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: Ocaratuzumab, previously known as AMG-317, is a humanized Fab- and Fc-engineered anti-CD20 antibody optimized for superior binding. Using single-point cell-surface determinants, focused of variabilities of the antibody complementary determining regions were scanned. Ocaratuzumab was selected from the various Bayesian improvements in its Fc, with bindings binding of the head group structure of the Fab, which has no impact on the binding affinity for CD20. Ocaratuzumab and neutralize to the same epitope, potentially resulting in improved competition and displacement between the two antibodies.

METHODS

Experiments were performed to assess the ability of ocaratuzumab’s measured affinity to improve binding to lymphoma cells in vitro compared to rituximab.

Results: In all, the concentration of ocaratuzumab was the amount of rituximab bound to the lymphoma cells. The results of a previous published work at 20 µg/mL showed that competition was optimally displaced at 20 µg/mL. Time-dependent displacement was assessed by determining biotinylated antibody using transitive avidin-phosphate conjugate and a competitive dual-wavelength assay. The final concentration of Ocaratuzumab was measured by assessing binding of biotinylated antibody using transitive avidin-phosphate conjugate and a competitive dual-wavelength assay.

Additional experiments were performed to determine the maximum concentrations of Ocaratuzumab (0.1-10 µg/mL) required to displace competition at levels of binding. The experiments suggest that the improved affinity of ocaratuzumab and its low rate can provide binding of a second antibody to these cells.

Conclusions: Ocaratuzumab is a humanized, next-generation anti-CD20 monoclonal antibody engineered for improved potency, compared to rituximab. Due to its faster Koff rate and slower Kcat rate, it has approximately 20-fold higher binding efficiency for the CD20 epitope (Figure 1).

METHODS

• For direct competition experiments, various concentrations of rituximab and ocaratuzumab were added to plates containing SKW-6 B-cells.
• The plates were incubated at 17°C for 2 hours, washed, incubated with NA API for 30 minutes, washed, developed, and read at OD 560.
• In displacement studies, titrations of biotinylated rituximab at 50 ng/µL, were first added to SKW-6 B-cells and incubated overnight at 17°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 much better at 74% (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 56%.

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 0.74 µg/mL) binding by 74% (Figure 3).
• Placed head to head at 20 µg/mL, no activity was seen.

DISPERSAL STUDIES: Ocaratuzumab is able to displace pre-bound rituximab even at levels half that of the competing antibody (Figure 4).

Furthermore, studies showed little differences resulting from ocaratuzumab concentrations ranging from 25-150 µg/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% reduction in pre-bound rituximab.

DISPERSAL STUDIES: Ocaratuzumab has the ability to significantly displace pre-bound rituximab from B-cells, even at concentrations half that of 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% (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 plateau 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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