Chemotherapy-refractory diffuse large B-cell lymphomas (DLBCL) are effectively killed by CD20 antibody-induced complement-mediated cytotoxicity
Abstract

Purpose: The combination of chimeric CD20 monoclonal antibody (mAb) rituximab (R) treatment with chemotherapy (CHOP) has significantly improved clinical outcome in patients with diffuse large B-cell lymphoma (DLBCL). However, the efficacy of R-CHOP is variable and DLBCL remains fatal in 30-40% of the patients. In this study we compared a novel human type I CD20 mAb ofatumumab with rituximab for the ability to induce complement dependent cytotoxicity (CDC) of cultured lymphoma cells of chemotherapy-refractory DLBCL patients.

Experimental design: Ten DLBCL patient samples and three DLBCL cell lines were investigated for their sensitivity to ofatumumab and rituximab using a highly sensitive flow cytometry method. Expression of CD markers and the apoptosis inhibitors Bcl-2 and XIAP was determined with immunohistochemistry, FACS analysis or RT-MLPA analysis.

Results: Ofatumumab and rituximab induced CDC in all DLBCL cell lines and all DLBCL patient cases tested. Ofatumumab was more effective in inducing CDC in nine out of ten DLBCL tumor samples compared to rituximab. Sensitivity of DLBCL to ofatumumab- and rituximab-induced CDC correlated with expression of complement defense molecule CD59, but not with expression of CD46, CD55 or apoptosis inhibitors Bcl-2 and XIAP. Functional inhibition of CD55 and CD59 demonstrated that ofatumumab-induced CDC was less inhibited by these complement defense molecules than rituximab-induced CDC.

Conclusions: We conclude that chemotherapy-refractory DLBCL cases are highly sensitive to ofatumumab- and rituximab-induced CDC, with the human mAb ofatumumab being more effective. Based on our results ofatumumab should be considered as a valuable alternative therapy option for patients with DLBCL.

Introduction

Diffuse large B-cell lymphomas (DLBCLs) represent the most frequent type of lymphoma and account for 40% of adult Non-Hodgkin lymphomas. For several decades the standard treatment for these lymphomas has been a combination of chemotherapy (cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP)). In the last decade, several independent studies have demonstrated that addition of the chimeric CD20 monoclonal antibody (mAb) rituximab to CHOP (R-CHOP) resulted in a significant increase in survival and therefore R-CHOP has become the standard therapy for DLBCL as well as for most other B-cell malignancies.

Although many patients benefit from R-CHOP treatment, the disease remains fatal in 30-40% of the patients. Previous studies have demonstrated that outcome in DLBCL patients is related to clinical characteristics. In addition, we and others have shown that the clinical response to chemotherapy and probably also to R-CHOP depends to a large extent on intrinsic cellular characteristics. In particular, high expression levels of anti-apoptotic genes involved in the intrinsic apoptosis pathway like Bcl-2 and XIAP, are strongly related to a poor clinical outcome.

Micro array expression profiling has demonstrated that DLBCL can be categorized in two major subtypes: DLBCL resembling germinal center B-lymphocytes (GCB-like DLBCL) and DLBCL resembling in vitro activated B-cells (ABC-like DLBCL). ABC-like DLBCL are characterized by constitutive activation of the NF-κB pathway with concomitant up-regulation of anti-apoptotic genes, thus explaining the relatively poor outcome in this patient group.

As inhibition of apoptosis is a major cause of chemotherapy resistance in DLBCL patients, it seems reasonable to use treatment methods that induce cell death independent of apoptosis. This notion is supported by the improvements in clinical outcome following the introduction of R-CHOP,
as rituximab primarily induces apoptosis-independent cell death via antibody-dependent cellular cytotoxicity (ADCC)\textsuperscript{21} and complement-dependent cytotoxicity (CDC).\textsuperscript{22} CDC involves a cascade of activating proteins that lead to the formation of the membrane attack complex, resulting in lysis of the target cells. CDC mediated lysis is regulated by expression of membrane-bound regulatory proteins, including CD55, CD59 and CD46. CD55 triggers the decay of both C3 and C5 convertases, CD59 binds to C8 and C9 and prevents pore formation by the membrane attack complex, whereas CD46 serves as a cofactor for the cleavage of C3b and C4b.\textsuperscript{23,24,25} Previous \textit{in vitro} studies in follicular lymphoma (FL), mantle cell lymphoma (MCL), prolymphocytic leukemia (PLL) and B-cell chronic lymphocytic leukemia (B-CLL), have shown that these proteins may inhibit cytotoxicity of rituximab.\textsuperscript{22,26}

CD20 mAb are divided in two major classes based on their mechanisms of action with type I mAb inducing strong CDC and ADCC but relatively poor apoptosis, and type II mAb activating apoptosis and ADCC but not CDC.\textsuperscript{27} We recently described and characterized a panel of human CD20 mAb including the type I mAb ofatumumab and the type II mAb 11B8.\textsuperscript{28,29} Ofatumumab is distinguished from rituximab by its remarkable potency in CDC.\textsuperscript{28} Currently, ofatumumab is in phase III clinical trials for the treatment of FL and B-CLL, and in phase II clinical evaluation for rheumatoid arthritis.\textsuperscript{30} In this study, we show that the human type I CD20 mAb ofatumumab is more efficient in inducing CDC than type I CD20 mAb rituximab and type II CD20 mAb 11B8 in DLBCL-derived cell lines and isolated lymphoma cells of chemotherapy-refractory DLBCL patients. Significant lysis by ofatumumab was achieved for all tumors tested. In addition, we demonstrate that expression levels of complement defense molecules CD55 and CD59, but not apoptosis inhibitors Bcl-2 and XIAP, negatively influenced type I CD20 mAb-induced CDC of chemotherapy-refractory DLBCL. Ofatumumab-induced CDC of DLBCL patient cells was less sensitive to expression of these complement defense molecules than rituximab-induced CDC.

\section*{Material and methods}

\textbf{DLBCL patient samples and cell lines}  
Ten lymphoma samples were obtained from our bank of isolated tumor cells. Tumor samples were diagnosed between 2001 and 2004 as DLBCL in the Comprehensive Cancer Center in Amsterdam, according to the World Health Organization (WHO) criteria.\textsuperscript{31} Both primary lymphomas and lymph node samples of secondary lymphomas were used. Most samples (n=7) were primary biopsies, also included were 2 biopsies of secondary lymphomas and 1 transformed lymphoma. All of the primary lymphoma samples were considered refractory because patients did not reach complete remission or obtained a relapse (according to standard clinical evaluation, including physical examination, chest x-ray, and computed tomography scanning of chest, abdomen and pelvis during a follow-up period of 9-43 months).

Lymphoma cells were isolated from tissue biopsies by a combination of mechanical and enzymatic dissociation and were selected as described previously.\textsuperscript{32} Cells were cultured in Iscove’s modified Dulbecco’s medium (BioWhittaker, Cambrex, Belgium) supplemented with 10\% fetal calf serum (Hyclone, Perbio, Sweden), 1\% penicillin and streptomycin (Gibco, Invitrogen, Grand Island, NY, USA) at 37 °C with 5\% CO\textsubscript{2} in a humidified atmosphere. The purity of the resulting tumor cell fractions was >95\% neoplastic CD20 positive cells, as determined by immunocytochemistry.
staining (CD3, clone CBC37, DakoCytomation, Denmark and CD20, clone L26, DakoCytomation). Approval of the study was obtained from the ethics review board of the VU University Medical Center. Informed consent was provided according to the Declaration of Helsinki. The DLBCL cell lines SUDHL4, SUDHL5 and HT (originally obtained from DSMZ, Braunschweig, Germany) were cultured in RPMI 1640 medium (BioWhittaker, Cambrex, Belgium) containing 10% fetal calf serum and 100 IU penicillin/100 µg/ml streptomycin.

Monoclonal antibodies
Ofatumumab (2F2, HuMax-CD20) and 11B8 are human IgG1, κ CD20 antibodies generated in HuMab mice.28 HuMab-KLH (human IgG1, κ) is a human antibody against an irrelevant antigen (keyhole limpet hemocyanin) and is used as an isotype control. Rituximab (Mabthera) is a chimeric IgG1, κ and was obtained from Roche (Basel, Switzerland).

Complement-dependent cytotoxicity assay
CDC assays were performed as described previously.28,33 Cells were washed and resuspended in RPMI/BSA 1%. Subsequently, cells were incubated with increasing concentrations of the mAb (0.1, 1 and 10 µg/ml, final concentrations) in the absence or presence of 10 µg/ml functionally blocking CD46 mAb (Chemicon, Temecula, CA, USA), CD55 mAb (Serotec, Oxford, UK), CD59 mAb (Serotec) for 15 minutes at room temperature. As negative controls, isotype matched antibodies were used. Normal human serum was added as source of complement and cells were incubated at 37°C. After 45 minutes, cells death was measured by flow cytometry.

Assessment of cell death
Sensitivity to CD20 mAb induced cell death was determined with a highly sensitive flow cytometry method.32 In brief, a standard number of fluorescent beads (Fluospheres, Becton Dickinson, USA) in combination with 7AAD (7-amino-actinomycin, ViaProbe, BD Pharmingen, Belgium) was used to determine the number of viable (7AAD negative) cells per 2500 beads for each individual experiment. Fluorescence was detected by the FACS Calibur flow cytometer and analyzed using CELLQuest software (both Becton Dickinson, San Jose, CA, USA). All tests were performed in triplicate.

Detection of CD20, CD55, CD59 and CD46 expression
Frozen tissue sections of DLBCL biopsies were stained with CD20 (clone L26, DakoCytomation, Denmark), CD46 (Chemicon, Temecula, CA, USA), CD55 (Serotec, Oxford, UK) and CD59 (Serotec). The antibodies were incubated overnight. For detection of CD20, CD46, CD55 and CD59, a standard highly sensitive Envision horseradish peroxidase system (DakoCytomation, Glöstrup, Denmark) was used. Antibodies were visualized with chromogen diaminobenzidine (DAB). Expression levels were quantified in relation to staining intensity observed in non-neoplastic lymphoid tissues that served as positive internal control for all tested antibodies.

For flow cytometric analysis of CD marker expression in DLBCL cell lines, cells were incubated with a fluorescence-labeled antibody against CD20 (L27, 0.5 µg/ml Becton Dickinson, San Jose, CA, USA) and unlabeled antibodies against CD46 (clone J4.48, 1 µg/ml, Chemicon), CD55 and CD59 (clone 67, MEM43, both 5 µg/ml, Serotec) for 15 minutes at room temperature. As negative controls, isotype matched antibodies were used. For detection of CD46, CD55 and CD59, cells were washed and incubated with a rabbit anti-mouse PE labeled antibody (DAKO, Denmark) for 15
minutes at room temperature. Subsequently, cells were washed and fluorescence was measured on a FACS Calibur and analyzed using CELL-Quest software (both Becton Dickinson). The percentage positively stained cells was determined and the Relative Fluorescence Intensity (RFI) was calculated as [Mean Fluorescence Intensity (MFI)] / [MFI isotype control].

**RT-MLPA analysis**

RNA of primary cells was prepared using RNbee solution (Tel-test Inc., Friendswood, TX, USA) according to the manufacturer’s recommendations. RT-Multiplex Ligation-dependent Probe Amplification (RT-MLPA) was performed on total RNA as described previously. Data were analyzed with Genotype and GeneScan software (Applied Biosystems, Warrington, UK). As internal reference, the housekeeping gene β-glucuronidase (GUS-B) was used to normalize and minimize possible effects of unequal amounts of mRNA.

**Statistical analysis**

The following statistical tests were used to analyze the data: Two-way Anova test, Mann Whitney-U test, and unpaired t-test using the Graph Pad Prism statistical software package. P-values below 0.05 were considered significant. LD$_{50}$ values were determined by linear regression of the logarithms of the data.

**Results**

**Ofatumumab is more effective than rituximab in CDC of DLBCL derived cell lines**

We first compared ofatumumab- and rituximab-induced CDC in DLBCL-derived cell lines: SUDHL4, SUDHL5 and HT. As expected, the negative control mAb HuMab KLH and 11B8 demonstrated low levels of CDC. Sensitivity to ofatumumab and rituximab differed strongly between the DLBCL cell lines. SUDHL4 and SUDHL5 were both highly sensitive (70-100%) to both ofatumumab and rituximab when compared to HT (20-40%) (Figure 1). Ofatumumab induced higher levels of CDC (Figure 1B,C) and was more effective at low concentration (0.1 μg/ml, Figure 1A), when compared to rituximab.

**Ofatumumab is more effective than rituximab in CDC of chemotherapy-refractory DLBCL lymphoma cells**

Next, we investigated whether ofatumumab and rituximab can induce CDC in cultured lymphoma cells isolated from 10 chemotherapy-refractory DLBCL. Treatment with the negative control HuMab KLH and 11B8 demonstrated low levels of CDC in all DLBCL samples. All patient samples were sensitive to type I CD20 mAb ofatumumab and rituximab.

CDC by ofatumumab and rituximab was concentration dependent. DLBCL lymphoma cells were more sensitive to ofatumumab, than to rituximab at all tested concentrations (two-way Anova, p=0.001, p=0.001, p=0.01, for increasing concentrations respectively, Figure 2A). As expected, levels of ofatumumab-induced CDC in DLBCL were significantly higher than mAb 11B8-induced cell death at all concentrations tested (Two-way Anova, p<0.001). For rituximab higher concentrations were required to achieve a significant difference with mAb 11B8-induced cell death (two-way Anova, p<0.001 for 1.0 and 10 μg/ml).
Figure 1. CD20 mAb-mediated CDC of DLBCL cell lines. SUDHL4 (A), SUDHL5 (B) and HT (C) were incubated with increasing concentrations of CD20 mAb: ofatumumab (●), 11B8 (♦), rituximab (▲) or HuMab-KLH (■). After addition of NHS as a source of complement and incubation at 37°C for 45 minutes, CDC was determined using 7AAD-beads FACS analysis.
Figure 2B showed the individual DLBCL patient samples analyzed at a concentration of 0.1 μg/ml. Nine out of ten cases were more sensitive to ofatumumab than to rituximab. This was also confirmed when the median lethal doses (LD₅₀) of ofatumumab and rituximab were compared for all ten patients. The median LD₅₀ of ofatumumab was < 0.1 ± 2.8 μg/ml whereas the median LD₅₀ of rituximab was 6.4 ± 4.9 μg/ml among the DLBCL samples (Mann-Whitney U test, p= 0.04).

Clinical characteristics as well as ABC/GCB subtype for each individual case are indicated in Figure 2B. Ofatumumab was particularly more effective than rituximab in induction of CDC in the 2 secondary DLBCL cases L17 and L20 at all concentrations tested. No differences in sensitivity to ofatumumab and rituximab were observed between GCB- and ABC-like DLBCL.

**Sensitivity to CDC by ofatumumab does not correlate with CD20 levels on DLBCL**

Expression of CD20 was determined in DLBCL samples using immunohistochemistry. No significant correlation was observed between ofatumumab- or rituximab-induced cell death and CD20 expression levels. However, two DLBCL cases (L7 and L13) with low expression of CD20 and one DLBCL case L23 with low CD20 intensity staining were relatively less sensitive to ofatumumab- and rituximab-induced cell death. In contrast, case L20 which was also less efficiently lysed by ofatumumab and rituximab showed high CD20 expression levels (Figure 2 & 3).

CD20-positive staining of the lymphoma samples was confirmed by flow cytometry (Figure 3C & 3D). There was also no clear correlation between the percentage CD20 positive cells or the RFI and sensitivity to ofatumumab- or rituximab-induced cell death. Cases L7, L13 and L23 that were less sensitive to ofatumumab- or rituximab-induced cell death tended to have lower percentages of CD20 expression and lower RFI values. DLBCL cell line HT that was less sensitive to ofatumumab- or rituximab-induced CDC also showed lower levels of CD20 (Table 1, Figure 1). However, case L20 which was also less efficiently lysed by ofatumumab and rituximab showed high CD20 expression levels and a high RFI value.

### Table 1. Expression of CD20, CD46, CD55 and CD59 in DLBCL cell lines

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**Sensitivity to CDC by type I CD20 mAb correlates with low expression levels of CD59 in DLBCL**

Expression of the complement defense molecules CD46, CD55 and CD59 was investigated using immunocyto-histochemistry and flow cytometric analyses to determine whether they predicted sensitivity to ofatumumab- or rituximab-induced CDC in DLBCL cell lines and DLBCL patient cells. SUDHL4, SUDHL5 and HT showed expression of CD46, CD55 and CD59 (Table 1). No clear
Figure 2. CDC mediated cell death by CD20 mAb in isolated DLBCL patient cells.
(A) Isolated lymphoma cells of ten chemotherapy-refractory DLBCL patients were treated with different concentrations of CD20 mAbs: ofatumumab (●), 11B8 (◆), rituximab (▲) or HuMab-KLH (□), followed by addition of NHS and incubation at 37°C for 45 minutes. CDC was measured using 7AAD-beads flow cytometric analysis. Shown are the mean ± SE values of ten individually tested cases.
(B) Percentage CDC-mediated lysis in each individual DLBCL sample after incubation with 0.1 ug/ml CD20 mAb ofatumumab (■) and rituximab (□). For each sample, the mean ± SD of CD20 mAb-mediated CDC is shown with the lethal dose (LD₅₀) for ofatumumab and rituximab. (P) primary; (S) secondary; (T) transformed; (GCB) Germinial Centre B-cell like; (ABC) Activated B-Cell like; according to Hans et al, 2004.41
Figure 3. CD20 expression levels do not correlate with sensitivity to CDC-mediated cell death by ofatumumab or rituximab in DLBCL

(A) CD20 expression was detected in ten DLBCL samples using immunohistochemistry. Expression of CD20 in a DLBCL sample (L13, top) with a high LD50 value for ofatumumab and a case (L24, bottom) with a low LD50 value are represented.

(B) Flow cytometric analysis for CD20 expression in DLBCL case L13 with low CD20 expression (top) and DLBCL case L24 with high CD20 expression (bottom). Black peaks depict isotype control staining and white peaks correspond to staining with CD20 antibody.

(C) Percentage positive CD20 stained cells in relation to % cell death of ten DLBCL samples tested, incubated with 0.1 μg/ml ofatumumab (■) or rituximab (▲), using flow cytometric analysis.

(D) Expression of CD20 in relation to % cell death of ten DLBCL samples tested, incubated with 0.1 μg/ml ofatumumab (■) or rituximab (▲), using flow cytometric analysis. The Relative Fluorescence Intensity (RFI) was calculated as Mean Fluorescence Intensity (MFI) of CD20 /MFI IgG1 isotype control.
correlation between expression of complement defense molecules and sensitivity to ofatumumab- and rituximab-induced CDC was found in these cell lines. However, HT cells showed a much higher expression of CD55 and were less sensitive to the type I CD20 mAb compared to the two other cell lines.

Immunohistochemistry was performed for all ten samples tested. Expression levels of CD46 and CD55 did not significantly correlate with the response to ofatumumab or rituximab (data not shown). DLBCL samples demonstrating no CD59 expression showed relatively low LD_{50} values for ofatumumab (Figure 4A, 4B left, unpaired t-test, p=0.02) suggesting that CD59 expression decreases sensitivity to ofatumumab-induced CDC. Also for rituximab, a correlation between CD59 expression and sensitivity to rituximab-induced CDC was observed (Figure 4B right, unpaired t-test, p=0.03). Together, these data suggest that CD59 is an important factor for indicating sensitivity to CDC in isolated DLBCL patient samples.

To further substantiate if surface-associated complement inhibitors regulate CDC in DLBCL we investigated the functional role of CD55, CD59 and CD46 by performing CDC assays in the presence of antibodies that functionally block these molecules. DLBCL patient sample L7 with high expression of CD46, CD55 and CD59 and high LD_{50} values for both ofatumumab and rituximab was tested. The blocking mAb were not able to induce CDC by themselves and isotype controls did not show an increase in CDC-mediated lysis in patient sample DLBCL L7 (data not shown).

Blocking of CD59 by addition of CD59 mAb increased ofatumumab- and rituximab-induced CDC in DLBCL case L7 (Figure 4C, left), whereas CD46 mAb did not. Combination of CD55 and CD59 mAb demonstrated an additional increase of cell death. Inhibition of CD59 and/or CD55 affected rituximab-induced CDC more than ofatumumab-induced CDC. Thus, the capacity to induce CDC by type I CD20 mAb partly depended on expression of complement regulatory proteins, especially CD59. In addition, these results demonstrate that ofatumumab-induced CDC of DLBCL is less inhibited by these complement defense molecules than rituximab-induced CDC.

Sensitivity to ofatumumab-induced CDC is independent from Bcl-2 and XIAP expression. We investigated the implication of apoptosis inhibitors Bcl-2 and XIAP in ofatumumab- and rituximab-induced CDC mediated lysis in DLBCL cells. Expression levels of Bcl-2 and XIAP were determined using RT-MLPA analysis. Levels of Bcl-2 and XIAP expression did not correlate with responses to ofatumumab or rituximab (data not shown).

**Discussion**

Clinical outcome in DLBCL is still frequently fatal despite intensive chemotherapy (CHOP) in combination with the chimeric CD20 mAb rituximab (R-CHOP) and stem cell transplantation as salvage therapy. Therefore, new therapeutic agents are currently being developed to improve outcome in those DLBCL patients. Recently, ofatumumab, a novel fully human mAb directed at CD20 has been described which is highly effective in CDC and may result in improvements in therapeutic outcome.

In this study, we demonstrated that type I CD20 mAb ofatumumab and rituximab induce high levels of CDC in chemotherapy-refractory DLBCL lymphoma cells and DLBCL derived cell lines. Nine out of ten chemotherapy-refractory DLBCL samples and all three DLBCL cell lines tested had a significantly better response to the fully human ofatumumab mAb than to the chimeric rituximab mAb. Especially at low concentrations ofatumumab was more potent in inducing cell lysis than rituximab. An explanation for the difference in sensitivity between rituximab and ofatumumab might
**Figure 4.** CD59 expression levels correlate with sensitivity to CDC-mediated cell death by ofatumumab or rituximab in DLBCL.

(A) CD59 expression was detected in 10 DLBCL samples using immunohistochemistry. Expression of CD59 in a DLBCL sample (L13, left) with a high LD$_{50}$ value for ofatumumab and a case (L24, right) with a low LD$_{50}$ value for ofatumumab are represented.

(B) Patient samples were divided into two groups based on their CD59 expression (negative or positive). The LD$_{50}$ values for ofatumumab (left) or rituximab (right) were plotted at the Y-axis. Horizontal lines represent median CD59 expression levels for each group.

(C) DLBCL case L7 was lysed with 10 µg/ml ofatumumab (■, left) or rituximab (■, right) and NHS in the presence or absence of 10 µg/ml blocking CD46 mAb, CD55 mAb and/or CD59 mAb. Cell death was measured using the 7AAD-beads FACS method. The blocking mAb did not induce CDC by themselves and isotype controls showed no increase in CDC-mediated lysis in both lymphomas (data not shown).
be the nature of the CD20 epitope. We recently showed that ofatumumab binds to a unique CD20 epitope, which is different from the rituximab binding site in that it binds to a discontinuous epitope comprising parts of the small loop and a region of the large loop located N-terminally of the rituximab epitope. In addition, ofatumumab dissociates much more slowly from cells than rituximab. These differences may explain the difference in CDC potency between the two mAb, and the increased efficacy in killing patient tumor DLBCL cells by ofatumumab.

To further investigate possible factors involved in sensitivity to ofatumumab and rituximab, we compared ofatumumab- and rituximab-induced CDC with expression levels of complement defense molecules and of CD20. For the DLBCL cell lines it was shown that the cell line expressing low levels of CD20 (HT) was less sensitive for ofatumumab- or rituximab-induced CDC. For DLBCL patient samples, however, no clear correlation was found between the percentage CD20 positive cells or the RFI values, and ofatumumab-induced or rituximab-induced cell death. This is in contrast with previous studies that showed that the CD20 expression levels on B-CLL, FL and transfected CEM cells correlated with sensitivity to rituximab-induced CDC.

Sensitivity to ofatumumab as well as rituximab correlated with absence of CD59 expression in DLBCL cells. However, in cases with high levels of CD59 expression, ofatumumab induced CDC more effectively than rituximab. These data are in agreement with previous studies in other tumors such as MCL, PLL and B-CLL. In addition, functional inhibition of CD55 and CD59 demonstrated that ofatumumab-induced CDC is less inhibited by these complement defense molecules than rituximab-induced CDC, further indicating that ofatumumab is more effective in killing of DLBCL cells with high levels of complement defense molecules than rituximab.

Many studies have indicated that inhibition of apoptosis plays an important role in chemotherapy resistance in DLBCL. Chemotherapy-refractory DLBCL often show high expression levels of the apoptosis inhibitory proteins including Bcl-2 and XIAP. In our study, sensitivity to ofatumumab and rituximab mediated cell death in DLBCL samples was not dependent on expression of XIAP and Bcl-2, consistent with previous studies on rituximab-induced CDC.

We conclude that chemotherapy-refractory DLBCL remain highly sensitive to CD20 type I mAb induced CDC in vitro. Ofatumumab is a more potent killer than rituximab in most chemotherapy-refractory DLBCL cases, probably due to its ability to bind to a unique CD20 epitope. Sensitivity to ofatumumab-induced CDC appeared to be independent of expression of Bcl-2 and XIAP, and relatively insensitive of CD55 and CD59 expression on DLBCL. Thus, based on the present results, ofatumumab might be a valuable alternative treatment for patients with DLBCL and may be useful as 2nd line treatment option.

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References


