Abstract #1936

Ocaratuzumab, a Fab-Engineered Anti-CD20 Antibody, Demonstrates Greater Affinity to CD20 and Ability to Bind to Rituximab-Coated B-Cells

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ABSTRACT

Background:
Ocaratzumab, previously known as AME-133v, is a humanized, Fab- and Fc-engineered anti-CD20 antibody optimized for superior binding. Using single-point codon-substitutions, thousands of variations of the antibody complementary determining regions were screened. Ocaratuzumab was selected from the variants because of its improvement in K_{off} rate, which prolongs binding of the antibody. Compared to the K_d constant of rituximab, ocaratuzumab has 13- to 20-fold greater binding affinity for CD20. Ocaratuzumab and rituximab bind to the same epitope, potentially allowing competition and displacement between the two antibodies.

Aims:
Experiments were performed to assess the ability of ocaratuzumab's increased affinity to improve binding to lymphoma B-cells pre-coated with rituximab.

Methods:
50 µg/mL of biotinylated rituximab was added to fixed SKW.4 lymphoma B-cells and incubated overnight at 37°C. Ocaratuzumab was added to each plate at half the concentration of rituximab (25 µg/mL) for 3, 6, and 24 hours. The amount of rituximab still bound to the B-lymphocytes was assessed by detecting biotinylated antibody using neutraAvidin alkaline phosphatase conjugate and a colorimetric substrate (optical density was measured at 560 nm in a spectrophotometer).

Results:
Even at half the concentration of rituximab, ocaratuzumab reduced the amount of rituximab bound to the lymphoma B-cells. The reduction in pre-bound rituximab was 8.44% at 3 hours, 16.32% at 6 hours, and peaked at 31.12% at 24 hours from baseline.

Additional experiments were performed, and higher concentrations of ocaratuzumab (75, 150 µg/mL) showed no additional displacement of rituximab, suggesting that low concentrations of the antibody are effective in maximally displacing rituximab bound to B-cells. Under controlled conditions, no spontaneous reduction in rituximab binding occurred, demonstrating the ability of ocaratuzumab to preferentially replace rituximab due to its faster K_{on} rate and its ability to remain bound longer due to its slower K_{off} rate.

Furthermore, independent experiments have shown pre-incubation of ocaratuzumab in primary chronic lymphocytic leukemia cells can significantly inhibit the binding of rituximab. This suggests that the improved affinity of ocaratuzumab to CD20 and its low K_{off} rate prevent binding of a second antibody to these B-cells.

Conclusions:
Ocaratuzumab (AME-133v) has the ability to bind to rituximab pre-exposed B-lymphocytes even at concentrations 50% lower than that of rituximab. Combined with Fc-engineering to improve antibody dependent cellular cytotoxicity, ocaratuzumab may improve clinical benefit in rituximab pre-treated patients with B-cell neoplasia.

BACKGROUND

Ocaratuzumab is a humanized, next-generation anti-CD20 monoclonal antibody engineered for improved potency, compared to rituximab. Due to its faster K_{on} rate and slower K_{off} rate, it has approximately 20 fold higher binding affinity for the CD20 epitope (Figure 1).

Figure 1. Ocaratuzumab Exhibits Optimized Binding Kinetics on Lymphoma Cells Compared to Rituximab

	Ocaratuzumab	Rituximab
K_d (pM)	97	2097
K_{on} (10^5/M/s)	7.8	4.7
K_{off} (10^{-5}/s)	7.6	98.6

METHODS

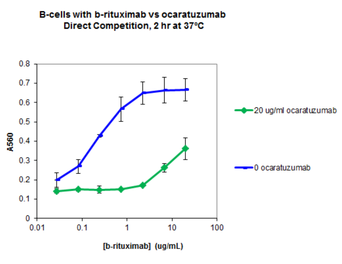
- For direct competition experiments, various concentrations of rituximab and ocaratuzumab were added to plates containing SKW6.4 B-cells.
- The plates were incubated at 37°C for 2 hours, washed, incubated with NA-AP for 30 min, washed, developed, and read at OD 560.
- In displacement studies, titrations of biotinylated rituximab at 50 µg/mL were first added to SKW6.4 B-cells and incubated overnight at 37°C.
- Ocaratuzumab was then added at various concentrations, incubated for multiple time points (3, 6, and 24 hours), washed, developed, and read at OD 560.

RESULTS

Competitive Binding Studies:

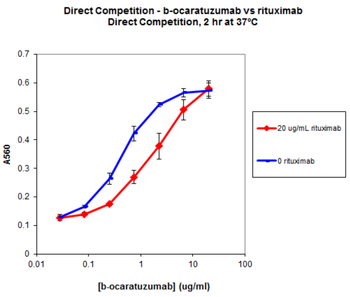
- When ocaratuzumab and rituximab were placed head to head at equal concentrations of 20 µg/mL, ocaratuzumab inhibited rituximab binding by 45.67% (Figure 2).
- This one-to-one competition demonstrates that circulating rituximab has no substantial effect on the ability of ocaratuzumab to bind to B-cells.
- Even at concentrations as low as 2.2 µg/mL, ocaratuzumab was able to competitively inhibit rituximab (20 µg/mL) binding by 5.63%.

Figure 2. Ocaratuzumab Inhibits Rituximab Binding at Substantially Lower Concentrations



- Due to its increased on-rate, ocaratuzumab effectively inhibits rituximab binding to CD20, even at much lower concentrations.
- In a competition study performed to measure the ability of rituximab to compete out the binding of biotinylated ocaratuzumab, rituximab showed much lower ability to displace the competing antibody.
- 20 µg/mL of rituximab inhibited ocaratuzumab (at 0.741 µg/mL) binding by 36.02% (Figure 3).
- Placed head to head at 20 µg/mL, no inhibition activity was seen.

Figure 3. At Concentrations Notably Higher Than That of Ocaratuzumab, Rituximab Shows No Substantial Inhibition Activity

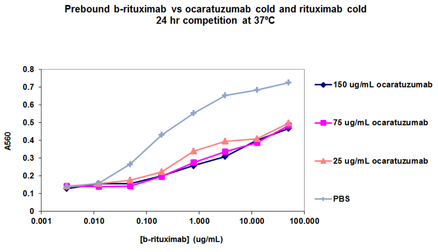


- When rituximab was at over 26-fold higher concentrations than ocaratuzumab, it failed to substantially inhibit ocaratuzumab binding.

Displacement Studies:

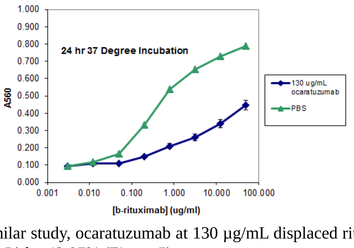
- Ocaratuzumab is able to displace pre-bound rituximab even at levels half that of the competing antibody (Figure 4).
- Displacement studies showed little differences resulting from ocaratuzumab concentrations ranging from 25-150 ug/mL, indicating a small dose of the antibody is efficient in optimally displacing rituximab pre-bound to B-cells.

Figure 4. Ocaratuzumab Displaces Rituximab Even at Low Concentrations



- At all concentrations of ocaratuzumab, the antibody displaced pre-bound rituximab in a time-dependent manner.
- Maximum displacement was achieved at 24 hours, reaching 31.12% reduction in pre-bound rituximab.

Figure 5. Displacement Effect of Ocaratuzumab Consistent at High Concentrations



- In a similar study, ocaratuzumab at 130 µg/mL displaced rituximab (at 50 µg/mL) by 43.07% (Figure 5).
- This further demonstrates the plateau effect of displacement that ocaratuzumab shows at concentrations higher than 25 µg/mL.

CONCLUSIONS

- Ocaratuzumab has the ability to significantly displace pre-bound rituximab from B-cells, even at concentrations half that of rituximab.
- Rituximab displacement occurs in a time-dependent manner and plateaus at 25 µg/mL, suggesting that even low concentrations of ocaratuzumab are effective at displacing competing antibody.
- Even at doses over 90 times that of ocaratuzumab, rituximab fails to impact the binding of ocaratuzumab to B-cells, suggesting that circulating rituximab will not effect ocaratuzumab binding.
- These data support ocaratuzumab's ability to be clinically effective in previously rituximab-treated patients, even refractory patients recently treated with rituximab. Previous clinical trials have demonstrated therapeutic benefit in rituximab relapsed and refractory follicular lymphoma patients.

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