enthusiastic to find a correlation between their findings and the outcomes of patients. Numerous prognostic markers have been described in different types of lymphoma. Because of the cost and complexity of their experiments, the number of patients included in each group is limited. These markers have to be correlated with other clinical data in large cohort studies to be sure that they are representative of the disease. There has not been a study of similar magnitude evaluating the International Prognostic Index (IPI) specifically in PTCLs; however, several retrospective studies have demonstrated that the clinical IPI is a useful prognostic tool in some PTCL subtypes. The IPI or modified IPI can be effective in identifying different prognostic risk groups in PTCL-U, ALK + ALCL, and NK/T lymphoma. The IPI was not useful in AITL,[5] and with the peculiar presentation of AILT, the finding that host/tumor interactions have a significant impact on survival makes sense. In fact, as T-cell lymphoma generally has a poor prognosis, the distinction in the high-risk population between poor and very poor becomes somewhat meaningless.

From this point of view, the most important point is how the biology can identify therapeutic targets for new drugs. We have not made significant progress in the therapy of T-cell lymphoma with conventional treatment, with only 20% to 30% of patients surviving 5 years. More encouraging survival data have been reported with autologous or allogeneic stem cell transplantation.[6] Based on

data from this paper, several pathways should be explored in therapy for AITL, such as inhibition of the NF-kB pathway, the anti-angiogenic pathway, microenvironment, and anti-IL6 blockade. Although some clinical studies are ongoing, the road is still long to find the right targets for PTCL. It is time to design prospective studies on T-cell lymphoma that incorporate up-front frozen material to ensure molecular characterization. This study is a step in that direction.

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● ● ● **CLINICAL TRIALS**

Comment on Heslop et al, page 925

EBV meets its match

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EBV-driven lymphoproliferative disease remains a significant clinical problem following HSCT. In this issue of *Blood*, Heslop and colleagues report their 15-year experience using EBV-specific T cells to prevent or treat this disease.[1]

Posttransplantation lymphoproliferative disease (PTLD) in this setting usually arises through Epstein-Barr virus (EBV)–transformed B lymphocytes expanding opportunistically in the T cell–compromised host. Disease risk increases with the degree of T-cell impairment; thus, T-cell depletion of the graft or using incompletely human leukocyte antigen

(HLA)–matched grafts that require greater immunosuppressive therapy will increase PTLD incidence, especially during the first 2 years after transplantation.[2] In most such cases of PTLD, the proliferating cells express the full spectrum of EBV-latent proteins. Collectively, these proteins not only drive cell growth but also constitute the target antigens through which, in

healthy EBV carriers, virus-transformed cells are recognized and destroyed by virus-specific T-cell surveillance. Accordingly, PTLD provides an ideal opportunity for the application of adoptive cell therapy using EBV-specific T cells.

Early attempts to treat PTLD using total peripheral blood mononuclear cells from the original, EBV-immune stem cell donor were marred by collateral graft-versus-host disease (GVHD).[3] Showing real forethought, Heslop, Rooney, and colleagues took existing laboratory protocols for the in vitro generation of EBV-specific cytotoxic T-cell (EBV-CTL) preparations, adapted them for the clinic, and, by 1995 (a year before others showed proof of principle in a SCID mouse model[4]) had completed their first small trial demonstrating effectiveness against PTLD.[5] The present report summarizes the wealth of clinical data that have been amassed in 3 clinical centers since that time.

With a median follow-up of 10 years, the authors report a total of 114 patients (mean age of 8 years; range, 0.5-38 years) who have received EBV-CTLs following hematopoietic stem cell transplantation (HSCT). Of these, 101 patients deemed to be at high risk for PTLD (through having a T cell– depleted graft, greater donor/recipient HLA disparity, or a history of immune deficiency) received T cells prophylactically. Such treatment led to a fall in EBV genome load in the circulating B-cell pool; more importantly, none of these patients developed PTLD, compared with an observed incidence of 11% in a control cohort receiving similarly T cell–depleted allografts. The remaining 13 patients received EBV-CTLs therapeutically for either biopsy-proven EBV-positive PTLD or probable disease diagnosed on radiologic and clinical grounds. Complete responses were confirmed in 11 of 13 patients, some with extensive extranodal disease including 1 patient with biopsy-proven monoclonal lesions in the central nervous system. Dramatic inflammatory reactions at the site of disease (shown to be mediated by infiltrating EBV-CTLs) occurred in 2 patients, both of whom fully recovered, but no other toxicity or GVHD attributable to EBV-CTLs was observed.

Interestingly, 26 patients received EBV-CTLs genetically marked with a retroviral vector encoding the neomycin resistance gene, enabling in vivo tracking of the number, durability, and safety of the infused cells. The authors now show that these gene-marked cells can persist in the circulating memory pool for as long as 105 months after CTL infusion and

can still expand in vitro in response to EBV antigen challenge. Importantly from a safety perspective, extensive PCR analysis of retroviral integration sites did not reveal any evidence for the emergence of dominant T-cell clones within these gene-marked populations.

Overall, this study clearly demonstrates the long-term safety and efficacy of EBV-CTL infusion in a post-HSCT setting. Nevertheless, questions as to its optimal role still remain, particularly since the advent of an effective alternative in the form of anti-CD20 monoclonal antibody treatment for PTLD.[6] Clearly EBV-CTLs can still fill an important niche, for example, in patients who do not respond to or relapse after antibody treatment. Methodological improvements have now shortened the time required to manufacture a CTL product from a specific donor, and the attractive concept of an "off-the-shelf" CTL product, from third-party HLA-compatible donors, has also been developed into an effective reality in the context of solid-organ transplantation.[7] Furthermore, the engineering of EBV-CTLs resistant to calcineurin inhibitors is an advance for those patients who require ongoing immunosuppressive therapy.[8]

However, the greater importance of this work currently lies in its heuristic value for the field of adoptive T-cell therapy, whether one is seeking to target other viral infections of the immunocompromised or other virus-associated malignancies. In this latter context, EBV-positive lymphomas such as Hodgkin and T/NK-cell lymphomas, where viral target antigen expression is much more limited, constitute the next battleground.[9] Looking further ahead, might T-cell preparations against such a common human virus be exploited for even greater

therapeutic benefit? That is as recipients of additional reactivities, conferred by T cell–receptor transfer in vitro, where the second specificity is directed against a target antigen of choice while the native EBV specificity guarantees retention of the cells in the virus-carrying host.

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● ● ● **PLATELETS & THROMBOPOIESIS**

The tale of two COXs

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In this issue of *Blood*, Dragani and colleagues provide evidence in ET for increased reticulated platelets and for a role of COX-2 in the enhanced thromboxane A_2 production. These findings highlight the need to rethink the optimum antithrombotic regimens in ET and other states with accelerated platelet generation.

A key feature of platelet biology in essential thrombocythemia (ET) patients is increased thromboxane production, as shown by enhanced excretion of the urinary metabolite of thromboxane A_2. These patients have an increased incidence of arterial thrombotic events. The most common therapy has been low-dose aspirin in addition to cytoreduction. The platelet mechanisms involved in the thrombotic events and the optimum therapeutic approaches remain unknown. The studies of Dragani et al shed new insights on these areas.[1]

Cyclooxygenase-1 (COX-1) and COX-2 isozymes catalyze the conversion of arachidonic acid to prostaglandin $(PG)H_2$, the initial step in prostanoid synthesis leading to the formation of several products including thromboxane A_2, PGE_2, and PGI_2. Aspirin acetylates Ser529 in human COX-1 and Ser516 in human COX-2. When given once daily at low doses, aspirin almost completely inhibits COX-1 in platelets, which lack the ability to replenish the irreversibly inactivated enzyme. Much higher doses are needed to inhibit COX-2, an inducible enzyme; this is influenced also by the ability of nucleated cells to rapidly regenerate the enzyme.

Under physiologic conditions, platelet thromboxane production is primarily driven by COX-1 and endogenous PGI_2 by COX-2. Less than 10% of normal platelets have COX-2.[2] Recent studies show that COX-2 is present in megakaryocytes, up-regulated during megakaryopoiesis, and expressed in young platelets.[2] These findings have drawn attention to the potential contribution of COX-2 to thromboxane production in diverse clinical conditions. Dragani et al show convincingly that younger thiazole-staining reticulated platelets are increased, indicating enhanced platelet generation, and that platelet COX-2 expression is up-regulated in ET patients.[1] This is also described in other states associated with increased platelet generation.[2-4] Dragani et al show that aspirin at doses that almost completely suppress thromboxane production in healthy subjects is not as effective in ET. In an open-label randomized study in ET patients who are taking daily aspirin, the authors show that the addition of a COX-2 inhibitor to aspirin decreases by approximately 30% both urinary excretion of a thromboxane metabolite (marker of endogenous production) and ex vivo thromboxane production in serum (measure of total synthetic capacity). These studies corroborate that COX-2 contributes to endogenous thromboxane production in ET. Of note, the addition of the COX-2 inhibitor did not abolish thromboxane production, suggesting the role of additional factors.