VTE such that they can be treated with an appropriate duration of antithrombotic therapy; such efforts are ongoing in the hospitalized medically ill with the direct oral anticoagulants.8,9

Conflict-of-interest disclosure: K.A.B. has received consulting fees from Janssen and Boehringer Ingelheim.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Jawhar et al, page 137

A molecular roadmap for midostaurin in mastocytosis

Jason Gottlib  STANFORD CANCER INSTITUTE

In this issue of Blood, Jawhar and colleagues describe the results of quantitative KIT D816V allele burden testing and next-generation sequencing of a panel of myeloid genes to characterize molecular correlates of response and progression on midostaurin therapy in patients with advanced systemic mastocytosis.1

On 28 April 2017, the US Food and Drug Administration approved the oral multikinase inhibitor midostaurin for the treatment of patients with newly diagnosed FLT3 mutation–positive acute myeloid leukemia in combination with chemotherapy and for patients with advanced subtypes of systemic mastocytosis (SM), such as aggressive systemic mastocytosis (ASM), SM with an associated hematoxic neoplasm (SM-AHN), and mast cell leukemia (MCL). Midostaurin’s target profile includes wild-type and D816V-mutated KIT.2 The oncogenic variant of KIT can be detected in 90% of SM patients with highly sensitive polymerase chain reaction assays.3 The identification of KIT D816V not only fulfills 1 minor diagnostic criterion for SM, but also informs the appropriate use of tyrosine kinase inhibitors for mast cell cytoreduction because this mutation is imatinib-resistant.

Patients with ASM, SM-AHN, and MCL share the common thread of reduced life expectancy, often related to progressive organ damage, complications of the associated hematologic neoplasm (AHN), or both. Neoplastic mast cells usually transit the blood as invisible marauders, plundering organs in their wake. The rarity of advanced SM and its protean manifestations of mast cell activation and organopathy often result in delayed diagnosis and treatment.

I think of advanced SM and late-stage myelofibrosis (MF) as “fraternal twins” because of their shared clinical and biological features. Patients often exhibit a hypercatabolic state with debilitating constitutional symptoms, hepatosplenomegaly, progressive organ impairment, and bone marrow failure with potential for transformation to acute myeloid leukemia (AML). The canonical driver mutations in SM and myelofibrosis, KIT D816V and JAK2 V617F, respectively, often coexist in a Darwinian ecosystem of multiple mutated clones. This is especially true in SM-AHN, whose genetic complexity partly reflects the marriage of 2 neoplasms, and stands in contrast to the comparatively bland clonal landscape of indolent SM that is usually restricted to KIT D816V.4 In both neoplasms, a similar set of genes influence prognosis: mutations in SRSF2, ASXL1, or RUNX1 (S/A/Rpos) adversely impact survival in SM5; in primary myelofibrosis, mutations in SRSF2, ASXL1, EZH2, or IDH1/2 are associated with worse overall and/or leukemia-free survival independent of the Dynamic International Prognostic Scoring System-Plus scoring system.6 Several groups have used these molecular data to generate mutation-enhanced clinical prognostic models to optimize risk stratification for patients with advanced SM7 and myelofibrosis.

If advanced SM and myelofibrosis are fraternal twins, then midostaurin and ruxolitinib may be linked as a band of brothers, waging Sisyphean battles to reclaim disease control amid a minefield of mutations (see figure). Ruxolitinib produces marked improvements in splenomegaly and MF-related disease symptoms, but meaningful reductions in bone marrow fibrosis or JAK2 V617F allele burden are uncommon.8 In the pivotal global trial of midostaurin, the overall response rate was 60%, of which 75% were major responses, indicating normalization of ≥1 SM-related organ damage finding.9 In addition, a majority of evaluable patients experienced reduction in splenomegaly and/or a >50% decrease in the serum tryptase level or bone marrow mast cell burden. Decreases in spleen size with ruxolitinib and reversion of MF are not uncommon in midostaurin-treated patients.10 A majority of midostaurin patients experience reductions in splenomegaly, SRSF2, ASXL1, EZH2, or IDH1/2 expression, with complete or near complete normalization of ≥1 SM-related organ damage finding.9

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Elucidating the biomarkers that predict response and progression with targeted therapies is a critical part of strategizing in these “myeloid wars.” In their report, Jawhar and colleagues analyzed 38 patients with advanced SM who were treated with midostaurin on the pivotal trial or via a compassionate use program. They found that the overall response rate, median duration of midostaurin treatment, and overall survival (OS) were significantly higher in patients with an S/A/Rnox mutation profile and in subjects with a ≥25% reduction in the KIT D816V allele burden (“KIT-responders”) vs <25% reduction (“KIT-nonresponders”) by using allele-specific quantitative reverse transcription-PCR. Notably, KIT-responder status was the strongest and only on-treatment marker that retained statistical significance in a multivariate analysis of OS. These molecular data complement a post hoc multivariate analysis of the pivotal trial, which showed that achieving a response according to modified Valent criteria or a ≥50% reduction in bone marrow mast cell burden on midostaurin therapy was associated with improved survival.9

The biological resemblance between advanced SM and MF also extends to the nature of progression on therapy. Among SM patients treated with midostaurin, 5 of 6 patients who developed secondary AML or MCL harbored the S/A/Rpos molecular profile.1 Irrespective of KIT-responder status, progression was also linked to the appearance of new mutations or an increase in the variant allele frequency of non-KIT D816V mutations (eg, K/NRAS, RUNX1, IDH2, and NPM1) that were present before starting midostaurin. This baseline genetic heterogeneity and secondary resistance owing to dynamic evolution of mutant subclones is the modus operandi of many myeloid neoplasms. It also closely mimics the experience of MF patients treated with ruxolitinib, where mutations in ASXL1, EZH2, or IDH1/2 (as well as the total number of mutated genes on a next-generation sequencing panel) negatively impacted spleen response, time to discontinuation, and OS.10

Interestingly, no additional mutations in KIT or JAK2 that confer resistance to midostaurin or ruxolitinib, respectively, have been identified to date.

How do we use the information generated by Jawhar and colleagues? First, the poor-risk S/A/Rpos profile should not engender nihilism about treating an already challenging advanced SM population. Although historical comparisons with unselected cohorts should be approached cautiously, they found that midostaurin-treated patients carrying these mutations experienced significantly longer survival than those who had not received midostaurin. The authors’ data also reinforce that SM-AHN should not be considered a monolithic entity, because outcomes within specific types of AHN may vary widely according to the molecular architecture of their hybrid disease. This mutational complexity not only forebodes challenges with midostaurin, but also with other therapies, such as cladribine and hematopoietic stem cell transplantation. Therefore, testing of novel agents or combination strategies with midostaurin will similarly benefit from dynamic assessments of the molecular determinants of response. Lastly, if deeper reduction of KIT D816V allele burden is a key biomarker of response and survival, then the next generation of selective KIT D816V inhibitors, such as BLU-285 and DCC-2618, may be able to build on the gains produced by midostaurin.

Unlike King Sisyphus who was eternally condemned to the futile task of rolling a huge boulder up a steep hill, only to have it perpetually roll back down as it reached the top,
patients with advanced SM have reason for optimism: the findings of Jawhar and colleagues provide a molecular footing to help navigate the steep mountain toward cure.

Conflict-of-interest disclosure: J.G. served as Chairman of the Study Steering Committee for the Novartis-sponsored global trial of midostaurin in advanced SM. He has received funding to conduct trials of midostaurin and BLU-285 in advanced SM. He has also served on advisory boards and received honoraria from Novartis, Blueprint Medicines, and DepiCera.

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Comment on Agathanggelou et al, page 156

Targeting deubiquitinases in CLL

Deepa Sampath THE OHIO STATE UNIVERSITY

In this issue of Blood, Agathanggelou et al demonstrate that inhibition of ubiquitin–proteasome protease 7 (USP7) compromises homologous recombination DNA repair in part by destabilizing the E3 ligase RAD18 leading to the accumulation of DNA damage which kills chronic lymphocytic leukemia (CLL) cells independently of ataxia telangiectasia mutated (ATM) and p53.1 Deregulation of the ubiquitin–proteasome pathway is common in cancer. USP7 is a deubiquitinase that removes ubiquitin groups from target proteins to protect them from proteosomal degradation. The role of USP7 gained prominence when it was identified as a novel regulator of the tumor suppressor protein p53 based on its ability to deubiquitinate and stabilize both p53 and its negative regulators Mdm2 and MdmX. In addition, it also antagonized the stability of FOXO4, phosphatase and tensin homolog, and TIP60, a p53-activating acetyltransferase while facilitating the function of oncogenic proteins such as claspin.2 These observations made USP7 a therapeutic target in cancer and initiated efforts to develop small-molecule inhibitors of USP7.2 CLL occurs due to the accumulation of slow-growing nonfunctional B lymphocytes that show enhanced survival. A hallmark of CLL is the presence of recurring chromosomal aberrations involving chromosomes 11q and 17p that result in the loss of 1 allele of the ATM and TP53 gene, respectively.3 Loss of ATM or p53 function results in impaired DNA-damage proapoptotic response and may in part contribute to the shortened responses to chemotherapy, clonal evolution, and inferior survival observed in CLL patients bearing these adverse prognosis markers.3,4 In addition to sensing DNA damage, ATM and p53 also play key roles in repairing DNA damage via the homologous recombination repair (HRR) pathway.5 In this issue, Agathanggelou et al observed that the USP7 deubiquitinase was overexpressed in CLL compared with normal donors. Inhibiting USP7 induced cytotoxicity in both quiescent and proliferating CLL cells regardless of their del11q or del17p status in vitro and in vivo using CLL cell line–derived xenograft mouse models. Mechanistically, inhibition of USP7 decreased the levels of RAD18, an E3 ligase necessary for the recruitment of the DNA repair proteins FANCD2 and RAD51 to sites of damage and successful HRR. Correspondingly, the loss in RAD18 levels was associated with loss of RAD18 foci and an increase in the levels of DNA damage marker γ-H2AX. In addition to suppressing HRR, inhibition of USP7 also led to hyperactivation of poly ADP-ribose polymerase 1 (PARP1), which activates apoptotic DNA fragmentation, depletes nicotinamide adenine dinucleotide (NAD+); leading to a loss of ATP, and activates necrotic cell death (as modeled in Figure 6 of the Agathanggelou et al article). Finally, USP7 inhibition synergized with DNA-damaging agents such as mitomycin C (MMC) as well as cyclophosphamide in vitro and in vivo.

These results are intriguing because they identify a p53-independent role for USP7 in regulating the HRR pathway by targeting the RAD18 E3 ligase. However, HRR is likely to be active only in the smaller proliferating fraction of CLL cells, which are located in protected niches within the bone marrow or secondary lymphoid tissue and are inactive in the quiescent fraction that makes up the bulk of circulating CLL lymphocytes2 raising additional questions that remain to be investigated. (1) Does USP7 regulate hitherto unknown proteins in the quiescent population of CLL cells such that its inhibition is sufficient to kill these cells? (2) In this study, USP7 inhibition synergized with the chemotherapeutic agents MMC and cyclophosphamide. DNA damage caused by these agents is repaired primarily by nucleotide excision repair in addition to HRR.4 If that is the case, does USP7 inhibition compromise nucleotide excision repair? (3) USP7 inhibition led to the hyperactivation of PARP and PARylation in their study. PARylation is known to regulate nonhomologous end joining (NHEJ) and base excision repair and to a lesser extent in HRR.5 Again, does USP7 inhibition compromise NHEJ and base excision repair? (4) USP7 inhibition was effective in targeting ibrutinib-relapsed cells. Presence of mutations...
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Jason Gotlib