A variant of obinutuzumab with abolished ADCC, ADCP and CDC is as efficient as rituximab in B cell depletion and antitumor activity

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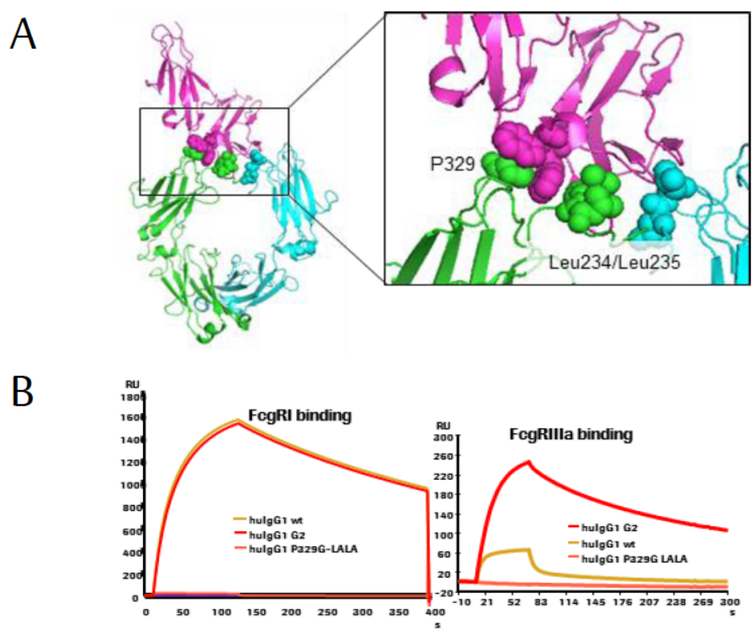
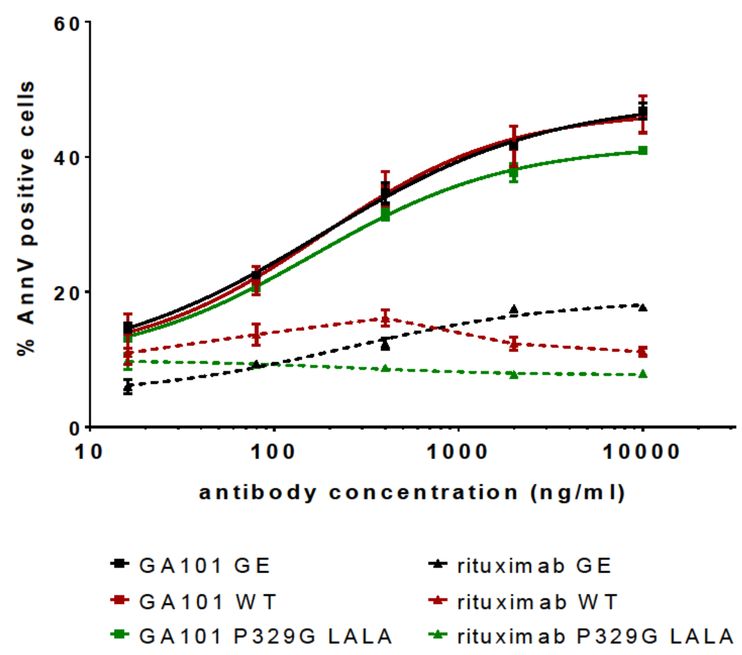
Engineering an immune effector-inactive version of obinutuzumab (GA101 PG LALA) by introduction of P329G LALA mutation in the Fc part



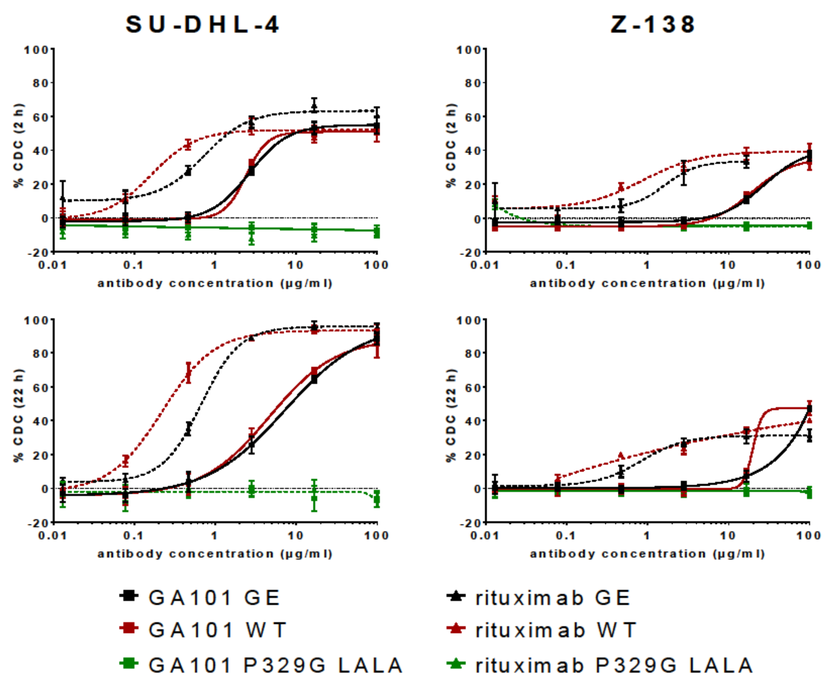
A) Cartoon of an Fc of human IgG1 (green and cyan) in complex with the FcgRIII (pink). The proline of the Fc forming a strong interaction with the receptor is indicated as P329. The two leucine residues of the hinge region are marked with Leu234/Leu235. Replacing those three amino acids with smaller ones, generates Fc variants with undetectable binding to the Fc gamma receptors.
B) SPR (Biacore) sensorgrams of IgG variants on the high affinity FcgRI and the low affinity FcgRIII.

PG LALA mutations do not affect direct cell death induction by obinutuzumab (GA101)



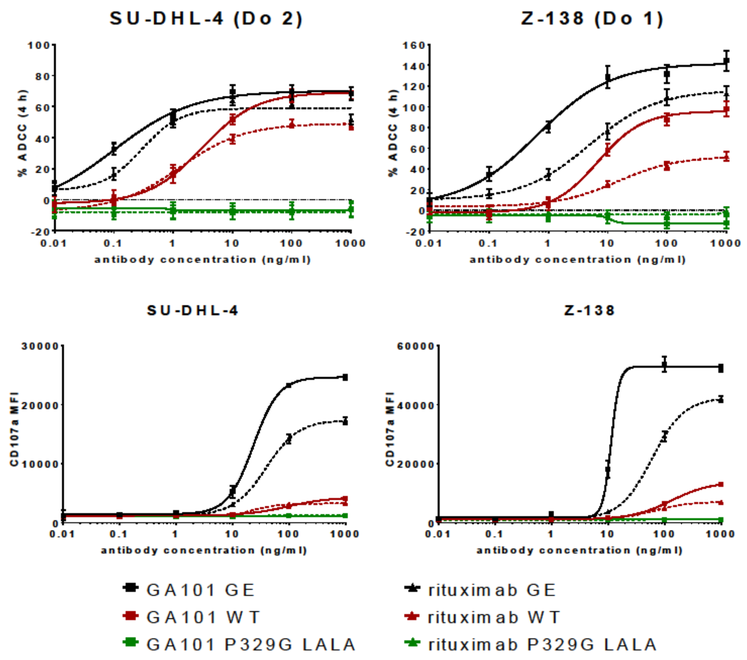
SU-DHL-4 or Z-138 cells were incubated for 22 h in the presence of anti-CD20 antibodies as indicated. Afterwards, cells were stained with Annexin V FLUOS and PI and analyzed by flow cytometry.

PG LALA mutations abolish CDC induction by obinutuzumab (GA101) and rituximab



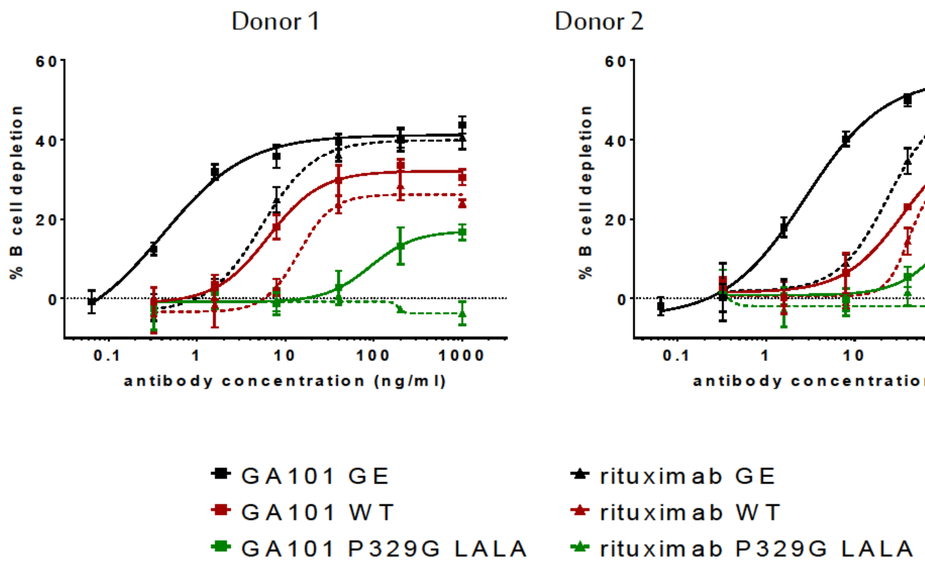
SU-DHL-4 or Z-138 cells were incubated with rabbit complement for 2 h or 22 h in the presence of anti-CD20 antibodies as indicated. CDC was calculated based on LDH release after 2 h or AlamarBlue readout after 22 h.

PG LALA mutations abolish ADCC induction and NK cell activation by obinutuzumab (GA101) and rituximab



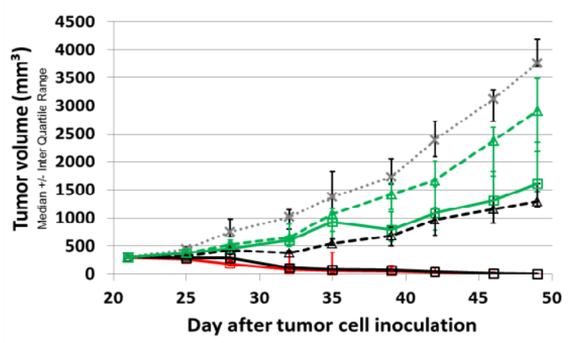
SU-DHL-4 or Z-138 cells were incubated with human PBMCs from two blood donors (E:T 25:1) for 4 h in the presence of anti-CD20 antibodies. Afterwards, ADCC was calculated based on LDH release (upper row) and degranulation of CD3-CD56+ NK cells was analyzed by flow cytometry measuring surface CD107a expression levels (lower row).

Immune effector-inactive obinutuzumab (GA101 PG LALA) mediates significant whole blood B cell depletion



B cell depletion in whole blood of healthy donors was determined after 20 h incubation in the presence of anti-CD20 antibodies as indicated. Results from two different blood donors are shown.

Immune effector-inactive obinutuzumab (GA101 PG LALA) mediates comparable anti-tumoral efficacy to rituximab in s.c. SU-DHL4 xenograft model in Scid beige mice



Treatment schedule 30 mg/kg; q7dx4; IP	TGI (%)	np TCR [95% CI] compared to vehicle		Tumor free Animals on Day 49
Vehicle	–	–	[–]	0
Obinutuzumab (GA101 GE)	>100	0	[0-0]	9
GA101 WT	>100	0	[0-0.006]	8
GA101 PG LALA	62	0.43	[0.30-0.60]	0
Rituximab	71	0.37	[0.29-0.50]	0
Rituximab PG LALA	26	0.73	[0.44-0.92]	0

Conclusions

- **Data from whole blood B cell depletion assays and the SU-DHL4 xenograft model support the contribution of enhanced ADCC/ADCP as well as enhanced direct cell death induction to the mechanism of action of obinutuzumab (GA101) in preclinical models as compared to rituximab**
- **These preclinical data also support a role of enhanced direct effects for the clinical mechanism of action of obinutuzumab (GA101)**

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