



¹Department of Immunopathology, Sanquin Research and Landsteiner Laboratory, Amsterdam UMC, University of Amsterdam
²Amsterdam Institute for Infection and Immunity
³Department of Paediatric Immunology, Rheumatology and Infectious Disease, Amsterdam UMC, University of Amsterdam
⁴Department of Neurology, Amsterdam UMC
⁵Antibodies and Immunogenicity, R&D, Sanquin Diagnostic Services
 all @ Amsterdam, The Netherlands
 abc@blood.nl

CD20 targeting therapeutic antibodies

The Journal of Immunology, 2024, 212: 15.

- B-cell depletion by ADCC and/or CDC
- Effective treatment for leukaemia and autoimmune diseases
- Anti-drug antibodies → lack of response and adverse events

- Rituximab, first in class since 1997(US)/1998(EU)
- Succeeded by ofatumumab, obinutuzumab and ocrelizumab
- Different epitope and binding mode and main B-cell depleting mechanism

- Anti-drug antibodies (ADAs) to target binding site might cross-react with alternative anti-CD20 treatments
- Cross-reactive ADAs could affect effectivity and safety of alternative drugs

	% human ¹⁾	binding mode	core epitope
rituximab chimeric	72		
ocrelizumab humanized	82 87	Type I	¹⁶¹ YINIYNCEP ANP SEKNSP ¹⁷⁸
obinutuzumab humanized	85 87	Type II	¹⁶¹ YINIYNCEP ANP-E NSP ¹⁷⁸
ofatumumab human	97 100	Type I	¹⁶¹ YINIYN EE AN PE SEKNSP ¹⁷⁸

¹⁾ resp. HC/LC V gene

Overview of anti-CD20 Abs used in this study.

	CDR1	CDR2
rituximab	QVQLQPGGA.ELVRF GASVVMSCAS	GTF...TSYN MHWVQTP GRGLEWIGA
ocrelizumab	EVQLVESGG.GLVQP GGSRLSCLAS	GTF...TSYN MHWVRQP GKGLEWVGA
obinutuzumab	QVQLVQSGA.EVKKP GSSVKVSCAS	GYAF...SYW INWVRQP GQGLEWGR
ofatumumab	EVQLVESGG.GLVQP GRSRLSCLAS	GTF...NDYA MHWVRQP GKGLEWVST

	CDR3
rituximab	SYNQRFK.G KATITADRS STAIQMLSSLS EDSAVYTC ARSTYYG.GDWTFNV WGAQTTVTVSA
ocrelizumab	SYNQRFK.G RFTISVDKSK NTLYLQMSLSRA EDTAVYTC ARVYYNSYTWFDV WQGTTLVTVSS
obinutuzumab	DTNGRFK.G RVTITADKST STAYMELSSLSR EDTAVYTC ARNVFD...GWLVTY WQGTTLVTVSS
ofatumumab	GYADSVK.G RFTISRDNK RSLYLQMSLSRA EDTALYTC AKDIQGNYYTGMVD WQGTTLVTVSS

	CDR3
rituximab	NLDSGVP.V RFSGG..SG TSYSLTISRVEA EDAATYTC QQWTS.....NPET FGGGTKEIK.
ocrelizumab	NLDSGVP.S RFSGG..SG TDFTLTISLQEP EDFATYTC QQWFS.....NPET FGGGTKEIK.
obinutuzumab	NLDSGVP.D RFSGG..SG TDFTLKISRVEA EDVGVYTC AQMLE.....LPT FGGGTKEIK.
ofatumumab	NRATGIP.A RFSGG..SG TDFTLTISLQEP EDFAVYTC QQRNS.....WPIT FGGGTKEIK.

Sequence alignment of H and L chain variable domain. Red residues are mismatches to closest matching germline V and J sequences. Boxes indicate sequence homology between rituximab and ocrelizumab within the CDRs.

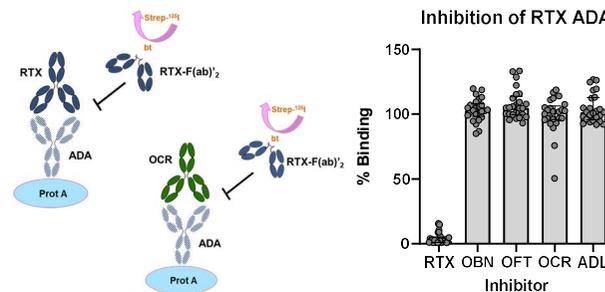


Figure 1: Cross-reactivity of ADAs with other drugs was tested in the depicted assay set-up.

Real-world samples from patients developing ADA to rituximab were tested in the rituximab ADA assay in the presence of different inhibitors (the three other anti-CD20 Abs and the anti-TNF drug adalimumab as negative control).

In 2/25 patient samples treated with rituximab ADA response could be blocked by addition of ocrelizumab. No cross-reactivity was seen for obinutuzumab or ofatumumab or anti-TNF drug adalimumab (negative control).

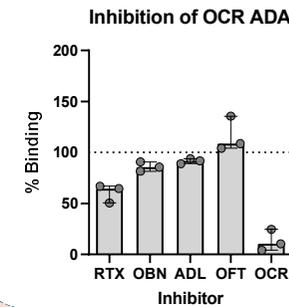


Figure 4: Detection of ocrelizumab ADA is inhibited by the addition of anti-CD20 rituximab and anti-CD20 ocrelizumab, but not with anti-CD20 Abs obinutuzumab and ofatumumab or negative control anti-TNF adalimumab.

Results

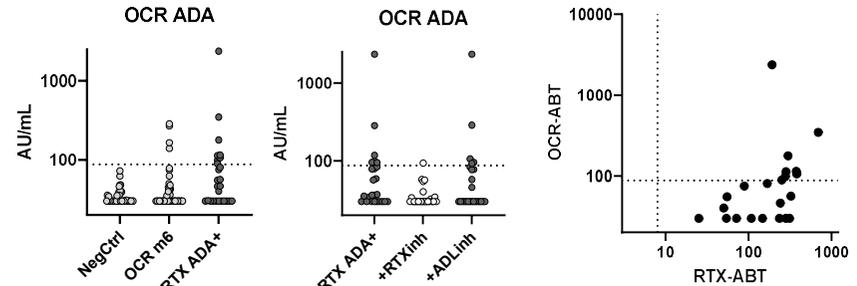
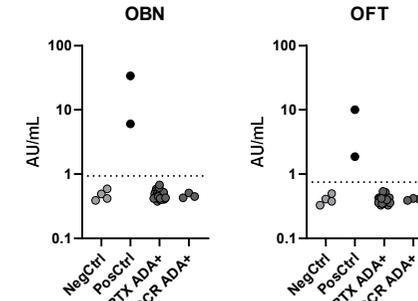


Figure 2: A portion of ocrelizumab and ADA+ rituximab treated patients is positive in the ocrelizumab ADA assay (left). Specificity is shown by block of signal after addition of rituximab and not with anti-TNF adalimumab (right).

Figure 3: Correlation between rituximab ADA level and signal intensity in the ocrelizumab ADA assay for samples from rituximab treated patients.



RTX: rituximab (anti-CD20) OBN: obinutuzumab (anti-CD20)
 OCR: ocrelizumab (anti-CD20) OFT: ofatumumab (anti-CD20) ADL: adalimumab (anti-TNF)

Figure 8: ADA in patient samples raised against either rituximab or ocrelizumab do not give a signal in anti-obinutuzumab or anti-ofatumumab assay. Positive control is anti-κ antibody. No patient samples containing ADA against Obinutuzumab or ofatumumab were available.

Conclusions

- ✓ ADAs raised against rituximab are cross-reactive with ocrelizumab and vice versa
- ✓ There is no cross-reactivity observed for the other two CD20 targeting therapeutic Abs
- ✓ Sequence alignment shows high homology of rituximab and ocrelizumab in CDRs
- ✓ Careful monitoring may be useful when switching between rituximab and ocrelizumab