These strategies require a large HLA-typed donor pool (~10 000 typed donors), and even then, there is no guarantee that full matching for patients with broad immunization or with rare haplotypes will be achieved. In addition, these approaches are time-consuming and expensive. Other strategies that have been suggested but which require further validation include the use of acid-treated platelets[8] or the use of platelets with consistently low expression of specific HLA class I antigens such as HLA-B8, -B12, or -B35 despite HLA mismatches.[9] Instead of matching at the antigen level (HSM platelets), it has been hypothesized that it may be more feasible to match at the epitope level (HEM platelets), which takes the characterization of short sequences of amino acids from linear or discontinuous regions of the HLA molecule into account (see figure). This approach may be more efficient because it would circumvent the need to maintain a large HLA-typed donor pool and reduce the costs. Indeed, HEM platelet transfusions have previously been described to improve PCIs. However, all of these studies have been retrospective and they lacked clinical outcomes (Marsh et al, supplemental Table 1). Therefore, Marsh et al undertook the first prospective, randomized, double-blind, noninferiority, crossover trial directly comparing HEM vs HSM platelet transfusions in 49 alloimmunized thrombocytopenic patients (acute myeloid leukemia, n = 26; aplastic anemia, n = 14; myelodysplastic syndrome, n = 9). Platelet refractoriness was defined as failure to achieve a 10-minute to 1-hour posttransfusion PCI of $>5 \times 10^9$/L on 2 successive occasions, using ABO-compatible fresh platelets <72 hours old. The patients received up to 8 prophylactic HEM and HSM platelet transfusions that were randomly administered. In the study, 219 adequate platelet transfusions were evaluated (HEM, n = 107; HSM, n = 112). The primary outcome of the trial was 1-hour posttransfusion PCI. Importantly, no significant differences were observed in the 1-hour PCI posttransfusion between the 2 groups. HEM platelet transfusions were concluded to be noninferior to HSM platelet transfusions. Furthermore, there were no differences in the secondary outcomes of bleeding events, platelet counts, and transfusion requirements. It was also found that for every additional 1-epitope mismatch, the probability of an adequate PCI decreased by 15%. One limitation of the study, however, is the relatively small number of patients who received at least 8 evaluable transfusions of 4 HSM and 4 HEM platelet transfusions (n = 14 of 49 randomly assigned patients).

In conclusion, Marsh et al address an important issue and significantly push the field forward by conducting the first prospective randomized controlled study of HEM platelet transfusions. They find HEM platelet transfusions to be no worse than HSM platelet transfusions in increasing posttransfusion platelet counts. Because HEM platelet transfusions are also associated with reduced costs and resource requirements, they should be considered as a potential alternative tool for managing HLA alloimmunized patients with platelet refractoriness. Larger prospective randomized studies are now warranted to further validate these promising results.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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GENE THERAPY

Comment on Gauthier et al, page 323

A second CD19 CAR T-cell infusion: yes or no?

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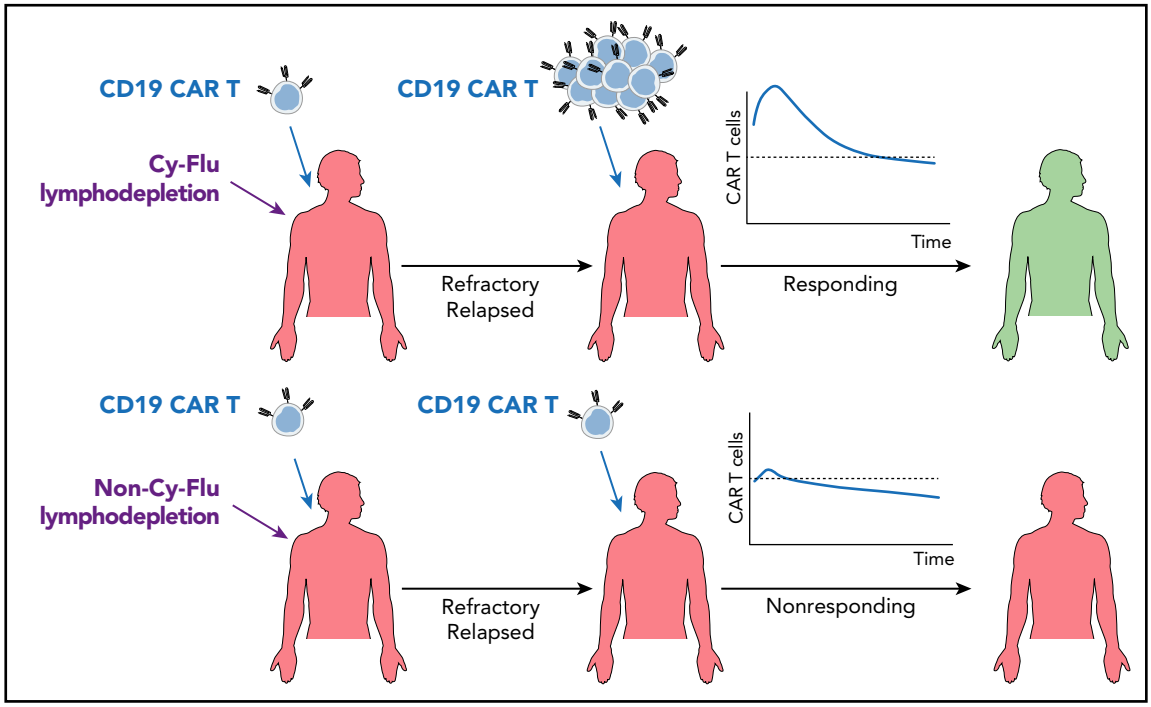
In this issue of *Blood*, Gauthier et al retrospectively analyzed the outcome of a second infusion of CD19 chimeric antigen receptor (CAR) T cells in patients with B-cell malignancies who relapsed or were refractory to the first infusion. The authors reported durable responses in a significant proportion of patients, with a low incidence of severe toxicity. They also identified actionable pretreatment factors associated with positive outcomes.[1]

CD19 CAR T-cell therapy has considerably changed the landscape of treatment options for B-cell malignancies. This therapy was developed as a single infusion of CAR T cells for individuals with relapsed/refractory diseases. However, the frequency of patients who fail to respond or eventually relapse after a partial or complete response is still high. The efficacy of a second infusion in patients unable to achieve durable remissions is still controversial,[2,3] and systematic analysis addressing specific clinical and biological factors in this unique setting has been missing.



Schematic illustration of the outcomes of a second infusion of CD19 CAR T cells in patients who failed to adequately respond to the first infusion. The addition of Cy-Flu lymphodepletion before the first infusion and an increased CAR T-cell dose for the second infusion are independently associated with durable responses.

Gauthier et al here describe the first study entirely dedicated to this topic and including the largest cohort of patients to date (n = 44). Responses were achieved in ~39% of patients (complete responses, 20%), irrespective of the refractory or relapsed status. However, there were differences across disease types. Despite a higher CAR T-cell dose employed for the second infusion compared with the first, relatively low rates of severe cytokine release syndrome (CRS) and neurotoxicity were reported (9% and 11%, respectively). Multivariable analyses revealed that durable responses were associated with the addition of fludarabine to cyclophosphamide-based (Cy-Flu) lymphodepletion before the first infusion and an increased CAR T-cell dose for the second round of treatment (see figure).

Extensive clinical experience has indicated that CAR T-cell expansion and persistence are required to achieve durable responses. This concept was confirmed here in the setting of a second CAR T-cell infusion. Indeed, Gauthier et al reported that responding patients had higher CAR T-cell peak expansion and longer persistence compared with nonresponding individuals. These results confirm that optimizing these features is mandatory to maximize efficacy. CAR T-cell fitness can be shaped by multiple factors. The first is the intrinsic "quality" of T cells retrieved from patients, which depends on age, tumor histology, and the extent of previous treatments.[4] The second parameter is the manufacturing platform, which has evolved over time to promote the enrichment of engineered T cells with stem and central memory phenotypes, endowed with improved proliferative and self-renewal capabilities. Accordingly, responses in chronic lymphocytic leukemia patients were found enriched in gene expression profiles associated with early memory T cells.[5] Besides T-cell differentiation, the CD4 and CD8 CAR T-cell composition was also shown to matter. Indeed, the key and synergistic contribution of both subsets in CAR T-cell–mediated responses is becoming clearer. The same group has already shown that formulating the final product at a 1:1 $CD4^+$ to $CD8^+$ CAR T-cell ratio results in superior efficacy and reduced toxicity.[2,6] Maintaining this ratio in the product can explain the low rates of severe CRS and neurotoxicity reported in this work. The third crucial aspect modulating CAR T-cell performances is the host environment, including tumor-derived immunosuppressive signals, homeostatic cytokines, and antitransgene immune reactions that may prematurely clear engineered T cells. In this article, Gauthier et al point to the crucial impact of lymphodepleting regimens on the host environment, with critical consequences on CAR T-cell dynamics in vivo. The same team has previously reported that Cy-Flu lymphodepletion was associated with better responses.[2,7] Consistently, in this work, they show that even the second round of treatment benefits from applying Cy-Flu before the first infusion. Cy-Flu lymphodepletion resulted in higher CAR T-cell expansion, persistence, and therapeutic efficacy. Besides the potential effect on homeostatic cytokines,[8] the data presented suggest that this may be due to a negative impact of Cy-Flu on the priming of endogenous T-cell responses against CAR T cells. The need to avoid premature CAR T-cell clearance may also explain why better therapeutic outcomes were observed with increased CAR T-cell doses. Overall, this study supports the relevance of proper lymphodepleting regimens and fully humanized CAR constructs, especially when planning multiple infusions, which may be the case when treating of solid malignancies.

Understanding why the first infusion failed while the second succeeded in inducing durable remissions can help to design strategies that maximize efficacy.

In this work, both CAR T-cell infusions employed cellular products derived from the same leukapheresis, suggesting that differential responses cannot be ascribed to intrinsic T-cell defects. Similarly, the protocol used for CAR T-cell manufacturing was the same, suggesting a negligible role for this aspect in explaining the outcomes. Alternatively, differences in the peculiar host environment and disease status that preceded the 2 infusions may impact the outcomes. Clinical trials designed to address the beneficial effect of a second CAR T-cell infusion should explore this crucial aspect.

Assuming that an allogeneic hematopoietic stem cell transplantation is recommended to consolidate long-term responses, this article shows that a second CAR T-cell infusion may help more patients achieve a favorable outcome. In this scenario, additional clinical factors will potentially impact treatment decisions. Tumor CD19 antigen expression profiling after the first treatment failure may impact the decision about whether to proceed with a second infusion of the same CAR T-cell product or opt for an alternative specificity, such as CD22.[6,9]

It has been recently reported that infusing CD22 CAR T cells in patients who achieved remission after CD19 CAR T-cell therapy is effective in consolidating responses,[10] but a formal comparison of a second infusion of CD19 CAR T cells with CD22 CAR T cells will clarify the cost-benefit ratio of this approach.

In summary, despite its retrospective nature and the small size of the patient cohort for each disease entity, this study will contribute to the design of future clinical trials for heavily pretreated patients who fail first CAR T-cell immunotherapy.

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HEMATOPOIESIS AND STEM CELLS

Comment on Okamoto et al, page 336

Fanconi anemia, put to sleep

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In this issue of *Blood*, Okamoto et al describe how reduced levels of *Schlafen* protein SLFN11 can rescue the DNA damage sensitivity in a Fanconi anemia (FA) cell line. They propose SLFN11 as a potential target for treatment of FA.[1]

FA is the most common inherited bone marrow failure syndrome and is also associated with an elevated predisposition to cancer. All of the phenotypes of FA are associated with unrepaired DNA interstrand crosslink (ICL) damage. Thus, FA cells are hypersensitive to endogenous byproducts of metabolism such as formaldehyde[2] or chemical agents such as cisplatin that act to covalently crosslink the 2 strands of DNA. ICLs are a potent barrier to DNA replication because the strands cannot be separated for duplication. This leads to stalled replication forks (see figure). In FA cells, something else happens; not only is replication blocked by the ICL, but newly synthesized DNA adjacent to the replication fork becomes actively degraded.[3] For this reason, it is thought that a major cellular function of FA gene products is fork protection.[4]

Although the exact mechanics of fork protection are enigmatic, they center on 2 processes. One process is the monoubiquitination-induced clamping of FANCD2:FANCI at DNA adjacent to the fork.[5] The other process is RAD51-, BRCA1-, and BRCA2-dependent homologous recombination–mediated stabilization of the recently synthesized DNA strands.[6] One or both of these processes are absent in all cases of FA,[4] meaning that stalled forks are no longer protected. DNA damage then accumulates, and the cells arrest in G_2 phase because of incomplete DNA replication.

But what exactly do the stalled forks need protection from? The main bad guys are nucleases, of which there are 2 kinds: endonucleases such as MRE11 and MUS81 that cut internally to DNA, and exonucleases such as DNA2 or EXO1 that chew up DNA from exposed ends. This is a problem because nuclease-mediated degradation leads to permanent loss of genetic information (which can drive either cell death or cancer-causing mutations, depending on the context of the genes affected). Importantly, short interfering RNAs or chemical inhibition of MRE11 or DNA2 nucleases can significantly reduce the degradation of stalled forks and