permits the therapeutic testing of novel inhibitors over an extended time frame [54]. Weekly intravitreal injection of compstatin in affected monkeys resulted in a clear amelioration of disease scores, as illustrated by a suppression of drusen formation and almost complete dissolution of the drusen in the retinas of compstatin-treated animals after 9 months. Of note, injection of concentrated compstatin solutions into the vitreous appears to cause local precipitation and may have favoured a slow-release mechanism that produced a sustained therapeutic effect over a long treatment period [54]. After licensing the first-generation compstatin technology from the University of Pennsylvania in 2006, Potentia clinically developed POT-4 for application in AMD. Phase I studies in patients suffering from severe wet AMD not only confirmed its tolerability and safety but also indicated clinical efficacy. Based on that promising profile, Alcon entered licensing options and conducted phase II trials of the compound, designated AL-78898A, for exudative/wet AMD in combination with Lucentis [61]. Unfortunately, for undisclosed reasons, a much-reduced dose of the compound was used in this study when compared to the phase I trials, and no therapeutic efficacy could be determined. Another phase II proof-of-concept study in GA (dry AMD) was subsequently terminated [62]. Despite these hold-ups, the encouraging results from the phase I trial of POT-4 and the announced progression of another alternative pathway inhibitor (anti-FD mAb; Lampalizumab, Genentech) to phase III trials show the potential of that treatment strategy. It will therefore be interesting to see whether improved formulation, dosage and inclusion criteria will lead to an improved therapeutic outcome for compstatin in AMD.

Sepsis. Severe septicaemia is a clinical disorder that 'derails' the host's inflammatory response to bacterial infection by causing massive activation of complement and also pronounced procoagulant responses that, if left unchecked, can lead to disseminated intravascular coagulopathy, multi-organ dysfunction and death [63]. The cytokine storm that ensues upon bacterial challenge causes a generalized impairment of innate immunity, leading to a severe disability to clear the pathogen and, ultimately, to high rates of mortality that still pose a serious challenge in clinical care [64].

Targeting C3 activation in models of human septicaemia has attracted considerable attention because of the cardinal role of this complement component in modulating cytokine-driven pathways and other innate immune effector systems. In this context, compstatin has shown consistent efficacy in blocking

inflammatory and procoagulant reactions in a human whole blood model of bacterial inflammation that closely resembles acute septicaemia [65–68]. Furthermore, recent studies have suggested that anti-CD14 treatment might confer additive protection against LPS shock or Gram-positive or polymicrobial bacterial challenge, when combined with C3 or C5 inhibition [69–71].

To translate these important findings into a clinically relevant context, C3 inhibition by compstatin treatment [analogue 4(1MeW)] was evaluated in a baboon model of *Escherichia coli*-induced sepsis (LD$_{50}$ model) that closely recapitulates the pathophysiology of clinical sepsis. In this primate model, the disease progresses from transient hypotension to multi-organ failure (MOF), which eventually leads to death [72,73]. Compstatin was administered to septic animals by i.v. infusion, according to two therapeutic strategies: an early 'prevention' regimen and a late 'rescue' regimen. Compstatin conferred significant protection from inflammatory and procoagulant complications and contributed to the protection of organs from the detrimental effects of ischemia-reperfusion (I/R) injury during the second stage of severe sepsis. Moreover, treatment with compstatin significantly reduced complement deposition in the kidneys, suggesting that endothelial injury and I/R-induced nephrotoxicity were decreased, resulting in improved clinical signs of acute kidney injury (AKI). In septic baboons, both the prevention and rescue regimens of compstatin treatment led to a recovery of white blood cells in the circulation and to lower plasma platelet consumption. Moreover, a clear inhibitory effect of compstatin on various sepsis-induced procoagulant markers was observed, and complement inhibition was associated with lower fibrin deposition through a modulation of the coagulation (TF) and fibrinolytic (PAI-1) pathways. Despite bacterial survival being a key target of antimicrobial therapeutics, it should be stressed that multi-organ dysfunction is the main cause of death in late-stage sepsis and trauma [63]. While bacteremia can be treated with antibiotics, there are no established therapies that provide organ protection. Most notably, C3 inhibition by compstatin not only restored systemic blood pressure in the septic baboons, in both the early and late therapeutic regimens, it also exerted a markedly protective effect on organ function that was evident as late as 6-h postbacterial challenge (e.g. no signs of thrombosis and lower leucocyte infiltration in the lungs, and protective effects in the liver, spleen and kidneys) [72]. The organ-protective effect of compstatin in late-stage sepsis raises awareness for a new therapeutic avenue that may be further explored, considering that no other therapy tested so far in the baboon model has shown protection when administered at such a late stage.

Taken together, these studies have provided new insight into the therapeutic potential of compstatin for systemic inflammatory disorders such as the systemic inflammatory response syndrome (SIRS) and sepsis, and they also open up avenues for clinical improvement through combined inhibition of C3 and CD14, as recently indicated in a porcine whole blood model of sepsis [74].

Paroxysmal nocturnal hemoglobinuria. PNH is a rare but chronic debilitating haematological disorder that is characterized by haemolytic anaemia, bone marrow failure and thrombosis [75]. Its main pathological attribute is the release into the circulation of mature blood cells (e.g. erythrocytes, platelets) that lack the GPI-anchored complement regulators CD55 and CD59 [76,77]. The absence of intrinsic complement regulation leads to persistent complement attack on the erythrocyte surface, dysregulated C3 opsonic turnover and MAC-dependent intravascular haemolysis [78]. The anti-C5 monoclonal antibody eculizumab (Soliris; Alexion Pharmaceuticals) is currently used in the clinic as an effective means of blocking complement-dependent intravascular haemolysis by preventing C5 cleavage and MAC formation on susceptible erythrocytes [79]. However, patients on anti-C5 therapy show variable responses and still face complications as the result of uninhibited amplification of C3 activation though the AP and persistent opsonization of erythrocytes [80]. This upstream pathway appears to lead to C3-mediated extravascular haemolysis, thereby limiting the therapeutic benefit of anti-C5 treatment [81]. Intervention at the level of C3 has therefore been considered a more comprehensive and effective treatment strategy. Whereas proof of concept has been shown with complement regulator-based protein therapeutics such as TT30 and mini-FH [82,83], small-sized inhibitors such as compstatin potentially offer additional benefits concerning cost-effectiveness and other factors. In a recent study, the second-generation analogue Cp40 and its PEGylated derivative (PEG-Cp40) were evaluated for therapeutic efficacy in blood from PNH patients [48]. Both unmodified Cp40 and PEG-Cp40 protected PNH erythrocytes from MAC-mediated intravascular haemolysis. In contrast to anti-C5, however, the compstatin derivatives also efficiently prevented C3 fragment deposition on PNH erythrocytes, which eventually could lead to immune cell recognition and extravascular haemolysis. These findings strongly suggest that compstatin may effectively intercept both intravascular and extravascular haemolysis in PNH, thus providing a therapeutic advantage over eculizumab treatment. Given the open questions concerning PEG-Cp40, it was important to demonstrate that sustained plasma inhibitor levels could be achieved even with unmodified Cp40 when injected subcutaneously into cynomolgus monkeys in a multidose regimen (at 12-h intervals). These findings suggest that Cp40 may prove a valuable option for long-term systemic treatment of PNH patients, potentially even allowing for self-administration by the patient

via a subcutaneous formulation [48]. AMY-101 (Amyndas Pharmaceuticals), a novel C3-targeted peptidic drug based on Cp40, has shown sustainable efficacy and favourable safety and PK profiles in NHP [84]. This novel therapeutic is undergoing clinical development for the treatment of PNH and other complement-mediated indications (Amyndas Pharmaceuticals). Its strong potential and unique clinical profile as a complement-based therapeutic have recently been endorsed by the European Medicines Agency (EMA) and the FDA, both of which accorded AMY-101 an orphan drug designation for PNH [85,86]

Haemodialysis-induced inflammation. Acute kidney injury (AKI) and end-stage renal disease patients undergo frequent cycles of haemodialysis as a standard option for treating renal failure [87]. However, haemodialysis evokes several clinical complications, including chronic inflammation, anaemia and elevated risk of thrombosis and cardiovascular disease, which arise from the contact of artificial filter surfaces with blood constituents. An important contributor to such complications is the biomaterial surface-triggered complement activation and subsequent inflammatory and procoagulant reactions that may have a profoundly negative impact on therapy outcome [88]. Whereas cellulose-based filters have long been known to invoke massive complement activation, the introduction of synthetic polymers was thought to have resolved this problem. However, both *in vitro* and *ex vivo* perfusion studies using whole blood have shown that even these 'biocompatible' filters activate complement and induce cytokine release, cell activation and expression of tissue factor; compstatin analogues have been shown to prevent these thromboinflammatory effects [68]. These investigations were extended in an NHP model of haemodialysis-induced inflammation, in which cynomolgus monkeys were subjected to haemodialysis sessions involving state-of-the-art paediatric filters and heparinization [89]. Even with these close-to-clinical conditions, significant complement activation could be observed during the 4-h session. Importantly, a single bolus injection of Cp40 prior to haemodialysis treatment proved to be effective in blocking complement activation throughout the entire procedure and in modulating haemodialysis-induced inflammatory markers such as IL-10 [89]. Controlling inflammatory triggers via C3 inhibition may improve the quality of life of the patient and may even beneficially influence the disease state. In any case, the availability of an add-on compound that can be produced in a cost-efficient manner and easily administered is of particular importance in a market in which cost control is of utmost importance.

Periodontal disease. Periodontitis is a chronic oral inflammatory disease that leads to the destruction of the tooth-supporting connective and bone tissue [90,91]. A disequilibrium between host immune factors and dysbiotic bacterial communities developing in tooth-supporting structures has been implicated as the underlying cause of this debilitating disease [92]. Recent studies have provided compelling evidence for a crucial role of complement and C5aR/TLR2 crosstalk in modulating the dysbiotic microbiota and inflammatory milieu that drive pathology in periodontitis [93–96]. Indeed, C5aR antagonism has been shown to suppress the inflammatory response that contributes to this disease and to afford significant protection from osteoclastogenesis and subsequent bone loss in mice [93,96–98]. In view of the broader therapeutic benefit afforded by upstream C3 targeting in many disease models, a recent study sought to determine the therapeutic efficacy of Cp40 in a NHP model of ligature-induced periodontitis [14]. Local, multidose administration of Cp40 within the interdental papillae of affected animals resulted in a significant protection from bone loss over a period of 6 weeks that was marked by a reciprocal reduction in osteoclasts recovered in bone biopsies, as compared to treatment with inactive peptide control, which resulted in progressive disease. The inhibitory effect of Cp40 on osteoclastogenesis was further underscored by a reduction in the levels of RANKL, a key osteoclastogenic factor, in gingival crevicular fluid. Moreover, Cp40 treatment significantly attenuated the proinflammatory response (e.g. TNF, IL-1β and IL-17), which was reflected by decreased values of the periodontal indices that measure clinical inflammation and tissue destruction. Important questions that remain to be addressed for this indication include the most suitable and effective form of administration and the required treatment period to prevent and/or treat inflammation and bone loss. Importantly, though, these findings strongly suggest that Cp40, and the clinically developed drug candidate AMY-101, could prove an effective therapeutic option for human periodontitis.

Transplantation. Complement activation has been implicated as a major pathogenic driver in acute antibody-mediated rejection (AMR) following allogeneic solid organ transplantation [99,100]. Sensitization of transplant recipients by donor-specific alloantibodies (DSAs) leads to complement activation that, together with a forceful cellular response to graft antigens, culminates in acute graft rejection or gradual deterioration of graft function. In this context, efforts to develop transplant protocols that could bypass the donor–recipient HLA or blood group (ABO) barrier have provided important insights into the mechanism by which complement affects graft survival and function [101,102]. Complement-mediated graft deterioration might involve both direct (i.e. inducing cell damage and activation) and indirect effects, such as increased cellular infiltration and inflammatory damage within the graft tissue [100]. Although the involvement of complement in acute graft rejection is well documented, there is still an ongoing debate as

to whether complement activation also has a direct impact on exacerbating chronic graft injury.

Therapeutic modulation of complement at the level of C5 (i.e. via C5 blockade) has been shown to ameliorate the complications of early AMR by prolonging graft survival and function in sensitized renal and pancreatic transplant recipients [103,104]. However, a significant proportion of graft recipients fail to respond to eculizumab treatment, suggesting that a broader and more comprehensive intervention is warranted [105]. In this context, systemic C3 interception might offer multiple benefits for improving graft accommodation and function in kidney transplant recipients. C3 inhibition can blunt the direct cytolytic and effector functions of complement that promote inflammatory damage to the graft endothelium and might also control the detrimental adaptive immune responses (i.e. dendritic cell function and alloantigen-specific T-cell activation) that perpetuate graft inflammation and cellular infiltration, leading to cumulative organ damage and chronic rejection [100]. The excellent clinical profile of next-generation compstatin derivatives makes them attractive C3-targeted therapeutics for modulating complement in settings of AMR in allogeneic organ transplantation. In this context, the Cp40-based therapeutic AMY-101 (Amyndas Pharmaceuticals) is currently being evaluated as a novel therapeutic option for attenuating complications of ABO-incompatible kidney transplantation.

The increasing demands for transplants and the shortage of human donor organs have pointed to the value of exploring additional therapeutic avenues. Alongside the use of HLA- or ABO-incompatible human organs, xenotransplantation is attracting considerable attention as an alternative intervention. In fact, xenotransplantation was among the first settings in which compstatin analogues were preclinically evaluated, as acetylated compstatin prolonged the survival of porcine kidneys that had been perfused with human blood [106]. Moreover, compstatin analogues showed promise in modulating the detrimental instant blood-mediated inflammatory reaction (IBMIR) that accompanies transplantation of porcine Langerhans islets, thereby indicating potential for applications in cell transplantation [107]. Interestingly, a recent study employing a xenogeneic porcine cell-to-human whole blood model has provided important insight into the mechanism by which xenogeneic tissue triggers innate immune responses that exacerbate graft survival, while also corroborating the therapeutic potential of C3 interception in a xenotransplantation setting [108]. In this model, Cp40 significantly attenuated both cellular and humoral activation, blocking neutrophil attachment to endothelial surfaces and also downregulating crucial adhesion and inflammatory markers. Overall, these findings suggest that C3 interception might be a viable new therapeutic option for attenuating adverse inflammatory complications invoked by xenografts.

Concluding remarks and outlook

Since its discovery in the mid-1990's, compstatin has been the focus of intense, cross-disciplinary investigation aiming to improve its complement inhibitory potency, while achieving a PK profile that is tailored for clinical use in both acute and chronic settings. The longitudinal optimization of compstatin has culminated in potent derivatives standing alongside a panel of complement-targeted biopharmaceuticals (antibodies, fusion-proteins, low-MW compounds) that also show promise as therapeutic options in expanding clinical indications.

A new class of compstatin derivatives (ranging from the first clinically developed analogue 4(1MeW) to the recently disclosed Cp40 and the Cp40-based therapeutic AMY-101) displays a robust panel of beneficial attributes that make them suitable for further clinical development and therapeutic translation in the clinic. They show promise as potent therapeutics for systemic application in complement-related diseases, display highly favourable PK and plasma stability profiles and also point to a more affordable treatment option for a wide spectrum of clinical disorders, given the significantly lower production costs associated today with peptide-based drugs [109]. Apart from the clinical conditions discussed in this review, these new compstatin derivatives show promise as alternative therapeutics for a broader spectrum of clinical indications in which uncontrolled or excessive C3 activation is a major pathogenic driver. These include I/R injury in the context of transplantation and also C3 glomerulopathies, a family of renal inflammatory disorders that manifest with pronounced C3 deposition in the glomeruli and are tightly linked to genetic or acquired C3 dysregulation [110]. Endorsing the emerging therapeutic potential of these C3-targeted inhibitors, both the EMA and the US FDA recently granted orphan drug designation to AMY-101 for the treatment of PNH.

The many lessons learned from the structure-guided optimization and preclinical evaluation of compstatin may provide valuable leads for developing tailored, small-molecule complement therapeutics that can be adapted to the distinct pathogenic signatures of a wide spectrum of complement-mediated diseases. Understandably, there is no 'magic bullet' in complement-based therapeutic design that will cater to all diseases and clinical manifestations. However, the paradigm of compstatin robustly illustrates the importance of consolidating structural, biochemical and preclinical findings in a system-wide perspective before advancing to the clinical stage of development.

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References

1 Seoane JA, Lopez-Campos G, Dorado J, Martin-Sanchez F. New approaches in data integration for systems chemical biology. *Curr Top Med Chem* 2013;**13**:591–601.

2 Ricklin D, Lambris JD. Progress and trends in complement therapeutics. *Adv Exp Med Biol* 2013;**735**:1–22.

3 Yang K, Dinasarapu AR, Reis ES, Deangelis RA, Ricklin D, Subramaniam S et al. CMAP: complement Map Database. *Bioinformatics* 2013;**29**:1832–3.

4 Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol* 2010;**11**:785–97.

5 Ricklin D, Lambris JD. Complement in immune and inflammatory disorders: pathophysiological mechanisms. *J Immunol* 2013;**190**:3831–8.

6 Pio R, Corrales L, Lambris JD. The role of complement in tumor growth. *Adv Exp Med Biol* 2014;**772**:229–62.

7 Carroll MC, Isenman DE. Regulation of humoral immunity by complement. *Immunity* 2012;**37**:199–207.

8 Kolev M, Friec GL, Kemper C. Complement – tapping into new sites and effector systems. *Nat Rev Immunol* 2014;**14**:811–20.

9 Ricklin D, Lambris JD. Complement in immune and inflammatory disorders: therapeutic interventions. *J Immunol* 2013;**190**:3839–47.

10 Ricklin D, Lambris JD. Complement-targeted therapeutics. *Nat Biotechnol* 2007;**25**:1265–75.

11 Qu H, Ricklin D, Lambris JD. Recent developments in low molecular weight complement inhibitors. *Mol Immunol* 2009;**47**:185–95.

12 Reis S, Falcao DA, Isaac L. Clinical aspects and molecular basis of primary deficiencies of complement component C3 and its regulatory proteins factor I and factor H. *Scand J Immunol* 2006;**63**:155–68.

13 Grumach AS, Kirschfink M. Are complement deficiencies really rare? Overview on prevalence, clinical importance and modern diagnostic approach. *Mol Immunol* 2014;**61**:110–7.

14 Maekawa T, Abe T, Hajishengallis E, Hosur KB, Deangelis RA, Ricklin D et al. Genetic and intervention studies implicating complement c3 as a major target for the treatment of periodontitis. *J Immunol* 2014;**192**:6020–7.

15 Mabbott NA, Bruce ME, Botto M, Walport MJ, Pepys MB. Temporary depletion of complement component C3 or genetic deficiency of C1q significantly delays onset of scrapie. *Nat Med* 2001;**7**:485–7.

16 Mosser DM, Edelson PJ. The third component of complement (C3) is responsible for the intracellular survival of Leishmania major. *Nature* 1987;**327**:329–31.

17 Subramanian AK. Antimicrobial prophylaxis regimens following transplantation. *Curr Opin Infect Dis* 2011;**24**:344–9.

18 Zhang Y, Nester CM, Holanda DG, Marsh HC, Hammond RA, Thomas LJ et al. Soluble CR1 therapy improves complement regulation in C3 glomerulopathy. *J Am Soc Nephrol* 2013;**24**:1820–9.

19 Pickering MC, Botto M, Taylor PR, Lachmann PJ, Walport MJ. Systemic lupus erythematosus, complement deficiency, and apoptosis. *Adv Immunol* 2000;**76**:227–324.

20 Strainic MG, Liu J, Huang D, An F, Lalli PN, Muqim N et al. Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naive CD4 + T cells. *Immunity* 2008;**28**:425–35.

21 Baudino L, Sardini A, Ruseva MM, Fossati-Jimack L, Cook HT, Scott D et al. C3 opsonization regulates endocytic handling of apoptotic cells resulting in enhanced T-cell responses to cargo-derived antigens. *Proc Natl Acad Sci USA* 2014;**111**:1503–8.

22 Davies KA, Schifferli JA, Walport MJ. Complement deficiency and immune complex disease. *Springer Semin Immunopathol* 1994;**15**:397–416.

23 Karsten CM, Kohl J. The immunoglobulin, IgG Fc receptor and complement triangle in autoimmune diseases. *Immunobiology* 2012;**217**:1067–79.

24 Sahu A, Kay BK, Lambris JD. Inhibition of human complement by a C3-binding peptide isolated from a phage-displayed random peptide library. *J Immunol* 1996;**157**:884–91.

25 Sahu A, Soulika AM, Morikis D, Spruce L, Moore WT, Lambris JD. Binding kinetics, structure-activity relationship, and biotransformation of the complement inhibitor compstatin. *J Immunol* 2000;**165**:2491–9.

26 Soulika AM, Morikis D, Sarrias MR, Roy M, Spruce LA, Sahu A *et al.* Studies of structure-activity relations of complement inhibitor compstatin. *J Immunol* 2003;**171**:1881–90.

27 Holland MC, Morikis D, Lambris JD. Synthetic small-molecule complement inhibitors. *Curr Opin Investig Drugs* 2004;**5**:1164–73.

28 Ricklin D, Lambris JD. Compstatin: a complement inhibitor on its way to clinical application. *Adv Exp Med Biol* 2008;**632**:273–92.

29 Sahu A, Morikis D, Lambris JD. Compstatin, a peptide inhibitor of complement, exhibits species-specific binding to complement component C3. *Mol Immunol* 2003;**39**:557–66.

30 Qu H, Ricklin D, Bai H, Chen H, Reis ES, Maciejewski M *et al.* New analogs of the clinical complement inhibitor compstatin with subnanomolar affinity and enhanced pharmacokinetic properties. *Immunobiology* 2013;**218**:496–505.

31 Lee DS, Yi TG, Lee HJ, Kim SN, Park S, Jeon MS *et al.* Mesenchymal stem cells infected with Mycoplasma arginini secrete complement C3 to regulate immunoglobulin production in b lymphocytes. *Cell Death Dis* 2014;**5**:e1192.

32 Sun Y, Yu W, Huang L, Hou J, Gong P, Zheng Y *et al.* Is asthma related to choroidal neovascularization? *PLoS ONE* 2012;**7**: e35415.

33 Janssen BJ, Halff EF, Lambris JD, Gros P. Structure of compstatin in complex with complement component C3c reveals a new mechanism of complement inhibition. *J Biol Chem* 2007;**282**:29241–7.

34 Soulika AM, Holland MC, Sfyroera G, Sahu A, Lambris JD. Compstatin inhibits complement activation by binding to the beta-chain of complement factor 3. *Mol Immunol* 2006;**43**:2023–9.

35 Janssen BJ, Huizinga EG, Raaijmakers HC, Roos A, Daha MR, Nilsson-Ekdahl K *et al.* Structures of complement component C3 provide insights into the function and evolution of immunity. *Nature* 2005;**437**:505–11.

36 Janssen BJ, Christodoulidou A, McCarthy A, Lambris JD, Gros P. Structure of C3b reveals conformational changes that underlie complement activity. *Nature* 2006;**444**:213–6.

37 Wiesmann C, Katschke KJ, Yin J, Helmy KY, Steffek M, Fairbrother WJ *et al.* Structure of C3b in complex with CRIg gives insights into regulation of complement activation. *Nature* 2006;**444**:217–20.

38 Rooijakkers SH, Wu J, Ruyken M, van DR, Planken KL, Tzekou A *et al.* Structural and functional implications of the alternative complement pathway C3 convertase stabilized by a staphylococcal inhibitor. *Nat Immunol* 2009;**10**:721–7.

39 Katragadda M, Magotti P, Sfyroera G, Lambris JD. Hydrophobic effect and hydrogen bonds account for the improved activity of a complement inhibitor, compstatin. *J Med Chem* 2006;**49**:4616–22.

40 Moretto A, Crisma M, Kaptein B, Broxterman QB, Toniolo C. N-methylation of N(alpha)-acylated, fully C(alpha)-methylated, linear, folded peptides: synthetic and conformational aspects. *Biopolymers* 2006;**84**:553–65.

41 Chatterjee J, Gilon C, Hoffman A, Kessler H. N-methylation of peptides: a new perspective in medicinal chemistry. *Acc Chem Res* 2008;**41**:1331–42.

42 Bose PP, Chatterjee U, Hubatsch I, Artursson P, Govender T, Kruger HG *et al.* In vitro ADMET and physicochemical investigations of poly-N-methylated peptides designed to inhibit Abeta aggregation. *Bioorg Med Chem* 2010;**18**:5896–902.

43 Qu H, Magotti P, Ricklin D, Wu EL, Kourtzelis I, Wu YQ *et al.* Novel analogues of the therapeutic complement inhibitor compstatin with significantly improved affinity and potency. *Mol Immunol* 2011;**48**:481–9.

44 Huang Y, Reis ES, Knerr PJ, van dDW, Ricklin D, Lambris JD. Conjugation to albumin-binding molecule tags as a strategy to improve both efficacy and pharmacokinetic properties of the complement inhibitor compstatin. *ChemMedChem* 2014;**9**:2223–6.

45 Craik DJ, Fairlie DP, Liras S, Price D. The future of peptide-based drugs. *Chem Biol Drug Des* 2013;**81**:136–47.

46 Knerr PJ, Tzekou A, Ricklin D, Qu H, Chen H, van der Donk WA *et al.* Synthesis and activity of thioether-containing analogues of the complement inhibitor compstatin. *ACS Chem Biol* 2011;**6**:753–60.

47 Jevsevar S, Kunstelj M, Porekar VG. PEGylation of therapeutic proteins. *Biotechnol J* 2010;**5**:113–28.

48 Risitano AM, Ricklin D, Huang Y, Reis ES, Chen H, Ricci P *et al.* Peptide inhibitors of C3 activation as a novel strategy of complement inhibition for the treatment of paroxysmal nocturnal hemoglobinuria. *Blood* 2014;**123**:2094–101.

49 McDonnell T, Ioannou Y, Rahman A. PEGylated drugs in rheumatology–why develop them and do they work? *Rheumatology (Oxford)* 2014;**53**:391–6.

50 Dennis MS, Zhang M, Meng YG, Kadkhodayan M, Kirchhofer D, Combs D *et al.* Albumin binding as a general strategy for improving the pharmacokinetics of proteins. *J Biol Chem* 2002;**277**:35035–43.

51 Qu H, Magotti P, Ricklin D, Lambris JD. Development of Compstatin Derivative-Albumin Binding Peptide Chimeras for Prolonged Plasma Half-Life. *21st American Peptide Symposium. Proceedings of the 21st American Peptide Symposium*, 219–20, 2009.

52 Lauffer RB, Parmelee DJ, Dunham SU, Ouellet HS, Dolan RP, Witte S *et al.* MS-325: albumin-targeted contrast agent for MR angiography. *Radiology* 1998;**207**:529–38.

53 Augustin AJ, Kirchhof J. Inflammation and the pathogenesis of age-related macular degeneration. *Expert Opin Ther Targets* 2009;**13**:641–51.

54 Chi ZL, Yoshida T, Lambris JD, Iwata T. Suppression of drusen formation by compstatin, a peptide inhibitor of complement C3 activation, on cynomolgus monkey with early-onset macular degeneration. *Adv Exp Med Biol* 2010;**703**:127–35.

55 Troutbeck R, Al-Qureshi S, Guymer RH. Therapeutic targeting of the complement system in age-related macular degeneration: a review. *Clin Experiment Ophthalmol* 2012;**40**:18–26.

56 Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P *et al.* Complement factor H variant increases the risk of age-related macular degeneration. *Science* 2005;**308**:419–21.

57 Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C *et al.* Complement factor H polymorphism in age-related macular degeneration. *Science* 2005;**308**:385–9.

58 Edwards AO, Ritter R III, Abel KJ, Manning A, Panhuysen C *et al.* Complement factor H polymorphism and age-related macular degeneration. *Science* 2005;**308**:421–4.

59 Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, Hardisty LI *et al.* A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci USA* 2005;**102**:7227–32.

60 Schramm EC, Clark SJ, Triebwasser MP, Raychaudhuri S, Seddon JM, Atkinson JP. Genetic variants in the complement system predisposing to age-related macular degeneration: a review. *Mol Immunol* 2014;**61**:118–25.

61 Alcon Research Evaluation of AL-78898A in Exudative Age-Related Macular Degeneration (RACE). ClinicalTrials.gov, NCT01157065, 2013.

62 Alcon Research A Multicenter, Proof-of-Concept Study of Intravitreal AL-78898A in Patients with Geographic Atrophy (GA)

associated with Age-Related Macular Degeneration (AMD). ClinicalTrials.gov, Identifier:NCT01603043, 2013.

63 Bosmann M, Ward PA. The inflammatory response in sepsis. *Trends Immunol* 2013;**34**:129–36.

64 Markiewski MM, Deangelis RA, Lambris JD. Complexity of complement activation in sepsis. *J Cell Mol Med* 2008;**12**:2245–54.

65 Mollnes TE, Brekke OL, Fung M, Fure H, Christiansen D, Bergseth G *et al.* Essential role of the C5a receptor in *E. coli*-induced oxidative burst and phagocytosis revealed by a novel lepirudin-based human whole blood model of inflammation. *Blood* 2002;**100**:1869–77.

66 Christiansen D, Brekke OL, Stenvik J, Lambris JD, Espevik T, Mollnes TE. Differential effect of inhibiting MD-2 and CD14 on LPS- versus whole *E. coli* bacteria-induced cytokine responses in human blood. *Adv Exp Med Biol* 2012;**946**:237–51.

67 Landsem A, Nielsen EW, Fure H, Christiansen D, Ludviksen JK, Lambris JD *et al.* C1-inhibitor efficiently inhibits *Escherichia coli*-induced tissue factor mRNA up-regulation, monocyte tissue factor expression and coagulation activation in human whole blood. *Clin Exp Immunol* 2013;**173**:217–29.

68 Brekke OL, Waage C, Christiansen D, Fure H, Qu H, Lambris JD *et al.* The effects of selective complement and CD14 inhibition on the *E. coli*-induced tissue factor mRNA upregulation, monocyte tissue factor expression, and tissue factor functional activity in human whole blood. *Adv Exp Med Biol* 2013;**734a**:123–36.

69 Egge KH, Thorgersen EB, Lindstad JK, Pharo A, Lambris JD, Barratt-Due A *et al.* Post challenge inhibition of C3 and CD14 attenuates *Escherichia coli*-induced inflammation in human whole blood. *Innate Immun* 2014;**20**:68–77.

70 Skjeflo EW, Christiansen D, Espevik T, Nielsen EW, Mollnes TE. Combined inhibition of complement and CD14 efficiently attenuated the inflammatory response induced by Staphylococcus aureus in a human whole blood model. *J Immunol* 2014;**192**:2857–64.

71 Huber-Lang M, Barratt-Due A, Pischke SE, Sandanger O, Nilsson PH, Nunn MA *et al.* Double blockade of CD14 and complement C5 abolishes the cytokine storm and improves morbidity and survival in polymicrobial sepsis in mice. *J Immunol* 2014;**192**:5324–31.

72 Silasi-Mansat R, Zhu H, Popescu NI, Peer G, Sfyroera G, Magotti P *et al.* Complement inhibition decreases the procoagulant response and confers organ protection in a baboon model of *Escherichia coli* sepsis. *Blood* 2010;**116**:1002–10.

73 Taylor FB Jr. Staging of the pathophysiologic responses of the primate microvasculature to *Escherichia coli* and endotoxin: examination of the elements of the compensated response and their links to the corresponding uncompensated lethal variants. *Crit Care Med* 2001;**29**:S78–89.

74 Thorgersen EB, Pharo A, Haverson K, Axelsen AK, Gaustad P, Kotwal GJ *et al.* Inhibition of complement and CD14 attenuates the *Escherichia coli*-induced inflammatory response in porcine whole blood. *Infect Immun* 2009;**77**:725–32.

75 Risitano AM. Paroxysmal nocturnal hemoglobinuria and the complement system: recent insights and novel anticomplement strategies. *Adv Exp Med Biol* 2013;**735**:155–72.

76 Varela JC, Brodsky RA. Paroxysmal nocturnal hemoglobinuria and the age of therapeutic complement inhibition. *Expert Rev Clin Immunol* 2013;**9**:1113–24.

77 Risitano AM. Paroxysmal nocturnal hemoglobinuria and other complement-mediated hematological disorders. *Immunobiology* 2012;**217**:1080–7.

78 Holguin MH, Fredrick LR, Bernshaw NJ, Wilcox LA, Parker CJ. Isolation and characterization of a membrane protein from

normal human erythrocytes that inhibits reactive lysis of the erythrocytes of paroxysmal nocturnal hemoglobinuria. *J Clin Invest* 1989;**84**:7–17.

79 Hillmen P, Young NS, Schubert J, Brodsky RA, Socie G, Muus P *et al.* The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med* 2006;**355**:1233–43.

80 Risitano AM, Notaro R, Luzzatto L, Hill A, Kelly R, Hillmen P. Paroxysmal nocturnal hemoglobinuria–hemolysis before and after eculizumab. *N Engl J Med* 2010;**363**:2270–2.

81 Hill A, Rother RP, Arnold L, Kelly R, Cullen MJ, Richards SJ *et al.* Eculizumab prevents intravascular hemolysis in patients with paroxysmal nocturnal hemoglobinuria and unmasks low-level extravascular hemolysis occurring through C3 opsonization. *Haematologica* 2010;**95**:567–73.

82 Schmidt CQ, Bai H, Lin Z, Risitano AM, Barlow PN, Ricklin D *et al.* Rational engineering of a minimized immune inhibitor with unique triple-targeting properties. *J Immunol* 2013;**190**:5712–21.

83 Risitano AM, Notaro R, Pascariello C, Sica M, Del VL, Horvath CJ *et al.* The complement receptor 2/factor H fusion protein TT30 protects paroxysmal nocturnal hemoglobinuria erythrocytes from complement-mediated hemolysis and C3 fragment. *Blood* 2012;**119**:6307–16.

84 Mastellos DC, Ricklin D, Yancopoulou D, Risitano A, Lambris JD. Complement in paroxysmal nocturnal hemoglobinuria: exploiting our current knowledge to improve the treatment landscape. *Expert Rev Hematol* 2014;**7**:583–98.

85 AMYNDAS Pharmaceuticals (Press Release). EMA grants orphan drug designation for PNH (EU/3/14/1327) to Amyndas Pharmaceuticals' novel complement inhibitor, 2014. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/orphans/2014/10/human_orphan_001417.jsp&mid=WC0b01ac058001d12b. Accessed on 20 February 2015.

86 AMYNDAS Pharmaceuticals (Press Release). FDA grants orphan drug designation for PNH to Amyndas Pharmaceuticals' novel complement inhibitor, 2014. Available at: http://www.accessdata.fda.gov/scripts/opdlisting/oopd/OOPD_Results_2.cfm?Index_Number=446714. Accessed on 20 February 2015.

87 Santoro D, Satta E, Messina S, Costantino G, Savica V, Bellinghieri G. Pain in end-stage renal disease: a frequent and neglected clinical problem. *Clin Nephrol* 2013;**79**(Suppl 1):S2–11.

88 Kourtzelis I, Markiewski MM, Doumas M, Rafail S, Kambas K, Mitroulis I *et al.* Complement anaphylatoxin C5a contributes to hemodialysis-associated thrombosis. *Blood* 2010;**116**:631–9.

89 Reis ES, Deangelis RA, Chen H, Resuello RR, Ricklin D, Lambris JD. Therapeutic C3 inhibitor Cp40 abrogates complement activation induced by modern hemodialysis filters. *Immunobiology* 2014; (in press).

90 Hajishengallis G, Lambris JD. Complement-targeted therapeutics in periodontitis. *Adv Exp Med Biol* 2013;**735**:197–206.

91 Hajishengallis G. Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. *Trends Immunol* 2014;**35**:3–11.

92 Hajishengallis G, Abe T, Maekawa T, Hajishengallis E, Lambris JD. Role of complement in host-microbe homeostasis of the periodontium. *Semin Immunol* 2013;**25**:65–72.

93 Abe T, Hosur KB, Hajishengallis E, Reis ES, Ricklin D, Lambris JD *et al.* Local complement-targeted intervention in periodontitis: proof-of-concept using a C5a receptor (CD88) antagonist. *J Immunol* 2012;**189**:5442–8.

94 Hajishengallis G, Lambris JD. Complement and dysbiosis in periodontal disease. *Immunobiology* 2012;**217**:1111–6.

95 Hajishengallis G, Liang S, Payne MA, Hashim A, Jotwani R, Eskan MA et al. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. *Cell Host Microbe* 2011;**10**:497–506.

96 Maekawa T, Krauss JL, Abe T, Jotwani R, Triantafilou M, Triantafilou K et al. Porphyromonas gingivalis manipulates complement and TLR signaling to uncouple bacterial clearance from inflammation and promote dysbiosis. *Cell Host Microbe* 2014;**15**:768–78.

97 Liang S, Krauss JL, Domon H, McIntosh ML, Hosur KB, Qu H et al. The C5a receptor impairs IL-12-dependent clearance of Porphyromonas gingivalis and is required for induction of periodontal bone loss. *J Immunol* 2011;**186**:869–77.

98 Wang M, Krauss JL, Domon H, Hosur KB, Liang S, Magotti P et al. Microbial hijacking of complement-toll-like receptor crosstalk. *Sci Signal* 2010;**3**:ra11.

99 Touzot M, Obada EN, Beaudreuil S, Francois H, Durrbach A. Complement modulation in solid-organ transplantation. *Transplant Rev (Orlando)* 2014;**28**:119–25.

100 Stegall MD, Chedid MF, Cornell LD. The role of complement in antibody-mediated rejection in kidney transplantation. *Nat Rev Nephrol* 2012;**8**:670–8.

101 Sacks SH, Zhou W. The role of complement in the early immune response to transplantation. *Nat Rev Immunol* 2012;**12**:431–42.

102 Biglarnia AR, Nilsson B, Nilsson EK, Tufveson G, Nilsson T, Larsson E et al. Desensitization with antigen-specific immunoadsorption interferes with complement in ABO-incompatible kidney transplantation. *Transplantation* 2012;**93**:87–92.

103 Stegall MD, Diwan T, Raghavaiah S, Cornell LD, Burns J, Dean PG et al. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. *Am J Transplant* 2011;**11**:2405–13.

104 Biglarnia AR, Nilsson B, Nilsson T, von Z-MB, Wagner M, Berne C et al. Prompt reversal of a severe complement activation by eculizumab in a patient undergoing intentional ABO-incompatible pancreas and kidney transplantation. *Transpl Int* 2011;**24**:e61–6.

105 Bentall A, Tyan DB, Sequeira F, Everly MJ, Gandhi MJ, Cornell LD et al. Antibody-mediated rejection despite inhibition of terminal complement. *Transpl Int* 2014;**27**:1235–43.

106 Fiane AE, Mollnes TE, Videm V, Hovig T, Hogasen K, Mellbye OJ et al. Compstatin, a peptide inhibitor of C3, prolongs survival of ex vivo perfused pig xenografts. *Xenotransplantation* 1999;**6**:52–65.

107 Goto M, Tjernberg J, Dufrane D, Elgue G, Brandhorst D, Ekdahl KN et al. Dissecting the instant blood-mediated inflammatory reaction in islet xenotransplantation. *Xenotransplantation* 2008;**15**:225–34.

108 Kourtzelis I, Ferreira A, Mitroulis I, Ricklin D, Bornstein SR, Waskow C et al. Complement inhibition in a xenogeneic model of interactions between human whole blood and porcine endothelium. *Horm Metab Res* 2015;**47**:36–42.

109 Vlieghe P, Lisowski V, Martinez J, Khrestchatisky M. Synthetic therapeutic peptides: science and market. *Drug Discov Today* 2010;**15**:40–56.

110 Barbour TD, Pickering MC, Terence CH. Dense deposit disease and C3 glomerulopathy. *Semin Nephrol* 2013;**33**:493–507.