P364

## Inhibiting MGAT1-mediated N-glycosylation reduces proliferation and adhesion of AML cells and increases affinities of anti-SLC3A2 directed immunotherapies

*Smit P.[1], Bühring-Uhle C.[1], Nather C.[1], Negraschus A.[1], Goldmann U.[2], Papadopoulou A.[3], Rivière J.[1], Morath V.[4], Henkel E.[1], Bromberger T.[5], Götze K.[1], Heinz L.[2], Fluhrer R.[3], Moser M.[5], Superti-Furga G.[2], Bassermann F.[1], Eichner R.[1]*

[1]Technische Universität München, Fakultät für Medizin, Klinikum rechts der Isar, Klinik und Poliklinik für Innere Medizin III, München, Deutschland, [2]CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Wien, Österreich, [3]Universität Augsburg, Medizinische Fakultät, Institut für Theoretische Medizin, Lehrstuhl für Biochemie und Molekularbiologie, Augsburg, Deutschland, [4]Technische Universität München, Fakultät für Medizin, Klinikum rechts der Isar, Klinik und Poliklinik für Nuklearmedizin, München, Deutschland, [5]Technische Universität München, Fakultät für Medizin, Klinikum rechts der Isar, Institut für Experimentelle Hämatologie, München, Deutschland

**Introduction:** SLC3A2 is a cell surface transmembrane glycoprotein, which is frequently overexpressed in acute myeloid leukemia (AML) and is crucial for integrin-mediated adhesion. Anti-SLC3A2 directed immunotherapies were shown to inhibit growth and propagation of leukemic cells in preclinical models. Despite its importance as a therapeutic target, no systematic screens on regulators of SLC3A2 have yet been reported.

**Methods:** Leukemia cell lines were infected with a genome-wide CRISPR/Cas9 knockout library, stained with anti-SLC3A2 antibody, and the populations with highest or lowest SLC3A2 signal were isolated by FACS. sgRNA distribution was analyzed bioinformatically. SgRNA constructs of main hits were cloned in lentiviral vectors, AML lines were infected and effects on SLC3A2 abundance, glycosylation and subcellular localization were assessed by western blot, flow cytometry and immunofluorescence. Binding of anti-SLC3A2 antibodies and anticalins was assessed by flow cytometry. Effects of main hits on proliferation and β1-integrin-mediated adhesion of leukemic cells were investigated using drop-out assays and plate-based adhesion assays.

**Results:** The genome-wide genetic screens identified the N-acetylglucosaminyltransferases MGAT1, 2 and 5 as SLC3A2 modifiers whose knockout increased SLC3A2 detection by anti-SLC3A2 antibodies. Mechanistically, we found loss of MGAT1, 2, or 5 to significantly reduce N-glycosylation levels of SLC3A2, without affecting SLC3A2 abundance or subcellular localization. The increased SLC3A2 detection in flow cytometry hence resulted from reduced glycosylation only. Target detection by an anti-SLC3A2 directed Anticalin was also improved upon knockout of MGAT1,2 or 5 and inhibitors of N-glycosylation like kifunensine hat comparable effects. Importantly, MGAT1 is overexpressed in AML and its knockout significantly reduced both the proliferative capacity and the adhesion of AML cells also independent of SLC3A2, thus underlining its potential as therapeutic target

**Conclusion:** Knockout and inhibition of the Golgi-resident N-acetylglucosaminyltransferases MGAT1, 2, and 5 reduces SLC3A2 N-glycosylation and increases affinities of anti-SLC3A2 directed immunotherapies. Inhibition of N-glycosylation has anti-cancer activities itself and reduces AML cell proliferation and adhesion. MGAT1 targeting could be hence be a promising approach in AML which additionally might synergize with anti-SLC3A2 directed therapies.

**Disclosure:** None.