Ublituximab, a Novel, Glycoengineered Anti-CD20 Monoclonal Antibody (mAb), Demonstrates Enhanced Antibody-Dependent Cellular Cytolysis (ADCC) Relative to Other Anti-CD20 mAbs

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OBJECTIVE

- The in vitro functional characterization of ublituximab relative to other anti-CD20 mAbs

KEY FINDINGS

- Ublituximab demonstrated enhanced Fcγ-receptor (FcγR) binding and ADCC compared with other commercially available anti-CD20 mAbs across all polymorphisms
  - FcγR binding affinity (K_D [nM]):
    - FcγRIIIa 158V: ublituximab, 64.1; ocrelizumab, 1025.8; ofatumumab, 1641.4; and rituximab, 1199.8
    - FcγRIIIa 158F: ublituximab, 680.3; ocrelizumab, 6762.9; ofatumumab, 14,815.2; and rituximab, 6960.9
  - ADCC activity (EC_{50} [pg/mL]): ublituximab, 2.4; ocrelizumab, 60.8; ofatumumab, 74.1; and rituximab, 5457.0
- All evaluated mAbs had comparable CD20 binding affinity (EC_{50} [µg/mL]): ublituximab, 0.063; ocrelizumab, 0.111; ofatumumab, 0.092; and rituximab, 0.133.

CONCLUSION

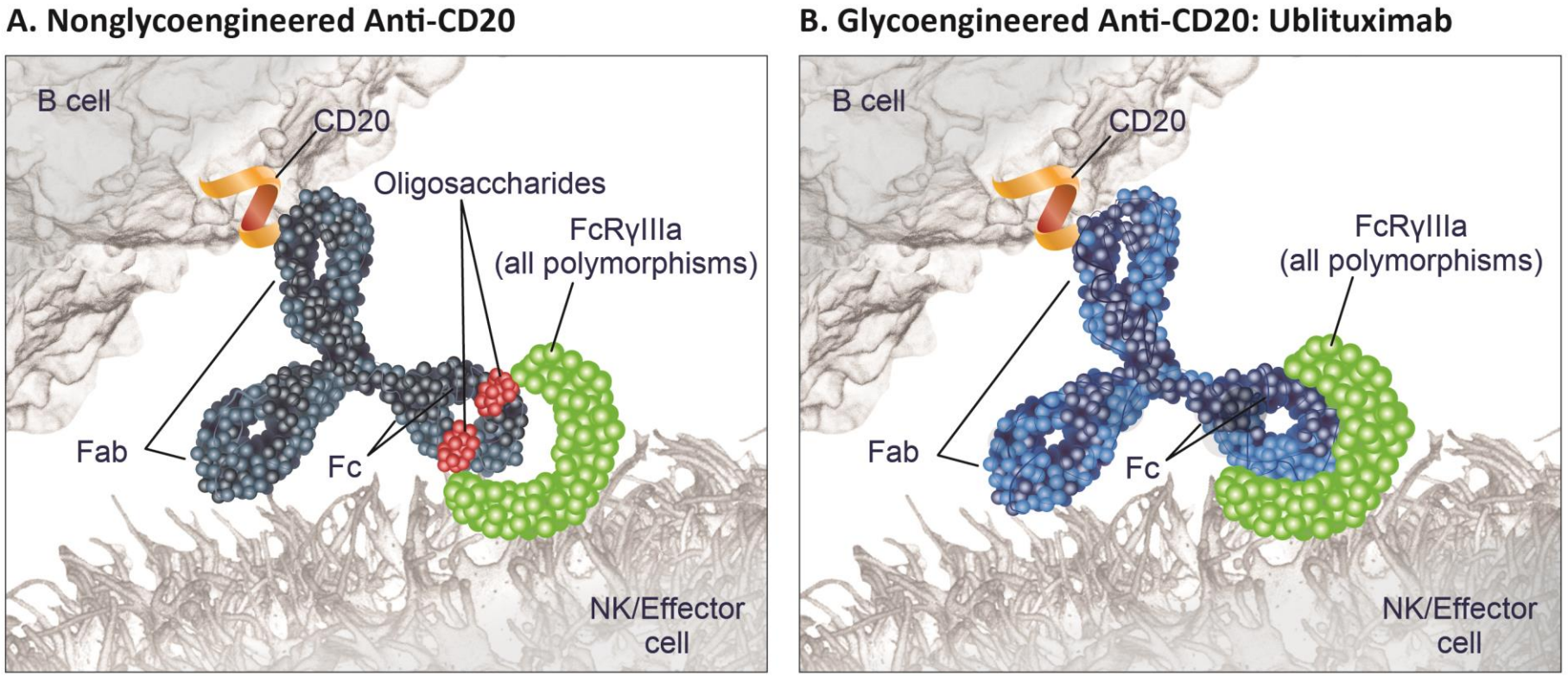
- In vitro studies demonstrated that ublituximab has higher FcγRIIIa binding and ADCC activity as a result of its glycoengineered Fc region compared with other commercially available anti-CD20 mAbs

EC_{50}, half maximal effective concentration; K_D, equilibrium dissociation constant.

BACKGROUND

- Anti-CD20 mAb–mediated B-cell depletion is induced by several mechanisms, including ADCC and complement-dependent cytolysis
- ADCC is independent of the complement system and is mediated by interactions between the Fc region of anti-CD20 mAbs and the FcγR on effector cells (eg, natural killer cells)[1]
- Polymorphisms of the FcγRIIIa at amino acid 158 (F/V) have resulted in impaired binding to immunoglobulin G (IgG), reduced effector-cell engagement, and reduced clinical response to rituximab in various disease states (eg, rheumatoid arthritis and neuromyelitis optica)[2,3]
- The FcγRIIIa 158F polymorphism, expressed in approximately 40% of the healthy population,[4] is more difficult to bind compared with FcγRIIIa 158V
- Ublituximab is a novel, chimeric, type 1 IgG mAb targeting a unique epitope of CD20 on B cells. Ublituximab is glycoengineered for reduced fucose on its Fc region, conferring greater affinity to FcγRs, leading to enhanced ADCC (**Figure 1**)

Figure 1. Ublituximab Is Glycoengineered to Enhance ADCC



(A) In nonglycoengineered anti-CD20 antibodies, the core fucose of Fc-linked oligosaccharides sterically blocks interaction with FcγRIIIa, reducing affinity.[5,6] **(B)** Ublituximab is glycoengineered to have a low fucose content in the Fc region, which allows for closer interaction and enhanced affinity for all variants of FcγRIIIa.[6-8]

ADCC, antibody-dependent cellular cytolysis; NK, natural killer.

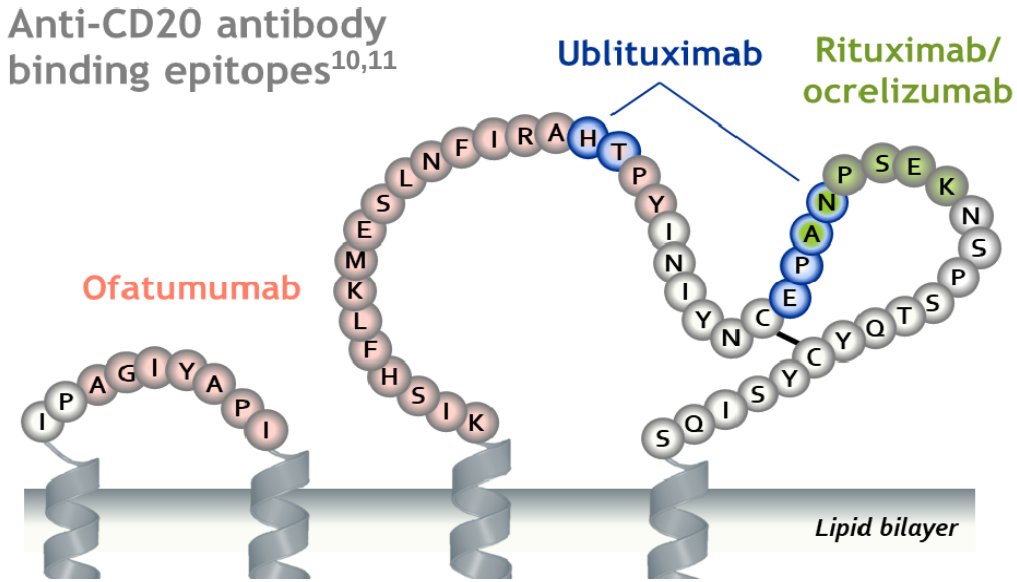
METHODS

- CD20 binding, FcγRIIIa-receptor binding, and ADCC were evaluated for ublituximab and other commercially available CD20 therapies (ocrelizumab, ofatumumab, and rituximab) with in vitro studies
- CD20 binding was evaluated using a CD20-expressing cell line (JeKo-1) and a Meso Scale Discovery electrochemiluminescence assay. FcγR affinity was evaluated using surface plasmon resonance. ADCC activity was evaluated using CD20-expressing Raji cells plus KILR® (CD16+) effector cells and diluted human serum samples in a luminescent cytotoxicity assay[9]

RESULTS

- CD20 binding affinity was similar for all evaluated antibodies (EC_{50} [µg/mL]): ublituximab, 0.063; ocrelizumab, 0.111; ofatumumab, 0.092; and rituximab, 0.133 (**Figure 2**)

Figure 2. CD20 Binding Affinity



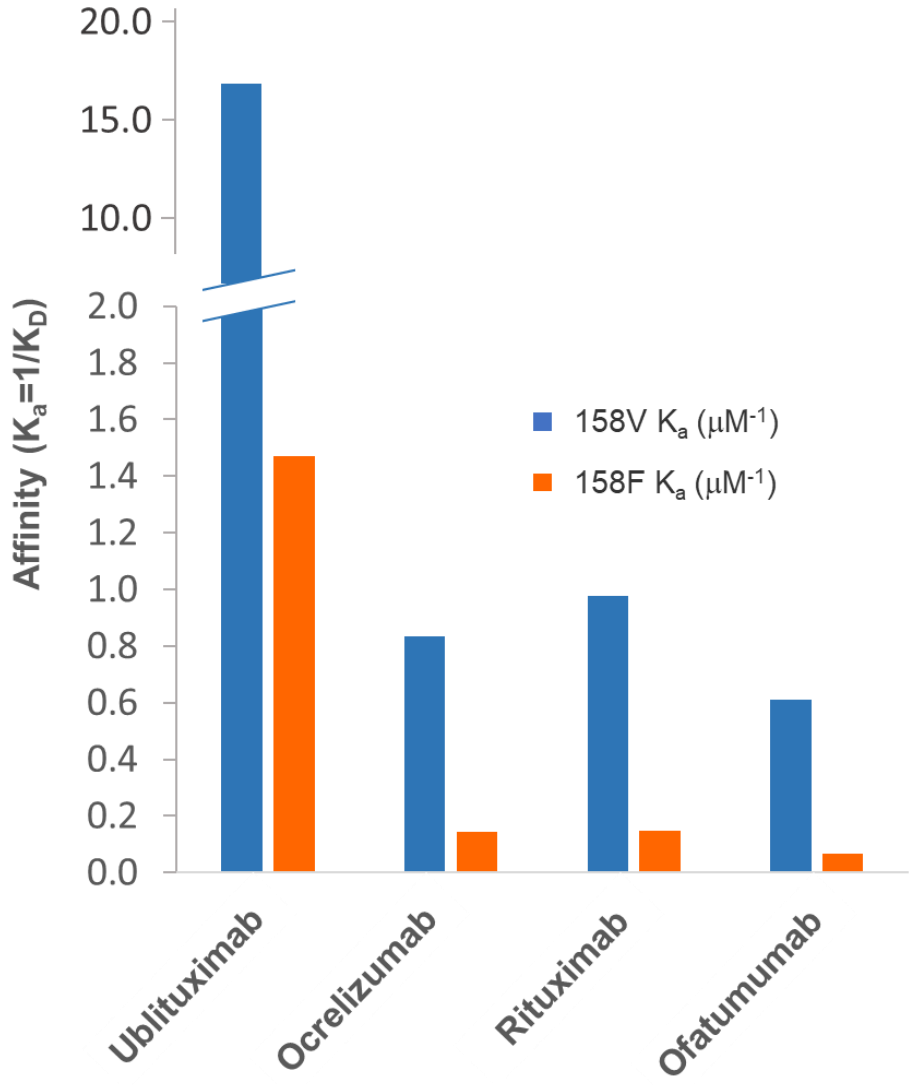
	EC_{50} (µg/mL)[9]
Ublituximab	0.063
Rituximab	0.133
Ocrelizumab	0.111
Ofatumumab	0.092

EC_{50}, half maximal effective concentration.
Adapted from Klein C, et al. *MAbs*. 2013;5(1):22-33.

RESULTS

- Ublituximab demonstrates the highest binding affinity (lowest K_D [nM]) to all FcγRIIIa polymorphisms: 16-25× greater for FcγRIIIa 158V and 10-22× greater for FcγRIIIa 158F compared with ocrelizumab, rituximab, and ofatumumab (**Figure 3**)

Figure 3. Ublituximab Has the Highest Binding Affinity to Polymorphisms of FcγRIIIa Receptor[9]



	158V		158F	
	K_D (nM)	K_a (μM^{-1})	K_D (nM)	K_a (μM^{-1})
Ublituximab	64	15.63	680	1.47
Ocrelizumab	1199	0.83	6960	0.14
Rituximab	1025	0.98	6762	0.15
Ofatumumab	1641	0.61	14,815	0.07

Ublituximab has the highest affinity to 158V and 158F

- **16-25×** higher binding affinity to FcγRIIIa 158V [high-affinity receptor] than ocrelizumab, rituximab, and ofatumumab
- **10-22×** higher binding affinity to FcγRIIIa 158F [low-affinity receptor] than ocrelizumab, rituximab, and ofatumumab

Ublituximab's affinity for 158F is higher than that of all other anti-CD20s to both 158V and 158F

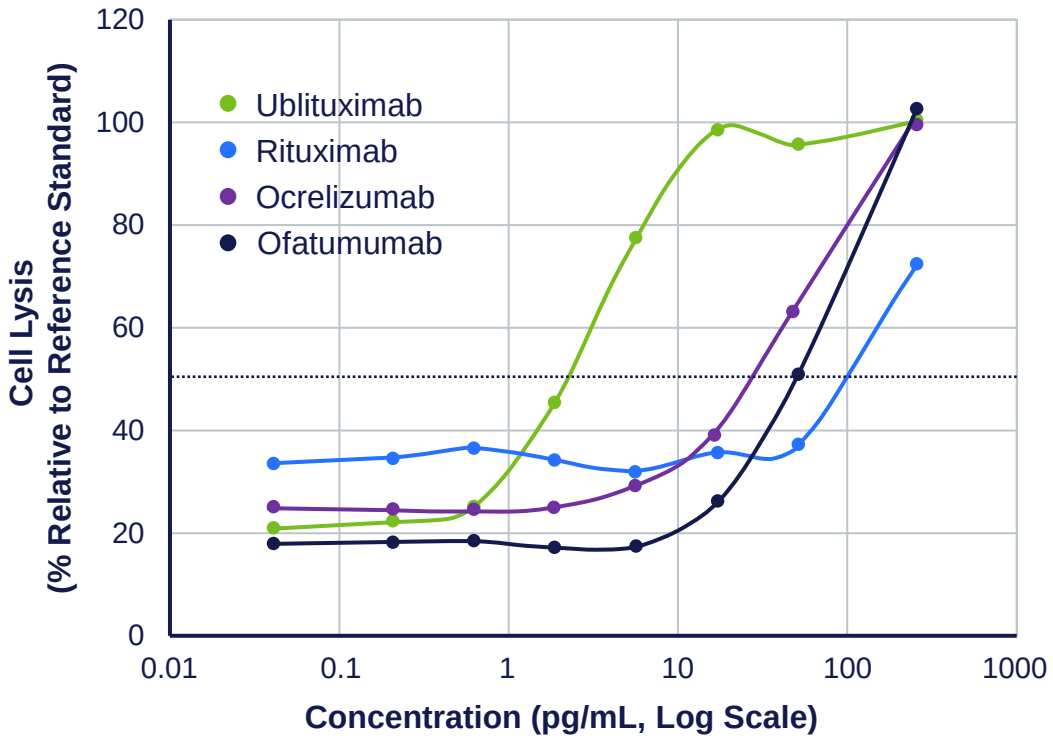
FcγRIIIa binding assay used a surface plasmon resonance (SPR)-based method. The equilibrium dissociation constants (K_D) of each sample is determined for each receptor using the rates of change of the SPR signal.

RESULTS

- Ublituximab has 25-30× the ADCC potential compared with ocrelizumab and ofatumumab and >2000× greater than that of rituximab (**Figure 4**)
  - ADCC activity (EC_{50} [pg/mL]): ublituximab, 2.4 versus ocrelizumab, 60.8; ofatumumab, 74.1; and rituximab, 5457.0

Figure 4. Ublituximab Has the Highest ADCC Activity Compared With Other Anti-CD20 Antibodies

ADCC Dose-Response Curve[9]



- ADCC activity was measured using CD20-expressing Raji cells in the presence of KILR (CD16+) effector cells and human serum samples diluted to 250-fold
- Cell viability was measured using CytoTox-Glo™

ADCC Activity[9]

	EC_{50}[a] (pg/mL)
Ublituximab	2.42
Rituximab	5457.0
Ocrelizumab	60.8
Ofatumumab	74.1

[a]The EC_{50} is the concentration effective in producing 50% of the maximal response and allows for comparison of drug potencies.[12]

EC_{50} of each testing sample was calculated based on a 4-parameter logistic fit curve.
ADCC, antibody-dependent cellular cytolysis; EC_{50}, half maximal effective concentration; KILR CD16+, single donor-derived human CD8+ T lymphocytes engineered to express CD16 (FcγRIII) on their plasma membrane surface.

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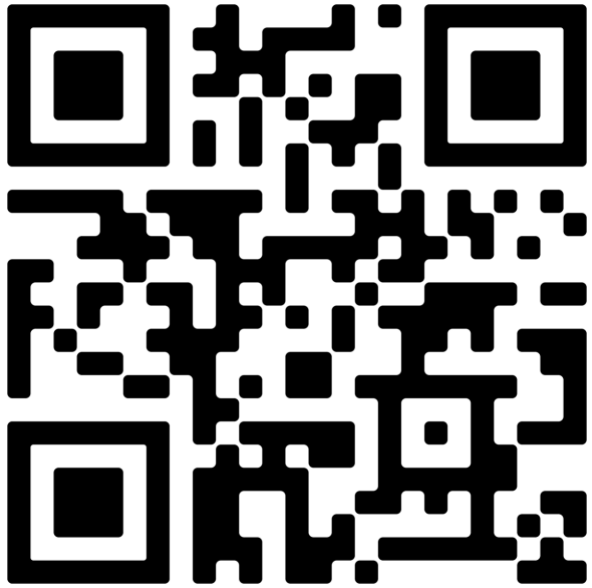

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