blood commentary

MYELOID NEOPLASIA

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BCL-2 inhibition and AML: can we best Darwin?

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In this issue of *Blood*, DiNardo and colleagues[1] provide fascinating details on the behavior of leukemic clones in less-fit patients treated for acute myeloid leukemia (AML) with the combination of venetoclax and either a hypomethylating agent or a low-dose cytarabine.

These data, derived from patient samples collected during 2 highly influential recent clinical trials,[2] not only describe the molecular correlates of disease response and resistance but also provoke important questions that should be addressed in the next wave of clinical studies investigating these backbone regimens. The authors make multiple interesting points. The following 3 take-aways stand out:

1. Patients with mutations in the nucleophosmin gene (NPM1mut) or in isocitrate dehydrogenase 2 (IDH2mut) have the best chance of a durable response with a venetoclax-based regimen. These are patients in whom we need to study time-limited therapy, including intensive or measurable residual disease–directed consolidation.
2. Sequential molecular studies on the remaining patients reveal confounding polyclonal patterns of resistance that emerge sometimes very early in treatment. These individuals (and their physicians) face a challenging game of whack-a-mole to control increasingly unmanageable leukemic subclones. Clinical research might focus on upfront combinations with other molecularly targeted treatments, with (or randomized against) dose-attenuated cytotoxic therapy or even novel chemoimmunotherapeutic approaches.
3. If we are going to depend on repetitive attempts at molecularly targeted therapy for disease control, we will need to develop molecular sequencing strategies that are accessible, validated, standardized, and cost-contained.

After years of therapeutic nihilism, the armamentarium for treating older individuals with AML is expanding. Since 2017, there has been a spate of drug approvals, none more widely heralded than the BCL-2 inhibitor venetoclax. Early-phase clinical studies combined it with low-dose cytarabine or with hypomethylating agents, and very encouraging results prompted Food and Drug Administration approval. Despite the absence of randomized data, there has been rapid clinical uptake of venetoclax-based combinations in older, possibly less-fit individuals.[3] It used to be that the challenge of facing a newly diagnosed, older patient was determining if they were robust enough for cytotoxic induction. That question remains, but now one needs to also ask: do I have all the cytogenetic and molecular testing I need to make a decision? Is my patient best served by cytotoxic induction, by CPX-351 (a gentler cytotoxic regimen), by a venetoclax-based regimen, by kinase or IDH inhibition, or with a clinical trial? Also, does this decision truly hinge on fitness? The absence of head-to-head studies makes picking a regimen feel like educated guess work.[4]

Enter the study published herein. Investigators studied sequential samples from 81 patients on 1 of the 2 prospective published venetoclax combination studies. Subjects were divided into 3 subgroups: those who achieved complete remission/complete remission with incomplete count recovery (CR/CRi) lasting >12 months (n = 18); those who achieved CR/CRi but who relapsed within a year (n = 25); and, finally, those who were refractory to treatment (n = 20). Interestingly, 18 patients achieved CR/CRi but came off the drug within a year for reasons other than relapse. Single-cell sequencing was performed in some instances to track clonal evolution along the treatment trajectory.

Some of the results are not surprising. Prior exposure to hypomethylating agents is associated with inferior survival. Response to therapy did not always mean that treatment continued. Adverse risk cytogenetics portends adverse risk.

Some of the results are provocative and compelling. Patients with NPM1 mutations (n = 11), IDH2 mutations (n = 7), or both mutations (n = 4) had a 2-year overall survival >70%. These data certainly provide a good argument for considering a venetoclax-based combination for less-fit individuals with NPM1mut or IDH2mut. With the caveat that we are talking about only 22 patients, a validation of this response in the ongoing randomized studies of venetoclax (NCT02993525 or NCT03069352) would be significant for patients in whom cytotoxic induction is truly too hazardous.

Some of the results are sobering. A total of 25 patients had disease that initially responded, but then relapsed at a median of 6.4 months. Serial analysis of samples as well as single-cell sequencing techniques illustrates that kinase activation, including FLT3-ITD, and clones that acquired biallelic silencing of *TP53* accounted for many of these clinical outcomes. What is the lesson? Selection pressure means that AML remains a wily disease. In some subjects, the resistant subclones that blossomed at relapse were present

at diagnosis. In other cases, they were novel. Importantly, they emerged quickly, sometimes just days to weeks into therapy.

Given these patterns of resistance, perhaps earlier combined targeted therapy is warranted (ie, triplet regimens that use FLT3 inhibition or *TP53* stabilization strategies)? However, that approach brings its own new questions: how good are our tests at detecting subclones? Do we need to target mutations that have not yet emerged? How many targets are good enough, and for how long? If a barrage of molecularly directed agents at the start of treatment guts the proliferation potential of the latent clones, well, that's excellent. However, I would be less enthusiastic about sequencing 1 agent after another, consigning patients to a permanent parade of consecutive treatments. I think it is a mistake to give up on a goal of true disease eradication. Combinations that use cytotoxic agents (possibly in attenuated doses or novel delivery mechanisms) or harness immune therapies alongside targeted therapies should be investigated. Evidence of polyclonal resistance supports the case that, as a research community, we need to focus on what the leukemic clones have in common and target those commonalities early on.[5]

Finally, a logistical point is presented. The authors argue that "serial molecular studies can identify patterns of drug sensitivity and resistance at the subclonal level." I fear this recommendation is premature. Are we ready to roll such a practice into prime time, given the costs, the lack of standardization, and the paucity of effective options for refractory disease?

DiNardo, Wei, and colleagues have done much to bring venetoclax to AML patients, a remarkable accomplishment. The data presented here show us that our work has only just begun.

Conflict-of-interest disclosure: L.C.M. served as a consultant to Novartis, Celgene, Incyte; previously held stock in Pfizer; and receives research funding from Jazz Pharmaceuticals. ■

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Liu et al, page 826

Checkmate for EBV-HLH

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In this issue of *Blood*, Liu et al describe the favorable response of adults with relapsed/refractory Epstein-Barr virus (EBV)-associated hemophagocytic lymphohistiocytosis (HLH) to treatment with nivolumab, a programmed cell death-1 (PD-1) inhibitor.[1] EBV-HLH presents a challenging clinical conundrum because only a minority of patients will achieve long-standing clinical remission with front-line therapy.[2] To further complicate matters, neither clinical nor pathologic-based criteria have been well established to differentiate which patients are likely to fail upfront HLH therapy with etoposide and dexamethasone.[3,4] Patients with relapsed/refractory disease have a dismal chance of survival because of high rates of disease-related mortality.[2,3]

Various salvage regimens have been used for relapsed/refractory EBV-HLH, including combination chemotherapy regimens, monoclonal antibodies targeting the host cellular reservoirs for EBV, and targeted agents aimed at controlling the systemic inflammatory syndrome that defines the disease pathophysiology.[3] Nivolumab presents a novel approach to EBV-HLH as it seeks to restore T-cell immune function against uncontrolled EBV infection, which is at the very root of this virally mediated disease process. Because immune checkpoint inhibition of PD-1 has proven a useful therapeutic option for relapsed/refractory EBV-related lymphomas, it offers an attractive novel option for EBV-HLH as well.[5] Although it is generally accepted that relapsed/refractory EBV-HLH will ultimately require allogeneic hematopoietic stem cell transplant (HSCT) to achieve a cure, the authors sought to explore whether restoration of immune function through PD-1 inhibition could lead to long-standing control of EBV infection and the associated HLH syndrome.[3]

In this case series of 7 adults with relapsed/refractory EBV-HLH, nivolumab monotherapy resulted in clinical complete remission in 5 patients with a median follow-up of 16 months. The clinical successes were corroborated by translational experiments using single-cell transcriptome analyses. These demonstrated baseline overexpression of inflammatory markers, including tumor necrosis factor, interleukin-1B, and CD163. They also demonstrated expansion of PD-1$^+$ T cells after treatment with nivolumab, which was associated with decreasing levels of interferon-γ and granzyme B (cytokines that drive the hyperinflammatory syndrome characteristic of HLH), enrichment of CD8$^+$ T cells in activation and degranulation pathways, and a correlative decrease in the EBV viral loads in 4 of the 5 patients who achieved clinical remission. Single-cell RNAseq analyses of CD8$^+$ T cells at baseline revealed underexpression of specific HLH-related genes, including STXBP2, UNC13, SH2D1A, and CD27, suggesting that such immune dysregulation may explain the vulnerability to EBV-related complications. Thus, immune checkpoint inhibition with nivolumab effectively restored T-cell immune competence against EBV, resulting in clinical improvement of the associated HLH (see figure).