more common in patients with an activated B-cell subtype (14/38, 37%) compared with patients with a germinal center B-cell subtype (1/20, 5%), as defined by gene expression profiling. Further, individual somatic mutations were predictive of response. As predicted from preclinical models, patients with *CARD11* mutations did not respond to ibrutinib (0/3, 0%). Patients with an underlying *MYD88* mutation also did not respond unless they also harbored a mutation in *CD79B* (4/5, 80%). This proof-of-principle trial led to a multinational phase 3 trial, testing the effect of ibrutinib added to standard frontline therapy for DLBCL.

The trial by Assouline et al identified *MEF2B* mutations as a molecular predictor of response to panobinostat. With the caveat that definitive conclusions cannot be drawn from small numbers, the observation supports a more rigorous assessment in an expanded cohort enriched with patients who harbor *MEF2B* mutations. Although only a minority of patients with relapsed DLBCL and transformed lymphomas may be eligible, such studies embody the essence of precision medicine.

Assouline et al also analyzed ctDNA as an early predictive biomarker of response. Recent data in DLBCL demonstrate that NGS-based assays for ctDNA can directly measure tumor response kinetics during combination chemotherapy and predict treatment outcomes.[5] In addition to measuring the dynamics of clearance, ctDNA can also be analyzed for tumor-specific mutations as a form of "liquid biopsy." Emerging assays for ctDNA can identify panels of genetic aberrations circulating at low allele frequencies in DLBCL, and may identify mutations not found in the tumor biopsy.[6-8] As a surrogate for the composite tumor genome, ctDNA as a liquid biopsy integrates all the genetic lesions from a tumor and can provide more comprehensive information than a single-tissue biopsy.[9]

It is notable that ctDNA was identified as an early molecular biomarker for response to panobinostat. The clearance of ctDNA after a few cycles of combination chemotherapy is predictive for response in DLBCL, but the observation of similar patterns with targeted therapy paves the way to test new agents using ctDNA as a surrogate translational end point. Two nonresponding patients who had a decrease in ctDNA at day 15 highlight another potential application of ctDNA. Could a liquid biopsy that incorporates a more extensive mutational panel or serial testing have discovered a treatment emergent resistant subclone?

As we pivot from conventional "one-size-fits-all" paradigms to treatment approaches that consider individual molecular variability, precision molecular monitoring becomes an important translational component. Certainly, both NGS and ctDNA monitoring will continue to evolve, expand, and improve. Although the clinical implications are constrained by the need for technical standardization and clinical validation, regulatory bodies recognize the importance of these approaches.[10] Indeed, Assouline et al deserve commendation for conducting a clinical trial that incorporates informative translational studies, and hence provides a far more nuanced result than response rates alone.

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

REFERENCES

1. Assouline SE, Nielsen TH, Yu S, et al. Phase 2 study of panobinostat with or without rituximab in relapsed diffuse large B-cell lymphoma. *Blood.* 2016; 128(2):185-194.
2. Roschewski M, Staudt LM, Wilson WH. Diffuse large B-cell lymphoma-treatment approaches in the molecular era. *Nat Rev Clin Oncol.* 2014;11(1):12-23.
3. Mehta-Shah N, Younes A. Novel targeted therapies in diffuse large B-cell lymphoma. *Semin Hematol.* 2015; 52(2):126-137.
4. Wilson WH, Young RM, Schmitz R, et al. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. *Nat Med.* 2015;21(8):922-926.
5. Roschewski M, Dunleavy K, Pittaluga S, et al. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. *Lancet Oncol.* 2015;16(5): 541-549.
6. Kurtz DM, Scherer F, Newman AM, et al. Dynamic noninvasive genomic monitoring for outcome prediction in diffuse large B-cell lymphoma [abstract]. *Blood.* 2015; 126(23). Abstract 130.
7. Scherer F, Kurtz DM, Newman AM, et al. Noninvasive genotyping and assessment of treatment response in diffuse large B cell lymphoma [abstract]. *Blood.* 2015;126(23). Abstract 114.
8. Rasi S, Monti S, Zanni M, et al. Liquid biopsy as a tool for monitoring the genotype of diffuse large B-cell lymphoma [abstract]. *Blood.* 2015;126(23). Abstract 127.
9. Roschewski M, Staudt LM, Wilson WH. Dynamic monitoring of circulating tumor DNA in non-Hodgkin lymphoma. *Blood.* 2016;127(25):3127-3132.
10. Blumenthal GM, Mansfield E, Pazdur R. Next-generation sequencing in oncology in the era of precision medicine. *JAMA Oncol.* 2016;2(1):13-14.

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● ● ● HEMATOPOIESIS AND STEM CELLS

Comment on Kim et al, page 204

A role for IFN during embryonic hematopoiesis

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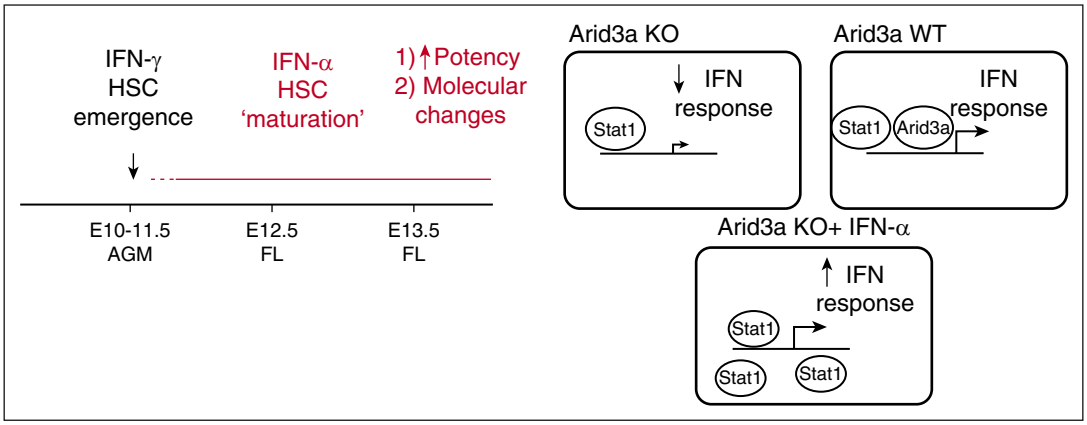
In this issue of *Blood*, Kim et al focused on the potential role of the proinflammatory cytokine interferon-α (IFN-α) in the developmental maturation of aorta-gonad-mesonephros (AGM) region hematopoietic stem cells (HSCs). They find that treatment of AGM HSCs with IFN-α increases long-term hematopoietic engraftment and donor chimerism. In addition, they identify adenine-thymine–rich interactive domain-3a (Arid3a) as an important transcriptional co-regulator of IFN-α signaling in embryonic HSCs.[1]

Traditionally, it was thought that inflammation and infection played only an indirect role in HSC biology in the bone marrow (BM) of adults. Recently, several studies have investigated the effect of proinflammatory cytokines such as interferons on HSCs and have found that HSCs can directly respond to IFN-α, IFN-β, IFN-γ, and tumor necrosis factor α (TNF-α) in vivo,[2-4] resulting in increased proliferation of these cells.

All of these studies focused on the link between inflammatory signaling and HSCs in the adult. It was only recently that focus shifted



The role of IFN-α during embryonic hematopoiesis. Although IFN-γ has been reported to play a role in promoting emergence of HSCs, Kim et al show that IFN-α promotes the next step in development of HSCs: the maturation of AGM HSCs. In this process, IFN-α signals parallel to Arid3a, with Arid3a being a transcriptional co-regulator of IFN effector genes. Therefore, when Arid3a is absent, IFN signaling in AGM HSCs is dampened. Exogenous IFN-α treatment of AGM HSCs will lead to saturation of the system with STAT1, overcoming this defect. See the complete Figure 7 in the article by Kim et al that begins on page 204.

to the role of proinflammatory cytokines during the development of the hematopoietic system. Studies that used mice and zebrafish have shown that IFN-γ signaling regulates the production of the first hematopoietic stem and progenitor cells in the AGM region in the embryo.[5,6] TNF-α signaling was also shown to play an important role in the emergence of the first HSCs in zebrafish.[7] These data have uncovered a role for inflammatory signaling in HSC production in the AGM region.

After HSCs arise in the AGM region, they migrate to the fetal liver (FL) before moving to the BM to maintain adult hematopoiesis. While progressing through the different embryonic development stages, HSCs alter molecularly and functionally. AGM HSCs, for example, have reduced repopulation capacity in adult BM transplantation compared with FL HSCs,[8] suggesting that AGM HSCs are immature compared with those that have already migrated to the FL. Little is known about the signaling pathways promoting this next step of developmental maturation of AGM HSCs.

Kim et al demonstrated an additional role for IFN signaling in the functional maturation of AGM HSCs. They performed several bioinformatics analyses on their previously published microarray data set of HSCs at different developmental stages[9] and predicted that the Jak-Stat signaling pathway, mediated by IFN-Stat1 signaling, would be low in AGM HSCs compared with FL HSCs, thus accounting for key differences between them. In addition, expression-level analysis confirmed higher levels of IFN-α in the FL. However, AGM HSCs did respond to treatment with IFN-α, despite the low levels of Jak-Stat1 signaling. In fact, pretreatment of AGM HSCs with IFN-α promoted the long-term engraftment of these HSCs, which was even more pronounced in secondary transplants. Further analysis that included limiting dilution assays showed that there is no significant difference in frequency or homing capacity of HSCs. The authors attribute the increase in engraftment to an increase in percentage of quiescent HSCs in the recipients, although the mechanism for this remains uncertain.

To find co-regulators of IFN-α during the maturation of AGM HSCs, Kim et al screened the previously published microarray of HSCs treated in vivo[2] and focused on Arid3a. Arid3a knockout (KO) mice are embryonic lethal as a result of defects in erythroid lineage differentiation.[10] They also have defects in FL HSCs; however, whether AGM HSCs were also impaired was unknown. HSCs do appear in the AGM region of Arid3a KO mice, although with lower frequency than in wild-type (WT) mice. Transplantation using Arid3a KO AGM HSCs shows lower chimerism. Surprisingly, in vivo treatment with IFN-α led to increased levels of Arid3a in adult HSCs,[2] whereas pretreatment of Arid3a KO AGM HSCs with IFN-α increased donor chimerism of KO HSCs. These data indicate parallel signaling via IFN-α and Arid3a in AGM HSCs.

Further analysis of Arid3a KO AGM HSCs revealed lower expression of IFNs and IFN receptors and downregulation of IFN target genes, all indicating deficient IFN-α signaling in these cells. The authors then used chromatin-immunoprecipitation sequencing analysis in human hematopoietic cell line K562 and identified overlap between *ARID3A* and *STAT1* binding sites, which implies that ARID3A is a transcriptional co-regulator of STAT1 and that Arid3a and IFN-α–Stat1 act in parallel pathways that converge on Stat1 (see figure).

Together, the data in the article by Kim et al show a role for IFN signaling during hematopoietic development not only at the stage of HSC emergence in the AGM region by IFN-γ and TNF-α, but also during the first maturation steps in AGM HSCs before they migrate to the FL via IFN-α. How the expression of the different IFNs is regulated during normal development and which cells are responsible for the production of IFNs at the different phases of development is likely complex and will require further investigation. In addition, the relationship between Arid3a and IFN signaling is quite complicated and needs to be explored during development as well as in adult HSCs.

Mice lacking the receptors for IFNs or TNF-α are viable, survive to adulthood, and show only limited hematopoietic defects. This indicates that even though these proinflammatory cytokines play an important role in the development of HSCs, reduced signaling does not prevent the development of the hematopoietic system. A current major challenge in this

field is to understand the regulation of inflammatory signaling in this new context, from development to adulthood.

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REFERENCES

1. Kim PG, Canver MC, Rhee C, et al. Interferon-α signaling promotes embryonic HSC maturation. *Blood.* 2016;128(2):204-216.
2. Essers MA, Offner S, Blanco-Bose WE, et al. IFNα activates dormant haematopoietic stem cells in vivo. *Nature.* 2009;458(7240):904-908.
3. Baldridge MT, King KY, Boles NC, Weksberg DC, Goodell MA. Quiescent haematopoietic stem cells are activated by IFN-γ in response to chronic infection. *Nature.* 2010;465(7299):793-797.
4. King KY, Goodell MA. Inflammatory modulation of HSCs: viewing the HSC as a foundation for the immune response. *Nat Rev Immunol.* 2011;11(10):685-692.
5. Li Y, Esain V, Teng L, et al. Inflammatory signaling regulates embryonic hematopoietic stem and progenitor cell production. *Genes Dev.* 2014;28(23):2597-2612.
6. Sawamiphak S, Kontarakis Z, Stainier DY. Interferon gamma signaling positively regulates hematopoietic stem cell emergence. *Dev Cell.* 2014;31(5):640-653.
7. Espín-Palazón R, Stachura DL, Campbell CA, et al. Proinflammatory signaling regulates hematopoietic stem cell emergence. *Cell.* 2014;159(5):1070-1085.
8. Arora N, Wenzel PL, McKinney-Freeman SL, et al. Effect of developmental stage of HSC and recipient on transplant outcomes. *Dev Cell.* 2014;29(5):621-628.
9. McKinney-Freeman S, Cahan P, Li H, et al. The transcriptional landscape of hematopoietic stem cell ontogeny. *Cell Stem Cell.* 2012;11(5):701-714.
10. Webb CF, Bryant J, Popowski M, et al. The ARID family transcription factor bright is required for both hematopoietic stem cell and B lineage development. *Mol Cell Biol.* 2011;31(5):1041-1053.

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IMMUNOBIOLOGY

Comment on Völkl et al, page 227

ALPS DNT cells: active senior living with mTOR

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In this issue of *Blood,* Völkl and colleagues report extended phenotypic and functional analysis of double-negative T cells in autoimmune lymphoproliferative syndrome (ALPS), and find that these cells not only resist cell death but also maintain increased mitotic activity dependent on mechanistic target of rapamycin (mTOR) signaling.[1]

ALPS is a condition characterized by defective apoptotic mechanisms that disrupt lymphocyte homeostasis. Failure to cull lymphocytes—particularly the characteristic TCRαβ$^+$ double-negative (CD4$^-$ CD8$^-$) T (DNT) cells—results in lymphadenopathy and hepatosplenomegaly, may lead to autoimmune disorders, and may also increase the risk of lymphoma. ALPS is most commonly associated with germline mutations in *FAS*, though a much smaller number of patients has been associated with inherited mutations in *FASLG* and *CASP10*, or somatic mutations in *FAS*. Approximately 20% of cases have no identifiable genetic abnormality.[2] The need for consensus statements with diagnostic criteria, including "definitive" or "probable" modifiers, indicates the evolving complexity of the ALPS diagnosis as an end point arrived at through many potential pathways and with variable penetrance.[3] Front-line therapy for clinically problematic lymphoproliferation has historically been corticosteroids.[2,4] Sirolimus (rapamycin), which targets the PI3K/Akt/mTOR signaling pathway,[5] has been demonstrated to have superior activity compared with other drugs in a mouse model of ALPS.[6] In a recent prospective clinical trial, treatment of ALPS patients with massive lymphoproliferation with sirolimus led not only to clinical improvement, but also to normalization of DNT cell populations.[7]

In this issue, Völkl and colleagues investigate the activity of DNT cells in ALPS. Surprisingly, they found that ALPS DNTs are not simply exhausted cells that refuse to die; these terminally differentiated cells exhibit substantial mitotic activity that is dependent on activation of Akt and mTOR. Sirolimus treatment in vivo reduced proliferation and abnormal differentiation of DNT cells. In addition, the authors identified mTOR-dependent proliferation in CD4$^+$ and CD8$^+$ DNT precursors, indicating some signs of mischief in Fas-deficient T cells, even before the DNT stage. These findings demonstrate that ALPS DNT cells are not simply accumulating in retiring senescence. Rather, ALPS DNT cells and DNT cell precursors remain active and proliferate under the influence of activated mTOR signaling (Figure 7 in Völkl et al). Although mTOR is activated in other conditions, such as *PIK3CD* gain-of-function mutations,[8] uncoupling of cell survival and proliferation appears unique to ALPS. These stubborn cells may provide an opportunity to further explore the roles of mTOR in T-lymphocyte differentiation and function.

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REFERENCES

1. Völkl S, Rensing-Ehl A, Allgäuer A, et al. Hyperactive mTOR pathway promotes lymphoproliferation and abnormal differentiation in autoimmune lymphoproliferative syndrome. *Blood.* 2016;128(2):227-238.
2. Rao VK, Oliveira JB. How I treat autoimmune lymphoproliferative syndrome. *Blood.* 2011;118(22):5741-5751.
3. Oliveira JB, Bleesing JJ, Dianzani U, et al. Revised diagnostic criteria and classification for the autoimmune lymphoproliferative syndrome (ALPS): report from the 2009 NIH International Workshop. *Blood.* 2010;116(14):e35-e40.
4. Teachey DT, Lambert MP. Diagnosis and management of autoimmune cytopenias in childhood. *Pediatr Clin North Am.* 2013;60(6):1489-1511.
5. Rao VK. Serendipity in splendid isolation: rapamycin. *Blood.* 2016;127(1):5-6.
6. Teachey DT, Obzut DA, Axsom K, et al. Rapamycin improves lymphoproliferative disease in murine autoimmune lymphoproliferative syndrome (ALPS). *Blood.* 2006;108(6):1965-1971.
7. Bride KL, Vincent T, Smith-Whitley K, et al. Sirolimus is effective in relapsed/refractory autoimmune cytopenias: results of a prospective multi-institutional trial. *Blood.* 2016;127(1):17-28.
8. Lucas CL, Kuehn HS, Zhao F, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110δ result in T cell senescence and human immunodeficiency. *Nat Immunol.* 2014;15(1):88-97.

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