Specifically, several studies have found that levels of circulating $CD4^+CD25^+Foxp3^+$ Tregs are inversely correlated with acute or chronic GVHD.[3-6] In acute GVHD, Tregs were found to express lower levels of granzyme A and chemokine receptors (eg, CCR5, CXCR3), suggesting that Treg-suppressive function and target tissue homing may also be impaired.[7] In chronic GVHD, impaired thymic generation of naive Tregs ($CD45RA^+$) and increased apoptosis sensitivity of proliferating memory/effector Tregs ($CD45RA^-$) selectively alter Treg homeostasis and contribute to the inability to maintain adequate numbers of Tregs relative to conventional effector $CD4^+$ T cells (Tcons).[8,9]

Using new methods to assess gene expression in single cells, Dong et al now present a close-up view of Tregs in human thymus, umbilical cord blood, normal adult blood, and during early reconstitution post-HSCT.[1] Characterizing $CD4^+CD25^+$ Tregs as naive ($CD45RA^+HLADR^-$), activated ($CD45RA^-HLADR^+$), and memory ($CD45RA^-HLADR^-$) subpopulations, they define a "Treg core" gene expression signature that not only distinguishes $CD4^+$ Tregs from $CD4^+$ Tcons but also differentiates each Treg subset. Single cells within each Treg subpopulation show remarkable stability of $Foxp3^+$ expression and lack of IL-2 expression, and also exhibit considerable heterogeneity in expression of various transcription factors, signaling molecules, and homing receptors. At the single-cell level, expression of interferon-γ or interleukin-17A (IL-17A) was very infrequent, suggesting little overlap with Th1 or Th17 lineages. In patients with acute GVHD, overall Treg frequencies and "Treg core" signature appeared to be preserved but there was a marked decrease in the naive Treg subpopulation with preservation of the activated Treg pool. However, residual activated Tregs retained suppressive capacity in in vitro assays.

What are some of the implications? Diversity is a hallmark of the adaptive immune system. The heterogeneity described within circulating Treg subpopulations is reminiscent of that seen in $CD4^+$ Tcons. While this report likely identifies only a fraction of the overall diversity in vivo that also includes T-cell receptor diversity, activation state, cytokine milieu, and target organ homing, etc, even this limited view is indicative of the extensive complexity and diversity of immune regulators that appear to match the diversity of immune effectors. Furthermore, the timing of naive Treg deficiency, that may precede acute GVHD, suggests that impairment of Treg thymic neogenesis is an early event. This is not entirely unexpected because the thymus has been shown to be an acute GVHD target organ, resulting in a decline in T-cell receptor excision circle ($TREC^+$) T cells and an oligoclonal T-cell repertoire that is observed even in grade 1 acute GVHD.

There may also be therapeutic implications. The observed stability of Treg $Foxp3^+$ expression and limited capacity for Th1 and Th17 cytokine secretion are potentially reassuring when considering adoptive Treg therapy, given the proinflammatory milieu in GVHD and its potential for inducing Treg lineage conversion to effector Tcons. However, adoptive Treg survival, activity, and lineage stability in the inflamed host receiving immunosuppressive agents for GVHD remain to be determined in the clinic. Additionally, low-dose IL-2 can induce clinical responses, augment Tregs in vivo, and restore Treg homeostasis in chronic GVHD due to enhancement of Treg thymic neogenesis, proliferation, survival, and function.[9,10] A similar impact on acute GVHD may be possible and should be considered. More speculatively, a combination of adoptive Treg infusion plus low-dose IL-2 may offer synergy in GVHD control. Treg-based GVHD therapies may also offer therapeutic potential in other disorders of impaired peripheral tolerance (eg, autoimmunity, solid organ transplantation). More Treg close-ups posttransplantation are in order.

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● ● ● CLINICAL TRIALS & OBSERVATIONS

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Superficial venous thrombosis: cause for concern

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In this issue of *Blood*, Leizorovicz et al show that progression of superficial venous thrombosis of the lower extremity is associated with a significant risk of

deep vein thrombosis and pulmonary embolism regardless of proximity to the saphenofemoral venous junction.[1]

Our approach to the evaluation and treatment of superficial venous thrombosis (SVT) has evolved over the past few decades. Until recently, it was generally believed that the course of SVT was benign and appropriately treated with local measures and/or antiinflammatory medications. However, we now know that up to 29% of patients presenting with acute SVT of the lower extremity have associated deep vein thrombosis (DVT) or symptomatic pulmonary embolism (PE).[2,3] These findings have changed practice recommendations for patients presenting with acute SVT.[4]

Given the thrombotic complications in patients with SVT, clinical trials to evaluate treatment approaches have been conducted. Three randomized trials separately evaluated 2 low-molecular-weight heparins (LMWHs) and fondaparinux compared with placebo or ibuprofen.[5-7] While the dosing intensity and duration of treatment varied, patients randomized to the LMWHs or fondaparinux treatment arms in each study had a decrease in thrombus extension, and longer treatment (30 or 42 days) was associated with a significant decrease in DVT, PE, and/or thrombus extension compared with the control populations.

In the Comparison of Arixtra in lower LImb Superficial vein Thrombosis with placebO (CALISTO) trial, 3002 patients with acute SVT ($\geq$5 cm by ultrasound) of the lower extremity were randomized in a double-blind fashion to receive fondaparinux (2.5 mg/day) or placebo injection for 45 days.[5] In the treatment arm there was an 85% reduction in the composite endpoint of symptomatic PE, DVT, recurrent SVT, or extension of SVT to within 3 cm of the saphenofemoral junction (SFJ). In the placebo arm, 5 patients developed PEs and 18 developed DVT compared with no PEs and 3 DVT in the treatment arm. However, the cost effectiveness of this treatment approach has been questioned, with a cost of $500 000 per quality-adjusted life year gained compared with no treatment.[8]

Our ability to define a higher-risk population could result in a more targeted and thus cost-effective approach to treatment of acute SVT. To that end, Leizorovicz et al used data on the 1500 patients randomized to placebo treatment in the CALISTO trial to further evaluate patients with SVT extension. In the placebo group subsequent thrombotic complications occurred more frequently if the involved veins were above the knee, if the subject had experienced venous thrombosis previously, or if the thrombus extended to within 10 cm of the SFJ. Symptomatic extension occurred in 7.3% of the placebo group (109/1500) compared to 1.1% of the fondaparinux-treated group (17/1502). Ninety-two percent of patients with extension had initial SVT involving the greater saphenous vein. Thus, extension into the SFJ, an accepted risk factor for deep venous propagation of SVT, would seem a likely risk factor for further complications.

In the report published in this issue of *Blood*, Leizorovicz et al performed a post hoc analysis of data from patients in the placebo arm of the CALISTO trial who experienced SVT extension by day 77, whether $\leq$3 cm or >3 cm from the SFJ. Surprisingly, there was no difference between the groups in incidence of subsequent DVT or PE, which occurred in approximately 9% of each group. There was also no difference in overall use of medical resources between the 2 groups.

Thus, patients with SVT extension are at significant risk of thrombotic complications, and proximity to the SFJ in patients with acute SVT of the greater saphenous vein should not be used as an indicator of greater risk of subsequent complications. Patients presumed to be at highest risk of complications were excluded from the initial CALISTO trial, including patients presenting with thrombus within 3 cm of the SFJ; those with cancer, recent SVT, or DVT/PE; and those with any condition favoring bleeding. Optimal treatment approaches for these patient populations, as well as for patients with upper extremity SVT, have yet to be determined.

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