Title: Ofatumumab exhibits enhanced in vitro and in vivo activity compared to rituximab in pre-clinical models of mantle cell lymphoma

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Running Title: Pre-clinical activity of ofatumumab in mantle cell lymphoma

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**Translational Relevance**

Mantle cell lymphoma is generally considered incurable with modern treatment regimens that incorporate rituximab with combination chemotherapy regimens. In pre-clinical testing, novel anti-CD20 monoclonal antibodies have demonstrated variable degrees of increased activity, when compared to rituximab, against a variety of CD20 expressing malignancies. In this study, we found that the anti-CD20 monoclonal antibody ofatumumab exhibits more potent activity compared to rituximab against mantle cell lymphoma cells *in vitro* and *in vivo*; including in cell lines with significant *in vitro* resistance to rituximab associated with high levels of expression of the complement inhibitory proteins CD55 and CD59 which have previously been correlated with decreased rituximab associated cytotoxicity. Our work supports the further development of ofatumumab in the treatment of mantle cell lymphoma.

**Abstract**

Purpose: Mantle cell lymphoma (MCL) is a mature B-cell lymphoma considered to be incurable with current treatments including: front-line rituximab in combination with multi-agent chemotherapy and for those eligible, high dose-chemotherapy and stem cell support or rituximab maintenance. On the other hand, achieving a complete remission by high-sensitive flow cytometry is associated with prolonged duration of remission, stressing the need to develop and/or incorporate novel agents into the management of MCL. To this end, we examined the activity of ofatumumab, an anti-CD20 monoclonal antibody with distinct binding and immunological properties than rituximab, in MCL pre-clinical models.

Experimental Design: MCL cells were labeled with $^{51}$Cr prior to incubation with rituximab or ofatumumab (10 µg/ml) plus human serum or effector cells. $^{51}$Cr-release was measured and the percentage of lysis was calculated. Surface CD20, CD55 and CD59 were measured by Imagestream analysis. SCID mice inoculated subcutaneously with Z138 cells were assigned to control versus 4 doses of ofatumumab or rituximab (10mg/kg/dose).
Results: Ofatumumab exhibited enhanced in vitro complement dependent cytotoxicity activity compared to rituximab in MCL cell lines, despite a high degree of in vitro resistance to rituximab associated with low CD20 levels and/or high expression of complement inhibitory proteins. Ofatumumab also delayed tumor progression and prolonged survival in a murine model of MCL.

Conclusions: Our results demonstrate that ofatumumab is more effective than rituximab in MCL pre-clinical models, including in the presence of rituximab resistance, and support the clinical investigation of ofatumumab in combination with standard systemic chemotherapy in MCL (NCT01527149).

Introduction
Mantle cell lymphoma (MCL) is a mature B-cell non-Hodgkin lymphoma (B-NHL) characterized by overexpression of cyclin D1 resulting from the translocation t(11;14) (1). MCL represents about 2-10% of B-NHL. Frequently presenting with advanced stage disease, MCL is generally considered to be incurable with current treatment regimens that incorporate high-dose cytarabine, rituximab, anthracycline-based multi-agent chemotherapy regimens (i.e. CHOP) and, for those eligible patients, high-dose chemotherapy with autologous stem cell support (HDC-ASCS). Despite a high response rate observed to up-front therapy and significant prolongation in survival with modern treatment regimens, the disease is still characterized by continuous relapses and acquirement of resistance to subsequent treatments resulting in a median survival of only 3-5 years post-diagnosis (2).

The addition of the anti-CD20 monoclonal antibody (mAb) rituximab to chemotherapy regimens has improved overall survival (OS) in indolent and aggressive B-NHL, though patients frequently relapse with disease that demonstrates variable degrees of sensitivity to re-treatment with rituximab containing regimens (3-6). The addition of rituximab to chemotherapy in treating MCL has also been shown to improve clinical outcomes, including OS (7) Despite the survival benefit with the addition of rituximab,
nearly all MCL patients eventually relapse and novel treatment approaches are needed to further improve outcomes.

In order to improve on the advances achieved in the era of rituximab in treating B-NHL, it is important to gain an understanding of factors that contribute to the anti-tumor activity of or to the development of resistance to rituximab. Potential factors that affect rituximab activity include genetic polymorphisms in the Fc receptor, decreased CD20 expression and increased complement inhibitory protein (CIP) expression (8-11). Multiple next-generation anti-CD20 mAbs with variable mechanisms of activity have been developed and are currently under evaluation in the treatment of B-NHL.

Ofatumumab is a humanized anti-CD20 mAb that is currently Federal Drug Administration (FDA) approved for the treatment of refractory chronic lymphocytic leukemia (CLL) (12). Ofatumumab binds to a unique, more membrane-proximal epitope on the CD20 antigen (13). In pre-clinical models, ofatumumab has demonstrated similar antibody-dependent cellular cytotoxicity (ADCC) activity with enhanced complement-dependent cytotoxicity (CDC) activity compared to rituximab and more efficiently induces B-cell depletion \textit{in vivo} (14-16). Ofatumumab is currently under investigation in clinical trials in both treatment naïve and relapsed/refractory indolent and aggressive B-NHL, including MCL.

We have previously reported on the enhanced \textit{in vitro} and \textit{in vivo} efficacy of ofatumumab against Burkitt’s lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL) pre-clinical models, including in the setting of acquired rituximab resistance (17). To further characterize the activity of ofatumumab against MCL cells, we performed pre-clinical testing in cytarabine-sensitive and –resistant MCL cell lines, primary patient-derived MCL cells and in \textit{in vivo} severe combined immunodeficiency (SCID) mouse xenograft models of human MCL.

**Methods**
MCL cell lines and cytarabine-resistant MCL cell lines

Experiments were conducted in several cytarabine-sensitive and cytarabine-resistant MCL cell lines. The sensitive cell lines Granta, HBL-2, Jeko-1, Mino and Rec-1 were purchased from DSMZ and the cytarabine-resistant MCL cell lines Granta-AraCR, HBL-2-AraCR, Jeko-1-AraCR, Mino-AraCR and Rec-1-AraCR generated by Dr. Pavel Klener (Charles University, Prague, Czech Republic) by culturing each respective parental cell line in escalating doses of cytarabine (18). The Z-138 and Raji cell lines were obtained from the American Type Culture Collection (ATCC) (Manassas, VA). Cell lines were authenticated by short tandem repeat analysis. All cell lines were routinely tested for mycoplasma contamination by PCR, microbial presence by microscopic observation, and antibiotic deprivation and morphology by microscopic observation. Cytarabine-sensitive cells were maintained in RPMI 1640 supplemented with Hepes 5mM, sodium pyruvate 1mM, penicillin and streptomycin (100 IU/ml) and 10% heat-inactivated fetal bovine serum (HI-FBS) (RPMI-1640). Cytarabine-resistant cell lines were initially defrosted and cultured in RPMI-1640 with cytarabine 50uM for 2 months then maintained in RPMI-1640. The rituximab-resistant Raji 4RH cell line was developed within our laboratory as previously described (8, 19).

Primary tumor cells derived from patients with mantle cell lymphoma

Neoplastic B-cells were isolated by MACS sorting (negative selection) from pre-treatment biopsy tissue obtained from patients with B-cell NHL treated at Roswell Park Cancer Institute (RPCI) procured under Institutional Review Board (IRB) RPCI protocols I42804 and I42904. Tissue specimens were placed in PBS-containing collagenase type IV (1mg/ml; Sigma-Aldrich, St. Louis, MO) and incubated for 15 minutes at 37°C, including manual agitation for five minutes. Next, samples were diluted with RPMI 1640-containing 10% fetal bovine serum (FBS) and the cell suspension filtered through a 100µm cell strainer to remove large clumps. Subsequently, lymphocytes were enriched by histopaque density centrifugation. B-cells were then isolated from enriched lymphocytes by MACS separation using a
human B-cell Isolation Kit II (Miltenyi Biotec, Gladbach, Germany). Cells were incubated with ofatumumab, rituximab, isotype or media with 25% human serum. After 48 hours, cell viability was determined by Cell-Titer Glo assay (Promega, Madison, WI).

**Functional cytotoxicity assays of ofatumumab and rituximab-induced ADCC and CDC**

Standard $^{51}$Cr release assays were performed to assess anti-CD20 mAb-mediated CDC and ADCC. For CDC and ADCC assays, $5\times10^5$ MCL cells were labeled for two hours at $37^\circ$C with $3.7$MBq of $^{51}$Cr ($100\mu$Ci). Subsequently, $1\times10^5$cells/well or $1\times10^4$cells/well were placed in 96 well plates for CDC or ADCC assays, respectively. MCL cells were then exposed to RPMI-1640, ofatumumab, rituximab, or isotype control at a final concentration of $10\ \mu$g/ml in combination with human serum (dilution 1:4, CDC assays) or peripheral blood mononuclear cells (PBMC) at an effector:target ration of 40:1 (ADCC assays). Pooled human serum and PBMCs were collected from healthy donors under RPCI IRB-approved protocol CIC 01-16. PBMCs were obtained by Ficoll-Histopaque centrifugation of whole blood as previously described (20). Cells were incubated at $37^\circ$C, 5% CO$_2$ for six hours. A set of $^{51}$Cr-labeled NHL cells were incubated in RPMI-1640 and then treated with 50$\mu$l of a 5% Triton solution to determine maximum cell lysis. Finally, the 96-well plates were centrifuged at 2000 rpm for five minutes and the supernatant of each well was collected; gamma emission was measured by the Packard Auto-Gamma Cobra II series counting system (IBM Inc.). Percentage of $^{51}$Cr release (lysis) was calculated using the standard formula: 

$$\%\text{lysis} = \frac{[(\text{test sample release} - \text{background release})/ (\text{maximum release} - \text{background release})] \times 100}{100}.$$ 

All samples were run in triplicate in three different sets of experiments. Results are reported as mean values with standard error bars.

**Direct Killing Assays**

The direct anti-tumor effect was assayed using an alamarBlue (Invitrogen, Grand Island, NY) reduction assay. MCL cell lines ($5\times10^4$/well) were incubated with rituximab, ofatumumab or isotype control at
10μg/mL in the presence of a cross-linking IgG antibody (1μg/mL). Cell viability and the induction of apoptosis were measured after 24, 48 and 72 hours of incubation.

**Surface expression of CD20 and complement inhibitory proteins (CD55 and CD59)**

To correlate anti-CD20 antibody activity in MCL cell lines to expression of CD20, CD55 and CD59, surface expression of each protein was measured by quantitative flow cytometry and surface density was determined by flow cytometry using Imagestream (Amnis). Flow cytometric analysis was performed using the FCS express software version 3 for windows (De Novo Software, Los Angeles, CA).

**Antibodies**

Rituximab (Biogen Idec, San Diego, CA and Genentech, San Francisco) was obtained from the RPCI Pharmacy Department at a stock concentration of 10mg/ml. Chimeric anti-human Her-2 neu (trastuzumab) provided by Genentech was used as an isotype control. Ofatumumab was provided by Genmab (Copenhagen, Denmark) or obtained from the RPCI Pharmacy Department. Unless otherwise specified, antibodies were used at a final concentration of 10μg/ml.

For phenotypic analysis of lymphoma cells, fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CD20, phycoerythin (PE)-conjugated mouse anti-human CD59 and Cy-Chrome-conjugated mouse anti-human CD55 mAbs were purchased from BD Pharmingen Inc (San Diego, CA). FITC-goat anti-mouse and PE-goat anti-human and APC-goat anti-human monoclonal antibodies were used as isotype controls (BD Pharmingen Inc. San Diego, CA).

**In vivo effects of ofatumumab and rituximab in mantle cell lymphoma**

For in vivo experiments, six- to eight-week old severe combined immunodeficiency (SCID) mice were bred and maintained at the Department of Laboratory Animal Resource (DLAR) facility at RPCI. The Institutional Animal Care and Use Committee (IACUC) at RPCI approved the experimental design under protocol P966M. All animals were housed and maintained in laminar flow cabinets or micro isolator...
units and provided with sterilized food and water. Six to eight-week old SCID mice were inoculated subcutaneously on the posterior flank with $10 \times 10^6$ Z-138 cells suspended in Matrigel. Mice were monitored for establishment of a subcutaneous tumor nodule. Tumor volume was determined by caliper measurement as previously described(21). Once the tumor volume reached approximately 250mm$^3$, mice were assigned to either a control group, ofatumumab (10mg/kg) or rituximab (10mg/kg). Monoclonal antibodies were delivered IV via tail vein injection on days +0, +3, +7 and +10 following tumor engraftment. Tumor volume was monitored by serial caliper measurement. Mice with a tumor exceeding 2cm in largest diameter were sacrificed as per RPCI IACUC requirements. Tumor size >2cm in largest diameter was used as the survival endpoint in a Kaplan Meier analysis. Differences in outcomes between treatment groups were compared by log rank analysis.

Statistics

In vitro experiments were performed in triplicates on three separate occasions. Data was plotted and analyzed using SPSS 16.0 for windows 2003 software (IBM, Chicago IL). For in vitro studies (ADCC and CDC assays), statistical differences between treatment groups and controls were determined by unpaired students t-test. In addition, in vivo survival curves were generated using the Kaplan-Meier method using SPSS software. Differences in survival were compared by log rank testing. A P-value of less than 0.05 was defined as having statistical significance.

Results

Ofatumumab activates complement and results in more potent mab-CDC than rituximab in MCL cells. Assays of CDC and ADCC activity were performed in a panel of MCL cell lines. Similar to previously reported results, ofatumumab exhibited greater induction of CDC induced cell lysis when compared to rituximab (17). Ofatumumab (10µg/mL) induced a higher percentage of cell lysis compared to rituximab (10µg/mL) in all but one of the cell lines tested (Granta cells), including in multiple cell lines (Mino, Rec-1
and Jeko-1) in which rituximab elicited minimal activity (Figure 1). Both rituximab and ofatumumab (10µg/mL) demonstrated lower levels of in vitro ADCC (effector:target 40:1) with little difference noted between the two antibodies (Figure 2). Cytarabine-resistance was not associated with any degree of resistance to either antibody.

Cytotoxicity assays were also performed in tumor cells isolated from MCL patient lymph node biopsy samples. A total of 4 MCL patients with lymph node tissue were available. In two patient samples, ofatumumab induced a slightly higher though overall similar degree of decrease in cell viability compared to rituximab when primary MCL cells were exposed to each antibody (10µg/mL) in the presence of human serum (Figure 3). In the other two samples, ofatumumab exposure resulted in a statistically significant decrease in viability when compared to rituximab, though one of the two had a dramatic response to both antibodies and thus, though statistically significant, the difference would be unlikely to be clinically relevant.

**Ofatumumab and rituximab exposure led to a similar degree of direct anti-lymphoma effect.** In MCL cell lines exposed to ofatumumab or rituximab, there was a time-dependent decrease in cell proliferation when compared to an isotype control. Overall, the direct effects of antibody exposure were modest and similar between ofatumumab and rituximab treated cells with the only statistically significant difference being noted in one of the cell lines tested (Mino) at the 72h time point (Figure 4).

**MCL cell lines express high levels of surface CD20 and complement inhibitory proteins (CIP) CD55 and CD59.** In order to investigate the possible mechanism(s) that could explain the differences in the anti-tumor activity between rituximab and ofatumumab in MCL cell lines, we tested the density of surface CD20 expression using Imagestream technology. Burkitt’s lymphoma cell lines known to be sensitive (Raji) or resistant (Raji-4RH) to rituximab were included for comparison. The Raji-4RH cell line was generated in our laboratory by the serial exposure of Raji cells to escalating doses of rituximab and
human serum as previously described (8). The MCL cell lines Mino, Jeko-1, Rec-1 and Z-138 all had surface CD20 density similar to the rituximab-sensitive Raji cell line as opposed to Raji-4RH cells known to have a low surface CD20 density, suggesting CD20 expression levels did not play a significant role in their intrinsic resistance to rituximab when compared to ofatumumab (Figure 5A). Meanwhile, each of the MCL cell lines demonstrated an increased level of surface CD55 and CD59 density compared to Raji cells. The degree of CD55/CD59 surface expression in MCL was comparable to that observed in Raji-4RH cells, suggesting that higher levels of CIPs may contribute to the rituximab-resistant phenotype in the MCL cell lines on in vitro testing (Figure 5B and 5C). Of note, Granta, the one cell line that was resistant to both rituximab and ofatumumab induced CDC, exhibited very high levels of CD55 and the highest levels of CD59, even exceeding those demonstrated in the rituximab resistant Raji-4RH cells, potentially explaining its high level of resistance to either of these antibodies.

**Ofatumumab more effectively controlled tumor growth and prolonged survival in an in vivo SCID mouse model of MCL.** Using a subcutaneous tumor model of MCL, the activity of ofatumumab and rituximab were investigated in Z-138 cell bearing SCID mice. Mice with established subcutaneous tumors treated with four doses of ofatumumab (10mg/kg) exhibited delayed early tumor growth compared to mice treated with rituximab at the same dose and schedule (Figure 6A). All mice in both the ofatumumab and rituximab groups had tumors that responded to their respective anti-CD20 therapy with a decrease in tumor volume. However, ofatumumab treated mice demonstrated a sustained response while rituximab treated mice eventually developed progressive disease as evidenced by the development of progressively larger tumors. Using a subcutaneous tumor size of 2cm in any dimension as the end-point for survival, ofatumumab significantly prolonged survival as compared to rituximab in this subcutaneous SCID mouse model at the dosing regimen tested (Figure 6B). Median survival in the rituximab treated mice was 127 days compared to the ofatumumab mice where the median survival was not reached (p=0.044).
Discussion

Our results indicate that ofatumumab exhibits more potent anti-lymphoma activity against human MCL cells and MCL mouse models when compared to rituximab. As previously reported, ofatumumab demonstrated a significantly higher degree of CDC associated cytotoxicity. It has been proposed that ofatumumab’s enhanced CDC activity may be related to its unique epitope binding, positioning the antibody more proximally to the cell membrane allowing it to more efficiently activate complement (15). Our findings also indicate that MCL cell lines may exhibit resistance to rituximab related to higher levels of CD55 and CD59 on the lymphoma cell surface. Differences between mAbs-targeting CD20 in their capacity to activate the complement cascade, could explain why MCL with higher levels of CIP are sensitive to ofatumumab but not rituximab. On the other hand, ADCC activity in vitro was noted to be comparable between the two antibodies. Using in vivo models of MCL, we demonstrated that ofatumumab was more effective than rituximab in controlling lymphoma growth. While, this could be explained by differences in complement activation in vivo, we previously demonstrated that the predominant mechanism of rituximab activity in our SCID mouse model is likely ADCC. Thus, while no significant differences in terms of ADCC was observed in vitro between rituximab and ofatumumab, it is possible that in vivo induction of ADCC is higher among ofatumumab-treated MCL bearing SCID mice when compared to those animals treated with rituximab.

While ofatumumab has increased potency in pre-clinical testing when compared to rituximab, it is currently unclear whether these results will translate into improved clinical responses or outcomes in lymphoma patients. In the initial Phase I/II trial of ofatumumab as a single agent in relapsed/refractory follicular lymphoma (FL), Hagenbeek et al. reported that ofatumumab was well tolerated and adverse events were primarily infusion related toxicities following the first antibody infusion (22). The best response rate was 43%, with an overall response rate (ORR) of 64% observed in patients previously...
treated with rituximab including 3 out of 4 patients previously deemed to be rituximab-refractory (22). In recently reported results from Czuczman et al, ofatumumab monotherapy resulted in an ORR of only 11%, though these patients were very heavily pre-treated and there was an ORR of 22% in patients refractory to rituximab monotherapy (23). There is limited data currently available on the clinical use of ofatumumab in MCL. Furtado et al recently reported on a phase II trial of single agent ofatumumab in relapsed/refractory MCL (24). In 12 patients, the overall response rate was only 8.3% with the best response being a PR in 1 patient while 6 patients (50%) had stable disease. Hunstig et al. also recently published a single case report of a patient with refractory MCL who achieved a complete response following single agent ofatumumab despite failing 5 prior treatment regimens. (25)

When combined with chemotherapy, ofatumumab has shown more promising response rates though with mixed results and no evidence of superiority over rituximab in the relapsed setting, albeit with limited data available. In a study of ofatumumab in combination with CHOP chemotherapy in treatment naïve follicular lymphoma patients, the O-CHOP combination exhibited a 90% ORR in the lower-dose 500mg group with 100% ORR in the 1000mg group including a 62% complete remission (CR) rate (26). In the setting of aggressive B-NHL, ofatumumab in combination with dexamethasone, cytarabine and cisplatin or ifosfamide, carboplatin and etoposide (DHAP or ICE) resulted in an ORR of 61% in patients with relapsed/primary refractory disease following at least one prior rituximab containing regimen (27). However, in a recently reported large international trial investigating ofatumumab head to head with rituximab in combination with DHAP in relapsed/refractory DLBCL, the ORR to O-DHAP was significantly lower at 38% and was similar to that of R-DHAP (42%) with no difference in progression free or overall survival between groups (28). Of note, in 19 previously untreated older (>65 years) patients with MCL, the combination of ofatumumab, bendamustine and dexamethasone resulted in an ORR of 94% with 89% of those achieving a CR (29).
Based on our results, ofatumumab represents a promising novel agent for the treatment of patients with MCL either up-front or in the setting of rituximab resistant disease, however the role of ofatumumab in treating MCL is unclear when considering other novel agents with potential promise in treating MCL such as other anti-CD20 mAb (e.g. obinutuzumab), proteasome inhibitors (e.g. bortezomib or carfilzomib), mTOR inhibitors (e.g. temsirolimus) or inhibitors of the B-cell receptor pathway (e.g. ibrutinib). While results from early phase trials have been somewhat mixed, ofatumumab continues to be investigated in trials incorporating de novo and relapsed/refractory B-NHL patients including an ongoing clinical trial sponsored by our institution investigating ofatumumab in combination with hyper-fractionated cyclophosphamide, doxorubicin, vincristine and dexamethasone (O-HyperCVAD) alternating with ofatumumab-high-dose cytarabine and methotrexate (O-MA) for patients with newly diagnosed Mantle Cell Lymphoma (NCT01527149).

Authorship

M.J.B designed and performed experiments, analyzed the data and wrote the manuscript; C.M. performed experiments, analyzed data and critically evaluated the manuscript; M.S.C. designed experiments and critically evaluated the manuscript; F.J.H. designed experiments and critically evaluated the manuscript.

References


**Figure Legends**

**Figure 1: Ofatumumab induces significantly higher levels of CDC associated cell lysis than rituximab.**

MCL tumor cell lines were tested for CDC cell lysis using a $^{51}$Cr release assay with human serum from
normal healthy donors as a source of complement. In all MCL cell lines tested, with the exception of Granta, ofatumumab was more effective than rituximab at inducing CDC lysis at a dose of 10ug/mL. (* p<0.05)

**Figure 2:** Ofatumumab and rituximab induce a similar degree of ADCC associated cell lysis. MCL tumor cell lines were tested for ADCC cell lysis using a $^{51}$Cr release assay using effector cells from normal healthy donors at a ratio of 40:1. In all MCL cell lines tested, ofatumumab and rituximab induced similar levels of ADCC lysis at a dose of 10ug/mL.

**Figure 3:** In MCL cells derived from patient samples, ofatumumab induced similar or more cell lysis by CDC compared to rituximab. B-cells were isolated from pre-treatment tumor samples (n=4) obtained from MCL patients and tested for monoclonal antibody response by CellTiter Glo assay using rituximab or ofatumumab at a concentration of 10ug/mL in the presence of human serum as a source of complement. Two of the 4 samples exhibit a statistically significant decrease in viability following ofatumumab exposure as compared to rituximab. The remaining samples show a similar response to each antibody.

**Figure 4:** Ofatumumab and rituximab exhibit modest direct anti-tumor activity against MCL cell lines. MCL cell lines were utilized in alamarBlue reduction assays to investigate the direct anti-lymphoma activity of each antibody. Each antibody exhibited a time-dependent decrease in MCL cell viability following exposure to 10ug/mL of each respective antibody in the presence of a cross-linking IgG antibody. With the exception of the Mino cell line at the 72h time point, all cell lines demonstrated similar degrees of direct anti-tumor activity with ofatumumab and rituximab at the dose tested. (* p<0.05)

**Figure 5:** Expression of CD20, CD55 and CD59 in MCL cell lines compared to rituximab-sensitive Raji and rituximab-resistant Raji 4RH cell lines. The surface density of CD20, CD55 and CD59 were
determined using Imagestream. MCL cell lines exhibit similar density of CD20 expression on their cell membrane when compared to the rituximab-sensitive Raji cell line. However, the complement inhibitory proteins CD55 and CD59 are present at increased surface density compared to Raji cells, similar to the rituximab-resistant Raji 4RH cell line.

**Figure 6: Ofatumumab delayed tumor growth and prolonged survival in an in vivo SCID mouse model of MCL.** SCID mice were inoculated subcutaneously on the posterior flank with $10 \times 10^6$ Z-138 cells. Mice with established tumors were assigned to control, ofatumumab 10mg/kg or rituximab 10mg/kg for 4 doses on days 0, 3, 7 and 10 following tumor establishment. Tumor volume was determined by caliper measurement. Ofatumumab slowed early tumor growth when compared to rituximab with a significant difference in tumor volume noted days 3 and 8 following the start of treatment. Mice were sacrificed when the subcutaneous tumor grew to 2cm in its largest diameter. Using this time-point as the survival endpoint in a Kaplan-Meier analysis, ofatumumab (median survival not reached) significantly prolonged survival compared to rituximab (median=127 days). (* p<0.05)
Figure 1

![Graph showing lysis percentage for different cell lines with different treatments. The x-axis represents cell lines: Mino, Mino AraCR, Rec-1, Rec-1 AraCR, Jeko-1, Jeko-1 AraCR, HBL-2, HBL-2 AraCR, Granta, Granta AraCR, and Z-138. The y-axis represents lysis percentage ranging from 0 to 100%. The treatments are indicated by different bars: Isotype (white), Rituximab (gray), and Ofatumumab (black). Asterisks (*) indicate statistically significant differences.](image-url)
Figure 2

Lysis (%)

- Isotype
- Rituximab
- Ofatumumab

Figure 3

% Viable Cells

0 25 50 75 100

68 71 82 93

- Isotype
- Rituximab
- Ofatumumab

* p<0.05
Figure 4
Figure 5

A: CD20 Density

B: CD55 Density

C: CD59 Density
Figure 6

A

B

Tumor Volume (mm$^3$)

Cum Survival

Day

Days

Control

Rituximab

Ofatumumab

* p<0.05
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