Utrecht University

Leids Universitair Medisch Centrum

Targeted modulation of complement activity to treat autoimmunity and cancer

Author: Jeroen van Beek (1145101)

Degree: Drug innovation (MSc)

Daily supervisors: Leendert Trouw

Examiner: Suzan Rooijakkers

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Abstract

The complement system plays a role in several autoimmune diseases and an upregulation of complement regulators is seen in some tumors. Antibody-based therapies can be employed to reduce complement activation or to induce complement dependent cytotoxicity of tumors. Currently, complement-based therapeutics are costly, require frequent dosing and have side effects. These drawbacks can possibly be overcome by specifically directing antibody therapies to a cell or tissue type, or a specific organ. This article discusses targeted approaches of therapeutics in complement related autoimmune diseases and complement activating antibodies in the treatment of cancer. Most approaches in the field of anti-autoimmunity drugs make use of fusion proteins that target areas of high inflammation or a specific organ. In the field of anti-cancer antibodies a variety of approaches are used, such as bi-specific antibodies, lipoplexes and conjugated antibodies. Some of these drugs target complement regulators to increase complement dependent cytotoxicity, which seems promising for efficient killing of tumor cells.

Layman's summary

The immune system is an extremely intricate system which protects the body against pathogens such as bacteria and viruses, but also from malignant cells, such as tumors. One of the components of the immune system is the complement system. This system consists of many proteins that circulate in the blood and are inactive until a pathogen is recognized. The activation can occur by three different triggers, which initiate three different biochemical reaction cascades, called the classical, alternative and lectin pathway. The triggers and biochemical reactions of these three pathways are different, but they all lead to the same immune reactions. The reaction begins when a pathogen is recognized by a molecule specific to the three pathways. Then, that molecule binds to other molecules and creates the enzyme C3 convertase, which is able to convert C3 that is always present in the blood, into C5 convertase. The C5 convertase enzyme cleaves C5, which is also present in the blood, and this results in the formation of the membrane attack complex. This is a structure of molecules that are assembled on the target pathogen and form a pore in the membrane, causing cell death. However, not just the membrane attack complex can kill pathogens. During the biochemical cascade of reactions, many side-products are created that each have their own function, such as opsonizing, or 'marking' the target cell and stimulating inflammation.

The complement system is often involved in diseases such as autoimmune diseases and cancer. In autoimmune diseases, the complement system may target healthy cells, causing inflammation and this can cause various symptoms. Drugs are already being used that inhibit certain complement enzymes, but seen as the complement system is present in the entire blood stream, this can cause some problems, such as a high dose required and side effects. Therefore, it would be useful if these drugs can target specific cells or tissues that are involved in the disease.

To fight cancer, sometimes antibodies are used that specifically bind to tumor cells and lead to an immunological reaction. One of the ways the antibody works is that it activates the classical pathway of complement. However, tumors often upregulate complement regulator proteins, which inhibit the function of certain complement enzymes, resulting in protection against the antibody treatment. Many new antibodies in development try to address this issue with various technologies. In this review, these technologies are discussed, as well as targeted complement antibodies in the field of autoimmune diseases.

Bispecific antibodies can be used to target both a tumor and a complement regulator. Another method is to use small interfering RNA (siRNA), which blocks the production of complement regulators in the cell. The siRNA is enveloped in a lipid membrane that is specific to the target tumor cell, preventing unwanted side effects. Lastly, antibodies can be bound to molecules that enhance complement activation or bind to areas of high complement activation. This increases the efficacy of the antibody treatment, but does not address the issue of increased complement regulator expression on tumor cells.

To make complement inhibitors for autoimmune disease more targeted, often fusion proteins are used. These fusion protein consist of a complement protein targeting antibody and a molecule that binds to a molecule of interest. This molecule can be an organ specific molecule, making the antibody bind specifically to the target in the target organ. Some therapies do not bind to a specific cell or organ, but to areas of high inflammation, which is the cause of autoimmune disease. However, not every inflammation process in a patient with an autoimmune disease should be targeted, for example when a wound in the skin causes an influx of pathogens. Here, an inflammation process is necessary for

efficient removal of pathogens, so side effects must be monitored when testing these types of drugs in humans.

1. Introduction

An important part of the immune system is the complement system. The complement system consists of over 30 proteins that circulate in the blood or are membrane-bound, and play a role in both the innate and adaptive immunity.[1] The goal of the complement system is to detect, mark and kill pathogens, injured tissue, immune complexes and more. The complement cascade begins when a pattern recognition protein (PRP) binds to a pathogen-associated molecular pattern (PAMP). This can happen in three ways, which initiate the three pathways of complement: the classical, alternative and lectin pathway (figure 1). When a PRP binds to antibodies, present in for example an immune complex,

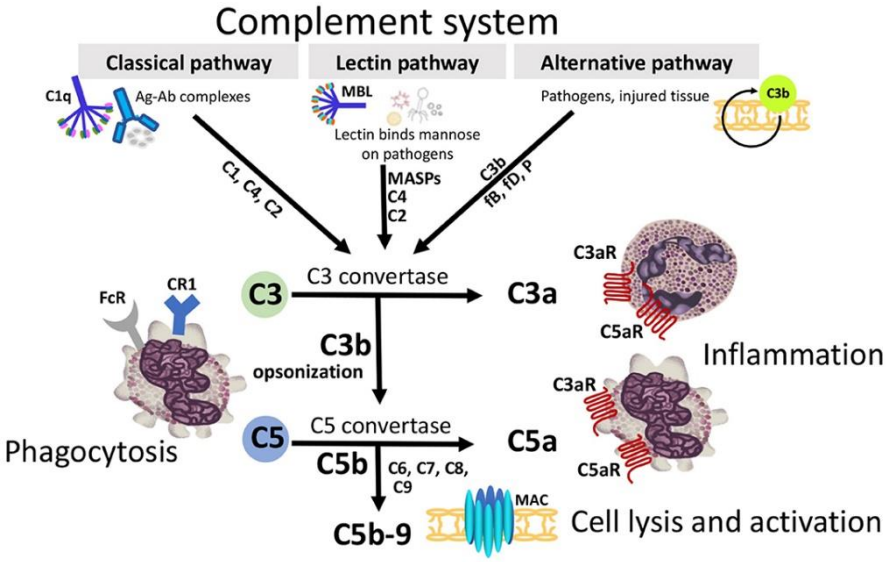

Figure 1 Schematic overview of the complement system. Source: Girardi et.al. [34]

the classical pathway is initiated. A PRP can also bind to a non-self-carbohydrate structure and initiate the lectin pathway. The alternative pathway of complement activation is triggered when C3b binds to a microbial surface. What all three pathways have in common is that they lead to the construction of the C3 convertase complex. This enzyme cleaves circulating C3 to C3a and C3b, which leads to amplification of the alternative pathway, opsonization, inflammation and the formation of the C5 convertase complex. This initiates the late steps of complement activation, where C5 is cleaved by C5 convertase, resulting in more inflammation and finally, the formation of the membrane attack complex (MAC), which induces osmotic lysis in the target cell.[2] Complement proteins are always present in the body, so to prevent complement activation damaging the host tissue, complement regulatory proteins exist. Some of the complement regulatory proteins are soluble and travel with the rest of the complement proteins in the circulation, while other complement regulators are membrane bound proteins that mediated complement regulation on the cell surface. A deficiency of them has been linked various immune-related diseases.[3]

The complement system is involved in several clinical conditions. For example, complement activation following the formation of autoantibodies is seen in systemic lupus erythematosus (SLE) and can cause inflammation of several tissues and organs. Also, deficiencies in complement regulatory proteins can cause diseases such as hereditary angioedema (HAE), atypical hemolytic uremic syndrome (aHUS) and diseases of the blood, such as paroxysmal nocturnal hemoglobinuria (PNH).[3]

For the treatment of aHUS and PNH, complement inhibitors have been developed. Currently, two therapeutic complement inhibitors are in clinical use. The C5 inhibitor eculizumab has been very

successful in the treatment of PNH, which is a disease where enterocytes lack two complement inhibitors This results in complement activation and the killing of enterocytes by the formation of a MAC complex. Eculizumab is able to stop MAC formation, protecting enterocytes.[4] However, treatment with eculizumab requires for patients to get a meningococcal vaccination, as the killing of the *Neisseria meningitidis* bacteria is primarily done by the formation of a MAC complex.[5] A tissue or cell targeted C5 inhibitor could overcome this side effect, but no such drug is on the market yet. The other type of complement inhibitors on the market are C1 esterase inhibitors, which are used for the treatment of HAE, a disease in which there is a mutation in the *SERPING1* gene, making the C1 inhibitor protein.[6]

Studies in animal models have shown that complement plays a crucial role in the effectiveness of monoclonal antibody (mAb) activity. For example, in a study investigating the effect of the mAb therapy with rituximab on tumor-growth, C1q-deficient mice responded worse to the treatment than mice that were not C1q-deficient.[7] This indicates the importance of complement-dependent cytotoxicity (CDC), which is a mechanism where C1q binds to IgM or IgG antibodies and initiate the classical pathway of complement, as opposed to antibody-dependent cellular cytotoxicity (ADCC), which is a mechanism in which effector cells are used to induce lysis of the target cell.[8] However, not every tumor is susceptible to killing by CDC. Several studies have shown that some tumors evade the complement system, primarily through secretion of membrane-bound complement regulatory proteins CD46, CD55, and CD59 on their cell surface.[9] There have been attempts to overcome this increase in complement regulatory proteins by modulating tumor-targeting mAbs so that complement regulatory proteins are inhibited. This review will discuss the latest literature concerning ways to target specific cells or organs in CDC-activating anti-tumor mAb therapy and targeted approaches for complement inhibition in autoimmune diseases.

2. Targeting complement with antibody treatment in cancer and autoimmune disease

2.1 Tumor targeting antibodies

Antibody mediated anti-tumor therapy can be employed in many different formats and with different effector mechanisms. Next to antibodies that impact on immune-checkpoints to modify the adaptive immune response, also directly cytotoxic anti-cancer antibodies are used. These antibodies bind to tumor cells and activate effector mechanisms, such as antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cell-mediated phagocytosis (CDCP) and complement-dependent cytotoxicity (CDC).[10]

Antibodies that mediate CDC kill the tumor cells by the insertion of the MAC. However, many tumor cells over-express membrane bound complement inhibitors (mCRPs) such as CD46, CD55, and CD59. These high levels of inhibitors put a constant brake on the efficiency of CDC.[11]

Table 1 Summary of drugs discussed for the treatment of cancer

Approach	**Reference**
siRNA enveloped in lipoplex	Cinci et al.[12], Mamidi et al.[13]
bsABs	Macor et al.[14], Gelderman et al.[15]
Conjugation to complement activating agent (CVF, C3b)	Gelderman et al.[16]
Co-administered fusion protein (CR2)	Elvington et al.[17]

To overcome the inhibitory effect of these mCRP, several (targeted) therapeutics have been developed. One can try to down regulate the expression of mCRPs using small interfering RNA (siRNA) like strategies, or one can try to cleave off the mCRPs or block their function with blocking antibodies. Importantly however, this needs to be done in a targeted, local fashion, to be safe and effective, as downregulation of mCRPs in normal tissue can cause unwanted side effects.

Mamidi and colleagues have provided compelling evidence that it is possible to use siRNA mediated down regulation of tumor cell associated mCRPs, CD46, CD55 and CD59. Indeed, the downregulation did have major consequences regarding the efficiency of complement activation and killing of the tumor cells. However, in this *in vitro* approach they could simply add the si-RNAs to the tumor cells, while for *in vivo* application, such a strategy would have to be targeted specifically to the tumor cells.[18] In a follow-up paper, Cinci et al now took a next step by encapsulating the anti-mCRP siRNA in transferrin-coupled lipoplexes, with the goal of targeted delivery to transferrin receptor (CD71)-positive tumor cells.[12] Results were successful, up to 90% silencing of all three mCRPs was achieved, dependent on CD71 expression. Also, the mCRP knockdown led to increased CDC on $CD71^{high}$tumor cells, and only slightly on $CD71^{low}$ cells. This indicates that the tumor cell targeted approach was effective in specifically targeting tumor cells and killing them. However, this study was done *in vitro*, so *in vivo* studies are needed to validate these results on their efficacy and toxicity. Problems could occur *in vivo* if for example there are cells other than the target tumor cells that express CD71 or if the tumor cells do not express sufficient CD71.

An elegant way to increase antibody binding specificity is to use a bispecific antibody (bsAb). bsAbs have two binding sites directed at two different antigens or even two epitopes on the same antigen. This technique is relatively new, but already there are three bsAb therapies in clinical use.[19] Macor and colleagues constructed two (bsAbs), with one arm of the antibody targeting CD20, an antigen

found on B-cell lymphomas, and the other arm targeting either the mCRP CD55 or CD59. To test the efficacy of tumor killing by the bsAbs, the results were compared to a control antibody with only a binding region to CD20 and no binding region to the mCRPs. The CD20-CD55 and CD20-CD59 antibodies were injected as a mixture in a mouse model of B-lymphoma and showed a 4-25 fold higher tumor killing than the control antibody. All mice in the treatment group survived the study period of 120 day.[14] However, a common issue with bsAbs is their ability to bind with only one of the two arms to a target other than the initial target, i.e. the tumor. In this case, the bsAb could bind to healthy cells expressing CD55 or CD59, causing these cells to be targeted by CDC. The authors could only test for this phenomenon in an *in vitro* assay, which concluded that binding affinity was driven by the CD20 part of the antibody. Still, this result should be confirmed *in vivo*, as it is possibly a major toxicity issue. An advantage of the bsAb strategy the authors used, is that antigens other than CD20 and other mCRPs can possibly be designed in the bsAb construct, allowing for a wide variety of applications.

Gelderman and colleagues also developed a bsAb, with one arm directed against Crry, which is a rodent-specific C3 regulatory protein functionally homologous to human CD46 and CR1, and the other arm directed against a tumor-associated antigen. Crry was chosen as it best represents human C3 mCRPs. The bsAbs were injected into a syngeneic lung metastases model of rat colorectal cancer and prevented almost completely the outgrowth of lung tumors. This was proven to be due to increased activation of complement.[15] The results of this study are promising, and these results are done in a model syngeneic for mCRP and complement, which increases their predictive results of the effect of the drug in a clinical setting. However, cross-reactivity could be an issue due to the double binding regions of the bsAb, and it is crucial that for clinical studies, the tumor-recognizing arm is of high affinity and the mCRP blocking arm should be of low affinity. The affinity of the mCRP blocking arm should not be too high, or it will cause cross-reactivity, but too low will cause insufficient blockage of the tumor-bound mCRP, resulting in inefficient CDC. This is a delicate balancing act and poses a challenge in the use of bsAbs.

A strategy to improve the activation of complement by mAb therapy against cancer, is to conjugate complement-activating agents to the therapeutic antibody. These agents can be for example C3b or cobra venom factor (CVF), which is a functionally and structurally equivalent of C3b. In a study by Gelderman et. al., an antibody against colorectal carcinoma was conjugated with either CVF or C3b. Both of these antibody conjugates increased C3 deposition and were able to activate complement more than the unconjugated antibody. The advantage of the CVF conjugate is that it is not inactivated by factor H and I in serum, as opposed to C3b. However, CVF has a complement-depleting effect, and could be immunogenic in humans.[20] The tumor-killing and complement activating effects of the two antibody-CV/C3b conjugates were compared to a bsAb directed against the colorectal cancer and CD55. The bsAb was equally or more efficient in complement activation.[16] The study was done *in vitro*, so efficacy and toxicity must also be determined *in vivo*, but the two techniques studied both seem promising for increasing CDC against tumors. The major advantage of the bsAb as opposed to the conjugated antibody, is that the bsAb targets mCRPs, which are known to be more abundant on some tumor cells. The conjugated antibodies do not tackle this issue.

Elvington and colleagues thought of ways different than the previous techniques described to enhance mAb-targeted complement activation on tumor cells. They created the fusion protein CR2Fc, consisting of a murine complement receptor 2 (CR2) targeting region linked to a murine IgG2a Fc complement activating region. This fusion protein was co-administered with a mAb against the a clinically relevant tumor antigen. The theory behind this approach is that the antitumor mAb activates complement and deposits C3d on a tumor cell, which is used as a ligand for the CR2Fc fusion protein. This leads to further activation of complement and opsonization on the tumor cell. The

coadministration of CR2Fc significantly improved the outcome of the mAb therapy in comparison to the mono-therapy with only mAb.[17]

To conclude, there are a variety of molecular techniques in which cytotoxic anti-cancer antibodies can be made more efficient and perhaps more importantly, more specific. siRNA constructs can be used to downregulate mCRPs and increase efficacy of the anti-tumor antibody, and encapsulate this in a lipoplex specific for tumor cells, to increase specificity. Moreover, bsAbs are used to target a tumor epitope and to neutralize an mCRP. Furthermore, complement-activating agents can be conjugated to an anti-tumor antibody, increasing CDC-mediated tumor killing, but not drug toxicity. Lastly, a fusion protein consisting of CR2 and an anti-tumor antibody is effective in tumor killing, as the CR2 part of the protein targets areas of high complement activation.

2.2 Targeted approaches in antibody therapy against autoimmune diseases

The complement system plays a big role in some autoimmune diseases. Uncontrolled activation of complement may cause autoimmune responses. For example, often in SLE, autoantibodies are produced against DNA and histones, causing the formation of immune complex following activation of the classical complement pathway. This can lead to autoimmunity, which presents itself as inflammation of various tissues and organs.[21] Besides this mechanism, complement regulator deficiency has also been identified as a cause of autoimmune disease. aHUS patients often have loss-of-function mutations in genes encoding for complement regulatory proteins, such as Factor I, CD46 and thrombomodulin. This can lead to excessive complement activity and can lead to endothelial cell injury, tissue ischemia and organ dysfunction.[22]

Currently, there are four different types of complement inhibitors on the market. These include C1r/C1s, C3, C5 and C5aR1 targeting drugs. Eculizumab, a mAb against C5 indicated for the treatment of aHUS and PNH was the first developed complement inhibitor. C1 inhibitors are indicated for the treatment of HAE. [23], [24] However, these drugs have side effects as a result of the systematic attenuation of the complement system. In the following section, several methods found in literature are explored that specifically target the organ involved in the pathological process, in order to reduce side effects.

Table 2 Summary of drugs discussed for the treatment of autoimmune diseases

Approach	**Reference**
C5 mAb fused to homing property peptide	Durigutto et al.[25]
Fusion protein (CR2 and factor H)	Risitano et al.[26]
Fusion protein (dansyl binding region)	Zhang et al.[27], [28]
Glycoprotein (sLEx moieties)	Mulligan et al.[29]

Durigutto and colleagues developed the recombinant protein Ergidina®, which consists of a neutralizing antibody to C5 fused to a cyclic-arginylglycylaspartic acid (RGD) peptide. This protein had a distinctive homing property for ischemic endothelial cells and was able to reduce tissue damage in a rat model of renal ischemia/reperfusion injury. A possible explanation for the high concentration of the drug in the kidney is that the RGD part binds to integrins expressing RGD-binding sites. Staining showed that these integrins were mostly present in the kidney. A quarter of the dose normally required when using a non-specific C5 inhibitor was enough to completely block the activation of C5 in the kidneys of the rats.[25] In conclusion, the addition of a small molecule to an already available C5 antibody is an elegant way to more specifically target the kidneys. However, the authors do not

mention further steps to be taken in order for the drug to go to clinical testing, and there are no specific diseases for which this drug should be indicated.

In PNH, the erythrocyte surface lacks the complement regulators CD55 and CD59, causing activation of the alternative pathway of complement which leads to intravascular hemolysis. In an *in vitro* study by Risitano and colleagues, a membrane-targeted delivery strategy was tested of a fusion protein consisting of the iC3b/C3d binding region of complement receptor 2 and the inhibitory domain of factor H, which is an inhibitor of the alternative pathway of complement. The drug was able to completely inhibit hemolysis of PNH erythrocytes and prevented C3 fragment deposition. This effect was dependent on binding to erythrocytes, which might indicate that this drug will not be reduce complement activation in other cells/tissues.[26] Another advantage of this novel drug compared to the conventional treatment with eculizumab, is that it acts upstream of C5, the target of eculizumab. This could provide useful for patients not responding to eculizumab treatment, as it is seen that in these patients there is still complement activation upstream of C5.[30]

Zhang et.al. used a different technique to cell-specifically inhibit the later stages of complement. They fused soluble CD59 with an antibody-combining site at the end of CH1, after the hinge, and after CH3 Ig regions. This made the fusion protein bind specifically to the antigen binding region of an IgG specific for 5-dimethylamino-naphthalene-1-sulfonyl (dansyl), which was also attached to the target Chinese hamster ovary cells. The fusion proteins all bound to target cells, but not to cells without dansyl and were able to prevent complement-mediated lysis.[28] However, these results should be tested *in vivo* to test for efficacy and toxicity. Also, this method is not representative for a real clinical situation, as target cells were labeled with dansyl, but in a clinical situation a suitable target must be found. The authors also published a study using the same method of binding dansyl to an antibody, in this case to decay-accelerating factor (DAF). Here, the results were positive too, as the fusion protein protected cells from complement lysis. The authors mention an advantage of using DAF as a complement inhibitor as opposed to CD59, is that DAF did not need to bind to the cell surface close to the site of complement activation.[27]

Mulligan and colleagues used soluble complement receptor 1(sCR1) bound to sialyl Lewis^x (sLE^x) moieties, resulting in a glycoprotein. These sLE^x moieties bind to adhesion molecules P- and E-selectin, which are molecules expressed during endothelial activation. In the early steps of inflammation, endothelial activation is required for the recruitment of leukocytes into tissues. By conjugating sLE^x to the sCR1, a higher binding specificity and affinity to regions of high inflammation is achieved. On top of that, the conjugated molecule also blocks leukocyte binding, resulting in reduced inflammation. In a rat model of selectin-dependent lung injury, the sLE^x conjugated molecules significantly improved the protective effect of sCR1 as compared to only sCR1 treatment.[29] The same strategy of conjugating sCR1 to sLE^x was tested in a mouse model of ischemic stroke. Here, again the conjugated molecule was superior, as neutrophil and platelet accumulation was inhibited more than in the unconjugated molecule.[31]

To conclude, there are several ways one can target the complement system in a tissue/organ specific manner in autoimmune diseases. Most of these techniques are based on fusing a complement inhibitor or mCRP to a molecule that binds to a specific cell type or tissue. One of these techniques is to fuse an anti-C5 antibody to a protein specific to ischemic endothelial cells, causing C5 to be blocked only in the organ of interest. Next, a complement inhibitor can be fused to CR2, which makes the drug specific for areas of high complement activation. Another fusion based technique is to fuse the mCRP CD59 or DAF to an antibody-combining site specific to a target molecule found on the target cells. Lastly, sLE^x moieties, which bind to molecules expressed during the early steps of inflammation can be bound to sCR1, increasing binding specificity to areas of high levels of inflammation.

3. Discussion and conclusion

As discussed in this paper, there are several studies that show that targeted complement inhibitors and CDC-inducing mAbs are more effective than untargeted therapeutics. However, most of the positive results in literature are seen in mouse studies and not much success is found in the clinical setting yet. The goal of targeted drugs in the setting of complement inhibition/activation is to decrease side effects, such as a higher risk of infection or complement activation on healthy tissues. On top of that, targeted drugs often result in higher efficacy, meaning that lower and less frequent doses are be necessary, which decreases costs of expensive complement inhibitors such as C5 inhibitors.

Tumor resistance to CDC poses a major challenge in the treatment of cancer with cytotoxic antibodies and there are not yet any therapeutic strategies in clinical trials that overcome this.[32] However, there are several drugs that show promising preclinical results, as described in this review. Two techniques seem to meet the criteria of being cell or tissue targeted and reducing mCRP mediated tumor resistance. These are the lipoplex delivered siRNA knockdown and bsAbs. By encapsulating an anti-tumor antibody in a lipoplex specific for tumor cells, healthy cells will be protected from complement activation. In the paper by Cinci et.al, the authors used a lipoplex system that delivered the antibody and siRNA knockdown of an mCRP to $CD71^{high}$ cells.[12] This showed great efficacy and specificity, but this system will only work on CD71 positive tumors and side effects were not researched by the authors, as their study was performed *in vitro*. The knockdown of mCRPs with siRNA is a promising long-term strategy, as the half-life of some siRNA drugs are several months.[33] Ultimately, using lipoplexes is effective in delivering siRNA and can also be modified to only infiltrate the target cell, meaning that this strategy increases both efficacy and specificity of cancer antibody therapy. Treatment with bsAbs is also aimed to achieve increased efficacy and specificity. One arm binds the tumor antigen and one arm an mCRP. The binding affinity to the mCRP must be fine-tuned, as too much affinity could cause cross-reactivity with healthy tissue expressing that mCRP, and too little affinity will result in insufficient blocking of mCRP activity. Therefore, extensive toxicity testing should be conducted *in vivo* to see if a balance can be achieved. If this is achievable, the bsAbs should be a very promising candidate for the treatment of cancer. The other two approaches discussed in this review also increase the efficacy of the antibody treatment, but not do not increase specificity to the tumor cells as much. The technique in which complement-activating agents are conjugated to the antibody increases complement activation at the tumor site, but disregards the upregulation of mCRPs that is often seen on tumors. In the study of Gelderman et.al., a conjugated antibody was compared to a bsAb, which did target an mCRP. The bsAb was equally or more efficient in inducing complement activation.[16] These results must be tested *in vivo* to provide more information on the efficacy of the two types of drugs, but it seems that addressing the upregulation of mCRPs is a crucial part in designing an efficient drug against tumors. The last method discussed, where an antibody is fused to CR2 also does not address upregulation of mCRPs, but is based on increased binding of the antibody to C3d rich areas. Elvington et.al. showed that this method increased tumor killing in a murine model of metastatic cancer, which is remarkable as this most successful *in vivo* studies are based on mCRP inhibition.[17]

Complement inhibitors have been quite successful in the treatment of various autoimmune diseases, such as PNH and aHUS. However, these treatments usually target the complement system in the whole body, resulting in a reduced immune defense and side effects such as higher susceptibility to certain infections. In this review, several novel drugs were described that aim to direct the effect of the drug to a cell or tissue type. These drugs aim to decrease drug toxicity and the dose required, as most complement targeting drugs are very expensive. Most of these drugs were fusion proteins, and

one was a glycoprotein, indicating that the coupling the complement-targeting drug to a molecule that binds specifically to a target of interest is a popular approach to decrease toxicity of complement-targeting drugs. However, a clear discrepancy can be observed in the choice of the target molecule that acts as a homing-property. In some studies, the complement inhibitor was coupled to CR2 or sLE^x moieties, which bind to molecules that are found in places of high inflammation. This approach could result in even more side effects that traditional complement inhibition, as inflammation is a process that can occur in the entire body, not limited to the target site of the autoimmune disease. Another approach uses a fusion protein that has a homing-property to the kidney, which could be useful in the treatment of aHUS. This approach is more precise than targeting inflammation processes in general, but clinical trials must be conducted to determine if this homing property of the drug is effective in humans.

In conclusion, there is progress being made in making complement-targeting drugs more cell/tissue specific, but most drugs are still in preclinical testing. Many different approaches are taken in the construction of tumor-targeting antibodies, such as bsAbs, siRNA conjugation and fusion proteins. In the treatment of autoimmune diseases with complement inhibitors, mostly fusion proteins are being developed. Ultimately, important aspects to consider for novel complement targeting-drugs are the presence of mCRPs, the exact disease mechanism and directing the drug to a cell or tissue of interest. This will hopefully reduce the cost and side effects of complement-targeting drugs, and increase the wellbeing of cancer and autoimmune disease patients.

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