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Review

The complement system in clinical oncology: Applications, limitations and challenges

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ABSTRACT

The complement system, a key component of innate immunity, is involved in seemingly contradictory aspects of tumor progression and cancer therapy. It can act as an immune effector against cancer and modulate the anti-tumor activity of certain therapeutic antibodies, but it can also contribute to a tumor-promoting microenvironment. Understanding this dual role should lead to the development of better therapeutic tools, strategies for cancer treatment and biomarkers for the clinical management of cancer patients. Here, we review recent advances in the understanding of the role of complement in cancer, focusing on how these findings are being translated into the clinic. We highlight the activity of therapeutic agents that modulate the complement system, as well as combination therapies that integrate complement modulation with existing therapies. We conclude that the role of complement activation in cancer is a rapidly evolving field with the potential to translate findings into new therapeutic strategies and clinically useful biomarkers

1. Background

Understanding the molecular mechanisms underlying tumor progression and the antitumor effects of cancer therapies is essential for the development of more effective treatments. In recent years, evidence has accumulated that the complement system, a master effector of innate immunity, plays an important role in influencing tumor biology. This role is diverse, context-dependent, and relies on the delicate balance between complement activation and inhibition.

Traditionally, activation of the complement system has been associated with effector activity leading to tumor cell destruction. Accordingly, a number of strategies have been proposed to enhance complement-dependent cytotoxicity (CDC) and phagocytosis (CDP) of complement-coated tumor cells. This has been specifically designed to enhance the antitumor activity of therapeutic antibodies, some of which include the complement system as part of their proposed mechanism of action [1]. Conversely, the current view of complement's role in cancer extends beyond the direct elimination of tumor cells. A growing body of evidence highlights the importance of tumor-associated complement activation in tumor progression. Cancer cells are able to modulate complement activity to their advantage without suffering its deleterious effects. This carefully regulated complement activity triggers tumor intracellular signaling pathways in tumor, stromal and immune cells and impacts not only tumor growth but also several crucial steps of the metastatic process [2,3]. Therefore, modulation of complement may be a valuable strategy in the treatment of cancer patients. Direct evidence for this comes from studies showing that modulation of complement activity enhances the capacity of several therapies applied to cancer patients, such as radiotherapy [4,5], chemotherapy [6,7] and immunotherapy [8–10]. These observations have led to the initiation of clinical trials evaluating complement modulation in combination with standard therapies. In addition, it is clear that tumor-associated complement activity affects the levels of complement proteins and/or complement activation fragments in cancer patients. Recent data suggest that complement-related proteins could therefore be used as biomarkers to predict prognosis and improve clinical management of cancer patients in specific clinical contexts [11].

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2. The complement system in cancer

2.1. The complement system

The complement system consists of a network of more than 50 soluble and membrane-bound proteins that interact in a highly coordinated manner to exert its diverse functions. While long recognized for its role in pathogen elimination, removal of immune complexes, and clearance of apoptotic cell debris, recent evidence has demonstrated a broader involvement of the complement system in key homeostatic and effector functions, including inflammation, adaptive immune responses, coagulation, metabolism, tissue regeneration, neural development, bone homeostasis, angiogenesis, and host-microbiota symbiosis [12,13].

Complement activation involves a multistep and sequential proteolytic cascade mediated by a number of serine proteases and active zymogens. Canonical complement activation is initiated by three distinct pathways - classical, lectin and alternative. The classical pathway is triggered by activation of the C1 complex (C1qC1rC1s) when C1q binds to antigen-antibody complexes, damaged cells, extracellular matrix proteins, amyloid deposits, C-reactive protein, pentraxins, prions or DNA, among others [14]. The lectin pathway occurs via binding of proteins homologous to C1q (mannose-binding lectin, collectins or ficolins) to carbohydrate structures or acetylated residues typically found on the surface of pathogens [15]. Finally, the alternative pathway is initiated by the spontaneous hydrolysis of C3 to C3(H_2O) on activating surfaces [16]. These three pathways have distinct activation mechanisms, but ultimately converge through the assembly of a C3 convertase that cleaves C3 into two fragments, C3a and C3b. The C3b fragment binds to the C3 convertase to form the C5 convertase, yielding the active fragments C5a and C5b. C5b binds to the cell surface and triggers the assembly of complement components C6 to C9 to form the cytolytic effector membrane attack complex (MAC) [16]. Non-canonical pathways of complement activation include the cleavage of C3 and C5 by proteases extrinsic to the complement cascade such as cathepsin L, renin, thrombin, coagulation factors XIa, Xa, and IXa, or plasmin [17–19], the C2 bypass pathway [20], and properdin-directed complement activation on microbial surfaces [21]. Recent insights into complement biology have also identified intracellularly active complement - the so-called complosome - which has been reported to influence fundamental physiological processes in the cell, including metabolism, cell proliferation and survival, and autophagy [22–24]

Proteolytic fragments generated during complement activation dramatically affect several effector and regulatory systems. These fragments include the opsonins C3b, iC3b, C3d, C4b, iC4b and C4d, which stimulate phagocytosis, and the anaphylatoxins C3a and C5a, which act as regulators of inflammation [25]. Sublytic MAC induces the activation of signaling pathways that influence cell homeostasis [26]. In addition, complement fragments modulate a variety of processes, including the initiation and regulation of B- and T-cell responses, coagulation, bone metabolism, angiogenesis, nervous system development, and tissue regeneration [12].

Complement activation is tightly regulated at multiple levels to protect host tissues from bystander damage. This is accomplished by soluble and membrane-bound complement regulators. Soluble regulators include factor I (CFI), factor H (CFH), factor H-like 1 (FHL-1), C1 inhibitor (C1-INH), C4b binding protein (C4BP), clusterin (CLU) and vitronectin (Vn), whereas membrane-bound complement regulators (mCRPs) include CD35, CD46, CD55, CD59 and complement receptor immunoglobin (CRIg) [27,28]. Despite this tight regulation, complement activation can be co-opted to exert different functions in a variety of pathophysiological contexts, including cancer.

2.2. Activation of the complement system in cancer

Considerable evidence has accumulated that tumor-associated changes in the composition and/or reorganization of the cell membrane can target tumor cells for complement recognition [29,30]. Consistent with this, *in vitro* experiments showed that tumor cells are more susceptible to recognition by complement than their non-malignant counterparts [29,31]. In cancer patients, elevated levels of complement activation fragments have been reported in a variety of human cancers (Table 1), and complement levels correlate with tumor burden [32]. Nevertheless, individuals with deficiencies in complement activation molecules do not have an increased incidence of tumors [1]. In addition, although complement can be activated on tumor cells, they are resistant to its deleterious effects. This has been attributed to the expression of mCRPs or soluble complement inhibitors. Expression of many of these regulators has been associated with poor survival in cancer patients (Table 1). Blockade of the mCRP CD55 can sensitize breast and prostate cancer cells to CDC [33]. Blockade of the mCRP CD59 has also shown this effect in a variety of tumors [34]. Expression and binding of the soluble complement inhibitors CFH, the major fluid-phase regulator of the alternative pathway of complement, and FHL-1 also protect tumor cells from complement activation and CDC [35–37]. Cancer cells can also control CDC by alternative mechanisms such as expression of proteases, which cleave complement components, elimination of MAC by endocytosis or vesiculation, or generation of sublytic doses of MAC that provide intracellular protection from complement attack [38]. The specific mechanisms by which tumor cells evade complement-mediated cytotoxicity appear to depend on the tumor type. For example, CD55 seems to protect breast and prostate cancer cells from complement-mediated cytotoxicity [33], whereas it appears to be dispensable for the protection of lung cancer cells [36]. Based on these observations, we proposed that tumor cells are able to balance complement activation and inhibition, taking advantage of complement initiation while minimizing its deleterious effects [39]. As discussed in the next section, this "controlled" complement activity in tumors may be a useful biomarker to aid in prognostication and clinical management. A meta-analysis of the expression levels of 50 complement-related genes in 30 human solid tumor types found high expression levels of classical pathway genes (C1QA, C1QB, C1QC, C1R, C1S, C4A and C2) in most tumor types, suggesting that this pathway plays a prominent role in tumor-associated complement activation [11]. Experimental data have confirmed the role of the classical pathway in tumor-associated complement activation. The classical pathway was the main contributor to complement activation in TC-1 engrafted mouse tumors [40]. Similarly, lung cancer cell lines bound C1q and activated the classical pathway in an antibody-independent manner, likely associated with changes in the phospholipid composition of lung cancer cell membranes [29]. *Kras*-mutant CMT167 lung tumor cells implanted in syngeneic mice also activated the classical pathway in the presence of IgM, again suggesting antibody-dependent classical pathway activation [41].

Activation of other canonical complement pathways has been associated with cancer. The expression levels of complement factor B (CFB) and complement factor D (CFD), elements of the alternative complement pathway, were found to be expressed by cancer cells in a heterogeneous and cell type-dependent manner [11]. The potential of the alternative pathway to amplify complement activation and the high expression of C3 observed in tumors suggest that this pathway may also be important for tumor-associated complement activation and tumor progression. Consistent with this idea, impairment of tumor development in C3-deficient mice has been reported in many murine tumor models [9,40,42–45].

Regarding the lectin pathway, the expression of genes encoding proteins of this pathway is low (e.g. MBL2, MASP2, FCN2) or heterogeneous (e.g. MASP1, FCN1, and FCN3) [11]. Nevertheless, certain glioma cell lines bind MBL to activate complement via the lectin pathway [46]. Recently, this pathway has also been implicated in pancreatic and sarcoma oncogenesis [9,47]. There is also evidence of low expression of the terminal pathway genes (C8A, C8B and C9) in most tumors [11]. Sublytic levels of C5b-9 have been found in certain tumor

Table 1
Complement-related biomarkers proposed for the diagnosis of cancer.

Type of biomarker	Biomarker	Biological sample	Tumor type	Utility	Reference
Complement proteins and/or complement activity	Ficolin−2 and ficolin−3	Serum	Ovarian cancer	Increased in cancer	[138]
	MBL and MASP−2 levels and MBL/MASP activity	Serum	Lung and colorectal cancer	Increased in lung cancer. Low levels predict pneumonia in colorectal cancer patients	[140,142]
	C9 levels	Plasma	Gastric cancer	Increased in cancer	[139]
Complement activation fragments	C4d-containing fragments	Plasma, tumor (IHC), bronchoalveolar lavage, sputum	Lung cancer	Increased in cancer. Predicts poor prognosis. Increased lung cancer risk in asymptomatic individuals. Improves the sensitivity of bronchoscopy	[29,130]
	C4d-containing fragments	Saliva, tumor (IHC)	Oropharyngeal squamous cell carcinoma	Increased in cancer. Associated with tumor stage	[133]
	C4d	Plasma, bronchoalveolar lavage	Lung cancer	Increased in cancer. Diagnosis of indeterminate pulmonary nodules	[131]
Complement regulators	Complement factor H	Urine	Bladder cancer	Increased in cancer (BTA-TRAK test)	[135]
	Complement factor H	Bronchoalveolar lavage and sputum	Lung cancer	Increased in cancer. Improves the sensitivity of bronchoscopy	[134]
	C4BP	Serum	Pancreatic cancer	Increased in cancer	[137]
	CD35	DNA	Hepatocellular cancer	Predicts cancer risk and poor prognosis	[151]
	Clusterin	Serum, plasma	Digestive system cancers	Increased in cancer. Predicts poor prognosis, especially in digestive system cancers	[150]
Complement gene genotypes	MBL-specific genotypes	DNA	Glioma, acute lymphoblastic leukemia and breast, ovarian, stomach, liver, gastric and colorectal cancer	Predicts cancer risk in a variety of cancers	[143–149, 169]
Complement-related autoantibodies	Complement factor H	Serum	Lung cancer	Increased in stage I	[136]
Complement-related signatures	C4c combined with CYFRA 21–1 and CRP	Plasma	Lung cancer	Increased in cancer. Increased lung cancer risk in asymptomatic individuals. Diagnosis of indeterminate pulmonary nodules	[132]

cells such as prostate and leukemia [48,49] and may maintain cancer-related transcription factor expression and cell cycle progression, while protecting malignant cells from apoptosis [50]. In addition, tumors can bypass the canonical recognition pathways to activate the complement system in a convertase-independent manner. This occurs through the activity of proteases that cleave C3 and C5. Human melanoma cells secrete the proteinase (pro)cathepsin L, which can cleave C3 [51,52]. During squamous cell carcinogenesis, urokinase $(uPA)^{+}$ macrophages activated plasmin to mediate C3-independent release of C5a [6]. *In vitro* studies showed that cancer cell lines can release C5a from C5 by the action of serine proteases [53,54]. In addition, thrombin, a protease capable of cleaving C5 and generating C5a, has been found to be expressed by several tumors.

Although few studies have reported intracellular complement activation in cancer cells, it is plausible to hypothesize that the intracellular cleavage of C3 and C5 reported in T cells [22] may also be important for cancer cell biology. In support of this, intracellular C3 and C5 have been reported in a variety of tumor cells. Using C5aR1-silenced lung cancer cells, we found that activation of the C5a/C5aR1 axis upregulates CXCL-16 to impair bone colonization in a model of lung cancer bone metastasis. Mechanistic analyses showed that these effects were mediated by both extracellular and intracellular C5a/C5aR1 signaling [55]. C1s and factor H also act in an intracellular, noncanonical manner to promote ccRCC progression by modulating the tumor cell phenotype [23,56]. These data suggest the potential importance of intracellular expression of complement proteins in tumor cell function.

3. Complement activation as an effector for cancer therapy

The complement system plays a dual role in cancer (Fig. 1). On the one hand, complement can attack and destroy tumor cells. On the other hand, complement can promote tumor growth and metastasis by modulating the tumor microenvironment (TME) and supporting cancer cell growth and migration [38]. This duality in the relationship between cancer and the complement system makes the complement system a complex target for cancer therapy. Preclinical and clinical research is exploring both ways to enhance the anti-tumor properties of complement activation, and to inhibit its tumor-promoting effects (Fig. 2).

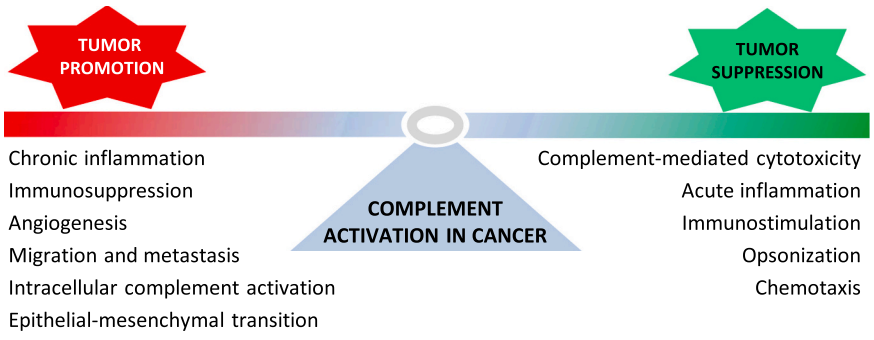

Fig. 1. Dual action of complement activation in cancer.

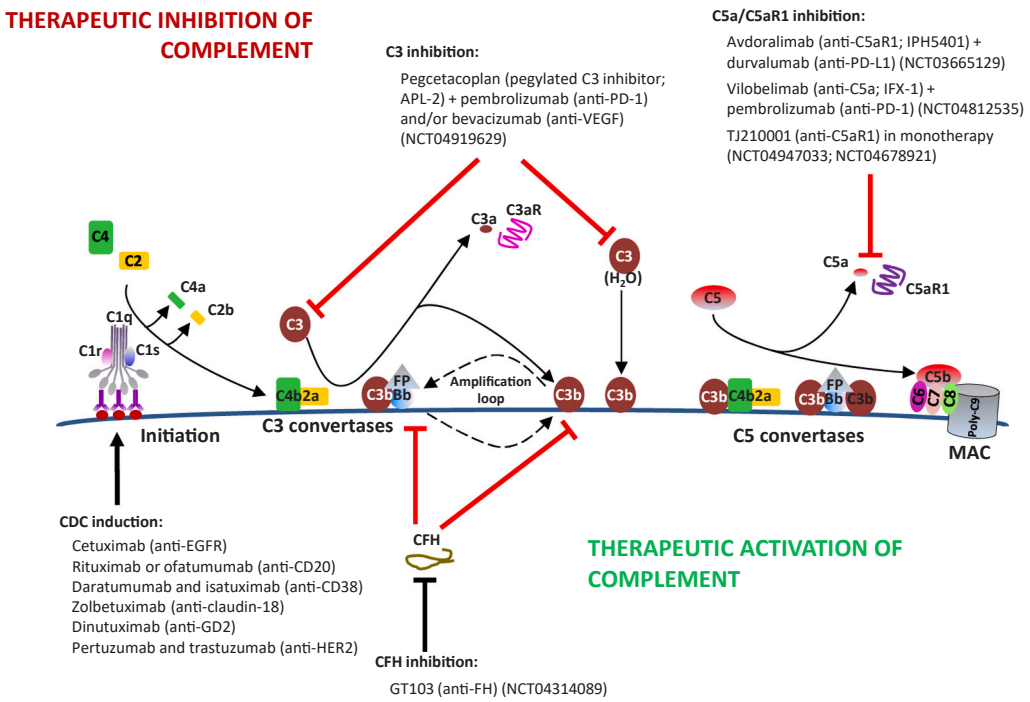

Fig. 2. Schematic of the complement cascade, highlighting the points of therapeutic intervention that have been or are being tested in the clinical setting of oncology.

3.1. Complement activation mediated by therapeutic antibodies

Clinically approved antibodies that activate the classical complement pathway on the surface of tumor cells include those directed against CD20: the type I antibodies rituximab, for the treatment of chronic lymphocytic leukemia (CLL) and non-Hodgkin's lymphoma (NHL), and ofatumumab, for the treatment of CLL; CD38: daratumumab and isatuximab, for the treatment of multiple myeloma; EGFR: cetuximab, for the treatment of head and neck cancer and colorectal cancer; claudin 18: zolbetuximab, for the treatment of gastric cancer); GD2: dinutuximab, for the treatment of neuroblastoma; and HER2: pertuzumab and trastuzumab, for the treatment of breast cancer [57–63]. Activation of the classical complement pathway by these antibodies can mediate CDC, but also interplay with antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and changes in signaling downstream of the target protein. In some cases, complement activation can enhance the activity of other antitumor effectors [12], but in others it can be detrimental [64].

Antibody-mediated complement activation occurs when sufficient Fc molecules bind to the six globular heads of C1q. The effectiveness of this process is highly dependent on the immunoglobulin isotype, post-translational modifications, and Fc-Fc interactions. In humans, the IgM, IgG1 and IgG3 isotypes are known to be the most potent complement activators, whereas IgG2 is a weak activator, and IgG4 fails to bind C1q [65]. All complement-fixing antibodies currently used in the treatment of cancer patients are of the IgG1 isotype, and thus most attention has focused on them, as recently reviewed [66]. Studies have shown that the glycosylation profile of the Fc domain is critical for the interaction of IgG with the complement system. For example, deglycosylation of the Fc domain, either enzymatically or by mutation of the N297 residue, abolishes C1q binding and subsequent complement activity [66]. In contrast, the addition of a terminal galactose to the glycans of the Fc fraction can potentiate the binding of the modified antibody to C1q, thereby enhancing its ability to induce CDC [67,68].

Structural data also show that Fc multimerization, and optimally hexamerization, is required for effective binding of C1q. Here, each Fc associates with a single head group of C1q, resulting in complement activity [69]. The amino acid substitution Glu345 to Arg within the Fc increases the homotypic interactions of cell-bound IgGs to enhance the formation of IgG hexamers that bind C1q and activate CDC more efficiently [69]. According to these findings, engineered anti-CD20 and anti-CD38 hexamers support faster and more robust CDC than their wild-type counterparts [70]. HexaBody-CD38 (GEN3014), a hexamerized human IgG1, has shown improved CDC in hematological cancer cell lines with lower CD38 expression levels compared to daratumumab [71]. A clinical trial is currently evaluating the antitumor activity of this antibody in refractory hematologic malignancies (NCT04824794). Zanidatamab, a bispecific anti-HER2 IgG1 antibody, forms hexamers upon binding to HER2, providing high avidity docking sites for C1q. Interestingly, zanidatamab shows improved functionalities compared to both trastuzumab and the combination of trastuzumab and pertuzumab [72].

Successful antibody-mediated complement activation also depends on the nature and characteristics of the target antigen. Selective targeting of tumor cells requires specific or predominant expression of the selected antigen on the tumor cells. The proximity of the epitope to the target cell membrane, the density of the antigen, the orientation imposed by the epitope positioning and the antibody-mediated movement of the antigen across the membrane are all factors that significantly influence antibody-mediated complement activation [73]. Antibodies targeting epitopes closer to the membrane trigger CDC more efficiently [74]. Antibody-mediated complement activation also correlates positively with antigen density. Higher levels of CD20 or EGFR promote more efficient antibody-mediated complement activation [75,76]. In some cases, the efficacy of complement activation also depends on the mobility of the antibody across the cell membrane mediated by the antigen. Type I antibodies against CD20, such as rituximab and ofatumumab, are characterized by their ability to stabilize CD20 in lipid rafts, leading to increased C1q binding and CDC activation [77]. This may be due to both an increase in the local density of anti-CD20 antibodies, which would facilitate effective C1q binding to antigen-antibody complexes, and the high cholesterol levels found in lipid rafts, which would provide a favorable microenvironment for MAC insertion [78]. Recent observations using cryo-electron microscopy and super-resolution

approaches of the cell surface distribution of CD20 and antibody molecules after binding are likely to provide further insight [79]. Interestingly, ofatumumab binds C1q with much higher avidity than rituximab [80], suggesting a more efficient Fc structural arrangement into hexameric platforms, in addition to its more cell surface proximal binding [81]. As a result, although both therapeutic antibodies have the same hIgG1 isotype and are potent complement activators, the complement activation capacity of ofatumumab is higher [82]. All these data highlight the importance of Fc structure and target epitope location in antibody-mediated complement activation.

Despite the ability of complement-fixing antibodies to activate complement, conclusive evidence regarding the role of complement in their therapeutic activity in cancer patients is lacking. In the case of type I anti-CD20 antibodies, conflicting results have been reported. On the one hand, CLL patients treated with ofatumumab and rituximab had partial B-cell depletion that coincided with reduced complement titers [83,84]. Similarly, polymorphisms in the C1qA gene may influence the clinical response and duration of response to rituximab therapy in follicular lymphoma [85]. On the other hand, expression of mCRPs on tumor cells does not predict clinical outcome after rituximab treatment in follicular lymphoma [86]. Experiments in transgenic mice expressing human CD20 showed that anti-CD20 antibodies engineered to lack complement-activating function were as effective as wild-type anti-CD20 antibodies in depleting B cells [87], suggesting that CDC is not required for the therapeutic activity of anti-CD20 antibodies in this experimental context. To further complicate the matter, NK cell activation and ADCC induced by rituximab-coated target cells was inhibited by C3b, indicating antagonism between potential effector functions [64]. In light of these studies, it is clear that different contexts may require different strategies to fully exploit the ability of antibodies to induce complement activation and elicit anti-tumor effects. There are still many aspects of antibody-dependent effector mechanisms that need to be explored to provide insight for designing the most efficient strategy in each context.

3.2. Enhancing complement activation mediated by therapeutic antibodies

Strategies proposed to improve antibody-mediated CDC, in addition to those described above, include exploiting the hexamerization activity of the IgM tail-piece [88], combination of therapeutic antibodies, inhibition of complement inhibitors, and preservation of complement activity using gain-of-function mutations.

Various combinations of antibodies have been proposed to improve CDC. When combined, anti-CD20 and anti-CD37 antibodies can form mixed hexameric antibody complexes to synergize their binding to C1q and induce superior CDC [89]. Similar CDC-enhancing effects have been observed for the combination of the anti-EGFR antibodies cetuximab and matuzumab in $EGFR^+$ cancer cells [90], the anti-HER2 antibodies trastuzumab and pertuzumab in $HER2^+$ breast cancer cells [63], the anti-folate receptor antibodies cMOV18 and cMOV19 in ovarian cancer cells [91], and the anti-CD20 ofatumumab and anti-CD52 alemtuzumab in CLL [92].

One element that interferes with the ability of complement-fixing antibodies to induce CDC is the presence of inhibitors of complement activation. Accordingly, a number of approaches based on neutralization of mCRPs in combination with therapeutic antibodies have been proposed. Blockade of CD55 and CD59 increased the susceptibility of lymphoma cells to CDC induced by rituximab [75,93] and of lung cancer cells by trastuzumab [94]. Blockade of CD46 and CD59 increased the CDC induced by the mixture of cMOV18 and cMOV19 in ovarian cancer cells [91]. To avoid the undesired effects of mCRP blockade in non-malignant tissues, strategies for selective delivery of mCRP-neutralizing antibodies to tumor cells have been proposed. Bispecific antibodies against CD20 and either CD55 or CD59 selectively targeted tumors and increased tumor cell susceptibility to CDC [95–97]. Similar results were observed with bispecific antibodies targeting CD38 (CD38xCD55) in lymphoma [98], renal cell carcinoma-associated antigen G250 (G250xCD55) in renal cell carcinoma [99], and HLA-I (HLA-IxCD55) and EpCam (EpCamxCD55) in cervical and colorectal cancer [100,101]. Downregulation of CD59 by herbal products such as curcumin and perillyl alcohol can sensitize rituximab-resistant B-lymphoma cells to CDC [102]. Genetic silencing of the mCRPs CD46, CD55 and CD59 using cationic lipoplexes enhanced the CDC activity of trastuzumab and pertuzumab in $HER2^+$ breast, lung and ovarian adenocarcinoma cell lines [103]. Blockade of soluble complement regulators can also enhance the antitumor activity of therapeutic antibodies. For example, genetic silencing of CFH enhanced the *in vivo* antitumor activity of cetuximab [61,104].

Under certain circumstances, complement activation can be saturated or depleted. For example, certain chemotherapeutic agents can reduce complement function in cancer patients [104] and, as discussed above, clearance of CLL cells by ofatumumab and rituximab is associated with complement consumption. *In vitro* experiments show that high doses of type I anti-CD20 antibodies reduce the efficacy of subsequent antibody administration, presumably due to the consumption of complement components [83]. One strategy to minimize complement consumption and maintain the antitumor efficacy of successive antibody infusions would be to reduce the antibody administration to the minimal dose required to induce CDC in tumor cells [83]. This strategy was attempted in the ARCTIC trial, by using low doses of rituximab in previously untreated CLL patients; however, the results were not as hoped and patients were rapidly returned to full-dose treatment [105]. Alternatively, the use of gain-of-function complement mutants capable of forming convertases that are insensitive to degradation by complement inhibitors has also been proposed. At a limited concentration of complement components, the addition of gain-of-function CFB mutants resulted in increased ofatumumab-mediated CDC in ofatumumab-resistant tumor cells [106]. In addition, CFB mutants were able to compensate for the loss of cytotoxic potential of serum collected from non-Hodgkin's lymphoma (NHL) and CLL patients after infusion of rituximab [107]. Gain-of-function C2 variants enhanced the cytocidal activity of rituximab and ofatumumab and reduced the antibody concentration required for efficient tumor cell lysis by NHL patient sera [107]. These variants may also act in concert with other therapeutic antibodies such as obinutuzumab, inotuzumab ozogamicin or daratumumab to overcome cancer cell resistance to CDC [107]. Other strategies that have been proposed to reinvigorate the activity of the complement system include supplementation with fresh complement components [108], the design of therapeutic antibodies that more efficiently to form hexamers and require lower complement titers to induce CDC [70], or the development of modular bispecific single-domain antibodies that simultaneously bind the desired surface antigen and C1 in an Fc-independent manner, thereby inducing potent complement activation [109]. However, as noted above, it remains to be seen whether increasing complement activity will lead to improved clinical responses, given the potential for modulation of other antibody effector functions. As recently shown, Fc modification leading to CDC augmentation, and resulting in strong complement deposition, negatively affected FcγR engagement, which would limit cellular effector mechanisms [110]. Finally, patient immune status, tumor heterogeneity, expression levels of CD20 and complement defense molecules, and dosing strategies are factors that can strongly influence the role of complement in the efficacy of anti-CD20 type I antibodies, as well as other therapeutic antibodies capable of activating complement.

3.3. Non antibody-mediated complement activation to inhibit tumor growth

In addition to the use of therapeutic complement-fixing antibodies, other strategies have been proposed to enhance complement activation in cancer patients. Blockade of CFH increased CDC and impaired non-small cell lung cancer (NSCLC) tumor growth *in vivo* [35,36].

Following these preclinical observations, a Phase 1B study (NCT04314089) is being conducted to evaluate the antitumor activity of a CFH inhibitor (GT103) in 25 heavily pretreated advanced NSCLC patients. Early clinical activity has been demonstrated, with stable disease seen in 24 % of patients. GT103 is a monoclonal antibody isolated from a single peripheral B cell from a patient with NSCLC [111]. This antibody has specificity for an altered conformational epitope of CFH on tumor cells, without binding to native soluble CFH or normal tissues [112]. Preclinical data indicated that GT103 induces CDC and ADCP, increases translocation of the danger-associated molecular pattern molecule calreticulin to the plasma membrane, and inhibits tumor growth *in vivo* [111,112]. This antitumor effect was accompanied by a reduction in regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSCs), and an increase in tumor-specific CD4 and CD8 T cells [113]. Initial reports from the NCT04314089 clinical trial indicate that GT103 is well tolerated, with stable disease observed in 24 % of treated patients [114].

Interestingly, the release of complement effectors during complement activation can potentiate the effects of some cancer therapies. C5a-activated neutrophils mediated the antitumor effect of a treatment based on the combination of tumor necrosis factor, CD40 agonist and a tumor-binding antibody in several tumor types [115]. C5a, and also C3a, plays a critical role in the antitumor activity of radiotherapy [4]; however, as noted below, conflicting results have been reported.

In summary, stimulation of complement activity may serve as a valuable tool in cancer treatment for enhancing the efficacy of therapeutic complement-fixing antibodies, or potentiating the action of other cancer treatments.

4. Complement inhibition as an effector for cancer therapy

4.1. Complement activity during tumor progression

The long-standing view of the complement system as an effector that only contributes to the destruction of tumor cells was challenged many years ago by evidence that complement activation can also promote tumor progression. As far back as 1975, Shearer et al. reported that complement promoted tumor growth when cells were treated with low concentrations of antitumor antibodies [116]. More recently, in 2008, Markiewski et al. found that binding of C5a to its cognate receptor, C5aR1, maintains an immunosuppressive milieu and promotes tumor growth [40], providing the foundation for subsequent studies that have corroborated the importance of tumor-associated complement activation in multiple facets of tumor cell biology. Complement-mediated downstream signaling in tumor cells has been implicated in cell proliferation, inhibition of apoptosis, epithelial-mesenchymal transition, and induction of migration and invasion capacities [38]. Complement activity also influences the stromal composition and immune responses in the TME, thereby promoting tumor growth and metastasis [3,117]. These findings have provided evidence for the potential utility of complement inhibition as an anticancer therapy [118].

Targeting complement components as a single treatment has shown some antitumor activity in a number of murine cancer models, but the general consensus in the field is that combinations with standard anticancer therapies would reveal the true potential of targeting complement. Accordingly, preclinical studies evaluating the ability of complement inhibition to enhance the antitumor efficacy of existing regimens have provided the framework for designing new clinical trials to test this novel therapeutic approach.

4.2. Complement inhibition to enhance the efficacy of cancer immunotherapy

Cancer immunotherapy harnesses the immune system to recognize and eradicate malignant cells, and has prolonged patient survival across multiple cancer types. To date, immune checkpoint blockade, specifically PD-1/PD-L1 blockade, is the most clinically successful immunotherapeutic strategy in cancer. However, the reality is that most patients either do not respond to treatment or develop resistance. Using cancer mouse models, we and others have shown that inhibition of complement-related proteins may provide a window of opportunity to enhance and/or overcome tumor resistance to cancer immunotherapy. In 2017, we demonstrated that inhibition of PD-1/PD-L1 synergizes with inhibition of C5a/C5aR1 in several preclinical models of lung cancer [8]. Similar results have been found in mouse models of other cancers such as melanoma, gastric, ovarian or colon tumors [8,119–121]. A common mechanism of C5a/C5aR1 blockade in all these models is the alleviation of immunosuppression within the TME and the induction of anti-tumor CD8 T cell activation. These data paved the way for the development of clinical trials combining PD-1/PD-L1 blockade with C5a/C5aR1 inhibition. A multicenter Phase I study NCT03665129 evaluated the combination of the anti-C5aR1 antibody avdoralimab (IPH5401) with the anti-PD-L1 antibody durvalumab in 14 patients with advanced hepatocellular carcinoma (HCC), urothelial carcinoma, renal cell carcinoma or NSCLC who had been received at least one line of systemic therapy. The treatment was safe and well tolerated and showed some encouraging responses in patients with lung and liver cancer [122]. In an extension of the study, 46 patients (21 NSCLC patients pretreated with anti-PD-L1, 21 naïve HCC patients and 4 HCC patients pretreated with anti-PD-L1) were enrolled. Minimal antitumor activity was observed, leading to the termination of the study [123]. The objective response rate was 4.8 % (95 % CI: 0.1–23.8) with a median duration of response of 8.3 (6.5–10.1) months in the HCC IO naïve cohort. No responses were observed in the other cohorts. Stable disease was observed in 12 naïve HCC and 13 previously treated NSCLC patients. A Phase 1 study was also conducted to evaluate the safety, tolerability and pharmacokinetics of TJ210001, an anti-C5aR1 monoclonal antibody, in patients with relapsed or refractory advanced solid tumors (NCT04678921). To our knowledge, no results have been reported. A Phase II trial combining the anti-C5a antibody vilobelimab (IFX-1) with the anti-PD-1 antibody pembrolizumab in patients with advanced cutaneous squamous cell carcinoma (NCT04812535) and a Phase I dose escalation trial of TJ210001 in patients with advanced solid tumors (NCT04947033) are ongoing. In addition to C5a and C5aR1, other complement inhibitors are being studied in combination with cancer immunotherapy. Pegcetacoplan (APL-2), a pegylated peptide targeting C3, is being tested in combination with pembrolizumab, an anti-PD-1 antibody, and/or bevacizumab, an anti-VEGF antibody, in recurrent ovarian cancer (NCT04919629). Preclinical studies have also suggested the potential of combining the blockade of C3a and PD-1/PD-L1 [9,42], a strategy that has yet to be translated into clinical trials.

4.3. Complement inhibition to enhance the efficacy of radiotherapy or chemotherapy

Recent studies have shown that targeting the complement system may affect the anti-tumor efficacy of radiotherapy. Most of the reported preclinical studies suggest that complement inhibition enhances the antitumor effects of radiotherapy. CR2-Crry-mediated complement inhibition significantly improved the antitumor efficacy of localized low-dose fractionated radiotherapy (RT) of murine subcutaneous lymphoma. This effect was associated with increased levels of apoptosis and inflammation [124]. In a preclinical mouse model of glioblastoma, radiotherapy combined with C1-INH impaired subcutaneous but not intracranial tumor growth [125], suggesting an important role of the metastatic environment in the efficacy of therapy. Concomitant administration of fractionated radiotherapy and blockade of C5a/C5aR1 enhanced radiosensitivity and antitumor immune responses in lung cancer [126]. C5a/C5aR1 blockade also improved the antitumor efficacy of single or equivalent fractionated radiation doses in colorectal cancer [5]. Mechanistically, C5aR1 targeting resulted in increased NF-κB-dependent apoptosis specifically in tumors and not in normal tissues, rendering cancer cells more susceptible to radiotherapy [5]. In

contrast, one study suggested that a single dose of local irradiation induced rapid and transient complement activation in both melanoma and colorectal tumors that enhanced antitumor CD8 T cell responses [4]. This contrasting result suggests a model dependence of the ability of complement to potentiate or inhibit the effect of radiotherapy.

To date, there is only limited information available on the interplay between complement activation and chemotherapy. Breast cancer patients treated with epirubicin/docetaxel-based neoadjuvant chemotherapy showed a reduction in circulating C3 and C4 levels that did not correlate with levels of the complement activation product C4d [127]. Defects in complement functionality that did not correlate with levels of complement activation markers, in this case C3d, were also observed in a cohort of cancer patients treated with chemotherapy [104]. In mouse models of squamous cell carcinoma, C5a/C5aR1 inhibition improved the antitumor activity of chemotherapy [6], suggesting that chemotherapy-induced complement downregulation may be beneficial for cancer patients.

5. Complement factors as biomarkers in cancer

5.1. Biomarkers of the complement system for the clinical management of cancer patients

Several prospective and retrospective studies have identified complement-related alterations as potential biomarkers, providing pathological information and/or influencing clinical decision making. The use of immunostaining for C4d, a cleavage product of the classical complement pathway, for the clinical diagnosis of antibody-mediated rejection in renal allografts exemplifies the potential of the complement system as a clinical biomarker [128]. In the case of cancer, molecular biomarkers with clinical utility include diagnostic biomarkers used for early detection, diagnosis, or cancer classification; prognostic biomarkers that provide information about the likely course of the disease; predictive biomarkers that predict the likely response to a particular treatment; pharmacodynamic biomarkers that indicate the biological response to a treatment; and monitoring biomarkers that are used to monitor disease status and detect recurrence. All of these biomarkers are critical to personalized medicine, enabling more precise and effective cancer treatment [129].

A number of studies have shown that cancer patients have altered complement profiles. These include alterations in the levels of complement proteins and regulators, the functional capacity and activation state of the various pathways, the levels of complement activation fragments, autoantibodies to complement proteins and the presence of specific genetic polymorphisms in complement proteins. Most of the complement-related biomarkers identified to date reflect altered complement activation in cancer and/or predict prognosis. Therefore, these biomarkers may provide valuable insights into the interplay between complement activity and cancer. For more clinical benefits, some of these biomarkers may help clinicians diagnose cancer at an early stage or select the best treatment option. In the next sections, we will review those complement-related biomarkers whose use has been proposed in specific real-world clinical oncology settings, such as diagnosis or prediction of response.

5.2. Diagnostic biomarkers

Several complement-related biomarkers have been proposed for the diagnosis of cancer (Table 1). Our group found that the measurement of C4 degradation products may be useful in the clinical management of lung cancer in three clinically relevant contexts: assessing lung cancer risk, aiding bronchoscopic diagnosis, and determining lung nodule malignancy. Thus, the detection of C4d-containing fragments in plasma may be useful in selecting high-risk individuals for inclusion in a computed tomography (CT) lung cancer screening program [29]. This marker has also been found to be elevated in bronchoalveolar lavage and sputum supernatants from patients with lung cancer, significantly increasing the sensitivity of conventional diagnostic methods such as bronchoscopy [130]. The specific determination of C4d may also be used to assess the malignancy of indeterminate lung nodules [131]. We developed a multivariable diagnostic model based on the quantification of complement-derived fragment C4c, cytokeratin fragment 21–1 (CYFRA 21–1) and C-reactive protein (CRP) in plasma. The model was able to discriminate between benign and malignant lung nodules with high specificity. In addition, the scores derived from the model were associated with a significantly higher risk of lung cancer in asymptomatic individuals enrolled in a CT screening program [132]. C4 activation fragments were also elevated in biological samples from oropharyngeal squamous cell carcinoma, although further analysis in clinically relevant cohorts is needed to assess their clinical utility [133].

A number of other complement-related biomarkers have been proposed for early diagnosis, differentiation of cancer from non-malignant conditions and prediction of disease progression. CFH is elevated in bronchoalveolar lavage and sputum from lung cancer patients and may be used as an adjunct to cytology in the diagnosis of malignant lung disease [134]. The use of the BTA stat and BTA TRAK tests, which detect human CFH and complement factor H-related proteins, has been proposed for the diagnosis of bladder cancer. These tests showed higher sensitivity than urine cytology for detecting bladder cancer, but lower specificity and a higher false-positive rate when tested in benign conditions [135]. The presence of autoantibodies to CFH in sera has been associated with early stage lung cancer [136]. The determination of serum C4BPA may be useful in the detection of early stage pancreatic ductal adenocarcinoma (PDAC) and in the differentiation of PDAC from pancreatitis and other gastroenterological cancers [137]. Circulating C9, ficolin-2/3 and MASP-2 levels can be used to differentiate cancer from benign disease in gastric, ovarian or lung cancer [138–140]. The diagnostic sensitivity of a panel consisting of serum β-2-glycoprotein 1, α-1-acid glycoprotein 2, complement C3 and α-fetoprotein (AFP) surpassed the diagnostic value of AFP, a well-known serum biomarker for hepatocellular carcinoma (HCC) [141]. Low preoperative MBL levels were predictive of pneumonia, which was associated with poorer survival in colorectal cancer patients [142]. Finally, MBL-specific genotypes have been shown to predict the risk of developing a variety of cancers [143–149]. The same clinical application has been proposed for certain CD35 polymorphisms and for circulating clusterin levels in digestive cancers [150,151]. Using humoral complementomics, an unbiased approach designed to study the global state of the complement system in pathological plasma samples across various disease contexts, elevated plasma levels of C4d and Bb at the time of surgery were found to correlate with poor prognosis, while autoantibodies against C3 and reduced FH correlated with a favorable outcome in renal cancer patients [152].

5.3. Predictive biomarkers

Predictive biomarkers are indicators of the likelihood of response to a particular therapy, allowing clinicians to identify which patients are more likely to benefit from certain treatments, thereby improving outcomes and minimizing unnecessary side effects. This is particularly relevant in the context of cancer immunotherapy. U.S. Food and Drug Administration (FDA)-approved biomarkers for the treatment with immune checkpoint inhibitors (ICI) include programmed cell death ligand 1 (PD-L1) expression, microsatellite status (i.e., microsatellite instability-high (MSI-H)), and tumor mutational burden (TMB) [153]. However, although useful, their utility is far from optimal as these biomarkers do not consistently predict response, underscoring the need for more accurate and reliable predictive tools. In this context, the use of complement-related proteins implicated in the therapeutic activity of ICIs may potentially improve the currently available predictive tools. To date, there is limited data on the potential clinical use of complement-related markers as predictive markers for ICIs, although

some promising results are emerging. High circulating C1q levels and tumor immunostaining for complement activation-derived products predicted response to immune ICIs in lung cancer patients [154,155]. In renal cancer patients, high levels of circulating C5, C5b-9 and complement factor I predicted a poor response to ICIs [156]. The opposite was observed for circulating CFH and complement factor D [156] These and other complement-related biomarkers associated with response to ICI are listed in Table 2.

Complement-related factors may also be associated with response to other therapeutic modalities. High levels of circulating C3 activation-derived fragments and tumor immunostaining of C3 activation-derived fragments and CD55 predicted response to chemotherapy in breast cancer patients [127,157]. Something similar was observed for circulating C4d-containing fragments in mesothelioma patients [158]. In esophageal cancer patients, circulating C3a and C4a and tumor immunostaining of CD59 were associated with response to radiotherapy or radiochemotherapy [159,160]. Circulating levels of C3 and C4 and tumor immunostaining for gC1qR predicted poor response to chemotherapy in ovarian cancer patients [161]. In contrast, in another study, circulating levels of C3 were down-regulated in chemoresistant ovarian cancers [162]. Finally, tumor immunostaining of C5aR1 predicted response to sorafenib or sunitinib in patients with renal cancer [163], and certain C1qA polymorphisms were associated with response to rituximab in patients with follicular lymphoma [85]. Table 2 details the biomarkers that have been specifically proposed to predict response to antitumor therapies.

6. Limitations and challenges

As noted in the previous sections, there are several limitations and challenges to the clinical implementation of complement-based therapies and biomarkers in oncology that warrant consideration. Efforts to overcome these challenges would undoubtedly expand the potential for harnessing the complement system in the clinical management of cancer patients.

A major limitation is the context-dependent role of the complement system in cancer [11], where it can have both pro- and anti-tumorigenic effects depending on numerous factors including tumor type, tumor stage, and elements present in the TME. For example, as we have discussed above, a molecule such as C5a can promote tumor progression but, in other contexts, can also contribute to anti-tumor immunity. This duality complicates the design of therapies that selectively harness the beneficial effects of complement while avoiding its deleterious effects. A deeper understanding of the role of the complement system in the specific contexts of cancer pathogenesis will help to identify which components or pathways are more relevant in each tumor type and individual patient.

Another limitation is the complexity of the complement system, which involves multiple activation pathways, each with numerous components and feedback loops. In this complex scenario, effective inhibition of complement at one point may inadvertently activate compensatory mechanisms or lead to unexpected outcomes in the TME. This is further complicated by the heterogeneity of complement activation on the surface of cancer cells. While we know that some tumor types initiate complement activation primarily through the classical pathway [29,40,41,164], others do so through the lectin pathway [9,46,47]. Evidence is also accumulating about the role of intracellular complement activation in cancer cell biology [56,164]. In addition, we still do not know what the particular targets and specific mechanisms of activation are in each case. All of this makes it challenging to determine the optimal point of intervention within the complement cascade in each tumor type. It also limits the design of therapies that combine complement modulation with other treatment modalities to maximize the efficacy of complement-targeted therapy.

Table 2
Complement-related biomarkers proposed for the prediction of response to cancer treatments.

Type of biomarker	Biomarker	Biological sample	Tumor type	Utility	Reference
Complement proteins and/or complement activity	C1q	Serum	Lung cancer	High levels predict good response to ICI	[155]
	C3 and C4	Plasma	Ovarian cancer	High levels of C4 and low levels of C3 predict poor response to chemotherapy	[162]
	C5	Plasma and tumor RNA	Renal cancer, melanoma and glioblastoma	High levels predict poor response to ICI in melanoma and good response in renal cancer and glioblastoma	[156,170]
	C5b−9	Plasma	Renal cancer	High levels predict poor response to ICI	[156]
Complement activation fragments	C3-derived fragments	Plasma	Breast cancer	High levels predict poor response to epirubicin/docetaxel chemotherapy	[127]
	C3-derived fragments	Tumor (IHC)	Breast cancer	High levels predict poor response to neo-adjuvant chemotherapy	[157]
	C3a	Serum	Esophageal cancer	High levels predict poor response to neoadjuvant chemoradiation	[159]
	C4d-containing fragments	Plasma	Mesothelioma	High levels predict resistance to platinum-based chemotherapy	[158]
	C4a	Serum	Esophageal cancer	High levels predict poor response to neoadjuvant chemoradiation	[159]
	C3- and C4-derived fragments and C5b−9	Tumor (IHC)	Lung cancer	High levels predict good response to ICI	[10]
Complement receptors	gC1qR	Tumor (IHC)	Ovarian Cancer	Resistance to cisplatin-based chemotherapy	[161]
	C5aR1	Tumor (IHC)	Renal cancer	High levels predict poor response to sorafenib or sunitinib	[163]
Complement gene genotypes	C1QA genotype	DNA	Follicular lymphoma	Predicts response to rituximab	[85]
Complement regulators	Complement factor H and complement factor D	Plasma	Renal cancer	High levels predict good response to ICI	[156]
	Complement factor I	Plasma	Renal cancer	High levels predict poor response to ICI	[156]
	CD55	Tumor (IHC)	Breast cancer	High levels predict poor response to neo-adjuvant chemotherapy	[157]
	CD59	Tumor (IHC)	Squamous esophageal carcinoma	High levels predict poor response to radiotherapy	[160]
Complement-related biomarker signatures	A protein signature associated with acute phase reactant, complement, and wound healing pathways	Serum	Melanoma	High levels predict poor response to ICI	[171]

The incorporation of complement-related predictive biomarkers to tailor complement-targeted therapies to individual patients will be also instrumental in bringing these therapies to the clinic. However, the limited knowledge about the mechanisms of complement activation and the specific function of individual complement mediators within the TME challenge our ability to select the best treatment option for each individual patient. In this sense, initiatives such as the development of the Humoral Complementomics platform, to assess a patient's complement status, are of great importance [152,165]. Such a platform would greatly assist in the design of clinical trials based on patient stratification.

In term of safety, targeting the complement system may lead to immune dysregulation and secondary complications [166]. Safety information from approved anti-complement drugs or those that have successfully completed human safety evaluations may be helpful in assessing this issue. Agents such as C5 inhibitors or C3 inhibitors may impair host defenses against infection, as seen in patients treated for non-oncologic conditions, underscoring the need for vigilant infection risk management. In oncology, where patients may already be immunocompromised, such risks may require closer attention. Clinical trials targeting complement in oncology remain sparse, with only a few studies available to evaluate this point. The Phase 1 trial of IPH5401, a C5a receptor antagonist, in combination with durvalumab, an anti-PD-L1 antibody, suggested good tolerability but some adverse event (AEs) to considered [123]. The most common AEs in this study were asthenia (50 %), cough (24 %) and pruritus (24 %). Sixty-seven percent of the patients had AEs considered to be related to C5a blockade, the most frequent of which were diarrhea, fatigue and pruritus (11 % each). Nine percent of patients discontinued treatment due to AEs.

The complexity of complement-targeting drug development is also a challenge [167,168]. The design of complement-targeting drugs must target the pathological complement activation without compromising immune homeostasis. Complement-fixing therapeutic antibodies should effectively balance complement activation with other effector mechanisms. For drugs that switch off complement activity, specific targeting applied in a well-defined time window appears to be a low-risk approach that could provide maximal therapeutic benefit [167]. Complement inhibitors are biologics associated with a high cost, which may limit accessibility, particularly in resource-constrained settings. To address these issues, innovative designs are needed to optimize safety and efficacy while ensuring affordability and scalability of complement-based therapies.

Finally, despite an exciting amount of high-quality discovery and validation work, none of the complement-related biomarkers proposed in the literature has yet entered oncology practice. To date, many of the identified complement-related biomarkers merely reflect altered complement activation in cancer or predict prognosis. The development of complement-related biomarkers for the clinical management of cancer patients should be based on their intended use in clinically relevant contexts. The implementation of standardized methods for the accurate measurement of complement-related analytes in the context of clinical oncology also remains a major challenge.

7. Concluding remarks

The role of complement activation in cancer is an exciting and rapidly evolving field with the potential to translate findings into new therapeutic strategies and clinically useful biomarkers. Complement has been extensively demonstrated to play a dual role in cancer progression and therapy. On the one hand, complement activation can promote cancer cell cytotoxicity and mediate the antitumor effect of certain antibody-dependent therapies. While this approach is promising, further studies are needed to define in detail the relevance of complement activation for the efficacy of therapeutic antibodies, as well as how it may interfere with other effector functions. On the other hand, in circumstances where pathological complement activation contributes to a tumor-promoting environment, complement inhibition may be a valuable therapeutic approach. In such scenarios, inhibition of complement activity may impede tumor progression and enhance the efficacy of other anticancer therapies. Based on this premise, a new generation of clinical trials targeting the complement system have been initiated. However, although promising, modulation of the complement system in cancer remains an emerging field and the number of trials in this area is still limited. The challenge now is to elucidate the impact of complement activation in specific contexts of cancer progression and treatment. This would facilitate the optimization of current treatments and the development of a new generation of reagents that precisely balance complement activation with other effector mechanisms. In parallel with the development of new anti-cancer strategies targeting complement, a number of complement-related biomarkers have been identified in cancer patients. However, despite the potential of the identified candidates, they have not been implemented in the clinic. The development of well-controlled studies to validate the clinical utility of the identified biomarkers in the specific contexts of intended use is essential to ensure their applicability in clinical practice.

CRediT authorship contribution statement

Mark S Cragg: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Conceptualization. **Daniel Ajona:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Funding acquisition, Conceptualization. **Ruben Pio:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Funding acquisition, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used DeepL Write in order to polish the language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of Competing Interest

RP and DA are inventors of two patents licensed to Amadix related to the use of complement C4 fragments as diagnostic and prognostic cancer biomarkers. RP has received consultant fees and research funding from Amadix. MSC is a retained consultant for BioInvent International and has performed educational and advisory roles for Baxalta and Boehringer Ingleheim. He has consulted for GSK, Radiant, iTeos Therapeutics, Surrozen, Hanall, Argenx and Mestag and received research funding from BioInvent, Surrozen, GSK, UCB and iTeos.

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