cell survival and proliferation and that it also plays a role in therapeutic responses both in vitro and in vivo. These latter findings are consistent with the earlier study and point to the potential utility of SMO antagonists in combination therapy in myeloma.[6] The possibility of using liver X receptor agonists as downstream inhibitors of Hh signaling as an alternative approach to block this pathway was also recently suggested and could provide a means to target this pathway when SMO antagonists are ineffective.[8]

Like most interesting studies, this one raises new questions that need to be addressed, most notably, Why have there been discrepancies in the findings regarding which cells are producing SHH? and Which cells are responsive to Hh signals? As for which cells produce the SHH, the Liu et al study clearly demonstrates that myeloma cells can be the source; however, it also argues that BMSCs are unlikely to be a source. Earlier studies pointing to BMSCs did not take into account the ratio of stromal cells to myeloma cells and therefore may have overrepresented the role of stroma. If the primary source of SHH is autocrine, then changes in this pathway are less likely to be a marker of stromal independence.

Regarding the use of Hh signaling in MM, we have one study stating that only the $CD138^-$ cells are involved,[4] a second stating that both $CD138^+$ and $CD138^-$ cells express the appropriate proteins for Hh signaling,[6] and now a third that claims it is only the $CD138^+$ cells.[1] Some of these issues are probably technical because different cell types, different growth conditions, and different functional assays are used throughout the studies. If one assumes that both $CD138^+$ and $CD138^-$ are responsive to Hh signaling, then the most likely model would be that Hh signaling has different consequences in cells at different stages of differentiation. In the case of the less mature cells, it appears to be important in self-renewal but not in survival because the cells differentiate when Hh signaling is inhibited. In the differentiated cells, the function appears to be more of a survival and proliferative control as evidenced by changes in expression of *Bcl-2*, *CCND1*, and differing responses to therapy. If this is the case, then targeting this pathway clinically becomes more important because it may function in the killing of the plasma cells as well as depleting a tumor initiating pool.

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● ● ● LYMPHOID NEOPLASIA

Comment on El Hajj et al, page 2072

ST1926 repression of Tax: ATL targeted treatment?

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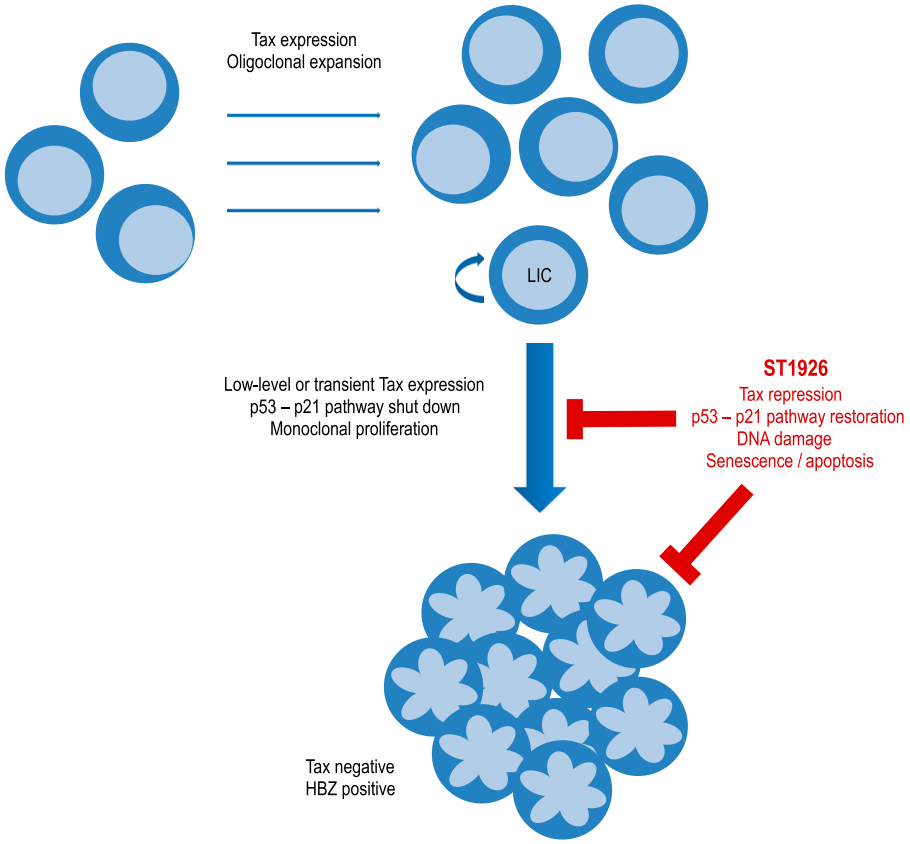
In this issue of *Blood*, El Hajj et al report that the synthetic retinoid ST1926 downregulates the oncoprotein Tax and induces apoptosis and growth arrest of adult T-cell leukemia (ATL) cells.[1]

ATL is an aggressive lymphoid proliferation caused by human T-cell leukemia virus type 1 (HTLV-1), which is also the etiologic agent of HTLV-1–associated myelopathy/tropical spastic paraparesis. The estimated lifetime risk of developing ATL in HTLV-1 carriers is 2% to 7%, and the disease usually occurs ≥20 to 30 years after HTLV-1 infection. ATL is classified as a peripheral T-lymphocytic malignancy of $CD4^+$ T phenotype. The diversity in clinical features and evolution has led to its classification into 4 clinical subtypes: smoldering, chronic, acute, and lymphoma-type ATL. Patients with acute or lymphoma forms have high-risk ATL (HR-ATL), and their poor prognosis is due to rapid proliferation, marked immunosuppression, and resistance to chemotherapy. Although the combination of zidovudine and interferon-α (IFN-α) improves response rate and survival,[2] almost all HR-ATL patients relapse. New therapeutics are therefore highly needed.

Retinoid acids are regulators of cellular proliferation and differentiation. Natural retinoids such as all-*trans* retinoic acid (ATRA) are currently used as therapeutic agents in human cancers, mainly acute promyelocytic leukemia.[3] Resistance to ATRA is frequent in ATL cells, and a pilot study showed only a partial response in less than half of ATL patients.[4] Synthetic retinoids, such as *N*-(4-hydroxyphenyl)retinamide (HPR), 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437), and (2E)-3-[3′-(1-adamantyl)-4′-hydroxy [1,1′-biphenyl]-4-yl]-2-propenoic acid (ST1926), have been developed to overcome resistance and attenuate side effects. These atypical retinoids are able to induce apoptosis in malignant cells through both retinoic acid receptor–dependent and –independent mechanisms. HPR has been shown to inhibit growth and induce apoptosis in HTLV-1–transformed cells.[5]

El Hajj et al evaluated the preclinical efficacy of ST1926 in ATL.[1] At pharmacologically relevant concentrations, ST1926 causes G_1 cell cycle arrest and massive apoptosis in HTLV-1–infected cell lines. A growth inhibition is observed with ATL primary cells but not peripheral



Schematic representation of ST1926 repression of Tax and apoptosis of ATL cells. Tax expression induces oligoclonal expansion of HTLV-1–infected CD4 T cells and deregulates signaling pathways including p53-p21 or DNA repair. ATL monoclonal proliferation occurs after decades of evolution in 2% to 7% of HTLV-1–infected patients. Cells having shut down Tax expression escape anti-Tax cytotoxic T lymphocytes and are preferentially selected. ATL cells have a Tax-negative and HBZ-positive phenotype. Transient or low-level Tax expression occurs, particularly in leukemia-initiating cells. ST1926 downregulates Tax expression and reactivates the p53-p21 signaling pathway. ST1926 induces massive and p53-independent apoptosis of leukemic cells.

blood mononuclear cells from healthy donors. ST1926-induced cell death is partially caspase dependent. The study provides further evidence that ST1926 is a DNA-damaging agent. ST1926 upregulates p53, but the growth suppressive effect is p53 independent. The authors also show that oral administration of ST1926 reduces leukemic burden and prolongs survival in a murine ATL model developed by Hasegawa et al.[6] The effect of ST1926 on ATL cells was more pronounced than those of HPR or CD437. Interestingly, among these synthetic retinoids, only ST1926 downregulates the levels of the viral oncoprotein Tax in HTLV-1–infected cell lines. ST1926-induced downregulation of Tax was confirmed by a reduction of Tax mRNA levels in spleen leukemic cells and Tax DNA in treated mice with ATL.

Tax transactivates viral expression but also deregulates apoptosis, cell cycle, and DNA repair. Tax supports the oligoclonal expansion of HTLV-1–infected T lymphocytes and plays a key role in the initiation of the multistep process of leukemogenesis (see figure). Paradoxically, Tax protein is usually not detectable in HTLV-1–infected peripheral blood mononuclear cells and ATL cells, and *tax* expression in vivo can only be assessed at the transcript level. In vivo dynamic studies support the persistence of continuous low-level or transient expression of Tax protein. The current view is that, Tax being the main target of the host's cytotoxic T lymphocytes, cells that have silenced Tax expression are preferentially selected during disease progression. Several mechanisms of Tax silencing have been described, including provirus deletion in the 5′ long terminal repeat, mutations in the *tax* gene, epigenetic modulation, and silencing by other regulatory viral proteins such as p30 or HTLV-1 bZIP factor (HBZ).

A question that has not been addressed is the effect of retinoids on the expression of HBZ, the other HTLV-1 oncogene. HBZ is consistently expressed in ATL primary cells, and evidence is accumulating about its critical role in the maintenance of HTLV-1–induced transformation.[7] Leukemic cells from the Tax transgenic murine model do not express HBZ, and the effect on Tax may not be sufficiently relevant in patients with HR-ATL.

The mechanism by which decreased Tax protein level is mediated by ST1926 action is also not yet elucidated. In a previous study, El Hajj et al reported that As_2O_3 and IFN-α trigger Tax proteolysis and target leukemia-initiating cells.[8] They suggest that ST1926 may downregulate Tax through proteasome degradation, with subsequent restoration of the Tax-inhibited p53-p21 pathways and apoptosis or senescence in quiescent leukemic cells.[1] They conclude that the association of ST1926 with As_2O_3 and IFN-α may abrogate ATL-initiating activity and favor long-term remissions.

The infected leukemic stem cell hypothesis has emerged recently as a potent mechanism for relapse in patients who have reached remission. Although the existence of chemotherapy-resistant cancer stem cells is supported by the Tax transgenic mouse ATL model,[9] their characteristics cannot be transposed to leukemic ATL-$CD4^+$ mature cells. Allogeneic hematopoietic stem cell transplantation may be the only long-term remission and potentially curative strategy.[10] Further studies on potential drugs, such as ST1926, should investigate the effect on leukemic stem cells and their use in the context of allogeneic transplant and maintenance therapy.

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

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THROMBOSIS & HEMOSTASIS

Comment on Couturaud et al, page 2124

VTE risk and family history: provocative findings

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In this issue of *Blood*, Couturaud and colleagues take us one step closer to identifying a root cause for unprovoked venous thromboembolism (VTE) by comparing the VTE risk in first-degree relatives of patients who had experienced unprovoked venous thrombosis to the corresponding risk in first-degree relatives of patients whose thrombotic event was provoked by an environmental factor such as surgery.[1]

VTE is a disease with many causes, and many environmental factors such as cancer, surgery, and estrogen use have been independently associated with an increased risk of venous thrombosis.[2] Although the genetic contribution to VTE risk is illustrated by families deficient in endogenous anticoagulant proteins such as protein C, protein S, and antithrombin,[3,4] our incomplete understanding of this disease is highlighted by the fact that many patients with apparently unprovoked VTE have no identifiable thrombophilia (inherited or acquired).

It is not surprising that Couturaud et al[1] found that first-degree relatives of VTE patients are themselves at increased risk to experience venous thrombosis. However, the observations that unprovoked (vs provoked) VTE and younger age at the time of first VTE are both associated with a higher risk of venous thrombosis in a first-degree relative have some important implications. One hypothesis that emerges from the findings of Couturaud et al is that patients with unprovoked VTE and a first-degree relative who had unprovoked VTE at a young age may be at higher risk for recurrent VTE than are otherwise similar patients. If confirmed in a prospective study, this sort of association could have implications for the recommended duration of anticoagulant therapy. Second, as the authors point out, the knowledge gained from this study may lead clinicians to recommend that patients with a first-degree relative who has experienced VTE avoid estrogen-containing contraceptive strategies and use aggressive VTE prophylaxis after surgery or hospitalization. Whether these common-sense suggestions should differ according to whether the first-degree relative's clot was provoked or occurred at an older age is not clear, but the finding that a family history of VTE, even if provoked, increases risk is important.

Since the only familial thrombophilia testing routinely performed in the Couturaud study was for factor V Leiden and prothrombin 20210A gene variants, some of the increased risk observed among these first-degree relatives of VTE patients is likely attributable to known, inherited thrombophilias such as deficiency of protein C, protein S, or antithrombin. However, because these congenital deficiencies are quite rare, even within VTE patient cohorts, the associations described in the study by Couturaud et al strongly suggest that additional as yet unidentified genetic or epigenetic factors contribute to the development of VTE. Like many pathologic conditions, venous thrombosis is almost always the result of more than one contributing factor. The study by Couturaud and colleagues suggests that the search for additional inherited thrombophilic variations in the human genome should continue.

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VASCULAR BIOLOGY

Comment on Sikora et al, page 2150

Intravascular hemolysis: the sacrifice of few…

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In this issue of *Blood,* Sikora et al demonstrate unequivocally that when erythrocytes are submitted to shear stress and hypoxia, only hemolysis contributes to the release of adenosine triphosphate (ATP), suggesting erythrocyte sacrifice as a primary mechanism for in vivo local purinergic signaling and blood flow regulation.[1]

Long ago, August Krogh arrived to the concept that "in the normal resting muscle a comparatively small number of capillaries… should be open, so as to allow the passage of blood while muscular work should cause the opening up of a larger number."[2] A century