Ocaratuzumab, an Fc-engineered antibody demonstrates enhanced antibody-dependent cell-mediated cytotoxicity in chronic lymphocytic leukemia

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Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; CDC, complement-dependent cytotoxicity; CLL, chronic lymphocytic leukemia; E:T ratio, effector to target ratio; ER, experimental release; i.v., intravenous; mAb, monoclonal antibody; MDM, monocyte-derived macrophages; MR, maximum release; s.c., subcutaneous; SR, spontaneous release

The enhanced ADCC of ocaratuzumab suggests that it may be effective at low concentrations. If supported by clinical investigation, this feature could potentially allow for subcutaneous dosing at low doses that could expand the potential of administering chemoimmunotherapy in developing countries.

Introduction

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in developed nations and may be more common in developing countries than currently known due to the lack of health care access and data recording in these countries. CD20 is a protein expressed on the B cell surface which is present on malignant, non-malignant, resting and active early pre-B cells through B cell development until differentiation into plasma cells. Several anti-CD20 monoclonal antibodies (mAbs), including rituximab...
(Rituxan®), ofatumumab (Arzerra®), and obinutuzumab (Gazyva™), are approved for the treatment of B cell malignancies. Antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC) have been identified as crucial mechanisms of action for these mAbs, although direct killing may also contribute in part to the cytotoxicity. Despite the high doses required when rituximab or ofatumumab is administered as single-agent CLL therapy, the single-agent activity is still less durable than that observed when administered in follicular lymphoma patients, which is potentially secondary to low density of CD20 expression on CLL cells. The higher dose requirements, inherent cost, and exclusively intravenous (i.v.) administration of rituximab and ofatumumab prohibit the use of the mAbs in developing nations. The need for cost effective and convenient administration of CLL therapy is apparent in these countries.

Ocaratuzumab (AME-133v, LY2469298) is a humanized IgG1 anti-CD20 mAb with Fc-engineering for more effective ADCC. In preclinical studies completed with SKW6.4 lymphoma cell line and primary B-lymphocytes, ocaratuzumab demonstrated a 13- to 20-fold increase in binding affinity for CD20 compared with rituximab. Additionally, ocaratuzumab was approximately 6-fold more potent than rituximab in ADCC assays. In monkey models, ocaratuzumab in i.v. and AME-133E (a closely related antibody) in subcutaneous (s.c.) formulation showed tolerability and dose-dependent B cell depletion.

Figure 1. Ocaratuzumab, rituximab, and ofatumumab mediated similar levels of direct cytotoxicity in the presence of a crosslinking antibody, antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC) in chronic lymphocytic leukemia (CLL) cells. (A) To assess direct cytotoxicity, CLL cells were treated with antibodies at 10 μg/mL and goat anti-human IgG crosslinker at 50 μg/mL for 48 h (n = 8). Error bars represent the range with central bar as mean. Boxes represent confidence interval. (B) ADCP by normal donor monocyte-derived macrophages of CLL cell targets coated with antibodies, as measured by flow cytometry (n = 16). Each dot represents a patient value. (C) CDC in CLL cells with 30% autologous plasma (either active (dark circles) or heat-inactivated (light circles)) using 10 μg/mL of antibodies (n = 9).
In two Phase 1 clinical studies for patients with previously treated follicular lymphoma, ocaratuzumab was well tolerated at all doses tested.8,11 Ocaratuzumab demonstrated a dose-dependent, rapid, and specific depletion of the B cells in all patients receiving at least 7.5 mg/m² of the mAb.8 Clinical activity was seen with response rates of 22–50% in these studies,8,11 even in patients previously treated with rituximab.11 Based on ocaratuzumab’s efficacy in pre-clinical investigation in B cell malignancy cells/cell lines and its tolerability in clinical trials for follicular lymphoma patients, we aimed to determine the pre-clinical efficacy of ocaratuzumab against primary CLL cells. As the mAb has demonstrated potency, our ultimate goal is to provide scientific rationale for development of a s.c. formulation that can be cost effectively administered at a low dose and conveniently administered to CLL patients in developed as well as developing nations.

Results

Direct cytotoxicity in the presence of a crosslinking antibody

Both rituximab and ofatumumab mediate minimal direct cytotoxicity in the presence of a crosslinking antibody.4 To evaluate whether the higher binding affinity of ocaratuzumab to CD20 would induce greater levels of direct cytotoxicity compared with other anti-CD20 mAbs in CLL cells, we treated CLL cells with ocaratuzumab, rituximab, or ofatumumab (10 μg/mL) and anti-human IgG crosslinker (50 μg/mL) for 48 h (n = 8); ocaratuzumab has very minimal activity when used without crosslinking antibody, as demonstrated in Figure S1. With ocaratuzumab treatment, a median of 70.6% (range = 42.9–128.5%) of CLL cells remained alive after normalizing values to a “crosslinker-alone” condition. Compared with rituximab, ocaratuzumab induced direct cytotoxicity at a similar level (difference = -4.35% with 95% CI = -12.8%, 4.13%; P = 0.81) and obinutuzumab (difference = -2% with 95% CI = -7.22, 3.22; P = 0.44) and at a lower rate than ofatumumab (difference = 19.66% with 95% CI = 14.1%, 25.2%; P < 0.0001). These data demonstrate that ofatumumab mediates superior CDC in primary CLL cells than ocaratuzumab, rituximab, or obinutuzumab.

Antibody-dependent cellular phagocytosis

In murine models, monocytes have been shown to be the dominant effector cells for anti-CD20-induced B cell depletion,12 and antibody-dependent cellular phagocytosis (ADCP) is thought to be an important cause of antibody-mediated cytotoxicity in human cancer cells. Ofatumumab has demonstrated significantly more ADCP than rituximab and obinutuzumab when tested in primary CLLs cells.9 We found that ocaratuzumab mediated ADCP at a rate of 12.3%, which was slightly less than ofatumumab (difference = -6.56 with 95% CI = -9.19, -0.06; P = 0.05) and similar to rituximab (difference = 1.15 with 95% CI = -3.41, -5.72; P = 0.61) and obinutuzumab (difference = 3.03% with 95% CI = -1.56, 7.62; P = 0.20). Results of the ADCP assay are detailed in Figure 1B. Therefore, we have demonstrated that ocaratuzumab, rituximab, and obinutuzumab mediate similar levels of ADCP, and ofatumumab-induced ADCP is slightly superior to that seen with ocaratuzumab in primary CLL cells.

Complement-dependent cytotoxicity

CDC is another mechanism of antibody-induced cytotoxicity. In primary CLL cells, ofatumumab has been shown to mediate superior CDC compared with rituximab and obinutuzumab.4 To compare cytotoxicity via initiation of complement between the mAbs, we evaluated complement-induced killing in primary CLL cells. CDC was detected by incubation of primary CLL cells with ocaratuzumab, rituximab, ofatumumab, or obinutuzumab (10 μg/mL) in 30% autologous plasma as described in the methods section (n = 9). As detailed in Figure 1C, ocaratuzumab mediated minimal CDC (0.11% cytotoxicity). This result was similar to the CDC caused by rituximab (difference = 0.66% with 95% CI = -4.9%, 6.2%; P = 0.81) and obinutuzumab (difference = -2% with 95% CI = -7.22, 3.22; P = 0.44) and at a lower rate than ofatumumab (difference = 19.66% with 95% CI = 14.1%, 25.2%; P < 0.0001). These data demonstrate that ofatumumab mediates superior CDC in primary CLL cells than ocaratuzumab, rituximab, or obinutuzumab.

Allogeneic antibody-dependent cell-mediated cytotoxicity

ADCC is thought to play another crucial role in antibody-induced cytotoxicity. Similarly to obinutuzumab, ocaratuzumab’s Fc portion was specifically optimized to mediate ADCC. In previous studies with CLL cells, at higher concentrations (5 μg/mL), obinutuzumab was shown to mediate superior ADCC than rituximab and ofatumumab. However, at decreasing concentrations (≤ 0.05 μg/mL), the enhanced ADCC with obinutuzumab was no longer apparent.4 We performed a comparison of ocaratuzumab with the other anti-CD20 mAbs in primary CLL cells using 51Cr-release assays. Using random donor NK cells with an effector to target (E:T) ratio of 6:1, ocaratuzumab mediated more effective ADCC than rituximab (0.1 μg/mL through 10 μg/mL; P < 0.03) and ofatumumab (1 μg/ml through 10 μg/ml; P < 0.005) at lower mAb concentrations as demonstrated in Figure 2. At low mAb concentrations (0.01 μg/ml through 1 μg/ml), ocaratuzumab and obinutuzumab mediated similar ADCC (n = 10). At the concentration of 10 μg/mL, ocaratuzumab demonstrated superior ADCC compared with rituximab or ofatumumab at all E:T ratios tested (E:T = 25:1; P < 0.001 for all comparisons; n = 6) as demonstrated in Figure 3A. Relative cytotoxicity at E:T = 25:1 was 43%, 15%, and 16% for ocaratuzumab, rituximab, and ofatumumab, respectively. Secondary to ocaratuzumab’s potency at low doses, an extended ADCC E:T titration with antibody concentrations at 0.1 μg/mL was also performed (Fig. 3B; n = 10). The results indicated that at E:T ratio of 0.4:1, ocaratuzumab induced 19.4% more cytotoxicity than rituximab (95% CI = 79.7%, 30.9%; P = 0.0066). At E:T = 1:5:1, ocaratuzumab induced 21.5% more cytotoxicity than obinutuzumab (95% CI = 10.04%, 32.98%; P = 0.0015). Ocaratuzumab induced similar ADCC to obinutuzumab at all E:T ratios tested (all P > 0.20). These data have effectively demonstrated that in CLL
cells Fc-engineered ocaratuzumab mediates superior allogeneic ADCC when compared with the non-Fc-engineered ofatumumab or rituximab at both lower antibody concentrations and smaller E:T ratios. These data show little difference in ADCC mediated by ocaratuzumab and obinutuzumab.

**Autologous antibody-dependent cell-mediated cytotoxicity**

Natural killer (NK) cell effector function in CLL patients has been shown to be diminished when compared with normal controls and decreases further as the cancer advances. To more closely replicate the interaction of ocaratuzumab with NK cells and CLL cells in the patients’ body, we performed ADCC assays using the patients’ own NK cells. In this setting, obinutuzumab has shown increased ADCC over ofatumumab and rituximab. In these autologous ADCC assays (Fig. 4), ocaratuzumab demonstrated superior cytotoxicity to ofatumumab and rituximab at all E:T ratios tested (E:T = 25:1, 12:1, 6:1; all \( P < 0.001; n = 8 \)). Again, ocaratuzumab and obinutuzumab mediated similar ADCC (all \( P > 0.28 \)). With these autologous assays from CLL patient samples, we have demonstrated that ocaratuzumab induces superior ADCC compared with ofatumumab and rituximab and similar ADCC compared with obinutuzumab.

**Discussion**

The data presented here describe preclinical results of ocaratuzumab, a glyco-engineered anti-CD20 mAb, in primary CLL cells in vitro. We highlight features of ocaratuzumab that make the antibody suited for use in the treatment of CLL and compare these features to the attributes of the current commercially available anti-CD20 mAbs, rituximab, ofatumumab, and obinutuzumab. These features include: (1) more effective ADCC than rituximab and ofatumumab at lower concentrations, lower E:T ratios and in autologous assays; (2) similar ADCC to the Fc-engineered obinutuzumab; (3) preserved monocyte activation and macrophage ADCP; and (4) higher binding affinity for the CD20 epitope than rituximab.
The most striking feature of ocaratuzumab is its ADCC activity in primary CLL cells. We demonstrate that ocaratuzumab showed more ADCC than rituximab or ofatumumab and similar ADCC to obinutuzumab when administered at low concentrations, even significant at the low dose of 0.1 μg/mL. This feature has important clinical implications. Given the requirement of higher doses of non-Fc-engineered antibodies such as rituximab for effective therapeutic benefit by this mechanism, the higher ADCC function of Fc-engineered ocaratuzumab observed at lower doses provides opportunities for both subcutaneous dosing that could make administration more feasible and reduce the costs by lowering the dose of antibody required for the same efficacy. A s.c. formulation of ocaratuzumab may be more convenient for patients than i.v. administration, and could potentially lead to self-administration of the drug in an out-patient setting.

The s.c. formulation of a closely related antibody (AME-133E) has been tested in monkeys and was found to be well-tolerated and demonstrated dose-dependent B cell depletion.10 Ocaratuzumab and AME-133E only differ by three amino acids in the Fc region. At position 332, AME-133E has glutamate while AME-133v has isoleucine. At position 247, AME-133E has proline while AME-133v has isoleucine. At position 339, AME-133E has alanine while AME-133v has glutamine. Studies of s.c. administration in monkeys (and other pre-clinical studies) were performed with AME-133E because this was the initial variant chosen for development. We found it reasonable that the AME-133E s.c. monkey studies may correlate with s.c. ocaratuzumab. Additionally, we noted that veltuzumab, another s.c. anti-CD20 mAb,16 is in development for B cell malignancies; however, a preclinical comparison of each antibody to rituximab suggests that ocaratuzumab has a nearly 80-fold increase in binding affinity compared with veltuzumab.8

Obinutuzumab, a glyco-engineered type II anti-CD20 mAb, is also the most recently approved by the US Food and Drug Administration for use in combination with chlorambucil for front-line therapy of CLL patients who are unfit for more aggressive regimens secondary to age or medical comorbidities. In the pivotal study,17 patients treated with obinutuzumab and chlorambucil achieved improved response and survival compared with patients who received chlorambucil alone. Additionally, patients treated with obinutuzumab and chlorambucil achieved improved progression-free survival than patients treated with rituximab and chlorambucil. Notably, 20.7% of patients who received obinutuzumab and chlorambucil on this study achieved a complete response with only 7.0% complete response rate in patients receiving rituximab and chlorambucil.17 Achievement of complete response to therapy with no minimal residual disease has been identified as an independent favorable prognostic factor.18 Until further clinical investigation is complete, it is unknown whether ocaratuzumab will have the same or better efficacy compared with obinutuzumab when used for treatment of CLL.

One limitation of our study is that the NK cells taken from the CLL patients were not tested for Fcγ receptor IIIa polymorphisms. These polymorphisms could have important implications for the effectiveness of mAb therapy as patients with follicular lymphoma with the CD16A-158V subtype were found to have better response rates and event-free survival than the patients with the CD16A-158F subtype when treated with rituximab.3,19,20 Preclinical and clinical data show that ocaratuzumab can bind effectively to either polymorphism and can induce responses in follicular lymphoma patients with both polymorphisms.8,11 Future study of ocaratuzumab in CLL patients should include the NK cell Fcγ receptor IIIa polymorphism status to further define if these polymorphisms play a role in differences of response between CLL patients.

The features of ocaratuzumab that we have described in our study with primary CLL cells, including enhanced ADCC activity, preserved monocyte and macrophage effector function, and improved CD20 binding affinity provide sound rationale for the use of ocaratuzumab in CLL patients. The efficacy at low concentrations and the s.c. animal study data with AME-133E contributed to the formation of the scientific rationale for an ongoing Phase 1 investigation of ocaratuzumab in s.c. formulation in patients with relapsed CD20+ B cell malignancies. An additional Phase 1 study for CLL patients with minimal residual disease following standard therapy is under development. If efficacy is confirmed in Phase 1/2 investigation, the s.c. formula could provide ease and convenience of mAb administration. Cancer centers in developing countries whose administration of other anti-CD20 mAbs is currently thwarted by the lack of infusion centers or the staff required to monitor i.v. infusion, could potentially have an effective therapeutic option with this mAb.
mAb. Additionally, the low doses needed may allow cost-effective manufacturing, which may also lead to wider availability of the mAb for underserved patients at an affordable price. Mentrik Biotech LLC, sponsor of the development of ocaratuzumab, has committed substantial resources to philanthropic work in developing countries with this mAb.

In conclusion, ocaratuzumab may provide an efficacious, convenient and inexpensive alternate to the currently available anti-CD20 mAbs in the treatment of CLL patients. Further plans for Phase 1 investigation of this agent in CLL patients are currently under development by our group.

**Materials and Methods**

**Production of ocaratuzumab**

Ocaratuzumab engineering was previously described. Briefly, relative to rituximab, ocaratuzumab has been optimized for increased affinity to CD20 (nine amino acid changes) and increased ADCC effector function (two amino acid changes).

**Patient sample processing and cell culture**

Blood was obtained from patients with informed consent in accordance with the Declaration of Helsinki and under a protocol approved by the Institutional Review Board of The Ohio State University. All patients examined in this series had immunophenotypically defined CLL and had no prior therapy for a minimum of 30 d at the time of collection. CLL peripheral blood mononuclear cells were isolated from freshly donated blood with Ficoll density gradient centrifugation (Ficoll-Paque Plus, Amersham Biosciences #17–1440–03). Enriched CLL fractions were prepared with the use of the Rosette-Sep B cell kit (StemCell Technologies #15064) according to the manufacturer’s instructions. Isolated cells were used in RPMI 1640 media (Invitrogen #21800–022) supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich #F12003–010) and 2 mM L-glutamine (Invitrogen #50056–021) also supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich #F12003–010) and 2 mM L-glutamine (Invitrogen #50056–021). RPMI 1640 media (Invitrogen #21800–022) supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich #F12003–010) and 2 mM L-glutamine (Invitrogen #50056–021). RPMI 1640 media (Invitrogen #21800–022) supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich #F12003–010) and 2 mM L-glutamine (Invitrogen #50056–021) was used for hypothesis testing.23 Holm’s method24 was used to determine ADCC activity was determined by standard 4 h 51Cr-release assay (Perkin Elmer #NE203001MC). 51Cr-labeled target cells (5 × 10^4 cells/ well) were incubated at 37 °C for 30 min with indicated concentrations of antibodies. Unbound antibodies were washed off and cells were placed in 96-well plates. Effector cells (NK cells from healthy donors or CLL patients) were then added to the plates at the indicated E:T ratios. After 4 h incubation, the supernatant was removed and counted on a Perkin Elmer Wizard gamma counter. The percentage of specific cell lysis was determined by: % lysis = 100 × (ER – SR)/(MR – SR), where ER, SR, and MR represent experimental, spontaneous, and maximum release, respectively.

**Complement dependent cytotoxicity assay**

CLL cells were suspended at 10^6/mL in RPMI 1640 media, media with 30% autologous plasma from the patient blood samples, or media with 30% heat-inactivated (56 °C, 30 min) plasma as negative control. Cells were then incubated with antibodies at 37 °C for 1 h, 200 μg PI added and CDC was measured by flow cytometry. Percent cytolysis was the percent death with heat-inactivated plasma subtracted from percent death with unheated plasma.

**Antibody-dependent cell-mediated cytotoxicity assay**

ADCC activity was determined by standard 4 h 51Cr-release assay (Perkin Elmer #NE203001MC). 51Cr-labeled target cells (5 × 10^4 cells/ well) were incubated at 37 °C for 30 min with indicated concentrations of antibodies. Unbound antibodies were washed off and cells were placed in 96-well plates. Effector cells (NK cells from healthy donors or CLL patients) were then added to the plates at the indicated E:T ratios. After 4 h incubation, the supernatant was removed and counted on a Perkin Elmer Wizard gamma counter. The percentage of specific cell lysis was determined by: % lysis = 100 × (ER – SR)/(MR – SR), where ER, SR, and MR represent experimental, spontaneous, and maximum release, respectively.

**Statistical methods**

As each patients’ CLL sample was treated with all conditions, data were analyzed by mixed effect models incorporating dependencies within each patient sample, and robust variances were used for hypothesis testing. Holm’s method was used to control familywise error rate at 0.05 when multiple comparisons were involved. SAS version 9.3 (SAS, Inc.) was used for data analysis.
Disclosure of Potential Conflicts of Interest
CC, DS, XM, SR, JB, JF, JJ, KM, ST, NM, and JCB have no conflicts of interest to disclose. AO and AR are employees of Mentrik Biotech, LLC.

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References
1. Mukibi JM, Nyirenda CM, Adeywu JO, Mzula EL, Magombo ED, Mbundula EM. Leukaemia at Queen Elizabeth Central Hospital in Blantyre, Malawi. East Afr Med J 2001; 78:349-54; PMID:11957257; http://dx.doi.org/10.4314/eamj.v78i6.9
2. Stashenko P, Nadler LM, Hardy R, Schlossman SF. Characterization of a human B lymphocyte-specific antigen. J Immunol 1980; 125:1678-85; PMID:657764
3. Carton G, Dacheux L, Salles G, Solal-Celigny P, Baroud P, Colonbat P, Watier H. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in Fcγ Fe receptor FcgammaRIIIa gene. Blood 2002; 99:754-9; PMID:11806974; http://dx.doi.org/10.1182/blood.V99.3.754
4. Rafiq S, Butchar JP, Cheney C, Mo X, Trotta R, Caligiuri M, et al. Comparative assessment of clinically utilized CD20-directed antibodies in chronic lymphocytic leukemia cells reveals divergent NK cell, monocyte, and macrophage properties. J Immunol 2013; 190:2702-11; http://dx.doi.org/10.4314/eamj.v78i7.9006
5. Byrd JC, Kitada S, Flinn IW, Aron JL, Pearson M, Lucas D, Reed JC. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 2002; 99:1038-43; PMID:11807010; http://dx.doi.org/10.1182/blood.V99.3.1038
6. Byrd JC, Murphy T, Howard RS, Lucas MS, Goodrich A, Park K, Pearson M, Wasedenko JK, Ling G, Grever MR, et al. Rituximab using a thrice weekly dosing schedule in B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma demonstrates clinical activity and acceptable toxicity. J Clin Oncol 2001; 19:2153-64; PMID:11304767
7. O’Brien SM, Kantarjian H, Thomas DA, Giles MJ, Murphy T, Howard RS, Lucas MS, Goodrich A, Park K, Pearson M, Wasedenko JK, Ling G, Grever MR, et al. Rituximab using a thrice weekly dosing schedule in B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma demonstrates clinical activity and acceptable toxicity. J Clin Oncol 2001; 19:2153-64; PMID:11304767
8. O’Brien SM, Kantarjian H, Thomas DA, Giles MJ, Murphy T, Howard RS, Lucas MS, Goodrich A, Park K, Pearson M, Wasedenko JK, Ling G, Grever MR, et al. Rituximab using a thrice weekly dosing schedule in B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma demonstrates clinical activity and acceptable toxicity. J Clin Oncol 2001; 19:2153-64; PMID:11304767
9. O’Reilly A, Davis T, Wayne J, Marulappa S, Jain Y. Ocatratuzumab, a fab-engineered anti-CD20 antibody, demonstrates greater affinity to CD20 and ability to bind to rituximab-coated cells. European Hematology Association Meeting Abstracts 2012:#1936
10. Marulappa S, Wayne J, Makori N, Shah HS, Watkins J. Subcutaneous administration of AME-133 demonstrated significant dose dependent B-cell depletion in cynomolgus monkeys (macaca fascicularis).ASH Annual Meeting Abstracts 2011; 118:2475.
11. Tobinai K, Obara M, Koyama Y, Uchida T, Watanabe T, Oyama Y, et al. Phase I study of LY2469298, an fc-engineered humanized anti-CD20 antibody, in patients with relapsed or refractory follicular lymphoma. Cancer Sci 2011; 102:432-8; http://dx.doi.org/10.1111/j.1349-7006.2010.01809.x
12. Uchida J, Lee Y, Hasegawa M, Liang Y, Bradney A, Oliver JA, Bowen K, Steeber DA, Haas KM, Poe JC, et al. Mouse CD20 expression and function. Int Immunol 2004; 16:119-29; PMID:14668067; http://dx.doi.org/10.1093/intimm/dxh009
13. Uchida J, Hamaguchi Y, Oliver JA, Ravech JV, Poe JC, Haas KM, Tedder TF. The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor-dependent mechanisms during anti-CD20 antibody immunotherapy. J Exp Med 2004; 199:1659-69; http://dx.doi.org/10.1084/jem.20040119; PMID:15210744
14. Ziegler HW, Kay NE, Zarling JM. Deficiency of natural killer cell activity in patients with chronic lymphocytic leukemia. Int J Cancer 1981; 27:321-7; PMID:6106660; http://dx.doi.org/10.1002/ijc.2910270310
15. Ziegler HW, Kay NE, Zarling JM. Deficiency of natural killer cell activity in patients with chronic lymphocytic leukemia. Int J Cancer 1981; 27:321-7; PMID:6106660; http://dx.doi.org/10.1002/ijc.2910270310
16. Negrea GO, Elstrom R, Allen SL, Rai KR, Abbas R, Farber CM, et al. Subcutaneous injections of low-dose veltuzumab (humanized anti-CD20 antibody) are safe and active in patients with indolent non-hodgkin’s lymphoma. Haematologica 2011; 96:567-73; http://dx.doi.org/10.3324/haematol.2010.037390
17. Goede V, Fischer K, Busch R, Engelke A, Eichhorst B, Wendtner CM, Chagorova T, de la Serra J, Dillhuudy MS, Illner T, et al. Obinutuzumab plus chlorambucil in coexisting conditions. N Engl J Med 2014; In press: http://dx.doi.org/10.1056/NEJMoa1313984; PMID:24401022
18. Bortcher S, Ritgen M, Fischer K, Strilgenbauer S, Busch RM, Fingerle-Rowson G, et al. Minimal residual disease quantification is an independent predictor of progression-free and overall survival in chronic lymphocytic leukemia: A multivariate analysis from the randomized GCLLSG CLL8 trial. J Clin Oncol 2012; 30:1038-43; http://dx.doi.org/10.1200/JCO.2011.36.9348
19. Weng WK, Levy R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol 2003; 21:3940-7; http://dx.doi.org/10.1200/JCO.2003.03.0513; PMID:12975461
20. Ghielmini M, Rufibach K, Salles G, Leoncini-Francisci L, Leger-Falandry C, Cogliatti S, Fey M, Martinelli G, Stahel R, Lozzi A, et al. Single agent rituximab in patients with follicular or mantle cell lymphoma: clinical and biological factors that are predictive of response and event-free survival as well as the effect of rituximab on the immune system: a study of the Swiss Group for Clinical Cancer Research (SAKK). Ann Oncol 2005; 16:1675-82; http://dx.doi.org/10.1093/annonc/mdi320; PMID:16030029
21. Zhao X, Lapalombella R, Joshi T, Cheney C, Gowda A, Hayden-Leibetter MS, Baum PB, Lin TS, Jarjoura D, Lehman A, et al. Targeting CD27-positive lymphoid malignancies with a novel engineered small modular immunopharmaceutical. Blood 2007; 110:2569-77; http://dx.doi.org/10.1182/blood-2006-12-062927; PMID:17440052
22. Verbeke G, Fieuws S, Molenberghs G, Davidian M. The analysis of multivariate longitudinal data: A review. Stat Methods Med Res 2014; 23:42-59; PMID:2410270310
23. Pan X, Li X, Jarjoura D. Hypothesis testing with common covariance structure. International Chinese Statistical Association Applied Statistics Symposium 2012: DOI: Boston, MA.
24. Holm S. A simple sequentially rejective multiple test procedure. Scand J Stat 1979; 6:65-70

Supplemental Materials
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