myeloid progenitors, and monocytes as well as non–leukemia cells, including colon and kidney epithelium. CD13 CAR T cells, although demonstrating potent anti-AML efficacy, almost completely eliminated HSCs, myeloid progenitors, and peripheral monocytes.

To develop a more tolerable and safer CAR T cell, the authors then developed a bispecific and split CAR (BissCAR) targeting CD13 and TIM3. TIM3 is a more specific marker with expression predominantly on exhausted T cells and LSCs, and limited expression on normal HSCs and myeloid progenitors. The BissCAR demonstrated elimination of leukemia as potently as seen with the anti-CD13 alone CAR T cell but with limited and reversible toxicity to normal HSCs, myeloid progenitors, circulating monocytes, and healthy organ systems. This suggests that dual targeting of optimally selected antibodies and tumor-associated antigens may be a new approach to develop a safer and more tolerable CAR therapy with maintained efficacy and is especially important in AML wherein preservation of normal HSCs and myeloid progenitors is likely to be critical to successful clinical CAR therapy development. This approach warrants clinical evaluation.

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REFERENCES

1. He X, Feng Z, Ma J, et al. Bispecific and split CAR T cells targeting CD13 and TIM3 eradicate acute myeloid leukemia. *Blood*. 2020;135(10):713-723.
2. Masarova L, Kantarjian H, Ravandi F, Sharma P, Garcia-Manero G, Daver N. Update on immunotherapy in AML and MDS: monoclonal antibodies and checkpoint inhibitors paving the road for clinical practice. *Adv Exp Med Biol*. 2018;995:97-116.
3. Daver N, Garcia-Manero G, Basu S, et al. Efficacy, safety, and biomarkers of response to azacitidine and nivolumab in relapsed/refractory acute myeloid leukemia: a non-randomized, open-label, phase II study. *Cancer Discov*. 2019;9(3):370-383.
4. Gojo I, Stuart RK, Webster J, et al. Multi-center phase 2 study of pembroluzimab (Pembro) and azacitidine (AZA) in patients with relapsed/refractory acute myeloid leukemia (AML) and in newly diagnosed (≥65 years) AML patients [abstract]. *Blood*. 2019;134(suppl 1). Abstract 832.
5. Sallman DA, Asch AS, Al Malki MM, et al. The first-in-class anti-CD47 antibody magrolimab (5F9) in combination with azacitidine is effective in MDS and AML patients: ongoing phase 1b results [abstract]. *Blood*. 2019;134(suppl 1). Abstract 569.
6. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science*. 2015;348(6230):62-68.
7. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med*. 2013;368(16):1509-1518.
8. Ritchie DS, Neeson PJ, Khot A, et al. Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia. *Mol Ther*. 2013;21(11):2122-2129.
9. Budde L, Song J, Kim Y, et al. Remissions of acute myeloid leukemia and blastic plasmacytoid dendritic cell neoplasm following treatment with CD123-specific CAR T cells: a first-in-human clinical trial [abstract]. *Blood*. 2017;130(suppl 1). Abstract 811.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Straus et al, page 735

The efficacy-toxicity conundrum: breaking the mold

Louis F. Diehl | Duke University Medical Center

Although the cure of advanced-stage Hodgkin lymphoma (HL) is a tremendous success, each new improvement in outcome has come with a significant price. Mechlorethamine, vincristine, procarbazine, and prednisone resulted in leukemia; doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) added possible fatal pulmonary toxicity; and bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone (BEACOPP) improved the cure but at a price of increased toxicity and increased leukemia. The ECHELON-1 study, reported by Straus et al in this issue of *Blood*, may be the first advance to break this pattern.[1]

Even more so today, physicians who are recommending treatment of patients with advanced-stage, classic HL (cHL) are faced with the dilemma of maximizing efficacy and limiting toxicity. This dilemma results from the fact that the 2 major chemotherapy regimens are at opposite poles in terms of toxicity and efficacy. Data from the German Hodgkin Study Group HD9 study support that, for cyclophosphamide, vincristine, procarbazine, and prednisone (COPP)/ABVD (a surrogate for ABVD), freedom from treatment failure (FFTR) was 64% and overall survival (OS) was 75%, whereas escalated BEACOPP (eBEACOPP) FFTR was 82% and OS was 86%.[2] On the other hand, acute and chronic toxicity were just the reverse. For COPP/ABVD vs eBEACOPP, grade 4 leukopenia was 19% vs 90%, grade 4 thrombocytopenia was 2% vs 47%, grade 4 infection was 1% vs 8%, grade 3 or 4 mucositis was 1% vs 8%, and grade 3 or 4 pain was 2% vs 9%.[3] The long-term toxicity of acute myeloid leukemia was 0.4% vs 3.0%, respectively.[2]

Contemporary studies have gone in 2 directions to address this efficacy/toxicity conundrum. One direction is to use the more toxic eBEACOPP regimen for the poor-prognosis patients whose positron emission tomography (PET) scan is positive after 2 cycles ($PET2^+$). Based on historically controlled data, advanced HL $PET2^+$ patients have progression-free survival (PFS) between 30% and 44%.[4] There have been 4 large studies that used dose escalation to BEACOPP to address this $PET2^+$ problem[5-8] (see table for the detailed results). Several conclusions emerge from examination of these data. When treatment is escalated to a form of BEACOPP in $PET2^+$ patients, the PFS is in the 60% to 70% range, which is significantly above the historical PFS of 30% to 40%. One of the critical data points in this update of the ECHELON-1 trial is that brentuximab vedotin (A) plus doxorubicin, vinblastine, and dacarbazine (AVD) (A+AVD) continued without change after $PET2^+$; the results for continuing A+AVD are in the same range of

PFS for PET2$^-$ and PET2$^+$ patients treated with various schedules of BEACOPP and patients treated on the ECHELON-1 trial continuing on A+AVD and ABVD

Treatment	PFS PET2$^-$, %	PFS PET2$^+$, %	Conclusion
• United Kingdom[5] • ABVD × 2 − PET2$^-$ then ABVD or AVD • ABVD × 2 − PET2$^+$ then BEACOPP-14 or eBEACOPP	84	68	The conclusion was that this rate was substantially higher than that observed in retrospective series in which patients continued ABVD and is similar to the rates in other series in which patients received BEACOPP
• GHSG HD 18[6] • eBEACOPP − PET2$^+$ then eBEACOPP × 6 or • eBEACOPP × 6 + rituximab	NA	91.4 93	The conclusion was that the PFS of the PET2$^+$ patients was much better than expected
• GITIL/FIL HD 0607[7] • ABVD × 2 − PET2$^-$ then ABVD ± XRT • ABVD × 2 − PET2$^+$ then eBEACOPP × 4 and standard BEACOPP × 4 ± rituximab	87	60	The conclusion again is that PET response-adapted treatment is a feasible, safe, and effective therapeutic strategy in advanced HL
• SWOG S0816[8] • ABVD × 2 − PET2$^-$ then ABVD × 4 • ABVD × 2 − PET2$^+$ then eBEACOPP × 6	76	66	The conclusion was that, in PET2$^+$ patients who received eBEACOPP, PFS was favorable but was associated with a high rate of second malignancies; there was 24% progression in PET2$^-$ at 5 y
• ECHELON-1[1]: ABVD × 2 − PET2 ± ABVD × 4 • ECHELON-1: A+AVD × 2 PET2 ± A+AVD × 4	79.5 85.8	51.5 67.7	A+AVD provides durable efficacy benefit regardless of patient status at PET2

A, brentuximab vedotin; AVD, doxorubicin, vinblastine, and dacarbazine; BEACOPP-14, standard dose BEACOPP every 14 days; GHSG HD, German Hodgkin Study Group HD9 study; GITIL/FIL HD, Gruppo Italiano Terapie Innovative nel Linfomi/Fondazione Italiana Linfomi HD study; NA, not applicable; XRT, radiation.

PFS as changing to BEACOPP in PET2$^+$. The initial comparator for these 4 studies was historically controlled data sets. Using the Deauville criteria, 3-year PFS for PET2$^+$ advanced-stage International Prognostic Index (IPI) ≤ 2 was 30%; and, for IPI ≥ 3, the PFS was 44%.[4] When measured against these historical controls of 30% and 44%, the PFS of PET2$^+$for the 4 escalated BEACOPP studies (68%, 91%, 60%, and 66%) appears to show an improvement in disease control.

The update of the ECHELON-1 study, reported in this issue, provides important data on the efficacy side of the question. Advanced-stage, cHL patients were randomized to A+AVD or ABVD. An interim PET after 2 cycles was required. The median follow-up is now 37 months. Although the 3-year PFS rates were 83.1% with A+AVD and 76% with ABVD, the critical point for PET2$^+$ patients was 69.2% with A+AVD and 54.7% with ABVD. Although this is not a randomized comparison between PET2$^+$ patients receiving eBEACOPP and A+AVD, it does provide strong evidence that the PET2$^-$ patients have better PFS with A+AVD than with ABVD and the PFS of the PET2$^+$ patients with A+AVD is better than with ABVD and in the same range as the eBEACOPP patients.

The second part of the conundrum is toxicity. To get the higher PFS of A+AVD, what is the cost in terms of toxicity? The major toxicities grade ≥3 were neutropenia (54% vs 39%), peripheral neuropathy (PN; 4% vs <1%), abdominal pain (3% vs <1%), infectious death (1.1% vs 0%), and pulmonary deaths (0% vs 1.7%) for A+AVD and ABVD, respectively. All of the infectious deaths in the A+AVD arm occurred in patients who had not received growth factors before neutropenia. At the 3-year follow-up, the residual toxicities for any residual PN, grade 1, grade 2, and grade ≥3 were: 25.6%, 14.2%, 7.8%, and 2.7%, respectively, for A+AVD and 11.5%, 7.3%, 3.6%, and <1%, respectively, for ABVD. What these data reveal is that if the infection complications are controlled with growth factor, the toxicity of A+AVD is minimally increased but the efficacy is a marked step forward. The efficacy-toxicity conundrum is very possibly changed.

The decision regarding how to treat advanced-stage cHL is still a difficult one, and individual patient characteristics still need to be considered; however, the treating physician now has sufficient information that A+AVD: has a higher PFS than ABVD, gets similar results to dose-escalated eBEACOPP studies, eliminates or reduces the bleomycin pulmonary toxicity, results in rare (3%) residual grade ≥3 PN, and may significantly reduce or eliminate the risk of infectious death with the upfront use of growth factors.

The authors' conclusions are fairly conservative in stating that A+AVD compares favorably to PET-adapted strategies and has a manageable and predictable safety profile. Historically, efficacy and toxicity had increased together, creating the efficacy/toxicity conundrum, but with A+AVD, many physicians may well decide that they can now offer their patients greater efficacy and decreased toxicity.

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REFERENCES

1. Straus DJ, Długosz-Danecka M, Alekseev S, et al. Brentuximab vedotin with chemotherapy for stage III/IV classical Hodgkin lymphoma: 3-year update of the ECHELON-1 study. *Blood.* 2020;135(10):735-742.

2. Engert A, Diehl V, Franklin J, et al. Escalated-dose BEACOPP in the treatment of patients with advanced-stage Hodgkin's lymphoma: 10 years of follow-up of the GHSG HD9 study. *J Clin Oncol.* 2009;27(27):4548-4554.

3. Diehl V, Franklin J, Pfreundschuh M, et al; German Hodgkin's Lymphoma Study Group. Standard and increased-dose BEACOPP chemotherapy compared with COPP-ABVD for advanced Hodgkin's disease [published correction appears in *N Engl J Med.* 2005; 353(7):744]. *N Engl J Med.* 2003;348(24): 2386-2395.

4. Oki Y, Chuang H, Chasen B, et al. The prognostic value of interim positron emission tomography scan in patients with classical Hodgkin lymphoma. *Br J Haematol.* 2014; 165(1):112-116.

5. Johnson P, Federico M, Kirkwood A, et al. Adapted treatment guided by interim PET-CT scan in advanced Hodgkin's lymphoma. *N Engl J Med.* 2016;374(25):2419-2429.

6. Borchmann P, Haverkamp H, Lohri A, et al. Progression-free survival of early interim PET-positive patients with advanced stage Hodgkin's lymphoma treated with BEACOPPescalated alone or in combination with rituximab (HD18): an open-label, international, randomised phase 3 study by the German Hodgkin Study Group. *Lancet Oncol.* 2017;18(4):454-463.

7. Gallamini A, Tarella C, Viviani S, et al. Early chemotherapy intensification with escalated BEACOPP in patients with advanced-stage Hodgkin lymphoma with a positive interim positron emission tomography/computed tomography scan after two ABVD cycles: long-term results of the GITIL/FIL HD 0607 trial. *J Clin Oncol.* 2018;36(5): 454-462.

8. Stephens DM, Li H, Schöder H, et al. Five-year follow-up of SWOG S0816: limitations and values of a PET-adapted approach with stage III/IV Hodgkin lymphoma. *Blood.* 2019; 134(15):1238-1246.

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PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Gollomp et al, page 743

"HIT"ing back against NETs

Shadi Swaidani and Keith R. McCrae | Cleveland Clinic

In this issue of *Blood*, Gollomp et al demonstrate that a monoclonal antibody (KKO) with properties resembling those of antibodies from patients with heparin-induced thrombocytopenia (HIT) can be deglycosylated and used therapeutically to improve outcomes in a murine model of sepsis.[1] The mechanism underlying this activity appears to involve contraction and stabilization of neutrophil extracellular traps (NETs) and suggests a novel approach to the management of sepsis, which is associated with a mortality of 30% and an annual cost exceeding 20 billion dollars in the United States.[2]

First described in 2004,[3] NETs have received considerable attention for their role in thromboinflammatory disorders. Initially thought to function simply to "trap" circulating bacteria, the physiologic and pathophysiologic consequences of NET formation and degradation have proven complex and remain incompletely understood (see figure). NETs are extracellular web-like structures consisting of decondensed neutrophil DNA to which proteins derived from neutrophil azurophilic and primary granules, including neutrophil elastase, cathepsin G, myeloperoxidase, and gelatinase, among others, are bound. NETs also bind nuclear proteins, such as histones, that are citrullinated by neutrophil PAD4.[4] Release of NETs from neutrophils may occur through specific pathways, such as suicidal, ROS-dependent NETosis, or vital NETosis, in which neutrophils remain viable. Activated platelets may also induce NET formation, a mechanism that may be relevant in sepsis, in which platelets are activated by proteins displaying pathogen-associated molecular patterns recognized by platelet Toll-like receptor 4.[5]

A critical mechanism underlying the pathogenesis of HIT is FcγRII-mediated platelet activation following binding of heparin-platelet factor 4 (PF4) complexes by HIT antibodies. However, it is now appreciated that HIT is an intensely proinflammatory and prothrombotic disorder associated with complement activation, as well as activation of multiple cell types, including platelets, endothelial cells, and monocytes.[6] In a previous study, Gollomp et al also demonstrate that activation of neutrophils leads to the formation of NETs in a passive murine HIT model in which mice are infused with KKO.[7] In that study, it was shown that PF4 bound to NETs and that KKO recognized NET-bound PF4 complexes. Binding of PF4, and to an even greater extent, PF4 and KKO, to NETs changed NET morphology by causing NET contraction and impaired the susceptibility of NETs to degradation by DNase. Bound KKO also promoted thrombus formation, presumably by providing an Fc-rich surface to further enhance immune activation of platelets and other cells.

In the current study,[1] these investigators used microfluidic approaches and in vivo models to assess the effects of KKO and deglycosylated KKO (DG-KKO) in another thromboinflammatory disorder, sepsis. As in their previous study, PF4 promoted NET contraction and resistance to DNase degradation, with lower levels of NDPs detected in plasma from wild-type mice compared with mice lacking PF4 (*cxcl4*$^{-/-}$); the inhibitory effects of PF4 on NET degradation were enhanced in the presence of KKO. NET stabilization by PF4 and KKO also increased bacterial capture. However, despite these seemingly beneficial effects, the presence of the intact Fc region of KKO led to worsened outcomes, including survival, in mice rendered septic in the cecal ligation and puncture (CLP) model; this was attributed to Fc-dependent activation of platelets and immune cells, as well as complement activation. DG-KKO, which has a reduced capacity to activate platelets and fix complement, blocked NET degradation and the release of toxic NET products, but, in contrast to KKO, it improved survival of CLP-treated mice. These studies are important because they provide new information concerning mechanisms of NET-induced toxicity. In particular, they suggest that in sepsis, at least in the murine CLP model, reduction of NDP levels and their well-described systemic toxicities plays an important role in improving outcomes, despite stabilization and persistence of NETs themselves. This hypothesis is consistent with human studies, in which levels of neutrophil-derived circulating cell-free DNA correlate with the multiple organ dysfunction score and other prognostic measurements of sepsis outcomes,[8] and levels of circulating DNA-myeloperoxidase complexes correlate with 28-day survival rates in septic patients.[9] In addition, Gollomp et al's data