

COMPARISON OF CELL LYSIS MEDIATED BY LFB-R603 (UBLITUXIMAB) WITH THAT MEDIATED BY OFATUMUMAB AGAINST CELLS EXPRESSING LOW LEVELS OF CD20

Abstract
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SUMMARY

In the study reported here, we further investigated the B-cell lysis activity of LFB-R603 (ublrituximab), a novel anti-CD20 monoclonal antibody (mAb), as compared with ofatumumab and rituximab on various cell lines and on PBMCs from CLL patients. In terms of CDC-mediated lysis, we observed higher CDC activity of ofatumumab when compared with LFB-R603 on several cell lines expressing high CD20 levels (data not shown). Rituximab displayed CDC activity slightly superior to that of LFB-R603. In contrast, CDC activity against CD20-low expressing cells, the SUDHL-8 cell line, and CLL patient-derived PBMCs was very low with all three mAbs, rituximab, ofatumumab and LFB-R603. Consistent with previous results, LFB-R603 was shown to mediate high ADCC against all cell lines tested, including CD20-low expressing cells, whereas both ofatumumab and rituximab mediated ADCC at low levels.

Altogether, these results support the fact that the therapeutic use of LFB-R603 may be advantageous over currently approved anti-CD20 mAbs in targeting malignant cells where surface CD20 molecules are known to be expressed at low levels such as in CLL and small lymphocytic lymphomas.

INTRODUCTION

Anti-CD20 mAbs, such as rituximab and ofatumumab, have several potential mechanisms of action, including **antibody-dependent cell cytotoxicity (ADCC)**, **complement dependent cytotoxicity (CDC)** and induction of **apoptosis**.

Their target is the CD20 molecule present almost exclusively at the surface of normal and malignant human B-cells. Nevertheless, it has been described that the level of CD20 can vary among the diseased populations. Indeed, CD20 surface antigen expression in chronic lymphocytic leukemia (CLL) is significantly lower than in other B-cell lymphoproliferative diseases⁽¹⁾, which may affect the degree of anti-CD20 antibody binding. Low density of CD20 expression on CLL cells may explain the minimal response rates exhibited with single agent anti-CD20 therapy.

LFB BIOTECHNOLOGIES has developed a third generation anti-CD20 mAb (LFB-R603) on the LFB EMABling[®] platform, capable of selecting antibodies with improved ADCC, based on their glycosylation pattern.

LFB-R603 has been described *in vitro* to have a potent ADCC activity, superior to that of rituximab.

MATERIALS AND METHODS

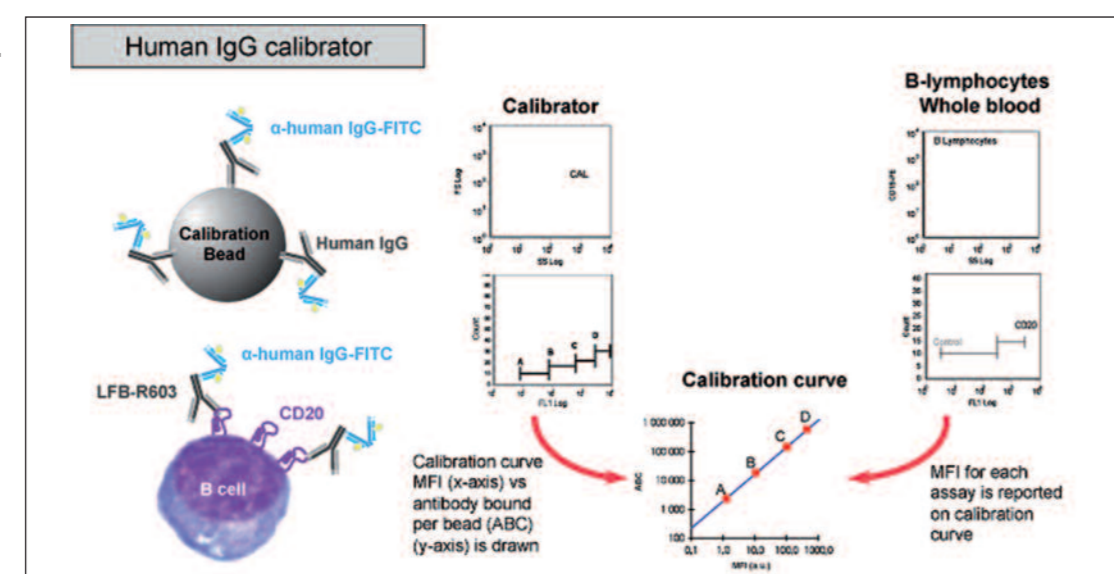
Cells - Human whole blood (WB) was obtained in EDTA for flow cytometry or citrate phosphate dextrose for PBMC preparation. PBMCs were isolated by centrifugation on density gradient, frozen and stored in liquid nitrogen. The target cell lines MEC-1 (data not shown) and SUDHL-8 are human B cell leukemia and human B cell lymphoma, respectively.

Flow cytometry (FC) - Phenotypic analyses were performed by FC. 100 µL of WB or cell line (3 x 10⁶ cells) were stained with human mAb according to protocol procedure of human IgG calibrator kit (BioCytex). For WB, cells were counterstained with CD19-PE and subjected to FC analysis (Cytomics FC500, Beckman Coulter).

Complement-dependent cytotoxicity assay (CDC) - Cell lines or enriched CLL cells in human C1q depleted serum were spiked 10 minutes at 37°C with human mAb at 10 µg/mL and C1q at various concentrations. Cell death was determined by intercalation of the DNA dye propidium iodide (PI) by FC.

Antibody-dependent cell cytotoxicity assay (ADCC) - After thawing, PBMC (source of NK cells) were washed and suspended in RPMI supplemented with 10% FCS. Target cells - cell lines or enriched B-cells from CLL patients - were incubated with human mAb at 20 µg/mL for 30 minutes at 4°C. After washing, cell lines or CLL cells were labeled with CFSE for 10 minutes. Labeled target cells were suspended in RPMI 1640 (+10% FCS) and mixed with PBMC at various effector/target (E/T) ratios. Cells were incubated 4 hours at 37°C, and analyzed by FC after staining with PI. Human B-CLL or human B lymphocytes were enriched from PBMC from CLL patients or healthy volunteers using kits from Miltenyi.

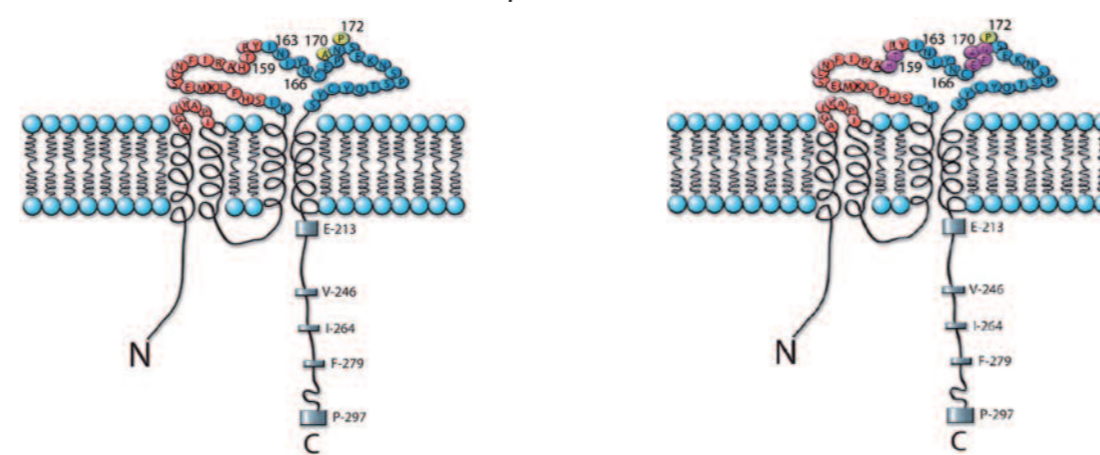
Calibration assay - Calibration beads are coated with known and increasing numbers of human IgG bound to their surface thus mimicking the binding of LFB-R603 to CD20 antigen expressed on the B-cells. Then, the calibration beads and B-cells are incubated with a polyclonal antibody FITC-conjugated anti-human IgG. The beads bear a specific number of sites and permit a calibration curve to be drawn that relates mean fluorescence intensity (MFI) into a number of receptors per cell. The MFI of the tested sample is reported on the calibration curve to determine the number of molecules expressed per cell. Non specific staining is evaluated using negative isotypic control and removed from ABC values to calculate the specific ABCs. ABC = Antibody binding capacity



RESULTS

Rituximab, ofatumumab and LFB-R603 epitope recognition

adapted from Ruuls *et al.*, 2008⁽²⁾



Left panel: Amino acids contributing to ofatumumab binding are indicated in red. Amino acids essential for rituximab, but not ofatumumab binding are indicated in yellow.
Right panel: Core amino acids of LFB-R603 epitope are shown in purple.

Quantification of CD20 molecules at the cell surface of cell lines and human PBMC from healthy subjects and CLL patients

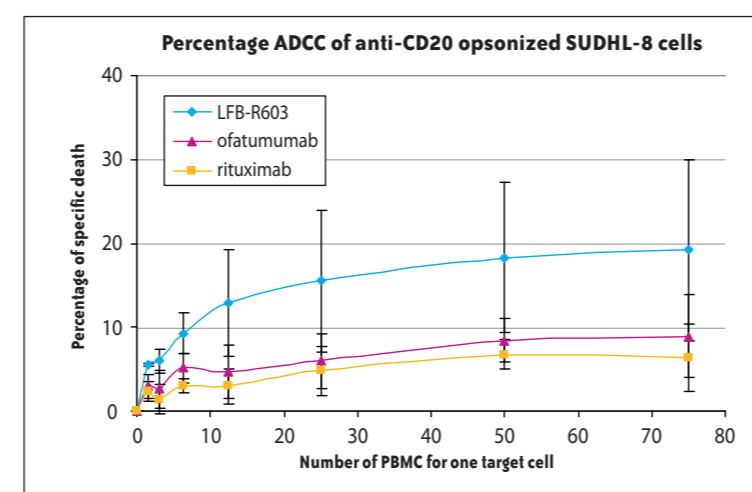
On cell lines expressing high and low levels of CD20 (mean of at least 4 assays)

	rituximab	ofatumumab	LFB-R603
MEC-1	527 164	519 175	484 497
SUDHL-8	18 514	22 950	18 721

On human B-lymphocytes in whole blood

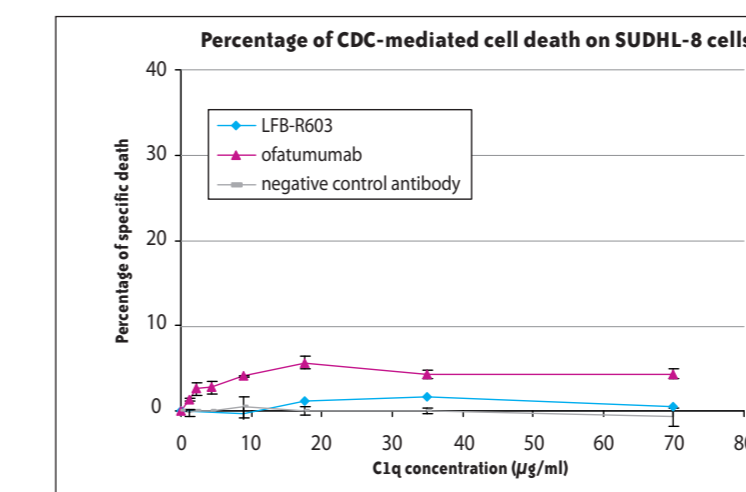
	rituximab	ofatumumab	LFB-R603
Healthy	Mean	138 488	124 034
	SD	21 822	19 851
	n	4	6
CLL	From 3 600 to 138 000 CD20/cell (n=8)		

ADCC on cell lines expressing low levels of CD20 : SUDHL-8



LFB-R603 induced superior ADCC levels on cells expressing low levels of CD20, illustrated by the SUDHL-8 cell line, in comparison with ofatumumab and rituximab.

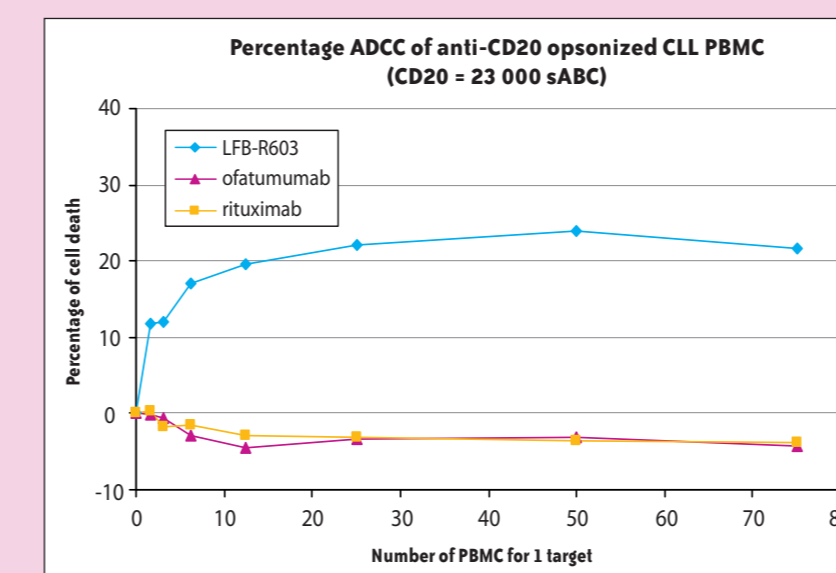
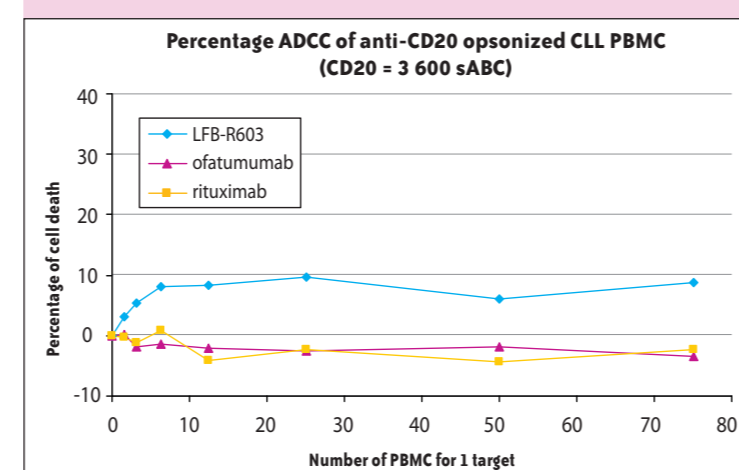
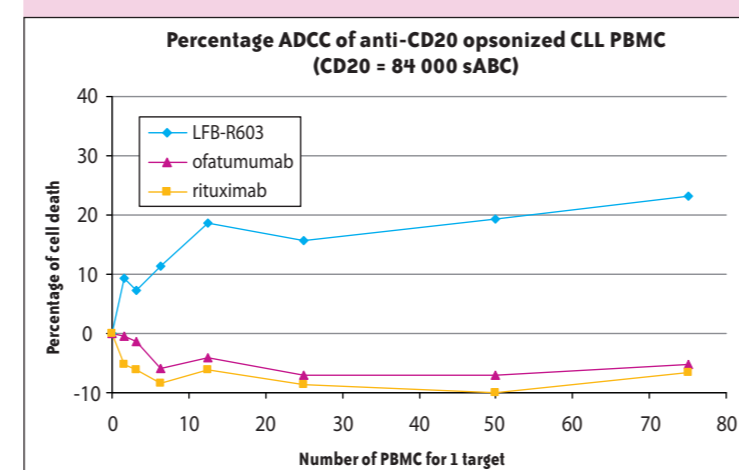
CDC on cell lines expressing low levels of CD20 : SUDHL-8



Ofatumumab induced very low CDC activity on cells expressing low levels of CD20, illustrated by the SUDHL-8 cell line, similar to LFB-R603 and rituximab.

ADCC of CLL primitive cells of three patients expressing different CD20 levels

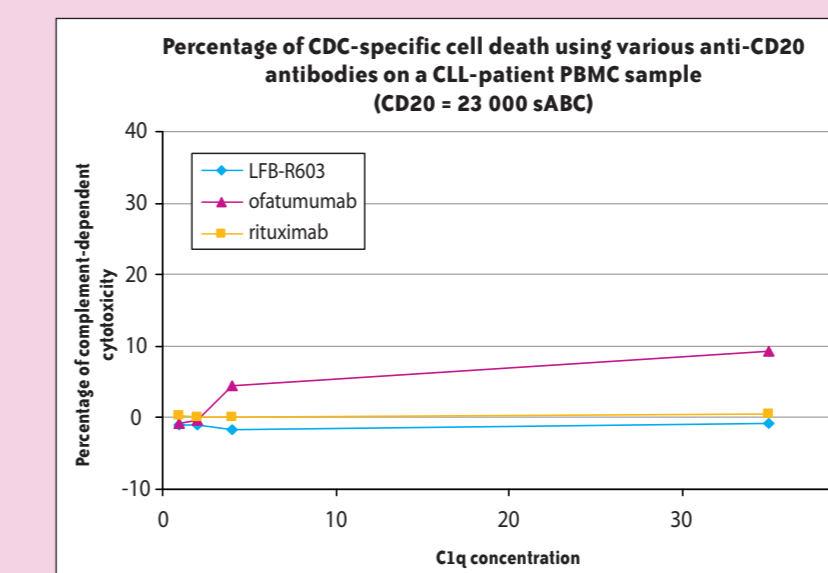
LFB-R603 mediated ADCC at a higher rate than other anti-CD20 mAbs on B-cells from 3 CLL patients even for cancer cells with very low CD20 density.



LFB-R603-mediated ADCC on CLL patient' PBMC with a CD20 expression comparable to the one described in the literature for CLL patients (approx. 23 000 molecules / cell).

CDC of CLL primitive cells of one patient with 23 000 CD20 at the cell surface

LFB-R603 and rituximab did not induce any complement-specific cell death on patient-derived CLL PBMC with low CD20 expression, comparable to the one described in the literature for CLL patients (approx. 23 000 molecules / cell). Ofatumumab mediated only a poor CDC (<10%), even at a high concentration of C1q.



CONCLUSION

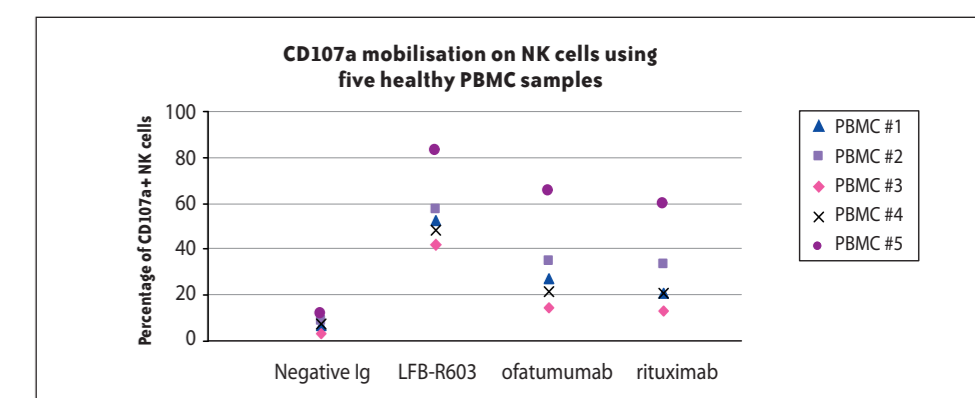
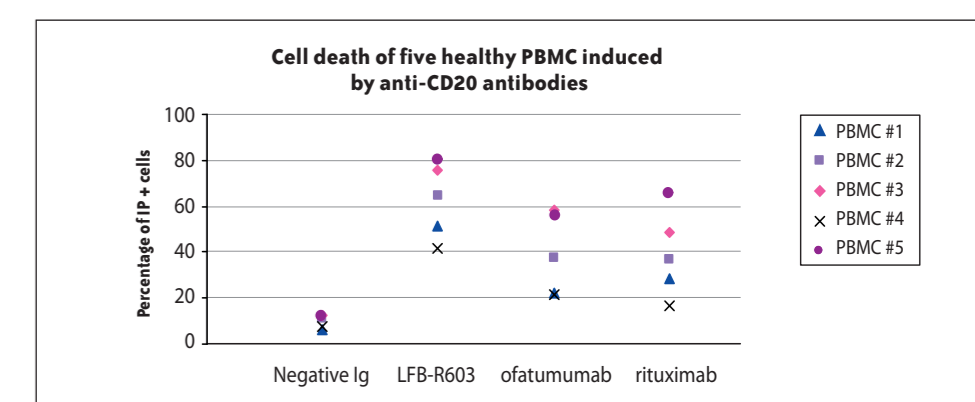
Anti-CD20 monoclonal antibodies can induce target cell killing by several mechanisms of action, including antibody-dependent cell cytotoxicity (ADCC), complement dependent cytotoxicity (CDC) and induction of apoptosis.

In this study, we have demonstrated that LFB-R603 is superior to not only rituximab but also ofatumumab, in terms of ADCC-mediated B-cell killing regardless of CD20 surface levels on target cells. Although ofatumumab presents superiority of CDC-mediated killing of target cells expressing high levels of CD20 (data not shown), this advantage has not been seen when target cells with low CD20 levels were assayed.

Altogether, these data support the theory that LFB-R603 could induce a higher killing of low-CD20 expressing target cells than ofatumumab and rituximab in CLL patients.

Perspectives

We showed *in vitro*, on healthy volunteers' (data below) and CLL patients' B-cells, a link between CD107a expression at the NK cell surface and ADCC activity.



We plan to confirm, in the next clinical study, that LFB-R603 is able *in vivo* to induce ADCC when administered to CLL patients, by studying CD107a expression on patients NK cells at various timepoints.

(1) Almasri NM, Duque RE, Iturraspe J, Everrett E, Braylan RC. Reduced expression of CD20 antigen as a characteristic marker for chronic lymphocytic leukemia. *Am J Hematol.* 1992 Aug;40(4):259-263
(2) Ruuls SR, Lammerts van Bueren JJ, van de Winkel JG, Parren PW. Novel human antibody therapeutics: the age of the Umabs. *Biotechnol J.* 2008 Oct;3(9-10):1157-71.