

Proceedings Article

# Copy Number Alterations in Myeloid Proliferative Neoplasms: Driver or Passenger of Leukaemic Transformation?

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Received 27 April 2026; Accepted 06 October 2026; Published online 07 October 2026

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## Abstract

Post-Myeloproliferative neoplasms Acute Myeloid Leukaemia (post-MPN AML) is commonly characterised by *TP53* mutations and a high burden of Copy Number Alterations (CNAs), yet the functional contribution of CNAs to leukaemic transformation remains unclear. In this study, single-cell RNA sequencing (scRNA-seq) data from 11 *JAK2V617F TP53*-mutant post-MPN AML patients were integrated with a *JAK2V617F Trp53*-loss mouse model to identify recurrent CNAs and their molecular consequences. We demonstrate that CNAs accumulate progressively across clonal stages and affect conserved pathways including PI3K/AKT/mTOR signalling, Myc targets, DNA repair, and apoptosis. Finally, synthetic lethal analysis reveal druggable vulnerabilities, highlighting potential therapeutic targets. Our results suggest that CNAs are not passengers but drivers of leukaemic transformation.

## 1. Introduction

MPNs are clonal blood forming (haematopoietic) stem cell disorders characterized by excessive production of mature myeloid blood cells (e.g red blood cell or platelets), most commonly driven by activating mutations in genes *JAK2* (V617F), *CALR*, or *MPL* [1]. Although many patients remain in a chronic phase for years, a subset progress to post-MPN AML, a clinically aggressive disease with median survival of less than six months [2]. Leukaemic transformation is a multistep process involving the accumulation of genetic and epigenetic alterations, most notably with loss of function mutations in the tumor suppressor gene *TP53*, which normally prevents tumor development by regulating cell division, DNA re-

pair, and programmed cell death (apoptosis). Loss of *TP53* functions are strongly associated with gains and losses of parts or whole chromosomes (chr) called CNAs and frequently observed in advanced myeloid malignancies [3], [4].

In post-MPN AML, recurrent abnormalities such as deletions of chr 5q, 7q, and 17p suggest a functional role for CNAs in disease evolution [4], [3], [2]. However, whether CNAs actively drive leukaemic transformation or arise as secondary consequences of genomic instability remains debated. Addressing this question is critical, as CNA-driven gene dosage changes may reshape transcriptional programs [3] and expose therapeutic vulnerabilities [5], [6].

In this study, we integrate single-cell transcriptomic data from human post-MPN AML with a genetically matched mouse model to assess the recurrence, functional impact, and therapeutic relevance of CNAs during leukaemic progression. We assess recurrent CNAs, their functions, and therapeutic relevance.

## II. Methods and materials

ScRNA-seq data from a median of 742 CD34+ haematopoietic stem and progenitor cells (HSPCs) from 11 *JAK2V617F TP53*-mutant post-MPN AML patients and seven healthy controls were obtained from [4]. Unpublished CNAs generated in the Lane Lab from two *Jak2V617F Trp53*-loss AML murine models were used for cross-species comparison.

Sc-data processing was performed using Seurat\_5.30.0, filtering out low quality cells (MT\_percent < 20%; min\_pc=19). The cell types were assigned using SingleR\_2.11.2 with the reference [7] and validated by marker gene expression and projection onto reference bone marrow atlas. CNAs were inferred from single-cell gene expression profiles using InferCNV of the Trinity CTAT Project (cutoff=0.1; analysis\_mode=subclusters). InferCNV applies a sliding-window approach in which gene expression is averaged across genomic regions and compared with healthy reference samples. Consequently, genes were classified as CN-lost based on their genomic coordinates lying within a predicted deleted region, not on a lack of expression in individual cells. To reduce technical artefacts, CNA calls were filtered based on recurrence across cells carrying CNAs in 30% of cells, and posterior confidence (posterior\_prob>30%); otherwise, default InferCNV parameters were used unless stated.

Differentially expressed genes (pValue<0.05) between *TP53*-multihit and wild-type (WT) were identified using edgeR\_4.7.2.. Pathway enrichment analyses were performed on recurrently CNA-affected genes and on ranked gene expression changes using enrichR\_3.4 with the Human MSigDB v2025.1.H. The underlying statistical test is Fisher's exact test. The p-value was used since the adjusted p-value was too stringent, and as an additional layer of validation the genes needed to be lost in at least three humans and two mice. Synthetic lethal interactions involving CNA-lost genes were identified using curated SynLethDB v2.0, filtered for high-throughput screens and evaluated for druggability.

## III. Results and Discussion

CNA prediction identified 9 chr (3, 5, 6, 7, 11, 12, 17, 19, 20) that are recurrently lost in at least three patients, and 15 chr recurrently gained (1, 2, 3, 4, 8, 9, 10, 11, 12, 13, 14, 18, 19, 21, 22). To further validate that we can predict CNAs from single cell gene expression data we assessed

if we can recover CNAs predicted from diagnostic gold standard methods like FISH and SNParrays. We could confirm that the reported del5q, del17p, chr20q11.22-13.2 loss, and chr17p13.1 CN-loss [4] can be recovered.

### III.I. CNAs drive clonal expansion

To see if the identified CNAs are associated with the expansion of the leukaemia driven population here *JAK2V617F TP53*-multihit as reported in [2], we compared the relative percentage of cells carrying CNAs in cells without mutations (WT), MPN-driver mutated cells (*JAK2V617F*), in preleukaemic (*JAK2V617F TP53* heterozygous) and leukaemic cell population. We found that loss of chr 5, 7 and 17 are significantly associated with the expansion of the cells carrying *JAK2V617F TP53*-multihit mutations (Fig. 1) which consists with previous reports [2] [3] [8].

### III.II. Mouse and human post-MPN AML HSPCs share CN lost genes

To further strengthen our hypothesis that CNAs are drivers of AML progression, we assessed if genes affected by CNAs are consistent in mouse and human post-MPN AML. Using the genes that are lost in three patients, and the two mice, we found four genes affected by CN-gains and 163 CN-lost genes.

### III.III. Identification of pathways and molecular functions

To assess if the recurrently lost genes in mouse and humans affect specific pathways, the 163 recurrently lost genes were used to perform the pathway analysis and were then compared to the gene set enrichment analysis (GSEA) of the differentially expressed genes, comparing WT with *TP53*-multihit. From the 163 genes 17 were enriched in pathways, including PI3K/AKT/mTOR signaling (p=0.0002), Myc Targets V1 (p=0.001), Apoptosis (p=0.01) and DNA Repair (p=0.03), with four (*CDK2*, *CDK4*, *DDIT3*, and *GPX4*) of the 17 genes being part of multiple pathways, see Table 1. Interestingly only two of the genes, *DDIT3* and *CDK4* are annotated in the cosmic database as known cancer genes.

To further resolve the putative functional consequences of CN-lost genes, we assessed the gene expression levels of WT vs *JAK2V617F TP53*-multihit cells within post-MPN AML samples. Only 11 of the 163 recurrently lost genes were downregulated. The lost and downregulated genes sit on chr 12 (n=6), and 19 (n=5) *MKNK2* is the only downregulated and lost gene that has an effect on the pathways.

We next identified deregulated pathways on the basis of differentially expressed genes using GSEA. We found significant activation (NES>0) of PI3K/AKT/mTOR Signaling (p=3.7e<sup>-6</sup>), Myc Targets (p=4.02e<sup>-4</sup>), DNA Re-

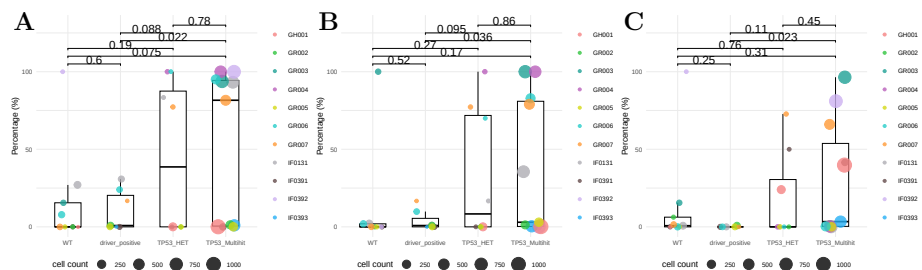


**Figure 1:** Boxplots of the percentage of WT, JAK2V617F (driver\_positive), JAK2V617F TP53 heterozygous (TP53\_HET), and JAK2V617F TP53 multi-hit (TP53\_Multihit) cells carrying a CNA loss in chr (A) 5 (B) 7 (C) 17. Each dot represents a MPN patient consistently coloured across the genotypes. Dot size is proportional to the number of cells. Statistical testing was performed with a two-sided Welch t-test.

pair ( $p=5.04e^{-4}$ ) and supression of apoptosis ( $NES<0$ ;  $p=0.019$ ), see Fig. 2.

| pathways                            | gene    | #Pat. | chr |
|-------------------------------------|---------|-------|-----|
| Apoptosis; DNA Repair               | GPX4    | 8     | 19  |
| DNA Repair                          | POLR2E  | 8     | 19  |
| PI3K/AKT/mTOR Sig.                  | MKNK2   | 8     | 19  |
| Apoptosis; Myc Targets V1;          | CDK2    | 7     | 12  |
| PI3K/AKT/mTOR Sig.                  |         |       |     |
| Apoptosis                           | GADD45B | 7     | 19  |
| Myc Targets V1                      | LSM7    | 7     | 19  |
| Myc Targets V1                      | PA2G4   | 7     | 12  |
| Apoptosis; PI3K/AKT/mTOR Sig.       | DDIT3   | 6     | 12  |
| DNA Repair                          | PRIM1   | 6     | 12  |
| Myc Targets V1; PI3K/AKT/ mTOR Sig. | CDK4    | 6     | 12  |
| Apoptosis                           | GNA15   | 5     | 19  |
| DNA Repair                          | ALYREF  | 5     | 17  |
| Myc Targets V1                      | PTGES3  | 5     | 12  |
| PI3K/AKT/mTOR Sig.                  | STAT2   | 5     | 12  |
| Myc Targets V1                      | XPOT    | 4     | 12  |
| PI3K/AKT/mTOR Sig.                  | TBK1    | 4     | 12  |
| Myc Targets V1                      | PWP1    | 3     | 12  |

**Table 1:** Displayed are 17 genes commonly CN-lost across mouse and human post-MPN AML that were associated with significantly enriched pathways as identified by enricher. Further is displayed the chromosomal location (chr) and the number of Patients (#Pat.) with a CN-loss of the gene.

III.IV. Synthetic lethal combinations deliver novel drug targets

While directly therapeutically targeting lost or non-expressed genes is impossible we can harness the unique vulnerability of synthetic lethality. Synthetic lethality is the combination of a lost gene and inhibition of the alternate pathway that results in cell death and has been proven effective [6], [5]. Here we assessed putative synthetic lethal targets to our recurrently lost genes by mining SynLethDB v2 that contains synthetic lethal combination predictions from functionally identified CRISPR/CRISPRi, DrugScreens, GenomeRNAi and RNAi Screen. From the 17 genes with functional consequences, eight genes have functional synthetic lethality. From those eight genes six have a noted inhibitor or drug as assessed by mining the guidetopharmacology DB. From the 48 possible target genes four are targets for multiple lost genes, see Fig. 3.

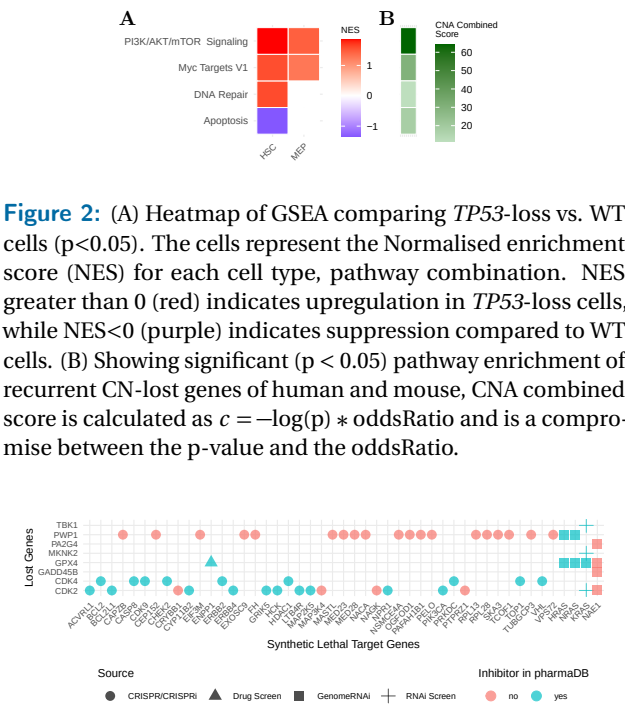


**Figure 2:** (A) Heatmap of GSEA comparing *TP53*-loss vs. WT cells ( $p<0.05$ ). The cells represent the Normalised enrichment score (NES) for each cell type, pathway combination. NES greater than 0 (red) indicates upregulation in *TP53*-loss cells, while  $NES<0$  (purple) indicates suppression compared to WT cells. (B) Showing significant ( $p < 0.05$ ) pathway enrichment of recurrent CN-lost genes of human and mouse, CNA combined score is calculated as  $c = -\log(p) * oddsRatio$  and is a compromise between the p-value and the oddsRatio.

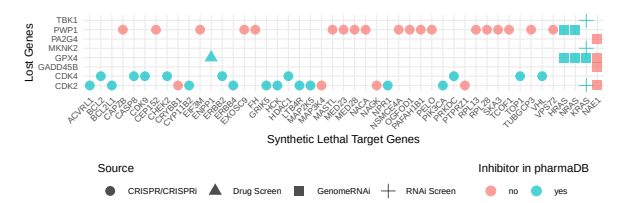


**Figure 3:** Dotplot of synthetic lethal targets to eight of the 17 recurrently lost genes. The turquoise dots, triangles, and crosses represent targets that have reported inhibitors in the guidetopharmacology DB.

III.V. Discussion

The steady increase across clonal expansion strongly suggests that the CNAs do not arise merely as a consequence of transformation but instead emerge early and confer selective advantages. This interpretation is consistent with the previously described clonal structure and complex karyotypes reported by McNamara et al., [9] and Rodriguez-Meira et al., [4]. Furthermore, Li et al., [3] showed that transformation originated in MEPs, this association could not be found here, which can be explained since our data were primarily annotated as HSCs. Also the significant association between the CNAs and

recurrent losses of chr 5, 7, and 17, has been reported in clinical studies of post-MPN AML [2], [4]. Interestingly, a subset of these recurrently lost genes clustered on chr 19 and appeared enriched in patients with an erythroid bias. This suggests that specific CNA patterns may contribute to lineage bias and clonal dominance during transformation [3].

The four enriched pathways collectively regulate fundamental decisions between proliferation and cell death. That all four pathways were differentially regulated indicates that CNA-driven gene dosage reduction directly contribute to functional rewiring of these programmes. Similar disruptions were observed by Li et al., [3], who demonstrated increased BMP2/SMAD pathway activity in transformed MEPs. That most apoptosis-related genes were downregulated, while pathways promoting survival and proliferation (Pi3K/AKT/mTOR, Myc targets) were upregulated, supports a model in which CNAs directly drive transcriptional reprogramming [3], and is consistent with a highly aggressive phenotype of post-MPN AML. That only a small number of recurrently lost genes are transcriptionally downregulated is biologically plausible, because many genes are expressed only during particular stages of the cell cycle and the expression can be cell-type specific.

The synthetic lethal combinations represent a promising opportunity to selectively target CNA-bearing leukaemic cells while sparing normal HSPCs. Previous studies have shown that post-MPN AML cells rely on BCL-xL (encoded in BCL2L1), and that combining its inhibition with p53 activation can overcome apoptosis resistance [5]. Our results support these findings and provide additional CNA-based rationale for such therapeutic strategies, while also nominating further candidate targets.

Key limitations of this analysis includes, that although inferCNV enables sensitive detection of CNAs from scRNA-seq data, the method's reliance on binned gene expression can obscure precise breakpoint resolution. Nevertheless, its resolution often exceeds that of SNP arrays or whole-genome sequencing [10]. While our validation against FISH and SNP-array data [4] confirmed several CNAs, differences in CNA boundaries were observed. Functional validation of the identified synthetic lethal candidates was beyond the scope of this project but will be essential in future studies. Another limitation is the limited number of cells available to determine cell-type-specific associations, for instance for MEPs. ScRNA-seq inferCNV is dropout-prone, so some of the 163 CN-lost genes may be passengers.

## IV. Conclusion

Taken together, our findings support the hypothesis that CNAs are not merely passengers of genomic instability but drivers of leukaemic transformation from MPN to post-MPN AML. They promote clonal expansion, re-

shape key survival pathways, and create exploitable therapeutic vulnerabilities. The analysis remains predominantly correlative, therefore, functional perturbation experiments are required to prove causality. Future work should integrate multi-omic single-cell approaches, combining DNA and RNA to refine CNA mapping and causality, and use CRISPR-based perturbation models to test candidate synthetic lethal interactions. Additionally, designing a boutique CRISPR screen focusing on the 472 candidate genes could further distinguish true drivers of transformation. Ultimately, this work provides a conceptual framework and a shortlist of targets for developing CNA-informed therapeutic strategies in post-MPN AML.

## Acknowledgments

The work has been carried out at QIMR Berghofer, Brisbane Australia and supervised by the Institute of Experimental Dermatology, Universität zu Lübeck.

## Author's statement

Conflict of interest: Authors state no conflict of interest. DeepL and ChatGPT were used for linguistic support.

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