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## Complement inhibition: a promising concept for cancer treatment

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### Abstract

For decades, complement has been recognized as an effector arm of the immune system that contributes to the destruction of tumor cells. In fact, many therapeutic strategies have been proposed that are based on the intensification of complement-mediated responses against tumors. However, recent studies have challenged this paradigm by demonstrating a tumor-promoting role for complement. Cancer cells seem to be able to establish a convenient balance between complement activation and inhibition, taking advantage of complement initiation without suffering its deleterious effects. Complement activation may support chronic inflammation, promote an immunosuppressive microenvironment, induce angiogenesis, and activate cancer-related signaling pathways. In this context, inhibition of complement activation would be a therapeutic option for treating cancer. This concept is relatively novel and deserves closer attention. In this paper, we will summarize the mechanisms of complement activation on cancer cells, the cancer-promoting effect of complement initiation, and the rationale behind the use of complement inhibition as a therapeutic strategy against cancer.

### Keywords

Complement system; cancer therapy; tumor microenvironment; inflammation; immunosuppression; angiogenesis

## 1. Introduction

Cancer is a major public health problem causing millions of deaths worldwide [1]. In the United States, one in four deaths is due to cancer [2]. Breast cancer in females and lung cancer in males are the most frequently diagnosed cancers and the leading causes of cancer death for each sex (Fig. 1). Despite substantial advances in the chemotherapeutic management of cancer, more than half of all cancer patients do not respond to therapy or relapse, dying from metastatic disease. In addition, cancer chemotherapy is usually

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accompanied by severe side effects. These facts have led to the belief that in some tumor types, traditional therapies have reached a “therapeutic plateau” [3]. More personalized therapies with higher tumor specificity and less toxicity are a clinical need.

A variety of strategies based on the understanding of the molecular events associated with cancer initiation and progression are now under intensive investigation. In fact, some rationally targeted therapies have already shown a remarkable effectiveness in selected populations [4]. Targeted therapy refers to a new generation of cancer drugs designed to interfere with a specific target that is believed to have a critical role in tumor cell proliferation and survival. Most of these strategies are based on the identification of targetable signaling proteins critical for tumor growth or progression. Alternatively, cancer therapies directed at immune modulation have also been pursued, but with only modest advances to date [5, 6]. A better understanding of the molecular interactions between tumors and the immune system should lead to better anticancer therapies. In this review we will present emerging data on the relationship between complement, an essential part of the innate immunity, and cancer progression. We will focus on the therapeutic potential of targeting complement activation in cancer, examining the rationale behind this strategy and the aspects that must be investigated before it can be considered an anticancer strategy that is ready for clinical testing.

## 2. The complement system

Complement has evolved as a first defense against non-self cells or unwanted host elements. The spectrum of complement-mediated functions ranges from direct cell lysis to the control of humoral and adaptive immunity. This system also regulates a number of immunological and inflammatory processes that contribute to body homeostasis [7]. Complement activities are mediated by more than 50 circulating or cell surface-bound proteins. There are three pathways of complement activation: the classical, the alternative, and the lectin pathways (Fig. 2). The three complement pathways differ in their mechanisms of target recognition but converge in the activation of the central component C3. After this activation, C5 is cleaved, and the assembly of the pore-like membrane attack complex (MAC) is initiated. The enzymatic cleavage of C3 and C5 leads to the production and release of anaphylotoxins C3a and C5a, two important inflammatory mediators and chemoattractants [8]. Complement is tightly controlled by several proteins whose main function is to prevent activation. Complement inhibitors are grouped into two categories, soluble regulators and membrane-bound regulators. These regulators naturally protect self cells and tissues from unwanted complement activation [9].

## 3. Complement activation in cancer cells

Malignant transformation is accompanied by the acquisition of genetic and epigenetic alterations that distinguish the transformed cells from their normal counterparts. This process dramatically changes cell-surface proteins, glycosylation, and phospholipids patterns [10–15]. Cancer-related membrane modifications can be recognized by innate and adaptive immune mechanisms that protect the host against the development of cancer [16]. This is the basis of the immune surveillance hypothesis, which proposes that the immune system surveys the body for tumor-associated molecules, eliminating many, if not most, emerging tumors [17]. The immune surveillance activity is one of the three phases proposed in the immunoeediting theory: elimination (immune surveillance), equilibrium, and escape [18]. If tumor cells are able to get pass the elimination phase, they enter an equilibrium period during which surviving tumor cells keep dividing and acquiring genetic and epigenetic abnormalities under the immunological pressure. This pressure contributes to the

selection of tumor cell variants that are resistant to immune effectors. During the escape phase, tumor cells effectively evade the immune system.

There is no direct evidence to support the contention that complement can eliminate nascent tumors. However, considering that complement is designed for the recognition of non-self elements, it is logical to assume that changes in the composition of tumor cell membranes make these cells a target for complement recognition. Consistent with this assumption, a number of clinical studies have reported an activation of complement in cancer patients [19–23]. Indirect evidence for a role of complement in immunosurveillance also comes from the fact that cancer cells develop a variety of strategies to avoid complement-mediated damage [24]. Their best-known escape mechanism is the overexpression of complement regulatory proteins, a subject that has been extensively reviewed [25–29]. According to the immunoediting hypothesis, this overexpression is indicative of a selective pressure created by complement activation in the tumor microenvironment that sculpts cancer cells to evade the harmful effects of complement.

Other indirect evidence of complement activation by cancer cells comes from the increased complement activity found in biological fluids from cancer patients [30–34]. Complement activation has also been observed in *in vitro* studies of cancer cell lines. Lung cancer cells deposit C5 and generate the active product C5a more efficiently than do non-malignant bronchial epithelial cells [35]. However, the antigens responsible for this activation and the pathway/s involved are not yet known. The classical pathway has been identified as the main contributor to complement activation on subcutaneously inoculated TC-1 cervical cancer cells [36]. *In vitro* studies have shown spontaneous activation of the classical complement pathway by two neuroblastoma cell lines [22]. In the case of primary tumors, there are few studies pointing to a specific activation pathway. Lucas et al. [21] have suggested that a tumor-specific immune response occurs in papillary thyroid carcinomas, with activation of the classical complement cascade. Follicular and MALT lymphomas also deposit elements of the classical pathway [23], and alterations in this pathway have been described in patients with chronic lymphocytic leukemia [37, 38]. In contrast, the results of other studies have suggested that lymphoma and myeloma cells activate the alternative pathway [19, 39–41]. Moreover, both the alternative and the classical pathway seem to be involved in some cases [42]. The lectin pathway of complement activation has been found to be significantly increased in colorectal cancer patients [33].

In general, the information about the pathways activated by cancer cells is fragmented. Most studies on this subject were published years ago, and results are confusing, most likely because of the high heterogeneity among different tumor types studied. Each tumor has its own unique antigenic identity and a characteristic profile of complement regulators. This variety of complement recognition molecules and regulators should result in a diversity of activation pathways. To make things more complicated, there are extrinsic complement activation pathways mediated by soluble and membrane-bound proteases, such as serine proteases of the coagulation and fibrinolysis systems [43–46]. Lung cancer cells can produce C5a in the absence of serum, likely through the action of an extrinsic pathway mediated by an uncharacterized trypsin-like serine protease [35]. Thus, a more systematic analysis of the pathways and mediators by which cancer cells activate complement is needed. Such studies would greatly improve our understanding of the dynamic interplay between complement and cancer and would offer the opportunity to identify new molecular biomarkers.

Complement components, or their activation products, have been proposed as markers in other pathologies in which this system is involved [47–49]. Lung cancer patients show significantly higher plasma levels of complement proteins and activation fragments than do control donors [32, 35], and elevated complement levels are correlated with lung tumor size

[30]. Complement-related proteins are also elevated in biological fluids from patients with other types of tumor [32–34, 50]. More interestingly, complement activity can be associated with clinical outcome. For example, a positive correlation has been observed between survival time and the initial activity of the classical pathway of complement in patients with chronic lymphocytic leukemia [51]. High MASP-2 levels in serum have been found to be an independent prognostic marker of recurrence and reduced survival in colorectal cancer [52]. High levels of complement regulatory proteins have also been associated with poor prognosis in different malignancies [53–55], and plasma complement components may also be useful as early predictive markers of response to chemotherapy [56].

#### 4. Promotion of cancer growth by complement

Recognition of cancer cells by the complement system has been traditionally associated with an effector activity that contributes to the destruction of the tumor cells. Accordingly, researchers have designed a wide variety of strategies to increase complement activation in the context of immunotherapy against tumors [29]. However, as early as in 1975, Shearer et al. reported that complement has the capacity to stimulate growth when cells are treated with low concentrations of antitumor antibodies [57]. More recent studies have demonstrated a tumor-promoting role of complement in mouse models [8, 58].

Although the finding that complement elements can act as tumor promoters may be considered unexpected, the idea is entirely consistent with the cancer immunoediting theory. Based on this theory, recognition of cancer cells by complement elements creates a selective pressure that leads to the expansion of new tumor populations that are able to control complement activation. In this context, cancer cells could take advantage of the convenient balance created between complement activation and inhibition (Fig. 3)

This new perspective on the role of complement in cancer was proposed in a syngeneic mouse model of cervical cancer [36]. Complement deficiencies in this model and pharmacological blockade of complement-related mediators have been associated with impaired tumor growth [36]. In particular, C5a, a potent chemoattractant and proinflammatory mediator generated locally in the tumor microenvironment, seemed to be an essential contributor to tumor growth. Later studies with mouse models have supported this seminal observation. C5a contributes to lung cancer progression [35], and genetic complement deficiencies, including C5aR knockdown, impair ovarian tumor growth in mice [59]. Contrasting results have been obtained in immunodeficient mice injected with human SKOV-3 ovarian adenocarcinoma cells transfected with mouse C5a. Tumor cells that overexpress C5a have been found to show a significant reduction in tumor progression [60]. More intriguingly, results obtained in a syngeneic lymphoma model suggest that the impact of C5a on tumor growth is concentration-dependent [60]. Taken together, all these animal models support the idea that complement activation is able to regulate tumor growth. A variety of cancer-related biological processes may be involved in this regulation. In the next sections, data on the potential role of complement in the modulation of chronic inflammation, immunosuppression, angiogenesis, and cancer cell signaling will be reviewed.

##### 4.1. Complement in tumor-associated inflammation

Inflammation is a protective biological process that removes harmful stimuli from the organism. Experimental data support the idea that chronic inflammation and adaptive immune responses are critically involved in tumor immunosurveillance [61]. Appropriately polarized inflammation triggered by the use of *Mycobacterium bovis* impairs bladder cancer progression [62]. Furthermore, infiltration of certain types of leukocytes within the tumor microenvironment is associated with anti-tumor T-cell responses and a good prognosis [63–65].

Nevertheless, the relationship between cancer and inflammation is complex and subject to opposing forces. In particular, tumor-associated inflammation can contribute to multiple hallmark capabilities by supplying bioactive molecules to the tumor microenvironment [66]. Thus, while acute responses are considered part of the defense against neoplastic cells, sustained inflammation in the tumor microenvironment increases the risk of neoplastic transformation and has many tumor-promoting effects [67]. Chronic exposure to irritants, including tobacco smoke, radon, and asbestos, considerably increases the risk of developing lung cancer [68]. Persistent inflammation is characterized by stromal accumulation of certain types of cytokines, chemokines, growth factors, matrix remodeling proteases, and reactive oxygen species [69]. These events critically compromise tissue homeostasis and create a tumor-supportive microenvironment.

The role of complement in inflammation is well known [70]. Complement breakdown reactions lead to the generation of powerful immune effectors, such as the anaphylatoxins C5a and C3a. Anaphylatoxins are potent chemoattractants for eosinophils, monocytes, and T lymphocytes [71, 72]. In addition, the interaction of these molecules with their receptors stimulates the release of granule-based enzymes, cytokines, eicosanoids, and reactive oxygen species, all of which contribute to innate immune functions and/or tissue damage [71–73]. Deregulated complement activity is involved in the pathogenesis of some chronic inflammatory diseases [74, 75].

In the case of cancer, transformed cells are recognized by complement but resist complement attack through the shielding role of complement inhibitors. This controlled tumor-mediated complement activation provides a permanent source of complement effectors that could create an inflammatory microenvironment favorable to cancer growth. Complement-bioactive molecules such as C3-, C4-, and C5-derived fragments, C1q, and MAC are prominent elements of the inflammatory tumor microenvironment [76], but, unfortunately, little is known about their function in tumorigenesis. In addition, C3-deficient mice exhibit an impaired production of the pro-inflammatory cytokine IL-6 after partial liver resection [77]. IL-6 is a multifunctional cytokine that displays a wide range of biologic activities in cancer: it inhibits apoptosis, stimulates angiogenesis, and increases drug resistance in patients with advanced tumors [78]. Activation of C3aR and C5aR increases IL-6 mRNA expression [79].

Complement activity also stimulates the expression of transforming growth factor  $\beta$  (TGF- $\beta$ ) [80, 81]. TGF- $\beta$  displays pro- and anti-tumor effects, depending on the cellular context [82]. In regard to the relationship between complement and other effectors of innate immunity, several studies have demonstrated a synergism between complement and toll-like receptors (TLRs). TLR engagement upregulates nitric oxide synthase-2 and cyclooxygenase-2 and contributes to tumor-associated inflammation [83]. Both C3a and C5a increase the activity of TLR2/6, TLR4, and TLR9 and hence the upregulation of the pro-inflammatory cytokines tumor necrosis factor, IL-6, and IL-1 $\beta$  [84]. Therefore, the role of complement activation in the stromal accumulation of cytokines and growth factors is consistent with its contribution to the inflammatory network in cancer.

Another important feature of tumors is their plasticity in adapting to different physiological conditions. A phenomenon closely related to tumor-associated inflammation is hypoxia [85]. Interestingly, under hypoxic conditions, there is an increase in complement activity because of a downregulation in the expression of factor H and factor I [86], which could lead to the generation of C5a and other proinflammatory molecules. This downregulation is a good example of how tumors engage strategies that not only help them to survive stress but also contribute to cancer progression. We can conclude that tumors are able to trick the anti-tumor effects of inflammation in order to polarize immune responses toward those effectors

that facilitate tumor growth. The biologically relevant interplay between complement and inflammation encourages the design of future studies to address the contribution of complement to the modulation of tumor-associated inflammation.

#### 4.2. Complement in immunoregulation

Tumor-mediated immune responses are different from those observed in other pathological conditions [87]. In the past, researchers considered that the immune responses observed within tumors were all the consequence of an immune attack. However, tumors also acquire the ability to circumvent immune recognition and even take advantage of it. Dense infiltrates of both innate and adaptive immune cells are a common feature of solid tumors and have an impact on the clinical outcome of cancer patients. The presence of M2-polarized tumor associated macrophages, myeloid-derived suppressor cells (MDSCs), type II natural killer T cells, regulatory/tolerogenic dendritic cells, and regulatory T cells (Tregs) is associated with cancer progression, whereas M1-polarized tumor associated macrophages, type I natural killer T cells, and effector T cells contribute to immune-mediated tumor eradication [88–92]. The delicate balance between these opposing cell populations determines the fate of a tumor (i.e., elimination or promotion).

The complement system is critical for the regulation of adaptive T-cell responses [93]. Deficiencies in complement proteins are associated with impaired CD4<sup>+</sup> and CD8<sup>+</sup> T-cell effector responses, suggesting that complement can sustain adaptive immune responses against tumors [94–98]. On the other hand, complement activation can also promote an immunosuppressive tumor microenvironment. C5a is a potent chemoattractant for MDSCs that is able to increase the number of highly suppressive polymorphonuclear MDSCs in the tumor stroma and to facilitate the tumor-immunosuppressive effects of mononuclear MDSCs. This activation of complement enhances the production of reactive oxygen and nitrogen species within tumor microenvironment, which is accompanied by a subsequent decrease in tumor cytotoxic CD8 T-cell responses [36]. In accordance with these functions, the blockade of C5a in mice bearing lung cancer cells significantly diminishes the number of polymorphonuclear MDSCs in the spleen and the intratumoral expression of immunosuppressive mediators [35].

Complement activation also affects the activity of Tregs, an important immunosuppressive population of T cells. Activation of C5a and C3a receptors in Tregs triggers pAKT-dependent phosphorylation of the transcription factor Foxo1, downregulating the levels of Foxp3 and, therefore, the suppressive Treg activity [99]. In addition, blockade of C3aR and C5aR in responder CD4<sup>+</sup> T cells can promote their differentiation towards a Treg phenotype [100]. However, C5a at high concentrations significantly promotes Treg differentiation, which suggests that this factor may regulate Treg differentiation in a concentration dependent manner [60].

Other complement effectors can also contribute to tumor-mediated immunoregulation. Crosslinking of CD46 with C3b or monoclonal antibodies combined with CD3 activation leads to the development of Tregs [101]. The ligation of the complement C3 activation product iC3b to CR3 on antigen-presenting cells results in antigen-specific systemic tolerance [102]. Furthermore, iC3b/CR3 interaction upregulates the expression of TGF- $\beta$ 2 and IL-10, two pivotal contributors of tumor-mediated immunosuppression [102]. Recently, it has also been demonstrated that iC3b promotes the generation of powerful immunosuppressing MDSCs [103]. In conclusion, there is evidence to support the hypothesis that complement modulates the immune response generated by tumor cells. However, only fragmentary results are available concerning the impact of complement activation on the immune tumor microenvironment (Table 1). Of note, the outcome with

respect to tumor growth can depend on the levels of complement effectors in the local tumor microenvironment [60].

#### 4.3. Complement and angiogenesis

Neoplastic tissues need to be able to grow new blood vessels in order to receive adequate oxygen and nutrients. Importantly, the aggressiveness of tumors is directly related to the density of their tumor microvasculature [104], and emerging cancer treatments are based on the suppression of angiogenesis [105]. The role of complement in angiogenesis has been studied in a wide range of diseases and experimental models. However, whether complement is pro- or anti-angiogenic is controversial, and the reported results have depended on the specific pathological features of the disease being studied.

On the one hand, activation of C5aR polarizes macrophages toward an angiogenesisinhibitory phenotype in a murine model of retinopathy of prematurity [106]. This phenotype is characterized by the upregulation of anti-angiogenic soluble vascular endothelial growth factor receptor-1. The secretion of this factor has also been observed in monocytes in an antibody-independent model of spontaneous miscarriage and intrauterine growth restriction [107].

On the other hand, C5a enhances endothelial cell migration and tube-like formation *in vitro* [35, 59, 108]. C5a triggers human umbilical endothelial cell activation through the upregulation of genes involved in endothelial adhesion, migration, and angiogenesis [109, 110]. In addition, C5a and C3a stimulate choroidal neovascularization, a common and severe complication of age-related macular degeneration (AMD) [111]. Also, laserinduced choroidal neovascularization is accompanied by the presence of C3 and MAC deposits and the upregulation of the angiogenic factors TGF- $\beta_2$ , vascular endothelial growth factor, and basic fibroblast growth factor [80]. The importance of complement in choroidal neovascularization is strongly supported by the involvement of the complement regulator factor H in AMD. Factor H is present in ocular tissues, and a polymorphic variation (Y402H) has been shown to be associated with an increased risk for AMD [112, 113].

Few studies have addressed the role of complement in tumor angiogenesis *in vivo*. No effect on tumor angiogenesis was observed after the blockade of C5aR in a murine cervical tumor model [36]. Similarly, in a lung cancer model, there were no differences in tumor vascular density when animals were treated with a C5aR antagonist, although the expression of basic fibroblast growth factor, a potent mitogen and chemotactic factor for endothelial cells, was significantly reduced within the tumors [35]. In contrast, genetic C3 and C5aR deficiencies have been found to be associated with reduced vascularization in a mouse model of ovarian cancer [59]. It is clear that the effect of complement on tumor angiogenesis is under the influence of uncharacterized variables that merit further investigation.

#### 4.4. Complement and cancer cell signaling

Complement participates in several transduction pathways involved in tumor progression. Tumor cells evade lysis by the use of protective mechanisms that limit the formation of functional MAC pores [114]. Non-lytic complement activation leads to the generation of bioactive products that may be involved in pro-oncogenic signaling pathways [115]. Deposition of sublytic doses of MACs induces ion shifts that promote proliferation, differentiation, and resistance to apoptosis [116]. Sublytic MACs activate cancer-associated signaling pathways involving MAPK, phosphatidylinositol 3-kinase, Ras, and p70 S6 kinase [117–119]. In addition, sublytic doses of complement inhibit apoptosis by blocking apoptosis drivers such as FLIP, caspase-8, Bid, and inducing the phosphorylation of Bad [116]. In oligodendrocytes, sublytic complement activation induces RGC-32 [120], a

molecule upregulated in several human malignancies that complexes with CDC2/cyclin B1 to positively regulate cell cycle activation [121, 122].

Other complement components can participate in the activation of relevant signaling pathways. Clq binding to frizzled receptors activates canonical Wnt signaling in a murine model of aging [123], and Wnt signaling pathways regulate various processes that are important in the pathogenesis of cancer [124]. The anaphylatoxins C5a and C3a trigger signaling pathways that mediate survival and antiapoptotic responses in a wide range of cells. They activate MAPK and AKT signaling in leukocytes [125, 126] and protect neurons against cell death [127, 128]. C5a upregulates the expression of the hepatocyte growth factor-c-Met axis in injured liver [129]. The activation of this pathway has mitogenic and anti-apoptotic effects and is deregulated in many tumors [130]. The C5a/C5aR axis also upregulates phospholipase C  $\beta$ 2 [131], phospholipase D [132], and cyclins E and D1 [129]. In addition, C5a enhances the transactivation of epidermal growth factor receptor [109], a therapeutic target in many tumors [133].

## 5. Complement inhibition for cancer treatment

Activation of the complement system by tumor cells was long believed to act only for benefit of the host. In fact, intense research has been devoted to the use of complement to improve the efficacy of anticancer therapies based on monoclonal antibodies [29]. The Fc regions of membrane-bound therapeutic antibodies interact with the heterooligomeric Clq complex and activate the classical pathway. Complement activation leads to formation of MAC and fosters opsonization. In addition, complement can synergize with other antibody-mediated mechanisms of action. Many therapeutic strategies designed to overcome the protection mediated by complement inhibitors or to improve complement-mediated effector responses have been developed and tested experimentally in both *in vitro* and animal models [26, 134–136]. However, recent findings proposing novel complement-mediated roles in tumor progression provide preliminary evidence of the potential utility of a distinct therapeutic option: complement inhibition as an anticancer therapy (Fig. 4).

Despite the ubiquitous presence of complement, relatively few side effects have been reported for complement-directed therapy [137, 138], in contrast to the high toxicity associated with traditional anticancer chemotherapeutics. A wide repertoire of chemical inhibitors targeting complement has been developed and are currently in preclinical or clinical development [138, 139]. In 2007, the US Food and Drug Administration approved the use of the first complement-specific drug eculizumab, an antibody against complement component C5. This approval was a breakthrough in the complement field that validated the complement system as a therapeutic target [139]. Since then, a multifaceted armory of therapeutic inhibitors has been proposed for the diverse array of complement-mediated pathologies [138]. However, when thinking about the best complement-related therapeutic options to treat cancer, many more questions than answers arise.

While the complexity of the complement response offers numerous potential targets, it also poses a major challenge. One of the most critical aspects to be considered is the optimal point of intervention within the complement cascade [140]. Although the effects of complement inhibition are still somewhat speculative, both advantages and disadvantages can be anticipated for the inhibition of complement at different steps of the activation cascade (Table 2). For example, inhibition of the Clq complex by the C1 inhibitor (C1-INH), a protease inhibitor approved by the FDA for the treatment of hereditary angioedema [141], would effectively shut down the activation of the classical pathway while preserving the beneficial functions of the other initiating pathways. Alternatively, a blockade further downstream would eliminate effector activities common to different activation pathways.



C5a has been shown to be a key mediator in the regulation of cancer growth by complement [35, 36, 59, 60]. Selective inhibition of the binding of C5a to its receptors could obstruct the protumoral microenvironment without depleting the defensive potential of complement activation. Drugs that inhibit C5a generation (e.g. eculizumab) or neutralize the C5a-C5aR interaction (e.g. PMX-53) are in different stages of clinical or preclinical development [142]. To date, little attention has been paid to the evaluation of their applicability against cancer. In addition, the involvement of other effector molecules, such as C3a and its receptor C3aR, has not yet been addressed and should not be neglected.

Compstatin (and its analogs) is a cyclic tridecapeptide that prevents the cleavage of C3 into its active fragments C3a and C3b and, therefore, affects the most central step in the complement cascade [143, 144]. Inhibition of complement at this level efficiently blocks all activation, amplification, and effector routes and should be seriously considered for cancer applications. Unfortunately, compstatin does not work on mouse C3 [145], restricting its evaluation in standard preclinical models of cancer. Therefore, for a comprehensive validation of this drug in the cancer field, alternative strategies need to be designed.

Irrespective of the treatment strategy, additional cancer models are needed to further validate the concept of complement inhibition for anticancer therapy. Most current *in vivo* models are based on heterotopic implantation of tumor cells. There is a need for testing orthotopic models, in which tumor cells are implanted into the organ of origin in models that allow a more suitable interaction between tumor cells and the surrounding stroma. Mouse models for spontaneous or chemically induced tumors can also provide more relevant information about the role of complement in the modulation of the tumor microenvironment.

Models of metastasis will also provide very useful information. Furthermore, the use of complement-directed drugs in combinatorial therapies should be considered. For example, given that complement inhibition can override tumor-dependent immunosuppression, this therapeutic approach could be used to supplement antitumor vaccines. It has been hypothesized that the deposition of sublytic doses of MAC induces protection from immune clearance [146]. In this context, therapeutic inhibition of complement may increase the efficacy of cell-based tumor immunotherapies [146].

There are many challenges and limitations that can impede progress in this field. A more detailed understanding of the intricate network established between complement and cancer is crucial to bridging the gap between promising preclinical trials and effective clinical treatments. For example, despite our accumulating knowledge about the role of complement in cancer, the activating molecules that initiate the complement cascade in cancer cells are essentially unknown. Due to the high heterogeneity of human cancer, it is possible that different activation pathways and mechanisms may be involved, and different strategies may be needed to treat different tumor types. The role of complement at particular stages of tumor progression must also be considered. In addition, translational problems between mouse and human systems are expected to be also encountered. It is well-known that bench results do not necessarily translate to bedside efficacy, and truly effective applications will be only demonstrated in the clinical setting (Fig. 5).

## 6. Final remarks

Based on a number of *in vitro* studies and preclinical models, the concept of blocking complement for the treatment of cancer is gaining recognition. However, the mechanisms and functional effects of complement-specific deregulation on the tumor microenvironment are still unclear. More work is needed to elucidate the complex processes by which cancer cells control complement activation and how complement effector activities influence tumor progression. Additional animal models have to be tested to define the best treatment

strategy, and biomarkers should be development to guide the selection of patients and individualize treatment. At present, we can conclude that complement-related anticancer strategies are a promising challenge and offer hope for successfully fighting cancer.

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## Abbreviations

<b>MAC</b>	membrane attack complex
<b>TGF-<math>\beta</math></b>	transforming growth factor $\beta$
<b>TLR</b>	toll-like receptor
<b>MDSC</b>	myeloid-derived suppressor cells
<b>Tregs</b>	regulatory T cell
<b>AMD</b>	age-related macular degeneration
<b>C1-INH</b>	C1 inhibitor

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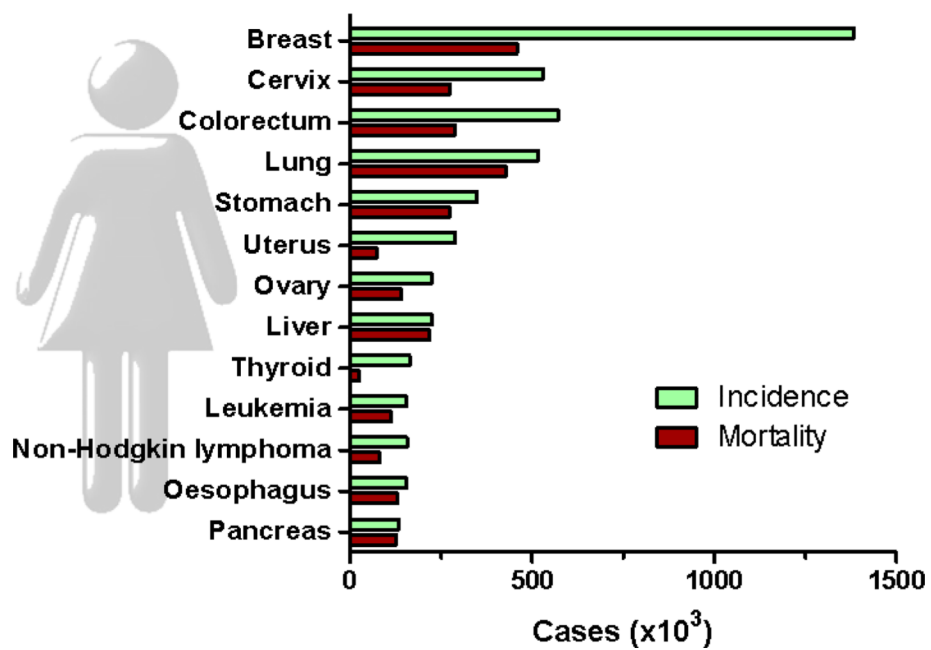
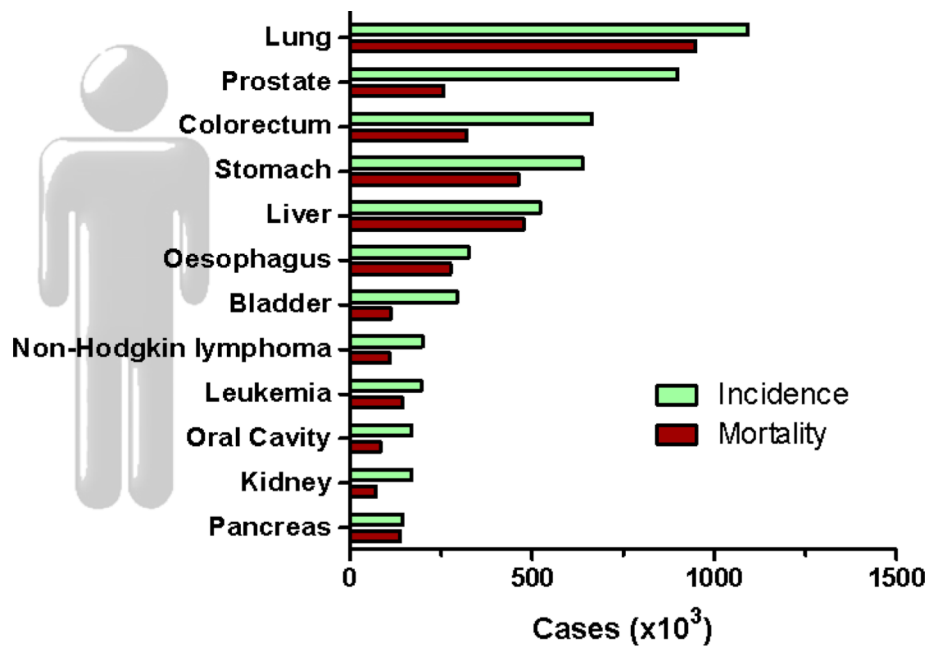
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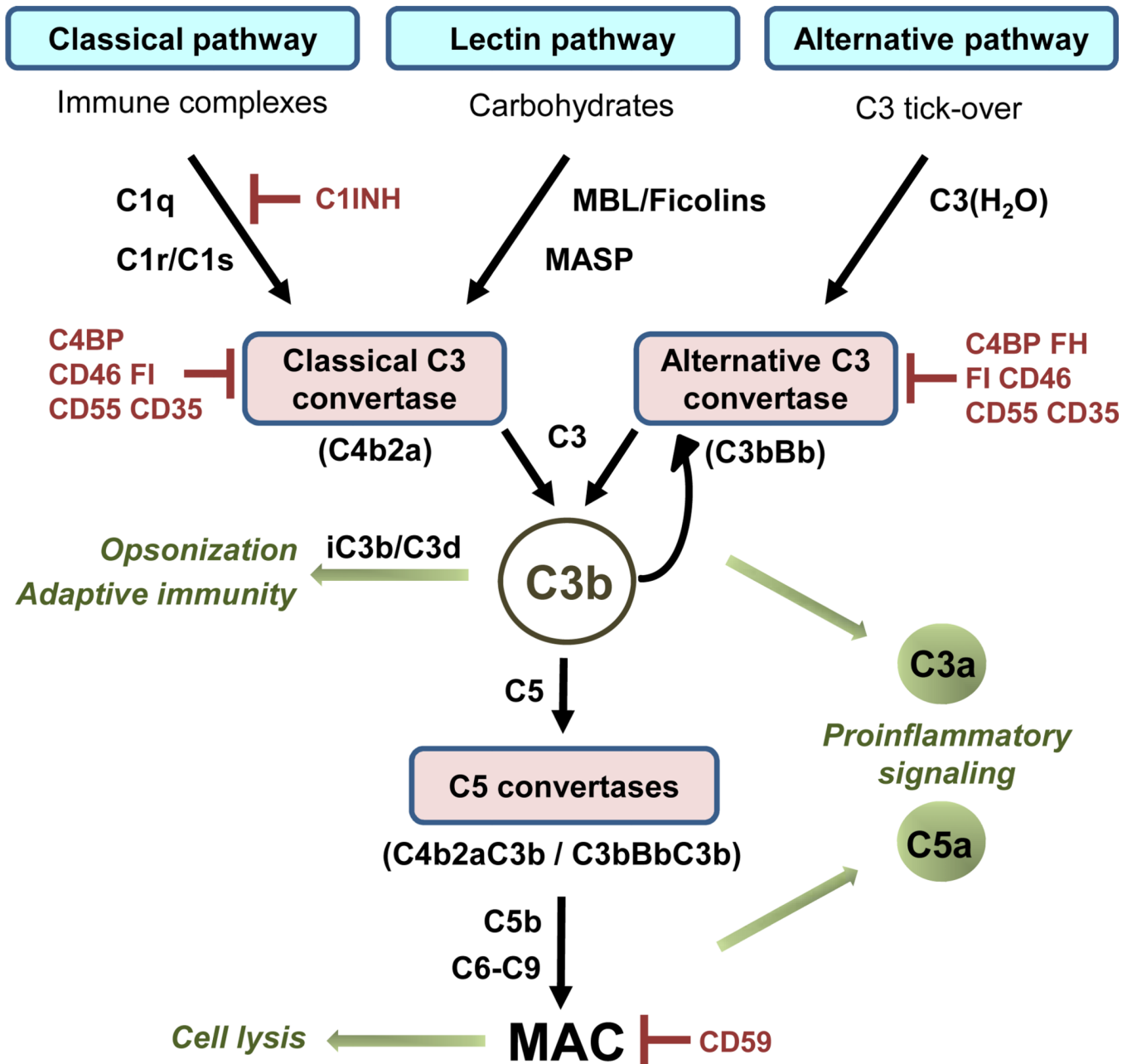
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### Highlights

- Several studies suggest that cancer cells efficiently activate complement
- Cancer cells establish a balance between complement activation and inhibition
- Complement activation exerts tumor-promoting activities
- Complement inhibition may serve as a therapeutic strategy against cancer

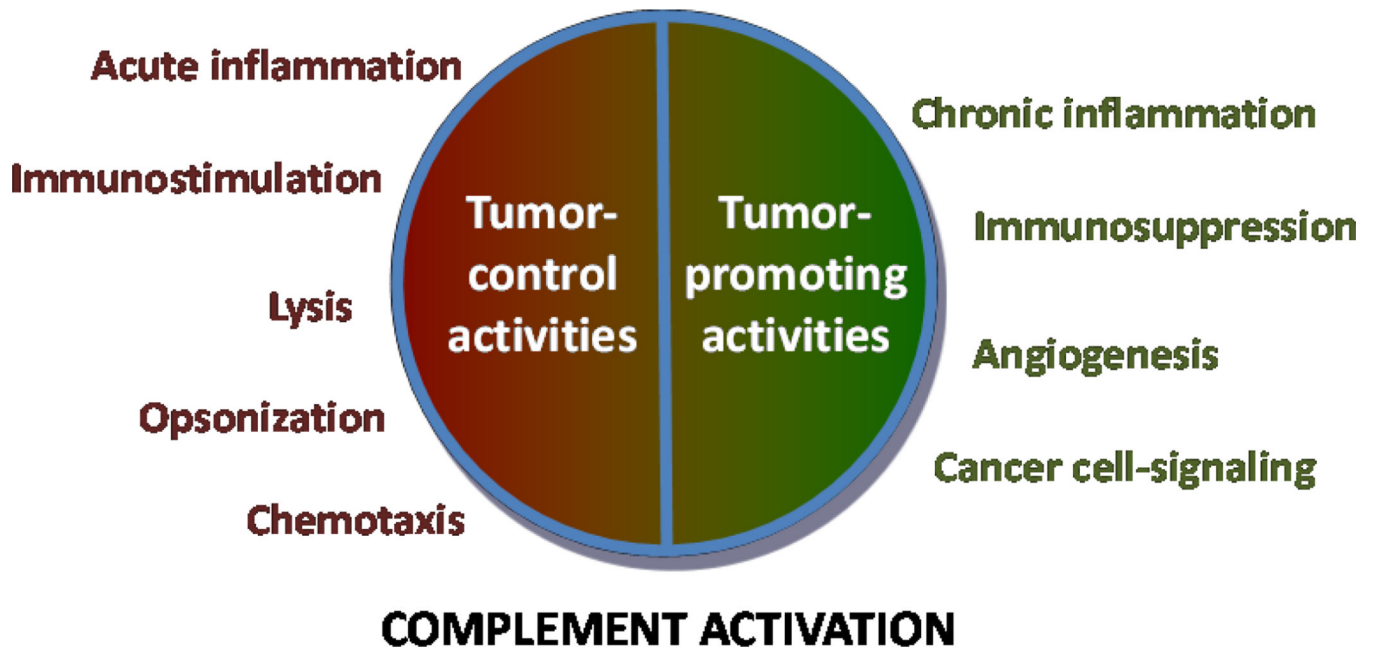


**Fig. 1.** Estimated new cancer cases (incidence) and deaths (mortality) for the leading cancer types worldwide in 2008. Source: Globocan 2008 (<http://globocan.iarc.fr/>)

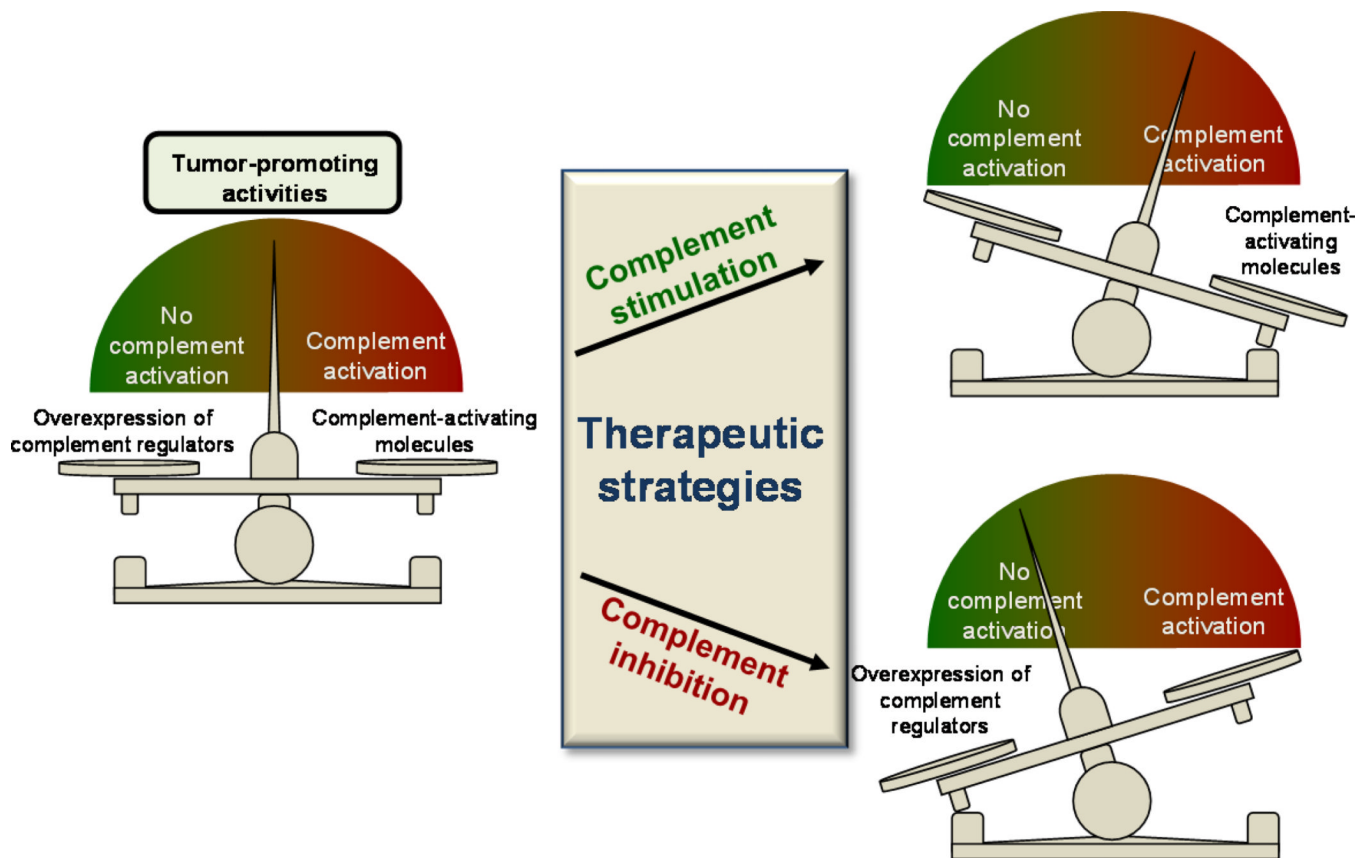


**Fig. 2.** Simplified scheme of the pathways of complement activation. Pattern recognition molecules such as C1q, MBL (mannose-binding lectin), or ficolins bind to surface structures and initiate the formation of the classical C3 convertase. The alternative C3 convertase results from spontaneous hydrolysis of C3 (tick-over). C3 convertases cleave C3 into C3b and C3a. Deposition of C3b leads to the generation of additional C3 convertases (self-amplification) and the C5 convertase, which cleaves C5 into C5a and C5b. In the terminal pathway, interactions between components C5b, C6, C7, C8, and C9 lead to the formation of the lytic membrane attack complex (MAC). The C3b degradation fragments iC3b and C3d participate in phagocytosis and are linked to adaptive immune responses. While the C3b and its degradation fragment iC3b participate in both phagocytosis and in adaptive immune response, the C3dg fragment is only involved in adaptive immune responses. The

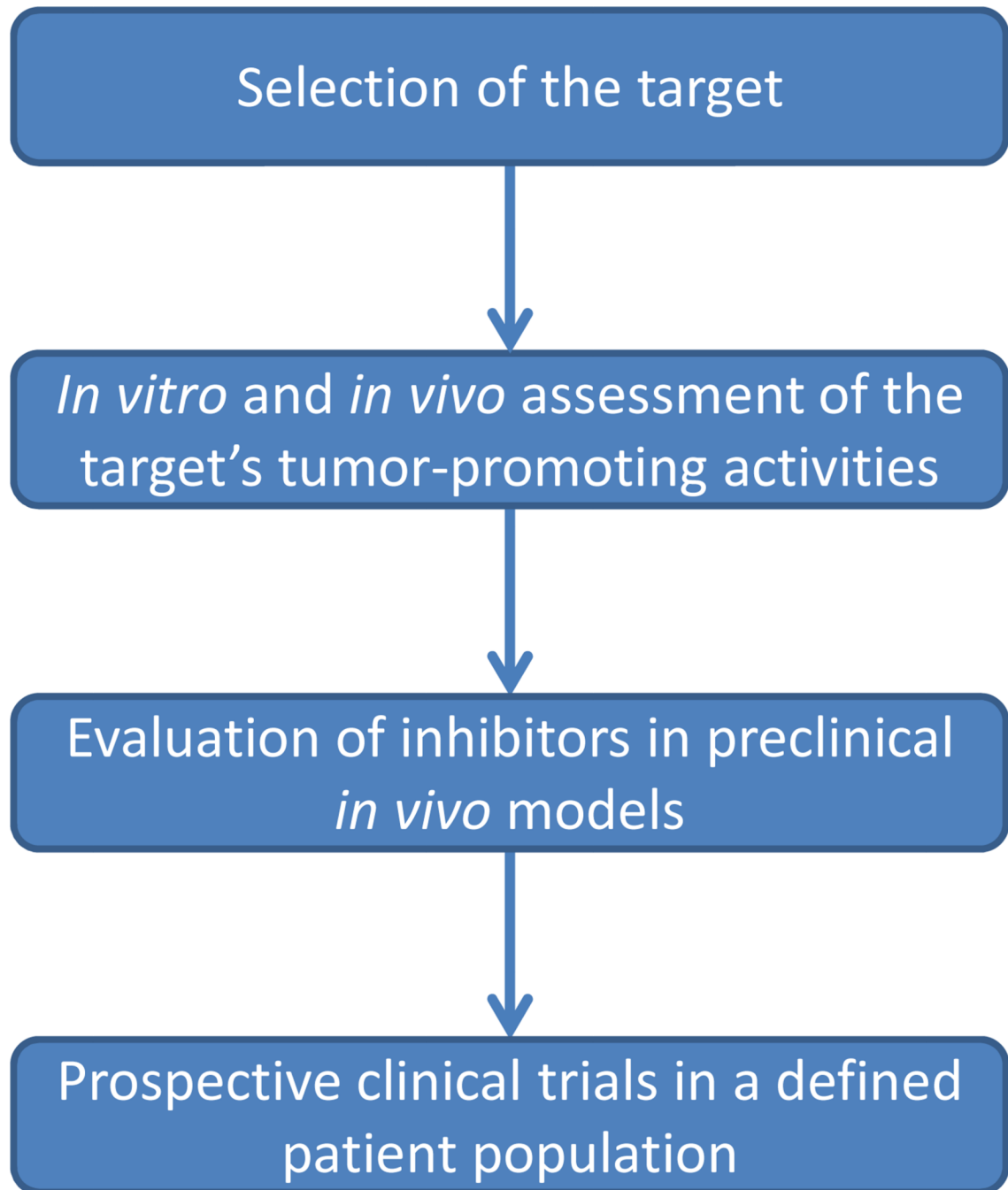
anaphylatoxins C3a and C5a trigger immune reactions upon binding to their receptors (C3aR, C5aR, C5L2). Complement regulators (indicated in red) prevent unwanted complement activation (C1INH: C1 inhibitor; C4BP: C4 binding protein; FI: Factor I; FH: Factor H).



**Fig. 3.** The complement system has a dual action in cancer. Experimental data support the idea that complement is activated by tumors. However, some studies also suggest that malignant cells evade the harmful effects of complement and make use of some complement effector molecules to promote cancer growth. Unfortunately, the exact mechanisms and consequences of this duality are not very well known.



**Fig. 4.** Potentially useful, although antagonistic, complement-based anti-cancer therapeutic strategies. Both activation and inhibition of complement can be proposed to treat cancer. In both cases, the cancer-promoting balance between activation and protection would be destroyed. For example, elimination of complement regulators would lead to an increase in tumor-control activities mediated by complement (e.g., lysis, opsonization, immunostimulation). On the other hand, complement inhibition would eliminate tumor-promoting activities, such as immunosuppression, chronic inflammation, or angiogenesis, which may be hampering other immune effector responses. In this context, combination with immunotherapies or chemotherapies would be advantageous.



**Fig. 5.** Sequential steps needed to validate anti-cancer targeted strategies before they can be translated to the bedside.



**Table 1**

Effects of complement on the immune tumor microenvironment of different murine cancer models<sup>1</sup>.

Tumor type <sup>2</sup>	Cervical cancer (TC-1)			Lung cancer (3LL)		Ovarian cancer (transgenic Tg <sup>1</sup> )			Ovarian cancer (SKOV-3)		Lymphoma (RMA)	
	C3 KO <sup>3</sup>	C5aR KO	C5aR AP	C5aR AP	C5aR AA	C3 KO	C3 Het.	C5aR KO	C5aR transfection (low expression)	C5aR transfection (high expression)	C5aR transfection (low expression)	C5aR transfection (high expression)
Tumor growth	↓	↓	↓	↓	↓ (n.s.)	↓	↓	↓	↓	↓	↓	↓
		↑ (n.s.)							↑ (n.s.)			
Total MDSCs												
MO-MDSC		=										
PMN-MDSCs		↓										
B cells												
Macrophages									t			
CD8 <sup>+</sup> T cells		↑ (n.s.)	↑ (n.a.)				↓					↓
CD4 <sup>+</sup> T cells												↓
NK												=
Tregs							↓					
RNS/ROS		↓										
iNOS												
Arginase-1			↑ (n.s.)									↓
LAG3				↓								
IL-6				↓	↓ (n.s.)							
IL-10				↓	↓ (n.s.)							
CTLA4				↓	=							
PDL1				↓	=							
TGF-β				=	=							
IL-12												
IFN-γ												
TNF-α				=	=							↓
Granzyme B				=	=							=
Perforin				=	=							=

Tumor type <sup>2</sup>	Experimental model	Cervical cancer (TC-1)			Lung cancer (3LL)		Ovarian cancer (transgenic Tg <sup>+</sup> )			Ovarian cancer (SKOV-3)		Lymphoma (RMA)	
		C3 KO <sup>3</sup>	C5aR KO	C5aR AP	C5aR AP	C5aR AA	C3 KO	C3 Het.	C5aR KO	C5aRtransfection (low expression)	C5a transfection (low expression)	C5a transfection (high expression)	
Spleen	Others <sup>4</sup>				=								
	Total MDSCs		↓		↓					↓ (n.s.)	=	↑	
	MO-MDSC				=								
	PMN-MDSCs				↓								
	Macrophages											=	
	CD8 <sup>+</sup> T cells				=						↑	↓	
	CD4 <sup>+</sup> T cells				=						↑	↓	
	NK									↑ (n.s.)		=	
	Tregs				=						=	↑	

<sup>1</sup>Data were obtained from Markiewski et al. [36], Corrales et al. [35], Nunez-Cruz et al.[59] and Gunn et al.[60]. The first three references correspond to models of complement inhibition. In contrast, the last study analyzed the effects of an increased expression of C5a (shaded boxes).

<sup>2</sup>Cell lines inoculated in mice are indicated in parenthesis.

<sup>3</sup>Abbreviations: KO: knockout; AP: antagonist peptide; AA: antagonist antibody; Het.: heterozygous; MDSCs: myeloid-derived suppressor cells; MO: mononuclear; PMN: polymorphonuclear; Tregs: regulatory T cells; NK: natural killers; RNS: reactive nitrogen species; ROS: reactive oxygen species; n.s.: differences did not reach statistical significance; n.a.: statistical differences no available.

<sup>4</sup>IDO, PD1, CCL17, CCL22, GITR, MCP1 and granzyme A.

**Table 2**

Potential points of therapeutic intervention to inhibit complement for cancer treatment.

<b>Intervention point</b>	<b>Drug example</b>	<b>Hypothetical advantages and disadvantages of each intervention point</b>
Pathway-specific Inhibition	Cl-INH	<p><i>Advantages:</i></p> <p>Preserve the beneficial functions of the other initiating pathways</p> <p>Supported by a genetic models of cervical cancer (C4 KO)</p> <hr/> <p><i>Disadvantages:</i></p> <p>More than one pathway may be involved in cancer progression</p>
C3 inhibition	Compstatin	<p><i>Advantages:</i></p> <p>Broadest effect</p> <p>Supported by genetic models of cervical and ovarian cancer (C3 KO)</p>
C5 inhibition	Eculizumab	<p><i>Advantages:</i></p> <p>Intact complement deposition at the C3 level</p> <p>Avoids the sublytic effect of MAC</p> <hr/> <p><i>Disadvantages:</i></p> <p>No tested in <i>in vivo</i> cancer models</p>
C5a/C5aR Inhibition	PMX-53	<p><i>Advantages:</i></p> <p>Experimental data supporting the protumoral activity of C5a</p> <p>Supported by preclinical models of cervical, ovarian, and lung cancer</p> <hr/> <p><i>Disadvantages:</i></p> <p>Only one effector molecule is inhibited, while others may also be important (e.g., C3a)</p>