

# Glyco-Optimized Rituximab Produced in *Lemna* Has Enhanced ADCC Activity and Decreased CDC Activity

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## Abstract

Monoclonal antibodies (mAbs) are one of the fastest growing classes of protein therapeutics. For many antibodies, the N-glycosylation status of the Fc region of the H-chain plays a significant role in the therapeutic function. The structure and extent of heterogeneity of these N-glycans are two of the distinguishing features in selecting a protein expression platform for a therapeutic antibody.

A glyco-optimized rituximab was expressed in the small aquatic plant *Lemna* (*LexOpt*). The optimized glycosylation was accomplished by co-expressing an interfering RNA (RNAi) construct targeting the endogenous alpha-1,3-fucosyltransferase (FucT) and beta-1,2-xylosyltransferase (XyIT) genes (Cox *et al.*, 2006). *LexOpt* rituximab contained a single major G0 N-glycan without any detectable xylose or fucose. In cell-based functional assays, *LexOpt* rituximab showed similar CD20 binding as Rituxan® (RTX) produced in mammalian cells with significantly enhanced antibody-dependent cellular cytotoxicity (ADCC), decreased complement-dependent cytotoxicity (CDC) and similar apoptotic activity. Preliminary data suggests *LexOpt* rituximab is more potent than RTX in causing B-cell depletion in whole blood.

## Overview

Rituxan® is a chimeric anti-CD20 antibody used in the treatment of non-Hodgkin's B-cell lymphoma (NHL). Although Rituxan is a key treatment for NHL, the patient response rate is only 50-60% and is significantly correlated with a FcγRIIIa receptor polymorphism (Carton *et al.*, 2002). More specifically, 90% of patients homozygous for valine at position 158 (~20% of the population) respond to Rituxan treatment whereas patients hetero- or homozygous for phenylalanine at position 158 have a considerably lower response rate. This lower response rate is likely the result of a lower affinity for FcγRIIIa phe<sup>158</sup> than for FcγRIIIa val<sup>158</sup> leading to lower ADCC activity, the primary mode of action for Rituxan. Recently, it has been shown that afucosylated IgG1 has a higher affinity for FcγRIIIa phe<sup>158</sup> and consequently higher ADCC activity than the corresponding fucosylated IgG1 (Shields *et al.*, 2002). An afucosylated rituximab could, therefore, be a potentially more potent and efficacious product regardless of the FcγRIIIa genotype.

In addition to ADCC, rituximab is thought to also mediate tumor cell killing through complement dependent cytotoxicity (CDC) (Cragg and Glennie, 2004). While the overall importance of CDC in the efficacy of rituximab is theorized, a much stronger case can be made for CDC playing a key role in the side-effects of rituximab treatment (Kolk *et al.*, 2001). It has been hypothesized that anti-CD20 therapy may be improved by reducing CDC activity while enhancing ADCC activity (Clark and Ledbetter, 2005). Since a positive correlation between the galactose content of rituximab N-glycans and CDC activity has been shown (Boyd *et al.*, 1995) it is reasonable to assume that a rituximab lacking galactose terminated N-glycans would have lower CDC activity.

The *Lemna* expression system (LEX System<sup>SM</sup>) is an ideal expression system for mAbs (Gasdaska *et al.*, 2003). Numerous proteins, including mAbs have been expressed at high levels. The LEX System offers rapid clonal expansion and full containment in a robust and well-controlled format. Recently, glycan optimization has been demonstrated in the LEX System (LEX<sup>Opt</sup>) with an anti-CD30 mAb (Cox *et al.*, 2006). Co-expression with an RNAi targeting the expression of fucT and xyIT resulted in a mAb with a single major G0 glycan without detectable xylose and fucose.

The results found in Figures 1-7 show that an afucosylated rituximab has been expressed in the LEX System with homogenous G0 glycans without affecting antigen binding. This LEX<sup>Opt</sup> rituximab has been shown to have enhanced ADCC activity with a decrease in CDC activity and similar apoptotic activity when compared to RTX. In whole blood, preliminary data suggests LEX<sup>Opt</sup> rituximab is more effective than RTX at causing B-cell depletion; the major mechanism of B-cell depletion appears to be cell mediated.

## Conclusions

• LEX<sup>Opt</sup> rituximab has been demonstrated to have the following characteristics:

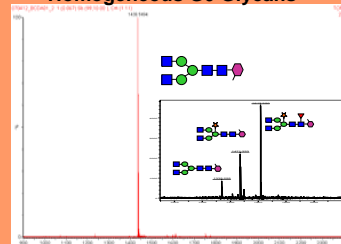
- Homogeneous G0 glycans
- Antigen binding and apoptotic activity similar to Rituxan
- ~20 to 200-fold higher ADCC activity than Rituxan
- ~10-fold lower CDC activity than Rituxan
- Demonstration of B-cell depletion in whole blood with preliminary indications of activity greater than rituximab

• LEX<sup>Opt</sup> rituximab may offer potential for:

- Increased efficacy and potency
- Decreased side-effects and reduced infusion time
- New indications

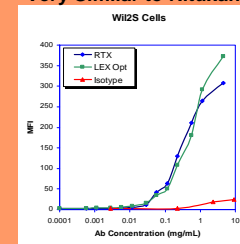
## Results

### LEX<sup>Opt</sup> Rituximab Contains Homogeneous G0 Glycans



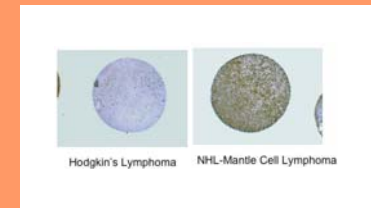
**Figure 1.** Mass spectrometry (MALDI-TOF) analysis of N-glycans labeled with 2-AA released from LEX<sup>Opt</sup> Rituximab. Structures are illustrated using the symbol nomenclature outlined by the Consortium for Functional Glycomics (<http://www.functionalglycomics.org>). Inset: Typical N-glycan profile from non-glycan optimized antibody in the LEX System.

### CD20 Binding of LEX<sup>Opt</sup> Rituximab is Very Similar to Rituxan



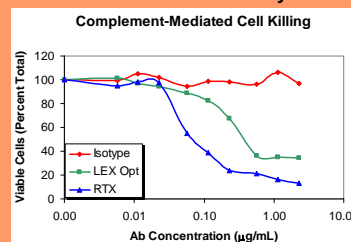
**Figure 2.** Antigen binding of glyco-optimized LEX<sup>Opt</sup> Rituximab (LEX Opt), Rituxan (RTX) and a glyco-optimized LEX System-produced isotype control to CD20 presented by W12S cells. CD20 binding by the primary antibodies (RTX and LEX<sup>Opt</sup>) is detected by fluorescence of a fluorochrome-labeled secondary anti-human IgG.

### Lex<sup>Opt</sup> Rituximab Binds to CD-20 Expressing Lymphoma Tissues



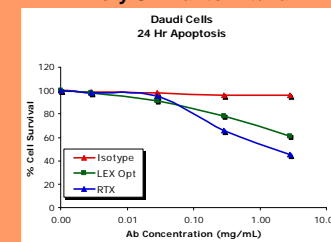
**Figure 3.** Formalin-fixed, paraffin-embedded tissue samples were treated with LEX<sup>Opt</sup> rituximab followed by a biotinylated anti-human IgG. Visualization (brown staining) was accomplished after incubation with HRP-conjugated streptavidin using diaminobenzidine as the substrate. The expression of CD20 in Hodgkins Lymphoma is not as ubiquitous as with NHL (Rassidakis *et al.*, 2002).

### LEX<sup>Opt</sup> Rituximab Has ~10X Lower CDC Activity



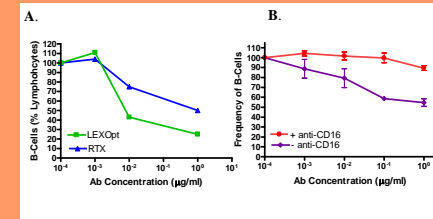
**Figure 4.** CDC activity of glyco-optimized LEX<sup>Opt</sup> rituximab and commercial Rituxan in Raji cells. Cell lysis is measured by uptake of a fluorescent dye where CDC-dependency is determined by dependency on human complement.

### Apoptotic Activity of LEX<sup>Opt</sup> Rituximab is Very Similar to Rituxan



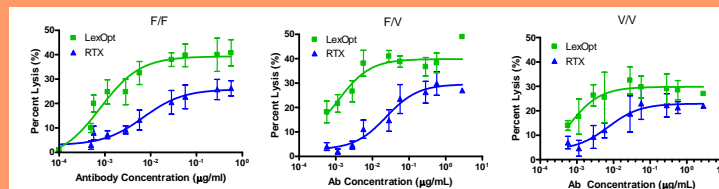
**Figure 5.** Antibody-induced apoptosis in Daudi cells. Apoptosis is measured by Annexin V-propidium iodide staining.

### LEX<sup>Opt</sup> Rituximab Leads to B-cell Depletion in Whole Blood Mediated Primarily by ADCC



**Figure 6.** B-cell depletion in whole blood where B-cells were measured by FACS using a fluorescent anti-CD19 antibody. **A.** Whole blood was treated with RTX and LEX<sup>Opt</sup> rituximab. **B.** Blocking of cell-mediated cell killing with an anti-CD16 antibody leads to a significant decrease in B-cell depletion after treatment with LEX<sup>Opt</sup> rituximab.

### LEX<sup>Opt</sup> Rituximab Shows Enhanced ADCC Activity for all FcγRIIIa Genotypes



**Figure 7.** ADCC activity of Rituxan (RTX) and glyco-optimized LEX<sup>Opt</sup> rituximab (LexOpt) in Raji cells. Percent cell lysis is determined by FACS analysis where target cells are pre-labeled with a green fluorescent dye which upon killing will lose the green dye and take up a red fluorescent dye.

## References:

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There are no relevant conflicts of interest to disclose.