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CLINICAL TRIALS AND OBSERVATIONS

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An "ATRA-ctive" new treatment of ITP?

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In this issue of *Blood*, Wu and colleagues[1] present encouraging results from one of the accumulating studies evaluating use of all-*trans* retinoic acid (ATRA) in adult patients with immune thrombocytopenia (ITP).

The most widely recognized use of oral ATRA is for treatment of acute promyelocytic leukemia, in which it has achieved the status of standard of care worldwide in combination with arsenic trioxide. Recently, data suggesting beneficial immunomodulatory effects and rescue of impaired thrombopoiesis in models of ITP[2-4] have supported further investigation into a potential therapeutic role for ATRA in ITP. Because of the heterogeneity of ITP pathogenesis and the potential for changes over time, the availability of agents with diverse mechanisms of actions for use in various combinations is needed for refractory ITP.[5]

Regarding ATRA, the few reports currently available explore combinations of ATRA with prednisone (10 mg twice daily)[4] or danazol[2] in patients with corticosteroid-resistant ITP. These studies reported response rates of ~55% to 60% with side effects of dry skin, dry mouth, and headaches. A recently published multicenter randomized controlled phase 2 trial of adults with newly diagnosed ITP in China demonstrated a higher sustained response rate (68%) in patients assigned to high-dose dexamethasone plus ATRA compared with high-dose dexamethasone alone (41%).[6]

Wu and colleagues had previously administered ATRA with danazol with the goal of using only oral agents and combining different mechanisms of action.[4] As addressed by the authors in the discussion, issues with liver disease, hirsutism, and uncertainty about long-term effects made danazol less than ideal. In this report, Wu and colleagues compare the combination of ATRA 20 mg/m^2 for 12 weeks plus low-dose rituximab (100 mg weekly for 6 weeks) with low-dose rituximab alone in adult patients with corticosteroid-resistant or relapsed ITP. The goal was to achieve "cure" (eg, 12-month sustained response), as well as faster onset of platelet increase with less toxicity. Notable findings included a higher overall response rate in the combination treatment arm (80%, 90/112) compared with monotherapy with low-dose rituximab (59%, 33/56), and a higher sustained response rate, 61% (68/112) vs 41% (23/56), respectively. Both groups had a median time to response of 28 days. Overall toxicity was low, mainly grade 1 to 2 adverse effects, of which dry skin, headache, and dizziness were reported in the ATRA group.

Several limitations to the study deserve mention, including inability to attain the planned sample size (188); 168 patients were enrolled and randomized, which could lead to underpowered analyses, potential impact of concomitant medications (14% to 18% in each arm), and limited follow-up. It is unclear if the promising results with a combination of ATRA with low-dose rituximab translate to long-term response. Per supplemental Figure 2, it appears that the platelet counts decline steadily toward the end of the study. It is also of interest that approximately three-fourths of the patients were entered at 1 study site.

Other issues, too, deserve consideration. First, both arms shared a time to response of 28 days. This is a relatively long time, particularly for patients with low platelet counts, and indeed, there was 1 case of intracranial hemorrhage (on day 27) in the combination arm. The authors address this and suggest utilization of a third agent in bleeding patients. Second, the investigators used low-dose rituximab, albeit for 6 weekly infusions instead of the usual 4 infusions. Higher-dose rituximab might have yielded faster and more durable results in both arms. Third, some of the platelet endpoints were not standard in that they have almost only been used for initial response[7]; this especially applies to sustained response where counts >50 000 per μL are more often used. Finally, the median duration of ITP in enrolled patients was 20 months. This combination may be better applicable to earlier-stage patients rather than ones with ongoing low platelet counts after >1.5 years from diagnosis. Given the current emphasis on not continuing steroids for >6 to 12 weeks, it seems like earlier might be a more appropriate time to explore the treatment combination described here.

Overall, this study supports the hypothesis that "two drugs are better than one"; however, which combination is optimal for use remains an open question. Consideration needs to be given to the mechanisms of effect of each treatment; cost of the agents; potential for overlapping toxicities; and the route of administration. Overall, the intriguing findings reported by Wu and colleagues further the possibilities of ATRA as a novel treatment of ITP and advance its utilization especially in combination perhaps with dexamethasone as recently reported[6] as well as with rituximab.

Conflict-of-interest disclosure: E.J.L. has served on the advisory board for Principia Biopharma. J.B.B. has served on advisory boards and/or consulted for Amgen, Novartis, Dova, Rigel, UCB, Argenx, Momenta, Regeneron, RallyBio, and CSL-Behring. ■

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HEMATOPOIESIS AND STEM CELLS

Comment on Fadlullah et al, page 343

Deciphering the continuum of hemogenic endothelium differentiation

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In this issue of *Blood*, Fadlullah et al[1] construct a comprehensive atlas of the endothelial-to-hematopoietic transition (EHT) continuum, as well as the subaortic niche cells in mouse embryonic aorta using a set of hemogenic endothelium (HE) reporter models. The atlas was then used to identify a novel surface marker of the HE continuum and decipher the precise cellular and molecular changes that are caused by deficiency of the pivotal transcription factors Runx1b and Gfi1/Gfi1b (see figure).

Hematopoietic stem cells (HSCs) are widely used in clinical treatment. Considering their limited numbers, generating HSCs in vitro has always been a major goal of regenerative medicine. The importance of a clear understanding of the cellular evolution and molecular program of HSC generation during development cannot be overstated. HSCs originate from vascular endothelial cells located predominantly in the ventral side of the aorta in midgestational mouse embryos. The transient and rare vascular endothelial cells with the ability to produce hematopoietic cells are called HE. The HSC-primed HE will form pre-HSCs localized mainly within the intra-aortic clusters through the EHT.[2]

To be able to isolate HE for further study, researchers have discovered several markers and established corresponding reporters to enrich HE, represented by Runx1, Gfi1, and newly identified Neurl3.[3-5] However, a single surface marker for HE enrichment in lieu of transgenic animal models has not been identified. The roles of Runx1 and its downstream target Gfi1 are well recognized; Runx1 is required for EHT, but not thereafter, and Gfi1 participates in the loss of endothelial properties during this process.[3,6] However, the precise cellular changes and molecular events that occur in the absence of these important transcription factors have not been deciphered at the whole transcriptomic level. The above issues are addressed in the study by Fadlullah et al.

In recent years, a series of studies at the single-cell level, involving single-cell transcriptomics and single-cell functional assays, have revealed the initial fate choice of HE from upstream arterial endothelium, uncovered the stepwise developmental path from HE to HSCs, identified several important intermediate cell populations, and revealed the molecular events underlying this EHT trajectory.[4,5,7,8] Fadlullah et al took this a step farther and isolated a series of phenotypic HE-related populations from the midgestational aorta region using heterozygous and homozygous *Runx1b* and *Gfi1/Gfi1b* reporter mouse models to perform high-precision full-length single-cell RNA sequencing (scRNA-seq) of nearly 1200 cells. The data set shows greater gene detection sensitivity than do previous studies using similar phenotypic populations.[5,8]

The investigators identified several clusters encompassing the whole EHT continuum, which were respectively annotated as pre-HE, HE, EHT, and intra-aortic hematopoietic cluster cells. Importantly, by profiling the cells from homozygous mice with both alleles replaced by the reporter, the exact molecular changes during the EHT procedure resulting from Runx1b or Gfi1/Gfi1b deficiency were revealed. *Runx1b* deletion led to obstruction of pre-HE to HE differentiation, consistent with a previous finding showing that Runx1 dosage regulates the efficiency of pre-HE to HE transition.[5] Specifically, the homozygous deletion of *Runx1b* resulted in the appearance of a distinct pre-HE population with unique characteristics. On the other hand, the deletion of *Gfi1/Gfi1b* led to the inability of HE to develop into EHT cells, with the Gfi1/Gfi1b-deficient HE diverted away from the canonical EHT developmental trajectory. In addition, by analyzing the Runx1b-expressing subaortic mesenchymal cells, Fadlullah et al identified 2 populations representing smooth muscle cells and Pdgfra-expressing mesenchyme. Moreover, *Runx1b* deletion resulted in an