otherwise normal ArhGAP15−/− mice. First, they saw that the numbers of circulating neutrophils and macrophages were significantly decreased. Furthermore, macrophages from ArhGAP15−/− mice showed an altered morphology but normal migration. Finally, they discovered that neutrophil motility was strongly altered, indicating an important cell-selective role of ArhGAP15 (see figure). Neutrophils from these knockout animals showed increased motility with primarily the directional migration and cell polarization affected, as was shown by time-lapse video. The investigators used agarose chemotaxis assays, where the cells have to squeeze underneath a solid agarose block to follow the chemotactic gradient, which causes strong flattening of the cells and thus more informative images. The enhancement of ArhGAP15−/− neutrophil migratory functions was accompanied by increases in phagocytosis, bacterial killing, and ROS generation. The enhanced ROS production seen in the knockout cells also depended on the stimulus employed: while ROS triggered by Fc receptors (ie, receptors of antibody-coated immune complexes) was normal in the knockout cells, the generation of oxygen species via iNOS or C3a (ie, G-protein coupled receptors) was strongly enhanced. Most importantly, the ArhGAP15−/− animals showed a clinically important phenotype because they were protected from severe polymicrobial abdominal sepsis. To this end, Costa et al had exposed wild-type and ArhGAP15−/− mice to a model of cecal ligation and perforation inducing sepsis. This resulted in a significantly increased neutrophil load in the cecum and in the peritoneum of the ArhGAP15−/− animals, while non-infected organs did not show any signs of altered neutrophil recruitment. Septic injury and mortality are strongly associated with exorbitant systemic inflammation and multiple cytokine production. Importantly, ArhGAP15−/− mice showed a dramatically reduced production of multiple cytokines during the experimental sepsis and the animals survived for extended periods of time while all control mice died within 48 hours.

Taken together these 2 papers provide novel and remarkable evidence for important negative regulatory roles of the GAP proteins ARAP3 and ArhGAP15 in neutrophil cell biology and pathophysiology. As has been shown for ArhGAP15, these findings might be exploited in the future in the clinical treatment of sepsis, for example, by the generation of specific, small molecular GAP inhibitors. Of course, there must be a reason for the evolution of such proteins, likely the mainten-
polymerization process, and HbF levels 
> 20% prevent most sickle cell disease clinical 
events.2-4 Examples of this ameliorating effect 
include infants with HbSS, who are well until 
after completion of the fetal to sickle globin 
gene switch, patients with S-HPFH (who have 
70% HbS and 30% HbF), or patients with the 
Saudi-Indian haplotype (HbF levels > 20%), 
who are asymptomatic or have only mild dis-
case.2-3 The Cooperative Study of Sickle Cell 
Disease documented that even incremental 
increases in HbF reduce clinical events.6 

In this report, Pomalidamide treatment of 
sickle transgenic mice increases HbF to levels 
equal to those induced by treatment with hy-
droxyurea (HU; see figure), and comparable to 
the average response observed in patients on 
the Multi-Center Study of Hydroxyurea.7 
There is reason to anticipate higher HbF in-
duction by Pomalidamide in humans than in 
mice, because mice normally switch from em-
byronic to adult globin without a developmen-
tal window of fetal globin gene expression. In 
contrast to the cytotoxicity observed in all he-
matoopoietic lineages in the HU–treated mice, 
Pomalidamide treatment resulted in a dou-
bling of erythroid cells in the marrow. Total Hb 
increased by 0.5 g/dL above controls and by 
1.5 g/dL above levels in HU–treated mice. 
These 2 beneficial effects comprise a holy grail 
for treatment modalities to reduce the primary 
pathology in sickle cell anemia, a disease for 
which only 1 drug has ever been approved, 
more than 15 years ago. Anti-inflammatory 
effects of Pomalidamide may add a third com-
ponent. Although there was no additive activ-
ity observed with simultaneous administration 
of HU and Pomalidamide, sequential dosing 
of the 2 agents might produce higher activity, 
as intermittent dosing regimens have in-
creased activity of other drugs, such as 
Butyrate.1

At least half of adult patients in most prac-
tices are considered responsive to HU, the 
only approved therapeutic available, and 
higher responses occur in children.2,3,8 This is 
a significant degree of benefit for any drug, as 
differences in drug metabolism alone render 
most therapeutics effective in only 25%-60% 
of patients.9 A recent 17.5-year follow-up 
study has shown there is still high mortality in 
sickle cell patients (43%), and prolonged HU 
therapy (at least 5 years) is needed for a sur-
vival benefit.10 Costs of supportive therapy for 
sickle cell disease are still high: analgesia, hos-
pitalizations, transfusions, chelation, orthope-
dic surgeries, and diagnostic studies consume 
billions of health care dollars annually, and 
these costs do not include the disabilities and 
compromised lives caused by the disease. 
Some patients who still suffer some vaso-
occlusive crises despite taking HU are wary of 
long-term use of a drug with a black-box warn-
ing regarding carcinogenicity. New HbF– 
inducing agents, particularly those with dist-
inct mechanisms of action such as 
Pomalidamide, could make a great impact on 
the treatment of sickle cell disease as single 
agents or in a combination chemotherapy 
approach.11 

Trials of therapeutics for sickle cell disease 
have been challenging, at least partially due to 
the use of unrealistic end points. No single 
drug can effectively reduce all disease manifes-
tations in a condition as pathophysiologically 
complex as sickle cell disease. Furthermore, 
the phenotypic heterogeneity of this global 
disease, affected by genetic modifiers only now 
being elucidated, make study populations di-
verse (reviewed in Akinsheye et al12). Trial end 
points that are meaningful (such as total Hb 
and HbF) and achievable within a reasonable 
time duration, and in patient numbers feasible 
to enroll for an orphan disease that is fre-
quently confounded by serious clinical events, 
should be accepted for drug approval. Trial 
end points that are more relevant to the mo-
lecular actions or target of the drug should be 
considered. For example, drugs that are not 
designed to affect hemoglobin polymerization 
and consequent sickling (such as IGA17403 
[Senicapoc] or Flocar) should not be held to an 
end point of reduction in secondary events 
such as pain crises for approval, when other 
meaningful, targeted end points were met. 
Indeed, drugs targeting aspects of sickle dis-
case other than pain crises could be highly 
beneficial additions to our limited therapeutic 
repertoire. Surrogate biochemical markers 
have been accepted by the FDA for other dis-
cases for many years (eg, cholesterol lowering 
for atherosclerosis), and elevations in HbF 
should be considered an acceptable surrogate 
end point for certain sickle cell therapeutic 
candidates.

Pomalidamide joins a short, sorely needed 
pipeline of new sickle cell therapeutic candi-
dates, which target HbF, HbS, inflammatory 
or adhesion molecules. As patients continue to 
suffer the pain and ravages of this complex 
condition, approval of new therapeutics can-
not come soon enough.
Conflict-of-interest disclosure: The author declares no competing financial interests.

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VASCULAR BIOLOGY

To sprout or “Notch” to sprout?

Natasha L. Harvey CENTRE FOR CANCER BIOLOGY, SA PATHOLOGY

In this issue of Blood, Zheng and colleagues demonstrate that the Notch signaling pathway is active in lymphatic endothelial cells and that inhibition of Notch signaling, in concert with vascular endothelial growth factor (VEGF) stimulation, promotes lymphatic endothelial cell sprouting. This work suggests that Notch signaling plays a key role in regulating the switch between lymphatic endothelial cell quiescence and sprouting, akin to the role played by Notch signaling during angiogenic sprouting of the blood vasculature, and has important implications for therapeutic targeting of the Notch pathway as a treatment for vascular disorders and cancer.

Notch signaling plays a variety of key roles in vascular development and physiologic and pathologic angiogenesis (the sprouting growth of blood vessels). The role of Notch signaling in lymphangiogenesis is just beginning to be explored; conflicting results in various model systems have made it difficult to ascribe a definitive role to Notch pathway activity in lymphatic endothelial cells. Recent work in zebrafish suggested that Notch signaling is important for the initial sprouting of lymphangioblasts from the axial vein; ablating Notch activity via a number of approaches interrupted formation of the thoracic duct. Moreover, work performed in that study suggested that after lymphatic endothelial cell fate specification, Notch signaling was important for guiding the navigation of lymphatic vessels along the intersomitic arteries.

In contrast to the study by Guedens et al, work in a mouse model demonstrated that Notch signaling is not required for the specification of Prox1-positive lymphatic endothelial progenitor cells in the embryonic cardinal veins. Srinivasan and colleagues inactivated Rdh8, a major transcriptional effector of Notch signaling, specifically in endothelial cells. While few embryos survived to embryonic day 10.5, reflecting the fact that Notch is required for early aspects of vascular development, the specification of lymphatic endothelial cells in the embryonic cardinal veins was not impeded. In this model, embryonic lethality precluded the analysis of the role of Notch signaling in later stages of lymphatic vascular development.

In the article by Zheng et al, the use of novel human lymphatic endothelial cell sprouting assays, together with a mouse model of in vivo sprouting lymphangiogenesis, has revealed a role for Notch signaling in maintaining quiescence of the lymphatic vasculature by suppressing lymphatic endothelial cell sprouting. Inhibition of Notch signaling using a soluble Dll4-Fc protein, or a chemical inhibitor of Notch cleavage and activation, promoted lymphatic vascular sprouting induced by VEGF in vitro and in vivo (see figure). Interestingly, in an in vitro sprouting assay, Notch inhibition selectively promoted sprouting induced by VEGF and low doses of VEGF-C, but at high doses of VEGF-C, lymphatic vascular sprouting was not further increased by Notch pathway inhibition. This observation goes some way toward explaining the enhanced effects of VEGF-C compared with VEGF in the promotion of lymphangiogenesis, although additional factors such as cell surface VEGFR-3 levels on lymphatic endothelial cells, together with the levels of other factors involved in Notch, VEGF and VEGF-C signal transduction may contribute to this effect. An intriguing finding of this work is that VEGFR-2 inhibition in vitro,
Switching globin, raising red cells

Susan P. Perrine