the recent identification of TET2 mutations in some *JAK2* V617F–negative myeloid precursors of *JAK2* V617F–positive patients indicates that the acquisition of TET2 mutations may predate the acquisition of *JAK2* V617F in a subset of CMPN patients, and, therefore, could be the initiating mutation.[7] Thus, it will be interesting to study the relationship between TET2 and polyclonal *JAK2* V617F–positive ET. The prediction is that these patients will have either no or different TET2 mutations. Moreover, CMPNs display a familial predisposition, suggesting that a heritable factor can predispose to the acquisition of *JAK2* V617F. Consistent with this, 3 groups independently identified a specific *JAK2* haplotype as a major risk factor for the development of MPN and showed that the *JAK2* V617F mutation preferentially arises on the allele bearing this susceptibility haplotype.[8-10]

Lambert et al propose that polyclonal ET should not be considered a malignancy. In fact, their previous work has shown that ET patients with polyclonal hematopoiesis have a decreased risk of thrombosis, suggesting that polyclonality may be a marker of a more benign disease course.[3] We think it is important to point out that polyclonality on its own is not synonymous with nonmalignancy. Thus, the independent acquisition of identical oncogenic mutations in the same gene has been observed in highly malignant multifocal solid tumors, such as lung and pancreatic cancers.[11,12] In ET, the fact that *JAK2* V617F may arise independently in multiple progenitor cells suggests that this progenitor cell pool may still be susceptible to acquiring additional mutations, such as TET2, which could lead to leukemic transformation.[13]

That a single type of point mutation is present in the majority of CMPN patients has been puzzling to the field ever since its discovery.[14] Clearly, there must be a selective advantage to the acquisition of *JAK2* V617F, and the observation that this particular mutation can occur at multiple independent times in the same individual makes this argument even more compelling. The big question is now what this selective advantage may be. The race is on.

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● ● ● CLINICAL TRIALS

Comment on Cushman et al, page 2878

High coagulant factors & venous thrombosis

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In this issue of *Blood*, results from the Longitudinal Investigation of Thromboembolism (LITE)[1] show that, of the coagulant factors IX through XIII, only elevated levels of procoagulant factor XI were associated with a first venous thrombosis. LITE is the first prospective cohort study reporting on all these factors. Factor IX initially appeared to be associated with thrombosis but, after adjustment for primarily body mass index, the association disappeared.

The annual incidence of venous thrombosis is 1 per 1000 persons and mortality among patients with pulmonary emboli is high. Patients may develop a postthrombotic syndrome and recurrent events occur frequently.[2] Major risk factors for venous thrombosis consist of acquired and genetic factors. Hypofibrinolysis appears to be a risk factor, but the role of individual fibrinolytic proteins is still unclear.[3] Hypercoagulability caused by an imbalance between anticoagulant and procoagulant systems increases the risk of venous thrombosis.[4] Deficiencies of antithrombin, protein C, and protein S are strong but rare risk factors for venous thrombosis. Factor V Leiden and the prothrombin 20210A mutation are more prevalent with a moderate increase in risk. High levels of factor VIII clearly increase the risk of venous thrombosis, but the results of studies investigating the effects of prothrombin, factor V, factor VII, and fibrinogen are inconsistent.[5] In studies in which associations between these latter factors and venous thrombosis were found, the effects were much less than those of elevated factor VIII.[4]

So far, the effects of coagulant factors IX through XIII have been investigated in only a few case-control studies, including the Leiden Thrombophilia Study (LETS)[5] and, more recently, the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study.[6,7] Prospective cohort studies have been lacking so far.[5]

In this issue of *Blood*, an efficient nested case-control design has been used within LITE, a prospective cohort study in individuals aged 45 to 100 years, with a median follow-up period of more than 9 years.[1] Included in the analyses were 462 validated cases of venous thrombosis and 1047 controls. Coagulation measurements took place in blood samples collected mostly at baseline. Levels were therefore measured long before the event, as opposed to case-control studies, in which

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.[1,5,8] 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.[1,5,7] High levels of factor IX were associated with an increased risk of venous thrombosis in all studies,[1,5,6,9] but after adjustment for body mass index (BMI), the risk in LITE attenuated to the null.[1] No adjustments for BMI were performed in the case-control studies.[5,6,9]

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.[10] 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.

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GENE THERAPY

Comments on Burns et al, page 2888

The hidden (and lazy) TCR

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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-affinity 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.[1] 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.[2] 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.[3] 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.[4] 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.[5,6] 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.[7]

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