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ORAL ABSTRACTS

631.MYELOPROLIFERATIVE SYNDROMES AND CHRONIC MYELOID LEUKEMIA: BASIC AND TRANSLATIONAL

Chromothripsis Orchestrates Leukemic Transformation in Blast Phase MPN through Targetable Amplification of DYRK1A

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Progression of myeloproliferative neoplasms to blast phase (BPMPN) is associated with lack of response to conventional therapies and dire clinical outcomes. Consequently, there is a major unmet need to develop new therapies for BPMPN. Chromothripsis, the process of catastrophic shattering and haphazard repair of chromosomes, is a key contributor to somatic variation in cancer, but this phenomenon has not yet been described in BPMPN. More broadly, whether chromothripsis might result in actionable molecular events that are amenable to targeting remains an open question.
To characterise the contribution of structural variants to BPMPN, we first performed integrated copy number and mutation profiling in 64 BPMPN patients by SNP karyotyping and targeted next generation sequencing. We observed a recurrent pattern of chromothripsis that involved chromosome 21, which together with other structural variants led to amplification of

a common region of chromosome 21 ('chr21amp') in ~25% of patients (GISTIC *q-val<0.01*, **Fig 1A**). Chr21amp was associated with *TP53* mutations and a higher number of copy number alterations. Patients with chr21amp had a particularly aggressive and treatment-resistant phenotype, with 0% surviving 12 months compared to 46% in the non-chr21amp pts (*p=0.0007*), retaining significance in multivariate analysis including after correction for *TP53* mutation status.

Whole genome sequencing confirmed that the chromosomal rearrangements resulting in chr21amp occurred by different mechanisms, ranging from simple amplification to highly complex chromothriptic events involving multiple chromosomes. There were no recurrent translocation partners or mutations. The minimally amplified region (MAR) spanned 2.7Mb and contained 24 genes, with a median copy number of 3.5 (range 2.7-8.3)

Single-cell transcriptomics combined with allelic resolution genotyping revealed that chr21amp was present in the dominant subclone and occurred subsequent to *JAK2V617F* and mut *TP53* acquisition. Chr21amp was detectable in phenotypic HSCs and throughout early stages of hematopoiesis, but not in mature erythroid cells, consistent with a differentiation block.

Of the 24 genes in the minimally amplified region, only one gene, *DYRK1A*, a serine threonine kinase linked to cell proliferation and survival, was both differentially expressed (single-cell and bulk RNAseq) and differentially accessible (ATACseq). To explore the functional role of *DYRK1A* in BPMPN we performed shRNA and CRISPR-mediated *DYRK1A*-knockdown and knockout (KO) in BPMPN cell lines (HEL and SET-2), which led to impaired cell proliferation. The DYRK1A inhibitors EHT1610 and GNF2133 also led to dose-dependent growth inhibition. *DYRK1A*-KO BPMPN HEL or SET2 cell clones showed a reduced ability to propagate leukemia *in vivo* with a significant survival advantage vs. wild type control mice. BPMPN chr21amp+ primary patient CD34+ cells were highly sensitive toDYRK1A inhibitors, while healthy control CD34+ cells were unaffected

Prior studies have shown that *DYRK1A* activates the DREAM complex, a transcriptional repressor of DNA-repair pathways. In chr21amp patient cells, the DREAM DNA repair gene signature was significantly downregulated (NES -1.74, *q-val <0.001*), while conversely in CRISPR *DYRK1A* KO SET2 cells the transcriptional DNA repair signature was upregulated (NES 1.76, *q-val <0.001*). In functional assays, CRISPR *DYRK1A* KO was protective against DNA damage, with a reduction in γ-H2AX foci after etoposide treatment or irradiation (*p<0.01* for both).

A second mechanism of leukemogenesis emerged from geneset enrichment analyses, which suggested enhanced JAK-STAT signaling in chr21amp BPMPN cells and downregulation in the CRISPR *DYRK1A* KO context. We validated this by showing that *DYRK1A* overexpression activates and potentiates STAT5B transcriptional activity in a luciferase reporter assay. Finally, we noted that the STAT target *BCL2* was selectively upregulated in chr21amp cells. BCL2 inhibition showed strong synergy with DYRK1A inhibitors for induction of BPMPN cell apoptosis (Bliss synergy score 15).

Collectively, these findings define the chr21amp event as a novel prognostic biomarker in BPMPN. We pinpoint *DYRK1A* amplification as a central driver of genomic instability and exacerbated JAK-STAT signalling, for the first time linking chromothripsis to a specific druggable target (**Fig 1B**).

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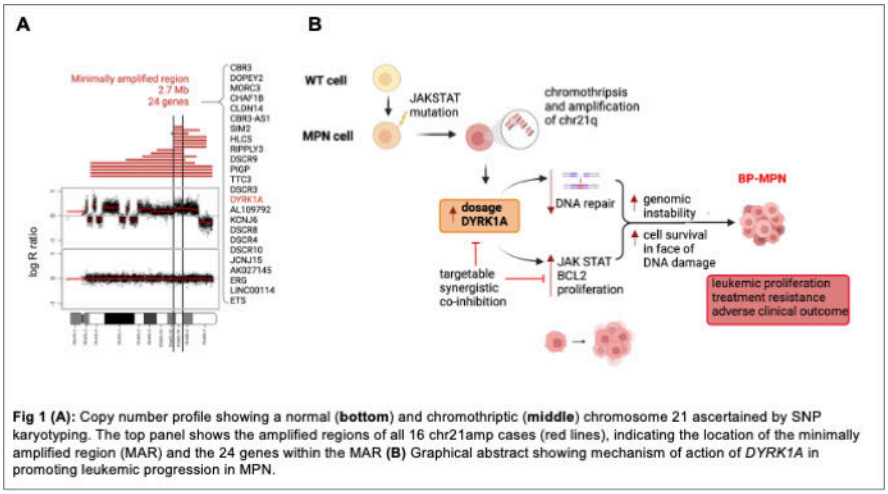

Fig 1 (A): Copy number profile showing a normal (**bottom**) and chromothriptic (**middle**) chromosome 21 ascertained by SNP karyotyping. The top panel shows the amplified regions of all 16 chr21amp cases (red lines), indicating the location of the minimally amplified region (MAR) and the 24 genes within the MAR **(B)** Graphical abstract showing mechanism of action of *DYRK1A* in promoting leukemic progression in MPN.

Figure 1

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