might provide durable disease control without the risk associated with high dose chemotherapy.

Many published and ongoing clinical trials are assessing the impact of the up-front use of regimens incorporating brentuximab and PD-1 inhibitors. Although the number of patients with primary refractory and relapsed disease will almost certainly decline over time as a result, further study is necessary to evaluate the long-term outcomes, including the OS and late effects compared with treatment with standard chemotherapy regimens. Because novel agents are being increasingly used in the frontline setting, strategies for salvage regimens may also need to change. Whether patients previously exposed to PD-1 inhibitors will remain sensitive to immunotherapy at relapse is an open question. Finally, many, if not most, patients relapsing after ASCT today will have already received both BV and PD-1 inhibitors. Treatment options for these patients are limited and novel treatment approaches are needed. Unlike non-Hodgkin lymphoma for which CD19 chimeric antigen receptor (CAR) T cells have proven to be an invaluable treatment option, autologous CAR therapies in Hodgkin lymphoma have so far failed to reliably achieve durable remissions. For patients relapsing after ASCT, allogeneic stem cell transplantation remains an important consideration and its use did not change across the 2 periods in this study.

As novel agents are used more frequently and in earlier phases in therapy, outcomes for patients with cHL have undeniably improved, but more work is needed to determine the optimal place for BV and PD-1 inhibitors in the treatment of cHL.

Conflicts-of-interest disclosure: R.W.M. provides consulting for Genmab, Adaptive Biotechnologies, Bristol Myers Squibb (BMS), AbbVie, Intellia, and Epizyme and received research funding from BMS, Merck, Genentech/Roche, and Genmab. A.S.L. provides consulting for Research to Practice and is on the advisory boards of Kite and Seagen. ■

REFERENCES

1. Spinner MA, Sica RA, Tamaresis JS, et al. Improved outcomes for relapsed/refractory Hodgkin lymphoma after autologous transplantation in the era of novel agents. *Blood*. 2023;141(22):2727-2737.
2. LaCasce AS, Bociek RG, Sawas A, et al. Brentuximab vedotin plus bendamustine: a highly active first salvage regimen for relapsed or refractory Hodgkin lymphoma. *Blood*. 2018; 132(1):40-48.
3. Moskowitz AJ, Schöder H, Yahalom J, et al. PET-adapted sequential salvage therapy with brentuximab vedotin followed by augmented ifosamide, carboplatin, and etoposide for patients with relapsed and refractory Hodgkin's lymphoma: a non-randomised, open-label, single-centre, phase 2 study. *Lancet Oncol*. 2015;16(3):284-292.
4. Merryman RW, Redd RA, Nishihori T, et al. Autologous stem cell transplantation after anti-PD-1 therapy for multiply relapsed or refractory Hodgkin lymphoma. *Blood Adv*. 2021;5(6): 1648-1659.
5. Moskowitz AJ, Shah G, Schöder H, et al. Phase II trial of pembrolizumab plus gemcitabine, vinorelbine, and liposomal doxorubicin as second-line therapy for relapsed or refractory classical hodgkin lymphoma. *J Clin Oncol*. 2021;39(28):3109-3117.
6. Advani RH, Moskowitz AJ, Bartlett NL, et al. Brentuximab vedotin in combination with nivolumab in relapsed or refractory Hodgkin lymphoma: 3-year study results. *Blood*. 2021; 138(6):427-438.
7. Moskowitz CH, Matasar MJ, Zelenetz AD, et al. Normalization of pre-ASCT, FDG-PET imaging with second-line, non-cross-resistant, chemotherapy programs improves event-free survival in patients with Hodgkin lymphoma. *Blood*. 2012;119(7): 1665-1670.
8. Ding K, Liu H, Ma J, et al. Tislelizumab with gemcitabine and oxaliplatin in patients with relapsed or refractory classic Hodgkin lymphoma: a multicenter phase II trial. Published online 26 January 2023. *Haematologica*. https://doi.org/10.3324/haematol.2022.282266

https://doi.org/10.1182/blood.2023020068

MYELOID NEOPLASIA

Comment on Krishnan et al, page 2738

Single cells tell multiple tales in CML

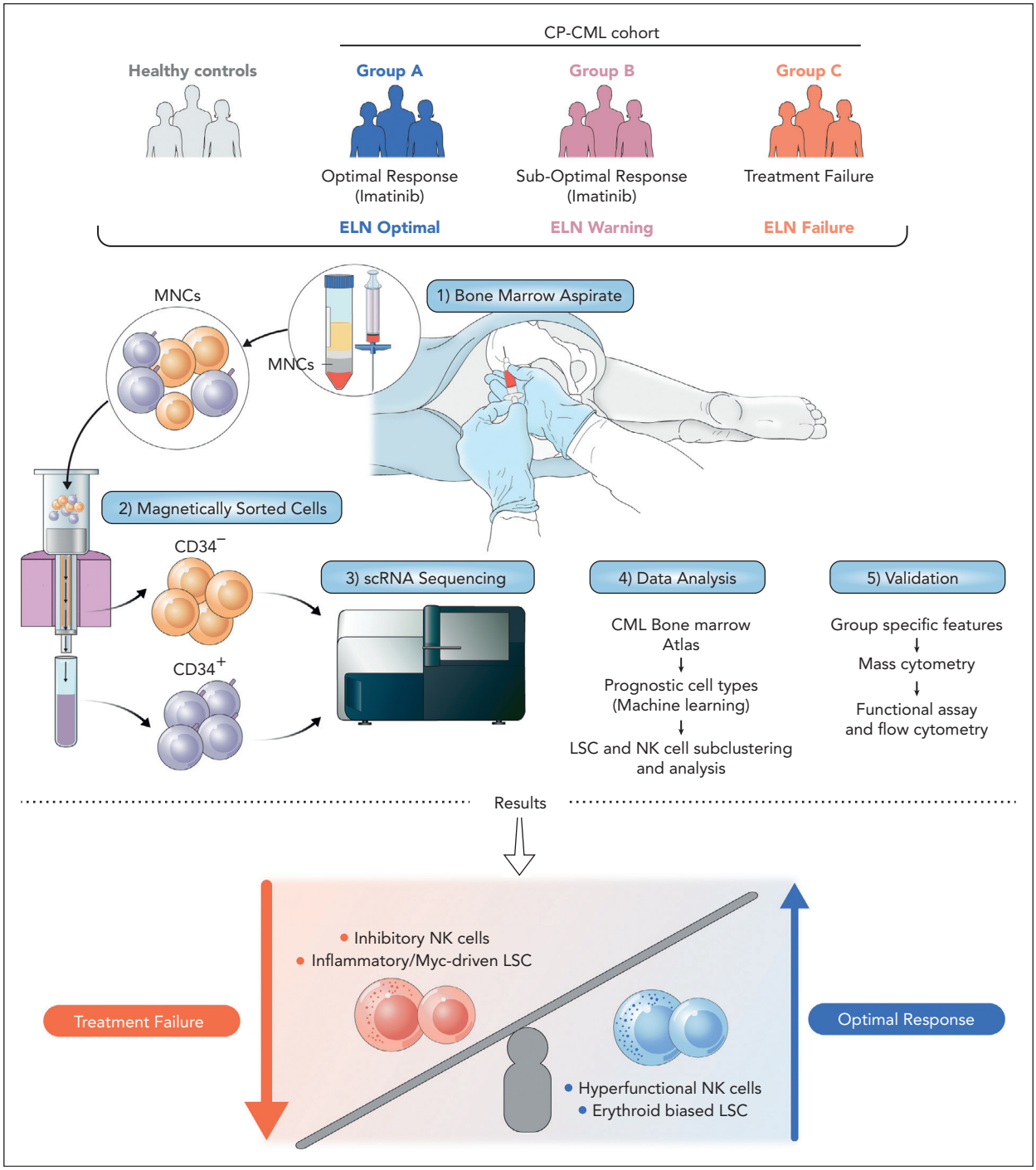
David M. Ross | South Australian Health and Medical Research Institute

In this issue of *Blood*, Krishnan et al describe a single-cell transcriptomic atlas of chronic myeloid leukemia (CML) at diagnosis.[1] By comparing groups with varying degrees of response or treatment failure on imatinib, they identified cellular features at diagnosis that were predictive of response to tyrosine kinase inhibitor (TKI) therapy (see figure).

CML has long been viewed as the paradigm of a monogenic cancer, defined by the *BCR::ABL1* fusion gene. The opportunity to target the founding lesion of CML with BCR::ABL1 TKIs has led to near-normal life expectancy for most people with CML, yet this headline result conceals striking heterogeneity of outcomes ranging from sustained treatment-free remission (TFR) in 20% to 30% of individuals to blast crisis in around 5% of individuals. Advances in sequencing methods have made it possible to interrogate the biological heterogeneity of CML with ever-increasing resolution so that novel factors that influence treatment response can be identified. Krishnan et al used de novo clustering to identify 32 cell populations from the transcriptomic data, comprising both $CD34^+$ cells and $CD34^-$ mononuclear cells. Using differential expression, they were able to distinguish *BCR::ABL1*-positive primitive cells from the remaining wild-type counterparts. A machine learning approach was used to identify gene expression signatures in individual cell types that were associated with outcome.[1]

In the leukemic stem and progenitor cell (LSC) compartment, an optimal response to TKI therapy was associated with erythroid differentiation bias.[1] In an important additional step, a flow cytometric approach was used to provide independent technical validation of the erythroid expression signature in LSCs. Conversely, an inflammatory and MYC-driven expression signature was associated with development of blast crisis,[1] in agreement with a prior study of single-cell transcriptomics in sorted LSCs from CML.[2]

In the immune cell compartment, favorable outcomes were associated with



Study design and key findings from the article by Krishnan et al that begins on page 2738. CP, chronic phase; ELN, European LeukemiaNet; LSC, leukemic stem and progenitor cell; MNC, mononuclear cells; NK, natural killer; scRNA, single cell RNA.

expansion of adaptive-like, hyperfunctional natural killer (NK) cells, whereas inhibitory NK cells were increased in patients who developed blast crisis.[1] Increased numbers of immune effectors and decreased immune suppressors measured in peripheral blood after an optimal response to TKI treatment are associated with a higher probability of TFR.[3,4] The work of Krishnan et al provides evidence that this immune balance may, at least in part, be predetermined, rather than being an outcome of TKI treatment, and a net inhibitory immune profile may also be associated with the emergence of blast crisis.

Risk-adapted therapy in CML is substantially driven by molecular response milestones during treatment. Slower decline in *BCR::ABL1* and failure to achieve early molecular response (EMR; *BCR::ABL1* ≤10% by 3 months) are associated with higher rates of treatment failure and reduced chance of TFR.[5] Although the first-line use of a more potent TKI reduces the incidence of EMR failure, the adverse impact of EMR failure on imatinib is only partially rescued by subsequent intensification of TKI treatment.[6] A small

proportion of patients develop early blast crisis even when treated first line with a more potent TKI, and for those individuals, novel therapies that might carry an increased risk of toxicity could be justified. In a machine learning logistic regression model, the authors developed a classifier for blast crisis with no false positives, and a sensitivity of 50% to 67%.[1] This cohort included only 6 patients, and the cases were diverse with respect to latency of blast crisis (76-2936 days) as well as lineage and genetics, so validation of these findings in a larger cohort is required. If patients destined for transformation or treatment failure could be identified at diagnosis, there would be an opportunity to design clinical trials of intensified first-line treatment to reduce this risk.

Recent studies have begun to dissect the relevance of genomic lesions in addition to *BCR::ABL1*, showing that some variants associated with blast crisis are already present at diagnosis.[7] Variants in *ASXL1* were found in 9% of 222 patients with chronic phase CML in a clinical trial using the more potent TKI, nilotinib, and were associated with a reduced incidence of major molecular response (*BCR::ABL1* ≤0.1%).[8] Variants in *ASXL1* were detected in 23.8% of patients by Krishnan et al (whose series was enriched for higher-risk individuals), but were identified across all response cohorts, including those with optimal response.[1] One of the limitations of bulk sequencing is that it cannot distinguish between the 3 cellular contexts in which a mutation might occur. Additional variants could represent clonal evolution (*BCR::ABL1* first), a preleukemic clone (*BCR::ABL1* second), or independent clonal hematopoiesis (*BCR::ABL1* wild type). In Ph-negative myeloproliferative neoplasms, it has been shown that the order of acquisition of mutations can influence disease biology.[9] Larger single-cell sequencing studies may help to determine whether the prognostic effect of an additional variant in CML is influenced by its clonal relationship to *BCR::ABL1*.

Broader application of single-cell sequencing for clinical purposes is currently limited by cost and analytical complexity. Recognizing the need for simpler, more widely applicable methods to validate and apply these findings, Krishnan et al showed that flow cytometry or mass cytometry could be used to detect the predictive signatures from their transcriptomic analysis. Although larger single-cell sequencing studies may be undertaken in the future, the data contained in this report present multiple hypotheses that can be tested now. Some of the biological pathways that influence optimal response and TFR overlap with those that lead to treatment failure and blast crisis. Understanding and targeting these pathways has the potential to lead to improved outcomes at both ends of the response spectrum in CML.

Conflict-of-interest disclosure: D.M.R. has received research funding from Novartis and BMS and honoraria from Novartis, BMS, Takeda, Keros, and Menarini. ■

REFERENCES

1. Krishnan V, Schmidt F, Nawaz Z, et al. A single-cell atlas identifies pretreatment features of primary imatinib resistance in chronic myeloid leukemia. *Blood*. 2023;141(22):2738-2755.
2. Giustacchini A, Thongjuea S, Barkas N, et al. Single-cell transcriptomics uncovers distinct molecular signatures of stem cells in chronic myeloid leukemia. *Nat Med*. 2017;23(6):692-702.
3. Ilander M, Olsson-Stromberg U, Schlums H, et al. Increased proportion of mature NK cells is associated with successful imatinib discontinuation in chronic myeloid leukemia. *Leukemia*. 2017;31(5):1108-1116.
4. Irani YD, Hughes A, Clarson J, et al. Successful treatment-free remission in chronic myeloid leukaemia and its association with reduced immune suppressors and increased natural killer cells. *Br J Haematol*. 2020;191(3):433-441.
5. Shanmuganathan N, Pagani IS, Ross DM, et al. Early BCR-ABL1 kinetics are predictive of subsequent achievement of treatment-free remission in chronic myeloid leukemia. *Blood*. 2021;137(9):1196-1207.
6. Yeung DT, Osborn MP, White DL, et al. TIDEL-II: first-line use of imatinib in CML with early switch to nilotinib for failure to achieve time-dependent molecular targets. *Blood*. 2015;125(6):915-923.
7. Branford S, Wang P, Yeung DT, et al. Integrative genomic analysis reveals cancer-associated mutations at diagnosis of CML in patients with high-risk disease. *Blood*. 2018;132(9):948-961.
8. Schonfeld L, Rinke J, Hinze A, et al. ASXL1 mutations predict inferior molecular response to nilotinib treatment in chronic myeloid leukemia. *Leukemia*. 2022;36(9):2242-2249.
9. Ortmann CA, Kent DG, Nangalia J, et al. Effect of mutation order on myeloproliferative neoplasms. *N Engl J Med*. 2015;372(7):601-612.

https://doi.org/10.1182/blood.2023020165

RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Qin et al, page 2756

Help on the way to unsilence HbF

Ann Dean | National Institute of Diabetes and Digestive and Kidney Diseases

In this issue of *Blood*, Qin et al[1] show that BMI1 (PCGF4), a polycomb group RING finger protein, is a repressor of fetal hemoglobin (HbF). They present strong evidence that the entirety of the effect of BMI1 is exerted by RNA-binding proteins LIN28B, IGF2BP1, and IGF2BP3.

Elevated HbF in adults moderates the symptoms of sickle cell disease and β-thalassemia and is a highly desirable therapeutic goal.[2] The significant discoveries of HbF repressors BCL11A and ZBTB7A (LRF) resulted in encouraging small clinical trials involving gene editing of mobilized patient hematopoietic stem cells ex vivo.[3] However, gene therapies are unlikely to be feasible for the vast patient population in less developed parts of the world because of their complexity and cost. Thus, there remains a strong incentive to develop other approaches, such as small-molecule inhibitors. The feasibility of this approach is illustrated by the rational targeting of components of the nucleosome remodeling and deacetylating complex, through which BCL11A and ZBTB7A work.[4] Qin et al enlarge the field of potential small-molecule targets by identifying polycomb repressive complexes 1/2 (PRC1/2) as indirect inhibitors of *HBG1/2* transcription.

PRC1 and PRC2 are crucial to establishment and maintenance of facultative