responsive to ibrutinib often develop resistance with disease progression and a poor prognosis.[5] Therefore, targeting this resistance is an area of active research. The reasons for resistance are multifaceted and include mutations, activation of signaling pathways, changes in the tumor microenvironment, and metabolic changes. OXPHOS is an active metabolic pathway in many solid tumors and lymphomas,[6] and several studies identified a shift toward OXPHOS metabolism being associated with ibrutinib resistance in MCL and ABC-DLBCL.[7-9]

Liu et al found that EGR1 protein levels are upregulated in ibrutinib-resistant ABC-DLBCL and MCL cell lines and even further upregulated when these resistant cell lines are exposed to ibrutinib. The latter was also seen in samples from patients with MCL upon ibrutinib treatment. Ibrutinib not only induced EGR1 expression, but further overexpression and knockdown experiments revealed that high EGR1 levels also contribute to ibrutinib resistance in ABC-DLBCL cell lines. The mechanism behind the EGR1 overexpression is EGR1 self-regulation by binding to its own promoter region. *EGR1* is a target of transcription factor 4 (TCF4), a known epigenetic driver for ibrutinib resistance. As previously described, metabolic reprogramming toward OXPHOS dependency is characteristic of ibrutinib resistance in MCL,[8,9] and Lui et al extended these findings to their ABC-DLBCL cell lines and saw augmented mitochondrial respiration. This is caused by increased PDP1 (a target of EGR1) expression via transcriptional activation, leading to enhanced pyruvate dehydrogenase activity and accelerated adenosine triphosphate production (see the online visual abstract from Liu et al[1]).

Apart from the detailed mechanism of OXPHOS upregulation in ibrutinib-resistant cells, the authors targeted this pathway with metformin as an OXPHOS inhibitor. Metformin treatment was able to restore ibrutinib sensitivity in resistant ABC-DLBCL cell clones and furthermore sensitized primary resistant ABC-DLBCL cell lines to ibrutinib. The in vivo model with metformin given with ibrutinib treatment showed a significant impact on tumor volume and weight only in ABC-DLBCL cell line xenografts. However, the more potent OXPHOS inhibitor IM156, a newly developed metformin derivative, was able to synergize with ibrutinib in all analyzed cells in vitro. Moreover, the drug combination of ibrutinib with IM156 achieved significant antitumor effects in a xenograft model derived from a patient with MCL compared with the monotherapy with either drug.

Based on these data, many questions arise. Will these findings be relevant only for patients who develop a resistance to ibrutinib, or, as part of the data suggests, might all patients who showed no initial response to ibrutinib benefit from combination therapy, targeting Bruton tyrosine kinase (BTK) and OXPHOS simultaneously? Since ibrutinib is no longer the only BTK inhibitor in use, can these findings be translated to other BTK inhibitors and to other disorders, which are currently treated with BTK inhibitors? One pressing question for the clinical applicability of these data is determining the optimal treatment strategy. To answer these and many other questions arising from the findings of Lui et al, further preclinical and clinical studies will be necessary as we await the sinking of ibrutinib resistance.

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

REFERENCES

1. Liu Y, Kimpara S, Hoang NM, et al. EGR1-mediated metabolic reprogramming to oxidative phosphorylation contributes to ibrutinib resistance in B cell lymphoma. *Blood*. 2023;142(22):1879-1894.
2. Dreyling M, Jurczak W, Jerkeman M, et al. Ibrutinib versus temsirolimus in patients with relapsed or refractory mantle-cell lymphoma: an international, randomised, open-label, phase 3 study. *Lancet*. 2016;387(10020):770-778.
3. Visco C, Di Rocco A, Evangelista A, et al. Outcomes in first relapsed-refractory younger patients with mantle cell lymphoma: results from the MANTLE-FIRST study. *Leukemia*. 2021;35(3):787-795.
4. Wilson WH, Wright GW, Huang DW, et al. Effect of ibrutinib with R-CHOP chemotherapy in genetic subtypes of DLBCL. *Cancer Cell*. 2021;39(12):1643-1653.e3.
5. Martin P, Maddocks K, Leonard JP, et al. Postibrutinib outcomes in patients with mantle cell lymphoma. *Blood*. 2016;127(12):1559-1563.
6. Monti S, Savage KJ, Kutok JL, et al. Molecular profiling of diffuse large B-cell lymphoma identifies robust subtypes including one characterized by host inflammatory response. *Blood*. 2005;105(5):1851-1861.
7. Choueiry F, Singh S, Sircar A, et al. Integration of metabolomics and gene expression profiling elucidates IL4I1 as modulator of ibrutinib resistance in ABC-diffuse large B cell lymphoma. *Cancers (Basel)*. 2021;13(9):2146.
8. Zhang L, Yao Y, Zhang S, et al. Metabolic reprogramming toward oxidative phosphorylation identifies a therapeutic target for mantle cell lymphoma. *Sci Transl Med*. 2019;11(491):eaau1167.
9. Fuhr V, Heidenreich S, Srivastava M, et al. CD52 and OXPHOS-potential targets in ibrutinib-treated mantle cell lymphoma. *Cell Death Discov*. 2022;8(1):505.

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PLATELETS AND THROMBOPOIESIS

Comment on Verdier et al, page 1895

Genetics of predicted platelet reactivity

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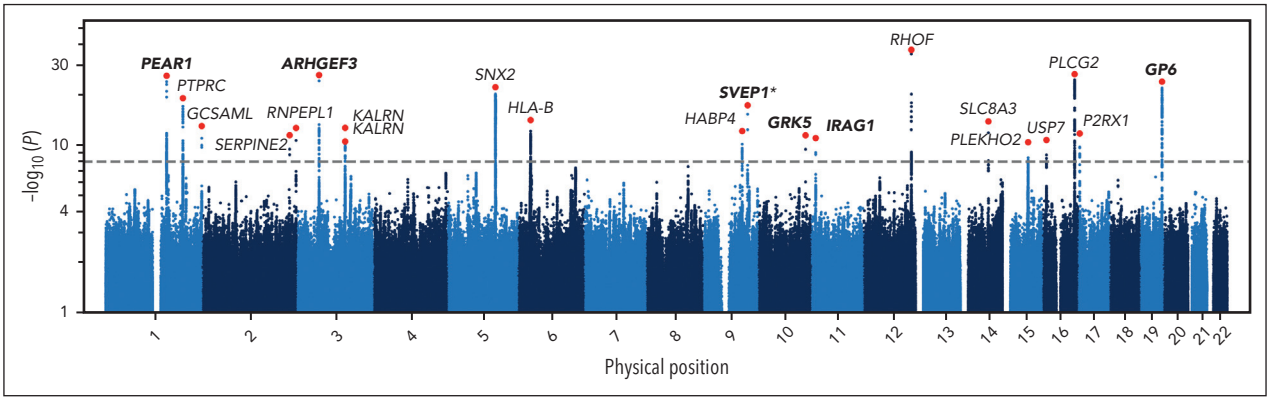
In this issue of *Blood*, Verdier et al pioneer a new method to predict platelet reactivity from complete blood count data, and they apply it to identify associated genetic variants and explore associations with thrombotic diseases.[1]

Platelets play a critical role in hemostasis and thrombosis, and antiplatelet drugs are widely used in cardiovascular disease prevention and treatment. There is interindividual variability in platelet reactivity, as measured by platelet aggregation responses to various agonists.[2] Identifying the genetic determinants of platelet reactivity could lead to a greater understanding of these differences and to the identification of additional targets for new antiplatelet drugs. Traditional methods for measuring platelet reactivity are difficult to implement at large scales,



A Manhattan plot showing the results of the genome-wide association study of predicted platelet reactivity. Each dot corresponds to an included genetic variant: the position on the x-axis indicates the chromosome number and genomic position of the variant, and the position on the y-axis indicates $-\log_{10}$ of the *P* value of the variant. The horizontal dashed line corresponds to the genome-wide significance threshold. The gene names indicate the protein-coding gene that is nearest to each of these associations. Regions associated with a platelet reactivity phenotype by a previous genome-wide association study are shown in bold. See Figure 2 in the article by Verdier et al that begins on page 1895 for further details.[1]

which has limited the platelet reactivity data that are available in epidemiological studies. Hypothesis-free genome-wide association studies have identified associated genetic variants in several genetic regions, including those near genes known to be relevant to platelets such as *GP6* and *PEAR1*.[2,3]

In this issue, Verdier et al devised a new approach to predict platelet reactivity to adenosine diphosphate using complete blood count scattergrams from widely implemented Sysmex analyzers.[1] The model was trained on 533 participants of the Cambridge Platelet Function Cohort. The predictive ability of this model was modest, with $r^2 = 0.26$. The authors then used the approach to predict platelet reactivity in 29,806 participants of the INTERVAL study. Strikingly, the large sample size of the INTERVAL study ensures that the *effective* sample size after accounting for the modest predictive ability of the model is still considerably larger than of the largest published genome-wide association study of platelet reactivity.

The authors identify 20 genetic regions containing variants associated with their predicted platelet reactivity phenotype (see figure), including 6 regions that have previously been identified by genome-wide association studies and 14 that have not. Many of the newly associated regions harbor candidate causal genes with plausible biological roles in platelet function. For example, *SERPINE2* encodes a potent thrombin inhibitor, thereby reducing platelet activation by thrombin. Other candidate genes, such as *PTPRC*, *GCSAML*, and *KALRN*, also have evidence linking them to platelet activation pathways. Interestingly, some of the candidate genes likely mediate platelet reactivity to agonists other than adenosine diphosphate, suggesting that the predicted platelet reactivity phenotype may serve as a broader readout of platelet reactivity beyond adenosine diphosphate. Previously published evidence that these candidate genes may regulate platelet activation in cellular and animal models does not diminish the impact of these findings, since the present study represents a powerful in vivo demonstration of their importance in humans.

Verdier et al then went on to use the results from their genetic study to identify significant associations between genetically determined platelet reactivity and risk of thrombotic diseases, including coronary artery disease, ischemic stroke, and venous thromboembolism. This causal inference approach, called Mendelian randomization, aims to provide causal effect estimates free from confounding and reverse causation by using genetic variants associated with an exposure (such as platelet reactivity) as "instruments" to assess the association of the exposure with disease. Mendelian randomization leverages the fact that genetic variants are fixed at conception and do not change as a result of confounders or disease. Importantly, these analyses are based on first occurrence of thrombotic diseases, emphasizing platelet reactivity as a target for prevention as well as treatment. These analyses highlight the importance platelet function in these 3 thrombotic diseases. The association with coronary artery disease is notable because the vast majority of identified genetic associations appear to be driven by atherosclerotic plaque development and not by the thrombotic response to plaque rupture.[4]

Although the insights gleaned from the first application of this approach are impressive in their own right, the true promise of this approach lies in future applications on even larger data sets, which would expand our understanding of the genetic architecture of platelet reactivity and potentially lead to the discovery of novel drug targets.

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REFERENCES

1. Verdier H, Thomas P, Batista J, et al. A signature of platelet reactivity in CBC scattergrams reveals genetic predictors of thrombotic disease risk. *Blood*. 2023;142(22):1895-1908.
2. Johnson AD, Yanek LR, Chen MH, et al. Genome-wide meta-analyses identifies seven loci associated with platelet aggregation in response to agonists. *Nat Genet*. 2010;42(7):608-613.
3. Keramati AR, Chen MH, Rodriguez BAT, et al. Genome sequencing unveils a regulatory landscape of platelet reactivity. *Nat Commun*. 2021;12(1):3626.
4. Aragam KG, Jiang T, Goel A, et al. Discovery and systematic characterization of risk variants and genes for coronary artery disease in over a million participants. *Nat Genet*. 2022;54(12):1803-1815.

https://doi.org/10.1182/blood.2023022302