genes incorporated into the platform. Patients with platelet function defects and a normal platelet count but without clues on evaluation to known entities are of specific interest. In such patients, the defects are largely unknown.

In the second study, Stritt et al combined exome sequencing, HPO coding, and phenotype similarity regression to identify a novel heterozygous variant in *DIAPH1* (p.R1213*) in 2 unrelated pedigrees with autosomal dominant MTP and early onset sensorineural hearing loss. Data were analyzed from 702 index BPD cases with unknown genetic mechanisms from the Biomedical Research Centres/Units Inherited Diseases Genetic Evaluation–BPD (BRIDGE-BPD) study[3] and 3453 control subjects. A total of 1073 genes had a rare variant in at least 2 cases and predicted to impact gene translation. The *DIAPH1* variant was not found within the 61 486 exomes in the Exome Aggregation Consortium database. Six affected pedigree members but not 3 asymptomatic members had the variant.

DIAPH1 encodes the protein that regulates cytoskeletal processes, such as actin assembly and microtubule stability. The *DIAPH1* variant predicts for truncation in the DIAPH1 diaphanous autoregulatory domain. The authors demonstrate reduced proplatelet formation from megakaryocytes and cytoskeletal alterations consistent with constitutive *DIAPH1* activation, and advance a causal link between the *DIAPH1* variant and MTP and hearing loss. To date, mutations in *MYH9* have been associated with MTP and sensorineural hearing loss. This report extends *DIAPH1* mutations to this constellation, although there were differences. Neutrophil cytoplasmic inclusions and renal failure noted in *MYH9* variants were not observed.

These 2 studies in this issue of *Blood* make important contributions to the field of platelet disorders. Since the advent of HTS, these and other studies[4–8] promise exciting times ahead in the quest to decode the secrets of nature underlying human bleeding disorders.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

REFERENCES

1. Simeoni I, Stephens JC, Hu F, et al. A high-throughput sequencing test for diagnosing inherited bleeding, thrombotic, and platelet disorders. *Blood.* 2016; 127(23):2791-2803.

2. Stritt S, Nurden P, Turro E, et al. A gain-of-function variant in *DIAPH1* causes dominant macrothrombocytopenia and hearing loss. *Blood.* 2016;127(23):2903-2914.

3. Westbury SK, Turro E, Greene D, et al; BRIDGE-BPD Consortium. Human phenotype ontology annotation and cluster analysis to unravel genetic defects in 707 cases with unexplained bleeding and platelet disorders. *Genome Med.* 2015;7(1):36.

4. Lentaigne C, Freson K, Laffan MA, Turro E, Ouwehand WH, on behalf of the BRIDGE-BPD Consortium and the ThromboGenomics Consortium. Inherited platelet disorders: toward DNA-based diagnosis. *Blood.* 2016;127(23):2814-2823.

5. Turro E, Greene D, Wijgaerts A, et al; BRIDGE-BPD Consortium. A dominant gain-of-function mutation in universal tyrosine kinase SRC causes thrombocytopenia, myelofibrosis, bleeding, and bone pathologies. *Sci Transl Med.* 2016;8(328):328ra30.

6. Leo VC, Morgan NV, Bem D, et al; UK GAPP Study Group. Use of next-generation sequencing and candidate gene analysis to identify underlying defects in patients with inherited platelet function disorders. *J Thromb Haemost.* 2015;13(4):643-650.

7. Watson SP, Lowe GC, Lordkipanidzé M, Morgan NV; GAPP Consortium. Genotyping and phenotyping of platelet function disorders [published correction appears in *J Thromb Haemost.* 2013;11(9):1790]. *J Thromb Haemost.* 2013;11(suppl 1):351-363.

8. Albers CA, Cvejic A, Favier R, et al. Exome sequencing identifies NBEAL2 as the causative gene for gray platelet syndrome. *Nat Genet.* 2011;43(8):735-737.

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HEMATOPOIESIS AND STEM CELLS

Comment on Vukovic et al, page 2841

The identity crisis of Hif-1α in HSC biology

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In this issue of *Blood*, Vukovic et al provide compelling data that hematopoietic stem cells (HSCs) do not require the transcription factor hypoxia-inducible factor 1α (Hif-1α) to duplicate themselves (ie, self-renew), reconstitute long-term hematopoiesis, or sustain hematopoiesis following injury.[1] These observations affect hematology because they challenge previous notions regarding the role of Hif-1α in HSC biology.[2]

Adult HSCs are multipotent progenitors that can sustain themselves as well as give rise to the various blood cell lineages for the lifetime of an organism.[3] Mammalian HSCs reside in a specialized microenvironment within the bone marrow (BM) called the HSC niche. HSC behaviors such as self-renewal, differentiation, or quiescence are dictated by the integration of external cues from the HSC niche through cell-intrinsic signals.[4] Though many HSC-regulating niche factors have been identified, the role of oxygen as a critical physiological factor governing HSC biology has been a recent topic of debate.

High-resolution imaging studies have shown that the BM is a low-oxygen organ[5] and that quiescent HSCs display molecular characteristics associated with hypoxia (a state of insufficient oxygen availability).[6] However, others have shown that HSCs display a molecular signature of hypoxia regardless of their surrounding oxygen availability.[7] Additional studies have shown that the BM is highly vascularized and that HSCs do not preferentially localize to low-oxygen niches[5] but rather reside close to blood vessels.[4]

Hif-1α is a transcription factor that is activated in hypoxic cells and drives transcriptional programs (eg, metabolic adaptation, angiogenesis, proliferation) that allow cells to negotiate hypoxic environments. Hif-1α is activated in mouse HSCs[2,7] and genetic ablation of *Hif-1α* diminishes the hematopoietic reconstitution abilities of mouse HSCs, suggesting that Hif-1α is an intrinsic regulator of HSC behavior.[2] Conversely, deletion of another hypoxia-inducible transcription factor, *Hif-2α*, either alone or in combination with *Hif-1α*, does not alter the ability of HSCs to reconstitute hematopoiesis.[8]

Using the same genetically engineered strain of *Hif-1α* floxed mice used in the Takubo et al[2] studies, Vukovic et al have found that deletion of *Hif-1α* in the hematopoietic system does not affect HSC survival or the ability of HSCs to reconstitute long-term, multilineage hematopoiesis. Using serial transplantation

Table: Comparison of the experimental designs between Vukovic et al and Tabuko et al

Models	Vukovic et al Hif-1α$^{fl/fl}$;Mx1-Cre and Hif-1α$^{fl/fl}$;Vav-iCre	Tabuko et al[2] Hif-1α$^{fl/fl}$;Mx1-Cre
Assessed HSC function when Hif-1α deletion occurs in both hematopoietic and nonhematopoietic tissues	No	Yes
Used serial transplantation assays to evaluate the cell-autonomous role of Hif-1α on HSC self-renewal	Yes	No
Used reconstitution assays to evaluate the hematopoietic-specific contribution of Hif-1α	Yes	Yes
Time of analysis post Hif-1a deletion	~0.5 and 6 months	~4 and 11 months
Time of analysis posttransplant	~2.5 and 8 months	~8 and 15 months
Impact of Hif-1α deletion on HSC responses to hematopoietic injury	Yes (no observed differences in survival)	Yes (observed differences in survival)
Dose of 5-FU	2 doses of 150 mg/kg	5-6 doses of 150 mg/kg
Time of first administration of 5-FU following post–Hif-1α deletion	Not specified	Not specified
Length of assays following Hif-1α deletion	~20-30 days	~50-80 days
Significant difference in survival following serial 5-FU treatment (*P* value)	No	Yes ($P < .001$)
Conclusion	Cell-intrinsic Hif-1α expression is not required for HSC survival and self-renewal	Hif-1α expression regulates HSC function

5-FU, 5-flurouracil.

assays, Vukovic et al also observed that the absence of Hif-1α does not affect HSC self-renewal and that the loss of Hif-1α expression does not significantly affect how HSCs or other hematopoietic progenitors respond to hematopoietic injury mediated by the myeloablative agent, 5-fluorouracil. The reports put forth by Vukovic et al and Guitart et al[8] establish a convincing argument that both Hif-1α and Hif-2α are not essential cell-intrinsic regulators of HSC function. However, the reasons the results of these studies conflict with those of Takubo et al[2] may potentially be due to subtle differences in study design between the 2 reports (see table).

Although both groups used the *Mx1-Cre* transgene to induce *Hif-1α* gene ablation (*Hif-1α$^{fl/fl}$;Mx1-Cre* mice) in the hematopoietic system, the time and specific location of *Hif-1α* deletion significantly varied between the 2 studies. Although *Mx1-Cre* efficiently recombines floxed alleles in the hematopoietic system, it is well-documented that it is expressed in components of the BM microenvironment[9] and sites of extramedullary hematopoiesis.[1] To circumvent the nonhematopoietic effects of the *Mx1-Cre* transgene, Vukovic et al transplanted whole BM cells from *Hif-1α$^{fl/fl}$;Mx1-Cre* mice into wild-type recipients and then induced *Hif-1α* deletion, thus selectively deleting *Hif-1α* in the hematopoietic system. In contrast, the vast majority of the HSC analyses carried out by Takubo et al[2] were performed in a context in which *Hif-1α* deletion was not restricted to the hematopoietic system.

In the subset of experiments in which Takubo et al[2] did assess the HSC-autonomous role of Hif-1α, they observed that the loss of Hif-1α expression enhanced multilineage reconstitution at 4 months postinduction of gene deletion. However, follow-up analysis at 11 months post-*Hif-1α* deletion (15 months posttransplant) revealed that the absence of Hif-1α significantly reduced multilineage reconstitution. These timelines differ from the HSC-autonomous studies conducted by Vukovic et al, who assessed multilineage reconstitution at 2 and 32 weeks following *Hif-1α* deletion (10 and 40 weeks posttransplant). Interestingly, studies evaluating the impact of an aging niche on HSC behavior[3,10] have shown that the HSC function is differentially affected by young and old niches, raising the possibility that Hif-1α may influence the behavior of HSCs exposed to aged niches.

Last, although Takubo et al[2] observed that unrestricted deletion of *Hif-1α* in both the hematopoietic system and the BM microenvironment reduces the frequency of functional HSCs in the BM, they also observed that the spleens of *Hif-1α*–deleted mice harbored significantly more functional HSCs than control mice. These observations are of particular interest when considering the well-recognized phenomena that extramedullary hematopoiesis often occurs in the presence of faulty BM microenvironment support.

The results of Vukovic et al have convincingly shown that Hif-1α is not an essential cell intrinsic regulator of relatively young HSC function. However, further studies are needed to determine whether *Hif-1α* deletion in nonhematopoietic BM microenvironment cell populations influences HSC behavior as well as if intrinsic Hif-1α expression is needed when HSCs are exposed to an aged niche. Determining how HSCs interact with their microenvironment and regulate their cell intrinsic status is critical to understanding the etiology of blood malignancies as well as to maximizing the application of HSCs in regenerative and transplantation medicine.

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

REFERENCES

1. Vukovic M, Sepulveda C, Subramani C, et al. Adult hematopoietic stem cells lacking Hif-1α self-renew normally. *Blood.* 2016;127(23):2841-2846.

2. Takubo K, Goda N, Yamada W, et al. Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. *Cell Stem Cell.* 2010;7(3):391-402.

3. Sykes SM, Scadden DT. Modeling human hematopoietic stem cell biology in the mouse. *Semin Hematol.* 2013;50(2):92-100.

4. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. *Nature.* 2014;505(7483):327-334.

5. Spencer JA, Ferraro F, Roussakis E, et al. Direct measurement of local oxygen concentration in the bone marrow of live animals. *Nature.* 2014;508(7495):269-273.

6. Parmar K, Mauch P, Vergilio JA, Sackstein R, Down JD. Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. *Proc Natl Acad Sci USA.* 2007;104(13):5431-5436.

7. Nombela-Arrieta C, Pivarnik G, Winkel B, et al. Quantitative imaging of haematopoietic stem and progenitor cell localization and hypoxic status in the bone marrow microenvironment. *Nat Cell Biol.* 2013;15(5):533-543.

8. Guitart AV, Subramani C, Armesilla-Diaz A, et al. Hif-2α is not essential for cell-autonomous hematopoietic stem cell maintenance. *Blood.* 2013;122(10):1741-1745.

9. Park D, Spencer JA, Koh BI, et al. Endogenous bone marrow MSCs are dynamic, fate-restricted participants in bone maintenance and regeneration. *Cell Stem Cell.* 2012; 10(3):259-272.

10. Vas V, Senger K, Dörr K, Niebel A, Geiger H. Aging of the microenvironment influences clonality in hematopoiesis. *PLoS One.* 2012;7(8):e42080.

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LYMPHOID NEOPLASIA

Comment on Dietrich et al, page 2847

BRAF inhibitor: targeted therapy in hairy cell leukemia

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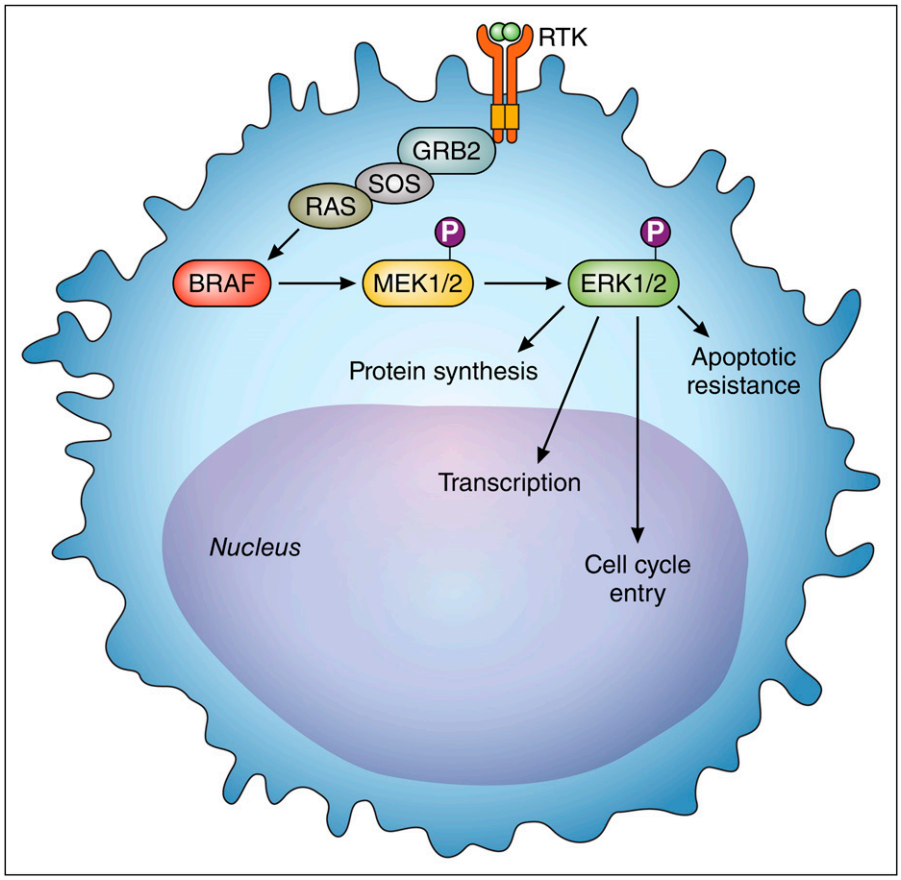
In this issue of *Blood*, Dietrich et al report that the low-dose BRAF inhibitor, vemurafenib, is highly effective in refractory hairy cell leukemia.[1]



The activated BRAF pathway provides oncogenic signaling to the leukemic hairy cell through the MEK-ERK cascade. Vemurafenib is an inhibitor of BRAF in this pathway. The activity of pERK is reduced as a consequence of BRAF inhibition. The reduced pERK results in decreased cell proliferation. This is measured by immunohistochemical analysis and is used as a pharmacodynamics biomarker of BRAF inhibition. P, indicates a phospho-group; RTK, receptor tyrosine kinase. Professional illustration by Patrick Lane, ScEYEnce Studios.

Despite enormous progress that has been made with purine nucleoside analogs as the initial treatment of patients with hairy cell leukemia, the relapse rate and eventual development of refractory disease mandate the continued search for effective new therapies. The discovery of the presence of the BRAF V600E mutation in the overwhelming majority of patients with classic hairy cell leukemia prompted the logical application of inhibitors of this oncogene for treating patients with relapsed or refractory disease.[2] Extensive work had previously been done to define a dose and schedule of vemurafenib for the treatment of malignant melanoma. Encouraging results indicated that this agent produced responses in patients with melanoma, but relapse and resistance were frequently encountered.[3,4] Likewise, studies using the same dose and schedule of vemurafenib in patients with hairy cell leukemia have recently shown that responses are observed, but unfortunately relapses are also routinely encountered.[5] Simply extrapolating the dose and schedule of this agent based on treatment plans established for patients with malignant melanoma underestimates the need to design specific therapeutic intervention based on the biology of the target in the patient with leukemia.

The current report provides important information on the dose-response relationship of vemurafenib in patients with refractory hairy cell leukemia, showing that the response rate and kinetics of response are independent of dosing. Furthermore, Dietrich et al[1] show that abrogation of phosphorylation of extracellular signal-related kinase (ERK) as a downstream target was consistently observed with low-dose administration. Identification of a genomic target in patients with hairy cell leukemia with a potential pharmacodynamics end point enables a rational approach to optimizing treatment. In the figure, the BRAFV600E pathway results in the expression of phospho-ERK (pERK). In recurrent or resistant melanoma, it is postulated that the reexpression of pERK and abnormalities in the MAPK pathway may be involved in the pathogenesis of progressive disease.[6] This pharmacodynamics end point will need to be further validated in other studies of hairy cell leukemia. Investigation of this oncogenic pathway will hopefully provide insight into new therapeutic strategies for treatment. Alternatively, other modalities may be incorporated into combination approaches to