levels are measured after the event, thus ruling out the possibility of reverse causation. A disadvantage is that levels may not reflect the situation immediately before the event, which, from an etiological point of view, would be the ideal situation. However, this is difficult if not impossible to achieve in any study design.

Although one might be inclined to expect differences in results between case-control studies and this cohort study simply because different populations were included or different assays used, the results were quite similar. Levels of factor X, factor XII, and factor XIII were not associated with risk of venous thrombosis. Elevated factor XI was associated with a nearly 2-fold increased risk for the top quintile or percentile, which is quite close to the risk of factor VIII. High levels of factor IX were associated with an increased risk of venous thrombosis in all studies, but after adjustment for body mass index (BMI), the risk in LITE attenuated to the null. No adjustments for BMI were performed in the case-control studies.

For some coagulation factors, the associated risk is stronger for deep vein thrombosis in the leg than for a pulmonary embolism. The opposite may be true for elevated factor XI in LITE, as the risk of pulmonary embolism combined with deep vein thrombosis appears to be larger than for deep vein thrombosis alone. However, the case classification in this study may not be reliable, preventing strong conclusions.

If these overall results are confirmed by other studies, further study into possible mechanisms for the increased risk of elevated levels of factor XI is warranted, which include increased fibrin formation and decreased fibrinolysis. Furthermore, this study raises the question whether elevated factor XI is also a risk factor for a recurrent venous thrombotic event, and if so, whether treatment prevents recurrent events. Until these results become available, measurement in clinical practice is not yet indicated.

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

REFERENCES

GENE THERAPY

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In this issue of Blood, Burns and colleagues analyzed samples from 18 patients with melanoma, treated with T lymphocytes gene-modified to express a tumor-specific TCR. Results show persistence of transduced cells, but early shutdown of TCR gene expression. Transgene down-regulation was not caused by epigenetic silencing and could be reversed by T-cell activation.

The adoptive transfer of tumor specific cytotoxic T lymphocytes (CTLs) to patients affected by melanoma have produced significant clinical results. However, the strategy is limited by the difficulty of isolating and expanding the rare, high-avidity tumor-specific cells, often deleted in patients due via central tolerance to self-antigens. To overcome these difficulties, high-avidity T-cell receptors (TCRs) isolated from tumor-specific CTLs have been genetically transferred into human T lymphocytes to redirect their specificity toward autologous tumor cells. In a pivotal clinical trial, Morgan and colleagues showed that the infusion of TCR gene-modified lymphocytes caused melanoma regression in a small (13%) fraction of treated patients. Although essential in proving the feasibility of this approach, clinical results of this initial work were suboptimal, especially if compared with the high rates (50%) of clinical responses reported by the same group with non–gene-modified tumor-specific lymphocytes. In this issue of Blood, Burns and colleagues analyzed samples from 18 patients treated with TCR-transduced cells and observed that, after an early contraction, gene-modified cells persist but lose TCR expression. Transgene down-regulation was not caused by epigenetic silencing. Until now, promoter-silencing in adoptively transferred human lymphocytes was only indirectly excluded by clinical trials using gene-modified lymphocytes. In the Burns study, transgene down-regulation was apparently related to the nature of the TCR gene, as suggested by the fact that the number of TCR transgene transcripts in circulating lymphocytes was similar to that of endogenous TCR-α and CD3 transcripts, and always lower than that measured in infused cells. Most importantly, Burns and colleagues showed that TCR expression could be rescued by T-cell activation. The close relationship between the activation status of gene-modified cells and the activity of the proviral LTR as well as viral promoters was previously known and identified as a major issue for TCR-gene transfer applications, but mainly in preclinical studies.

Thus, this work is important for the following reasons: (1) it rules out DNA methylation as a putative limitation of T-cell–based gene therapy by a thorough and comprehensive analysis of treated patients; (2) it underscores the need to combine adoptive with active immunotherapy to sustain the function of gene-modified T cells, and possibly optimize clinical results; and (3) it highlights the need to move the focus from persistence of adoptively gene-transferred cells, a mission accomplished by gene therapists, to transgene expression.
and function in vivo. When applied to TCR gene transfer, emphasis should be given to the use of high-affinity TCRs, the identification of appropriate promoters, and the development of novel approaches aimed at reducing mispairing between tumor-specific and endogenous TCR, a relevant issue that might ultimately lead to reduced efficacy of TCR-transferred cells.

Some questions and issues remain open: (1) This work hypothesizes that transferred cells undergo early quiescence in vivo, resulting in exogenous and endogenous TCR downregulation. Early quiescence of lymphocytes in patients conditioned with a lymphodepleting regimen is an unexpected result, in contrast with previous works suggesting a proliferative advantage of transferred cells in lymphodepleted hosts. A more extensive characterization of the activation status of circulating cells after immunotherapy could provide hints to reconcile these observations. (2) Has the tumor environment a role in reducing the activation, and TCR expression of transferred cells? (3) Most importantly, have these observations clinical relevance? It is intuitive to assume that a reduced expression of tumor-specific TCR should result in reduced efficacy. However, the specific role of TCR expression could be demonstrated only by a direct comparison of results obtained in patients with clinical responses and patients who failed.

Adoptive cellular immunotherapy has the potential to become a major specific and effective modality for the cure of cancer. TCR gene transfer is the simple solution to several limitations encountered by the field. Many of the questions raised by this work will undoubtedly be addressed in the upcoming years and will be critical to translate the potential into a clinical reality.

Conflict-of-interest disclosure: C.B. is a consultant of MolMed SpA. V.R. declares no competing financial interests.

REFERENCES

Schematic representation of the signaling events that are initiated on binding of collagen, convulxin, or collagen-related peptide (CRP) to platelet GPVI receptor complex leading to dense granule release.

Lyn and PKCδ order SHIP1 embargo

Ulhas P. Naik University of Delaware

In this issue of Blood, Chari and colleagues provide a novel mechanism for the unique negative regulatory role of PKCδ in platelet dense granule release downstream of collagen signaling.

Upon vascular injury, platelets adhere to collagen and release dense granule content to recruit more circulating platelets to an injured site. This process is initiated by signaling through glycoprotein VI (GPVI), a major platelet collagen receptor, leading to intracellular calcium mobilization and the activation of protein kinase C (PKC) isoforms. Of the 7 PKC isoforms expressed in platelets, PKCδ differentially regulates dense granule release in platelets; it positively regulates dense granule release by thrombin and negatively regulates it by collagen.1

Chari et al provide convincing evidence regarding the negative regulation of dense granule release downstream of GPVI signaling and reveal a new association of PKCδ with Lyn and SHIP1. Lyn is an important member of the Src tyrosine kinase family that functions downstream of GPVI, whereas SHIP1 is a...
The hidden (and lazy) TCR

Chiara Bonini and Vincenzo Russo