

To Bridge, Blossom, or Boost: That Is the Question

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(See the Major Article by Zhao et al on pages 1429–37.)

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When cytomegalovirus (CMV) was nicknamed the “troll of transplantation” in 1979, diagnostics were still rather primitive and no effective therapy was yet available [1]. Today, with adequate screening tools and effective, albeit toxic, antiviral drugs at our disposal, CMV still deserves that pejorative title. Especially after allogeneic hematopoietic stem cell transplantation (HSCT), with the associated high risk of graft-vs-host disease (GVHD), CMV remains a significant cause of morbidity and mortality [2–5]. Current strategies vary from CMV prophylaxis to preemptive therapy, but ultimately the virus must be controlled by the immune system. Treatment failures and toxicity of antiviral drugs have led to studies using adoptive transfer of donor-derived CMV-specific T cells (ATC) to restore immunity to CMV. Clinical studies, all phase 1/2, varied regarding inclusion criteria, manufacturing protocols, and timing of ATC therapy. However, ATC therapy was consistently safe with no excess GVHD and was

generally suggested to be effective on the part of the recipients.

In this issue of *Clinical Infectious Diseases*, Zhao and colleagues report on a clinical phase 1/2 study of preemptive ATC therapy after allogeneic haploidentical stem cell transplantation, and on data from a humanized mouse model [6]. The results indicated that early ATC was safe and lowered the risk of late or persistent CMV infection compared to a high-risk control group. However, CMV infection in the setting of stem cell transplantation is a complex problem in which many factors play a role. Even though no single factor will determine the outcome, a concise discussion of the elements will reveal a pattern, within which the added value of the study by Zhao can be estimated.

CMV SEROSTATUS

Donor/recipient CMV serostatus is an important determinant of the risk of CMV infection after allogeneic HSCT. Leaving apart D⁻/R⁻ recipients, the risk increases steeply from D⁺/R⁻ to D⁺/R⁺, with the highest risk for D⁻/R⁺ transplantations [7]. The study by Zhao et al was limited to concordant seropositive donor/recipient pairs, thus a high-risk group.

D⁻/R⁺ recipients are not eligible for studies with donor-derived ATC, although they have the highest risk of CMV infection and disease [8]. The risk is high because the graft only contains undifferentiated progenitor cells that still need to undergo thymic programming

to naive CMV-specific T cells, but no memory pool that can rapidly expand if so required. To overcome this problem, use of third-party ATC “off the shelf” or production of CMV-specific T cell receptor (TCR) transgenic cells were studied [8–10].

CONDITIONING AND T-CELL DEPLETION

Myeloablative conditioning will more effectively eliminate memory T-cell populations in the graft recipient. In contrast, nonmyeloablative conditioning allows participation of recipient T cells in the immune response to CMV. In the study by Zhao et al, the conditioning regimen could be either type but was not explicitly reported.

It is generally thought that a stem cell graft consists of stem cells but, in fact, only a few percent are actually (CD34⁺) progenitor cells; the remainder consist of the peripheral blood repertoire of mononuclear cells, of which roughly half are T cells. Thus, undepleted grafts contain CMV-specific memory T cells, which occur with high frequency in healthy CMV-positive adults.

Some haploidentical transplant protocols use nondepleted grafts and treatment of the recipient with, for example, antithymocyte globulin, as in the study by Zhao et al; others use T-cell depletion of the graft, for example, by alemtuzumab (anti-CD52) “in the bag.” In both cases, the depleting antibody ends up in the recipient and will exert its effect for at least 6 weeks,

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making ATC in this period ineffective. Nevertheless, some cells escape elimination, implicating that memory T cells from the donor can be available to the recipient already at the time of transplantation. This might explain why some patients at very high risk still never develop CMV complications.

DONOR LYMPHOCYTE INFUSION

Transplant protocols may include donor lymphocyte infusion (DLI) several months after transplantation, aimed at a graft-vs-leukemia effect. The high risk of GVHD following DLI is mainly explained by the presence of naive alloreactive cells. However, DLI also contains the whole repertoire of memory T cells of the donor, including CMV-specific cells. Because DLI was an exclusion criterion in the study by Zhao et al, it cannot have contributed to antiviral immunity.

HUMAN LEUKOCYTE ANTIGEN MATCHING

In the study by Zhao et al, only recipients of haploidentical grafts were included, thus at high risk of GVHD and thereby of immunosuppression-induced CMV reactivation. ATC was produced from the original stem cell donor and therefore also haploidentical. However, T cells restricted to a specific human leukocyte antigen (HLA)–peptide combination, of which the HLA molecule is absent in the recipient, are useless from a functional point of view as these cells will never encounter their cognate target in the recipient. This “useless crowd” can even be disproportionally large; for example, HLA-B7–restricted CMV-specific T-cell responses are notoriously dominant. Thus, if the donor is HLA-B7 positive whereas the recipient is not, ATC therapy will be largely off-target. This might explain the failure of ATC in certain donor/recipient pairs.

GRAFT-VERSUS-HOST DISEASE

CMV infection and GVHD are intricately related [11]. In all previous studies

of ATC therapy, patients with clinically significant GVHD requiring immunosuppressive therapy were excluded. In contrast, the occurrence of GVHD before CMV reactivation was the main selection criterion in the study by Zhao et al. All (haploidentical) graft recipients received GVHD prophylaxis and part of the ATC-treated cohort used corticosteroid doses up to 0.5 mg/kg at the time of ATC, but still no excess GVHD occurred following ATC infusion. The safety of ATC in the setting of GVHD is an important novel finding. However, given the proapoptotic effect of steroids on lymphocytes, it is unclear to what extent the adoptively transferred cells persisted in patients using steroids. With the aim to overcome this problem, an interesting preclinical study describes protection of ATC from the proapoptotic effects of corticosteroids by ingenious genetical engineering [12].

ANTIGEN REPERTOIRE AND FUNCTIONALITY OF ATC

The antiviral effect of ATC depends on the composition, which is determined by the production protocol. Until now, the number of different protocols used roughly equals the number of studies done. In a nutshell, early studies used tetramer-based selection and extensive in vitro stimulation, resulting in terminally differentiated, effector CD8⁺ T cells, which serve to temporarily bridge a period with low immunity [13]. Later studies used more broad antigen panels and selection based on interferon- γ production, resulting in T-cell lines containing both CD4⁺ and CD8⁺ cells targeting a broader antigen repertoire and proliferating in vivo [14–18]. The trade-off between effector function and replicative potential of T cells has been demonstrated [19].

In the study by Zhao et al, ATC was produced by stimulation with a high concentration of cytokines and anti-CD3 (without costimulation), followed by 2 rounds of peptide stimulation. We believe this led to mainly terminally differentiated effector cells that had lost

reproductive potential. However, the main study result was a lower rate of late and persistent CMV. Based on data from the mouse model and TCR spectratyping in 3 patients, this effect was purportedly caused by enhanced recovery of graft-derived cell-mediated immunity rather than persistence of ATC. The mechanism of this boosting effect was not studied, but T-helper function of the minority of CD4⁺ T cells in the ATC might have caused this effect.

TIMING

Manufacturing of ATC takes time and is costly, so selection of candidates and timing of ATC production is critical. In the study by Zhao et al, only patients with GVHD before CMV reactivation were eligible. ATC was used as early first-line therapy together with antiviral drugs. The authors did not report on general T-cell recovery, but it is notable that 14 of 35 recipients of ATC had already cleared CMV within 1 week after ATC therapy [6]. This suggests that graft-dependent immune reconstitution was already ongoing and that ATC may not have been contributive in this subgroup.

TO BRIDGE, BLOSSOM, OR BOOST—AND DOES IT MATTER?

Figure 1 provides a simplified conceptual model of the possible different components of ATC and the period during which it may be effective. For containment of actively replicating CMV, CD8⁺ cytotoxic T cells with an immediate effector function are needed (bridge). In a less urgent setting, cells with a broad repertoire and proliferative potential are needed, and part of these may even persist as memory pool (blossom). The study by Zhao et al now suggests a third mechanism: stimulation of CMV-specific responses by the graft (boost) [6].

To discriminate reliably between adoptively transferred and graft-derived CMV-specific T cells is a challenge because they originate from same donor. From a practical point of view, it probably makes no difference whether the

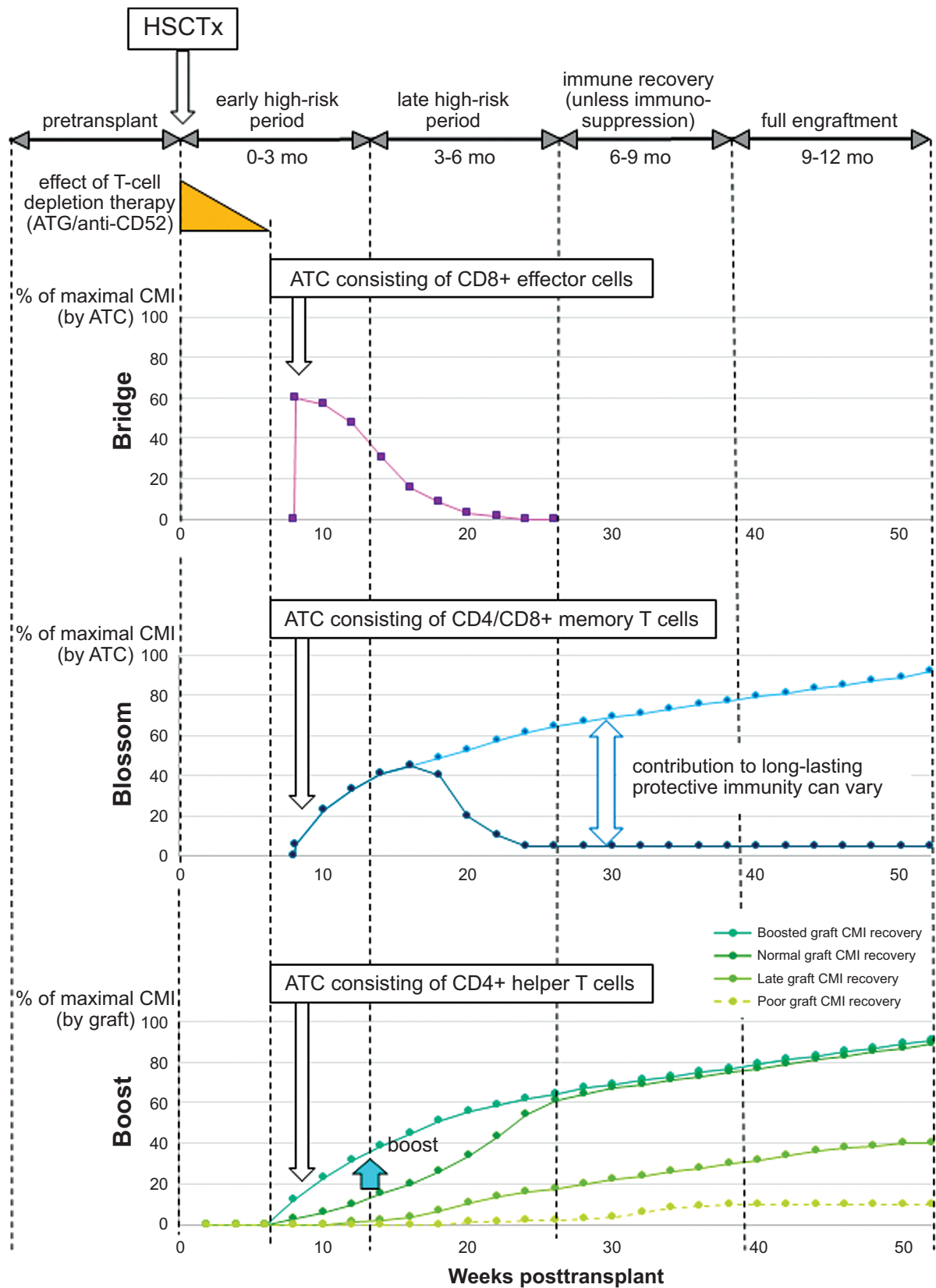


Figure 1. Simplified conceptual model of the possible contributions of adoptive cytomegalovirus (CMV)-specific T-cell therapy to posttransplantation anti-CMV immunity. All cell types mentioned in the graphs represent CMV-specific T cells. Abbreviations: ATC, adoptive T-cell therapy with cytomegalovirus-specific T cells; ATG, antithymocyte globulin; CMI, cell-mediated immunity; CMV, cytomegalovirus; HSCTx, hematopoietic stem cell transplantation.

clinical effect results from ATC or from enhanced graft function, as long as durable protective immunity is achieved. Nevertheless, further studies of the mechanisms that lead to recovery of cell-mediated immunity are justified. These can help to optimize ATC manufacturing, resulting in effector cells that can bridge an immune gap, early memory (CD62L⁺) T cells with potential for in vivo expansion, and/or helper T cells to boost graft function, as desired. ATC with multivirus specificity holds even more promise [16, 20].

At present, implementation of ATC therapy awaits a randomized phase 3 trial, which will start this year as part of the Horizon 2020 project TRACE (EudraCT number 2018-000853-29). Other developments include US Food and Drug Administration approval for prophylactic use of letermovir, a nontoxic oral drug active against CMV [21]. A possible drawback may be development of resistance due to a low genetic barrier [22]. Several studies evaluated monitoring of CMV-specific immunity [23, 24]. Finally, novel strategies to prevent GVHD without immunosuppression are under investigation.

In conclusion, the study by Zhao et al indicates that ATC was safe when deployed as first-line therapy in patients with GVHD followed by CMV reactivation. The data further tentatively suggest enhanced recovery of graft-dependent CMV-specific immunity.

In *Hamlet*, Act III, Scene I, William Shakespeare (1564–1616) wrote:

To be, or not to be: that is the question:
Whether 'tis nobler in the mind to suffer
The slings and arrows of outrageous fortune,

Or to take arms against a sea of troubles,

And by opposing end them?

This soliloquy now seems surprisingly appropriate to CMV as posttransplantation trouble-maker, with ATC as arms to oppose the problem. In our model, different arms perform different roles in the battle. Further studies that lead to recipes for ATC with dedicated functions may help to improve the prognosis of our transplant recipients.

Note

Potential conflicts of interest. The authors: No reported conflicts of interest. Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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