

resolution to study the emergence of resistance and identify new targets. The authors combined bulk whole genome sequencing data with single-cell RNA and assay for transposase-accessible chromatin sequencing (ATAC-seq) data with longitudinal samples from 15 patients with relapsed/refractory disease to reconstruct the clonal structures at multiple time points.

For more than a decade, scientists have evaluated the multiple myeloma (MM) genome from patients with precursor conditions to heavily treated relapsed refractory disease using genomic interrogation.^{2,3} Although these studies have helped clinicians and scientists identify new biomarkers, targets, and resistance pathways, they have also revealed significant patient heterogeneity.^{4,5} When does this heterogeneity arise? Are the alterations present at initial diagnosis or are they acquired? Because these studies used bulk sequencing platforms, in which all cells from a patient are pooled together before profiling, it was difficult for scientists to explore these questions. However, the tools used for genomic studies are also evolving,^{6,7} as demonstrated by Poos et al.

The authors identified mitochondrial mutations using the chromatin accessibility data (single-cell ATAC sequencing) and used those mutations to refine the subclone evolution patterns predicted based on copy number alterations and mitochondrial DNA mutations.⁸ The branches in the resulting evolution tree provide a clearer picture of the epigenomic and transcriptomic differences across clones and time points, and it suggest that converging adaptation of existing subclones before treatment might be responsible for acquired resistance, which is independent of treatment.

By comparing shrinking and growing clonal populations after treatment, the authors identified the upregulation of CD44, a cell adhesion molecule, as a potential target for the MCL1 inhibitor.⁹ This finding was validated in another patient data set and by analyzing the interaction between microenvironment cells and MM cells. The critical point was that not all subclones had the same level of interactions with different cell types in the bone marrow.

This study showed that combining techniques provides substantial advantages for in-depth evaluations^{7,10} and opens doors

to many future applications, such as evaluating cells from precursors to newly diagnosed MM, evaluating the effects of specific treatments, evaluating the effect of MM cell targeting therapies vs immunotherapies, and comparing in vivo and in vitro models. However, there are still technical areas needing refinement. The authors had difficulty studying branching evolution patterns because of the limitations of existing single-cell platforms. Some of these difficulties may improve by simply sequencing more cells or improving strategies for combining MM cells and bone marrow microenvironment interactions. Alternatively, spatial technologies may be found to be useful in future studies. Mutations, which may have a significant role in MM subclonal structures, were not studied here because of the limitations of single-cell methodologies; however, combining whole-transcriptome sequencing with ATAC-seq or combining DNA and RNA data at a single-cell level may help future studies of mutation-driven subclones.¹⁰ This study is a glimpse of the future, in which single-cell studies will help in overcoming MM.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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MYELOID NEOPLASIA

Comment on [Senapati et al](#), page 1647

Changing treatment changing prognosis of mutations

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In this issue of *Blood*,¹ Senapati et al suggest that recurrent mutations in genes encoding RNA splicing factors may not confer an adverse prognosis in patients with acute myeloid leukemia (AML) treated with venetoclax-based therapies.

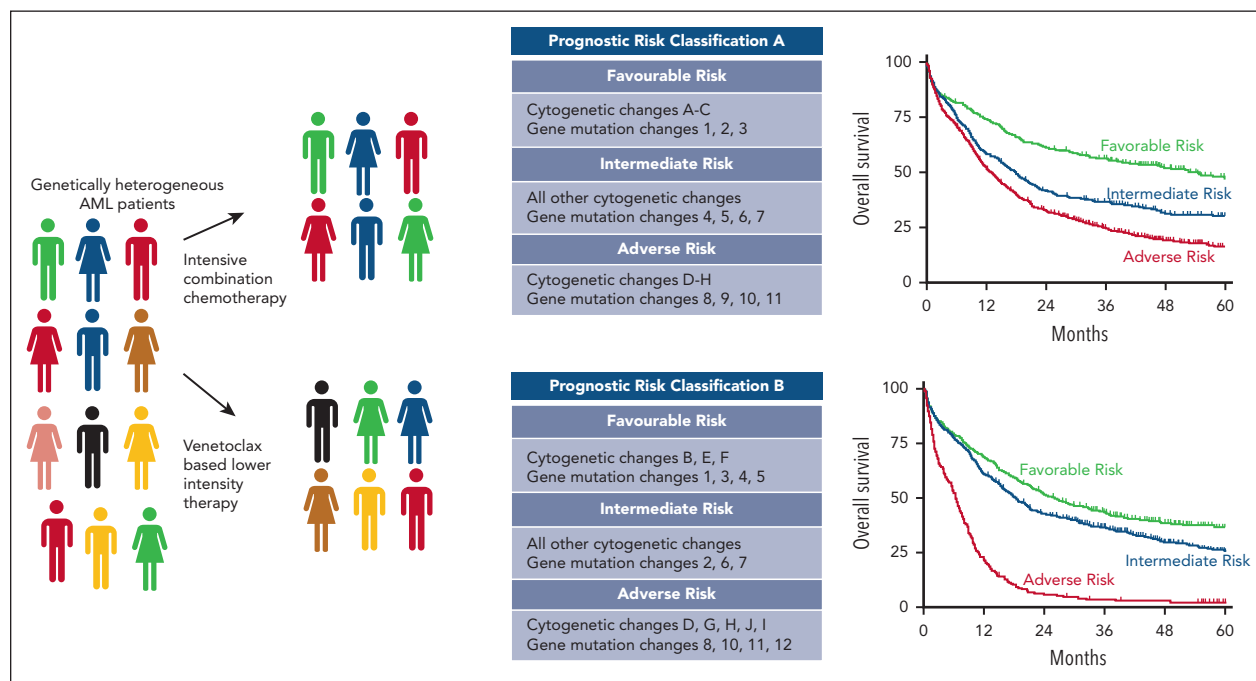
In many blood cancers, acquired recurrent genetic changes are used as prognostic biomarkers. As biomarkers, these changes often form the basis of prognostic classifications for newly diagnosed patients that can dictate their treatment. As an example, the commonly used European LeukemiaNet (ELN) recommendations on the diagnosis and management of AML include a classification of prognostic risk, with 3 prognostic groups (favorable, intermediate, and adverse).^{2,3} These recommendations are widely used to guide discussions with patients on prognosis and treatment. In the revised 2022 recommendations, it was recognized that recurrent genetic mutations commonly seen in myelodysplasia correlate with an adverse outcome.³ This was true regardless of whether the patient had an antecedent clinical history of myelodysplasia or had myelodysplastic-related cytogenetic changes in their AML cells. These myelodysplastic-related gene mutations included those in genes encoding the RNA splicing factors *SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2* (hereafter referred to as splicing factor mutations, or *SF^{mut}*). However, a caveat with the ELN risk classification is that it has been developed with data from patients treated with intensive combination chemotherapy. As the incidence of myelodysplastic-related gene

mutations increases with age, AML patients with these mutations are likely to be older and more likely to receive venetoclax and hypomethylating agents (HMA).⁴ This raises the obvious, and clinically important, question of whether the ELN risk classification applies to the majority of patients with *SF^{mut}* who are treated with venetoclax-based, lower-intensity treatments and not intensive combination chemotherapy.

To address this question, Senapati et al examined the clinical outcome of 994 newly diagnosed AML patients treated between 2017 and 2022 from a single tertiary referral institution. The patients had extensive molecular and cytogenetic data at diagnosis. The median follow-up for the cohort was 26 months. Sixty-six of 994 patients with core-binding factor AML were excluded from the analysis; 928 patients were studied for most of the analyses. Of those, 266 patients had *SF^{mut}* and 662 patients did not have splicing factor mutations (hereafter referred to as *SF^{wt}*). Overall, the *SF^{mut}* and *SF^{wt}* groups were not matched for age, cytogenetics, ELN high-risk mutations besides mutations in genes encoding splicing factors (*TP53*, *RUNX1*, and *ASXL1*), and incidence of secondary and therapy-related AML. Of

the 928 patients, 617 received lower-intensity treatments and 311 were treated with higher-intensity regimens. There was considerable heterogeneity with respect to treatment (up to 46 different treatments were given, but venetoclax-HMA-based treatments were most common among lower-intensity treatments).

Given the heterogeneous and retrospective nature of this cohort coupled with imbalances in risk factors between the *SF^{mut}* and *SF^{wt}*, analyses of these data need to be interpreted with caution. Starting first with patients who received intensive combination chemotherapy, the authors confirmed that *SF^{mut}* patients (53/662) compared with *SF^{wt}* patients (258/662) had an inferior overall survival (15.9 months compared with 26.7 months; $P = .06$) and relapse-free survival (9.6 months compared with 21.4 months; $P = .04$). However, when they examined patient groups who had received venetoclax combined with combination chemotherapy (29/53 *SF^{mut}* and 131/258 *SF^{wt}* patients), the difference in overall survival and relapse-free survival between *SF^{mut}* and *SF^{wt}* patients disappeared. This was the first hint that addition of venetoclax (ie,



Frontline treatment of a genetically heterogeneous group of patients newly diagnosed with AML who received either intensive combination chemotherapy or venetoclax-based lower-intensity therapy. Treatment- and patient-specific factors will determine the prognostic biomarkers that may differ between treatment groups (middle panel), which produce survival curves relevant to each treatment group.

treatment change) may alter the prognostic impact of SF^{mut} .

Turning to patients who received lower-intensity treatments, the authors conducted a number of analyses, such as on patients above and below the age of 60 and comparing patients with de novo AML with those with a clinical history of therapy-associated AML and secondary AML, and in both cases stratifying patients by whether they received venetoclax or not in their treatment schedules. The clinical outcomes that were compared were overall survival and relapse-free survival. In all analyses, SF^{mut} was not associated with an adverse outcome. To pick an example of these analyses in patients over the age of 60 years, who more commonly receive lower-intensity treatment, overall survival and relapse survival in the SF^{mut} and SF^{wt} patient groups were 12.3 months versus 8.5 months and 9.3 months versus 7.7 months, respectively. In patients who received venetoclax as part of lower-intensity therapy, overall survival and relapse survival in SF^{mut} and SF^{wt} patient groups were 14.1 months versus 9.6 months and 9.8 months versus 9.1 months, respectively. In univariate analysis and multivariate analysis, SF^{mut} did not affect attainment of overall response in both intensively treated patients and patients who received lower-intensity treatments. Finally, Cox regression analysis was used to identify factors predictive of survival in prestratified groups. In patients older than 60 treated with lower-intensity regimens, on univariate analysis SF^{mut} did not affect the hazard of relapse or overall survival. This was also true in multivariate analysis. By contrast, in both univariate and multivariate analyses, the ELN 2017 adverse-risk category (which includes a number of poor-risk cytogenetic subgroups and mutations in the genes encoding *TP53*, *RUNX1*, and *ASXL1* but not SF^{mut}) was associated with significantly poorer overall survival and relapse-free survival, whereas addition of venetoclax to therapy and stem cell transplant was associated with improved overall survival and relapse-free survival.

As mentioned previously, though these data have important caveats and need to be validated, they remind us that any prognostic biomarkers need to be assessed in the context of the treatment

given (see figure). Most clinicians would agree this is self-evident, but the data from Senapati and colleagues are a timely reminder of this truism. Specifically, in AML, where the standard of care has changed for most patients with SF^{mut} to venetoclax-based lower-intensity regimens, the field needs to agree on a new validated prognostic classification for this group of patients as a priority. Progress on this front is beginning.⁵

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Wang et al, page 1658

PCBP1 is essential for proper iron absorption

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In this issue of *Blood*, Wang et al shed light on a new role for the cytosolic iron chaperone protein poly rC binding protein 1 (PCBP1) in regulating intestinal absorption and systemic iron homeostasis.¹ Iron is an essential micronutrient required by all cells for diverse cellular functions, including mitochondrial respiration, DNA replication, and protein synthesis. Beyond cellular metabolism, iron plays a critical role in systemic oxygen delivery through hemoglobin-mediated erythrocyte function.² Previous studies have explored manipulating regulatory pathways, such as dietary changes or iron chelator administration, to modulate iron uptake.

Systemic iron balance involves intricate communication among various cell types, regulating intestinal iron absorption, erythropoiesis, and recycling of iron from senescent red blood cells. Dietary iron is absorbed in the duodenum through specific transporters on intestinal epithelial cells (IECs). Nonheme iron is first converted from ferric (Fe^{3+}) to ferrous (Fe^{2+}) via duodenal cytochrome b before being transported into enterocytes via the divalent metal transporter 1 (DMT1; gene name *Slc11a2*).^{3,4} Within enterocytes, absorbed iron can be stored as ferritin or exported into the bloodstream via the sole mammalian iron transporter, ferroportin (FPN; gene name *Slc40a1*).⁵

Iron absorption in the intestine is synchronized with systemic iron levels and erythropoietic demands via hepatic regulation. Hepcidin (gene name *HAMP*), a hepatic hormone, suppresses intestinal iron absorption by inducing degradation or blocking ferroportin function.⁶ However, during iron deficiency or increased erythropoietic demand, hepcidin expression is suppressed, allowing for increased iron absorption.⁷ Mutations affecting hepatic hepcidin and intestinal ferroportin are associated with hereditary hemochromatosis.⁸

PCBP1, part of a family of adaptor proteins binding cytosine-rich RNA, DNA, and ferrous complexes, plays a role in