(however, other iron chelators were not studied).[1] Increased HIF-2α messenger RNA (mRNA) and protein were found in neutrophils from patients with chronic obstructive pulmonary disease (COPD) and inflammatory arthritis (IA). This chronic HIF-2α expression may elicit unique biological effects not seen in normoxic human neutrophils from patients with a HIF-2α gain-of-function mutation.

Inflammatory cytokines and Toll-like receptor ligands present in COPD and IA may be inducing HIF-2α expression independently of hypoxia. Consistently, patients with gain-of-function HIF-2α mutations express more HIF-2α target genes at steady state, suggesting that hypoxia is not always required for the induction or function of HIF-2α in neutrophils. HIF-2α can antagonize the expression of HIF-1α in nonhematopoietic cells, but it is not known if similar regulatory mechanisms exist in neutrophils.[6] Previous studies in macrophages demonstrate that lipopolysaccharide can induce HIF-1α mRNA expression but that hypoxia is a prerequisite for HIF-1α protein accumulation via the inhibition of prolyl hydroxylases.[7] Like HIF-1α, the accumulation of HIF-2α protein at sites of inflammation may therefore be dependent on factors controlling both gene transcription and HIF-2α protein half-life.

The key role of HIF-2α in the regulation of macrophage inflammatory cytokine production introduces a possible caveat to this study because the mice used in these studies lacked HIF-2α in neutrophils and macrophages. This was addressed using a fractionated irradiation regimen (3 fractions of 1 Gy/day for 4 days) to ablate bone marrow–derived myeloid cells but not wild-type resident lung macrophages. Following acute lung injury, HIF-2α–deficient neutrophils were recruited to lungs containing wild-type resident macrophages. This recruitment occurred independently of HIF-2α, but the subsequent resolution of neutrophilic inflammation was accelerated in the absence of HIF-2α. Conversely, expression of gain-of-function HIF-2α mutants in zebra fish delayed the clearance of neutrophils from a tail fin wound (see figure). Moreover, HIF-2α deficiency affects neutrophil life span but not phagocytosis or the respiratory burst, suggesting that the diverse functional roles of HIF-2α in macrophages do not extend to neutrophils.[8] Inflammatory cytokine production by HIF-2α–deficient macrophages and neutrophils was also not altered in an acute lung injury model, supporting the conclusion that the alterations in neutrophil life span are cell intrinsic.

This study reports that human HIF-2α gain-of-function mutations increase basal expression of prolyl hydroxylase-3 (PHD3), an enzyme that helps neutrophils adapt to hypoxia. PHD3 is upregulated in peripheral blood neutrophils from patients with rheumatoid arthritis.[9] This study demonstrates that HIF-2α is also strongly upregulated in peripheral blood neutrophils and lung biopsies from patients with rheumatoid arthritis and COPD, respectively, suggesting that HIF-2α regulates PHD3 expression in these human cells. PHD3 regulates Bcl-x_L, a prosurvival protein whose expression is responsive to hypoxia.[9] However, in this study, no perturbations were noted in Bcl-x_L expression in HIF-2α–deficient neutrophils. More studies are needed to determine if Bcl-x_L, or other prosurvival proteins such as Mcl-1, are HIF-2α targets during the adaptation to hypoxia. Neutrophil-specific conditional gene-targeting approaches will be essential to identify host factors that shift the balance between neutrophil life and death.

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● ● ● THROMBOSIS & HEMOSTASIS

Comment on Bouvier et al, page 404, and on Bouvier et al, page 414

Preventing pregnancy loss

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In this issue of *Blood*, 2 articles by The Nîmes Obstetricians and Hematologists–Antiphospholipid Syndrome (NOH-APS) Study Group give us new information about the effects of low-molecular-weight heparin (LMWH) on pregnancy complications in women with prior pregnancy loss and either purely obstetric antiphospholipid syndrome (APS) or inherited thrombophilia. The results better define women at risk, suggest a role for LMWH, and confirm the need for further investigation.[1,2]

APS has been linked to pregnancy complications including fetal loss at all stages of gestation, preeclampsia, eclampsia, placental insufficiency, and premature birth. The inherited thrombophilias, factor V Leiden (FVL) mutation and prothrombin gene (PTG) mutation G20210A, have variable associations with nonthrombotic pregnancy complications; they are believed to play a minimal role in spontaneous abortion or placenta-mediated complications and only a modest role in second- or third-trimester fetal loss.

The NOH-APS study is a large prospective observational study of 6318 women with

Recommended treatment approach

	Treatment	
Diagnosis	**Early loss**	**Late loss**
FVL/PTG	0*	LMWH
Obstetric APS	LMWH	LMWH
	ASA	ASA

ASA, low-dose ASA; LMWH, prophylactic-dose LMWH.
*Informed discussion on risks/benefits and minimal data.

a history of pregnancy loss. Women with unexplained pregnancy loss, no past history of thrombosis, and meeting criteria for either the diagnosis of obstetric APS (n = 517) or the inherited thrombophilias FVL or PTG (n = 279) were followed during subsequent pregnancies. Rates of recurrent pregnancy loss and other morbidities were compared in separate analyses for patients with obstetric APS or with thrombophilia, with a common control group of women (n = 796) with prior pregnancy loss lacking these diagnoses. Patients identified as having pure obstetric APS received aspirin alone (ASA), 100 mg, when not pregnant; prophylactic-dose enoxaparin (LMWH) was started at the time of a positive pregnancy test and ASA was continued. Women in the thrombophilia group were treated with prophylactic LMWH if they had had 1 prior fetal loss after 10 weeks gestation (late loss); they were not treated with LMWH if they had a history of 3 losses prior to 10 weeks (early loss). In the analyses, patients with obstetric APS, thrombophilia, or the control group were subdivided based on whether prior pregnancy loss manifested as 3 recurrent spontaneous losses before 10 weeks gestation or 1 late fetal loss; the women were followed for subsequent pregnancy outcomes.

For women with inherited thrombophilia, LMWH decreased both fetal death and placenta-mediated complications after first late pregnancy loss compared with controls with the same pregnancy history. The NOH-APS thrombophilia study hints that LMWH heparin may benefit women with recurrent early loss, as these untreated women had higher rates of late pregnancy loss and complications than control women, although numbers were small and not statistically significant. For women with pure obstetric APS, LMWH decreased early and late fetal loss in women with a history of late fetal loss compared with controls but did not decrease placenta-mediated complications to control levels. Although women with obstetric APS and recurrent early loss treated with LMWH had similar early loss rates as control women, they had increased late pregnancy loss. All obstetric APS women had higher rates of placenta-mediated complications than control women.

Studies assessing the role of LMWH in decreasing pregnancy loss and complications have yielded inconsistent results, in part due to study design, small sample size, and heterogeneous definitions of both patient populations and outcomes. Many studies have grouped women with a range of previous obstetrical complications, thrombotic history, and types of thrombophilia.[3,4]

Heparin therapy was initially predicated on the concept that thrombosis in the utero-placental circulation was responsible for pregnancy loss. Early studies suggested a beneficial role for LMWH in decreasing miscarriage in APS[5,6] leading to the more widespread use of LMWH in women with pregnancy loss without APS in an understandable effort to prevent recurrence. Two recent studies, however, have failed to confirm that LWMH or LMWH/ASA decreased miscarriages in women without APS but with a history of 2 prior losses prior to 20 weeks[7] or 24 weeks[8] gestation. Evaluation of LMWH in preventing placenta-mediated complications is also limited. A pilot study of women without APS or thrombophilia suggests that LMWH can decrease both placenta-mediated complications and late pregnancy loss, but the numbers of both patients and events are extremely small.[9]

The strengths of the NOH-APS studies lie in the patient sample size and the careful classification of both patients and outcomes, providing much needed information on the natural history of women with previous pregnancy loss and subsequently identified obstetric APS, FVL, or PTG. Excluding women with prior thrombosis and distinguishing between pregnancy loss at <10 weeks gestation vs later than 10 weeks gestation, in accordance with criteria for diagnosing APS, may yield more consistent results as etiology for pregnancy loss may be similar in these more closely related groups.

The results confirm the current generally accepted practice of administering LMWH in women with FVL or PTG and prior late pregnancy loss. They raise questions about the recommendations not to use LMWH in women with FVL or PTG with prior pregnancy early loss or complications,[10] especially in light of animal models that suggest heparin may ameliorate nonthrombotic pregnancy complications associated with FVL.[11]

For women with pure obstetric APS, management is less clear. As Bouvier et al suggest, other positive effects of heparin on interrupting complement activation, facilitation of trophoblast invasion, and placentation raise concerns that the current prophylactic-dose LMWH may be insufficient. For now, current practices should continue based on underlying diagnosis of either obstetric APS, or FVL and PTG, and thorough discussion of the risks and benefits of treatment with LMWH for women with a history of pregnancy loss. Based on available information, recommended treatment approaches are listed in the table.

The NOH-APS Study Group has set the stage for future studies by rigorously identifying patient populations at risk, both by defining the underlying disease process and stratifying by time of pregnancy loss. Similar approaches should be used in further randomized controlled trails to improve outcomes in these patients.

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TRANSPLANTATION

Comment on Zhang et al, page 428

New molecule for mobilizing marrow stem cells

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In this issue of *Blood*, Zhang et al describe an exciting new small-molecule antagonist of CXCL12-CXCR4 binding with a potent ability to mobilize hematopoietic stem cells (HSCs).[1] Further clinical development of this new drug, Me6TREN, may have broad applications in stem-cell mobilization for cancer and in regenerative medicine.

The field of HSC transplantation has been strongly impacted by the clinical approval of plerixafor, a CXCR4 antagonist that has been shown to be effective in mobilizing stem cells from the bone marrow into circulation in the blood.[2] The clinical use of plerixafor in combination with 5 days of granulocyte colony-stimulating factor (G-CSF) administration has allowed collection of $CD34^+$ stem cells from patients with lymphoma and myeloma undergoing autologous stem cell transplantation in fewer days and in higher numbers than had been possible when mobilizing patients with G-CSF alone.[3,4] A limitation in the clinical use of plerixafor is the need to combine it with G-CSF, thus subjecting potential donors to toxicities of 2 drugs. In addition, while the addition of plerixafor to the armamentarium of drugs for stem-cell mobilization has decreased the incidence of mobilization failure from 10% to 40% to <7%,[5] there are still patients in whom attempts at stem-cell collection fail, and these patients are unable to proceed to autologous stem-cell transplantation. Thus, scientists studying hematopoiesis and stem-cell transplant physicians are interested in new agents that could increase the efficiency of stem-cell mobilization and collection. To address this need, Zhang et al identified a small molecule, Me6TREN, that potently mobilized HSCs from bone marrow to peripheral blood in mice. Using flow cytometry and in vitro colony assays, they found that a single Me6TREN injection mobilized phenotypically defined $lineage^-$ sca^+ $c\text{-}kit^+$ (LSK) stem cells and hematopoietic colony-forming cells, and that the blood content of circulating progenitor cells remained elevated for 4 days after Me6TREN treatment.

The natural question, when faced with a new drug, is whether it is more effective than existing drugs. Based upon their chemical structures, it would appear that these drugs might have different mechanisms of action, because plerixafor is a 1,1′-[1,4-phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] molecule with bilateral symmetry, whereas Me6TREN is a tris[2-(dimethylamino)ethyl]amine molecule with trivalent symmetry (see figure). To compare the activity of Me6TREN with G-CSF and plerixafor as single agents for HSC mobilization, the authors measured the content of high-proliferative–potential colony-forming units (HPP-CFU) in the blood of mice 1 hour following plerixafor administration, 12 hours after Me6TREN administration, and after 5 days of G-CSF administration and performed competitive repopulation assays by transplanting mobilized blood from mice in different treatment groups into irradiated recipients in combination with a fixed number of congenic bone marrow cells. In these experiments, mice treated with Me6TREN had significantly more HPP-CFU in their blood and superior competitive repopulating activity when transplanted into irradiated mice compared with blood mobilized with plerixafor or G-CSF. Me6TREN-mobilized progenitors contributed to long-term durable multilineage hematopoietic reconstitution in recipient mice after secondary transplantation of marrow from mice engrafted with mobilized cell, thus fulfilling the criteria for mobilization of a self-renewing HSC.

Some questions regarding this interesting new compound remain. Although Me6TREN antagonized SDF-1–induced migration of murine and human hematopoietic progenitors, antagonistic binding of Me6TREN to CXCR4 has not been established. The schedule of plerixafor administration used by Zhang et al was based upon work from Broxmeyer et al, who showed that single-agent plerixafor resulted in a 5- to 10-fold mobilization of CFU-GEM into blood 1 hour after drug administration.[2] In contrast to the schedule of plerixafor used in these murine experiments, our clinical experience with plerixafor indicates that optimal mobilization of HSCs in humans usually occurs >8 hours and up to 17 hours following a subcutaneous plerixafor injection.[6] Although plerixafor appears to be a pure CXCR4 antagonist and thus acts directly on CXCR4/CXCL12 binding, the authors show that Me6TREN activates the phospho-Akt, mitogen-activated protein kinase, and phospho-extracellular signal-regulated kinase pathways and induces MMP9 expression. Using radiation chimeras engrafted with MMP9-knockout bone marrow, they show that much of the mobilization induced by Me6TREN is dependent upon expression of MMP9 by hematopoietic cells. Local production of metalloproteases is implicated in disruption of adhesion molecules that tether HSCs to stromal cells, including VLA4 (see figure). It would be of interest to test whether Me6TREN acts indirectly on the CXCR4/CXCL12 axis by modulating stromal-cell expression of messenger RNA for CXCL12, the ligand for CXCR4. Thus, differences in