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# POSTER ABSTRACTS

## 602.MYELOID ONCOGENESIS: BASIC

### Nuclear Condensates Are a Therapeutic Vulnerability in NPM1-Mutant Acute Myeloid Leukemia

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The development of targeted therapies against drivers of malignancy requires a robust mechanistic understanding of the pathogenic axis underlying disease. Several subtypes of acute myeloid leukemias (AML), such as those driven by mutations in *IDH1/2*, *FLT3*, and *KMT2A*, have benefitted from the development of novel therapeutics that disrupt their transcriptional program. In contrast, there are no approved therapies that target the mutant version of Nucleophosmin (NPM1), which is the most common driver in adult AML. Mutations in *NPM1* result in export of the mutant protein (NPM1c) to the cytoplasm, prompting the suggestion that aberrant localization leads to neomorphic and pathogenic functions of NPM1c in the cytoplasm. In parallel, recent data from several groups has suggested that NPM1c directly regulates gene expression within the nucleus. Despite this progress, a well-defined mechanism for NPM1c-mediated gene regulation is lacking, hindering the development of NPM1c-targeted therapies.

To investigate the function of NPM1c, we generated over a dozen AML cell lines expressing unique NPM1c truncations and characterized their impact on the leukemic transcriptome and cell morphology. To our surprise, we found that although the majority of these truncated proteins reside in the cytoplasm, most were unable to recapitulate the transcriptional program driven by full-length NPM1c, and cells subsequently underwent differentiation and growth arrest. We further explored the chromatin binding ability of these truncated proteins to key genomic regions such as the *HOXA* cluster and *MEIS1* through CUT&RUN analysis. Next, we identified a region of the NPM1c protein critical for active nuclear import (NLS). Strikingly, disruption of this region enforces cytoplasmic sequestration of the truncated NPM1c protein. However, cells harboring this truncation fail to maintain leukemic gene expression and differentiate. Together these results suggest that aberrant localization and/or shuttling of NPM1c between cellular compartments is insufficient to maintain leukemic gene expression and cell-stemness.

We next considered whether NPM1c had a de-novo function that may explain its role in gene regulation. We have recently shown that NPM1c forms phase-separated condensates in the nucleus that regulate *HOX* gene expression and are necessary for stem-ness. These NPM1c condensates recruit multiple gene regulatory factors including XPO1, NUP98, and KMT2A **(Figure 1)**. Through selective degradation of NPM1c, or pharmacological inhibition of XPO1, we found that disruption of nuclear condensates abrogates recruitment of these protein co-factors. We further explore the effect of drug compounds known to inhibit NPM1-mutant AML on condensate behavior and chromatin binding. This condensate behavior is a defining feature of heterotypic phase separation, a process in which two or more biomolecules form multivalent interactions to create a condensate. Importantly, we found that significant overexpression of NPM1c causes an imbalance in the ratio between NPM1c and its binding partners, disrupting normal condensate formation, and causing subsequent differentiation and growth arrest.

Collectively, these results suggest that therapeutic targeting of NPM1c, or a host of interacting partners, may disrupt condensate formation and the leukemic transcriptional program, providing an exciting new therapeutic vulnerability in *NPM1*-mutant AML.

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

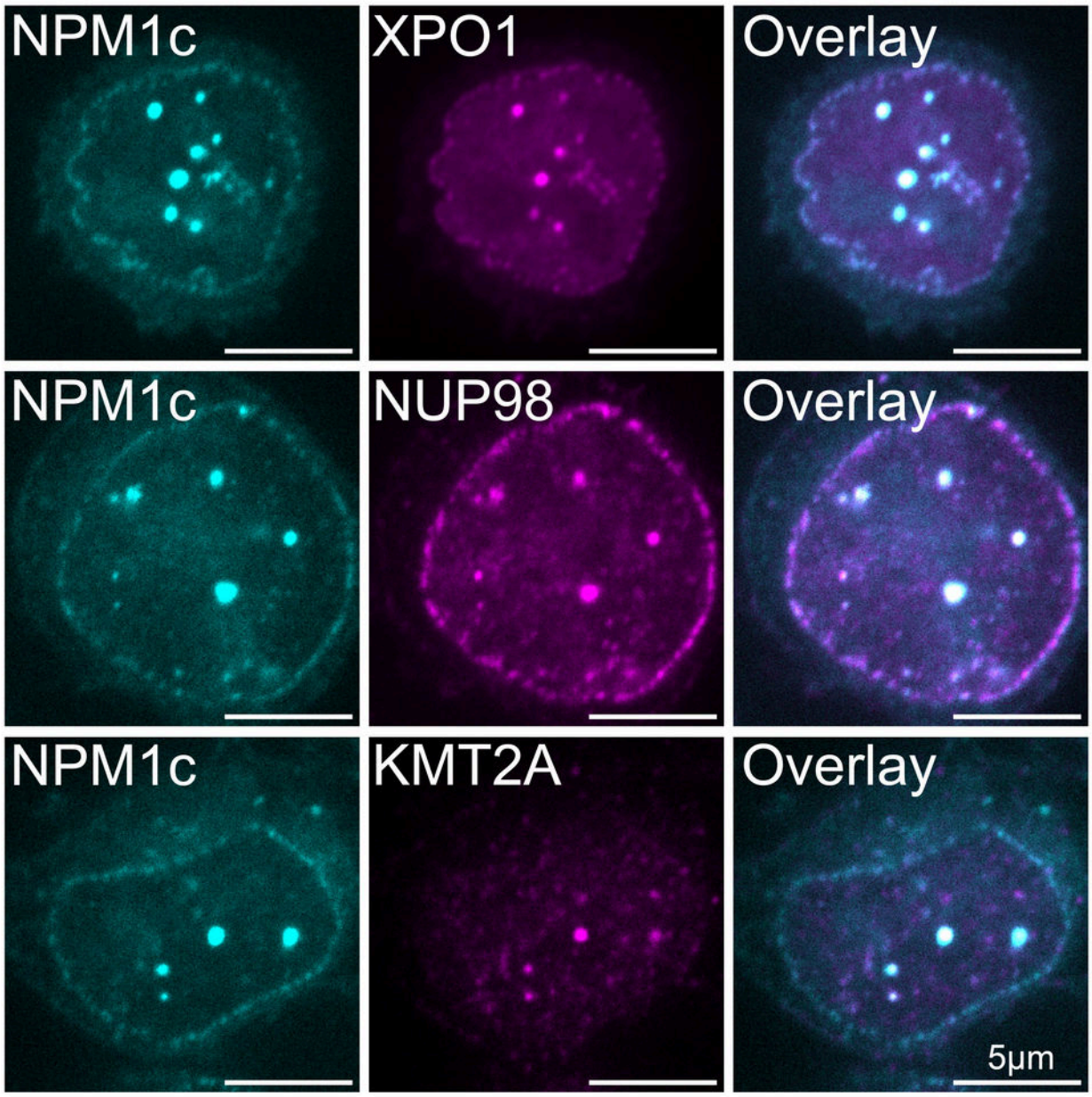


**Figure 1: NPM1c condensates recruit multiple protein interactors**
**Left Column** - Fixed OCI-AML3 cells expressing NPM1c-muGFP fusion proteins shown in cyan. **Center Column** - Immunofluoresence staining of multiple proteins shown in magenta. **Right Column** - Overlay of NPM1c and interacting proteins.

**Figure 1**

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