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### 09 Monoclonal Gammopathies & Multiple Myeloma

#### CHARACTERIZATION OF THE BIOLOGICAL AND MOLECULAR RELEVANCE OF NONO PROTEIN IN MULTIPLE MYELOMA

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**Introduction** Paraspeckles (PSs) are a new class of nuclear ribonucleoprotein organelles, whose relevance in multiple myeloma (MM) pathogenesis has been well documented. PS assembly relies on the binding of the lncRNA NEAT1 with several PS proteins (PSPs), among which NONO.

NONO is a multifunctional protein deregulated in many cancer types. Data concerning NONO involvement in MM are virtually absent. We recently reported its overexpression in CD138+ MM cells as compared to healthy plasma cells, also highlighting its correlation with poor outcome in MM patients. Moreover, NONO expression in human MM cell lines (HMCLs) is significantly higher than in other haematological non-HMCLs.

**Methods** Gymnotic delivery of specific LNA-gapmeR (g#NONO) was used to silence the expression of NONO in a panel of 4 HMCLs. Dose-effect curves were obtained by Trypan Blue exclusion counts. Cell viability was assessed through CCK-8 assay. Clonogenic potential was evaluated by methylcellulose assay. Cell cycle phases distribution and apoptosis induction were investigated by FACS analysis. PSs integrity was analyzed by confocal microscopy analysis of combined NEAT1 RNA-FISH and NONO IF. WB was used to study PSPs levels.

**Results** All the tested HMCLs, albeit at different levels, showed high sensitivity to NONO silencing starting from the 3<sup>rd</sup> day of gapmeR exposure (median IC<sub>50</sub> value | 6. 5µM). Growth curves retrieved from CCK-8 assay confirmed a significant reduction in the number of viable cells in samples treated with sub-cytotoxic concentration of g#NONO (5µM) until the 7<sup>th</sup> day of exposure. Modulation in the proliferative behavior of NONO-depleted cells (NONO-KD) was confirmed by the 2-fold decreased number of colonies as compared to controls. In line with FACS results showing an increase of the % of cells distributed in the subG0/G1 phase of the cell cycle in NONO-KD samples (>10% for all HMCLs), we demonstrated apoptosis induction from the 4<sup>th</sup> day of gapmeR treatment (≈2-fold). From a molecular point of view, along with the significant downregulation of NONO (silencing efficiency >80% for all the HMCLs), we also showed a significant reduction in the expression level of the essential PS scaffold NEAT1 (50-70%, depending on the HMCL). The reduction of both NONO and NEAT1 fluorescence intensity and co-localizing signals was also confirmed by confocal microscopy analysis, demonstrating a strong PSs structure impairment. Of note WB analysis showed a 2-6-fold increase in the expression levels of two other PSPs, SFPQ and PSPC1, suggesting the presence of a compensatory mechanism between NONO and other PS elements.

Conclusion| Our results clearly demonstrates that NONO silencing in HMCLs leads to PSs structure impairment and results in an anti-proliferative and pro-apoptotic effect. Overall, a better elucidation of NONO relevance in MM could highlight it as a therapeutically valuable target for the development of novel pharmacological approaches for this incurable disease.

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