therapeutic implications. Firstly, expression of *BAX* mutations conferred resistance not only to venetoclax, but also to other BCL2 inhibitors such as S55746 as well as to the MCL1 inhibitor S63845. These data suggest the possibility that BAX loss-of-function may underlie therapeutic resistance to multiple classes of BH3-mimetic compounds in the treatment of AML. Furthermore, *BAX* mutations were almost never seen at baseline in patients with AML or in the setting of relapse following cytotoxic chemotherapy, indicating that these mutations are acquired because of a selective advantage conferred in the setting of venetoclax therapy specifically. Consistently, BAX-deficient AML cells in vitro, although resistant to venetoclax and other BH3 mimetics, were still sensitive to conventional cytotoxic agents such as cytarabine or anthracyclines. These findings support the emerging concepts of combining venetoclax with cytotoxic chemotherapy, and it would be interesting to see if *BAX* mutations also arise in this drug combination that elicits distinct mechanisms of leukemic cell death. Lastly, it would be important to identify genetic and pharmacological vulnerabilities in BAX-deficient AML cells, with the goal of identifying novel targets for patients with AML upon relapse to venetoclax.

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TRANSPLANTATION

Comment on Patterson et al, page 659

Peritransplant CAR-T cells

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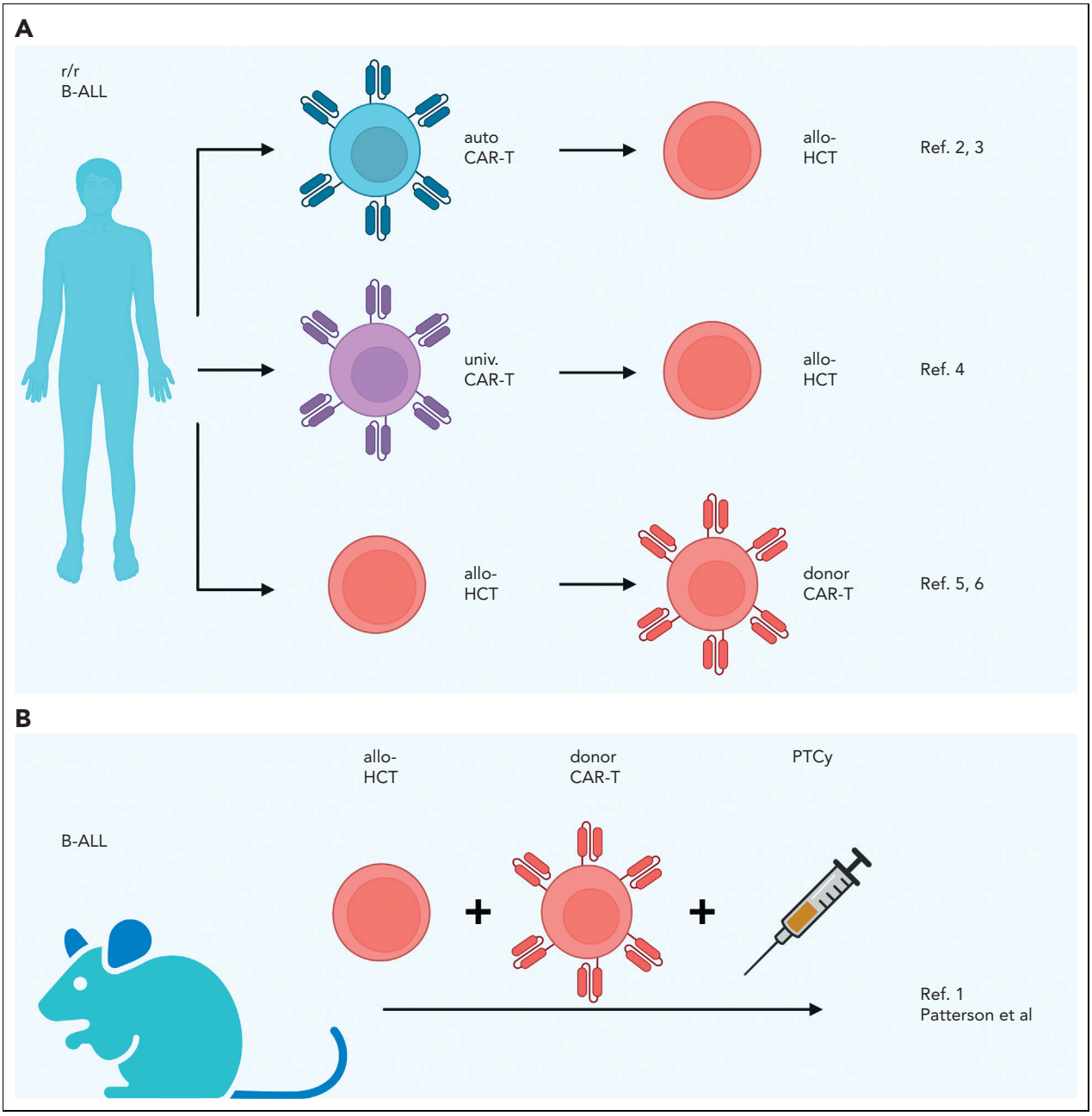
An increasing number of patients with relapsed or refractory B-cell leukemia and lymphoma receive both allogeneic hematopoietic cell transplantation (allo-HCT) and chimeric antigen receptor (CAR)-T cell therapy over the course of their disease. Yet little is known about the optimal sequence and how to best integrate these 2 treatment modalities. In this issue of *Blood*, Patterson et al[1] demonstrate in murine studies that allo-HCT with posttransplantation cyclophosphamide (PTCy) can effectively be combined with allogeneic CD19 CAR-T cell treatment. Surprisingly, CAR-T cells given just before or shortly after cyclophosphamide graft-versus-host disease (GVHD) prophylaxis exert stronger antileukemic effects than CAR-T cells administered later.

The many possibilities for combining allo-HCT and CAR-T cells to treat aggressive hematologic malignancies are currently best illustrated by relapsed or refractory B-cell acute lymphoblastic leukemia (B-ALL). Data from early clinical trials and clinical series in this patient population indicate that the following combined approaches may be safe and effective: first, consolidative allo-HCT in patients who have achieved complete remission after autologous CD19 CAR-T but remain at high risk of relapsing[2,3]; second, allo-HCT in patients who have responded to bridging therapy with universal CAR-T cells[4]; and third, donor CD19 CAR-T in patients who relapse after allo-HCT.[5,6] In their preclinical study, Patterson et al investigated a different integrated approach in which allo-HCT and donor CD19 CAR-T cells are given in short succession (see figure). To study how to prevent the allogeneic CAR-T cells from causing GVHD, they explored a major histocompatibility complex (MHC)-haploidentical allo-HCT model with PTCy prophylaxis. To discern the effects of PTCy on CAR-T cells, they developed a model of pre-B-ALL (E2a-PBX tumor cell line with a B6 background not expressing major histocompatibility antigens different from those expressed on either recipient or donor strain) in which only the donor CD19 CAR-T cells can exert an antileukemic effect but not the allogeneic



Combination strategies for allogeneic hematopoietic cell transplantation and CAR-T cells previously reported (A) and developed by Patterson et al (B). allo, allogeneic; auto, autologous; r/r, relapsed or refractory; univ., universal.

T cells in the graft. Testing different administration schedules, they found CAR-T cells given before or shortly after PTCy eradicate leukemia more efficiently than CAR-T cells given later after PTCy. The finding that direct in vivo exposure of the CAR-T cells to cyclophosphamide not only spares but enhances their antileukemic effect was unexpected and prompted the authors to study the possible underlying mechanisms. The investigators found that administration of CAR-T cells before PTCy resulted in earlier CAR-T cell expansion, higher phenotypic CAR-T cell activation, and fewer CAR-T regulatory cells compared with administration after PTCy. In addition, transcriptional profiling indicated increased activation of $CD4^+$ CAR-T cells and more cytotoxic $CD8^+$ CAR-T cells for CAR-T cell application before PTCy as opposed to a later application. A possible explanation for this observed PTCy effect is that it transiently rests the CAR-T cells, resulting in better long-term antileukemia potency, as reported for other rest-inducing modalities in CAR-T cells.[7]

A few critical points remain that will be relevant for a future clinical translation of this approach combining allo-HCT and donor CAR-T cells. A limitation of the MHC-haploidentical HCT model used is that the CAR-T cells themselves do not induce GVHD. Although the data clearly indicate that PTCy does not ablate the CAR-T cells' antileukemia effect, it remains to be assessed whether PTCy can prevent human donor CAR-T cells from inducing GVHD. Furthermore, the authors used a CD19 CAR with a CD28 costimulatory domain throughout the study.

Because the potential of donor-derived CD19 CAR-T cells to induce GVHD has been shown to differ depending on their costimulatory domain, it is important to evaluate the feasibility of integrating CAR-T cells with different costimulatory domains into allo-HCT strategies with PTCy prophylaxis in future studies.[8]

In conclusion, this study identifies a promising strategy for leveraging the complementary antileukemic effects of polyclonal alloreactive T cells and antigen-specific CAR-T cells. The data support the clinical translation of allo-HCT with PTCy in combination with donor CD19 CAR-T cells to evaluate its safety and efficacy. Integration of these 2 immune-based approaches shows potential to advance relapse prevention and treatment for patients with aggressive hematologic malignancies.

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