

**[229] Novel 3<sup>rd</sup> Generation Humanized Type II CD20 Antibody with Glycoengineered Fc and Modified Elbow Hinge for Enhanced ADCC and Superior Apoptosis Induction. Session Type: Oral Session**

*Pablo Umana, Ekkehard Moessner, Peter Bruenker, Gabriele Unsin, Ursula Puentener, Tobias Suter, Roger Grau, Carla Schmidt, Christian Gerdes, Adam Nopora, Monika Patre, Samuel Moser, Peter Sondermann, Luise Wheat, Martin J.S. Dyer, Sibbrand Poppema, Sabine Bauer, Pamela Strein, Thomas Friess, Christian Klein GLYCART Biotechnology AG, (a Member of the Roche Group), Schlieren, Zurich, Switzerland; University of Leicester & MRC Unit, Leicester, United Kingdom; University of Groningen Medical Center, Groningen, Netherlands; Research Oncology, Roche Diagnostics GmbH Pharma, Penzberg, Germany*

**Background:** Treatment of B-cell non-Hodgkin lymphoma (NHL) with antibodies targeting CD20 in conjunction with combination chemotherapy is standard clinical practice. Two different types of CD20 MAb differing significantly in their mode of CD20 binding and biological activities have been identified (Cragg and Glennie. *Blood* 103: 2738-2743, 2004): type I antibodies, as rituximab, are potent in complement mediated cytotoxicity, whereas type II antibodies, as tositumomab, effectively initiate target cell death via caspase-independent apoptosis with concomitant phosphatidylserine exposure. GA101 is a humanized and optimized, third generation, type II CD20 IgG1 antibody that exhibits enhanced ADCC and superior caspase-independent apoptosis induction in comparison with currently available CD20 MAbs. **Material and Methods:** GA101 was humanized by grafting CDR sequences from the murine monoclonal antibody B-ly1 on framework regions with fully human IgG1-kappa germline sequences. During humanization different elbow hinge sequences in the variable region were studied for their capability to induce apoptosis.

Furthermore, the Fc region-carbohydrates were glycoengineered using GlycoMab™ technology leading to bisected, afucosylated Fc region-carbohydrates. **Results:** The humanized GA101 antibody bound CD20 as type II antibody with nanomolar affinity. Its glycoengineered Fc region bound with 50-fold higher affinity to human FcγRIII receptors compared to a standard, non-glycoengineered antibody. Increased FcγRIII binding led to a 10-100-fold increase in ADCC against CD20-expressing NHL cell lines. Modification of elbow hinge sequences within the antibody variable framework regions resulted in a strong apoptosis-inducing activity of GA101 upon CD20 binding on target cells. Direct comparison to other CD20 antibodies GA101 showed enhanced apoptosis induction in both a panel of NHL cell lines and *ex vivo* in samples from patients with a variety of B-cell malignancies. Furthermore, in B-cell depletion assays with whole blood from healthy donors and B-cell leukemic patients, an assay combining ADCC-, CDC- and apoptosis-mediated mechanisms of action, GA101 was significantly more potent and efficacious than other CD20 antibodies, including rituximab and Fc-variants of rituximab that have increased ADCC. Finally, the *in vitro* superiority of GA101 also translated into superior efficacy *in vivo*. In NHL xenograft models of different histological origin, including aggressive DLBCL and MCL, treatment with GA101 results in complete tumor remission and long-term survival (cure) compared to tumor stasis, at best, for rituximab. **Conclusion:** Compared to existing CD20 antibodies GA101 represents a novel, third generation antibody with significantly enhanced efficacy in a variety of *in vitro* and *in vivo* preclinical models. GA101 constitutes the first type II CD20 antibody successfully engineered for increased ADCC. Based on these data, GA101 is a promising therapeutic antibody candidate for the treatment of B-cell malignancies.

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