

New monoclonal antibodies for non-Hodgkin's lymphoma

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introduction

The anti-CD20 rituximab represents the first widely available antibody for the treatment of cancer based on significant single-agent activity in indolent lymphoma [1]. In diffuse large B-cell lymphoma (DLBCL), the incorporation of rituximab with standard chemotherapy improves survival [2]. However, while it is clear that the use of rituximab has had a significant impact in improving outcomes in B-cell lymphoma, primary and secondary resistance remains an issue. Given the proof of principle for antibody-based lymphoma therapy with rituximab, a number of groups have pursued the development of monoclonals which either target CD20 in a potentially enhanced fashion or are directed against novel targets. Many of these efforts have demonstrated early promise; however, the challenges of developing new treatments in this era remain substantial.

properties of antibodies for effective anti-B-cell therapy

Several target antigen characteristics are relevant to the development of an effective antibody for B-lymphoma therapeutics. It is desirable that a candidate target antigen is preferentially expressed on malignant cells, is not modulated or secreted and serves an important biologic function. The fact that CD20 meets only some of these criteria, yet is effective, demonstrates that a non-ideal target antigen can still be valuable. In addition to the target, it is critical that anti-cancer antibodies are associated with effective anti-tumor mechanisms. Possibilities include antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), vaccine-like effects, direct cytotoxicity through induction of target cell signaling and delivery of a radioactive or toxic molecule to target cells (an area separate from the focus of this review). One can also envision that depending on the dominant pathway, improvements in the antibody with respect to choice of antigen, degree of binding, enhancement of immune-based mechanisms or co-administration of other agents all might provide better efficacy.

Several modifications are under assessment in novel antibodies undergoing preclinical and clinical studies. Antibody binding to effector cells through activating Fc receptors (FcR,

R11, CD16) is an important aspect of the activity of rituximab and some new agents have improved binding. Additionally, therapeutic antibodies include two major subtypes, Fc type I which fixes complement, and type II which does not [3]. The importance of CDC in rituximab-mediated elimination of cancer cells is unclear, and likely depends on the tumor subtype and the specific treatment regimen. Several efforts (including new anti-CD20 agents in CLL) are focused on the delivery of enhanced CDC. Other studies have suggested that the rituximab-induced apoptosis of tumor cells can induce a secondary immune response resulting in a vaccine-like effect. Direct cytotoxicity of monoclonal antibodies relates to the properties of the target, and certainly novel agents against new antigens may more effectively mediate these therapeutic effects. Finally, antibody engineering away from murine protein structure (as well as chimeric agents) toward humanized or human antibodies offers potential for improved pharmacokinetics that could result in enhanced therapeutic effects although the clinical importance of immunogenicity remains to be fully defined.

novel anti-CD20 antibodies

Several novel 'second generation' anti-CD20 antibodies are undergoing preclinical and clinical development. To capitalize on the potential for CDC to kill tumor cells of atumumab (human CD20) is associated with increased complement activity relative to that of rituximab. This IgG1 antibody also has type 1 characteristics, meaning that when it binds to CD20 it induces its translocation into detergent-insoluble lipid rafts associated with complement activation. This agent has potentially important differences from rituximab, in that it has a fully human sequence, it binds to an epitope on CD20 closer to the cell membrane and has a slower off-rate. The latter two properties may increase complement activation [4].

Ofatumumab is the subject of several ongoing clinical trials. A phase I/II study in patients with recurrent follicular lymphoma includes four weekly doses from 300 to 1000 mg [5]. Toxicity was similar to that of rituximab, and responses occurred in 15 of 36 evaluable patients, including 8 of 14 patients previously treated with rituximab. An area of focus for this agent has been in chronic lymphocytic leukemia (CLL), where enhanced CDC may be of value. In a phase I/II trial, CLL

patients received four weekly infusions of up to 2000 mg of ofatumumab with substantial response rates (as high as 50%). While this is better than data reported with rituximab in this setting, whether these findings reflect an improved antibody versus a higher dose remains unclear since higher than standard rituximab doses can induce more responses. A second study is ongoing in patients with CLL who have progressed after fludarabine and alemtuzumab, in addition to other ongoing trials in various settings. Another novel anti-CD20 is veltuzumab (hA20), a humanized IgG1 monoclonal that targets the same epitope as rituximab [6]. Preclinical studies demonstrated activity similar to that of rituximab; while in the clinic it is noteworthy that veltuzumab has delivered objective responses in indolent lymphoma patients at doses lower than those generally used with rituximab. A phase I/II study of 68 patients with relapsed B non-Hodgkin lymphoma (NHL) evaluated dosing from 80 mg/m² to 750 mg/m² weekly for 4 weeks, and at all dose levels objective responses (including half of follicular subjects) were observed [7]. While the dose–response curve for rituximab has not been well defined, it is possible that effects in different settings or with different agents may be altered based on the relevant mechanism. A study of low doses of veltuzumab administered subcutaneously is ongoing.

Since ADCC is an important mechanism of action for rituximab, numerous efforts are attempting to enhance antibody binding to activating Fc receptors to potentially improve potency. The ability of IgG1 antibodies to mediate ADCC is related to asparagine-linked oligosaccharides associated with the Fc region. As many monoclonals (including rituximab) are produced in Chinese hamster ovary (CHO) cells with high levels of fucosylation 1–6 fucosyltransferase interferes with binding to activating FcR3 receptors while removal of fucose improves ADCC [8]. GA101 is a type II antibody generated from CHO cells that have been engineered to have higher levels of β -1,4-*N*-acetylglucosaminyl transferase, resulting in antibodies with bisected, afucosylated, Fc region carbohydrates. This agent also has an altered hinge region associated with greater apoptosis-inducing activity [9]. Preclinical studies demonstrated enhanced ADCC and apoptotic effects, and ongoing clinical studies will determine whether these modifications result in improved efficacy. Other efforts are underway to evaluate different strategies to enhance ADCC. AME-133 is a human IgG1 antibody with high affinity for CD20 and an Fc region capable of binding to CD16 with 5- to 10-fold greater affinity than rituximab [10]. Preclinical studies suggest that AME-133 was more effective than rituximab at natural killer-cell activation and can overcome the effects of less favorable FcR polymorphisms. A phase I/II trial is underway in patients with relapsed follicular lymphoma (non-rituximab refractory) and at least one allele corresponding to the low-affinity Fc γ R3A. The hypothesis is that these antibody modifications will result in improved efficacy in patients with suboptimal immune effector-cell status.

antibodies against targets other than CD20

CD22 is widely expressed on normal and malignant B cells, and its function appears to relate to B-cell activation and adhesion.

Epratuzumab is a humanized IgG1 anti-CD22 antibody associated with both ADCC and direct cytotoxicity in preclinical studies. Phase I/II studies demonstrated objective responses across various dose levels in both follicular and DLBCL [11]. Toxicity was manageable and consisted primarily of infusion-related reactions. Phase II studies have been conducted of concurrent administration of epratuzumab and rituximab [12]. Objective responses were noted in about two-thirds of patients with follicular lymphoma and about half of a smaller number of DLBCL subjects, including some complete responses. Comparative studies are necessary to determine whether effects can be attributed to one or both antibodies, while toxicities of the combination are comparable to those of single-agent rituximab. Ongoing studies are currently evaluating the use of epratuzumab in combination with CHOP chemotherapy plus rituximab in DLBCL, while the Cancer and Leukemia Group B (CALGB) has activated a trial to evaluate the combination of epratuzumab + rituximab (combined biologics without chemotherapy) in the initial treatment of follicular lymphoma.

Another relevant target for immunotherapy of B-cell lymphoma is CD40, a member of the tumor necrosis factor receptor family. It is detected by immunohistochemistry on malignant T and B cells and has a role in cellular proliferation and differentiation. SGN-40 is a humanized IgG1 antibody with high affinity for CD40, which has been associated with anti-lymphoma effects in preclinical studies. A phase I study in patients with recurrent B-cell NHL demonstrated an acceptable toxicity profile and five objective responses [13]. Activity in DLBCL was particularly encouraging, and led to two ongoing phase II studies in this area, one as a single agent and one employing the combination of SGN-40 with cytotoxic chemotherapy.

CD80 represents an additional target for lymphoma therapy, given its presence on the surface of various B-cell and Hodgkin lymphomas and its role in activation of T and B cells. Antibody binding of CD80 has been associated with various pro-apoptotic effects. Galiximab is a human–primate chimeric anti-CD80 antibody with excellent tolerability and single-agent activity in recurrent follicular lymphoma [14]. Since preclinical data suggest a potential role for the combination of rituximab and galiximab, a phase I/II trial was conducted which demonstrated a 64% response rate and a 12.1 month median progression-free survival [15]. A phase III trial comparing rituximab with the combination of rituximab plus galiximab in patients with relapsed follicular lymphoma is ongoing, while an additional CALGB study is currently evaluating the combination of rituximab and galiximab as initial treatment in follicular NHL.

conclusions

Therapeutic monoclonal antibodies have provided significant benefit for patients with NHL. Virtually all patients with B-cell lymphoma receive rituximab at multiple times over their treatment. Radiolabeled antibodies can be effective in rituximab-resistant and chemotherapy-resistant disease. While significant efforts continue in this area, the logistics, hematologic toxicity and other factors have limited their use

and largely preclude their concurrent use with standard chemotherapy. Novel anti-CD20 agents offer the potential for enhanced activity relative to that of rituximab, while agents directed against different targets offer the possibility of combination with rituximab. However, many challenges exist in the clarification of the optimal use of such novel agents. Whether new anti-CD20s are better than rituximab requires randomized comparative trials or demonstration of effectiveness in rituximab-refractory patients. Novel combinations can only be vetted through comparative studies with rituximab alone. Whether or not such combinations are effective, and warrant the associated costs, remains to be seen. Nonetheless, the promise of antibody-based therapeutics in lymphoma suggests that development of such agents offers the potential for significant clinical benefit for patients.

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references

- McLaughlin P, Grillo-Lopez AJ, Link BK et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J Clin Oncol* 1998; 16: 2825–2833.
- Feugier P, Van Hoof A, Sebban C et al. Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: A study by the Groupe d'Etude des Lymphomes de l'Adulte. *J Clin Oncol* 2005; 23: 4117–4126.
- Cragg MS, Glennie MJ. Antibody specificity controls in vivo effector mechanisms of anti-CD20 reagents. *Blood* 2004; 103: 2738–2743.
- Teeling JL, French RR, Cragg MS et al. Characterization of new human CD20 monoclonal antibodies with potent cytolytic activity against non-Hodgkin lymphomas. *Blood* 2004; 104: 1793–1800.
- Hagenbeek A, Plesner T, Johnson P et al. HuMax-CD20, a novel fully human anti-CD20 monoclonal antibody: results of a phase I/II trial in relapsed or refractory follicular non-Hodgkin's lymphoma. *ASH Annual Meeting Abstracts* 2005; 106: 4760.
- Stein R, Qu Z, Chen S et al. Characterization of a new humanized anti-CD20 monoclonal antibody, IMMU-106, and its use in combination with the humanized anti-CD22 antibody, epratuzumab, for the therapy of non-Hodgkin's lymphoma. *Clin Cancer Res* 2004; 10: 2868–2878.
- Morschhauser F, Leonard JP, Coiffier B et al. Phase I/II results of a second-generation humanized anti-CD20 antibody, IMMU-106 (hA20), in NHL. *ASCO Meeting Abstracts* 2006; 24: 7530.
- Shinkawa T, Nakamura K, Yamane N. The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. *J Biol Chem* 2003; 278: 3466–3473.
- Umana P, Moessner E, Bruenker P et al. Novel 3rd generation humanized type II CD20 antibody with glycoengineered Fc and modified elbow hinge for enhanced ADCC and superior apoptosis induction. *ASH Annual Meeting Abstracts* 2006; 108: 229.
- Weiner GJ, Bowles JA, Link BK, Campbell MA, Wooldridge JE, Breitmeyer JB. Anti-CD20 monoclonal antibody (mAb) with enhanced affinity for CD16 activates NK cells at lower concentrations and more effectively than rituximab (R). *ASH Annual Meeting Abstracts* 2005; 106: 348.
- Leonard JP, Coleman M, Ketas JC et al. Phase I/II trial of epratuzumab (humanized anti-CD22 antibody) in indolent non-Hodgkin's lymphoma. *J Clin Oncol* 2003; 21: 3051–3059.
- Leonard JP, Coleman M, Ketas J et al. Combination antibody therapy with epratuzumab and rituximab in relapsed or refractory non-Hodgkin's lymphoma. *J Clin Oncol* 2005; 23: 5044–5051.
- Advani R, Forero-Torres A, Furman RR et al. SGN-40 (anti-huCD40 mAb) monotherapy induces durable objective responses in patients with relapsed aggressive non-Hodgkin's lymphoma: evidence of antitumor activity from a phase I study. *ASH Annual Meeting Abstracts* 2006; 108: 695.
- Czuczman MS, Thall A, Witzig TE et al. Phase I/II study of galiximab, an anti-CD80 antibody, for relapsed or refractory follicular lymphoma. *J Clin Oncol* 2005; 23: 4390–4398.
- Friedberg JW, Leonard JP, Younes A et al. Updated results from a phase II study of galiximab (anti-CD80) in combination with rituximab for relapsed or refractory, follicular NHL. *ASH Annual Meeting Abstracts* 2005; 106: 2435.