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Niclas Kiepe, Paul Zimmermann, Irene Pauls, Tobias Zeller, Sophie Kreissig, Christian Wichmann, Matthias Peipp, Andreas Humpe, Lennart Lenk, Christian Kellner

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P028. Targeting FMS-like Tyrosine Kinase 3 (FLT3) in B-Cell Acute Lymphoblastic Leukemia with an Fc-engineered antibody

[1]Niclas Kiepe, [1]Paul Zimmermann, [1]Irene Pauls, [2]Tobias Zeller, [1]Sophie Kreissig, [1]Christian Wichmann, [3]Matthias Peipp, [1]Andreas Humpe, [4]Lennart Lenk, [1]Christian Kellner

[1]Division of Transfusion Medicine, Cell Therapeutics and Haemostaseology, LMU University Hospital, LMU Munich, Munich, Germany, [2]Hematology and Oncology, Faculty of Medicine, University of Augsburg, Augsburg, Germany, [3]Division of Antibody-Based Immunotherapy, Department of Internal Medicine II, Christian Albrechts University of Kiel and University Hospital Schleswig-Holstein, Kiel, Germany, [4]Department of Pediatrics I, ALL-BFM Study Group, Christian Albrechts University of Kiel and University Hospital Schleswig-Holstein, Kiel, Germany.

Background: FMS-like tyrosine kinase 3 (FLT3) represents an appreciated immunotherapeutic target in acute myeloid leukemia. FLT3 is also expressed in the majority of B cell acute lymphoblastic leukemia (B-ALL) cases and certain subtypes including *KMT2A*-rearranged B-ALL over-express the antigen. Here, we analyzed FLT3 as immunotherapy target on B-ALL cells using an Fc-engineered FLT3 antibody optimized for Fcγ receptor engagement.
Methods: The Fc-engineered variant of the FLT3 antibody EB-10 "FLT3-DE" (modifications: S239D/I332E) and the fusion protein Sia-Fcσ, which consisted of a sialidase and a human Fc domain with mutated FcγR binding sites, were produced in eukaryotic cells and purified by affinity chromatography. Flow cytometry was employed to analyze antigen expression, sialylation and antibody binding. Antibody-dependent cellular phagocytosis (ADCP) was determined by fluorescence microscopy or live cell imaging. Calcein-release assays were employed to analyze antibody-dependent cell-mediated cytotoxicity (ADCC).
Results: Among the analyzed B-ALL cell lines, SEM expressed the highest FLT3 cell surface levels (mean specific antibody binding capacity: 54,000) followed by RS4;11 (5,000) and REH (2,000). In ADCC experiments with human mononuclear cells, FLT3-DE induced killing of SEM, REH and RS4;11 cells in a dose dependent manner with half maximum effective concentrations between 0.4 and 0.9 nM. Moreover, FLT3-DE mediated ADCP by macrophages. ADCP was increased by antibody blockade of the "Don´t Eat Me" signal molecule CD47, which was expressed by all three cell lines. Regarding the inhibitory function of sialic acids in the regulation of immune cells, the antibody FLT3-DE was combined with the sialidase-Fc fusion protein Sia-Fcσ. Sia-Fcσ sensitized B-ALL cells for cellular cytotoxicity by cleaving both α2,3-linked and α2,6-linked sialic acids from B-ALL cells. Importantly, when combined with the antibody FLT3-DE, Sia-Fcσ enhanced ADCC of B-ALL cells in a synergistic manner.
Conclusion: The antibody FLT3-DE eliminated B-ALL cells by ADCP and ADCC and its efficacy was further enhanced by combination with a CD47 antibody or a Sialidase-Fc fusion protein. Thus, targeting FLT3 using Fc-engineered antibodies may represent an attractive strategy for the treatment of B-ALL and deserves further *in vivo* testing.
Keywords: FLT3, B-ALL, immunotherapy, Fc engineering
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Corresponding Author: Christian Kellner, https://orcid.org/0000–0002-2562–6902