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POSTER ABSTRACTS

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

Chromosome 9p Duplication Promotes T-Cell Exhaustion and Enhances Stem Cell Clonogenic Potential in JAK2-Mutant Myeloproliferative Neoplasms

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Myeloproliferative Neoplasms (MPNs) are a diverse group of clonal hematopoietic disorders originating from a single hematopoietic stem cell, causing an excessive production of mature blood cells. Classic forms of MPNs include polycythemia vera, essential thrombocythemia, and myelofibrosis. The most common mutation is a gain-of-function point mutation in the *JAK2* gene, known as JAK2V617F. This mutation leads to constant activation of the JAK-STAT signaling pathway, resulting in the overgrowth of MPN cells. Furthermore, the variant allele frequency (VAF) of JAK2V617F has a significant impact on the severity and phenotype of the disease. Alongside many described genetic mutations, cytogenetic abnormalities are commonly observed in MPNs, particularly involving chromosome 9. As the *JAK2* gene is located on the short arm of this chromosome, we hypothesized that chromosome 9 copy number abnormalities might be a disease modifier in JAK2V617F-mutant MPN patients.

To characterize the biological effects of chromosome 9 copy number abnormalities on JAK2-mutated MPN cells, we analyzed circulating CD34+ hematopoietic stem and progenitor cells (HSPCs) as well as monocytes and granulocytes from 32 MPN patients. Through Next Generation Sequencing, we categorized the patients into three main groups based on *JAK2* mutation and copy number status: patients with two copies of the *JAK2* gene with either heterozygous (VAF between 20% and 60%; n=10) or homozygous (VAF > 60% n=10) JAK2V617F mutation, or patients carrying mutant JAK2 and gene amplification (n=12).

In-depth analysis of JAK2-amplified patients revealed that the amplification involved the entire chromosome 9p, thus including other gene loci like *CD274*, which encodes programmed death-ligand 1 (PD-L1). Further investigation of the order-of-events and clonal hierarchies through droplet digital PCR on CD34+ cell-derived colonies showed that most colonies of 9p-duplicated patients had three copies of *JAK2*, with 2 out of 3 alleles harboring the JAK2 mutation and that point mutations are frequently the initial pathogenic event in clonal evolution, followed by amplification of the *JAK2*-mutated allele. Functionally, CD34+ cells from +9p patients displayed high clonogenicity and gave rise to a greater number of primitive colonies (Colony Forming Unit-Granulocyte, Erythrocyte, Monocyte, Megakaryocyte, CFU-GEMM).

As JAK2 hyperactivation had been previously reported to lead to increased PD-L1 expression, we further explored the functional significance of *CD274* amplification in +9p patients. Our analysis showed increased PD-L1 messenger RNA and protein levels in +9p patient CD14+ monocytes compared to those from patients carrying only two copies of chromosome 9. Moreover, immunofluorescence analysis demonstrated significant re-localization of PD-L1 to the cytoplasmic membrane in monocytes from +9p patients, but not in JAK2V617F-homozygous patients (panel A).

Increased levels of PD-L1, an immune checkpoint known to curb T cell activation, led us to analyze the T cell compartment, which resulted enriched in CD3+/CD8+/CD57-/PD-1+ exhausted T-cells in +9p patients compared to other MPN patients and healthy donors (panel B).

In conclusion, our comprehensive characterization of the molecular interplay between JAK2V617F and chromosome 9 alterations, along with their immunological implications due to PD-L1 hyperactivation, fills a critical knowledge gap and provides valuable insights into the disease progression of 9p-MPNs. Further analysis is ongoing to explore the associations between 9p duplication and hematological parameters in 9p-MPN patients for a better understanding of the clinical implications of this genetic abnormality in MPNs.

Disclosures Mora: *Novartis:* Speakers Bureau. **Luppi:** *Novartis:* Membership on an entity's Board of Directors or advisory committees; *Abbvie:* Membership on an entity's Board of Directors or advisory committees; *Gilead Sci:* Membership on an entity's Board of Directors or advisory committees, Other: Travel Grant; *Sanofi:* Membership on an entity's Board of Directors or advisory committees; *Grifols:* Membership on an entity's Board of Directors or advisory committees; *Daiichi-Sankyo:* Membership on an entity's Board of Directors or advisory committees; *MSD:* Membership on an entity's Board of Directors or advisory committees; *Jazz Pharma:* Membership on an entity's Board of Directors or advisory committees. **Guglielmelli:** *GSK:* Speakers Bureau; *Novartis:* Other: Other member of advisory board, speaker at meeting, Speakers Bureau; *Abbvie:* Other: Other member of advisory board, speaker at meeting, Speakers Bureau. **Passamonti:** *Novartis, GSK, Bristol Myers Squibb, Celgene, Sierra Oncology, AbbVie, Janssen, Roche, AOP Orphan, Karyopharm, Kyowa Kirin, MEI, Sumitomo:* Honoraria; *BMS:* Consultancy, Honoraria, Research Funding; *Abbvie:* Consultancy, Honoraria; *Roche:* Consultancy. **Vannucchi:** *AOP:* Honoraria; *Roche:* Honoraria; *Abbvie:* Honoraria; *BMS:* Honoraria; *GSK:* Honoraria; *Novartis:* Honoraria; *Incyte:* Honoraria.

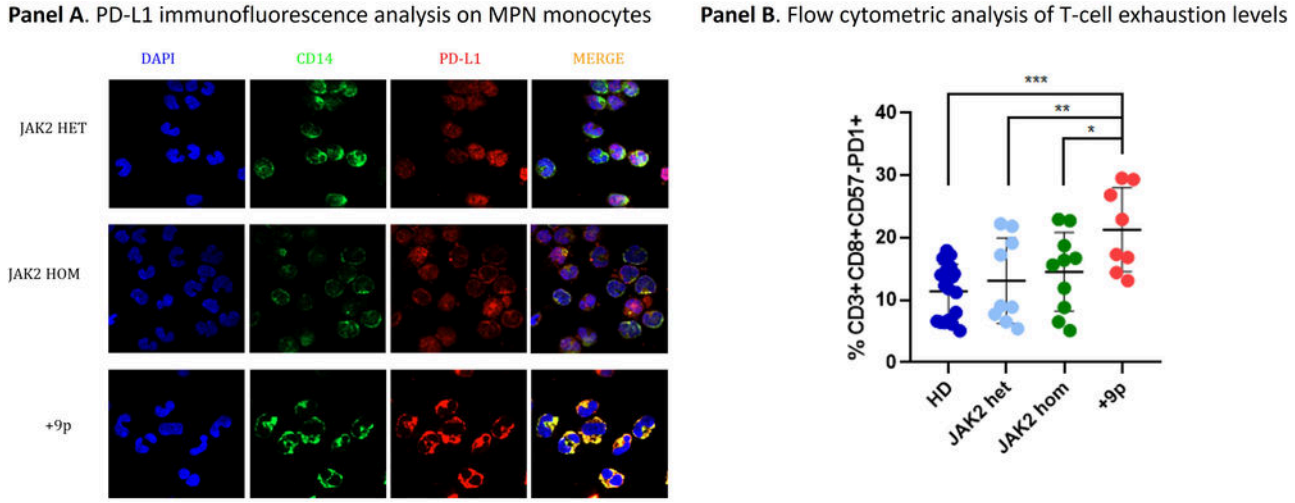

Figure 1

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