in identifying high-risk patients. Interestingly, the dFL subtype showed a near 3-fold enrichment of grade 3A FL cases. This finding suggests value in the traditional histologic grading system based on centroblasts per high-power field and supports a potential difference in the mutation patterns among FL grades.

Other large-scale sequencing studies in FL have also recently been published.[5] Crouch et al used a panel of nearly 300 genes and reported 3 genetic subtypes that differed significantly with regard to prevalence of aSHM, but the subtypes did not correlate to FLIPI or histologic transformation. However, differences in aSHM were observed between subgroups and *CREBBP* mutations clustered with *STAT6* and *TNFRSF14* mutations, both of which trended toward significance in the cFL subtype reported by Dreval et al. Crouch et al conclude that the genetic subtypes they found had limited prognostic clinical significance. How then do we reconcile these different conclusions? First, the approaches were different. Crouch et al used panel deep sequencing (~500×) of formalin-fixed, paraffin-embedded FL biopsies, whereas Dreval et al used whole genome sequencing (~50×) with paired normal from newly sequenced and historic cohorts of FL and DLBCL. Thus, the patient populations, controls, and genomic features detected for the 2 models were different. Second, of the overlapping genetic features, *CREBBP* mutations were all treated similarly by Crouch et al, whereas Dreval et al distinguished missense mutations within the KAT domain from other mutations. This small but important distinction appears to be a critical nuance in predicting transformation of FL. The distinction of domain-specific genetic features is also critical for predicting genetic subtypes of DLBCL.[2] However, like the FL predictor, the full complement of genetic features is required to achieve maximum sensitivity, specificity, and accuracy. Future studies with large replication cohorts will be needed to establish the minimum set of genetic alterations required to appropriately predict genetic subtypes of lymphoma.

As machine learning approaches continue to gain traction and offer new insights into the heterogeneity of FL, it will be critical to thoroughly annotate the research cohorts being studied. Roughly 20% of transformed FL cases are of the activated B-cell (ABC) type by gene expression and have a lower incidence of *BCL2* rearrangement, compared with GCB cases.[4] Prior studies also have identified subsets of FL that more closely resemble ABC-DLBCL, with high expression of interferon regulatory factor 4 (IRF4) and low expression of CD10; high expression of IRF4 is a risk for early transformation.[4,9] Notably, such cases are generally negative for the *BCL2* translocation and should be distinguished from conventional FL. Recognizing that FL is >1 disease, we must ensure that future models take this heterogeneity into consideration.[10]

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

REFERENCES

1. Dreval K, Hilton LK, Cruz M, et al. Genetic subdivisions of follicular lymphoma defined by distinct coding and noncoding mutation patterns. *Blood*. 2023;142(6):561-573.
2. Wright GW, Huang DW, Phelan JD, et al. A probabilistic classification tool for genetic subtypes of diffuse large B cell lymphoma with therapeutic implications. *Cancer Cell*. 2020;37(4):551-568.e14.
3. Garcia-Ramirez I, Tadros S, Gonzalez-Herrero I, et al. Crebbp loss cooperates with Bcl2 overexpression to promote lymphoma in mice. *Blood*. 2017;129(19):2645-2656.
4. Kridel R, Mottok A, Farinha P, et al. Cell of origin of transformed follicular lymphoma. *Blood*. 2015;126(18):2118-2127.
5. Crouch S, Painter D, Barrans SL, et al. Molecular subclusters of follicular lymphoma: a report from the United Kingdom's Haematological Malignancy Research Network. *Blood Adv*. 2022;6(21):5716-5731.
6. Solal-Celigny P, Roy P, Colombat P, et al. Follicular lymphoma international prognostic index. *Blood*. 2004;104(5):1258-1265.
7. Pastore A, Jurinovic V, Kridel R, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. *Lancet Oncol*. 2015;16(9):1111-1122.
8. Casulo C, Byrtek M, Dawson KL, et al. Early relapse of follicular lymphoma after rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone defines patients at high risk for death: an analysis from the National LymphoCare Study. *J Clin Oncol*. 2015;33(23):2516-2522.
9. Karube K, Guo Y, Suzumiya J, et al. CD10-MUM1+ follicular lymphoma lacks BCL2 gene translocation and shows characteristic biologic and clinical features. *Blood*. 2007;109(7):3076-3079.
10. Campo E, Jaffe ES, Cook JR, et al. The international consensus classification of mature lymphoid neoplasms: a report from the Clinical Advisory Committee. *Blood*. 2022;140(11):1229-1253.

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MYELOID NEOPLASIA

Comment on Qiu et al, page 574

Food for thought (and CML survival)

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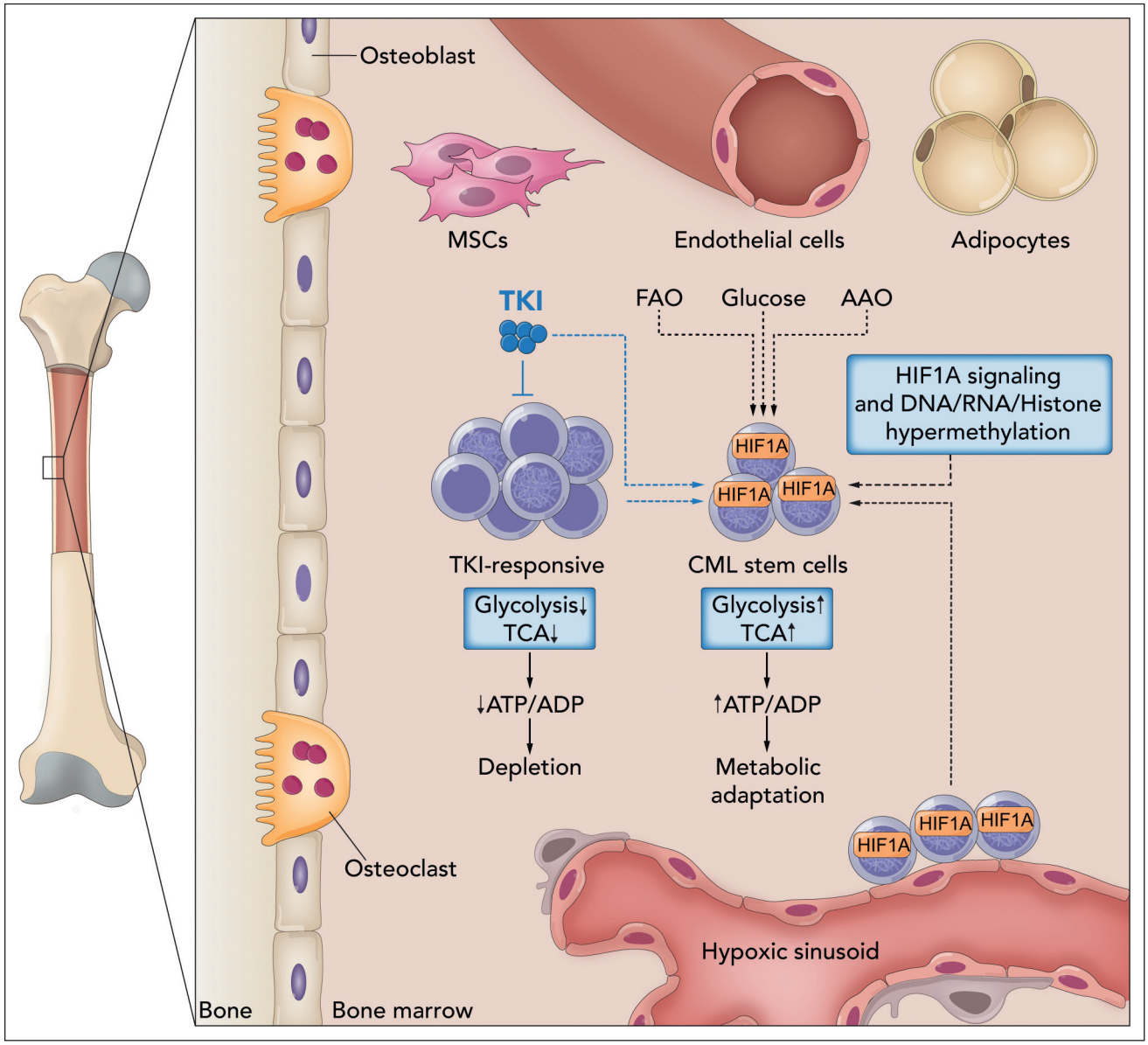
In this issue of *Blood*, Qiu et al[1] shed light on the metabolic changes that arise in a population of chronic myelogenous leukemia (CML) stem cells in a mouse model that allow them to better adapt to continued tyrosine kinase inhibitor (TKI) treatment through HIF1A activity.

CML is a myeloproliferative disease, characterized by leukocytosis and uncontrolled proliferation of granulocytes. The hallmark of CML is the Philadelphia chromosome, in which a reciprocal translocation of t(9;22) results in the BCR-ABL fusion gene and constitutively active tyrosine kinase activity of the resulting oncoprotein. The incorporation of small-molecule TKIs as the first-line treatment for CML has revolutionized prognosis for patients, elevating median survival from a dismal 3 to 5 years from initial diagnosis to that of the general population.[2] Even so, cases of TKI monotherapies unable to completely eradicate CML persist; imatinib reports molecular relapse in 30% to 60% of patients, and 25% of patients have TKI intolerance.[3,4]



TKI-persistent CML stem cells upregulate glycolysis and TCA cycle-related signatures and metabolically adapt via HIF1A activity. Other intrinsic and extrinsic pathways that can be potentially targeted (dotted lines) to block either the source of metabolic adaptation (ie, from the BM microenvironment [FAO, AAO]) or HIF1A signaling/stabilization (via hypoxia) and DNA, RNA, and histone hypermethylation are depicted. AAO, amino acid oxidation; ADP, adenosine diphosphate; ATP, adenosine triphosphate; FAO, fatty acid oxidation; MSC, mesenchymal stromal cell. Professional illustration by Somersault18:24.

This has been attributed to a population of residual leukemic stem cells within CML that functionally share similarities with normal hematopoietic stem cells (HSCs). They are a self-renewing and quiescent population, exhibiting engraftment potential when serially transplanted, and their inert status is thought to contribute to the inadequate clearance of CML and TKI resistance.[5] The exact details, however, remain elusive. Lifelong therapy imposed on patients with CML undoubtedly calls for better treatment options. In this study, Qiu et al address the metabolic basis for TKI resistance in CML and leave ample room for further speculation on potential intrinsic and extrinsic pathways of therapeutic intervention.

The authors first identified key metabolic changes that occur upon TKI treatment using the double transgenic tetracycline-inducible CML mouse model under the control of the murine stem cell leukemia gene, SCL-tTA/BCR-ABL. After 2 days of treatment with nilotinib, their bioenergetics analysis consistently pointed toward reduced rates of glycolysis and oxidative phosphorylation (OXPHOS) in CML c-Kit$^+$ progenitor cells compared with vehicle-treated controls. This was reversed, however, after 2 weeks of treatment, where metabolites of the glycolytic and glutaminolytic cycles and trichloroacetic acid (TCA) intermediates bounced back in CML progenitor cells to levels similar to controls. The authors concluded that CML cells adapt to continued TKI exposure by restoring energy metabolism. To understand the basis for their observations, cells labeled

with [U-$^{13}C_6$]-glucose were tracked for their glycolytic end products. The authors found reduced glycolytic (pyruvate, alanine) and TCA cycle intermediates (α-ketoglutarate, fumarate, malate) in CML progenitor cells after 2 days of nilotinib treatment, whereas cells from mice treated for 2 weeks showed increases in lactate and restored glycolytic flux and TCA cycle activity. [U-$^{15}C_5$]-glutamine labeling confirmed increase use of glucose carbons for amino acid synthesis.

Single-cell RNA sequencing of untreated Lin$^-$Sca-1$^+$c-Kit$^+$ BM cells showed clustering of quiescent HSC (qHSC) populations with reduced OXPHOS, glycolysis, and nucleotide metabolism and enhanced quiescence gene signatures. These cell populations were enriched after 2 weeks of TKI treatment together with partial restoration of OXPHOS, MYC, and E2 transcription factor following the initial slight reduction observed after 2 days of treatment. Among the metabolic genes upregulated in qHSCs were classical HIF1A targets. Using pySCENIC, a computational method for gene regulatory network (regulon) reconstruction, the authors found significant increase of HIF1A regulon in qHSCs, among various others involved in metabolic regulation. This prompted the authors to investigate the role of HIF1A in maintaining TKI-persistent cells. Combination therapy of nilotinib with the HIF1A inhibitor, echinomycin significantly reduced CML stem and progenitor cells compared with nilotinib treatment alone. This potency was also confirmed for human CML cells, whereby CML CD34$^+$ xenografts in immunodeficient NOD/RAG1$^{-/-}$IL2Rγ$^{-/-}$ mice expressing human IL3, CSF2, and KITLG showed reduced CD34$^+$CD38$^-$CD90$^+$ stem cells upon combined nilotinib and echinomycin treatment, validating a critical role of HIF1A activity in maintaining TKI-persistent CML stem cells.

Here, the authors identify HIF1A activity as a key adaptive mechanism that can be targeted to eliminate TKI-persistent CML stem cells, but targeting HIF1A can essentially mean intervention on many other levels, both intrinsic and extrinsic, given its multifaceted role in various physiological pathways (ie, cell proliferation and survival, angiogenesis, innate and adaptive immune activation). The importance of HIF1A activity on stem cell regulation has been established before by Prost et al, who showed that combination therapy of imatinib with the peroxisome proliferator-activated receptor-γ (PPARγ) agonist glitazone effectively eroded CML stem cells by decreasing STAT5 and its downstream target HIF2A.[6] Furthermore, HIF1A can be stabilized by hypoxia and oxygen-independent pseudohypoxia, both of which are highly relevant for cancer pathobiology.[7] Regarding the latter, pseudohypoxia-mediated HIF1A dysregulation alone was found to be both essential and sufficient for the pathology of myelodysplastic syndromes.[8] Of note, hypoxia can also alter epigenetic networks in tumor cells. CML stem cells appear to present a unique pattern of epigenetic hypermethylation, such as upregulating proliferation and antiapoptotic oncogenes via N^6-methyladenosine as a way to prevent TKI-mediated killing.[9] In addition, the possible contribution of the bone marrow (BM) microenvironment toward CML persistence should be noted. The authors show upregulation of fatty acid and amino acid oxidation in CML cells, which implies the utilization of other fuel sources besides glucose to supplement their energy demands (ie, from the BM). BM adipocytes could be an ample source of fatty acids for CML stem cells to fuel OXPHOS, as previously reported for acute lymphoblastic leukemia.[10] Alternatively, CML stem cells could actively seek hypoxic surroundings such as the BM sinusoidal network to stabilize HIF1A and thereby promote stem cell function (see figure).

Adaptation is an essential program for survival, whether through intrinsic or extrinsic means. Here, CML stem cells "step up" their metabolism to fend off TKI toxicity. In summary, Qiu et al establish a critical link between HIF1A signaling and metabolic status that can be further explored to search for alternative intrinsic and extrinsic avenues for CML stem cell eradication and treatment-free remission.

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REFERENCES

1. Qiu S, Sheth V, Yan C, et al. Metabolic adaptation to tyrosine kinase inhibition in leukemia stem cells. *Blood*. 2023;142(6):574-588.
2. Brunner AM, Campigotto F, Sadrzadeh H, et al. Trends in all-cause mortality among patients with chronic myeloid leukemia: a Surveillance, Epidemiology, and End Results database analysis. *Cancer*. 2013;119(14):2620-2629.
3. Hayashi Y, Yokota A, Harada H, Huang G. Hypoxia/pseudohypoxia-mediated activation of hypoxia-inducible factor-1α in cancer. *Cancer Sci*. 2019;110(5):1510-1517.
4. Hayashi Y, Zhang Y, Yokota A, et al. Pathobiological pseudohypoxia as a putative mechanism underlying myelodysplastic syndromes. *Cancer Discov*. 2018;8(11):1438-1457.
5. Hochhaus A, Baccarani M, Silver RT, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. *Leukemia*. 2020;34(4):966-984.
6. Ito K, Ito K. Leukemia stem cells as a potential target to achieve therapy-free remission in chronic myeloid leukemia. *Cancers*. 2021;13(22):5822.
7. Prost S, Relouzat F, Spentchian M, et al. Erosion of the chronic myeloid leukaemia stem cell pool by PPARγ agonists. *Nature*. 2015;525(7569):380-383.
8. Saussele S, Richter J, Guilhot J, et al. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. *Lancet Oncol*. 2018;19(6):747-757.
9. Tucci J, Chen T, Margulis K, et al. Adipocytes provide fatty acids to acute lymphoblastic leukemia cells. *Front Oncol*. 2021;11:665763.
10. Yan F, Al-Kali A, Zhang Z, et al. A dynamic N(6)-methyladenosine methylome regulates intrinsic and acquired resistance to tyrosine kinase inhibitors. *Cell Res*. 2018;28(11):1062-1076.

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