cumulative incidence of CNS relapse of 1.6% treated with R-ACVBP vs 4% following R-CHOP, R-CHOEP, or doseescalated R-CHOEP (R-MegaCHOEP).<sup>9</sup> Although the difference was not significant, the low rate of CNS relapse after R-ACVBP may suggest that the combination of aggressive first-line therapy, IT MTX, and systemic administration of multiple drugs crossing the bloodbrain barrier may reduce the incidence of CNS relapse.

Although patients with low or intermediate CNS-IPI can be spared CNS prophylaxis because of the low relapse rates reported, CNS imaging and fluorescenceactivated cell sorter analysis of the cerebrospinal fluid will identify CNS involvement at diagnosis in some patients with high CNS-IPI. Patients with high CNS-IPI but no evidence of CNS involvement by modern screening technologies remain prime candidates for CNS prophylaxis. In light of the devastating prognosis of CNS relapse, and before results of studies involving alternative molecules (lenalidomide, Bruton tyrosine kinase, or BCL2 inhibitors) become available, this small group of patients (<<10% of DLBCL patients) may benefit from the combination of aggressive first-line therapy, IT MTX, and systemic administration of multiple drugs crossing the blood-brain barrier.

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

## REFERENCES

- Orellana-Noia VM, Reed DR, McCook AA, et al. Single-route CNS prophylaxis for aggressive non-Hodgkin lymphomas: realworld outcomes from 21 US academic institutions. *Blood.* 2022;139(3):413-423.
- NCCN Clinical Practices Guidelines in Oncology (NCCN Guidelines), B-cell lymphomas. 2021;Version 4.2021.
- Schmitz N, Zeynalova S, Nickelsen M, et al. CNS International Prognostic Index: a risk model for CNS relapse in patients with diffuse large B-cell lymphoma treated with R-CHOP. J Clin Oncol. 2016;34(26): 3150-3156.
- Klanova M, Sehn LH, Bence-Bruckler I, et al. Integration of cell of origin into the clinical CNS International Prognostic Index improves CNS relapse prediction in DLBCL. *Blood*. 2019;133(9):919-926.
- Ollila TA, Kurt H, Waroich J, et al. Genomic subtypes may predict the risk of central nervous system recurrence in diffuse large B-cell lymphoma. *Blood.* 2021;137(8): 1120-1124.

- Boehme V, Schmitz N, Zeynalova S, Loeffler M, Pfreundschuh M. CNS events in elderly patients with aggressive lymphoma treated with modern chemotherapy (CHOP-14) with or without rituximab: an analysis of patients treated in the RICOVER-60 trial of the German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL). *Blood.* 2009;113(17): 3896-3902.
- Boehme V, Zeynalova S, Kloess M, et al. Incidence and risk factors of central nervous system recurrence in aggressive lymphoma – a survey of 1693 patients treated in protocols of the German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL). Ann Oncol. 2007;18(1):149-157.

## MYELOID NEOPLASIA

Comment on Davis et al, page 424

- for central nervous system relapse in patients with diffuse large B-cell lymphoma: the impact of the addition of rituximab to CHOP chemotherapy. Ann Oncol. 2010;21(5):1046-1052.
  9. Thieblemont C, Altmann B, Casasnovas O, et
  - al. CNS relapse in DLBCL patients below 60 years treated with R-ACVBP, R-CHOEP, or R-CHOP: a joint analysis of LYSA and GLA/ DSHNHL [abstract]. J Clin Oncol. 2021;39 (15 suppl). Abstract 7543.

8. Villa D, Connors JM, Shenkier TN, Gascoyne RD,

Sehn LH, Savage KJ, Incidence and risk factors

DOI 10.1182/blood.2021014043

© 2022 by The American Society of Hematology

Driving differentiation: targeting APA in AML

**Pia Sommerkamp<sup>1</sup> and Andreas Trumpp<sup>2</sup>** | <sup>1</sup>German Cancer Research Center; <sup>2</sup>Heidelberg Institute for Stem Cell Technology and Experimental Medicine

In this issue of *Blood*, Davis et al show that the posttranscriptional alternative polyadenylation (APA) process contributes to acute myeloid leukemia (AML) pathogenesis and that targeting the APA regulator FIP1L1 in AML cell lines promotes differentiation.<sup>1</sup> The authors quantify changes in APA patterns by performing 3' untranslated region (UTR) extraction and deep sequencing (3' READS) (see figure) on samples from patients with primary AML and on hematopoietic stem and progenitor cells (HSPCs) from healthy donors. Mechanistic analysis suggests that targeting APA promotes differentiation of AML cells.

More than 70% of all genes harbor more than 1 polyadenylation site, and differential usage of these sites is termed APA.<sup>2</sup> Depending on the exact localizations of the different polyadenylation sites, this process can affect the 3' UTR as well as the coding DNA sequence (CDS). APA can influence RNA stability, RNA output, protein localization, and protein isoform expression. Occurring within the 3' UTR, APA determines the length of the UTR, which directly affects the presence or absence of binding motifs and regulatory sequences. In contrast, differential usage of polyadenylation sites within exons, introns, and alternative 3' UTRs leads to changes in the CDS of the transcript.

Large-scale sequencing of transcriptomes in different cancer entities and the application of novel 3' sequencing methods have revealed that changes in APA are a common feature of malignant transformation.<sup>3</sup> Although multiple studies have dealt with the prevalence of APA in solid tumors, few reports have focused on hematologic diseases and leukemia. Recently, APA was studied in healthy murine HSPCs and samples from patients with primary acute lymphoblastic leukemia, chronic lymphocytic leukemia, and AML.<sup>4-7</sup> These studies suggest that APA plays a key role in the development of hematologic diseases. To date, however, mechanistic insights into how APA is involved in leukemia pathogenesis have remained elusive, which has also limited the potential for therapeutic exploitation. The study by Davis et al serves as a resource for APA patterns in samples from patients with AML and from HSPCs from healthy individuals, provides a comprehensive mechanistic analysis of the signaling pathways deregulated by APA in AML, and introduces APA as a putative target for AML therapy.

Davis et al performed 3' READS on primary CD34 $^+$  AML blasts from patients



Downloaded from http://ashpublications.net/blood/article-pdf/139/3/317/1861285/bloodbld2021013814c.pdf by guest on 18 May 2024

3' READS was performed on human AML samples with and without t(8;21) that were enriched for CD34<sup>+</sup> cells and on healthy CD34<sup>+</sup> HSPCs to define global APA profiles. In AML samples, shortening of 3' UTRs and lengthening of CDSs was detected. Mechanistic analysis revealed FIP1L1 as a major regulator of APA in AML that mediates global 3' UTR shortening. In AML cells with t(8;21), this leads to increased levels of AML1-ETO, whereas in AML cells without t(8;21), mTORC1 and MYC levels are increased. This mechanism contributes to the characteristic block of AML cell differentiation. Knockdown of FIP1L1 reversed the characteristic APA patterns and induced differentiation of AML cells. dPAS, distal poly(A) site; pPAS, proximal poly(A) site.

with or without a t(8;21) translocation, which leads to the generation of an AML1-ETO fusion oncoprotein, using healthy CD34<sup>+</sup> HSPCs as controls. Comparison of the data sets revealed global shortening of 3' UTRs and lengthening of CDSs in samples from patients with AML. The genes affected by APA are involved in processes such as cell cycle, differentiation, and oncogenic signaling, and they include targets known for their role in blocking AML cell differentiation. Thus, several specific genes are deregulated by APA in AML and thereby potentially contribute to AML pathogenesis. Davis et al identified FIP1L1 as a global regulator of APA in AML. FIP1L1 is part of the polyadenylation machinery, and it promotes the use of proximal polyadenylation sites to maintain globally short 3' UTRs.<sup>8</sup> By using t(8;21) Kasumi cells as a model system, the authors demonstrated that knockdown of *FIP1L1* reverses the AML APA phenotype, leading to global lengthening of 3' UTRs and shortening of CDSs and accompanied by leukemic cell differentiation. This seminal result reveals that targeting of APA regulators can induce differentiation of AML cells. Mechanistically, *FIP1L1* knockdown induces 3' UTR lengthening of the AML1-ETO fusion transcript, which results in lower protein levels. Knockdown of *FIP1L1* in AML cell lines without t(8;21) leads to attenuation of mTORC1 signaling and reduced levels of MYC protein, ultimately promoting differentiation and pointing to a broader role of *FIP1L1* in regulating APA in AML.

The results by Davis et al are pivotal and provide a solid resource data set that can be exploited to identify additional targets and modulators of APA in AML. Of particular interest are the potential clinical applications. All-trans retinoic acid and arsenic trioxide have successfully been used in patients with acute promyelocytic leukemia, and isocitrate dehydrogenase (IDH) inhibitors have been used in IDH-mutant AML as prodifferentiation therapies. In the Davis et al study, targeting of APA regulators such as FIP1L1 is presented as an additional way to induce differentiation of AML cells. For clinical applications, the use of FIP1L1 inhibitors is conceivable. However, additional studies must be conducted to analyze potential adverse effects on healthy HSPCs. In the future, direct targeting of global APA patterns could represent an alternative to targeting APA regulators, and it might be beneficial in treating leukemia and other cancer entities. Different small molecules and morpholino oligomers have recently been shown to transiently regulate APA profiles, which opens new avenues for therapeutic uses.<sup>9,10</sup>

Overall, the study by Davis et al shows how APA deregulation can make AML cells more aggressive and immature by blocking their differentiation. Additional studies on APA in primary samples from other AML subtypes and leukemias may contribute to a comprehensive understanding of the functional role of APA in these cancers. It will be important to analyze bulk samples, to compare APA between leukemic stem cell and non-stem cell populations, and even perform single-cell resolution analysis. Because previous studies have shown both quantitative and qualitative changes in APA within differentiation hierarchies, these processes might also be differentially regulated between leukemic stem cell and blast fractions and could represent a druggable vulnerability that merits further investigation.

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

# REFERENCES

- Davis AG, Johnson DT, Zheng D, et al. Alternative polyadenylation dysregulation contributes to the differentiation block of acute myeloid leukemia. *Blood.* 2022;139(3): 424-438.
- Sommerkamp P, Cabezas-Wallscheid N, Trumpp A. Alternative polyadenylation in stem cell self-renewal and differentiation. *Trends Mol Med.* 2021;27(7):660-672.
- Gruber AJ, Zavolan M. Alternative cleavage and polyadenylation in health and disease. Nat Rev Genet. 2019;20(10):599-614.
- Sommerkamp P, Altamura S, Renders S, et al. Differential alternative polyadenylation landscapes mediate hematopoietic stem cell activation and regulate glutamine metabolism. *Cell Stem Cell.* 2020;26(5):722-738.e7.
- Nordlund J, Kiialainen A, Karlberg O, et al. Digital gene expression profiling of primary acute lymphoblastic leukemia cells. *Leukemia*. 2012;26(6):1218-1227.
- 6. Lee SH, Singh I, Tisdale S, Abdel-Wahab O, Leslie CS, Mayr C. Widespread intronic

polyadenylation inactivates tumour suppressor genes in leukaemia. *Nature*. 2018;561(7721):127-131.

- Ye C, Zhou Q, Hong Y, Li QQ. Role of alternative polyadenylation dynamics in acute myeloid leukaemia at single-cell resolution. RNA Biol. 2019;16(6):785-797.
- Lackford B, Yao C, Charles GM, et al. Fip1 regulates mRNA alternative polyadenylation to promote stem cell self-renewal. *EMBO J*. 2014;33(8):878-889.
- Araki S, Nakayama Y, Sano O, et al. Decoding transcriptome dynamics of genome-encoded polyadenylation and autoregulation with small-molecule modulators of alternative polyadenylation. *Cell Chem Biol.* 2018;25(12):1470-1484.e5.
- Vorlová S, Rocco G, Lefave CV, et al. Induction of antagonistic soluble decoy receptor tyrosine kinases by intronic polyA activation. Mol Cell. 2011;43(6):927-939.

DOI 10.1182/blood.2021013814

© 2022 by The American Society of Hematology

# RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Coffey et al, page 439

# Erythroferrone in iron regulation and beyond

Jodie L. Babitt | Massachusetts General Hospital–Harvard Medical School

In this issue of *Blood*, Coffey et al<sup>1</sup> generate a new mouse model overexpressing the erythroid hepcidin regulator erythroferrone (ERFE) to demonstrate that increased ERFE levels have multifactorial effects, not only to suppress hepcidin and load iron, but also to impact the development and homeostasis of several organ systems.

How does the body ensure enough iron is available for erythropoiesis? In 2014, ERFE was discovered as a signal that communicates the iron demand of erythroid cells in the bone marrow to the liver and other organs to regulate the iron supply.<sup>2</sup> Produced and secreted by erythroblasts in response to erythropoietin, ERFE acts on the liver to suppress production of the iron regulatory hormone hepcidin.<sup>2</sup> In the absence of its ligand hepcidin, the iron exporter ferroportin is stabilized on the surface of duodenal enterocytes, macrophages, and hepatocytes, thereby increasing iron release into circulation from dietary sources and body stores.<sup>3</sup> This pathway is not only induced as a physiologic response to anemia but also as a pathophysiologic response to ineffective erythropoiesis, which contributes to dietary iron hyperabsorption and iron overload in  $\beta$ -thalassemia.<sup>4</sup> Recently, ERFE was proposed to suppress liver hepcidin production by acting as a ligand trap to sequester bone morphogenetic proteins (BMPs), key signaling molecules responsible for regulating hepcidin expression via SMAD transcription factors.<sup>5,6</sup> However, the physiologic functions of BMPs extend well beyond hepcidin regulation.<sup>7</sup> This raises the possibility that chronic overproduction of ERFE, as seen in  $\beta$ -thalassemia and other disorders of ineffective erythropoiesis, could contribute to other pathologies beyond iron overload.

In the present study, Coffey et al generate several transgenic mouse lines with graded overexpression of *Erfe* in erythroid cells to gain a better understanding of ERFE function independent of any effects of anemia or erythropoietin. As