The paper by Yao et al now establishes Shh as an autocrine factor for MC motility, fostering chemotactic movement toward the PDGF-BB–releasing endothelium. PDGF-BB in turn acts upstream of Shh in MCs during this process, and both pathways feed into the phosphatidylinositol 3-kinase (PI3K) and extracellular signal-regulated kinase 1/2 (ERK1/2) cascades via specific kinase isoforms.1

Herein, Shh appears to play the role of a delayed reinforcement of the PI3K and ERK1/2 signal, possibly retaining pathway activity on an elevated level when the PDGF-BB boost has already vanished in the haze (see figure). When putting these novel findings in the context of published data, they intriguingly fit to the described arteriolarizing role of Shh that was suggested to act via upregulation of VEGF in somites that in turn leads to Notch pathway activity in the endothelium.4 Yao et al describe that VEGF-induced PDGF-BB release from the endothelium activates Shh expression in MCs so that one could envision a self-sustaining circle leading to vascular stability (see figure). Although this aspect was not touched on by Yao et al, a maintenance function in the vasculature might be suggestive, because Shh is important for blood–brain barrier integrity, which also relies on PDGF–BB–mediated pericyte recruitment.8,9

Besides the insufficiently understood cell-autonomous effects of Hh in the endothelium, these findings raise a number of important questions. For example, with which players other than PDGF–BB, such as angiopoietin/Tie, Dll4/Notch, and transforming growth factor β, does Shh interact during MC recruitment? Because the Wnt/β-catenin pathway has been suggested to regulate PDGF–BB in the endothelium, leading to MC recruitment to tumor vessels,10 it remains to be deciphered if this would contribute to the PDGF–BB–driven function of Shh in MCs observed by Yao et al. Furthermore, the contribution of other sources of Shh (ie, astrocytes in the brain) to MC recruitment needs to be clarified (see figure).

In conclusion, the study by Yao et al has successfully exposed that autocrine Shh signaling in MCs is crucial for their recruitment to endothelial cells and consequently for vessel stability. Future studies are required to determine whether targeting the Shh pathway in MCs might have beneficial effects in diseases involving pathologically increased or decreased MC recruitment, such as in regenerative and tumor angiogenesis.

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

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A WHIM satisfactorily addressed

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In this issue of Blood, McDermott et al present the intriguing, clinically relevant, and perhaps unexpected findings for the efficacy and safety of long-term administration of low-dose plerixafor treatment of patients with warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome.1

WHIM is a rare disorder of primary immunodeficiency associated with warts, hypogammaglobulinemia, retention of neutrophils in the bone marrow, and recurring bacterial infections. Until this study (ClinicalTrials.gov identifier: NCT00967785),1 there was no treatment that allowed for long-term control of infections and warts in this syndrome. The rationale for using plerixafor (also known as AMD3100 and Mozobil) was to take advantage of the peripheral blood neutrophil and other leukocyte-mobilizing capacity of AMD3100/plerixafor to increase numbers of leukocytes in the circulation2 to alleviate some symptoms of this disease. This was a reasonable approach as a majority of patients with WHIM have an autosomal-dominant mutation of CXCR4, a chemokine receptor, believed to increase intracellular signaling that allows retention of neutrophils and other leukocytes at their tissue sites. The leukocyte-mobilizing effects of CXCR4 antagonism are relatively rapid, usually within minutes to hours, and cessation of administration of the antagonist is quickly (usually within hours) followed by decreased numbers of blood cells.2,3 Hence, maintaining increased levels of circulating leukocytes would require frequent dosing of patients with the antagonist. Frequent dosing, however, has implications for possible problems involving desensitization of the receptor to the antagonizing effector, and induction of serious side effects and safety concerns. In fact, CXCR4 is a coreceptor for HIV, and attempts at continuing antagonism of CXCR4 by AMD3100 to prevent or decrease HIV infection were associated with severe side effects.4 AMD3100 has been used successfully to mobilize hematopoietic stem (HSC) and progenitor (HPC) cells to the blood for collection for use in hematopoietic cell transplantation (HCT),3,5,6 but due to the rapid and effective mobilization of HSCs/HPCs, and its synergy in this effect with granulocyte colony-stimulating factor, the AMD3100...
needed only to be administered short-term to donors for efficient mobilization of enough cells for effective HCT.\(^6\)

The strength of the article by McDermott et al.\(^1\) is that the 3 patients with WHIM assessed in this study were given plerixafor at low dose (twice a day by self-administration) for 6 months without side-effects (and with greatly decreased infectious episodes) and, in combination with imiquimod, improved control of warts (see figure). In retrospect, the design of this long-term study is quite impressive; the effectiveness of long-term low-dose administration of plerixafor was not necessarily predictable, even though it followed low-dose administration of plerixafor was not impressive; the effectiveness of long-term low-dose administration of plerixafor was not impressive; the effectiveness of long-term low-dose administration of plerixafor was not impressive; the effectiveness of long-term low-dose administration of plerixafor was not impressive; the effectiveness of long-term low-dose administration of plerixafor was not impressive; the effectiveness of long-term low-dose administration of plerixafor was not impressive; the effectiveness of long-term low-dose administration of plerixafor was not impressive; the effectiveness of long-term low-dose administration of plerixafor was not impressive; the effectiveness of long-term low-dose administration of plerixafor was not impressive; the effectiveness of long-term low-dose administration of plerixafor was not impressive; the effectiveness of long-term low-dose administration of plerixafor was not impressive; the effectiveness of long-term low-dose administration of plerixafor was not impressive; the effectiveness of long-term low-dose administration of plerixafor was not impressive; the effectiveness of long-term low-dose administration of plerixafor was not impressed with the understanding that this work is only a beginning to proving the clinical efficacy of plerixafor for treatment of some of the health issues associated with WHIM. It may not be a cure, but it clearly demonstrates health benefits for patients with WHIM syndrome, and follow-up confirmatory studies are warranted.

This study\(^1\) brings up a number of questions and possibilities (see figure) that could be experimentally evaluated in context of further clinical trials in this and other disorders associated with decreased circulating leukocytes, and in animal models of disease, as AMD3100 works in mice, and higher animals, as well as in humans. An intriguing finding was the apparent lack of desensitization of CXCR4 to the leukocyte-mobilizing effects of plerixafor, which is interesting because CXCR4 is a G-protein–linked 7-transmembrane spanning receptor that manifests desensitization to a natural ligand, stromal-derived factor-1 (SDF-1)/CXCL12. It has been reported that rapid mobilization of HPCs by AMD3100 in mice may be mediated at least in part by CXCR4-dependent release of SDF-1/CXCL12 from stromal cells in the bone marrow.\(^9\) In this context, it would be of interest to assess serum levels of SDF-1/CXCL12 in patients being treated with long-term low-dose plerixafor. Perhaps it is CXCR4-dependent release of SDF-1/CXCL12 that is in part responsible for the increase in circulating leukocytes and their sustained levels for a while after cessation of plerixafor. It would also be of interest to see whether, along with plerixafor-induced increases in circulating leukocytes, there is also an increase in phenotypically defined and functionally active subsets of long- and short-term repopulating human HSCs and HPCs, as well as other cells which may play a role in regenerative medicine, such as endothelial colony-forming cells (ECFCs) and mesenchymal stem/stromal cells (MSCs). Although mobilization of HSCs, HPCs, ECFCs, and/or MSCs may not play a role in helping to ameliorate some of the problems inherent in patients with WHIM syndrome, such information could be of importance for other diseases states, as endogenous tissue repair from certain stress conditions may reflect the enhanced circulation of these stem/progenitor cell populations.

It is becoming clear that levels of circulating blood cells may be under control of circadian oscillations, as has recently been reported for HSCs,\(^10\) and correct timing of mobilization of such cells may greatly enhance the numbers of cells mobilized. Hence, studies of modified timing of low-dose plerixafor may increase efficacy. Whether clinical use of long-term administration of low-dose plerixafor can be adequately translated for treatment of other disorders associated with low blood leukocyte counts is of interest; this will require that there are enough leukocytes available in bone marrow and other tissue sites for potential mobilization, and the leukocytes in these patients would need to function normally, as in the evaluated 3 patients with WHIM syndrome. What the study by McDermott et al.\(^1\) (and those publications preceding this work that established AMD3100/plerixafor as a mobilizing agent) demonstrates is that ingenuity, creativity, and flexibility in experimental design, starting at a basic science level and preceding through preclinical investigation, can lead to efficacious and safe manipulations that improve health care. This is what the scientific process is about.

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Comment on Nogami et al, page 2420

**FV and APC resistance: the plot thickens**

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In this issue of *Blood*, Nogami et al report on a novel factor V (FV) gene mutation (FV Trp1920→Arg, FV\(^{Nara}\)) associated with activated protein C (APC) resistance and a severe thrombotic phenotype in a young Japanese patient.\(^3\) Since the affected amino acid residue is located in the light chain of FV, far from the known APC-cleavage sites, this discovery may afford new insights into the molecular mechanisms of APC resistance.

The serine-protease APC plays a major anticoagulant role by proteolytically inactivating factors Va (FVa) and VIIIa (FVIIIa), the essential cofactors of the prothrombinase and intrinsic tenase complexes, respectively. Both reactions are greatly stimulated by anionic phospholipids and by the APC-cofactor protein S. APC cleaves FVa at Arg306, Arg506, and Arg679, all located in the heavy chain of the protein, whereas the light-chain anchors FVa to the membrane surface. On the other hand, APC also cleaves the inactive precursor FV (particularly at Arg506), converting it into a still poorly defined anticoagulant cofactor that stimulates the inactivation of FVIIIa by the APC/protein S complex. Therefore, FVa is both a substrate and a cofactor of APC (reviewed in Castoldi and Rosing\(^2\))

In 1993, a reduced anticoagulant response of plasma to APC (APC resistance) was first described in a thrombophilic family\(^3\) and quickly recognized as the most common risk factor for venous thrombosis in the Caucasian population. Soon afterward, the FV Arg506→Gln (FV\(^{Leiden}\)) mutation was identified as the underlying genetic defect.\(^4\) This mutation eliminates the APC-cleavage site at Arg506, thereby hampering not only the APC-mediated inactivation of FV\(^{Leiden}\) (which relies entirely on the protein S–dependent cleavage at Arg506), but also the conversion of FV\(^{Leiden}\) into an APC cofactor for FVIIIa inactivation. The FV\(^{Leiden}\) mutation is present in ~5% of Caucasians, but is virtually absent in the indigenous populations of Africa, America, Eastern Asia, and Australia. More recently, other FV gene mutations associated with APC resistance have been identified in various populations, including Arg306→Thr (FV\(^{Cambridge}\)), Arg306→Gly (FV\(^{Hong Kong}\)), Ile359→Thr (FV\(^{Liverpool}\)), and Glu666→Asp (reviewed in Castoldi and Rosing\(^2\)). Remarkably, all of these variants predict amino acid changes at or close to the APC-cleavage sites in the heavy chain of FV(a), and their mechanisms of action can be rationalized in terms of reduced cleavage at these sites.

The interesting study by Nogami et al describes a 13-year-old Japanese boy who developed recurrent venous thrombosis during oral anticoagulant treatment.\(^5\) The patient had reduced FV levels (40 IU/dL antigen, 10 IU/dL activity) and pronounced APC resistance (see figure), prompting FV gene sequencing. This revealed a novel homozygous missense mutation (Trp1920→Arg, FV\(^{Nara}\)) in the CI domain of FV, which was not found in 50 healthy Japanese people. In line with the observations made in the patient, recombinant FV\(^{Nara}\) showed reduced expression in conditioned media (~50% of wild-type FV) and conferred APC resistance to reconstituted FV-deficient plasma. Moreover, detailed characterization of the mutant in model systems indicated that: (1) the APC-mediated inactivation of FV\(^{Nara}\) is severely impaired and hardly sensitive to stimulation by protein S; and (2) FV\(^{Nara}\)

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**Thrombosis & Hemostasis**

**THROMBOSIS & HEMOSTASIS**

![A](image1.png) ![B](image2.png)

Thrombin generation curves obtained in platelet-poor plasma from a normal control (A) and from the FV\(^{Nara}\) homozygous patient (B) in the absence and presence of APC. Plasma from the FV\(^{Nara}\) homozygous patient is completely insensitive to the anticoagulant action of APC. Professional illustration by Marie Dauenheimer.
A WHIM satisfactorily addressed

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