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POSTER 4: Consequences of RNA epigenetic dysregulation on translation in a mutant IDH2 context

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Scientific background: Acute myeloid leukaemia (AML) is a hematopoietic malignant disease characterized by differentiation arrest and an uncontrolled rapid growth of myeloid stem/progenitor cells. Approximately 20% of AMLs harbour an isocitrate dehydrogenase *(IDH) 1* or *IDH2* mutation. These genes code for enzymes of the TCA cycle which convert isocitrate into α-ketoglutarate (αKG). *IDH2* mutations result in the production of neomorphic enzymes able to convert αKG into an oncometabolite, R-2-hydroxyglutarate (R-2HG). Through its similar structure, R-2HG competitively inhibits enzymes dependent on αKG, notably DNA and RNA demethylases. This is true for Ten-Eleven Translocation (TET) enzymes which demethylate cytosines by catalysing the hydroxylation of DNA and RNA 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). It has been shown that DNA/RNA methylation is a key epigenetic mark for gene expression. However, the importance of RNA modifications in regards to stability and expression in *IDH*-mutant AMLs have yet to be defined.

Working hypothesis: We hypothesized that when *IDH2* is mutated in AML cells, multiple enzymes dependent on αKG are inhibited, leading to aberrant RNA modifications. These modified marks could impact transcription and translation processes and be the key to understanding molecular mechanisms of differentiation arrest, abnormal proliferation, and even molecular mechanisms of response and therapy resistance. Our research aims to elucidate the epigenetic mechanisms by which *IDH2* mutations affect mRNA stability, splice and translation.

Preliminary results: Consistent with our hypothesis, quantification of RNA modifications using liquid chromatography-mass spectrometry (LC-MS) indicates an increase in 5mC on mRNA and a general increase in epigenetic marks on tRNA in a mutant context. In parallel to ongoing RNA methylome analysis, polysome profiling and fractionation as well as puromycin incorporation were performed to study the impact of *IDH2*m on translation. Study of the ribosomal landscape shows an increase in 80S monosomes in an *IDH2*m context whilst quantification of active translation with puromycin indicates a hyperproliferation state. These observations highlight a defect in regards to protein synthesis. In addition, RNA collected from the ribosomal fractions was sequenced to determine which mRNAs are being actively translated.

Further studies are being pursued to decipher the molecular mechanisms behind our observations and to bridge our understanding on the consequent effect on translation processes in an *IDH2* mutant context.