EXPERT OPINION

Modulators of complement activation: a patent review (2008 – 2013)

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Introduction: The architecture of the complement system has evolved during the last 600 – 700 million years to become an amazingly efficient and highly versatile alerting and cell killing device. Under physiological conditions, this system acts as a well-regulated cascade, protecting the organism against pathogens and participating during the initial defensive steps of humoral and cellular response. The unregulated activation of this system may cause or even aggravate diseases; therefore, its modulation is currently considered of high importance.
Areas covered: This review is a critical examination on patent literature published between 2008 and 2013. An insight is provided about the discovery and development of novel therapeutic agents. These include macromolecules, polysaccharides and proteins, specific antibodies, and hybrid or chimeric products. Peptides and low molecular weight organic compounds (natural products, their derivatives and fully synthetic molecules) are covered as well.
Expert opinion: The search of specific inhibitors of the complement cascade has become one of the Holy Grails of Medicinal Chemistry for the last 30 – 40 years, with very few cases of success. Some highly specific macromolecules are currently available as modulators of the complement. However, there is still a marked need to find new, more specific, efficient and convenient alternatives, especially suited for chronic administration, including novel inexpensive small molecule inhibitors. Analogously, despite the initial success with specific monoclonal antibodies, a vast territory is awaiting to be explored and conquered, regarding the regulation of complement activation by antibody-mediated blockage of specific polypeptides or receptor sites.

Keywords: alternative pathway, aptamers, chimeric proteins, classical pathway, complement inhibitors, complement-mediated diseases, complement therapeutics, lectin pathway, natural products, specific monoclonal antibodies

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1. Introduction

The complement system has long been recognized as a potentially useful therapeutic target, and its modulation has been repeatedly mentioned as a promising action plan for drug discovery. Hence, a large number of strategic approaches and the corresponding therapeutic agents have been developed during the last 25 years. Nevertheless, to place in the market a complement-directed drug turned out to be a much more difficult task than originally expected.

Fortunately, however, in 2007 the US FDA approved eculizumab [1], a humanized mAb against the key complement component C5. This landmark was shortly followed by another breakthrough, the approval of nanofiltered C1INH in 2008.

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Article highlights.

- Intellectual property documents on therapeutic inhibition of complement activation from the United States patent and trademark office and Espacenet databases (2008 – 2013) have been evaluated.
- Inhibition of complement activation was approached with low molecular weight natural and synthetic compounds, polypeptides and macromolecules.
- Increased tendency was observed to specifically inhibit complement activation with fusion, chimeric and hybrid proteins, and monoclonal antibodies.
- Recognition and activation stages, as well as central C3 and C5 levels and complement receptors/regulators have been the main targets for inhibition of complement activation.
- Improved structural and functional knowledge of the complement system components is progressively yielding more specific and powerful inhibitors.

This box summarizes key points contained in the article.

Furthermore, in one of the latest advances, the FDA granted the 'orphan drug' designation to OMS721, a human mAb targeting the mannose binding lectin (MBL)-associated serine protease 2 (MASP-2), for prevention of complement-mediated thrombotic microangiopathies. These landmarks signal a resurgence of the interest in the potential of complement therapeutics for the treatment of disease.

Together, they have distinguished the complement system as a suitable therapeutic target and paved the way for new developments, soon to take place. A review of the recent patent literature in the field may shed light on the upcoming innovations and help to preview this bright-looking future.

1.1 The complement system

The complement system is a phylogenetically conserved defense element, which appeared about 600 – 700 million years ago, preceding the emergence of vertebrates and also before the development of antibodies. Even the primitive sea urchins, which appeared some 700 million years ago, have a fully functioning complement system [2]. The system evolved to be a sophisticated, distinct and important part of the innate immune system, relevant for protection against alien contamination and non-self. Human beings born with highly defective complement systems do not live long before succumbing to infection. Furthermore, transcending its role in innate immunity, the complement system has forged functional associations with multiple pathways and networks that modulate basic biologic processes. The complement system has also links with adaptive immunity, participates in protein-protein interactions involving numerous ligands and has associations with signaling cascades and cellular networks that affect both inflammatory and noninflammatory processes [3].

The complement system is composed of slightly more than 30 plasma and membrane components, including factors, regulators and receptors located on cells, most of which belong to the immune system, among which over 20 constituents are proteins found in blood serum. These are linked in unidirectional biochemical cascades, known as the classical (CCP), alternative (ACP) and lectin (LCP) complement pathways [4], which share some of its components and modes of action (**Figure 1**).

New discoveries on the complex physiology of the complement system are continuously appearing in the literature and some controversies still exist about the role of novel components being described; however, a relatively detailed and accurate picture of its mode of action can be drawn. Accordingly, the complement system can be triggered through the above three different pathways. They are analogous in the sense that each one is designed as a sophisticated mechanism able to distinguish potentially harmful strange or foreign agents, by detecting surface structures through pattern-recognition proteins [5]. In addition, each pathway has a unique set of regulators; however, some of the elements of one set may be shared by more than one pathway.

The CCP recognizes the antibodies when they are bound to antigens and take part of immune complexes. The initiating component is C1q, which upon proper interactions is induced to associate the serine proteases C1r and C1s to form C1, a large enzymatic complex that activates the CCP cascade. The C1 complex is able to sequentially cleave C4 and in turn C2, producing C4b and C2a, respectively, which assemble to form the C3 convertase of the CCP.

The recognition elements of the LCP are ficolins and the MBL [6]. Both recognize specific chemical repeating carbohydrate structures exposed on the surface of antigens, pathogens and target cells and trigger the complement cascade. Higher organisms lack these molecular architectures, under normal conditions. Activation of the LCP takes place upon complexation with MASP-2, which effects the sequential cleavage of C4 and C2, leading to the assembly of C4b. C2a, a C3 convertase [7] analogous to that of the CCP. This pathway has also been described as operative in cases of ischemia-reperfusion of systemic organs, a situation which results in exposure of self-antigens, which bind to MBL [8].

The ACP is the most ancient pillar of the complement system. It is always ticking with consumption of C3, which is spontaneously activated by hydrolysis of an internal thioester bond, generating $C3(H_2O)$ [9]. This slow but permanent C3 degradation keeps a low level of complement activity, which exerts a sort of sensing of homeostasis and surveillance against intrusion. However, the C3 degradation rate becomes markedly enhanced upon recognition of microorganisms and various exposed elements found on foreign surfaces. Then, $C3(H_2O)$ is capable of activating the ACP by binding and assisting factor D in cleaving factor B into Ba and Bb. This process generates $C3(H_2O)Bb$, which in turn is able to cleave additional molecules of C3 into C3a and C3b [10] and form the ACP C3 convertase.

Properdin promotes the association of C3b with factor B and provides a focal point for the assembly of C3bBb on

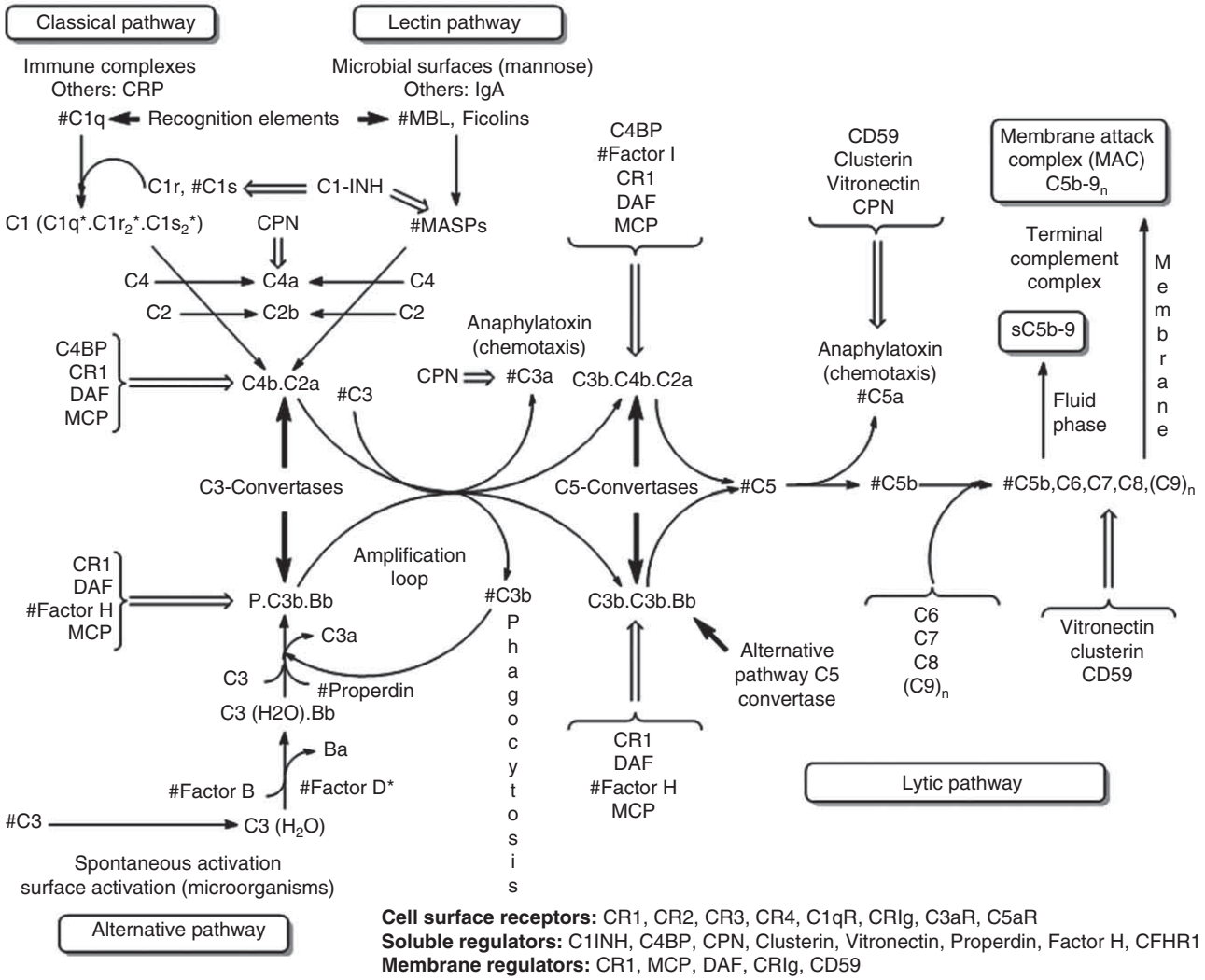

Figure 1. Overview of the main components, activation pathways, regulation points (underlined) and potential therapeutic targets (marked with #) within the complement system.

C3aR: C3a receptor; C4BP: C4-binding protein; CFHR: Complement factor H receptor; CPN: Carboxypeptidase N; CR: Complement receptor; CRIg: Complement receptor of the immunoglobulin superfamily; CRP: C-Reactive protein; DAF: Decay acceleration factor; MASPs: MBL-associated serine proteases; MBL: Mannose-binding lectin; MCP: Membrane cofactor protein; sC59-9: Soluble (serum) C5b-9 complex.

a surface; it also binds to preformed ACP C3 convertase [11] having a C3 convertase-stabilizing role [12].

The ACP may also act as an 'amplification loop' (**Figures 1** and **2**) for activation of the complement system, because regardless the pathway of its origin, any C3b fragment generated may bind factor B, allowing further propagation of C3 activation, through formation of C3bBb [13]. Factor I prevents excessive generation of C3b, by promoting its cleavage to iC3b and other fragments, only when C3b is complexed to factor H (**Figure 2**).

In each of these proximal pathways, the recognition stage precedes activation, which yields a C3 convertase (C4b2a in the CCP and LCP, and C3bBbP in the ACP), a serine protease that ultimately cleaves the central complement protein C3 into C3a and C3b.

Analogously, incorporation of C3b yields the C5 convertases (C4bC2aC3b in the CCP and LCP, C3b*n*Bb in the ACP) [14], which can split C5 into C5a and C5b [15]. In turn, C5b complexes with C6 and C7, which recruit C8 and trigger binding and polymerization of C9 to form C5b-9, the membrane attack complex (MAC). This sequence (lytic pathway, LP) is common for all the activation pathways. The MAC creates pores in membranes that are not protected by complement regulators, causing their lysis, and promotes destruction of pathogenic organisms or immune complex-coated cells.

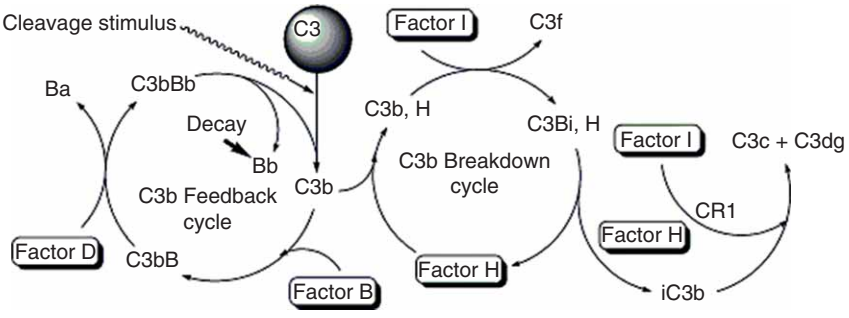

Figure 2. Mechanisms of complement activity amplification and control through C3b.
CR1: Complement receptor 1.

Table 1. Relevant complement regulators and receptors for effector proteins.

Regulator	Point of action	Ligand	Regulator	Point of action	Ligand
Soluble regulators and effectors			*Surface-bound regulators and effectors*		
Factor H*,‡,§	ACP	C3b, C3d	CR1*,‡,§	C3	C3b, iC3b, C4b, C1q
FHL1	ACP	C3b	CR2*	C3	C3dg, C3d, iC3b
Properdin	ACP	C3	CR3	C3	iC3b, factor H
Carboxypeptidase N	CCP, LCP	C3a, C4a, C5a	CR4	C3	iC3b
C4BP*,‡,§	CCP, LCP	C4, C4b	CRIg	C3	C3b, iC3b, C3
C1INH	CCP, LCP	C1r, C1s, MASP2	MCP*,§	C3	C3b, C4b
CFHR1	TCP	C5 convertase, TCC	DAF*,‡	C3	C3 convertases C4b2b, C3bBb
Clusterin	TCP	C7, C8β, C9, TCC	Protectin	TCC	C8, TCC
Vitronectin	TCP	C5b-7, TCC			
Receptors for complement effector proteins					
C3aR	C3	C3a	C5aL2	C5	C5a
C5aR	C5	C5a	C1qR	CCP	C1q

*Coded in the regulator of complement activation gene cluster.
‡Promotes dissociation of the elements of the convertase (decay acceleration).
§Displays cofactor activity.
ACP: Alternative complement pathway; C3aR: C3a receptor; C4BP; C4-binding protein; CCP: Classical complement pathway; CFHR: Complement factor H receptor; CRIg: Complement receptor of the immunoglobulin superfamily; DAF: Decay acceleration factor; LCP: Lectin complement pathway; MASP2: MBL-associated serine protease 2; MCP: Membrane cofactor protein.

The peptides C3a and C5a, produced and released during complement activation, are anaphylatoxins, powerful chemoattractants for leukocytes, which may cause important tissue damage.

The complement system is regulated through a series of interrelated mechanisms. Two general mechanisms account for inhibition of its destructive components. The proteins involved in them (**Table 1**) are encoded by a region of the genome designated as the 'regulators of complement activation' (RCA) gene cluster [16].

The first one is known as 'decay acceleration' and involves the facilitated and reversible dissociation of the C3 convertases (C3b from Bb and C4b from C2a); it includes reversible binding of the antagonist proteins to C3b or C4b components, preventing their re-association.

The dissociation of the C3 convertases is effected by the C4-binding protein (C4BP) and factor H, two plasma proteins, and by the decay acceleration factor (DAF) and the complement receptor 1 (CR1), two membrane proteins. Reversible binding to C4b is carried out by C4BP, DAF and CR1, while reversible binding to C3b takes place with factor H, DAF and CR1.

The second mechanism consists in the proteolytic cleavage of the C3 convertase components C3b or C4b by factor I, a serine protease. This inactivation process is irreversible and takes place only in the presence of a cofactor (factor H and C4BP in plasma and CR1 and the membrane cofactor protein [MCP] at the cell surface). Factor H, CR1 and MCP exert cofactor activity for cleavage of C3b, while C4BP, CR1 and MCP are the cofactors for cleavage of C4b. Possibly, the CR2 has also this kind of cofactor activity at the cell surface. Interestingly, these general regulatory mechanisms also apply to the inhibition of the C5 convertases.

The mission of the complement system is twofold. On one hand, to protect the organism against self or foreign infecting agents; on the other, to participate as one of the initial steps in

Table 2. Main complement effects and their corresponding complement mediators.

Effect	Component(s) involved
Cell lysis	C5b-9_n (MAC)
Inflammatory response	
Degranulation of eosinophiles	C3a, C5a
Degranulation of mast cells and basophils	C3a, C4a, C5a
Extravasation of leukocytes at the inflammation site	C3a, C5a, C5b-7
Increased expression of CR1 and CR3 on neutrophils	C5a
Inhibition of monocyte/macrophage migration and spreading	Bb
Release of hydrolytic enzymes from neutrophils	C5a
Release of neutrophils from bone marrow	C3c
Neutralization of virus	C3b, C5b-9 (MAC)
Opsonization of particulate antigens	C3b, C4b, iC3b
Solubilization and clearance of immune complexes	C3b

MAC: Membrane attack complex.

the generation of humoral and cellular immunological answers to an immune aggression [17].

Under physiological conditions, this complex mission is accomplished employing different strategies, which depend on the nature of the triggering agent: i) tagging pathogen cells to promote their phagocytosis; ii) generating anaphylatoxic responses, which result in local inflammatory reactions; iii) promoting membrane attack on pathogenic cells, which causes their lysis; iv) assisting the solubilization of immune complexes, which helps their clearance from circulation; v) promoting an antibody response by helping the immune system to select the appropriate antigens; and vi) helping the body to eliminate self-reactive B cells. The nature of its mission explains why the complement system is so ubiquitous within living species and why it must remain constantly activated. **Table 2** contains a short list of complement effects and their corresponding mediators [18].

1.2 The complement system and disease

The complement system has the shape of an aggressive proteolytic cascade, which once triggered is unable to discriminate between proper and alien vital structures. Its architecture was perfected over time to yield an extremely powerful device for lysing cells. Therefore, activation of complement may result in a double-edged sword that can destroy harmful cells, but also entails the potential to affect harmless cells, including those of the host. Nature has taken care of these characteristics by designing complex mechanisms of functionally related membrane-bound and soluble proteins, which tightly regulate the complement system at different levels [19].

However, when the delicate protective mechanisms that regulate this sophisticated enzymatic system are unbalanced, the complement system may cause tissue inflammation and damage. Lack of balance can be a consequence of deficiencies of these regulatory components [20], and complement deficiencies have been associated with an increased risk to develop autoimmune disorders [21].

Lack of balance can also be a result of the system being overwhelmed because of excessive activation in response to certain pathological conditions. Such unbalanced activation has been associated with the pathogenesis and clinical manifestations of several autoimmune diseases [22], such as systemic lupus erythematosus, Fuchs' dystrophy [23], vasculitis, Sjögren's syndrome, anti-phospholipid syndrome, systemic sclerosis, dermatomyositis, rheumatoid arthritis and other conditions [24], pathological disorders and situations taking place during medical interventions [25], some of which are shown in **Table 3**. Since complement plays an important role in the pathogenesis of autoimmune diseases, treatment of the latter in most cases includes affecting the complement system.

Controlled activation of the complement system is critical for a proper response. On the contrary, the autologous activation of the complement may lead to disease complications, resulting in significant tissue damage with devastating effects [26]. This is often a result from incomplete biocompatibility of materials during hemodialysis, use of artificial hearts and the like.

The unregulated complement activation leading to acute inflammation and tissue damage has been implicated in the pathogenesis of many disease states [27]. Activation of the classical pathway has been implicated in hereditary angioedema [28], vascular leak syndrome [29], humorally mediated graft rejection [30,31] and acute respiratory distress syndrome [32].

Complement activation is also an important event during allograft rejection, turning the use of complement inhibitors a requirement for long-term graft survival in allotransplantology [33]. There is a growing body of evidence that the LCP is highly involved in allograft rejection [34] as well as in ischemia-reperfusion injury [35], and that lectin molecules increasingly seem to underpin the injury in different organs.

These and other often life-threatening conditions, including preeclampsia [36], hyperacute rejection of xenografts [37], necrosis of infarcted heart tissue [38], brain damage [39] and autoimmune tissue lesions [40], can be ameliorated by complement inhibition [41-43]. Several autoimmune conditions where the complement system is a rational therapeutic target have been listed [44].

In addition, the involvement of the complement system in the early phases of the inflammatory response and the wide array of pro-inflammatory consequences of complement activation [45] have made this system an attractive target for therapeutic intervention. This has resulted in the isolation, synthesis and preparation of a host of complement

Table 3. Conditions in which the complement system has been found to be implicated.

Disease/intervention	Process	Evidence of complement involvement
Immune-mediated diseases	Rheumatoid arthritis	Consumption of complement in acute disease; activation products in synovial fluid and on synovial membrane; systemic complement inhibition suppresses disease in animal models; complement deficiency suppresses disease in mouse models
Membranoproliferative diseases	Glomerulonephritis	Abundant deposits of complement activation products, including membrane attack complexes, in glomeruli; complement inhibition suppresses disease in models; intrarenal administration of complement regulators suppresses disease in models
	Multiple sclerosis	Activation products found in CSF and around areas of demyelination in CNS; complement inhibition suppresses disease in animal models; complement deficiency suppresses disease in rodent models
	Myasthenia gravis	Abundant activation products at motor end plate during attacks; complement deficiency or depletion abrogates disease in rodent models; complement inhibition (CVF; sCR1) suppresses disease in models
Degenerative diseases	Alzheimer's disease	Complement activation products in and around plaques in the CNS; anti-inflammatory therapy appears to slow progression
Ischemia-reperfusion injuries	Myocardial infarct stroke	Reperfusion associated with activation in ischemic area; abundant deposits of activation products in and around infarct; complement inhibition at time of reperfusion in models reduces size of infarct
Interventional activation	CP bypass hemodialysis	Complement activation products in plasma; 'coating' circuits to reduce complement activation improves outcome

CVF: Cobra venom factor; sCR1: soluble complement receptor type 1.

inhibitors [46], as well as the design of new molecular entities and useful bioactive structures [38,47].

Furthermore, activation of the complement system has effect on other systems, including kinin-kallicrein, prostaglandin and blood-clotting. Therefore, factors affecting the normal functioning of the complement may yield serious consequences. **Table 4** lists a series of clinical conditions where intervention with inhibitors of complement activation may be beneficial.

2. Overview of the complement IP literature. Selection of the documents

The authors searched the United States Patent and Trademark Office (USPTO) and Espacenet (developed by the European Patent Office) databases at the end of 2013. After manually removing duplicate publications (employing the 'also published as' feature in Espacenet) and out-of-scope items (e.g., those covering nucleic acids coding for specific proteins, purification of complement elements or their use for diagnostics), it was found that since 2008, 42 patents and 66 patent applications contained the word 'Complement' in their title, totaling 108 documents (18 items/year). Interestingly, a wider and more complete survey on complement patents, covering from 1976 to 2011, has been recently published [48].

A similar search of the Espacenet database highlighted 156 valid articles, covering the period 2008 – 2013. Together with their duplicated publications, these amounted to 1081 records. Although the set of documents may not contain all of the publications related to the subject, its size turns it representative of the main developments and advances that took place in the field during the covered period.

These suitable documents were analyzed according to two main criteria: i) industrial indicators; and ii) chemical and biological characteristics of the molecules involved. The specific indicators considered relevant for the industrial sector were: a) the year of the documents, which helped to suggest a temporal evolution of the interest in the field; b) the citizenship of the inventors; c) the companies and academic places that invested and/or performed the research, which helped locating where the technology was developed; and d) the countries where the IP was filed or deposited, as an indication of the markets aimed to be protected.

On the other hand, the specifically relevant chemical and biological characteristics considered for the molecules involved were: i) point of action, within the complement cascade; ii) mode of action; and iii) type of molecule, according to its size and class.

2.1 Analysis of the results. Indicators relevant for the industrial sector

2.1.1 Year of the documents

Analysis of the IP documents becomes complicated due to duplication of the filings and because many items (particularly non-US/European filings [EP]/World [WO]) represent copies or newer versions of previously published patents or applications, most of them belonging to the period before 2008. **Figure 3** shows bar plots of the relevant US documents, without taking into account duplicate publications. It can be

Table 4. Potential clinical conditions for intervention with complement activation inhibitors.

Alternative complement pathway	Classical complement pathway
Acute myocardial infarction	Auto immune diseases
Adult respiratory distress syndrome	Bullous diseases
Adverse drug reactions	Cryoglobulinemia
Cerebral infarction (stroke)	Glomerulonephritis
Crohn's disease	Hemoglobinuria
Crush injury	Hemolytic anemia
Drug allergy	Hyperacute allograft
Hyperacutexenograft rejection	Inflammatory bowel diseases
Hypovolemic shock	Inflammatory disorders
IL-2-induced vascular leakage syndrome	Multiple sclerosis
Inflammatory disorders	Myasthenia gravis
Intestinal ischemia	Paroxysmal nocturnal
Multiple organ failure	Rheumatoid arthritis
Pancreatitis	Septic shock and endotoxemia
Post-pump syndrome (bypass and hemodialysis)	Systemic lupus erythematosus
Radiographic contrast media allergy	Transplant rejection
Reperfusion injury	Urticaria
Sickle cell anemia	Vasculitis
Thermal injury (burn and frostbite)	
Transplant rejection	

observed that the amount of records in the USPTO database has been slowly but steadily increasing during the analyzed period.

When the whole Espacenet set was analyzed starting from 2008, it revealed that around 90 documents/year were published, except during 2010 and 2011 (70 items/year), probably due to the world crisis. However, the number of filings has now regained its level, indicating that there is still a strong interest in R&D in the area complement inhibitors/regulators.

2.1.2 *Countries/citizenships of the inventors*

Analysis of the 156 selected documents revealed that their applicants were 325 inventors, from 20 different nationalities, totaling 528 authorships (average = 3.4 inventors/item). Almost two-thirds of the authorships were made by inventors declaring US citizenship. Together with Chinese inventors, they accounted for approximately 75% of the authorships.

Grouped continent-wise, American (US and Canada) and Asian (China, Taiwan and Israel) inventors accounted for approximately 80% of the authorships. European countries were responsible for almost 20% of the authorships, and < 1.5 of the document authors were Australian. In 11% of the documents, the inventors were of more than one citizenship and in two cases, inventors were of three different nationalities.

Most of the documents are continuations of previous items or have been published in different years or countries, (~ 200 of them before 2008) in 925 additional uniquely numbered documents. This number increases 10 – 20% when filing for multiple classes is considered.

2.1.3 *Countries where filing was made*

The countries where the documents were filed suggest potential markets, where the technology can be competitively developed, and therefore protection is required. In many cases, inventors protected their intellectual property in several agencies and countries. The distribution of the countries/blocks where the set of considered items were filed is: USA (17.1%), Australia (10.8%), Japan (8.7%), Canada (6.9%), China (5.3%), Korea (3.2%), Mexico (2.5%), Russia (2.2%) and New Zealand (2%). South America (Argentina, Brazil, Chile, Colombia, Peru, Uruguay, Cuba) amounted to 4.5% of the documents, while individual European countries had a share of 6.2% records and the remaining individual Asian countries (Malaysia, Israel, Hong Kong, Taiwan) participated with 1.4%. In addition, there were 14.9% EP and 14.2% WO records.

2.1.4 *Assignees. Companies and academic institutions that financed the research*

Between 2008 and 2013, about 30 companies, mostly US and European based, filed or were granted patents related to complement inhibitors and their use. During the same period, the Academia strongly financed research work, filing many applications. Numerous patents related to complement inhibitors and their use were assigned to academic and health institutions. In 35 cases, the assignees were the inventors themselves.

2.2 Analysis of the results. Chemical and biological characteristics

Figure 1 depicts the complement system and its most relevant components targeted during the analyzed period. The sites aimed for inhibition were mainly the recognition/initiation stages of the three pathways (C1q/C1s, MBL/MASPs, and factors B and D of the ACP), the central components C3 and C5 and the RCAs including CR1, MCP, DAF, factor H and CD59.

2.2.1 *Strategic points for inhibition*

Before evaluating the protected IP, analysis should be made on the targets that may have therapeutic potential within the complement system. In view of the particular design of the complement system and taking into account its complex functions, it can be anticipated that no single therapeutic complement activation inhibitor will be able to solve all the problems caused by the uncontrolled activation of the complement system.

However, strategies may focus on four main objectives, which include the recognition and activation steps, the central

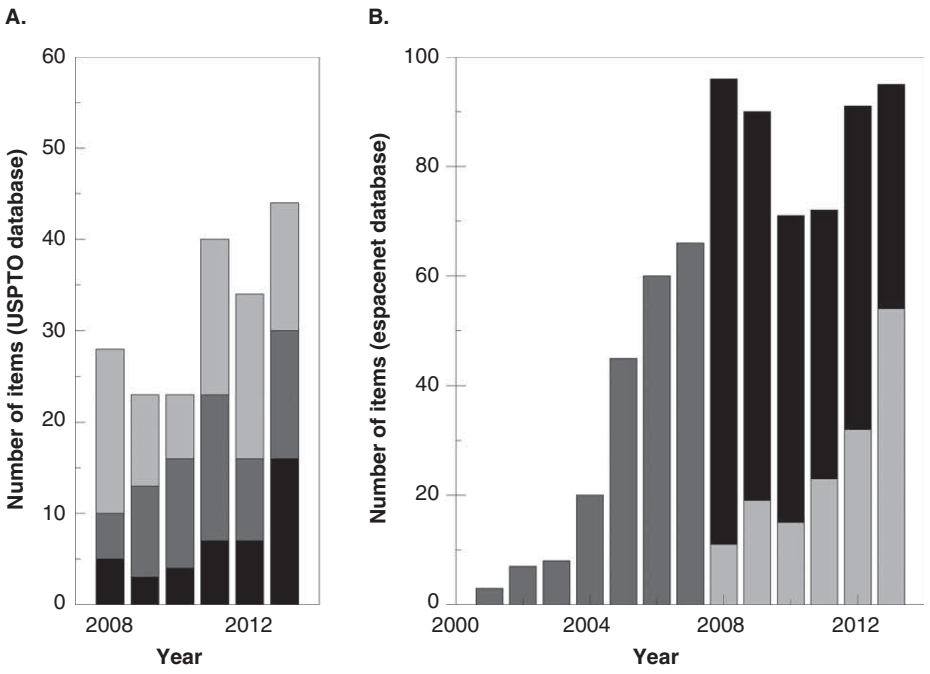

Figure 3. Yearly distribution of intellectual property on complement. A. Non-duplicated US patents (black) and patent applications (dark gray) found in the USPTO database with the keyword 'Complement' in the title; total of items, including duplicate documents (gray). **B.** Relevant non-duplicated patents from the database Espacenet (gray), total number of relevant patents, including duplicates (black). The years before 2008 include only documents published again in the period 2008 – 2013 (dark gray).
USPTO: United States patent and trademark office.

C3 and C5 convertases and also the regulation stages, affecting the interaction between specific compounds derived from activation of the complement and their corresponding receptors or targets. The following is a brief rationale of either alternative.

2.2.1.1 *Inhibition of the initiation stages*

There are at least three such stages related to the CCP, LCP and ACP. These can be blocked independently; however, in order to decide the best strategy, their mechanisms and the effect of a given activation on the physiopathology of the condition should be known. Different clinical conditions involve different pathways (**Table 4**).

2.2.1.1.1 *Classical pathway*

Blocking activation of C1 preserves the beneficial functions of the other initiating pathways; however, activation of C1 is antibody-dependent and more than one pathway may be involved in some diseases.

2.2.1.1.2 *Alternative pathway*

Its activation may also be part of the amplification mechanism of the complement, even when triggered by the other two pathways. Factor D is the rate-limiting step of this route; therefore, its inhibition may cause slowdown of any of the routes, but it will not block important functions, such as C1q and MBL-mediated opsonization and receptor-binding capabilities. Blockade of the ACP leaves the other alternatives to fight pathogens and protect the host from infection.

2.2.1.1.3 *Lectin pathway*

This pathway is associated to several conditions previously ascribed to the CCP, such as injuries resulting from ischemia-reperfusion. Specifically blocking MBL or the MASPs should significantly lower organ damage, while leaving intact many other functions of the complement system.

2.2.1.2 *Inhibition of the central components C3 and C5. Inhibition of the convertases*

2.2.1.2.1 *Blocking at the activation of C3*

Due to its central place in the complement cascade, blocking the activation of C3 has the broadest effect and is equivalent to applying an ample inhibition to the whole system. The rationale behind this strategy is that if complement activation is detrimental to the host, its inhibition should be somehow beneficial.

2.2.1.2.2 *Blocking at the stage of C5*

This will inhibit the formation of the C5b-9 complex and avoid sublytic effects of MAC. Selective blockade at C5 allows activation of C3, which still enables the organism to defend itself against foreign invaders.

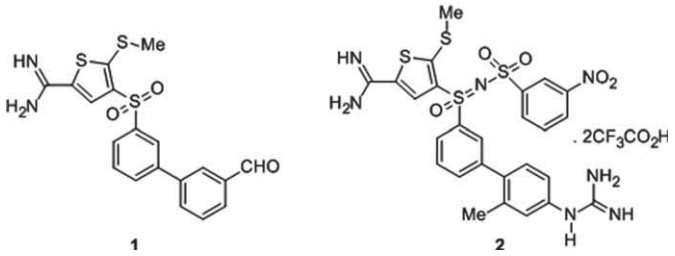

Figure 4. Examples of classical complement pathway C1s inhibitors. Thiophene sulfone-amidines and sulfoximines.

2.2.1.3 *Hindering the interaction of specific activation by-products with their corresponding receptors by blocking one of them*

The anaphylatoxins (C3a and C5a) are an example of this strategy. The mission can be accomplished using specific antibodies (anti-C3a) or antagonists of the C3a receptor (C3aR), which leave intact other physiological functions of C3. However, only one effector molecule is inhibited with each antibody, leaving uninhibited others important effectors. One scenario for this action is airway hyperresponsiveness. Analogously, C5a formed during inflammations associated with systemic complement activation may be inhibited, leaving the ability of C5b to form C5b-9 and kill bacteria. However, risk/benefit studies must also be taken into account in order to preserve the equivalent of a normal level of complement activity.

2.2.1.4 *Affecting RCA*

Complement control proteins work in concert to regulate the system and keep it from damaging host tissue. Use of RCAs or modifying their activity level may influence the performance of different aspects of the complement system, depending on the affected RCA.

2.2.2 *Inhibitors and their mode of action*

Without aiming toward a strict classification, for a more comprehensive perspective, the set of relevant IP records regarding complement inhibition has been grouped according to pathways and molecular targets affected, and also with regard to their chemical nature. The mode of action of the different agents, as well as their potential therapeutic indications, is also informed when data are available.

2.2.2.1 *Classical complement pathway*

Wound healing assisted by a complement-inhibiting hydrophilic sulfonic polymer was proposed [49]. The new material was inspired in heparin, a sulfonylated polymer that inhibits the CCP. A treatment for glaucoma with unspecified C1q inhibitors was protected [50], and use of anti-C1q antibodies has been disclosed as a treatment for myasthenia gravis [51].

Small molecules such as the thiophene sulfone-amidines (**1**, K_i = 0.006 μM) [52] and sulfoximines (**2**, K_i = 0.010 μM) [53], which specifically inhibit the C1s protease (**Figure 4**), have been disclosed. Other low molecular weight compounds able to bind and inhibit the recognition molecules of the CCP and LCP, conjugated through suitable linkers to PEGs as high molecular weight carriers, were proposed as serine protease inhibitors [54].

mAb175-62 is an mAb that binds to C2a, blocks its functional activity in complement activation and inhibits CCP-mediated hemolysis [55].

The *Vaccinia virus* complement control protein (VCP) has been proposed for prophylaxis of atherosclerosis and reperfusion injury. It is a strong inhibitor of the three complement pathways, acting on both C4 and C3. VCP is a secreted product of the *Vaccinia virus* containing four short consensus repeats (SCRs) that display great sequence homology with several RCAs, including C4BP, MCP and DAF. It also shares high functional similarity with CR1 [56].

2.2.2.2 *Lectin complement pathway*

Novel ficolin-associated polypeptides and related compounds were protected for treatment of conditions associated with inflammation, apoptosis, autoimmunity, coagulation, and thrombotic and coagulopathic diseases [57]. Antibodies against Ficolin-3 inhibit complement activation and were also suggested for treatment of these conditions [58].

The use of MBL inhibitors, including legume-derived lectin and keratin-binding molecules, has been proposed to regulate the LCP and ameliorate hyperglycemic myocardial damage [59].

Complement inhibition by unglycosylated folded C-terminal fragments of a multidomain serine protease of the complement cascade (MBL or C1) obtainable by expression in a bacterial host has been disclosed [60].

CP1 is a 30-amino-acid peptide derived from human Astrovirus coat protein. A method for depleting plasma of functional complement activity was devised, which reduces cell lysis and peptide mediators of inflammation. CP1 binds and inactivates MBL and C1, inhibiting formation of iC3b and activation of the LP [61]. Being able to regulate the LCP and CCP, almost without affecting the ACP, it can be

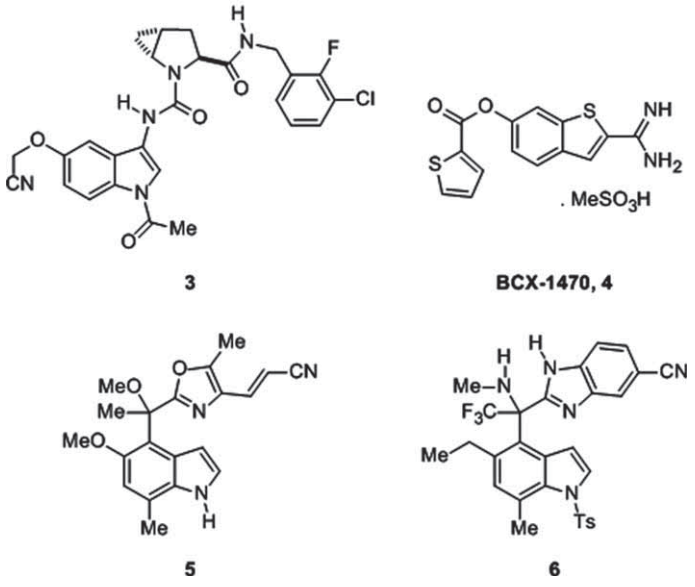

Figure 5. Examples of low molecular weight factor D (3 and 4) and factor B (5 and 6) inhibitors.

used for treating diseases mediated by dysregulated activation of these pathways [62].

Antibodies that bind to and inhibit MBL and MASP1-3-were proposed for preventing or treating tissue damage associated with ischemia-reperfusion injury and thoracoabdominal aortic aneurysm [63].

MASP-2 was characterized as a new serine protease that acts in the LCP [64,65]. This protease shows some homology with MASP-1 and Clr and Cls, two Clq-associated serine proteases of the CCP. Anti-MASP-2 antibodies [66], which selectively inhibit MASP-2-dependent complement activation without substantially inhibiting C1q-dependent complement activation, may be used in conditions associated with MASP-2 related complement activation [67-69].

INSP052 is an immunoglobulin domain-containing cell surface recognition molecule that interacts with complement proteins including MBL-C, MASP-l, MASP-2, properdin and factor H. It was proposed for treatment and/or prevention of diseases related to activation of the LCP or ACP [70].

Inhibitory agents were suggested for inhibiting the MASP-3-dependent complement activation in subjects suffering from paroxysmal nocturnal hemoglobinuria (PNH). Combinations of MASP-1, MASP-2 and MASP-3 inhibitors were also proposed as agents effective to increase the survival of red blood cells [71]. Interestingly, it was shown that MASP-depleted MBL compositions are superior at activating the complement cascade when compared with MBL purified in complex with its associated MASPs [72].

2.2.2.3 *Alternative complement pathway*

A method of inhibiting the adverse effects of complement activation was disclosed. It entails administering dextran sulfate, which effectively inhibits formation of ACP activation products [73]. The polysaccharide PS-1, isolated from the water extract of a Chinese herbal preparation, inhibits activation of both ACP and CCP, with no anticoagulative effect [74].

Factor D is a suitable target for inhibiting amplification of the complement pathways because its plasma concentration in humans is very low (about 1.8 fg/ml), and it is the limiting enzyme for activation of the ACP.

Anti-factor D antibodies such as mAb166-32 [75,76], which block the functional activity of factor D in complement activation, were invented for diagnostic, prophylaxis and treatment (in the form of whole antibody, Fab fragment or single-domain antibody [77,78]) of conditions associated with excessive or uncontrolled complement activation [79,80], including ocular diseases such as age-related macular degeneration (AMD, the leading cause of blindness), diabetic retinopathy and ocular angiogenesis.

The inhibition of the ACP and particularly of factor D, in patients suffering from conditions like AMD, diabetic retinopathy and related ophthalmic diseases, employing indole inhibitors has also been disclosed. The most potent inhibitors have IC_{50} = 1.0 nM [81]. The factor D inhibitor BCX-1470 (**Figure 5**) took part of a method for treating AMD patients [82].

MoAb71-110 is an anti-properdin antibody that binds to a region on properdin that is only involved in activation of the ACP [83]. It is able to inhibit factor D-mediated cleavage of C3bB and PC3bB complexes, and block the ACP activation without affecting the CCP. Treatments for ischemia-reperfusion injury and for preventing symptoms of physiological damage resulting from traumatic brain or spinal cord injury [84] containing anti-properdin antibodies were proposed in a patent application. Blocking the action of properdin [85],

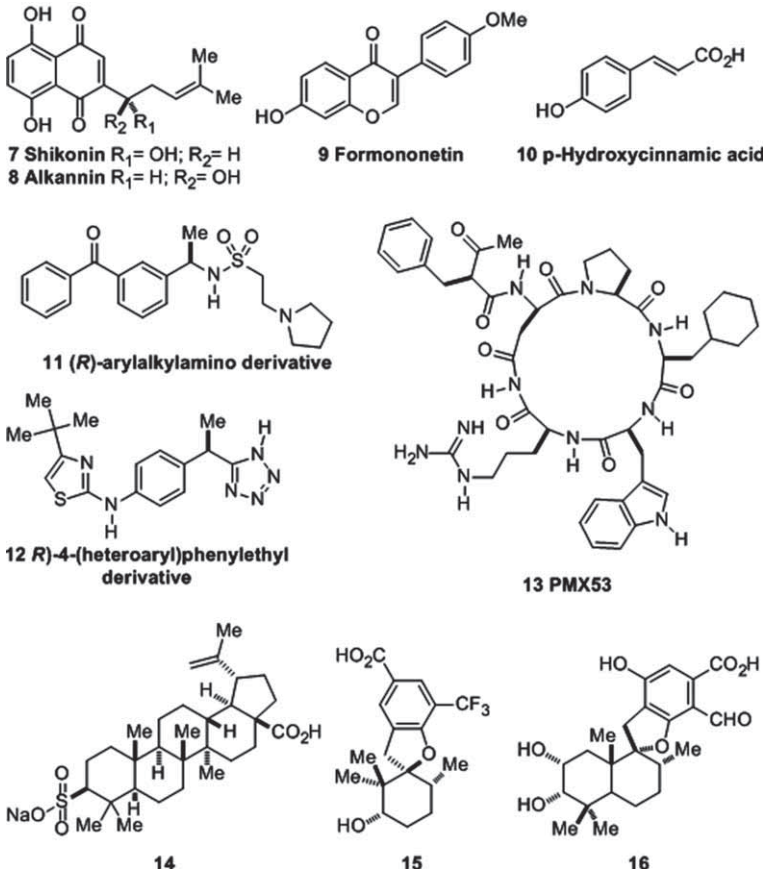

Figure 6. Natural products (7 – 10), low molecular weight synthetic compounds (11, 12), cyclic peptides (13) and semi-synthetic natural product derivatives (14 – 16) with complement inhibition activity.

factor H, and other components of the pathway up to C5b-9 with antibodies has been proposed for treating eye diseases [86].

Cell expression of factor B can be modulated by administering tannic acid [87]. Other small molecule inhibitors of factor B such as indole derivatives 5 and 6 (IC_{50} = 3 μM) have been disclosed (**Figure 5**) [88]. Mutation or removal of a free cysteine improves the yield and thermostability of a properly folded factor B protein analog, and/or reduces its aggregation, not affecting its biological activity profile [89].

Prevention and treatment of complement-associated eye conditions, such as choroidal neovascularization (CNV) and AMD, can be achieved by administration of certain anti-factor B antibodies [90]. Other anti-factor B antibodies were proposed to reduce or prevent other diseases and conditions where the ACP has an active role, including airway hyperresponsiveness, airway inflammation and ischemia-reperfusion injury [91]. Humanized anti-factor B antibodies, which bind factor B in the third SCR domain and selectively inhibit activation of the ACP by preventing formation of the C3bBb complex, were disclosed [92].

Administration of anti-factor Bb [93] and anti-factor Ba (that specifically binds to factor Ba sequences involved in factor B binding to C3b or factor B cleavage into Ba) [94] selectively inhibited formation of ACP activation products C3a, C5a and C5b-9. A method of inhibiting complement activation mediated by inhibitors of factor Bb was suggested. The agent prevents binding of at least one of the Bb molecules to factors B and properdin, inhibits C3 cleavage, formation of C3a, C5a and MAC, and inhibits the activation of neutrophils, monocytes and platelets [95]. On the other hand, antibodies 3E7 and H17 have been prepared, which inhibit binding of factor B and factor H to C3, C3(H_2O), and C3b [96].

2.2.2.4 *Modulation of C3, C3a/b and C3 convertase*

Recombinant C3 [97] and modified C3 by substitution with a corresponding portion of a cobra venom factor (CVF) protein results in a C3 protein with CVF functions and substantially reduced immunogenicity. It provides a C3 convertase with increased stability and resistance to the actions of factors H and/or factor I. The combination of long intrinsic half-life and resistance to regulation, allowing CVF to continuously activate C3 and C5, ultimately results in depletion of the serum complement activity.

Opposite to complement-oriented drug development attempts, based on inhibiting the activation of complement, CVF-C3 hybrids act through a new concept, by depleting

complement in serum. These proteins may be useful in treating complement activation-related conditions [98].

A staphylococcal complement inhibitor (SCIN)-based protein that acts on the ACP and CCP convertases has been proposed for treating inflammatory conditions [99]. SCIN is a member of a large family of compounds with activity on complement. SCIN-B and SCIN-C inhibit solely the ACP, not affecting CCP and LCP.

Peptides and peptide analogs, which mimic the structure and activity of secreted *Staphylococcus aureus* proteins extracellular fibrinogen binding protein (Efb) and SAV1 155, capable of binding C3 and inhibiting complement activation, were disclosed [100].

A polypeptide isolated from the saliva of the hematophagous tick *Ixodes scapularis* was claimed to reduce the activity of ACP, by binding to properdin and thus accelerating the decay of C3 convertase [101].

The C3 convertase of the ACP and the assembly of the MAC can be selectively inhibited by a low molecular weight aurin tricarboxylic acid synthetic complex and its derivatives. This may have impact in the treatment of various conditions [102].

A patent application on the structure of C3c in complex with the C3 inhibitor Compstatin suggests how this information can be used for rational design or identification of complement-inhibiting drugs [103]. Compstatin, its analogs, derivatives and peptidomimetics were claimed to modulate C3, and as inhibitors to treat sepsis [104], organ failure resulting from sepsis [105], chronic respiratory disorders such as chronic obstructive pulmonary disease [106,107] and to alleviate tissue damage resulting from trauma [108]. When delivered intranasally, a Compstatin analog was proposed for treating far chronic rhinosinusitis or nasal polyposis [109].

A peptide highly homologous with C3a was employed to agonize C3aR. Dimeric peptides based on the C-terminal sequence of C3a inhibit secretory responses of mast cells and basophils; they may be useful in the treatment of allergic disorders [110].

A method of inhibiting complement activation mediated by C3b with anti-C3 antibodies, including humanized and chimeric versions, has been put forward. The antibodies inhibit C3 cleavage, blocking the formation of C3a, C5a and the MAC, as well as the activation of neutrophils, monocytes and platelets [111,112].

A complement modulator comprising a regulatory region (factor H, SCRs 1 – 4) connected by a poly-glycine flexible linker of at least 12 residues in length to a multifunctional binding region (factor H, SCRs 19 – 20) that enables binding to C3b activation/inactivation and/or oxidation end products, as well as to polyanionic surface markers on host cells was disclosed. Methods of using this complement regulator were also given [113].

The complement receptor of the immunoglobulin superfamily is a polypeptide that binds C3b and iC3b and inhibits the C3 convertase. It was proposed for prevention and treatment of complement-associated eye conditions (AMD, CNV) [114].

Aptamers that bind C3 and a bifunctional aptamer construct that clamps specifically with C3b or iC3b, and another target proteins can be used to direct the opsonization process [115].

Most natural RCA are large molecules (> 100 kDa) that act at the level of C3b, the central component of the complement convertases. Since these are difficult to develop as therapeutic agents, anti-C3b antibodies [116] are a possible solution. Hence, inhibition of complement activation was achieved with anti-C3 antibodies targeting different parts of the molecule.

The development of molecules that specifically recognize C3b and not native C3 avoids their consumption by non-activated C3 [117]. In addition, anti-C3d antibodies, useful for inhibiting host humoral immune response [118], and an anti-C3c antibody to C3b have been disclosed [119].

The roles of complement components C3, as well as C5 and their receptors C3aR and C5aR, were investigated and potential uses for their modulation were proposed. These include promoting wound healing [120], preventing the onset of polyp formation and subsequent colon cancer [121], and treating injuries derived from intracerebral hemorrhage [122]. These inhibitors were also proposed as solutions to deplete sera from biomaterial-induced procoagulant activity in blood subjected to extracorporeal circulation [123].

2.2.2.5 *Inhibition at the level of C5 (C5a/b) and the C5 convertase*

An immunological adsorber consisting of monoclonal anti-C5a antibodies supported on an organic or synthetic polymer was designed for treating inflammations and eliminating complement factors, being useful for extracorporeal detoxification [124].

Eculizumab, pexelizumab and TNX-558 (anti-C5a) were identified as potential candidates to affect C5. Eculizumab, a humanized monoclonal anti-C5 antibody that hinders formation of C5a by impeding cleavage of complement C5 and inhibits terminal complement activation, was patented as a safe alternative for chronic therapy for PNH [125]. Pexelizumab is a single-chain version of eculizumab. A single amino acid change in pexelizumab confers significant physicochemical advantages to the antibody [126], improving its solubility.

Methods of prolonging survival of allotransplanted cells, tissues or organs in cases where the recipient has an ABO mismatch with the allografts were presented. These include chronic administration to the recipient of an antibody or antibody fragment (pexelizumab, eculizumab), together with one or more immunosuppressants [127].

Anti-C5 antibodies [128] have also been proposed for treating ocular diseases including AMD [129], for treating or preventing asthma [130], glomerulonephritis [131] and neuropathies [132], and for prolonging survival of allografts [133]. Monoclonal antibodies against C5 and/or its fragments have been disclosed. MoAb137-26 binds to a shared epitope of

human C5 and C5a, without preventing activation of C5 and formation of C5b. This inhibitor may be a tool to treat conditions mediated by excessive or uncontrolled production of C5a [134].

Related monoclonal antibodies MAb137-76 and MAb137-30 also bind to and inhibit C5. They block type II endothelial cell activation, with suppression of E-selectin and may be useful in treatment of delayed xenograft rejection or acute vascular rejection [135]. In addition, an antibody that binds to the alpha chain of C5, inhibiting complement activation and the binding of C5 to either C3b or C4b, was disclosed. This antibody does not bind to C5a [136].

Compositions containing an anti-C5, and anti-C5a/C5b antibodies, or antigen-binding fragments as human complement inhibitors were patented for treating or preventing Degos' disease [137]. Polypeptides able to bind C5 and comprising different C5-binding motifs were also proposed for use in therapies affecting C5 [138].

Complement inhibitors of CCP and ACP were proposed to treat severe injuries [139]. The *Ornithodoros moubata* complement inhibitor EV576 (OmCI) was isolated from the salivary glands of this soft hematophagous tick. This substance was claimed to inhibit both the CCP and ACP at the C5 convertase level. EV576 does not affect C3 activation and preserves the immune clearance and opsonization functions of complement, which depend on C3b [140]. The protein was modified in order to increase its specificity [141]. Treatment with rEV576 was proposed as a solution for myasthenia gravis [142] and respiratory [143] and peripheral nerve [144] disorders.

Aptamer therapeutics, where an anti-Complement aptamer modulates a function of a complement component [145], has been proposed as a useful approach for treating disorders associated with complement activation, particularly ocular disorders. ARC1905 is an experimental aptamer capable of binding C5. The inventions provide nucleic acids and methods for their therapeutic use in these disorders [146].

2.2.2.6 Modulation of the LP

Mutated Fc monoclonal antibodies specific to C5, C5 convertase, C5a or C5b-9 that do not activate the complement cascade were put forward for treatment of pulmonary conditions [147]. On the other hand, bi-specific antibodies able to bind two or more different epitopes of two or more different proteins, among them C5a, C5b, the C5b-9 complex, and a component or intermediate of the LP, were proposed as inhibitors of this pathway and for treatment of complement-associated disorders [148].

Antagonist oligomers (10 – 60 nucleotides) encoding for C6 with > 80% homology and capable of reducing the level of C6 mRNA were designed to inhibit or block expression of C6 and proposed for enhancing nerve regeneration following acute or chronic nerve damage and treatment of multiple sclerosis [149].

An inhibitor of the formation of the MAC was disclosed for treating cerebral amyloid angiopathy-related conditions or diseases affecting cerebrovasculature. Inhibition was also found sufficient to decrease the incidence or prevent cytolysis of the smooth muscle cells [150].

2.2.2.7 Complement modulation involving complement receptors

A soluble complement receptor type 1 (sCR1), which inhibits all activation pathways, also proved useful and was proposed for treating chronic nephropathies [151]. sCR1 inhibits *in vitro* the consequences of complement activation such as neutrophil oxidative burst, hemolysis, and production of C3a, C5a and the MAC. It has been shown that inhibition of complement activation in brain-dead organ donors with an sCR1 polypeptide, prior to harvesting the organ for transplant, improves organ functionality in the recipients [152].

A pharmaceutical combination comprising three elements, one of them a soluble human complement inhibitor, was proposed for treatment or prevention of insulin-producing cell graft rejection. TP-10 was among the suitable C3/C5 soluble complement inhibitors. This is a recombinant protein that blocks C3 and C5 activation by all three activation pathways, which embodies a modified CR1 molecule lacking the transmembrane and cytoplasmic domains. TP-10 binds C3b and C4b, blocking their interaction with other proteins in the complement cascade, also acting as a cofactor in the enzymatic degradation of C3b and C4b to their inactive forms [153].

The three dimensional structure of the crystalline complex between human CR2 protein and C3d has been disclosed [154]. Building fusion proteins by linking an RCA (MCP, DAF, CD59) to a CR2 has been put forward as a strategy for targeting inhibition to sites of complement activation [155,156] and preventing diseases such as post-traumatic and degenerative arthritis [157]. A CR2-FH chimeric molecule comprising fragments of both proteins was proposed for treatment of conditions (AMD, rheumatoid arthritis, ischemia-reperfusion) involving activation of the ACP [158,159].

CR3 is a human cell surface receptor that binds to C3b, found on polymorphonuclear leukocytes, particularly neutrophils. Microorganisms causing systemic disease employ a variety of complement evasion mechanisms; acquisition of complement regulators is a frequently adopted paradigm. The glucose transporter HGT1 from *Candida albicans* (CaHGT1P) is involved in evasion of phagocytosis; it may act as a CR3 analogue. Therefore, an invention directed to inhibiting or masking CaHGT1P was disclosed, in order to overcome evasion of phagocytosis by the yeast [160].

PMX-53 and neutrazumab are C5aR antagonists. Inhibition of C5aR with an anti-C5aR antibody that specifically binds to an extracellular loop was proposed for treating bleeding-related inflammation [161]. Administration of an inhibitor of C5aR signaling in the tumor microenvironment was proposed as a cancer treatment [162].

It has also been suggested that C5a may be influencing the fine local inflammatory balance of the bone healing process. Accordingly, methods for promoting fracture healing in the presence of other traumatic injuries, which involve administration of a complement inhibitor to block systemic C5aR signaling, were disclosed [163]. A set of small molecules was claimed to selectively bind anaphylatoxin C5a receptor, thus modulating the ACP response [164].

Complement factor H receptor proteins and related materials are specific C5 convertase inhibitors. They have been suggested as agents for preventing inflammatory reactions, precluding complement activation during transplantation and dialyses, and for coating devices that enter in contact with blood or body fluids, especially implants [165].

2.2.2.8 *Complement modulation involving regulatory proteins*

Analogs of RCAs with altered specificities and affinities for their targets (C3b and/or C4b) have been described. They were obtained by substituting amino acids that affect the complement inhibitory activities or by substituting, rearranging or adding SCRs or SCR regions to the proteins, deleting amino acid sequences, and combinations of these strategies [166].

Chimeric molecules containing RCA proteins [167], such as a ficolin-associated polypeptide and a modulator of complement activity (factor H, C1INH, C4BP, factor I, DAF, etc.), capable of inhibiting complement activation were disclosed [168].

Complement inhibition may help axonal regeneration [169]. Blockade of the CCP with recombinant C1INH proved activation of this pathway after nerve injury.

MCP has a higher affinity for binding C3b than for C4b, resulting in increased inhibition of convertase formation by the ACP; CD59 (protectin) is a regulatory protein and DAF regulates all three complement pathways by binding and accelerating the decay of the C3 convertase in the CCP and ACP, preventing downstream MAC deposition on biological surfaces.

A recombinant chimeric soluble terminator of activated complement (STAC) protein was constructed with the complement regulatory domains of each MCP, DAF and the native secretory signal of CD59 at the N-terminus. The STAC protein negatively modulates CCP and ACP; therefore, a method for regulating or treating a complement-related condition was proposed [170]. Alternatives employing CD59 alone have also been suggested [171].

A fusion protein (scFv-DAF) was obtained by coupling an acetylcholine receptor-resistant single-chain antibody to the amino terminal of DAF (SCR1-4) through a connecting peptide. This protein blocks the complement cascade, protects the acetylcholine receptor and eliminates immune injury caused by complement activation. It was proposed as a biological agent for treating myasthenia gravis [172].

Other fusion proteins with anti-inflammatory action involving a single-chain anti-P Lectin coupled to complement receptor 1-related protein Y [173] and to DAF [174] were disclosed.

A missing or significantly reduced function of factor H has been demonstrated in diseases that finally harm kidney function, like the atypical hemolytic uremic syndrome or the type II membranoproliferative glomerulonephritis (also known as dense deposit disease or C3 glomerulopathy). Thus, employing factor H as a therapeutic agent for the protection of cellular membranes lacking endogenous membrane-anchored regulators is a new concept. Therefore, its use as a supplement in chronic nephropathies taking place with or without proteinuria, including in antibody-independent tubulointerstitial fibrosis, was proposed [175].

Polymorphisms and haplotypes in the complement factor H gene are associated with development of AMD. Factor H containing isoleucine at residue 62 and tyrosine at residue 402 has been proposed for its treatment [176]. In addition, factor H-binding peptides that impede the complement inhibitory activity of factor H were disclosed. When immobilized onto the surface of a biomaterial, these peptides recruit factor H, resulting in a substantial inhibition of biomaterial-induced complement activation in substances exposed to biomaterials [177].

A chimeric recombinant polypeptide containing partial sequences of factor H and a complement activation inhibitor like Compstatin, Efb, second immunoglobulin-binding protein or SCIN was put forward as an agent for treatment of complement-mediated diseases [178].

Factor I has C3b-inactivating and iC3b-degradating activity. Raising factor I above the physiological level can be used to treat diseases in which the underlying pathology is linked to overactivity of the C3b-feedback cycle and the generation of iC3b [179].

2.2.3 *Classes of inhibitors, according to their size, origin and structure*

The molecules involved in the intellectual property documents were low molecular weight natural products, semisynthetic and synthetic compounds, cyclic peptides, polypeptides and mostly macromolecules. The latter included tailor-made proteins (mainly monoclonal antibodies, Fc fragments and single chains, hybrid and chimeric proteins) and aptamers, as well as polysaccharides and synthetic polymers.

2.2.3.1 *Low molecular weight natural and synthetic compounds, and cyclic peptides*

Bioactive small molecules were disclosed for their anti-Complement activity (**Figure 6**). Shikonin (7) is a phenolic compound extracted from a Boraginaceae plant. It inhibits the CCP and ACP, with CH_{50} = 0.16 – 0.63 mM, and AP_{50} = 0.24 – 0.41 mM [180,181]. Shikonin tetramers also display complement inhibitory activity [182], as does alkannin (**8**) dimer, a related quinone isolated from the same Boraginaceae [183].

A flavan obtained from commelina and fagopyrum exhibited inhibition values of CH_{50} = 0.13 – 0.78 mM and

AP_{50} = 0.12 – 1.00 mM [184]. An acylated flavonoid glycoside demonstrated to possess inhibitory activity toward complement activation [185]. The isoflavone formononetin (**9**) has CH_{50} = 0.09 ng/ml and AP_{50} = 0.42 mg/ml [186]. Vanillic acid and *p*-hydroxycinnamic acid (**10**) have also been proposed as anti-Complement agents, through the CCP [187].

(*R*)-arylalkylamino (**11**) [188] and (*R*)-4-(heteroaryl)phenylethyl (**12**) derivatives useful in the inhibition of the chemotactic activation induced by the fraction C5a of complement were proposed for treatment of autoimmune hemolytic anemia, psoriasis, bullous pemphigoid, rheumatoid arthritis, ulcerative colitis, acute respiratory distress syndrome, idiopathic fibrosis, glomerulonephritis and in the prevention and treatment of injury caused by ischemia-reperfusion [189].

Cyclic peptidic and peptidomimetic compounds such as PMX53 (**13**) are useful for neurodegenerative diseases, neuroimmunological disorders, diseases arising from dysfunction of the blood brain barrier and stroke [190].

Betulinic acid has demonstrated to inhibit complement activation at the level of C1q. Several Russian patents were granted to the isolation of betulinic acid sulfate (**14**), disulfate and its sodium salts [191-194]. In addition, tricyclic complement inhibitor **15** was disclosed [195], as a simplified analog of the recognized inhibitor K76-COOH (**16**) [196-200].

Hydroxylamines and/or their pharmaceutical compositions were disclosed for the treatment of angiogenesis, hepatitis, complement-mediated pathologies, drusen-mediated pathologies, macular degeneration and certain other ophthalmic conditions, inflammation, arthritis and related diseases, and for the inhibition of complement activation [201].

3. Conclusion

This review is based on patents and applications published between 2008 and 2013. The steady pace of increase in the number of patents granted to complement-related issues clearly indicates that this is an active field and a fertile area for the discovery of new therapeutic alternatives and solutions. The complement system is involved in a wide array of clinical conditions, many of them chronic, and some of them of a life-threatening nature. The possibility of developing treatments for some of these diseases with inhibitors of the activation of complement cascade represents a long explored but still promising area of research within modern medicinal chemistry.

High-affinity monoclonal antibodies are one of the most exploited general approaches toward site-specific inhibition; however, some low molecular weight Natural products have also been discovered to possess ability to inhibit the complement cascade and their mode of action awaits to be studied. On the other hand, completely synthetic products also compete to bridge the gap between what Nature provides and what humankind needs. Despite the huge advances in this area of medicinal chemistry, which were crowned with episodes like the approval of eculizumab for treating PNH, there is still much to do with regard to the development of relevant and novel drugs.

4. Expert opinion

The architecture of the complement has evolved during the last 600 – 700 million years to furnish a sophisticated cell killing device, a complex transduction mechanism able to detect and respond to alien structures and interconnect to other body systems, and an amazingly efficient and highly versatile arm of the immune system.

However, the complement system has two main functional drawbacks, which stem from its own characteristics and design. On one hand, its action is non-specific because its need to attack widely different alien targets, protecting its host; on the other, the complement system operates as a precise clockwork piece, which under imbalance can become the enemy of its host at any time.

To understand the basics of the complement system's biology has been the initial step toward achieving sustained progress in this area. Identifying and validating complement as a therapeutic target has allowed allocation of resources to experimentally unlock the potential of complement modulators as an approach for treating some of the most life-threatening and debilitating diseases.

There is an increasing awareness of the need for complement activation inhibitors. It stems from a constantly improving knowledge of complement-mediated conditions and diseases and the development of increasingly complex medical interventions, including organ transplants, which demand efforts toward minimizing damages due to the ischemia-reperfusion syndrome and inflammatory responses associated with these and other processes.

Science has sought for a long time to create therapeutic drugs to specifically inhibit complement activity at specific stages, finding that it is a target-rich area. The search for specific inhibitors of the activation of the complement cascade has become one of the Holy Grails of modern medicinal chemistry for the last 30 – 40 years, still with very few cases of success. Although some small molecules and unspecific inhibitors of complement activation are currently available for various indications, there is still a marked need to find new, more specific and efficient alternatives. Analogously, despite initial success with specific monoclonal antibodies, there is a vast territory to be explored and conquered regarding the RCA by antibody-mediated blockage of specific polypeptides or receptor sites.

The critical analysis of the documents filed during 2008 – 2013 revealed a strong and growing interest in the complement system as a therapeutic target. This is manifested not only among the more than 30 industrial companies, mostly US based, which still lead the effort in developing new solutions, but also among companies based in other places of the world, particularly in China and Europe. Furthermore, many of these companies have complement inhibitors in their pipelines, aiming to treat a wide array of inflammatory and immune diseases.

Despite the ever-increasing budgets demanded by current drug development regulations, the Academia and academic health institutions are still heavy generators of intellectual property, and fruitful sources of new ideas. These are headed by US-based universities, but with an important contribution from European and Chinese institutions, which speaks about the international character of this research topic.

Analysis of the last decade clearly revealed a growing trend toward a more intense globalization of the research and development effort. On one hand, inventors from several nations and citizenships have filed patent applications; on the other side, many inventors and companies have sought to protect their developments, including relatively minor advances, in numerous countries abroad, and also through international agencies, resulting in an important amount of extended and duplicate publications.

Among the intellectual property documents, precision targeting of the complement system with the use of macromolecules as inhibitors of complement activation has now taken a clear lead over the design and development of low molecular weight organic molecules. Only recently the first fruits of these efforts have started to be collected. As research on this paradigm continues, they still hold the promise of being just the first wave of new and potent drugs as well as interesting breakthroughs. This tendency seems to be more accentuated when comparisons are made taking the last 20 years. This is a result of several recent advancements in molecular biology, biotechnology and genetic engineering, but it also stems from a better knowledge of the inner works of the complement system itself.

The resulting inhibitors are nowadays more structurally complex and more specific than ever before; despite this complexity, in many cases their main site of action is more precisely known, and is usually indicated in the documents, since it has been at the heart of the design of the inhibitor. Many of these novel molecules are tailor-made, responding to a handful of different but efficient working paradigms. Copying and imitating Mother Nature (e.g., the use of sCR1 and receptor antagonists) are still two of them, and very successful ones.

The remaining challenge is still translating this knowledge to the development of product candidates, to populate pipelines of targeted therapies with the potential to prevent or ameliorate the severe tissue damage that takes place in a range of diseases and conditions, including kidney (PNH and membranoproliferative glomerulonephritis) and ocular diseases (especially AMD), organ transplants and ischemia-reperfusion syndrome. Fulfilling this aim will have impact in areas such as hematology, nephrology, neurology and ophthalmology, and also in different markets, including those of inflammatory, genetic and rare diseases.

The expectations are that with a continuously increasing interest in the field, fueled by vigorous industrial and academic research worldwide, the dreams of the last 25 years, partly fulfilled by the arrival to the market of the first macromolecular complement inhibitors, may crystallize in the near future, with the approval of more new entities. Most probably, these will include mainly monoclonal antibodies and their fragments, hybrid, chimeric and fused proteins, aptamers and, to a lesser extent, peptides, synthetic functionalized polymers and low molecular weight compounds, including natural products and their derivatives.

It is not difficult to foresee an increased importance of targeted protein therapeutics, with new indications for currently approved drugs (e.g., esterase inhibitors for trauma, inflammation, endotoxemia, sepsis, neuromyelitis optica, multiple organ dysfunction syndrome, graft rejection, hereditary angioedema and other genetic disorders), and the expression of specific fragments of valuable molecules in new bacteria and even plants to eliminate the need for expensive post-translational modifications, resulting in more homogeneous products.

On the other hand, deeper exploration of current approaches and paradigms, especially monoclonal antibodies and tailor-made proteins, will aim to fulfill the multifaceted arsenal of therapeutics required to provide suitable solutions to the number of complement-mediated pathologies, and their diverse underlying mechanisms.

Despite a few small molecules are now on clinical trials, research in low molecular weight compounds has lost much of its initial impulse; however, it must be borne in mind that proteins lack many of the advantages of these small molecules, including low cost, high chemical stability, absence of immunogenicity, ease of formulation and convenient dispensation. Small molecules also have better ability to penetrate in tissues and can be administered orally, a unique feature that should be very useful for treating chronic conditions. These are powerful reasons to keep alive research in small molecule complement inhibitors. Since no such class of molecules is marketed today, to be the first is also a powerful incentive to develop research programs in this direction.

Declaration of interest

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