Targeting gene dependencies in MYC overexpressing Multiple Myeloma

Lama Hasan Bou Issa[*1], Léa Fléchon[1], William Laine[1], Aicha Ouelkdite[1], Guillaume Escure[2], Silvia Gaggero[1], Tiziano Ingegnere[1], Romanos Sklavenitis Pistofidis[3], Irene M. Ghobrial[3], Thierry Facon[2], Suman Mitra[1], Jérôme Kluza[1], Bruno Quesnel[1, 2], Salomon Manier[1, 2]

[1]Univ Lille, Canther, INSERM UMR-S1277 - CNRS UMR9020, [2]CHU Lille, Department of Hematology, Lille, France, [3]Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, United States of America

Background. Multiple myeloma (MM) is an incurable plasma cell malignancy in which *MYC* alterations play an essential role in the progression and correlate with adverse outcome. However, MYC is not therapeutically targetable.

Hypothesis. To overcome this, we hypothesized that the proliferative advantage promoted by MYC overexpression induces differential genomic dependencies that can be exploited therapeutically.

Methods. We searched for genomic vulnerabilities associated with *MYC* overexpression using genome-scale pooled short hairpin RNA screening data (Project Achilles). We generated an isogenic model of MYC overexpression (OE) in U266 cell line. We performed RNA-seq, quantitative proteomics and a drug screening of ~2000 small molecules. For validation, we performed pharmacological inhibition of glutaminolysis and shRNA-mediated GLS1 knockdown. CellTiter-Glo® was used to assess viability in both drug screen and validation tests. To understand the differential metabolic rewiring, we performed metabolomic and Seahorse XF analysis.

Results. Achilles analysis revealed main dependencies associated with *MYC* overexpression specifically *GLS1* (glutaminase) and *SLC1A1* (glutamine transporter). Our drug screen spotlighted NAD synthesis inhibitors and mTORC1, which rely on intracellular glutamine pool, had preferential effect on U266/MYC. Both RNA-seq and quantitative proteomics showed no significant upregulation of glutaminolysis-related genes, suggesting a non-oncogenic dependency. Our data showed that MYC OE cells failed to proliferate under glutamine deprivation and had higher sensitivity to CB-839 and V-9302 inhibiting GLS1 and SLC1A5, respectively. Using the Seahorse Bioanalyzer, we observed higher oxygen consumption rate (OCR) in U266/MYC which was induced by glutamine. Furthermore, CB-839 had a potent effect on U266/MYC observed at basal and maximal OCR highlighting the glutamine role controlling mitochondrial OXPHOS in MYC OE cells. The metabolomic analysis showed higher GLS1 activity in U266/MYC with elevated glutamine to glutamate flux. Notably, CB-839 in U266/MYC results in more pronounced changes in TCA cycle and energy debt. This effect was blunted by co-incubation with αKG. Interestingly GLS1 inhibition was not limited to this, but extended to redox balance and amino acid biosynthesis.

Conclusion. A comprehensive understanding of these vulnerabilities can provide a powerful roadmap to guide innovative therapeutic approaches. In this prospective, our results are important in finding new strategies to target MYC in clinic.