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Complement in Monoclonal Antibody Therapy of Cancer

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Abstract

Monoclonal antibodies (mAb) have been used as targeted treatments against cancer for more than a decade, with mixed results. Research is needed to understand mAb mechanisms of action with the goal of improving the efficacy of currently used mAbs, and guiding the design of novel mAbs. While some mAb-induced tumor cell killing is a result of direct effects on tumor cell signaling, mAb opsonization of tumor cells also triggers activation of immune responses due to complement activation and engagement of antibody receptors on immune effector cells. In fact, complement has been shown to play an important role in modulating the anti-tumor activity of many mAb through complement-dependent cytotoxicity (CDC), antibody-dependent cytotoxicity (ADCC), and through indirect effects by modulating the tumor microenvironment. Complement activity can have both agonistic and antagonistic effects on these processes, and which mechanisms are most responsible for effective elimination of malignant cells remain unclear. In this review, we discuss the mAbs currently approved for cancer treatment, and examine how complement can impact their efficacy with a focus on how this information might be used to improve the clinical efficacy of mAb treatment.

Keywords

monoclonal antibody; CDC; ADCC; complement; cancer therapy

THERAPEUTIC MONOCLONAL ANTIBODIES IN CANCER

A major goal of cancer therapy research is to develop therapies that specifically kill cancer cells while sparing normal cells. The idea of using monoclonal antibody (mAb) therapy to treat cancer is attractive because antibodies have the ability to specifically target antigens associated with cancer cells. Rituximab, the first mAb used extensively for the treatment of cancer, was approved by the FDA in 1997 and produced an impressive 48% overall response rate in patients with relapsed low-grade non-Hodgkin's B-cell lymphomas [1]. Rituximab is now also used routinely and effectively for the treatment of other B-cell malignancies. The clinical success of rituximab bolstered the development of other mAbs against different targets and tumor types (Table 1) [2]. Unfortunately, not all mAbs have been as successful as rituximab and development of drug resistance is common, highlighting the need to improve understanding of mAb mechanisms of action [3–5].

In general, mechanisms contributing to mAb-induced tumor cell killing include direct effects on tumor cell signaling, complement-dependent cytotoxicity (CDC), antibody-dependent cytotoxicity (ADCC), and indirect effects modulating the tumor microenvironment. Complement activity has an obvious impact on CDC, but also affects each of the other mechanisms of action of mAb, and can have both additive and antagonistic effects. Moreover, which mechanism dominates mAb's clinical efficacy and how complement impacts on this efficacy are also subjects of debate. In this review, we discuss the mAbs currently approved for cancer treatment, examine how complement can impact their efficacy (Figure 1) with a focus on how this information might be used to improve the clinical efficacy of mAb treatment.

THE COMPLEMENT PATHWAY

Antibody (including mAb) bound to a cell surface activates complement through the classical pathway (Figure 1) when complement component C1 recognizes the Fc portion of Igs and becomes activated, cleaving C4 into C4a and C4b [6]. Activated C1 also cleaves C2 into C2a and C2b. C4b and C2a together form the C3 convertase (C4b2a), which enzymatically cleaves complement component C3 into C3a and C3b. C3a is a weak anaphylatoxin, which can recruit immune effector cells to the site of complement activation through interaction with the C3a receptor expressed on a variety of cell types. C3b is deposited on the target cell surface. This opsonization stimulates phagocytosis and cytotoxic killing, thereby “complementing” adaptive immunity [7–9].

In addition to functioning as an opsonin, C3b is also incorporated into the classical C3 convertase to form C5 convertase (C4b2a3b), which cleaves C5 into C5a and C5b. Similar to C3a, C5a acts as a strong anaphylatoxin and binds immune cells expressing the C5a receptor (C5aR), inducing inflammation and phagocytosis. Like C3b, C5b also gets deposited on the target cell surface. Upon C5b deposition, subsequent complement proteins C6–C9 form the terminal complex, also called the membrane attack complex (MAC). These generate pores in the target cell surface, thus lysing the cell in a process called complement dependent cytotoxicity (CDC).

Complement component C3b also feeds back into the alternative complement pathway, thereby amplifying the initial activation generated by antibody binding. In the alternative pathway, C3b interacts with Factor B to form the alternative C3 convertase (C3bBb), the function of which is also to cleave C3 into C3a and C3b. Generation of more C3b allows formation of the alternative C5 convertase (C3bBb3b) and subsequent production of C5b and CDC.

To prevent uncontrolled amplification and activation of complement, there are a variety of negative regulators that limit complement effects. Membrane bound regulators of complement activation (mRCA) include CD46 (membrane cofactor protein), CD55 (decay accelerating factor, DAF) and CD59 (protectin). These function to prevent MAC formation and lysis of nonpathogenic host cells. CD46 serves as a cofactor for Factor I and promotes C3b degradation to its inactive form, iC3b, so that it no longer contributes to CDC. However, iC3b and other C3b degradation products can still contribute to ADCC through

interaction with various complement receptors expressed on immune effector cells. CD55 prevents CDC by inhibiting the C3 convertase and thus C3b generation. There is also evidence that CD55 can negatively regulate T cell-mediated immunity [10, 11]. CD59 prevents MAC formation downstream of C5b deposition.

In addition to the membrane-bound complement regulators, there are soluble regulators including, but not limited to, C1 inhibitor, C4 binding protein (C4BP), Factor I, Factor H, and Factor H Related proteins (CFHR1-5). C1 inhibitor is a protease inhibitor that hinders the activity of C1 components C1r and C1s, thereby preventing the first step in complement activation by antibody [12]. C4BP inhibits the classical C3 convertase by competitively binding the C4 component. Factor I regulation occurs downstream of C1 inhibitor, and Factor I degrades soluble and surface bound C3b into inactive breakdown products. C3b degradation often involves the help of Factor H or CFHR3, both of which exhibit cofactor activity for Factor I [13]. In addition to cofactor activity, Factor H also displays decay acceleration activity that promotes dissociation of the alternative pathway C3 convertase. The functions of CFHR1-5 proteins are less studied, but it has been demonstrated that CFHR1 can inhibit the C5 convertase and reduce C5b deposition on target cell surfaces [14]. Due to high sequence homology, it is proposed that the other CFHR proteins share similar complement regulatory functions.

COMPLEMENT DEPENDENT CYTOTOXICITY

The vast majority of attention related to the role complement plays in mAb therapy has focused on CDC mediated by the classical complement cascade. Human IgG1 and IgG3 are particularly effective at fixing complement to the target cell surface, and many of the currently approved therapies are of the IgG1 isotype. A variety of cell-based assays have demonstrated the ability of mAb to recruit complement components *in vitro*, but the efficiency of CDC to kill tumor cells *in vivo* is less clear, particularly for solid tumors, in part because tumor cells themselves express membrane bound complement regulators (CD46, CD55, and CD59). These regulators limit MAC formation and lysis of normal and cancer cells alike. In fact, these proteins are overexpressed in a number of tumor types, and their upregulation has been postulated to contribute to mAb resistance *in vivo* [15, 16]. As such, recent efforts investigating the effect of mRCA down-modulation on mAb efficacy have demonstrated that CD55 and CD59 blockade can enhance rituximab efficacy in a xenograft model [17], suggesting an inherent resistance of tumor cells to mAb-induced CDC.

Circulating malignant cells coated with immune complexes that include mAb and complement may have unique characteristics that can result in additional resistance of tumor cells to CDC. In a series of elegant studies, Taylor and colleagues have demonstrated that such complexes on the surface of chronic lymphocytic leukemia cells can be “shaved” off the surface of the cell when the cell passes through the liver or spleen, leaving viable malignant cells that lack the target antigen [18–20], a mechanism that may lead to therapy failure.

Nevertheless, there is considerable evidence supporting CDC as an important *in vivo* mechanism of action for mAb treatment of lymphoma. C1q-deficient mice exhibit poor response to some mAb therapies, and tumor clearance occurred similarly regardless of whether ADCC effector cells were depleted [21, 22]. In chronic lymphocytic leukemia patients, serum complement is quickly consumed upon administration of rituximab, indicating complement activation and potential CDC [23]. In addition, we found that germline variations in complement component *CIQA* and complement regulatory genes *CFH* and *CFHR5* correlate with differential patient response to rituximab, suggesting functional complement is important for antitumor effects [24, 25].

CDC induction is also important in alemtuzumab action, as resistance of chronic lymphocytic leukemia cells to alemtuzumab therapy correlates with decreased complement activation [26]. While trastuzumab alone induces minimal CDC *in vivo*, increased CDC induction has been proposed as the explanation for why trastuzumab and pertuzumab combination therapy is more effective than either mAb alone [27, 28]. Because of these data, many mAb optimization efforts have focused on increasing complement fixation. For example, mAbs with greater C1q affinity have been developed with the goal of increasing CDC [29]. One example is ofatumumab, an alternative anti-CD20 antibody to rituximab that enhances killing by CDC both *in vitro* and in preclinical xenograft models [30, 31]. Clinical trials are ongoing comparing rituximab efficacy to ofatumumab (ClinicalTrials.gov NCT01200589). However, enhanced CDC may not be a requirement for improved clinical efficacy. For example, obinutuzumab, an anti-CD20 antibody that has reduced ability to induce CDC performed better than both rituximab and ofatumumab in xenograft models [32]. Obinutuzumab is also showing promise compared to rituximab in clinical trials for chronic lymphocytic leukemia patients and has been approved by the FDA [33].

ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY

Complement is an effective means of quickly and locally eliminating pathogenic threats and apoptotic cells, particularly in the circulation. However, cancer cells are inherently protected as “self” from complement mediated destruction due to cell surface expression of complement regulatory proteins. Furthermore, concentrations of complement proteins may be less in the tumor microenvironment than in the circulation. This has led some to argue that antibody dependent cellular cytotoxicity (ADCC) may be the central mechanism of action for many anti-cancer mAbs. ADCC occurs when the Fc region of the bound mAb interacts with an Fc receptor on an immune effector cell, resulting in the production of pro-inflammatory cytokines like IFN γ and release of cytotoxic compounds such as perforin and granzyme that can then kill the target cell via an immunologic synapse. Natural killer (NK) cells are generally considered as the main ADCC effector cell type, although macrophages, dendritic cells, neutrophils and eosinophils also express stimulatory Fc γ receptors [34].

The importance of ADCC in mAb efficacy *in vivo* was highlighted in a xenograft model where tumor-bearing Fc γ R-deficient mice treated with mAb exhibited inferior response compared to wild type mice treated with the same mAb [27]. In humans, germline variation in Fc γ RIII correlates with rituximab treatment outcome. Specifically, individuals expressing higher affinity receptors experience increased clinical response to rituximab [35, 36].

Moreover, the high affinity variants are associated with increased NK cell activation, suggesting that ADCC may be more active in individuals expressing these variants [37]. Similar evidence exists for trastuzumab and cetuximab, further supporting the *in vivo* significance of FcγR-mediated mechanisms of anti-tumor action [38, 39].

In humans, stimulatory Fcγ receptors include FcγRI, FcγRIIA, FcγRIIC and FcγRIIIA. In mice, FcγRI, FcγRIII, and FcγRIV are stimulatory IgG receptors [40]. Differences in IgG binding affinities and cellular expression patterns exist between humans and mice, so direct functional correlation between the mouse and human systems can be problematic. Therefore, it is important that the appropriate homolog is selected in mouse knockout studies (for reference, see [40]). Furthermore, data generated by mouse models, such as cell types involved in tumor cell clearing, must be interpreted with caution.

Despite these challenges, effort has been made to increase the ability of therapeutic mAb to induce ADCC. For example, alterations to the Fc region of the mAb have been engineered to increase affinity for the FcγR [41, 42]. Our lab found that less mAb is required to activate NK cells when the binding affinity of mAb Fc to FcR is stronger [43]. In addition, preliminary clinical trials directly comparing two mAbs against the same antigen, but with differing FcγR affinities suggest that the mAb with greater affinity may be more effective clinically [33]. These data suggest ADCC plays a critical role in mAb therapy *in vivo*.

COMPLEMENT REGULATORS AND MAB EFFICACY

Complement regulatory proteins add yet another layer of complexity. Their presence in the tumor microenvironment is generally thought to inhibit mAb induced CDC through the mechanisms described earlier. This led to the experiments knocking down membrane-bound CD55 and CD59 expression with the goal of increasing mAb-induced CDC. Surprisingly, CD55 and CD59 blockade also enhanced ADCC, even in the absence of complement [17, 44]. Similarly, a rodent-specific membrane-bound complement regulator, Cr1, was found to inhibit NK cell-mediated ADCC in the absence of active complement [45]. This suggests that complement regulators can inhibit ADCC, in addition to CDC. Earlier studies suggest this may be mediated by interaction with receptors on lymphocytes [46, 47].

While the membrane-bound complement regulators may decrease ADCC, complement regulators can have additional effects. It was recently found that soluble regulators Factor H and CFHR1 are able to bind complement receptors on human neutrophils [48]. This interaction induces release of cytotoxic granules by neutrophils. Assuming these regulators are able to produce similar effects by binding complement receptors on other effectors, such as NK cells, they could serve to augment mAb-induced ADCC.

THE BALANCE BETWEEN CDC AND ADCC

While many studies of mAb mechanism tend focus on either CDC or ADCC, these processes are interrelated and can influence each other. For example, complement activation by mAb can also serve to recruit immune effector cells through the release of chemotactic components C3a and C5a. In addition, complement and its various breakdown products deposited on the tumor cell surface can activate complement receptor signaling, increasing

phagocytosis and CR-dependent cytotoxicity [15]. Therefore, complement activation can promote both CDC and ADCC, and these mechanisms can act synergistically.

Paradoxically, our lab has demonstrated that active complement can also directly inhibit Fc receptor-mediated functions, and so contribute to reduced ADCC. Specifically, active complement can disrupt the interaction of mAb and the Fc γ R, and also decrease mAb-induced NK cell activation. In a series of experiments, we found that C3b was the complement component likely responsible for inhibiting Fc γ R interaction with rituximab. Our data would suggest that, while complement activation can result in tumor cell killing through CDC and complement receptor-mediated signaling, Fc γ R-mediated ADCC processes might simultaneously be disrupted. In this way, complement fixation may actually compete with ADCC [49].

The primary mechanism of action likely varies depending on the mAb, and could even vary from patient to patient after treatment with the same mAb. There are many factors that could influence whether CDC or ADCC would have greater benefit, one of which is immune complex concentration. It has been shown that the amount of tumor cell lysis by CDC directly correlates with antigen expression, and that having more mAb bound to the target cell surface results in more efficient activation of CDC [50]. Interestingly, ADCC did not directly correlate with target antigen expression in this study, suggesting ADCC might act synergistically with CDC, especially in tumor environments with low antigen expression. Further, if tumor cells down regulate their targets, then the mAb efficacy may depend less on CDC, and more on ADCC, to exert their anti-tumor activity [51].

Another factor influencing mAb mechanism is tumor location. For example, in human genetic studies associating Fc γ R polymorphisms with clinical response to mAb therapy, the high affinity Fc γ R genotype conferred better clinical response to rituximab treatment of nodal disease [36]. Yet, a different study found that Fc γ R affinity for antibody had no impact on clinical response of chronic lymphocytic leukemia (CLL) to rituximab [52]. One explanation might be that CDC is more efficient in the vascular system, while ADCC is more effective in tissues outside the vascular compartment including the tumor microenvironment. This could result from variation in complement component abundance if, for example, complement components are not as readily available in extravascular tumors or are not replenished rapidly when consumed. While not extensively studied, there have been examples where the components necessary for MAC formation are less abundant in bronchioalveolar lavage fluid than in serum [53]. We also found reduced levels of some complement components in transudative pleural and ascites fluid obtained from patients who did not have known malignancy or active infection [49]. More data on complement expression levels will be required to determine whether this proposed phenomenon truly influences mAb action.

The tumor microenvironment at different sites may have attributes other than complement abundance that contribute to sensitivity to mAb therapy. Choice of preclinical models, therefore, is highly important. Injections of cell lines in syngeneic models have the advantage of allowing for study of an immunologic therapy in a model with an intact immune system, but site of injection can influence outcome. For example, orthotopic

injection of renal, colon, and prostate cell lines showed considerably less response to mAb therapy than the same cell lines injected subcutaneously, highlighting the contribution of tumor location, and likely the tumor microenvironment to mAb-induced immune responses [54]. In fact, heterogeneity of the tumor itself can influence response to therapies. Injection of cell lines, even if injected orthotopically, do not recapitulate the genetic diversity of a spontaneous tumor [55]. Such tumors also grow so quickly that the resulting tumor microenvironment may be very different from that found in spontaneous cancer. For all these reasons, results of studies in preclinical models should be interpreted with caution.

COMPLEMENT AND DEVELOPMENT OF ACTIVE IMMUNITY

Findings that complement gene polymorphisms correlate with the outcomes of various diseases ranging from acute infection to cancer to autoimmunity highlight the complexity of complement in immune regulation [56, 57, 25, 58]. There is indirect evidence suggesting that tumor lysis during mAb therapy can lead to cross-presentation of tumor antigens and development of active anti-tumor immunity, and that complement components might contribute to this process. Several complement fragments can enhance the anti-tumor immune response by recruiting antigen-presenting cells to the tumor microenvironment, by enhancing release of pro-inflammatory mediators and by modulating the magnitude of lymphocyte activation [59–61]. These observations conform to the traditional view of complement as pro-inflammatory component and project complement as a positive regulator of anti-tumor immunity.

On the other hand, recent results challenge the concept that complement's role in cancer is predominantly pro-inflammatory. These studies suggest that complement fragments can also suppress the adaptive immune response by various mechanisms. For example, components of activated complement can inhibit the anti-tumor immune response by reducing lymphocytic infiltration in the tumor, and by inhibiting T cell proliferation [62, 63]. Complement can also skew the fine balance between effector and immunosuppressive cells and create an immunosuppressive tumor microenvironment. Complement fragments C3b and C5a have such effects and can enhance myeloid suppressor cell recruitment, or increase conversion of effector T cells to immunosuppressive Treg-like cells [64, 65].

While these studies suggest that active complement can have both pro-inflammatory and immunosuppressive roles, the regulatory mechanisms governing which will predominate in the tumor microenvironment are not well understood. This balance appears to depend, in part, upon which receptors are engaged by activated complement components. For example, a pro-inflammatory environment is produced when C5a binds to C5aR. However, C5a binding to alternative receptor C5L2 seems to produce an anti-inflammatory effect instead, by promoting Treg differentiation [61, 66]. Better understanding of the differential effects complement receptors have on the development of successful tumor-specific memory responses will be important for improving current therapeutic strategies.

CONCLUSIONS

Complement impacts on mAb action, but whether it helps or hurts with respect to the efficacy of mAb therapy of cancer is less clear. The mouse complement system is very different from that of the human. Preclinical models evaluating anti-cancer mAb mechanisms of action have relied heavily upon immunodeficient xenograft models, or inoculation of immunocompetent mice with rapidly growing syngeneic cell lines. Neither approach accurately recapitulates the heterogeneity of the tumor itself, or the microenvironment surrounding a spontaneous tumor. There are a number of complement therapeutics in development that might prove successful in combination with mAb therapy [67], and the evaluation of such combinations could help us determine more definitively whether complement inhibition or activation is desired.

References

1. McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 1998; 16(8):2825–33. [PubMed: 9704735]
2. Sliwkowski MX, Mellman I. Antibody therapeutics in cancer. *Science*. 2013; 341(6151):1192–8.10.1126/science.1241145 [PubMed: 24031011]
3. Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 1999; 17(9):2639–48. [PubMed: 10561337]
4. Villamor N, Montserrat E, Colomer D. Mechanism of action and resistance to monoclonal antibody therapy. *Seminars in oncology*. 2003; 30(4):424–33. [PubMed: 12939711]
5. Brand TM, Iida M, Wheeler DL. Molecular mechanisms of resistance to the EGFR monoclonal antibody cetuximab. *Cancer biology & therapy*. 2011; 11(9):777–92. [PubMed: 21293176]
6. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nature immunology*. 2010; 11(9):785–97.10.1038/ni.1923 [PubMed: 20720586]
7. Vetvicka V, Thornton BP, Wieman TJ, Ross GD. Targeting of natural killer cells to mammary carcinoma via naturally occurring tumor cell-bound iC3b and beta-glucan-primed CR3 (CD11b/CD18). *Journal of immunology*. 1997; 159(2):599–605.
8. Klein E, Di Renzo L, Yefenof E. Contribution of CR3, CD11b/CD18 to cytolysis by human NK cells. *Molecular immunology*. 1990; 27(12):1343–7. [PubMed: 1980339]
9. Li B, Allendorf DJ, Hansen R, Marroquin J, Ding C, Cramer DE, et al. Yeast beta-glucan amplifies phagocyte killing of iC3b-opsonized tumor cells via complement receptor 3-Syk-phosphatidylinositol 3-kinase pathway. *Journal of immunology*. 2006; 177(3):1661–9.
10. Liu J, Miwa T, Hilliard B, Chen Y, Lambris JD, Wells AD, et al. The complement inhibitory protein DAF (CD55) suppresses T cell immunity in vivo. *The Journal of experimental medicine*. 2005; 201(4):567–77.10.1084/jem.20040863 [PubMed: 15710649]
11. Varela JC, Imai M, Atkinson C, Ohta R, Rapisardo M, Tomlinson S. Modulation of protective T cell immunity by complement inhibitor expression on tumor cells. *Cancer research*. 2008; 68(16): 6734–42.10.1158/0008-5472.CAN-08-0502 [PubMed: 18701498]
12. Davis AE 3rd. Biological effects of C1 inhibitor. *Drug news & perspectives*. 2004; 17(7):439–46. [PubMed: 15514703]
13. Skerka C, Chen Q, Fremeaux-Bacchi V, Roumenina LT. Complement factor H related proteins (CFHRs). *Molecular immunology*. 2013; 56(3):170–80.10.1016/j.molimm.2013.06.001 [PubMed: 23830046]

14. Heinen S, Hartmann A, Lauer N, Wiehl U, Dahse HM, Schirmer S, et al. Factor H-related protein 1 (CFHR-1) inhibits complement C5 convertase activity and terminal complex formation. *Blood*. 2009; 114(12):2439–47.10.1182/blood-2009-02-205641 [PubMed: 19528535]
15. Gelderman KA, Kuppen PJ, Okada N, Fleuren GJ, Gorter A. Tumor-specific inhibition of membrane-bound complement regulatory protein Crry with bispecific monoclonal antibodies prevents tumor outgrowth in a rat colorectal cancer lung metastases model. *Cancer research*. 2004; 64(12):4366–72.10.1158/0008-5472.CAN-03-2131 [PubMed: 15205353]
16. Watson NF, Durrant LG, Madjd Z, Ellis IO, Scholefield JH, Spendlove I. Expression of the membrane complement regulatory protein CD59 (protectin) is associated with reduced survival in colorectal cancer patients. *Cancer immunology, immunotherapy : CII*. 2006; 55(8):973–80.10.1007/s00262-005-0055-0
17. Macor P, Tripodo C, Zorzet S, Piovan E, Bossi F, Marzari R, et al. In vivo targeting of human neutralizing antibodies against CD55 and CD59 to lymphoma cells increases the antitumor activity of rituximab. *Cancer research*. 2007; 67(21):10556–63.10.1158/0008-5472.CAN-07-1811 [PubMed: 17975000]
18. Williams ME, Densmore JJ, Pawluczkoawycz AW, Beum PV, Kennedy AD, Lindorfer MA, et al. Thrice-weekly low-dose rituximab decreases CD20 loss via shaving and promotes enhanced targeting in chronic lymphocytic leukemia. *Journal of immunology*. 2006; 177(10):7435–43.
19. Beum PV, Lindorfer MA, Taylor RP. Within peripheral blood mononuclear cells, antibody-dependent cellular cytotoxicity of rituximab-opsonized Daudi cells is promoted by NK cells and inhibited by monocytes due to shaving. *Journal of immunology*. 2008; 181(4):2916–24.
20. Beum PV, Peek EM, Lindorfer MA, Beurskens FJ, Engelberts PJ, Parren PW, et al. Loss of CD20 and bound CD20 antibody from opsonized B cells occurs more rapidly because of trogocytosis mediated by Fc receptor-expressing effector cells than direct internalization by the B cells. *Journal of immunology*. 2011; 187(6):3438–47.10.4049/jimmunol.1101189
21. Di Gaetano N, Cittera E, Nota R, Vecchi A, Grieco V, Scanziani E, et al. Complement activation determines the therapeutic activity of rituximab in vivo. *Journal of immunology*. 2003; 171(3):1581–7.
22. Golay J, Cittera E, Di Gaetano N, Manganini M, Mosca M, Nebuloni M, et al. The role of complement in the therapeutic activity of rituximab in a murine B lymphoma model homing in lymph nodes. *Haematologica*. 2006; 91(2):176–83. [PubMed: 16461301]
23. Kennedy AD, Beum PV, Solga MD, DiLillo DJ, Lindorfer MA, Hess CE, et al. Rituximab infusion promotes rapid complement depletion and acute CD20 loss in chronic lymphocytic leukemia. *Journal of immunology*. 2004; 172(5):3280–8.
24. Charbonneau B, Maurer MJ, Fredericksen ZS, Zent CS, Link BK, Novak AJ, et al. Germline variation in complement genes and event-free survival in follicular and diffuse large B-cell lymphoma. *American journal of hematology*. 2012; 87(9):880–5.10.1002/ajh.23273 [PubMed: 22718493]
25. Racila E, Link BK, Weng WK, Witzig TE, Ansell S, Maurer MJ, et al. A polymorphism in the complement component C1qA correlates with prolonged response following rituximab therapy of follicular lymphoma. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2008; 14(20):6697–703.10.1158/1078-0432.CCR-08-0745 [PubMed: 18927313]
26. Baig NA, Taylor RP, Lindorfer MA, Church AK, Laplant BR, Pavey ES, et al. Complement dependent cytotoxicity in chronic lymphocytic leukemia: ofatumumab enhances alemtuzumab complement dependent cytotoxicity and reveals cells resistant to activated complement. *Leukemia & lymphoma*. 2012; 53(11):2218–27.10.3109/10428194.2012.681657 [PubMed: 22475085]
27. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. *Nature medicine*. 2000; 6(4):443–6.10.1038/74704
28. Mamidi S, Cinci M, Hasmann M, Fehring V, Kirschfink M. Lipoplex mediated silencing of membrane regulators (CD46, CD55 and CD59) enhances complement-dependent anti-tumor activity of trastuzumab and pertuzumab. *Molecular oncology*. 2013; 7(3):580–94.10.1016/j.molonc.2013.02.011 [PubMed: 23474221]
29. Idusogie EE, Wong PY, Presta LG, Gazzano-Santoro H, Totpal K, Ultsch M, et al. Engineered antibodies with increased activity to recruit complement. *Journal of immunology*. 2001; 166(4):2571–5.

30. Li B, Shi S, Qian W, Zhao L, Zhang D, Hou S, et al. Development of novel tetravalent anti-CD20 antibodies with potent antitumor activity. *Cancer research*. 2008; 68(7):2400–8.10.1158/0008-5472.CAN-07-6663 [PubMed: 18381448]
31. Barth MJ, Hernandez-Ilizaliturri FJ, Mavis C, Tsai PC, Gibbs JF, Deeb G, et al. Ofatumumab demonstrates activity against rituximab-sensitive and -resistant cell lines, lymphoma xenografts and primary tumour cells from patients with B-cell lymphoma. *British journal of haematology*. 2012; 156(4):490–8.10.1111/j.1365-2141.2011.08966.x [PubMed: 22150234]
32. Herter S, Herting F, Mundigl O, Waldhauer I, Weinzierl T, Fauti T, et al. Preclinical activity of the type II CD20 antibody GA101 (obinutuzumab) compared with rituximab and ofatumumab in vitro and in xenograft models. *Molecular cancer therapeutics*. 2013; 12(10):2031–42.10.1158/1535-7163.MCT-12-1182 [PubMed: 23873847]
33. Goede V, Fischer K, Busch R, Jaeger U, Dillhuydy MS, Wickham N, et al. Chemoimmunotherapy with GA101 plus chlorambucil in patients with chronic lymphocytic leukemia and comorbidity: results of the CLL11 (BO21004) safety run-in. *Leukemia*. 2013; 27(5):1172–4.10.1038/leu.2012.252 [PubMed: 22936013]
34. Nimmerjahn F, Ravetch JV. Fcγ receptors: old friends and new family members. *Immunity*. 2006; 24(1):19–28.10.1016/j.immuni.2005.11.010 [PubMed: 16413920]
35. Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, Colombat P, et al. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcγRIIIa gene. *Blood*. 2002; 99(3):754–8. [PubMed: 11806974]
36. Weng WK, Levy R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2003; 21(21):3940–7.10.1200/JCO.2003.05.013 [PubMed: 12975461]
37. Veeramani S, Wang SY, Dahle C, Blackwell S, Jacobus L, Knutson T, et al. Rituximab infusion induces NK activation in lymphoma patients with the high-affinity CD16 polymorphism. *Blood*. 2011; 118(12):3347–9.10.1182/blood-2011-05-351411 [PubMed: 21768303]
38. Musolino A, Naldi N, Bortesi B, Pezzuolo D, Capelletti M, Missale G, et al. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2008; 26(11):1789–96.10.1200/JCO.2007.14.8957 [PubMed: 18347005]
39. Bibeau F, Lopez-Crapez E, Di Fiore F, Thezenas S, Ychou M, Blanchard F, et al. Impact of Fc{γ}RIIa-Fc{γ}RIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2009; 27(7):1122–9.10.1200/JCO.2008.18.0463 [PubMed: 19164213]
40. Bruhns P. Properties of mouse and human IgG receptors and their contribution to disease models. *Blood*. 2012; 119(24):5640–9.10.1182/blood-2012-01-380121 [PubMed: 22535666]
41. Shields RL, Namenuk AK, Hong K, Meng YG, Rae J, Briggs J, et al. High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. *The Journal of biological chemistry*. 2001; 276(9):6591–604.10.1074/jbc.M009483200 [PubMed: 11096108]
42. Wright A, Morrison SL. Effect of glycosylation on antibody function: implications for genetic engineering. *Trends in biotechnology*. 1997; 15(1):26–32.10.1016/S0167-7799(96)10062-7 [PubMed: 9032990]
43. Bowles JA, Wang SY, Link BK, Allan B, Beuerlein G, Campbell MA, et al. Anti-CD20 monoclonal antibody with enhanced affinity for CD16 activates NK cells at lower concentrations and more effectively than rituximab. *Blood*. 2006; 108(8):2648–54.10.1182/blood-2006-04-020057 [PubMed: 16825493]
44. Finberg RW, White W, Nicholson-Weller A. Decay-accelerating factor expression on either effector or target cells inhibits cytotoxicity by human natural killer cells. *Journal of immunology*. 1992; 149(6):2055–60.

45. Caragine TA, Imai M, Frey AB, Tomlinson S. Expression of rat complement control protein Crry on tumor cells inhibits rat natural killer cell-mediated cytotoxicity. *Blood*. 2002; 100(9):3304–10.10.1182/blood.V100.9.3304 [PubMed: 12384431]
46. Hamann J, Vogel B, van Schijndel GM, van Lier RA. The seven-span transmembrane receptor CD97 has a cellular ligand (CD55, DAF). *The Journal of experimental medicine*. 1996; 184(3): 1185–9. [PubMed: 9064337]
47. Shenoy-Scaria AM, Kwong J, Fujita T, Olszowy MW, Shaw AS, Lublin DM. Signal transduction through decay-accelerating factor. Interaction of glycosyl-phosphatidylinositol anchor and protein tyrosine kinases p56lck and p59fyn 1. *Journal of immunology*. 1992; 149(11):3535–41.
48. Losse J, Zipfel PF, Jozsi M. Factor H and factor H-related protein 1 bind to human neutrophils via complement receptor 3, mediate attachment to *Candida albicans*, and enhance neutrophil antimicrobial activity. *Journal of immunology*. 2010; 184(2):912–21.10.4049/jimmunol.0901702
49. Wang SY, Veeramani S, Racila E, Cagley J, Fritzinger DC, Vogel CW, et al. Depletion of the C3 component of complement enhances the ability of rituximab-coated target cells to activate human NK cells and improves the efficacy of monoclonal antibody therapy in an in vivo model. *Blood*. 2009; 114(26):5322–30.10.1182/blood-2009-01-200469 [PubMed: 19805620]
50. van Meerten T, van Rijn RS, Hol S, Hagenbeek A, Ebeling SB. Complement-induced cell death by rituximab depends on CD20 expression level and acts complementary to antibody-dependent cellular cytotoxicity. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2006; 12(13):4027–35.10.1158/1078-0432.CCR-06-0066 [PubMed: 16818702]
51. Pohlmann PR, Mayer IA, Mernaugh R. Resistance to Trastuzumab in Breast Cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2009; 15(24):7479–91.10.1158/1078-0432.CCR-09-0636 [PubMed: 20008848]
52. Farag SS, Flinn IW, Modali R, Lehman TA, Young D, Byrd JC. Fc gamma RIIa and Fc gamma RIIa polymorphisms do not predict response to rituximab in B-cell chronic lymphocytic leukemia. *Blood*. 2004; 103(4):1472–4.10.1182/blood-2003-07-2548 [PubMed: 14563637]
53. Bolger MS, Ross DS, Jiang H, Frank MM, Ghio AJ, Schwartz DA, et al. Complement levels and activity in the normal and LPS-injured lung. *American journal of physiology Lung cellular and molecular physiology*. 2007; 292(3):L748–59.10.1152/ajplung.00127.2006 [PubMed: 17071722]
54. Devaud C, Westwood JA, John LB, Flynn JK, Paquet-Fifield S, Duong CP, et al. Tissues in different anatomical sites can sculpt and vary the tumor microenvironment to affect responses to therapy. *Molecular therapy : the journal of the American Society of Gene Therapy*. 2014; 22(1): 18–27.10.1038/mt.2013.219 [PubMed: 24048441]
55. Kerbel RS. Human tumor xenografts as predictive preclinical models for anticancer drug activity in humans: better than commonly perceived-but they can be improved. *Cancer biology & therapy*. 2003; 2(4 Suppl 1):S134–9. [PubMed: 14508091]
56. Racila DM, Sontheimer CJ, Sheffield A, Wisniewski JJ, Racila E, Sontheimer RD. Homozygous single nucleotide polymorphism of the complement C1QA gene is associated with decreased levels of C1q in patients with subacute cutaneous lupus erythematosus. *Lupus*. 2003; 12(2):124–32. [PubMed: 12630757]
57. Sontheimer RD, Racila E, Racila DM. C1q: its functions within the innate and adaptive immune responses and its role in lupus autoimmunity. *The Journal of investigative dermatology*. 2005; 125(1):14–23.10.1111/j.0022-202X.2005.23673.x [PubMed: 15982298]
58. Chen M, Daha MR, Kallenberg CG. The complement system in systemic autoimmune disease. *Journal of autoimmunity*. 2010; 34(3):J276–86.10.1016/j.jaut.2009.11.014 [PubMed: 20005073]
59. Carroll MC. The complement system in regulation of adaptive immunity. *Nature immunology*. 2004; 5(10):981–6.10.1038/ni1113 [PubMed: 15454921]
60. Carroll MC. The complement system in B cell regulation. *Molecular immunology*. 2004; 41(2–3): 141–6.10.1016/j.molimm.2004.03.017 [PubMed: 15159059]
61. Strainic MG, Shevach EM, An F, Lin F, Medof ME. Absence of signaling into CD4(+) cells via C3aR and C5aR enables autoinductive TGF-beta1 signaling and induction of Foxp3(+) regulatory T cells. *Nature immunology*. 2013; 14(2):162–71.10.1038/ni.2499 [PubMed: 23263555]

62. Gunn L, Ding C, Liu M, Ma Y, Qi C, Cai Y, et al. Opposing roles for complement component C5a in tumor progression and the tumor microenvironment. *Journal of immunology*. 2012; 189(6): 2985–94.10.4049/jimmunol.1200846
63. Wagner C, Ochmann C, Schoels M, Giese T, Stegmaier S, Richter R, et al. The complement receptor 1, CR1 (CD35), mediates inhibitory signals in human T-lymphocytes. *Molecular immunology*. 2006; 43(6):643–51.10.1016/j.molimm.2005.04.006 [PubMed: 16360013]
64. Markiewski MM, DeAngelis RA, Benencia F, Ricklin-Lichtsteiner SK, Koutoulaki A, Gerard C, et al. Modulation of the antitumor immune response by complement. *Nature immunology*. 2008; 9(11):1225–35.10.1038/ni.1655 [PubMed: 18820683]
65. Barchet W, Price JD, Cella M, Colonna M, MacMillan SK, Cobb JP, et al. Complement-induced regulatory T cells suppress T-cell responses but allow for dendritic-cell maturation. *Blood*. 2006; 107(4):1497–504.10.1182/blood-2005-07-2951 [PubMed: 16239430]
66. Weaver DJ Jr, Reis ES, Pandey MK, Kohl G, Harris N, Gerard C, et al. C5a receptor-deficient dendritic cells promote induction of Treg and Th17 cells. *European journal of immunology*. 2010; 40(3):710–21.10.1002/eji.200939333 [PubMed: 20017191]
67. Ricklin D, Lambris JD. Progress and trends in complement therapeutics. *Advances in experimental medicine and biology*. 2013; 735:1–22.

Figure 1. Complement and mAb Efficacy

Antibody therapy activates complement through the classical pathway, with subsequent amplification through the alternative pathway, and complement regulators (red text) act on many levels. The components that impact mAb anti-tumor activity (circled in blue) have complex effects, and a summary is given below.

C1_{active}: Promotes mAb induced CDC [21, 22, 24].

C3: Inhibits T cell proliferation through CR1 signaling [63].

C3b: Prevents ADCC by disrupting mAb interaction with FcγR [49].

C3a and C5a: Promotes CDC, but Induces inflammatory (and possibly tumor-permissive) microenvironment [59–62, 64].

DAF (CD55), MCP (CD46), and CD59: Prevent CDC, but may also directly block ADCC [15–17, 44, 46–47].

Table 1

Currently approved monoclonal antibodies for cancer therapy

FDA-Approved Antibodies	Antigen	Indications	Company	Complement Fixation
rituximab	CD20	B-cell malignancies (NHL, CLL)	Roche	Y
trastuzumab	HER2	Breast, Gastric	Genentech	Y
cetuximab	EGFR	Colorectal, Head and Neck SCC	Bristol-Myers Squibb	Y
panitumumab	EGFR	Colorectal	Amgen	N
pertuzumab	HER2	Breast	Genentech	Y
bevacizumab	VEGF	Colorectal, NSCLC, Glioblastoma, Kidney	Genentech/Roche	Y
ipilimumab	CTLA4	Melanoma	Bristol-Myers Squibb	Y
ofatumumab	CD20	CLL	GlaxoSmithKline	Y
alemtuzumab	CD52	CLL	Genzyme	Y
obinutuzumab (GA101)	CD20	CLL	Roche	N
brentuximab vedotin	CD30	Hodgkin's Lymphoma	Seattle Genetics	N
ado-trastuzumab emtansine	HER2	Breast	Genentech	Y
⁹⁰ Y-labeled ibritumomab tiuxetan	CD20	Follicular Lymphoma	IDEC Pharmaceuticals	N
¹³¹ I-labeled tositumomab	CD20	Follicular Lymphoma	GlaxoSmithKline	Y

Abbreviations: NHL, non-hodgkin's lymphoma; CLL, chronic lymphocytic leukemia; SCC, squamous cell carcinoma; NSCLC, non-small cell lung cancer; Y, yes; N, no.