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## 651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

# The NAD $^{+}$ Metabolic Dependency Restriction Overcomes Drug-Resistance Phenotype of Multiple Myeloma CELLS

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**Introduction:** We previously reported that Nicotinamide Adenine Dinucleotide (NAD $^{+}$), a master regulator of catabolic metabolism and several enzymatic activities, plays a crucial role for Multiple Myeloma (MM) cells growth; indeed, small molecules targeting the rate-limiting NAD $^{+}$ producer enzyme NAMPT, have potent anti-MM activity. However, targeting dysregulated metabolic landscape of tumors has proven to be a major challenge as suggested by existence of alternative NAD $^{+}$ production routes which confer resistance to NAMPT inhibitors. As result here we focused on whole NAD $^{+}$ metabolic dependency of MM cells by investigating relevance of a comprehensive NAD $^{+}$ metabolic restriction as strategy to enhance activity of currently used anti-MM strategies.
**Methods:** whole transcriptomic analysis of the NADome signature was performed on MM patients by probing MMRF CoMMpass study; its correlation with clinical data (OS and PFS) was also investigated. Next, GSEA algorithm on biological modules was run on MM patients carrying low- vs high-NADome signature. Chemical and genome editing-approaches using CRISPR-Cas9-based strategy were both used to investigate relevance of our findings. Then, an anti-MM drugs screen was used to challenge fully-NAD depleted MM cells using different viability tools. Mechanistic studies were also performed using qPCR, western blot, immunofluorescence analyses, targeted enzymatic assays and *in-vivo* xenograft MM models. Finally, clinical benefits of our findings were accomplished by challenging publicly available dataset on ASCT-treated MM patients.
**Results:** RNAseq analysis on CoMMPass dataset sharply divided MM patients according to their comprehensive NADome signaling landscape: high-NAD producers showed poorer outcome than those expressing low mRNA levels, both in terms of PFS and OS as well. In line with these data, a panel of MM cell lines showed that pervasive NAPRT levels reduced NAMPT inhibitors activity thus supporting a complete NAD $^{+}$-depletion for more effective strategies also in *in-vivo* xenograft models. PROGENy analysis revealed an increased NF-KB activity in NAPRT-depleted cells which in turn resulted in higher oxygen reactive species generation from these cells. Indeed, NAPRT KO cells showed oxidative damage accumulation, with increased malondialdehyde (MDA) activity and lower endogenous antioxidant defenses as suggested by glutathione reductase, glutathione peroxidase and ratio of reduced GSH to oxidized glutathione (GSSG) ratio measurements. Next, mitochondrial-energetic status was also tested by measuring electron transport chain (ETC) Complex I and Complex II activities: a shift towards oxidative metabolism was observed. Overall these data phenocopy the energetic status observed in drug-resistant cells; as result NAPRT-KO makes MM cells resistant to Melphalan. In such a context, the use of NAMPT-inhibitors was able to overcome drug-resistance by re-sensitizing cells to anti-MM activity of DNA damaging agents. Finally, a focused analysis on ASCT-treated MM patients, revealed poor outcome for "high-NAD $^{+}$ producers" thus supporting clinical relevance for NAD $^{+}$ metabolic restriction in these patients.

**Conclusions.** Our data provide evidence that $NAD^+$ biosynthesis shapes metabolic phenotypes of MM cells, with its impairment resulting in metabolic reprogramming and cells viability injury. As result, a comprehensive $NAD^+$ depletion with dual NAPRT and NAMPT inhibition primes MM cells to alkylating agents which still represent the backbone strategy for ASCT-eligible patients.

**Disclosures** No relevant conflicts of interest to declare.

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