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Clonal Architecture in Myeloproliferative Neoplasms: Old Dog, New Tricks?

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In a recent issue of *HemaSphere*, Luque Paz and colleagues[1] described the reconstruction of clonal architecture in myeloproliferative neoplasms (MPN) using single-cell DNA sequencing (scDNAseq). This approach not only had similar power to traditional methodologies based on the growth and sequencing of individual hematopoietic colonies, but distinct patterns of clonal hierarchy were also reported to show prognostic significance.

The ease of studying single-cell-derived hematopoietic colonies, most often grown from peripheral blood, has been a long-standing foundation and strength of MPN biological research. Early colony studies of patients with *JAK2* mutations, for example, identified the differing patterns of *JAK2 V617F* heterozygosity and homozygosity between patients with essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF)[2] and confirmed the presence of mutations in almost all patients in PV.[3] Following the identification of other gene mutations in myeloid disease, analysis of comutation patterns within colonies then illuminated the clonal dynamics of stable and progressive MPN: that complex, branching or oligoclonal substructures could exist even in chronic phase[4–7]; that the same 2 mutations acquired linearly but in different orders could have variable biological consequences[8]; and that specific subclonal genomic events could drive disease transformation.[7,9]

Although a backbone of many important MPN studies, sequencing of many thousands of individual hematopoietic colonies is labor-intensive and could never be applicable to routine clinical practice. Luque Paz and colleagues used the alternative method of scDNAseq (Tapestri technology), used previously in other myeloid malignancies particularly acute myeloid leukemia (AML),[10] to sequence the individual subclones present in MPN peripheral blood samples.[1] A total of 50 MPN patients with *JAK2 V617F* and additional mutations were studied with traditional liquid or methylcellulose colony assays and the clonal substructures delineated were compared with results from scDNAseq in 22 of these. Although almost all mutations originally identified in bulk DNA were detected by both methods, there was 80% concordance in detection of individual clones and subclones. Although scDNAseq seems less reliable in distinguishing between heterozygous and homozygous mutant subclones, a broad scDNAseq panel can detect additional low-level clones whose variants might not be detected when sequencing bulk granulocyte DNA. scDNAseq also avoids the risk of differential clonal efficiencies in culture leading to overrepresentation or underrepresentation of specific genotypes. scDNAseq can, therefore, comprehensively decode the subclonal architecture of a broad panel of variants from a single peripheral blood sample without the labor-intensive processes involved in culturing and genotyping numerous colonies.

Based on the clonal architecture data from all 50 patients, 4 patterns (clusters) were identified in an unsupervised clustering analysis, differing in number of mutations, architectural complexity including comutation within subclones, order of mutation acquisition, and presence of *JAK2 V617F* homozygosity. The group with the most complex clonal architecture (cluster 4) showed the highest rate of transformation to AML/MDS and a reduced overall survival, which was independent of MPN subtype, age at diagnosis, and the presence of high molecular risk mutations. By contrast, a group (cluster 1) who carried additional mutations but mostly in a different clone to the *JAK2 V617F*—although these included high-risk mutations such as in *ASXL1* or *EZH2*—had the best overall survival.

Genomic data have become an integral part of prognostic modeling in all MPN subtypes[11–13] (Figure 1). Previous studies have demonstrated that a higher total number of mutations is associated with increased transformation and poorer survival[7]; a higher number of high molecular risk mutations is also associated with reduced survival in myelofibrosis.[11] This new study now delivers the clear message that the prognosis of a MPN does not simply reflect the sum of its genomic abnormalities, but the way in which these interact within a clonal substructure. In particular for cluster 1, in whom most additional mutations were not found within the *JAK2*-mutant clone, the predictive power of the total number of mutations was actually improved when these mutations outside the *JAK2*-mutant clone were discarded altogether. Moreover, the clonal architecture-based classification outperformed the Sanger risk calculator[13] in predicting outcome for this group.

It is well recognized that mutations can cooperate biologically in myeloid disease, making it logical that patterns of comutation are highly relevant clinically. Certain genes show increased likelihood of comutation in AML, myelodysplastic neoplasms, or MPN.[13,22,23] With *JAK2 V617F*, combination with *TET2* loss, *EZH2* loss or gain of mutant *IDH1/2* have all been shown to have synergistic effects, driving more severe myeloproliferative phenotypes in mouse models through effects on hematopoietic stem and progenitor cell function and differentiation.[24–26]

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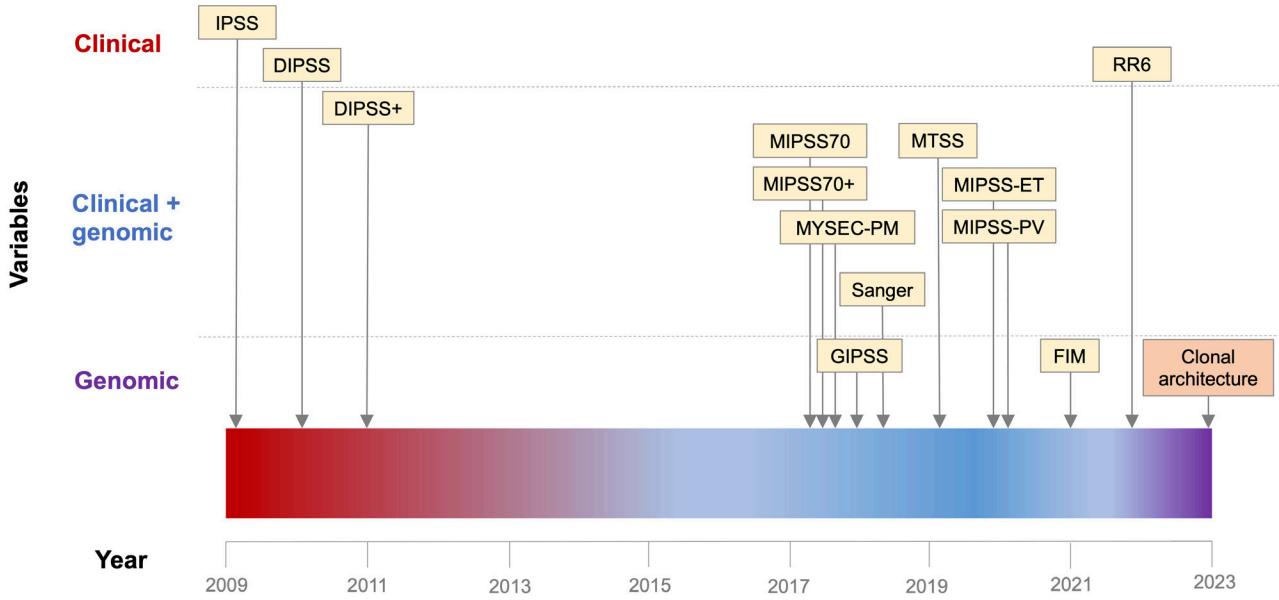

Figure 1. Evolution of prognostic modeling in the myeloproliferative neoplasms.[11–21]

Moreover, the phenomenon of clonal hematopoiesis of indeterminate potential, where small hemopoietin subclones carrying isolated mutations in genes such as *ASXL1* can exist in the absence of any definite hematological neoplasm,[27] is consistent with the finding that such subclones, if entirely separate to the *JAK2*-mutant clone, might not adversely affect the outcome of a patient with MPN at all.

How might these findings be translated to clinical practice? The prognostic model requires independent validation and it is important to note that only a subgroup of MPN patients were covered, specifically those with *JAK2 V617F* and additional mutation(s). The utility of such a model in patients with *CALR*- or *MPL*-mutant disease requires further exploration and no patients with a single driver were included. The incremental benefit of the clonal architecture model was also mainly seen in a group of patients (cluster 1) that included many with ET and PV, in whom comprehensive prognostic profiling is often not clinically necessary at present. Besides these caveats, cost would currently prevent the widespread use of scDNAseq, although in recent years other technologies previously considered prohibitively expensive have transitioned to routine diagnostic processes.[28]

Cancer diagnostics is increasingly moving toward establishing each individual patient's full genomic profile and using this as a surrogate for disease biology to inform diagnosis, personalized prognostic predictions, and, where possible, personalized therapy. In other fields, broad techniques including whole genome sequencing have advanced clinical practice through the ability to map a cancer's genomic lesions comprehensively.[28] Chronic myeloid malignancies have some particular considerations: clinical practice includes decisions about whether to treat at all and therapeutic studies are already considering whether treatments might drive regression, outgrowth, or evolution of disease subclones.[29] In this context, the ability to generate an unbiased snapshot of clonal architecture from peripheral blood on diagnosis, follow-up, and progression is appealing. If affordable and deliverable clinically in future, scDNAseq technologies could therefore not only have a role in further evolution from current prognostic models (Figure 1), but also facilitate studies of how best to tailor therapy for individual MPN patients.

AUTHOR CONTRIBUTIONS

ALG conceptualized and wrote the article.

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