addition, Miller et al present the long-term (neurologic) outcomes of HCT and the impact of adjuvant treatment with NAC (N-acetyl-L-cysteine) on survival, and they speculate on how to improve outcomes further. These are all very important issues to move the field forward.

Further improvement of the outcomes (survival and neurologic) of this very rare, devastating disease (which should also apply for other rare IMDs) can only happen when we as a transplant community: (1) centralize and coordinate the cellular therapies (such as HCT and gene-therapy) for these rare diseases and (2) tighten the international collaboration through a well-functioning international network.

The most important argument for these recommendations is the rarity of the disease. With the exception of the University of Minnesota, there are no other centers in the world transplanting these numbers of X-linked ALD (X-ALD) patients (60 in 6 years). In the study by Peters et al, only 126 patients were included from 43 centers between 1989 and 1999. This demonstrates that it is very difficult to perform single-center studies (for studying novel therapies), gain experience in clinical care, provide multidisciplinary follow-up (because most patients continue to have residual disease burden), and recognize typical disease-specific, post-HCT complications. For X-ALD, future topics of interest include:

1. How can we improve survival rates and neurologic outcome?
2. Can toxicity be further reduced using nonmyeloablative conditioning regimens, which may result in better survival and neurologic outcome?
3. Does mixed-chimerism, which may be a result from reduced-intensity conditioning, influence the long-term results as it does in other IMDs (eg, Hurler disease)? If it does, it may have implications for gene-therapy trials as well.
4. Can we optimize the outcomes with adjuvant therapies, such as NAC, which was shown by Miller et al in a previous report to impact the survival in neurologically affected patients before HCT? As previously mentioned, there is need for further study of the effect of adjuvant therapies, such as NAC, to further improve outcomes.
5. Patients (and parents) may profit from well-organized, dedicated, disease-specific, multidisciplinary teams. As early diagnosis and early transplantation influence outcome, early recognition of certain problems, which may be part of residual disease burden, is of importance to prevent more severe morbidities. This can be done best by experienced teams in specialized centers.

For X-ALD (which may also apply to other IMDs), nationwide, regional, or state-centralized care may improve outcomes after HCT. It would substantially increase the experience of the multidisciplinary teams caring for these rare diseases, increase data collection for international studies, and promote uniform treatment protocols or guidelines. These centers should be organized as clinical and research networks. In The Netherlands there has been 1 national referral center (UMC Utrecht) for HCT in IMDs since 2004. Within a multidisciplinary team the indication for HCT is made and after transplantation there is a standardized, multidisciplinary follow-up program for these patients. This has definitely impacted the process of care for this vulnerable patient group. In the past decade, international collaboration, such as the introduction of transplantation guidelines for IMD within the European Group for Blood and Marrow Transplantation, has resulted in better outcomes (higher survival and lower mortality rates). For example, the survival rate of HCT in Hurler syndrome increased from 50% to > 90%. And currently, an international long-term follow-up study, including the majority of the European– and North American– transplanted Hurler syndrome patients, is in its analyses phase. We have to build on these collaborations to continue to improve treatment in X-ALD and other IMDs. Greater international collaboration will lead to better outcomes in this interesting and challenging field.

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Comment on Cheng et al, page 1998

Channeling the homocysteine chapel

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Hyperhomocysteinemia (HHcy), a potent risk factor for cardiovascular disease, is associated with impaired endothelium-dependent vasodilation. In this issue of Blood, Cheng et al report that severe HHcy causes oxidation and tyrosine nitration of small and intermediate conductance Cα2–3-activated potassium channels, resulting in impaired endothelium-derived hyperpolarizing factor (EDHF)--mediated relaxation of resistance arterioles.

When functioning normally, the endothelium regulates vasomotor tone and local hemostasis by releasing vasodilator substances (nitric oxide, prostacyclin, or EDHF mediators) and vasoconstrictor substances (endothelin-1, angiotensin II, thromboxane A2, or free radicals). Linking HHcy to specific potassium channel dysfunction and impaired EDHF-mediated relaxation points to new therapeutic targets with potentially broad relevance in HHcy-related atherothrombotic disease (see figure).

Loss of endothelial responsiveness, an important aspect of overall endothelial dysfunction, is an early event in the pathogenesis of atherosclerosis, and maintaining endothelial...
Summary of known and novel adverse effects of hyperhomocysteinemia on vascular cells.

- **Known**
  - EDHF-mediated vasorelaxation
  - Ca²⁺-induced hyperpolarizing K⁺ current: IK₁, SK₃ channels

- **New**
  - Hyperoxygen species Peroxynitrates

Smooth muscle cell hyperpolarization mediated through endothelial cell potassium channel opening is a fundamental mechanism for vasodilation; it is particularly important at the arteriolar level for control of tissue perfusion, and often compensates for loss of other dilator mechanisms in disease settings.

Although HHcy has previously been linked to endothelial dysfunction via effects on both endothelial nitric oxide synthase (eNOS) and prostacyclin production, Cheng et al focus on the effect of severe HHcy on EDHF-mediated relaxation. It is now recognized that EDHF-mediated responses, characterized first in the 1980s as alternative to the cyclooxygenase and the NO synthase pathways, can be mediated by a variety of agents, not just the single diffusible factor implied by the name. Regardless of triggering agent, the small and intermediate conductance potassium channels, SK₃ and IK₁, are key components of EDHF-mediated relaxation.

Cheng et al performed ex vivo studies of small mesenteric arterioles (150-200 μm in diameter) derived from control mice, or transgenic mice with severe HHcy, to characterize vascular relaxation in response to a battery of agonists and inhibitors, and also to provide material for biochemical and immunohistologic studies. The central finding is that EDHF-mediated relaxation is compromised in the mice with severe HHcy; interestingly, despite robust increases in arterial SK₃ and IK₁ expression, the net function of these channels is significantly impaired in the setting of severe HHcy. This pathologic condition corresponds to increased superoxide and nitrotyrosine in the vascular wall, and Cheng et al show also that superoxide scavengers, peroxynitrite inhibitors, or the K⁺ channel opener NS309, which robustly increases channel Ca²⁺ sensitivity, restore most of the EDHF-mediated relaxation.

While rescue of relaxation by these interventions is intriguing, the target(s) of these untoward reactive species within the arterial wall is not entirely clear. Cheng et al studies of cultured endothelial cells show that homocysteine-mediated stress increases expression of IK₁ and tyrosine nitration of both SK₃ and IK₁, but how these modifications interfere with channel function remains to be determined. Large conductance Ca²⁺-gated potassium channels (BKCa) become more active with exposure to superoxide and hydrogen peroxide, but less so with peroxynitrite; little information is available on how these compounds affect SK or IK channel function.

Likewise, the increase in channel expression coincident with decreased function is also of interest. In one possible scenario, the HHcy-induced modifications that impair channel function might also stabilize the channels by interrupting their normal processing. Alternatively, a more complicated feedback loop may increase channel expression in response to decreased function, but be unable to compensate in full. Further detailed studies of direct channel modifications, with assessment of corresponding function and stability, as well as gene activation could yield insight on these points.

The findings of Cheng et al also bear on clinical HHcy. Pharmacologic rescue of EDHF-mediated vasorelaxation in severe HHcy, commonly defined by a serum Hcy concentration of > 100 μM, has clear potential therapeutic value for children with homocystinuria, a highly morbid autosomal recessive syndrome affecting 1 in 200,000 live births in the United States and accompanied by atherothrombosis of medium and small arterial vessels, in addition to optic discoloration, premature osteoporosis, and mental retardation. Going forward, it will also be important to assess how EDHF-mediated mechanisms fare in the setting of the less extreme elevations of Hcy that are far more prevalent in our society; such increased levels of Hcy can occur as a result of other, less severe genetic mutations, inadequate dietary intake of folate and vitamins B₆ and B₁₂, or as a consequence of some medications including methotrexate. It has been suggested that cardiovascular disease risk increases nearly 20% for each 5 μM increase in serum homocysteine level, so if the threshold at which HHcy can impair EDHF-mediation relaxation falls in the range of moderate or intermediate HHcy, this newly identified mechanism may contribute much more broadly to the pathologic consequences of HTN, diabetic vascular disease, endothelial dysfunction in cardiovascular interventions, and small vessel disease known to cause end-organ damage.

Finally, the insights offered by Cheng et al may inform new strategies for more effective manipulation of HHcy. Recent clinical trials of combined folic acid and B-vitamin therapy have achieved biochemical goals by lowering Hcy levels, but failed to show clinical benefit in terms of cardiovascular disease outcomes. This lack of clinical effect may reflect the complexities of folate and other B-vitamin biochemistry: folic acid supplementation not only decreases Hcy, but also indirectly impairs eNOS function, in effect nullifying the vascular protective effect of Hcy lowering. Potentially, more nuanced understanding of the pathogenic mechanisms provoked by HHcy may point toward therapeutic approaches that can discretely interrupt its deleterious effects.

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they found that the angiogenic defect observed in total rap knockout mice can be recapitated by an endothelial-specific knock-out of rap genes, and that rap1a and rap1b play nonredundant roles in angiogenesis, which rules out the possibility that the angiogenic defect was because of a general loss of an angiotropic factor in tissues that require vascularization.

Rap1b appears to be a stronger mediator of developmental angiogenesis than rap1a. Given these findings, the study by Lakshmikanth et al focuses on the role of rap1b on VEGFR2 signaling and integrin activation. Rap1b appears to have both integrin β3-dependent and -independent events. Both Rap1b and β3 knockdown appear to reduce the magnitude of VEGFR2 phosphorylation in response to VEGF stimulation. However, the magnitude of stimulation appears to be relatively similar, with the differences in magnitude of final stimulation being dependent on the basal levels of VEGFR2 phosphorylation. This suggests that β3 integrins and rap1b act as rheostats that modify basal levels of VEGFR2 phosphorylation. These results suggest that dual down-regulation of β3 integrin and rap1b would be superior as an antiangiogenic than inhibition of either molecule alone, and suggests that chronic down-regulation of either β3 integrin or rap1b in the clinical setting might lead to a compensatory up-regulation of the other molecule.

Blockade of rap1b has physiologic significance. Down-regulation of rap1b by morpholinos in zebrafish leads to a distinct defect in the number and length of intersomitic vessels of the midtrunk, which is phenocopied in a more severe form by a VEGFR2 kinase inhibitor. This also suggests a developmental gradient, in which midtrunk vessels are more susceptible to rap1b blockade than anterior vessels.

How can we target rap1b? Two studies that should be performed are xenograft studies and carcinogenesis studies. The major question is, how does endothelial rap1b deficiency impact on pathologic angiogenesis? Both xenograft and carcinogenesis studies are suggested because while both of these are examples of pathologic angiogenesis, they have different contributions of local versus bone marrow-derived endothelial contributions. Carcinogenesis experiments are a good measure of local endothelial recruitment, while xenograft studies rely on recruitment of bone marrow precursors, as elegantly demonstrated by the lack of xenograft growth in Id1/Id3 deficient mice.1,3

Finally, abnormalities of rap1 signaling are present in human disease. Loss of Kirit1, a rap1 effector, leads to cerebral cavernous malformation, which is associated with uncompensated rhoA activation.1,4 Conversely, hemangiomias are associated with elevated ral1/reactive oxygen signaling (see figure).1,4 The lessons from the study by Lakshmikanth et al suggest that rap1 signaling is required for normal vasculature, and that a careful balance of rap1 signaling results in tonic VEGFR2 activation, which allows normal vessels to respond rapidly to an angiogenic stimulus.

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