A Comprehensive Review on the Complement System in Cancer: From Accelerating Tumor Development to Therapeutic Strategies

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Cancer immunotherapy has traditionally focused on boosting existing immune pathways to enhance antitumor responses. However, the role of the complement system in cancer development and treatment has been largely overlooked. The complement system is an immune related system composed of a variety of proteins, mostly found within the blood. The complement system can be activated by pathogens to create inflammation and signal immune cells towards the site of infection. However, complement-mediated inflammation has been implicated in accelerating tumor development. Herein we discuss the specific effects of complement activation on tumorigenesis and how tumors can manipulate the complement system into creating an immunosuppressive microenvironment. We also discuss existing complement inhibiting drugs, therapeutics that are currently in development, and 2 clinical applications of complement inhibition in cancer therapy. However, the complement system's exact interactions with both immune pathways and tumors are currently unknown and complement activation can simultaneously aid and hinder tumor development. Further research is needed to determine the nature of these interactions. Furthermore, inhibiting the entire complement system can result in life threatening infections. More research is required to further refine complement inhibiting therapeutics to specifically target the harmful molecules.

Keywords: Cancer immunotherapy, complement system, anaphylatoxins, complement inhibition, immunoediting, tumorigenesis, tumor microenvironment, immune cell polarization

Introduction

Cancer is currently the second leading cause of death globally, and is expected to rise to first by 2060[1] despite advances in cancer therapies. The increasing prevalence of cancer necessitates enhanced treatment methods. Immunotherapy has emerged as a promising approach to treating cancer by bolstering existing immune pathways to provoke an anti-tumor response. While immunotherapy has traditionally focused on targeting specific immune molecules/checkpoints, targeting complement system in immunotherapy has not been adequately explored.

The complement system was first discovered in 1891. It was originally thought to be a single protein that could 'complement' the antibacterial activities of antibodies. The complement system is now understood to be a complex system made up of a variety of inactive plasma proteins that can react with each other to aid in the immune response against pathogens[2].

The human complement system serves a vital role in both the innate and adaptive response systems, and its effects are widespread throughout the body. Complement proteins have the ability to directly attack pathogens themselves, while simultaneously summoning stronger immune cells towards the site of infection. The complement system is made of 3 branches, all of which have similar effects/end results: opsonization, inflammation, chemoattraction[2]. Each pathway is defined by a distinct trigger, followed by an enzyme cascade. It is possible for multiple pathways to be triggered simultaneously, but it is currently unclear whether this helps or hinders immune response[3]. While complement proteins can help the body in fighting tumors, they often have the opposite effect. Excessive or inappropriate complement activation has been proven to have detrimental impacts for cancer progression[4]. Proinflammatory cytokines released through complement activation have several pro-tumor effects, including the polarization of immune cells and the promotion of angiogenesis and metastasis. Furthermore, tumor cells have the ability to manipulate complement activation to create a more pro-tumor inflammatory microenvironment. While complement-based cancer therapies have been utilized to some extent, the realm remains largely unexplored. There is a significant lack of clinical trial data and literature regarding the role of complement activation in cancer immunotherapy or complement system modification for therapies. This paper aims to provide a comprehensive review of role of complement activation in tumor development and cancer immunotherapies to highlight the potential of complement utilization in future therapies.

This paper will discuss the 3 complement activation pathways, complement activation's effect on the tumor microenvironment

(TME), current cancer immunotherapy approaches in relation to the complement system, complement evasion mechanisms by tumors, and clinical uses of complement inhibition in cancer treatment.

Complement Pathways

Classical Pathway

The classical pathway is activated by specific antibodies binding to the surface of foreign substances found within the body. Antibodies IgG and IgM bind to pathogens' membranes. They then recruit additional proteins C1s, C1r, and C1q to form the C1 complex. After the C1 complex forms, it recruits additional complement proteins C4 and C2 to form a larger complex named C3 convertase. At this point, the classic pathway converges with the other pathways, as both the alternative and lectin pathways also lead to the formation of C3 convertase. The full cascade is displayed in Figure 1.

C3 is a ubiquitous complement protein that naturally breaks down into C3a and C3b at a somewhat slow rate. C3 convertase has the ability to cleave C3 into more C3a and C3b, which increases the breakdown rate of C3. C3 convertase formation creates a positive feedback loop that accelerates the production of C3 convertase. The newly formed convertase complexes all bind to pathogens and help with opsonization, which is the process of flagging a pathogen for consumption by a phagocyte[2].

Alternative Pathway

The alternative pathway was named the alternative pathway because it was discovered second and was considered to be the 'alternative' to the classical pathway[5]. In contrast to the lectin and classical pathways, the alternative pathway is not dependent on pathogen-specific proteins for initiation, and only requires the spontaneous cleavage of C3[5], which gives it an advantage. The alternative pathway is activated by a molecule called C3b, which is formed by the breaking down of complement protein C3. C3b binds to carbohydrates, proteins, or lipids on the membranes of foreign organisms. After the initial binding, additional molecules bind to C3b, named factors B and D. This leads to the formation of C3 convertase. Plasma properdin is another key component that stabilizes the complex by extending its lifespan and preventing premature decay. The alternative pathway then converges with the classical and lectin pathways[2].

Lectin Pathway

The lectin pathway is very similar to the classical pathway, and only differs in initiation. Due to this similarity, the lectin pathway was the last to be discovered in 1987. The lectin pathway is especially prominent during early childhood, indicating its importance in innate immunity before the adaptive immune system is properly developed[5]. The lectin pathway is activated by proteins called lectins (a recognition molecule). Specific serine proteases named MASP 1, 2, and 3 bind to the original recognition lectin to create a MASP complex. MASPS are molecules that can cleave proteins. The newly formed MASP complex cleaves complement protein C2 into its subcomponents C2a and C2b and cleaves C4 into its subcomponent C4a. C2 and C4 are both common complement proteins that can be found in the blood throughout the body, and both play an important role in activating C3 proteins by helping to form C3 convertase. After this, a variation of C3 convertase is formed that is different in conformation from the alternative pathway, but the same as the classic pathway (C4bC2aC3b vs C3bBbC3b). The lectin pathway then converges with the other pathways[2].

Membrane Attack Complex

The membrane attack complex (MAC) is the final step of all 3 pathways. It is an assembly of proteins that forms on the membranes of pathogens and creates a hole. In sufficient quantities, the MAC is able to lyse pathogens[6]. In order for MAC formation to occur, additional C3 molecules bind to C3 convertase to convert it into a bigger complex named C5 convertase. C5 convertase can create additional C3b and C3a by cleaving C3, as well as creating protein C5a, which has a similar function to C3a and is made by cleaving complement protein C5. All of these molecules have important functions. C3b is an opsonin and can summon phagocytes and help mark which cells must be consumed and destroyed. C3a and C5a are anaphylatoxins and chemoattractants. They trigger inflammation, which helps to bring more immune cells to the area of infection[2]. Following the formation of the C5 convertase complex, C6, C7, C8, and C9 proteins can bind and create the membrane attack complex (MAC depicted in Figure 2), which creates a hole in the pathogen membrane and can cause cell lysis in sufficient quantities[6].

Complement System and Tumor Immunology

The tumor microenvironment (TME) is crucial for cancer development, and the complement system plays a critical role in TME development and maintenance. The complement system has several tumor-promoting effects within the TME, including promoting the dysfunction of neutrophils, macrophages, MDSCs, and inflammatory T cells, as well as directly aiding in tumor growth through the stimulation of angiogenesis and metastasis[7]. The complement system's role in the TME can directly aid in tumor growth progression. Certain key complement proteins, such as C3 and C5, greatly contribute to the development of the TME during formation, and the stability of the TME after formation. Blockading these proteins shows potential to be a future option

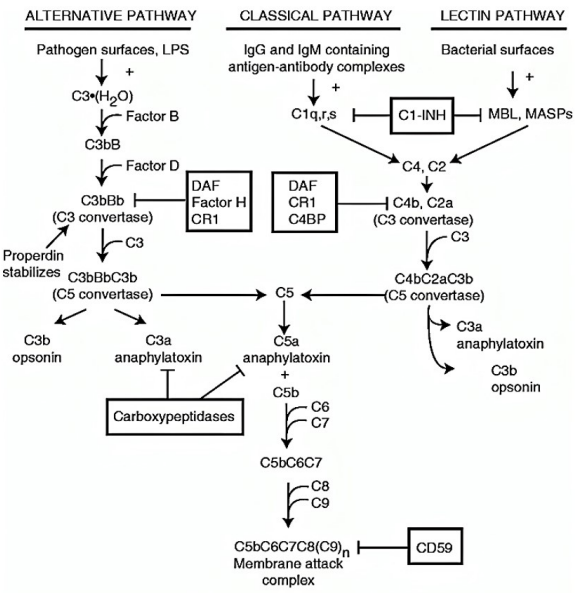

Fig. 1 A schematic overview of the 3 complement system pathways[2]. This figure displays the different molecules associated with each pathway and the cascade mechanism that occurs. The initiating triggers and points of divergence are also shown.

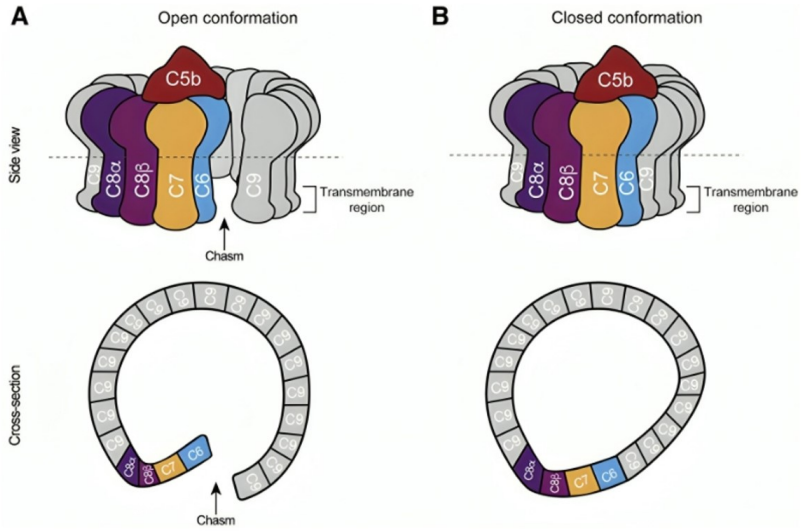

Fig. 2 The membrane attack complex has 2 different conformations[6]. The closed conformation typically is the pre-pore complex stage, while the open conformation is the pore-forming complex and can damage pathogens.

for cancer treatment[8]. Therefore, understanding the connection between the TME and the complement system is crucial because it allows the possibility of finding new cancer treatment options by targeting these key complement components.

Tumor-Associated Macrophages

Macrophages are an important component of the innate immune response and are part of the initial response to pathogens. They can act as phagocytes to destroy pathogens, as well as signaling other immune cells towards the site of infection, especially T cells[9]. Complement proteins aid in the recruitment/polarization of tumor-associated macrophages (TAMs), which can have a

variety of tumor-promoting effects. C1q, which is a complement protein that helps form the C1 complex in the classical pathway, can cause macrophage polarization. The binding of C1q changes the original phenotype and gene expression of a macrophage and alters its original function [10]. These polarized macrophages have increased levels of PD-L1 and PD-L2, which are programmed death-ligands and serve as immune checkpoint molecules, which have been proven to aid in tumor growth and promote disease progression [11]. The increased amount of immune checkpoint molecules suppresses the creation of inflammatory T cells and favors the production of regulatory T cells [7]. Inflammatory T cells are responsible for actively fighting pathogens, such as cancer, while regulatory T cells (Tregs) are responsible for suppressing immune responses. The switch from inflammatory T cells to Tregs promotes the creation of immunosuppressive niches and helps tumor development.

Tumor-Associated Neutrophils

Neutrophils are another key immune cell within the innate response. Neutrophils make up 50-70% of immune cells in the bloodstream, and are dormant until they encounter a pathogen [12]. They can be converted into tumor-associated neutrophils (TANs) by complement protein C5a, which primarily serves as an anaphylatoxin. C5a stimulates endothelial and epithelial cells to release a chemokine called LTB4, which attracts neutrophils to the region [13]. C5a then causes neutrophil dysfunction and polarization by binding to C5a receptors (C5aR). Polarized TANs exhibit a pro-tumor phenotype known as N2-type TANs, which actively help tumors, rather than fight against them like anti-tumor N1 neutrophils. C5a also has the ability to promote the production of tissue factor (TF) in peripheral blood neutrophils, which bolsters tumor growth and metastasis [14].

Myeloid-derived Suppressor Cells

Myeloid cells are a family of cells found within the bloodstream that originate in bone marrow tissue. Under normal conditions, they can protect tissue from damage caused by extensive inflammation due to an uncontrolled immune response [15]. However, MDSCs can be recruited by cancer using complement proteins to protect cancer cells from the immune system and aid in immune cell polarization. C5a can attract MDSCs to tumors, which creates a favorable TME for cancer progression. C5a does this by inducing the production of arginase-1 (Arg-1), IL-10, TGF-beta 1, cytotoxic T lymphocyte antigen 4, and PDL-1, which serve as immunoregulatory molecules [15]. These molecules trigger the differentiation of MDSCs and favor cancer progression by inhibiting T cells. MDSCs can suppress T cell function by inhibiting their antigen-specific responses. MDSCs can also promote the formation of regulatory T cells (Tregs) and TAMs, which protect cancer cells from the immune system and immunotherapy [16]. It has been proven that when the C5a protein was inhibited, Treg production decreased [17].

Angiogenesis

Although largely implicated in immune related processes, the complement system may additionally have a role in facilitating angiogenesis. Angiogenesis is an important step in tumor growth, and tumors often secrete angiogenic factors to promote the growth of vascular networks. Inhibition of C3 or C5aR has been proven to alter endothelial cells, and changes the expression of vascular endothelial growth factor, or VEGF [18]. VEGF is released by TAMs and MDSCs that have been polarized by C3a or C5a. C1q has also been shown to be very effective in promoting angiogenesis in endothelial cells [19]. However, there is contradictory evidence regarding the complement system and angiogenesis. Blockading C5aR has been shown to impair T cells' ability to enter tumors and control progression. Furthermore, local complement activation has the ability to disrupt the tumor endothelial barrier, which attracts T cells to the site and stimulates an immune response [20]. More research is required to accurately determine the role of the complement system in angiogenesis.

Metastasis

During the later stages of cancer development, tumors can spread from their original location into other locations in a process called metastasis. Imbalanced complement and inflammation can trigger metastasis by boosting cancer cell mobility, altering the TME, degrading the extracellular matrix (ECM), and breaking tissue barriers. C3a promotes metastasis by suppressing T cell responses and preventing immune intervention, as well as promoting Treg proliferation [17]. C5a also promotes metastasis by increasing expression of MMP-1 and MMP-9, which help to degrade the ECM and make it easier for cancer cells to migrate [21]. Complement protein C1q can also independently promote metastasis, even if the complement system has not been activated [22].

Proinflammatory Cytokines

Cytokines are small proteins that help to regulate immune processes. When secreted under normal conditions by non-polarized immune cells, they aid in T-cell priming and activation, and play a role in anti-cancer immunity. However, proinflammatory cytokines are often secreted by polarized immune cells such as TAMs, MDSCs, and CAFs (cancer-associated fibroblasts). Examples of secreted cytokines include tumor necrosis factor alpha (TNF-alpha), transforming growth factor beta (TGF-beta) interleukin-1 beta (IL-1beta), IL-23, and IL-6, all of which aid in the production of Th17 cells. Th17 cells are a form of helper T cell that are responsible for restricting immune responses and

are prevalent in several autoimmune disorders[23]. Cytokines secreted in the TME by polarized cells are responsible for several pro-tumor processes, including epithelial-mesenchymal transition (EMT) and enhanced growth and invasion abilities[24]. EMT is a process in which cells detach themselves from their localized environment, and this process is a precursor to metastatic disease. While the behavior of cytokines can be variable, they often have proinflammatory effects, which play a significant role in furthering tumor growth and immune cell polarization. Cytokines are released by polarized immune cells, which are converted by complement activation, implicating the complement system in protumor cytokine activity. Furthermore, proinflammatory cytokines can also be released by tumor cells in response to the formation of MAC complexes on the cell membrane, further implicating complement activity in the release of proinflammatory cytokines.

Current Cancer Immunotherapy Approaches

The current main goal of cancer immunotherapy is to strengthen the immune system's natural antitumor responses. Cancer immunotherapy methods attempt to enhance immune response efficiency, and include using cancer vaccines, oncolytic viruses, adoptive T-cell therapy, and chimeric antigen receptor T-cell therapy. The complement system has varying effects on these methods.

Cancer Vaccines

Cancer vaccines attempt to improve tumor antigen presentation to enhance the immune response. There are 2 main types of cancer vaccines. Specific peptide antigen vaccines reintroduce tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs) to be presented by antigen-presenting cells (APCs), such as dendritic cells or macrophages. Vaccine antigens help to stimulate T cells, which can increase antitumor responses[25]. TSA/TAA vaccines are also administered with an adjuvant immune stimulant. The other main type of cancer vaccine is whole tumor cell vaccines, which inject whole tumor cells to be used as antigens rather than specific proteins. This allows dendritic cells to process and present multiple antigens, which can elicit a stronger polyclonal T cell response. Multiple subsets of T cells can each respond to individual tumor-related antigens[26]. However, cancer vaccines are often limited to patients with very specific HLA haplotypes/immune cell genetic makeups, and do not work on all patients. Many tumors can also mutate very fast and create new antigens, which necessitates personalized vaccines to target these specific mutant neoantigens[27]. Complement proteins play important roles in coordinating the adaptive immune response and are being considered as adjuvants to be administered alongside cancer vaccines. C5a has the ability to improve the antigen processing and presentation ability of dendritic cells[28]. C3d is another possible adjuvant for cancer treatment and can improve adaptive immune responses and tumor control[29].

Oncolytic Viruses

Oncolytic virus therapy is a new class of cancer therapy and utilizes viruses to directly kill cancer cells. They can also induce a stronger immune response by causing the release of damage-associated molecular patterns (DAMPs) such as calreticulin, high-mobility group protein B1, and TAAs[30]. These TAAs can be used to induce the adaptive immune response. However, oncolytic viruses (OVs) are often destroyed by immune responses, limiting their efficacy[31] Therefore, using OVs in combination with treatments that restrict the expression of genes that block antiviral defense mechanisms has potential to be a more effective alternative[27]. Complement activation usually results in virus destruction upon detection, which necessitates a mechanism to evade complement-mediated destruction. Complement inhibiting proteins can be used to prevent complement activation against OVs. Similarly, viruses can be given altered coats to avoid complement activation. For example, Adenovirus uses a polyethylene glycol shield to reduce complement activation, and expresses CD59 to prevent MAC formation[32,33]. CD59 is a membrane-bound complement inhibitor that prevents the MAC from forming, which helps to keep the virus protein coat intact and prevent destruction.

T-cell Therapy

Adoptive T cell therapy (ACT) is a treatment method that artificially increases the number of T cells, which can lead to enhanced immune efficiency and tumor elimination. Existing Tregs and competing mechanisms are first eliminated to improve treatment effectiveness[34]. Then T cells that respond to tumor antigens are enriched to have more potency and introduced into the patient. Using genetic engineering, T-cell receptors (TCRs) with specificity for TAAs can be expressed to increase T cell efficiency. However, T cell recognition of TAAs is dependent on major histocompatibility complex (MHC) molecules, and tumors can downregulate their expression of MHC molecules[35]. Furthermore, the diversity of peptides makes it difficult for standardized production of T cells for generalized treatment. This makes it necessary to either individualize production of TCRs or make many subsets of T cells that recognize many different TAAs.

Chimeric antigen receptors (CARs) were designed to overcome the dependence on MHC molecules. They are made up of 2 parts: the exodomain and the cytoplasmic domain (CAR anatomy displayed in Figure 3). The exodomain can recognize specific antigens found on the surface of cancer cells and can be modified to target different antigens. The cytoplasmic domain

receives the signal from the exodomain and initiates a series of signaling events that help to activate the T cell and initiate its response[27]. CARs can include costimulatory signals to strengthen T cells, and can be transfected into many other types of effectors cells[36]. Unlike regular TCRs, CARs can recognize antigens independently of MHC. Limitations to CARs include scarcity of TAAs, an inability for CAR-T cells to reach the TME, and an immunosuppressive TME[37].

Complement activation has beneficial effects for T cell activation and responses and can have positive effects on both CAR-T and ACT therapy. In order for T cells to be able to respond correctly to pathogens/foreign molecules, they require antigens to be presented to them by APCs. APCs, such as macrophages and dendritic cells (DCs) express many complement receptors and regulators and are very receptive to complement signals. For example, C3aR and C5aR expression on DCs can stimulate production of cyclic adenosine monophosphate (cAMP), extracellular signal-regulated kinases (ERKs), and NF-kB[39–41]. These drive production of IL-12, IL-23, IL-6, and TGF-beta, all of which enhance Th1, Th2, and Th17 T cell responses[42]. Anaphylatoxin receptors can also influence toll-like receptor (TLR) activity on APCs. TLRs are important for recognizing antigens and initiating an immune response[43]. Furthermore, C1q is a complement protein that can interact with DCs to increase expression of IL-12p70, which can promote Th1 T cell responses. C1q also increases expression of Cluster of Differentiation 83 (CD83), Cluster of differentiation 86 (CD86), human leukocyte antigen-DR (HLA-DR), and C-C chemokine receptor type 7 (CCR7) on cell surfaces. All of these molecules can boost immune responses. CD83 helps immune cells coordinate and communicate with each other by marking mature DCs. CD86 is a costimulatory molecule that can aid in T cell activation. HLA-DR is an MHC molecule that augments antigen presentation to T cells. CCR7 is a receptor protein on the surfaces of T cells and DCs that can guide them towards specific locations[44]. Therefore, while complement activation effects on T cells are context-dependent, T cells can benefit in many ways from complement activation.

Prevalent Immunotherapy Alternatives

Chemotherapy

Other cancer therapy approaches attempt to directly target tumor cells rather than utilizing the immune system. Chemotherapy is commonly used during cancer treatments and utilizes drugs that specifically target cells that divide very quickly, such as cancer cells. Chemotherapeutic agents can alter the TME and make tumors more likely to trigger immune responses. Chemotherapy is an effective method to treat many types of cancer, but its efficacy is often limited by complement activation. For example, the 5 year survival rate for pancreatic cancer is abysmally low (10%) mainly due to chemoresistance to gemcitabine, which is the main chemotherapeutic drug used to treat pancreatic cancer[45]. Chemoresistance to gemcitabine can arise in 4 main pathways: the nuclear factor kappa B (NF-kB) pathway, the c-mesenchymal-epithelial transition factor (C-MET) pathway, or the signal transducer and activator of transcription 3 (STAT3) pathway. NF-kB is a transcription factor that can cause angiogenesis, tumorigenesis, and chemoresistance when introduced into the nucleus[46]. C3a and C5a, which are produced by the complement system, stimulate production of TNF-alpha and IL-1 beta, both of which lead to the activation of NF-kB[45]. Once NF-kB enters the nucleus, it stimulates the release of CXCL14, which promotes angiogenesis and tumor growth[47]. NF-kB also inhibits human concentrative nucleoside transporter 1 (hCNT1), which is important for allowing gemcitabine to enter cells. hCNT1 inhibition blocks gemcitabine from entering cells and contributes to chemoresistance[48]. TNF-alpha and IL-1 beta also have the ability to activate STAT3, which is a gene that upregulates VEGF and FGF expression, both of which stimulate angiogenesis. STAT3 can also upregulate Cyclin D1 and Cyclin B1, which are proteins that inhibit apoptosis[45]. Once activated STAT3 affects ATP-binding cassette (ABC) membrane transporters, which prevents gemcitabine from being taken into the cell[49]. In the C-MET pathway, hepatocyte growth factor (HGF) binds to membrane-bound receptor tyrosine kinases (RTKs), which are coded by the C-MET gene. This interaction causes VEGF expression, which encourages angiogenesis, and downregulates apoptosis-inducing factors[50]. C-MET is expressed much more in cancerous tissue than healthy tissue, and aberrant C-MET activation is the result of anaphylatoxins released by complement activation. Therefore, although chemotherapy is a widely used treatment strategy to treat cancer, its efficacy is often limited by complement activation.

Radiotherapy

Radiation therapy is another common cancer treatment method that is used in over 50% of cancer patients and will only increase in prevalence in the future. Targeted radiation is projected onto tumors, which can directly kill tumor cells while also initiating both the innate and adaptive immune responses[51]. When tumor cells are exposed to radiation, their DNA sustains damage, which can lead to apoptosis, mitotic catastrophe, or senescence[52]. Tumor antigens are exposed from tumor cell death, which allows adaptive immune cells to respond appropriately. Radiation causes the release of DAMPs, which activates the innate immune response. Radiotherapy also results in complement activation, and can trigger the classical and alternative pathways[51]. Unlike other treatment methods, complement activation pairs well with radiotherapy, and improves anti-tumor responses. Complement activation leads to the increased expression of C3a/C3aR and C5a/C5aR on dendritic cells, which

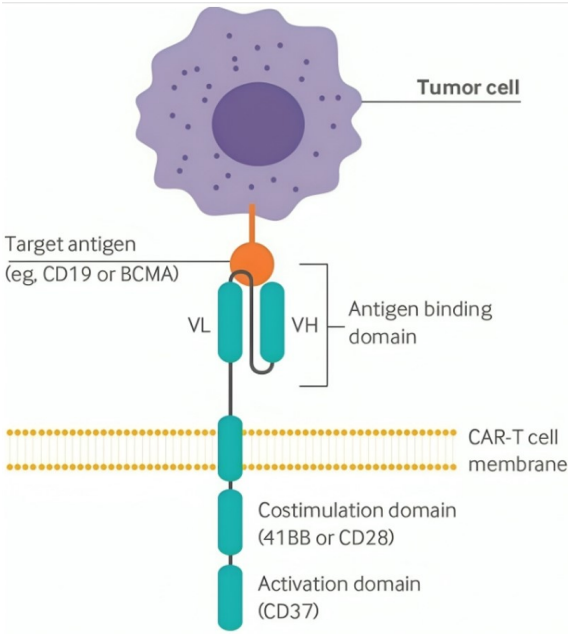

Fig. 3 The above diagram of a chimeric antigen receptor displays the relevant structures[38]. The antigen binding domain binds to pathogens, such as tumor cells, and signals to the intracellular costimulation and activation domains, triggering an immune response.

allows for enhanced dendritic cell activation. Dendritic cells are an important part of the adaptive immune system and help to activate T cells. Complement activation paired with radiotherapy also helps to restrict the proliferation of Tregs. Without C3a or C5a present, radiotherapy is much less effective and can lead to a worse prognosis[53]. Although complement activation has negative implications for many other forms of cancer therapy, it is necessary for radiotherapy's success.

Complement and Immune Evasion by Tumors

Tumors often express complement-inhibiting proteins to protect themselves from complement-mediated damage. There are two main types of evasion mechanisms: basal and induced. Basal mechanisms include membrane-bound complement regulatory proteins (mCRPs), soluble complement regulatory proteins (sCRPs), chondroitin sulfate proteoglycans, ecto-proteases, and ecto-protein kinases (ecto-PKs). Induced mechanisms are expressed when there is a sub lytic quantity of MACs on tumor cell membranes, which leads to higher complement resistance. Expression of these mechanisms is often a result of selection pressure placed upon tumor cell populations upon primary recognition by complement components, and mechanisms that are expressed are a result of the immunoediting theory (explained further in section 7).

Basal Evasion Mechanisms of Complement Activation

All cells express some degree of mCRPs, but cancer cells tend to have significantly elevated levels. This increased expression is due to the immunoediting process. Immunoediting is a mechanism in which immune responses favor the development of certain traits in tumors that can aid in tumorigenesis (discussed further in section 7). Neoplastic cells with stronger mCRP expression are favored by an evolutionary process within the TME, causing the entire population to exhibit higher levels of mCRPs[54]. Common mCRPs include CD35, CD46, CD55, and CD59. CD35, CD46, and CD55 interfere with C3 convertase generation earlier on in the complement cascade, while CD59 interferes with MAC formation much later in the complement cascade. CD35 can accelerate the decay of C3b and C4b, both of which are crucial components of the complement cascade[55]. CD46 can bind to C3b to prevent formation of C3 convertase by degrading C3b, similarly to CD35[56]. CD55 can also prevent C3 convertase function, and can also destroy existing C3/C5 convertases[57]. CD59 is another mCRP that can prevent cytolysis by binding to C8 and C9 and preventing C9 polymerization, which is an essential step in MAC formation[58]. Higher expression of these mCRPs can lead to higher rates of cancer differentiation and progression and cause higher levels of complement resistance. mCRP expression can further be enhanced by the activation of protein kinase C (PKC), which is activated by phorbol myristate acetate (PMA).

sCRPs have a similar form and function to mCRPs but are

found in body fluids rather than bound to cell membranes. One common sCRP is C1 inhibitor. C1 inhibitor blocks C1 function, which is important for initiating the classical pathway. Factor I (fI) and Factor H (fH) are also common sCRPs that are both expressed on tumor cells[55]. fI can cleave C3b and C4b, both of which are important for the continuation of the complement cascade. Clusterin and S-protein are also sCRPs that are heavily upregulated in response to cell injury and are associated with attempts for cell survival following complement-mediated damage. They compete with the C5b-7 complex for the same binding sites and prevent its binding, which regulates the later portion of the complement cascade and prevents MAC formation[59]. sCRPs can also be formed when mCRPs are released and do not find a secondary location to bind to. Furthermore, sCD46, which is the soluble version of CD46, can augment fI-mediated cleavage[60].

However, a clarification is required for the role of CRPs (including mCRPs and sCRPs) in cancer progression. Complement activation has been heavily associated with increased tumor invasiveness. Therefore, it would be plausible to assume that tumors suppressing complement activity with CRPs would be helpful rather than harmful and would be beneficial for patient prognosis. To clarify this, it would be better to consider CRP overexpression as a biomarker for tumor progression. Normal levels of expression of CRPs is not inherently harmful; it is the overexpression of CRPs that is bad. Thus, CRP overexpression via immunoediting and complement activation can both contribute to enhanced tumor progression and a worse patient prognosis[61].

Proteochondroitin (CSPG) is a type of chondroitin sulfate proteoglycan that is secreted by malignant B cells. It has a similar conformation to C1 inhibits, can bind to C1q and inhibit activity by blocking its binding to receptors. CSPG can inhibit the C1 complex, and can specifically protect tumor cells from antibodies[62].

Ecto-proteases are enzymes that cleave complement components and modulate complement responses. Activating C3 is necessary for MAC formation, and proteases are able to cleave C3 and disrupt MAC formation, which protects tumor cells from MAC-mediated damage. p57, p65, and p39 are common ecto-proteases that are upregulated in tumors and can cleave C3[55].

Ecto-PKs are membrane-bound protein kinases, which are enzymes that use ATP available in body fluids. A subcategory of ecto-PKs called ser/thr-ecto-PKs is able to phosphorylate C9 and reduce its activity. C9 is crucial for MAC formation, and ecto-PKs are able to prevent MAC formation and prevent complement-mediated lysis of tumor cells[63].

Induced Evasion Mechanisms of Complement and Immune Responses

Complement resistance can be increased when there is a sub lytic number of MACs attached to the cell membrane. MAC complexes are essentially pores that create holes in the cell membrane and allow substances to freely enter and exit the cell. The introduction of MACs allows calcium ions [Ca^{2+}] to enter the cell, drastically increasing the concentration[55]. Elevated calcium ion concentration is strongly associated with resistance to complement-mediated lysis, as a reduction in calcium ion concentration within cells enhances complement-mediated cell lysis[64]. This change is known as an induced mechanism because it is induced by MAC formation and binding.

Induced mechanisms can also include enhanced resistance to the traditional immune response rather than solely resistance to the complement response. Sub lytic levels of MACs can cause secretion of proinflammatory cytokines such as IL-8 and MCP-1, resistance to apoptosis, entrance into the cell cycle, and increased expression of adhesion molecules and eicosanoids[55,65,66]. Eicosanoids are molecules that regulate pro-inflammatory processes, and adhesion molecules facilitate interactions between tumor cells and the ECM, thereby promoting metastasis. These processes augment tumor cell invasiveness and destructiveness. A sub lytic amount of MACs also activates G-proteins and stimulates generation of diacylglycerol kinase phosphorylates (DGKs) within tumor cells, which causes increased complement resistance[67]. G-proteins have been implicated in enhancing cancer invasiveness and aiding in processes such as angiogenesis, metastasis, immune evasion, and drug resistance, as well as playing a role in tumor initiation[68]. Similarly, DGKs are highly expressed in many types of cancer, including melanoma, hepatocellular carcinoma in the liver, and glioblastoma in the spinal cord. DGKs are heavily implicated in aiding apoptosis and proliferation, as well as inducing a nonresponsive state in T cells known as anergy[69].

Current Methods of Complement Inhibition

It was traditionally thought that complement activation has more benefits for the host than for tumor cells[54]. Therefore, many cancer therapies regarding the complement system have attempted to strengthen complement responses. However, more recent evidence shows that complement recognition allows tumors to develop mutations that allow them to better evade immune responses through the immunoediting process. Thus, several more recent complement-targeted therapies have focused on complement inhibition rather than promotion in an effort to prevent these mutations from occurring[54].

Immunoediting Theory

The formation of neoplasms includes the alteration of surface proteins and phospholipid conformation, which distinguish neoplastic cells from normal cells. These changes are recognizable by the complement system and can trigger activation. Complement activation exhibits a directional selection pressure upon the neoplastic population. Tumor cells that highly express CRPs (complement regulatory proteins) or other evasion methods (discussed in depth in section 6) are selected for and have a higher chance of avoiding complement-mediated destruction, while tumor cells with lower expression of mCRPs have a lower chance of survival. The selection of mCRP-expressing tumor cells promotes the creation of new tumor populations that are able to completely bypass complement-mediated lysis. This process is known as the immunoediting theory, which states that the immune system can drive tumor development and stimulates developmental changes that allow cancerous cells to escape immune attacks[54]. Immunoediting takes place in 3 main stages. The first stage is elimination, and neoplasms are destroyed immediately upon detection. The second stage is equilibrium, in which the neoplastic cells are able to evade immune-mediated destruction and proliferate. The final stage is escape, which can lead to invasive and metastatic disease (stages illustrated in Figure 4).

While immunoediting nullifies the complement system's ability to eliminate neoplastic cells, it still allows for complement recognition of tumors and inflammation, thereby allowing for a permanent source of a tumor-promoting microenvironment. Harmful inflammation is primarily caused by the production and usage of C3a/C5aR and C5a/C5aR. Anaphylatoxins can have other tumor-promoting effects as well as promoting inflammation. The consistent activation of C3aR and C5aR increases IL-6 expression, which can promote tumors by inhibiting apoptosis, stimulating angiogenesis, and increasing resistance to anti-cancer drugs[70]. Prolonged inflammation also stimulates polarization of immune cells, and can contribute to the creation of TAMs, MDSCs, Tregs, regulatory dendritic cells, and type 2 natural killer (NK) cells, all of which promote an immunosuppressive TME and stimulate further tumor growth (further discussed in section 3). Therefore, most complement inhibitory drugs target anaphylatoxins. Since anaphylatoxins are integral to the complement cascade, selectively blockading C3a/C5a can inhibit the entire complement system.

The immunoediting theory has a substantial amount of evidence to support it. The elimination stage has been illustrated in experiments with murine models[71–73]. Genetically engineered mice that were deficient in the RAG2 gene[71], interferon-γ[73], or perforin[72] developed spontaneous tumors more often than immunocompetent mice. The RAG2 gene codes for the RAG complex, which is important for B cell and T cell function. Similarly, interferon- and perforin are responsible for modulating a number of immune responses. These immunodeficient mice developed spontaneous tumors more often than immunocompetent mice, demonstrating that developed immune responses are responsible for inhibiting early tumor development. The equilibrium and escape stages of immunoediting were demonstrated in a computational model study[74]. Neoplastic cells grow until spatial constraints prevent them from growing further. A weak immune response kills some cells and relaxes the spatial constraints, allowing for further growth and promoting eventual metastatic growth, representing the equilibrium phase. Only with a sufficiently strong immune response can tumor growth be successfully limited. However, the residual isolated cancer cells will develop into a more malignant form of cancer with the eventual result of metastatic disease, representing the escape phase of the immunoediting process.

Although the immunoediting theory has been widely accepted for many years, new theories have surfaced challenging it. One such contradictory theory is that the escape phase of tumor development is driven by resource competition between tumor cells and immune cells within the TME[75]. Tumor cells leave the TME and spread through metastasis due to limited glucose availability and resource competition, rather than being able to manipulate and overpower immune pathways according to the immunoediting theory. Similarly, other researchers have suggested that the effects of immunoediting are relatively weak compared to neutral evolution[76]. The immunoediting process entails strong sweeping eradications, which would result in a relatively homogeneous tumor cell population that would have specific favorable genetic traits. In reality, according to an experiment conducted on mouse models, tumor cells have varied genotypes, implying that the development of tumor cells populations is more dependent on neutral evolution, with almost all traits being equally favorable, rather than selection for specific traits[76]. Therefore, although the immunoediting theory has been widely accepted for many years, there is still some debate among researchers regarding the extent of immunoediting effects.

Current and Future Complement Inhibitory Drugs

Eculizumab was the first complement inhibitor to receive FDA approval in 2007, and validated complement inhibition as a valid treatment option for various illnesses/autoimmune disorders. Ravulizumab is a more recent drug that was approved in 2018 that serves a similar function to eculizumab. Both are injectable complement inhibitors that target C5 activity, thereby preventing the assembly of the C5b-9 complex. Pegcetoplan is another injectable complement inhibitor that targets C3 activity and was approved in 2021. Sutimlimab was approved in 2022 that blocks C1 activity. Avacopan is an orally administered C5aR inhibitor that can help prevent C5a-mediated neutrophil polarization, and was approved in 2021. Further research is currently being conducted on all of the mentioned drugs to

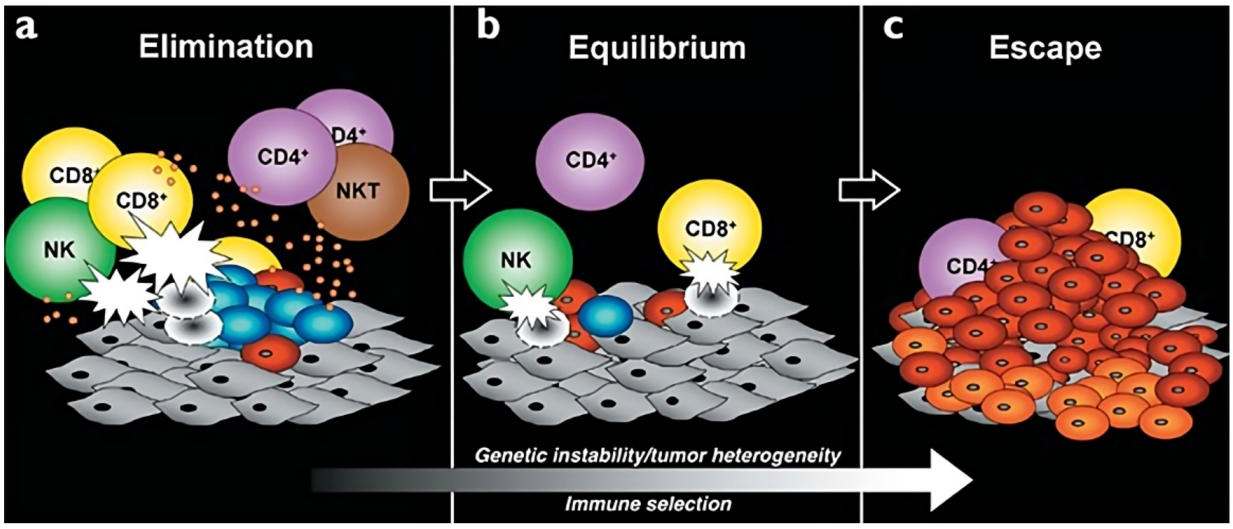

Fig. 4 The figure shows the 3 stages of immunoediting exhibited by the complement system upon neoplasms[38]. The tumor mutates due to selection pressure, and is eventually able to survive immune attacks and escape/metastasize.

determine efficacy and appropriate use cases.

However, complement inhibition can have grave side effects. Most inhibitors target the classical, lectin, and terminal pathways, all of which are vital for immune surveillance. Blocking these pathways can significantly increase the risk of serious infection, which can be potentially life-threatening. Due to these risks, eculizumab, ravulizumab, and Pegcetoplan are only available via a REMS (Risk Evaluation and Mitigation Strategy) program. Newer drugs that are currently in development attempt to selectively target the alternative pathway, allowing for immune surveillance to occur via the classical and lectin pathways (complement drugs in development are depicted in Figure 5) to hopefully mitigate some of the risks of complement inhibition.

Although the development of new therapeutic drugs is potentially extremely rewarding, there are a number of obstacles that must be addressed[77]. Identifying biomarkers, or key molecules found within certain mechanisms, to target with treatments is a key part of developing new drugs. However, not all of the biological mechanisms exhibited by the complement system have been entirely discovered or understood. The clinical efficacy of the biomarkers being utilized in treatments must be validated through extensive clinical trials to ensure the safety of the patients. Furthermore, continuous research into the mechanisms at a molecular level is needed to continue the search for the best possible biomarkers to be used for more targeted treatments, which could potentially reduce some of the grave side effects of complement inhibition drugs. An additional problem that researchers face is the potential lack of translation of efficacy of drugs between murine models and human subjects during experiments. Diseases in mice occasionally correlate poorly with disease progression in humans, rendering research using murine models inaccurate (81). This necessitates translational medical research to accurately segue results with murine models into treatments with humans.

Clinical Trials and Generalization to the Population

Eculizumab has had some degree of success in improving patients' quality of life. One such example is with a 69-year-old female who had been suffering from metastatic relapsing breast cancer for 2 years[78]. According to the case study, the patient had been receiving chemotherapy and was suffering from thrombotic microangiopathy as a side effect (TMA), which is the destruction of red blood cells. The patient's condition was not improving from the chemotherapy and the patient was becoming increasingly dependent on dialysis, severely degrading her quality of life. After treatment with eculizumab, the patient's TMA symptoms and renal function improved, allowing her to stop dialysis and improving her quality of life.

However, complement inhibition is still a relatively new field, and still requires further research to be a viable option. In 2021, a case study by MacDougall et al. was done regarding a 47-year-old male with stage IV pancreatic cancer who developed gemcitabine-induced thrombotic microangiopathy (GiTMA) as a result of his chemotherapy treatments. GiTMA is a life-threatening complication that can arise from gemcitabine usage and has a mortality rate ranging from 50-90%. After the patient was admitted into the intensive care unit due to his drastically declining levels of platelets and red blood cells, the decision was made to treat him with ravulizumab. After treatment, the patient had an initial improvement, but continued to experience declining blood counts, and suffered persistent

Table 1 The below table displays several examples of complement inhibition drugs in their various stages of development (62). The specific mechanisms targeted by the experimental drugs are also demonstrated, as well as their method of administration.

Drug name	Mechanism	Developmental Stage	Effects
Iptocopan	Orally administered small-molecule factor B inhibitor	Currently undergoing Phase 3 clinical trials	Highly selective for factor B, blocks C3 convertase activation without blocking the classical or lectin pathways
IONIS-FB-L	Subcutaneously injectable antisense oligonucleotide, factor B inhibitor	Currently undergoing Phase 3 clinical trials	Reduces factor B levels in plasma, and blocks alternative pathway at the level of C3 convertase
Danicopan	Orally administered small-molecule factor D inhibitor	Currently undergoing Phase 2 clinical trials	Blocks the entire alternative pathway by inhibiting the enzymatic activity of factor D
Vemircopan	Orally administered factor D inhibitor	Currently undergoing Phase 2 clinical trials	Effective and sustained inhibition of the alternative pathway through factor D inhibition
BCX9930	Orally administered small-molecule reversible factor D inhibitor	Currently undergoing Phase 3 clinical trials	Almost complete suppression of the alternative pathway
GT005	Single subretinal injection, gene therapy, factor I agonist	Currently undergoing Phase 1/2 clinical trials	Increases production of factor I to decrease levels of downstream complement components within the alternative pathway

hemolysis for the next 10 days, requiring frequent red blood cell transfusions. The patient was soon admitted into hospice[79]. However, ravulizumab cannot be ruled out as a failure, as new treatments often take weeks or months to show effect. More research is required to determine how complement inhibitors can be effectively used along with other treatment options.

At first glance, it may seem unlikely that case studies done on single patients can be generalized to the population. It is exceedingly improbable that the symptoms of any single patient would exactly match those of the subjects of these 2 studies. However, single case studies are important for building off knowledge gained from large-N studies and help in precision medicine. Single case studies can be generalized through direct and systematic replication[80]. Direct replication is when a single case study is replicated under the same conditions as the original study to confirm the reliability of the results. Systematic replication is when the conditions are altered from the original study to evaluate the results of the treatment under different circumstances. When a systematic replication fails under a particular condition, it is known as the 'boundary' of the effect. A series of single case studies creates a 'line of research' when the treatment has been replicated enough times that the outcome can be reasonably predicted. Thus, single case studies are an important point of reference for precision medicine. Healthcare providers often assign treatments to patients using the line of research and single case studies as reference points because they provide details about the performance of the treatment under a variety of different conditions.

However, large-N studies have several advantages. Large-N studies allow for the most accurate overall generalizations to the population because they are far less prone to bias. The large sample size tends to average out the effects of any confounding

variables that may exist. The downside of this is that the larger number of subjects eliminates any individual differences that patients may have. Because the results of large-N studies are far less variable than single-case studies, they are almost always used as the core reference point for any experimental drug or treatment. Therefore, while group designs are efficient and very useful for generalizing to the population, they are often needed in parity with single-case studies in order to have a solid understanding of the effects and efficacy of a particular treatment.

Although these case studies are single-case studies, they hint towards where complement inhibitors can be effective. Complement inhibitors were more effective in stage IV breast cancer, while they had disastrous effects for stage IV pancreatic cancer. Therefore, it is possible that complement inhibitors may be significantly more effective for breast cancer than for pancreatic cancer. Furthermore, complement inhibitors were ineffective when used as an immediate treatment option, but where more successful at improving patient quality of life over a longer period of time. This hints that complement inhibitors may be an effective adjuvant treatment method that can aid in long-term patient comfort and health, but would not be effective at improving patients' short term critical conditions.

Due to the fact that clinical trials involving the complement system can be potentially hazardous to the patient, stringent standards must be complied with in order to assure that the risk-reward ratio remains beneficial to the patient for the duration of the study. Ethical codes such as the Nuremberg Code 1947 and the various amendments to the Helsinki declaration are widely used and implemented into modern clinical trials, and are extremely important for maintaining the integrity of clinical research. Firstly, the concept of informed consent is widely practiced, where patients' consent is required after understanding all potential risks/benefits of the experimental treatment in question. In the majority of cases, children will not be able to give informed consent and therefore should not be included in clinical trials. Furthermore, the safety of the individual must always take precedence over the benefit of society. Ethical clinical trials are required to assume equipoise between all modes of treatment that patients are given, meaning that there is not assumed to be any inherent benefit to one treatment over another[81]. If, at any point during a clinical trial, equipoise can no longer be safely assumed, the trials must be discontinued immediately if possible. The use of placebos in clinical trials must also be closely monitored, as the use of treatments that are known to not work can be considered unethical. Rather, clinical trials must be conducted against the existing medical best practices whenever possible. Only with these practices and considerations in place can ethical clinical trials be performed regarding the complement system and cancer immunotherapy

Conclusion

Complement activation has been implicated in causing many different tumor-promoting effects, and selective complement inhibition is a promising treatment method. While many of the complement system's interactions with tumors are unknown, there is a clear correlation between complement activation and tumorigenesis. Furthermore, tumors can evade complement-mediated damage and manipulate complement activation into creating a constant source of tumor-promoting inflammation according to the immunoediting theory. Clinical trials are currently being conducted on several complement inhibition drugs, including iptocopan, vemircopan, and danicopan. Experimental treatments like this hold promise for the future of complement inhibition in cancer immunotherapy and may be available in coming years. However, there are currently no viable complement-inhibition based treatments that can be mass-produced. Complement-inhibition drugs that are currently available are only usable for patients with specific prerequisites and often cannot be applied to a wider scope of use cases. More research is needed to develop drugs that can safely be applied to a variety of cancers. Furthermore, while complement activation has a strong tumor-promoting effect, it can also exhibit an anti-tumor response, resulting in a dual role for the complement system in cancer. The exact mechanisms and effects for this dual role are currently unknown, and further research is required to determine the mechanisms involved in tumor suppression or promotion. Such research into complement interactions with tumors would yield a better understanding of the relevant molecular biomarkers involved and would provide a basis for future treatments. Having a knowledge of specific molecules that have been proven to be the most influential in pro-tumor responses could potentially be targeted with future therapies. For example, treatment could target only specific complement receptor-ligand complexes that are overexpressed by tumor cells. Inhibiting the entire complement cascade, which has traditionally been a common approach, would cripple patients' protection from external pathogens and would leave them in an immunocompromised state. Therefore, therapies targeting specific molecules have the potential to solely inhibit the destructive effects of complement activation while preserving the protective benefits. Further research is needed in this direction before complement inhibition can be a safe and effective treatment method.

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