is known to carry a ubiquitin-binding domain that could promote the direct interaction of both proteins.[7] Second, from a mechanistic point of view, it will be important to decipher how $As_2O_3$ initiates the interaction of p62/SQSTM1 with BCR-ABL. Along this line, the phytoalexin resveratrol has been shown to promote the interaction of p62/SQSTM1 with microtubule-associated protein light chain 3 (LC3), thus favoring autophagy.[8]

The study by Goussetis et al paves the way for the possibility of new therapeutic intervention in CML. TKIs that target BCR-ABL are currently the leading compounds for patients suffering CML, leading to complete remission in a majority of cases. However, although they inhibit the tyrosine kinase activity of BCR-ABL, TKIs failed to eliminate the so-called leukemic initiating cells (LICs) that are critically involved in the reinitiation of the disease in a non-negligible proportion of the treated patients. Conversely to TKIs, $As_2O_3$, ATRA, or resveratrol, all drugs that are susceptible to target LICs could represent new therapeutic options in the treatment of CML. This should be achieved using combination of TKIs with the above-mentioned compounds.

Furthermore, BCR-ABL promotes the activation of the mammalian target of rapamycin (mTOR) pathway and as such acts as a potent inhibitor of autophagy, either directly or via inhibition of the adenosine monophosphate kinase. $As_2O_3$ and resveratrol are both capable to inhibit the mTOR pathway and to trigger CTSB-dependent BCR-ABL degradation.[8,9] In the future, we must build on these dual effects of $As_2O_3$ for a better approach to CML treatment.

In conclusion, it is clear that the therapeutic modulation of autophagy represents a new avenue for the treatment of leukemia. The discovery that different clinically well-characterized therapeutic drugs trigger their effects via the autophagic degradation of oncogenic fusion proteins is of outstanding importance in oncohematology. Accordingly, the article by Goussetis et al sheds new light on the regulation and function of BCR-ABL, representing the premises for the use of these drugs in combination with TKIs in chronic phase CML and as a single therapy in TKI-resistant patients.

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

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● ● ● THROMBOSIS & HEMOSTASIS

Comment on Feys et al, page 3611, and on Callewaert et al, page 3603

# Blocking VWF platelet binding to treat TTP

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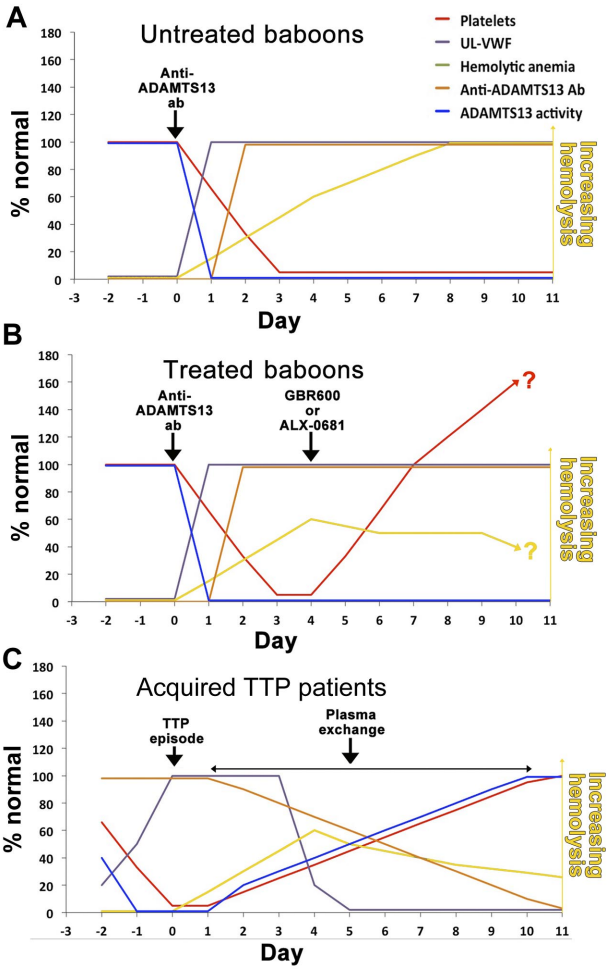
Two articles in this issue of *Blood* from Feys et al and Callewaert et al, respectively, have employed very similar and elegant strategies in attempts to ameliorate the symptoms of thrombotic thrombocytopenic purpura (TTP).[1,2]

TTP is associated with severe deficiency in ADAMTS13, the metalloprotease that regulates von Willebrand factor (VWF) multimeric size and its platelet-tethering function.[3,4] ADAMTS13 deficiency induces the persistence of pathogenic ultra-large (UL)–VWF in plasma that precipitates the formation of microvascular platelet-rich thrombi that variably cause microangiopathic haemolytic anaemia, thrombocytopenia, neurologic abnormalities, fever, and renal dysfunction. TTP is most frequently an acquired autoimmune disorder, arising through the formation of inhibitory antibodies against ADAMTS13. Untreated, the mortality rate of TTP is approximately 90%.

TTP is currently most effectively treated by plasma exchange, which serves to remove anti-ADAMTS13 antibodies and UL-VWF, and also replenish plasma ADAMTS13 activity. Steroids are used as immunosuppression to attain complete remission and, more recently, the use of rituximab has also proved highly efficacious in treating acquired TTP.[5] Despite the appreciable reduction in mortality (to 10%-20%) using these therapeutic approaches, treatments are individualized and may be associated with further risks and complications. Therefore, new safer and simpler strategies to treat TTP are desirable.

In mice, ADAMTS13 deficiency alone is insufficient to precipitate TTP-like symptoms, making it a difficult model in which to study novel therapeutic approaches.[6] To circumvent this, and to better mimic the human scenario, Feys et al recently developed a baboon model of acquired TTP by infusing an anti-ADAMTS13 monoclonal antibody that efficiently inactivates plasma ADAMTS13.[7] In baboons, this causes elevated plasma UL-VWF, platelet-rich thrombi in the microvasculature resulting in thrombocytopenia and haemolytic anaemia (see figure panel A). Although this nicely models early-stage human TTP, baboons do not develop life-threatening disease, or signs of neurologic or renal dysfunction. The clinical features of TTP are primarily linked to elevated plasma UL-VWF. For this reason, both Feys et al and Callewaert et al rationalized that targeting the glycoprotein Ib binding site in the VWF A1 domain might specifically prevent TTP.[1,2]

Feys et al employed a humanized mouse monoclonal antibody against the VWF A1




**Schematic representation of parameters in the baboon model of TTP (A), with treatment with either GBR600 or ALX-0681 (B), or in patients with acquired TTP receiving plasma exchange therapy (C).**

domain (termed GBR600), whereas Callewaert et al used a humanized bivalent nanobody recognising the same domain in VWF (termed ALX-0681). Both GBR600 and ALX-0681 avidly block VWF binding to glycoprotein Ib on the surface of platelets. Feys et al and Callewaert et al demonstrated that administration of GBR600 or ALX-0681, respectively, at the same time as the anti-ADAMTS13 antibody effectively inhibited UL-VWF function in baboons and prevented the onset of thrombocytopenia. Hemolytic anemia (as measured by the reduction in haptoglobin and the appearance of schistocytes) was also appreciably reduced using GBR600 or ALX-0681. Together these results demonstrated the UL-VWF-dependence of the TTP features seen.

These settings do not mirror the human situation where a patient presents with preexisting symptoms. To model this, both studies allowed symptoms of TTP to develop for 4 days before administration of either GBR600 or ALX-0681. After treatment, both therapies caused a rapid normalization of platelet count after 3 days that continued to rise further over the following 3 to 4 days, suggesting that further platelet thrombi development and platelet consumption had been effectively inhibited (panel B). Improvement of hemolytic anemia was also evident through the gradual reduction in the numbers of schistocytes and signs of increases in plasma haptoglobin by the end of the 11-day study period. Crucially, there were no signs that blocking the VWF A1 domain caused appreciable risk of severe bleeding.

Together, these results are particularly encouraging and suggest that targeting VWF may be a highly effective strategy to prevent further development of symptoms in TTP. However, some important considerations remain in taking these studies further. The question arises as to whether blocking VWF is as effective as plasma exchange in ameliorating TTP in humans with more severe symptoms and, therefore, whether, in conjunction with immunosuppression, it has the potential to replace it. In acquired TTP patients, plasma exchange rapidly reduces antibody titer, UL-VWF and restores plasma ADAMTS13 activity to nonpathologic levels, enabling platelet counts to recover (panel C). It is likely that the restoration of ADAMTS13 activity by plasma exchange provides important therapeutic benefit for TTP patients that would be lacking with a VWF blocking approach alone, which allows the anti-ADAMTS13 antibodies and plasma UL-VWF to persist (panel B). Although both GBR600 and ALX-0681 showed signs of improvement of hemolytic anemia, Callewaert et al also showed that ALX-0681 did not alter the number of platelet-rich thrombi in treated baboons. Neither GBR600 nor ALX-0681 can dissociate preformed VWF-platelet complexes,[1,2] which could be a limitation in treating TTP patients with more severe symptoms. Recently, Crescente et al demonstrated the thrombolytic potential of ADAMTS13 in a murine model of thrombosis, suggesting that ADAMTS13 activity might play an important role in the dissolution of platelet thrombi.[8] Conceptually, this could be of particular benefit to TTP patients in ameliorating the effects of occluded vessels.

The 2 studies by Feys et al and Callewaert et al provide excellent evidence for the safety and efficacy of targeting VWF in TTP,[1,2] which is also corroborated by some of the data using an aptamer (ARC1779) that similarly blocks the VWF A1 domain.[9] However, the immune pathophysiology of TTP must still be treated separately. This is illustrated both by the tendency toward thrombocytopenia and hemolytic anemia after cessation of ALX-0681 in the baboons where the disease-inducing antibody 3H9 remains in circulation, and by data from the initial experience with ARC1779 in TTP patients.[9] Whether it is feasible to employ the baboon model to make a comparison of GBR600 or ALX-0681 therapy with plasma exchange is unclear, but this could certainly enable the investigators to ascertain whether their therapeutic agents compare favorably with current treatment as well as defining whether plasma exchange does indeed carry important benefits associated with clearance of existing thrombi. This could determine whether VWF inhibition might in fact complement plasma exchange in TTP patients, in particular with respect to the time to

platelet recovery and number of required plasma exchanges.

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

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● ● ● TRANSPLANTATION

Comment on Hassan et al, page 3615

# HCT survival in ADA-SCID: what's the buzz?

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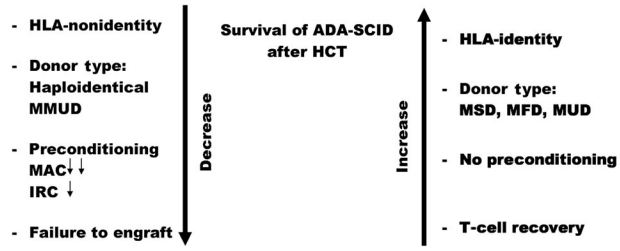
In this issue of *Blood*, Hassan et al have turned the spotlight on hematopoietic stem cell transplantation (HCT) of adenosine deaminase (ADA)–deficient severe combined immunodeficiency (SCID).[1] They opened up the curtain of beliefs on this therapy that enables facts to be separated from fiction.




**Risk factors for survival of adenosine deaminase deficient (ADA) patients with severe combined immunodeficiency (SCID) treated with hematopoietic stem cell transplantation (HCT). MMUD indicates mismatched unrelated donor; MAC, myeloablative conditioning; IRC, intensity reduced conditioning; MSD, matched sibling donor; MFD, matched family donor; and MUD, matched unrelated donor. Double small arrows MAC indicate lower survival versus the single small arrow with IRC.**

Hassan et al show what common beliefs concerning stem cell immunoreconstitution of ADA-SCID are true or not. To be helpful to the expectations of parents of such children, they also raise the critical issue of quality of life and educational and employment accomplishments as important goals not being sufficiently assessed.[2,3] The results of this study are powerful and persuasive, as it is the only definitive analytical study of a large number of ADA-SCID children given HCT. ADA enzyme replacement and ADA gene therapy are also considered alternate therapies. The authors point out that the ADA deficiency affects many cells and the remedy for the immune cell lineages may not apply to other cell types, such as the central nervous system and respiratory system.

One hundred six ADA-SCID patients were entered into this multicenter analysis of the outcomes of their HCT therapy. The most prominent risk factor for a good outcome is the lack of perfect HLA matching as noted by an earlier publication.[4] In the present study, HCT of ADA-SCID patients with these donors yielded the following overall survival (OS) results: mismatched unrelated donors, 29%; haploidentical donors, 43%; matched unrelated donors, 67%; matched family donors, 83%; and matched sibling donors, 86%.[1] These results confirm the common belief that HLA matching is the most important risk factor for OS for ADA-deficient SCID children treated with HCT (see figure).

This large study also addressed the controversial question of preconditioning of SCID children before HCT.[5] Myeloablative preconditioning was shown to be a risk factor for OS (56%), whereas reduced-intensity conditioning displayed a trend toward improved survival (67%) but was not significantly different from unconditioned transplants (78%), whereas myoablative therapy was. We must remember that almost all unconditioned transplants had the benefit of HLA matching so the superior survival is not unexpected, and ADA-deficient cells may be more susceptible to toxic effects of preconditioning agents. Unconditioned haploidentical transplants fared much worse in a small cohort with 1 of 6 survivors. Thus, this report has something for proponents on both sides of the debate on whether or not to precondition SCID patients before transplantation.

This study refutes the belief that prior ADA enzyme therapy is a risk factor for OS of patient after HCT.[6] Those patients who were treated with ADA enzyme did as well as those not receiving ADA enzyme at the time of HCT. Similarly, this study does not support previous findings that early transplantation enhances overall outcome of patients,[7] although it seems intuitive that a prolonged pretransplant period in an unprotected environment would favor acquisition of a potentially fatal virus infection. The HCT approach for ADA-SCID needs to be balanced with the concept of gene therapy where spectacular results (100% survival) have been achieved by Aiuti et al.[8]

As an indication of the fundamental difference between ADA-SCID and other forms of SCID, the immunoreconstitution of B cells and ability to produce and secrete immunoglobulins and specific antibodies in evaluable immunoreconstituted ADA-deficient patients is striking. Some of these patients, while not showing donor B-cell engraftment, were still able to make normal humoral immune