

of AML, opening up new avenues for therapeutic intervention in AML.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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<https://doi.org/10.1182/blood.2022019244>

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TRANSPLANTATION

Comment on *Lohmeyer et al*, page 1755

New mechanisms of GVHD suppression by Tregs

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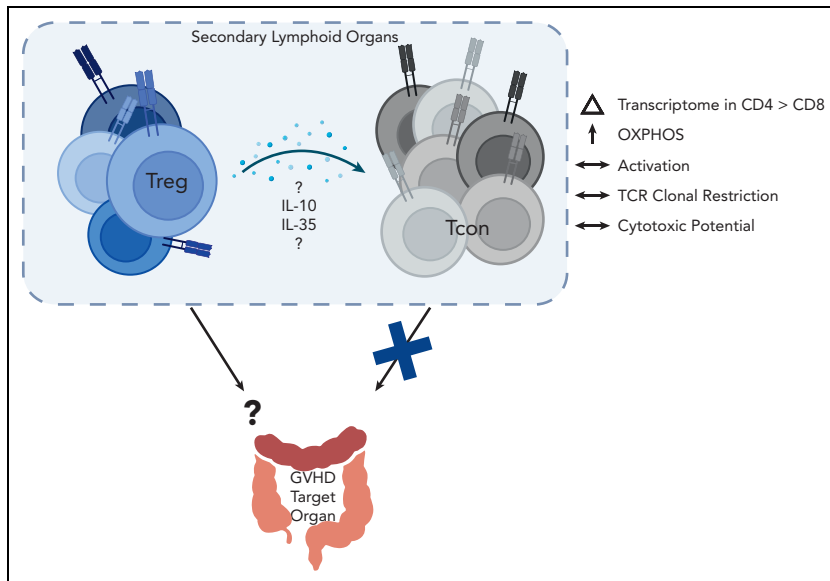
In this issue of *Blood*, Lohmeyer et al uncover how regulatory T cells (Tregs) affect the transcriptomic, metabolic, and functional states of pathogenic effector T cells to blunt graft-versus-host disease (GVHD) in mouse models of allogeneic hematopoietic cell transplantation (allo-HCT).¹

The infusion of Tregs has emerged as an attractive approach to mitigate GVHD, which is the main life-threatening and life-altering immune complication of allo-HCT. In preclinical mouse models, Tregs prevent severe GVHD without eliminating the potent graft-versus-tumor effects of allo-HCT, a desirable pattern of immunomodulation that has inspired clinical translation.²⁻⁴ Recent reports in patients include promising strategies of early Treg administration ahead of conventional T cells (Tcons) that mediate GVHD, in some cases without any pharmacological immunosuppression.⁵ Yet, it remains unknown how Tregs keep pathogenic T cells on a tight leash to achieve beneficial immunomodulation in vivo.

To gain new insights, Lohmeyer et al used a well-established mouse model of major histocompatibility complex (MHC)-mismatched allo-HCT, deploying a combination of transcriptional profiling and T-cell receptor (TCR) clonality index analysis in Tregs vs CD4⁺ and CD8⁺ Tcons purified from syngeneic or allo-HCT recipients (see [figure](#)).¹ Key comparisons focused on Tcons transplanted in the presence or absence of Tregs, and on Tregs before and after transplantation. Two nonmutually exclusive models of protection were considered: a quantitative impact of Tregs on the activation of alloreactive T-cell clones and/or qualitative effects on their pathogenic functions. The authors observed an

increased clonality index both in Tcons and in Tregs after transplantation into allogeneic as compared with syngeneic hosts, consistent with alloantigen-driven selection of a restricted TCR repertoire in both populations. Interestingly, Treg administration did not affect the clonality index of Tcon populations recovered from allo-HCT recipients. Instead, Tregs exerted transcriptional effects on CD4⁺ and, to a lesser extent, CD8⁺ Tcons, enhancing the expression of some anti-inflammatory and Th2 signature genes and favoring an oxidative phosphorylation program at the expense of glycolysis, among other changes. Paired transcriptomic analysis showed increased transcripts encoding interleukin-10 (IL-10) and IL-35 components in Tregs, as well as upregulated IL-10- and IL-35-mediated gene expression signatures in alloreactive Tcons exposed to Tregs in vivo, indicating that these pathways are potential mediators of GVHD suppression. Treg administration also correlated with decreased T-cell accumulation in the gut, a key GVHD target organ. Importantly, Tregs did not prevent alloantigen-driven Tcon activation or upregulated expression of cytotoxic effector genes essential for T-cell-mediated antitumor activity. Altogether, Lohmeyer et al made interesting predictions about Treg activation and functions after allo-HCTs that will need mechanistic testing and should inspire human investigations, particularly when Tregs are administered without interference from calcineurin inhibitors or other pharmacological agents.

In terms of the 2 models of Treg-mediated GVHD protection that the authors intended to test, the data were most consistent with a model implying qualitative effects of Tregs on Tcon pathogenic functions after transplantation rather than a quantitative impact on Tcon activation. Indeed, Treg administration preserved overall T-cell activation/differentiation per transcriptomic criteria and did not interfere with alloantigen-driven Tcon clonal restriction, thus maintaining the full breadth of the alloreactive Tcon repertoire. Together with the preserved induction of cytotoxic effector gene programs, the broad pool of alloreactive Tcon arising in the presence of Tregs may account for the previously reported capacity of Treg administration to spare beneficial graft-versus-tumor effects.²⁻⁴



Immunomodulatory functions of Tregs to control GVHD. In a mouse GVHD model, CD4⁺ and CD8⁺ Tcons profiled in secondary lymphoid organs were transcriptionally and metabolically altered upon Treg administration but showed preserved activation, TCR clonal restriction, and cytotoxic gene programs.¹ Treg-derived IL-10 and IL-35 emerged as candidate pathways to mediate these effects. The presence of Tregs also blunted T-cell accumulation in the gut, which is a key GVHD target organ. OXPHOS, oxidative phosphorylation.

In terms of Tregs, Lohmeyer et al observed an increased clonal restriction of the TCR repertoire after allo-HCT as well as profound transcriptional changes indicative of Treg activation. These findings suggest that host alloantigen-driven Treg activation and differentiation underly the activity of Tregs after allo-HCT, which is consistent with previous data showing that donor-derived or third-party— but not host-derived Tregs could prevent GVHD.⁶ However, Lohmeyer et al observed only a partial overlap in TCR sequences in donor-derived CD4⁺ Tcons and Tregs, suggesting that they may respond to different pools of MHC class II alloantigens (provided sampling and sequencing depth captured a representative repertoire).¹

Interestingly, Treg administration had a more profound transcriptional impact on CD4⁺ Tcons than on CD8⁺ Tcons.¹ It remains to be determined whether these findings reflect the unique features of the MHC-mismatched allo-HCT mouse model used in this study, in which GVHD pathogenesis is highly dependent on CD4⁺ T cells. Alternatively, it will be important to assess whether the dominant impact of Tregs on CD4⁺ Tcons extends to other preclinical GVHD models and/or human recipients of allo-HCT. Notably, this study

focused on the interaction of Tregs with Tcons in secondary lymphoid organs based on the critical importance of Treg administration at the time of T-cell priming to confer GVHD protection, although Treg activity during the early infiltration of GVHD target organs cannot be discounted. Among the multiple potential molecular mechanisms of Treg-mediated immunomodulation, Lohmeyer et al identified IL-10 and IL-35 as the 2 prime candidates that mediate the effects of Tregs on Tcons in GVHD. For IL-10, past work established that Treg-derived IL-10 contributes to mitigate GVHD, although other cellular sources exist.² For the heterodimeric IL-35 cytokine, a recently discovered member of the IL-12 family, data in mouse GVHD models are limited, so far, to exogenous IL-35 administration, showing a protective effect.⁷ Assessing the impact of Treg-derived IL-35 will require inactivation of IL-35 subunits specifically in Tregs. Interestingly, clinical-grade ex vivo expanded Tregs are enriched for IL-10 and IL-35-expressing cells.⁸ Treg administration also affects other critical processes in Tcons, including Notch (a key pathogenic pathway in GVHD) and cellular metabolism (known for its complex regulation and impact on T-cell function after allo-HCT).^{9,10} More work is needed to characterize Treg-mediated Tcon

metabolic reprogramming beyond transcriptomics, connect it mechanistically to Treg activity, and assess its relative importance in GVHD protection.

Altogether, Lohmeyer et al provided an important body of work to help illuminate the mechanisms that underlie the efficacy of Treg administration in reducing GVHD after allo-HCT while preserving graft-versus-tumor effects.¹ New predictions from mouse models are timely because clinical translation now includes protocols in which Tregs are given without pharmacological immunosuppression, an attractive platform to track Treg activation, differentiation, and function in human allo-HCT recipients.⁵

Conflict-of-interest disclosure: I.M. received research funding from Regeneron Pharmaceuticals and Genentech and is a member of the scientific advisory board for Garuda Therapeutics. This funding is unrelated to the contents of this commentary. K.L. declares no competing financial interests. ■

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<https://doi.org/10.1182/blood.2022019396>

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