at diagnosis. In other cases, they were novel. Importantly, they emerged quickly, sometimes just days to weeks into therapy.

Given these patterns of resistance, perhaps earlier combined targeted therapy is warranted (ie, triplet regimens that use FLT3 inhibition or *TP53* stabilization strategies)? However, that approach brings its own new questions: how good are our tests at detecting subclones? Do we need to target mutations that have not yet emerged? How many targets are good enough, and for how long? If a barrage of molecularly directed agents at the start of treatment guts the proliferation potential of the latent clones, well, that's excellent. However, I would be less enthusiastic about sequencing 1 agent after another, consigning patients to a permanent parade of consecutive treatments. I think it is a mistake to give up on a goal of true disease eradication. Combinations that use cytotoxic agents (possibly in attenuated doses or novel delivery mechanisms) or harness immune therapies alongside targeted therapies should be investigated. Evidence of polyclonal resistance supports the case that, as a research community, we need to focus on what the leukemic clones have in common and target those commonalities early on.[5]

Finally, a logistical point is presented. The authors argue that "serial molecular studies can identify patterns of drug sensitivity and resistance at the subclonal level." I fear this recommendation is premature. Are we ready to roll such a practice into prime time, given the costs, the lack of standardization, and the paucity of effective options for refractory disease?

DiNardo, Wei, and colleagues have done much to bring venetoclax to AML patients, a remarkable accomplishment. The data presented here show us that our work has only just begun.

Conflict-of-interest disclosure: L.C.M. served as a consultant to Novartis, Celgene, Incyte; previously held stock in Pfizer; and receives research funding from Jazz Pharmaceuticals. ■

REFERENCES

1. DiNardo CD, Tiong IS, Quaglieri A, et al. Molecular patterns of response and treatment failure after frontline venetoclax combinations in older patients with AML. *Blood*. 2020;135(11):791-803.
2. Wei AH, Strickland SA Jr., Hou JZ, et al. Venetoclax combined with low-dose cytarabine for previously untreated patients with acute myeloid leukemia: results from a phase Ib/II study. *J Clin Oncol*. 2019;37(15):1277-1284.
3. Winters AC, Gutman JA, Purev E, et al. Real-world experience of venetoclax with azacitidine for untreated patients with acute myeloid leukemia. *Blood Adv*. 2019;3(20):2911-2919.
4. Estey E, Karp JE, Emadi A, Othus M, Gale RP. Recent drug approvals for newly diagnosed acute myeloid leukemia: gifts or a Trojan horse? [published online ahead of print 8 January 2020]. *Leukemia*. 2020;
5. Pollyea DA, Stevens BM, Jones CL, et al. Venetoclax with azacitidine disrupts energy metabolism and targets leukemia stem cells in patients with acute myeloid leukemia. *Nat Med*. 2018;24(12):1859-1866.

DOI 10.1182/blood.2019004757

IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Liu et al, page 826

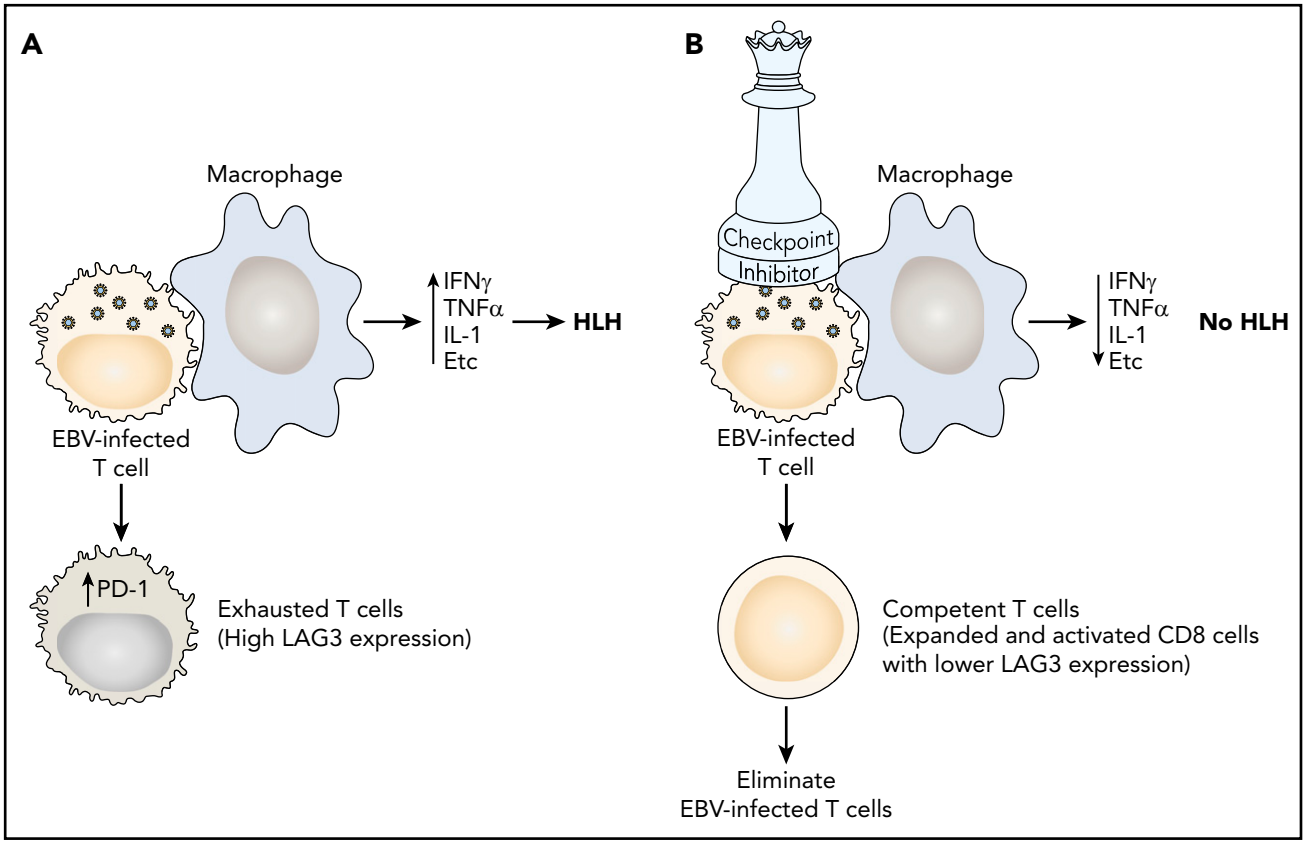
Checkmate for EBV-HLH

Nader Kim El-Mallawany and Kenneth L. McClain | Baylor College of Medicine

In this issue of *Blood*, Liu et al describe the favorable response of adults with relapsed/refractory Epstein-Barr virus (EBV)-associated hemophagocytic lymphohistiocytosis (HLH) to treatment with nivolumab, a programmed cell death-1 (PD-1) inhibitor.[1] EBV-HLH presents a challenging clinical conundrum because only a minority of patients will achieve long-standing clinical remission with front-line therapy.[2] To further complicate matters, neither clinical nor pathologic-based criteria have been well established to differentiate which patients are likely to fail upfront HLH therapy with etoposide and dexamethasone.[3,4] Patients with relapsed/refractory disease have a dismal chance of survival because of high rates of disease-related mortality.[2,3]

Various salvage regimens have been used for relapsed/refractory EBV-HLH, including combination chemotherapy regimens, monoclonal antibodies targeting the host cellular reservoirs for EBV, and targeted agents aimed at controlling the systemic inflammatory syndrome that defines the disease pathophysiology.[3] Nivolumab presents a novel approach to EBV-HLH as it seeks to restore T-cell immune function against uncontrolled EBV infection, which is at the very root of this virally mediated disease process. Because immune checkpoint inhibition of PD-1 has proven a useful therapeutic option for relapsed/refractory EBV-related lymphomas, it offers an attractive novel option for EBV-HLH as well.[5] Although it is generally accepted that relapsed/refractory EBV-HLH will ultimately require allogeneic hematopoietic stem cell transplant (HSCT) to achieve a cure, the authors sought to explore whether restoration of immune function through PD-1 inhibition could lead to long-standing control of EBV infection and the associated HLH syndrome.[3]

In this case series of 7 adults with relapsed/refractory EBV-HLH, nivolumab monotherapy resulted in clinical complete remission in 5 patients with a median follow-up of 16 months. The clinical successes were corroborated by translational experiments using single-cell transcriptome analyses. These demonstrated baseline overexpression of inflammatory markers, including tumor necrosis factor, interleukin-1B, and CD163. They also demonstrated expansion of PD-1$^+$ T cells after treatment with nivolumab, which was associated with decreasing levels of interferon-γ and granzyme B (cytokines that drive the hyperinflammatory syndrome characteristic of HLH), enrichment of CD8$^+$ T cells in activation and degranulation pathways, and a correlative decrease in the EBV viral loads in 4 of the 5 patients who achieved clinical remission. Single-cell RNAseq analyses of CD8$^+$ T cells at baseline revealed underexpression of specific HLH-related genes, including STXBP2, UNC13, SH2D1A, and CD27, suggesting that such immune dysregulation may explain the vulnerability to EBV-related complications. Thus, immune checkpoint inhibition with nivolumab effectively restored T-cell immune competence against EBV, resulting in clinical improvement of the associated HLH (see figure).



(A) In EBV-HLH, the viral infection causes a hyperinflammatory interaction with T cells and macrophages, resulting in excessive production of interferon-γ (IFNγ), tumor necrosis factor α (TNFα), interleukin-1 (IL-1), and other cytokines leading to HLH. Increased expression of PD-1 renders cytotoxic T cells incapable of controlling the infection (exhaustion). (B) Nivolumab binds to PD-1, rendering T cells competent to control the EBV infection and extinguish HLH.

Although immune checkpoint inhibition has recently garnered widespread excitement for its role in anticancer therapy, elegant in vivo experiments demonstrating the potential immunological role in the treatment of viral infections was reported 14 years ago.[6] These studies showed that PD-1 expression was upregulated in exhausted T cells, and that PD-1 inhibition helped restore the dysfunctional CD8 T-cell immune response against viral infection.[6] More recent work has highlighted the impact of T-cell exhaustion in other infections (eg, malaria, HIV, hepatitis B virus) and the role of immune checkpoint blockade in treating infectious diseases as well.[7] In addition, immune checkpoint blockade has been evaluated in patients with virus-associated malignancies, in particular, in patients living with HIV and cancer, and has generated encouraging results leading to several ongoing prospective clinical trials.[8]

This case series of adults with EBV-HLH treated with nivolumab offers a glimmer of hope for improving the treatment of EBV-HLH, particularly for those patients with relapsed/refractory disease. Although the authors did not expand their discussion to the closely related spectrum of diagnoses that fall under the category of systemic EBV-associated T- and natural killer (NK)-cell lymphoproliferative diseases, there may be some potential overlap in the utility of PD-1 inhibition for this extremely challenging subset of diseases.

The updated World Health Organization classification of lymphoid neoplasms has categorized systemic EBV-associated T- and NK-cell lymphoproliferative diseases as a closely related group of diagnoses with extensive clinical and pathological overlap. Included in this umbrella of diagnoses are EBV-HLH, systemic T- and NK-cell chronic active EBV, and the systemic EBV-positive T-cell lymphoma of childhood.[9,10] Patients who have HLH and EBV viremia may follow one of the following clinical scenarios: (1) HLH secondary to EBV infection that is effectively cured with front-line HLH therapy, (2) HLH driven by EBV infection in the context of an underlying genetic predisposition to EBV-associated lymphoproliferative disease that requires allogeneic HSCT for cure, (3) HLH driven by EBV as the clinical manifestation of what eventually is diagnosed as T- or NK-cell chronic active EBV, (4) HLH driven by EBV as the clinical manifestation of the systemic EBV-positive T-cell lymphoma of childhood.[9,10] The latter 2 diagnoses are associated with extremely high rates of disease-related mortality and are only cured through allogeneic HSCT.[9,10] This group of EBV-associated T- and NK-cell lymphoproliferative diseases are in desperate need of novel therapeutic approaches to offer hope for improved outcomes.

The work of Liu et al presents exciting preliminary data on the potential role of immune checkpoint inhibition for the treatment of EBV-HLH. Although it must be validated with larger, prospective cohorts of patients with longer off-therapy follow-up, it offers the possibility that anti-PD-1–targeted therapy may restore immune function against a disease mediated by EBV infection. It might also offer a potential therapeutic avenue for the treatment of systemic T- and NK-cell chronic active

EBV and the systemic EBV-positive T-cell lymphoma of childhood, disease processes that are closely related to EBV-HLH.

Conflict-of-interest disclosure: K.L.M. is on the medical advisory board for Sobi Corporation. N.K.E.-M. declares no competing financial interests. ■

REFERENCES

1. Liu P, Pan X, Chen C, et al. Nivolumab treatment of relapsed/refractory Epstein-Barr virus–associated hemophagocytic lymphohistiocytosis in adults. *Blood*. 2020;135(11):826-833.
2. Yoon JH, Park SS, Jeon YW, et al. Treatment outcomes and prognostic factors in adult patients with secondary hemophagocytic lymphohistiocytosis not associated with malignancy. *Haematologica*. 2019;104(2):269-276.
3. La Rosée P, Horne A, Hines M, et al. Recommendations for the management of hemophagocytic lymphohistiocytosis in adults. *Blood*. 2019;133(23):2465-2477.
4. Smith MC, Cohen DN, Greig B, et al. The ambiguous boundary between EBV-related hemophagocytic lymphohistiocytosis and systemic EBV-driven T cell lymphoproliferative disorder. *Int J Clin Exp Pathol*. 2014;7(9):5738-5749.
5. Kim SJ, Hyeon J, Cho I, Ko YH, Kim WS. Comparison of efficacy of pembrolizumab between Epstein-Barr virus–positive and –negative relapsed or refractory non-Hodgkin lymphomas. *Cancer Res Treat*. 2019;51(2):611-622.
6. Barber DL, Wherry EJ, Masopust D, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature*. 2006;439(7077):682-687.
7. Wykes MN, Lewin SR. Immune checkpoint blockade in infectious diseases. *Nat Rev Immunol*. 2018;18(2):91-104.
8. Cook MR, Kim C. Safety and efficacy of immune checkpoint inhibitor therapy in patients with HIV infection and advanced-stage cancer: a systematic review. *JAMA Oncol*. 2019;5(7):1049-1054.
9. Kim WY, Montes-Mojarro IA, Fend F, Quintanilla-Martinez L. Epstein-Barr virus-associated T and NK-cell lymphoproliferative diseases. *Front Pediatr*. 2019;7:1-18.
10. Cohen JI, Iwatsuki K, Ko YH, et al. Epstein-Barr virus NK and T cell lymphoproliferative disease: report of a 2018 international meeting [published online ahead of print 13 December 2019]. *Leuk Lymphoma*. 2019; doi:10.1080/10428194.2019.1699080.

DOI 10.1182/blood.2020005041

LYMPHOID NEOPLASIA

Comment on Odabashian et al, page 834

Sugar-coated BCR kept during FL clonal evolution

Sandrine Roulland | Aix-Marseille University, CNRS, INSERM, CIML, Marseille, France

In this issue of *Blood*, Odabashian et al[1] interrogate by deep sequencing the evolution patterns of N-glycosylation (N-gly) motifs introduced by somatic hypermutation (SHM) into the variable region of the B-cell receptor (BCR) in sequential follicular lymphoma (FL) biopsies taken before and after progression. This phylogenetic study recognizes the maintenance of N-gly motifs from the early stages until disease progression, seemingly independent of the intratumoral (epi)genetic variability, thereby supporting a model for a conserved dependence for this mode of constitutive BCR triggering signal through clonal evolution.

FL is a germinal center (GC)-derived B-cell neoplasia characterized by a long asymptomatic preclinical course followed by remission/relapse cycles becoming eventually refractory to treatment or transforming to a more aggressive lymphoma.[2] Clonal evolution studies, including longitudinal (diagnosis vs relapse/transformation) and spatial profiling, provide insights into the genetic basis of FL and clonal dynamics at progression. By analyzing the hierarchy of mutations, it was inferred that FL progression commonly arises through a divergent evolution process, emerging from a less evolved common precursor cell (CPC) that is responsible for propagating each new FL episode with the acquisition of independent mutations.[3,4] Additional evidence for FL CPCs comes from the detection of precursors in "healthy" individuals years before FL develops[5] and the demonstration that those committed precursors can transfer the disease in bone marrow transplants.[6] As all FL patients ultimately relapse, this indicates that current therapies are unable to eradicate the reservoir of FL CPCs and that targeting key CPC founding alterations may represent an attractive therapeutic strategy to prevent progression and relapse.

Phylogenetic studies have started to unravel the set of molecular alterations contributing to CPC genesis. Besides the founder t(14;18) translocation allowing the BCL2^{pos} cells to recirculate as premalignant precursors years before FL onset, mutations in epigenetic modifiers, such as *KMT2D* or *CREBBP*, have been suggested as cofounding lesions.[3,4] A third key player is the ability of FL cells to coopt BCR signaling pathways and establish a strong dependence toward microenvironment signals. A remarkable feature of FL BCRs, affecting >80% of patients, relies on the introduction during SHM of sequence motifs into the BCR variable region that create novel N-linked glycosylation acceptor sites, a modification rarely seen in normal B cells.[7,8] Loaded with atypical oligomannose glycans, these sugar moieties trigger activation of BCR signaling pathways through interaction with endogenous mannose-binding lectins like DC-SIGN expressed by dendritic cells and macrophages, thereby providing an alternative tumor-supportive mechanism independent from antigen recognition.[7,8] However, it remains largely unexplored when BCR N-gly motifs arise during FL ontogeny, whether they are stable, and how they clonally diverge and evolve during FL clinical course. This study by Odabashian et al. addresses these goals.

To reconstruct the evolutionary patterns of N-gly sites during progression, the authors used deep sequencing of BCR repertoire to profile sequential tumor biopsies paired in space and time from patients with variable clinical course and treatments. By comparing thousands of FL subclones, they identified that N-gly motifs are universally present within the dominant subclones of the first disease event and are retained in >96% of subsequent relapse/transformation subclones, with no further N-gly sites gained that