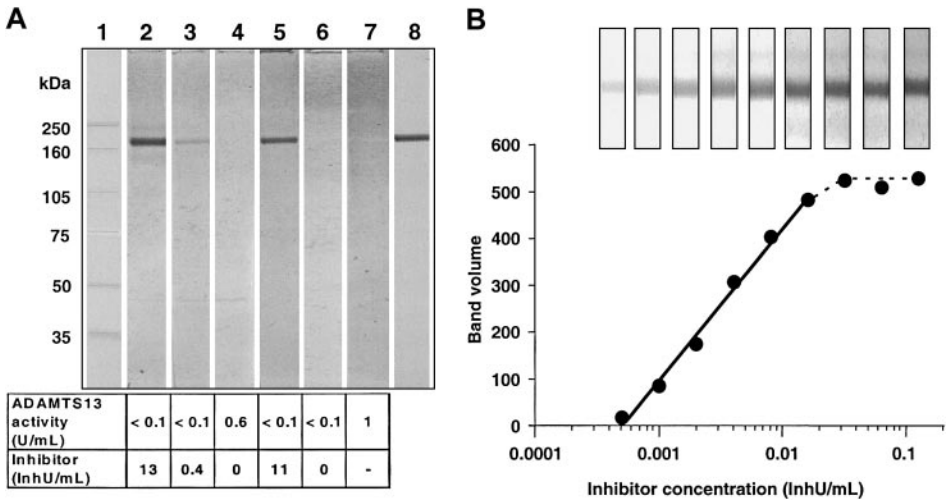

Figure 1. Immunoblot for detecting autoantibodies against ADAMTS13 in plasma samples. (A) Comparison of different plasma samples with a monoclonal anti–human ADAMTS13 antibody. Lane 1: molecular mass marker (full-range rainbow marker, Amersham Pharmacia). Lanes 2-4: plasma samples from a patient[6] with antibody-induced TTP taken at different stages during her disease course. Lane 5: plasma sample from a patient with antibody-induced TTP. Lane 6: plasma sample from a patient with hereditary TTP. Lane 7: pooled normal plasma. Lane 8: monoclonal anti–human ADAMTS13 antibody applied in 1/1000 dilution. The plasma samples used for staining lanes 2-7 were diluted 1/200 with 20 mM Tris (tris(hydroxymethyl)aminomethane), 130 mM NaCl, pH 7.2 buffer, containing 0.05% (wt/vol) Tween 20 and 10% (vol/vol) blocker casein in Tris-buffered saline (TBS; Pierce, Rockford, IL). The bottom panel shows the ADAMTS13 activities and the inhibitor concentrations measured by the collagen-binding assay. (B) Quantitative evaluation of the immunoblots. The intensity of the ADAMTS13 bands in the Western blots developed with serial dilutions of a plasma sample from patient A were plotted against the applied inhibitor concentration. The inset shows the ADAMTS13 bands as they appeared on the immunoblots.

developed with the monoclonal antibody against the rADAMTS13 preparation (lane 8).

The results from this immunoblot assay correlated well with the quantitative determination of inhibitors by the collagen-binding assay.

The sensitivity of inhibitor detection was determined by using serial dilutions from 1/100 to 1/25 600 of a plasma sample from a patient with an inhibitor titer of 13 inhibitor units (InhU)/mL (Figure 1B). When the blots were quantified by densitometry, there was a linear correlation between the inhibitor concentrations of the samples and the band intensities in the range of 0.0005 to 0.010 InhU/mL. Taking into account that the plasma samples were diluted at least 100-fold, we estimated that the method detected inhibitory antibodies at a plasma concentration of 0.05 InhU/mL, which is far below the detection limit of currently available assays. In addition to the functional inhibitor assays, the immunoblotting can also detect noninhibitory antibodies, which will further explain the role of ADAMTS13 proteases and the pathogeneses of TTP.

The high specificity and sensitivity of this immunoblotting procedure renders the assay a valuable tool complementary to quantitative ADAMTS13 inhibitor assays and thus facilitates screening patients for antibody-induced TTP.

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We are grateful for the excellent technical assistance of Manfred Billwein.

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To the editor:

Bcr-Abl mutations, resistance to imatinib, and imatinib plasma levels

Corbin and colleagues analyze in their report[1] the biochemical and biologic effects of several mutations in the kinase domain of the Bcr-Abl protein identified in patients who developed resistance to imatinib. Some mutations (6 of 9 studied here) confer only a modest resistance index (less than 2-fold increase in IC_{50}), with IC_{50} values lower than plasma values commonly observed in patients treated with standard dosages of imatinib. Therefore, the authors suggest that these mutations could not represent the cause of resistance.

In our opinion, some quality criteria (ie, analysis of clones instead of polymerase chain reaction products, detection of a

minimum number of positive clones, genomic confirmation of the mutation) should be used to better assess the nature of these mutations. However, since the mutants were detected in resistant patients and were present at very low frequency before treatment,[2] one must conclude that it is unlikely that so many mutants represent just laboratory artifacts.

We present here an alternative interpretation of Corbin et al's data. A considerable amount of preclinical and clinical information shows that plasma imatinib concentrations do not represent a reliable estimate of intracellular or tissue concentrations of this drug.[3-7] In addition, it is evident that a substantial fraction of imatinib present in plasma is not biologically active.[5] In our opinion, the likely although not universally accepted[8,9] explanation for this phenomenon resides in the strong binding of imatinib to alpha 1 acid glycoprotein (AGP[3]). Our interpretation of Corbin et al's data states that in the cases in whom IC_{50} values were close to the wild-type (WT) one, selection of mutants probably happened at (active) imatinib concentrations higher than the WT IC_{50} but lower than those present in mutants (0.7-1.6 μM), and also lower than the usually observed imatinib plasma concentrations.[6,10]

Two studies were published on imatinib pharmacokinetics. One was performed in patients with chronic myeloid leukemia in chronic phase[10]; the second one mostly involved patients in accelerated phase/blast crisis.[6] The average imatinib concentration obtained at steady state, 24 hours after the last dose, ranged in the 2 studies between 1.46[10] and 1.96[6] μM (400-mg dose level), with average peak concentrations of 4.6[10]-7.52[6] μM. The 600-mg dose level produced substantially higher concentrations.[6] We think it is important for the scientific community to know that imatinib plasma levels cannot be used to estimate the amount of the drug present inside cells, since plasma imatinib concentration is dependent also on the AGP plasma level.[6]

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Response:

Imatinib resistance may be more complex than impaired inhibitor binding

Our recent study examined several Bcr-Abl kinase domain mutants that have been detected in association with clinical resistance to imatinib.[1] We identified a group of mutants with IC_{50} values that are close to wild-type Bcr-Abl and below or just above steady state trough plasma concentrations in patients on 400 mg imatinib daily.[2] As the title of our manuscript indicates, the crucial point is that some mutants retain sensitivity to concentrations of imatinib that are achievable in the plasma of patients. In individuals with these mutants, response may be recaptured by dose escalation, whereas this is less likely in patients with highly resistant mutants. Moreover, although IC_{50} values provide some orientation, it is not known which level of inhibition in vivo determines clinical efficacy. Pharmacodynamic assays to accurately measure residual kinase activity in cells expressing the various mutants, correlation with induction of apoptosis, and careful evaluation of the effect of dose escalation in patients will help to devise rational treatment strategies.

As we discussed, studies such as these may conclude that impaired drug binding of imatinib to this group of mutants may not be sufficient to explain clinical resistance. Therefore, we proposed that more complex consequences of the mutations, like altered protein interactions or coincidence with other mechanisms of resistance, may play a role. However, we did not claim that these mutations simply represent laboratory artifacts.

We agree with Gambacorti-Passerini and colleagues that slightly increased IC_{50} values in combination with less bioavailable imatinib could theoretically favor the outgrowth of these mutant clones. However, as they concede, the role of acid alpha 1 glycoprotein in impairing the bioavailability of imatinib is controversial.[3] Furthermore, the effective imatinib concentrations in cellular assays in vitro[4] are better correlated with total than with free in vivo plasma concentrations of imatinib.[5] It still remains to be shown that by reducing the protein-bound fraction of imatinib one could indeed regain response to therapy.

In addition, it is important to distinguish between the phenomena of in vivo selection and clinical resistance. In vivo selection of mutant clones may occur without clinical relapse. In line with this concept, cytogenetic clonal evolution has been observed in patients who maintained response to imatinib.[6] Thus, Bcr-Abl mutants with marginally reduced sensitivity to imatinib may be selected over time, while response is maintained. The fact that mutant clones are detectable prior to imatinib therapy in some cases is circumstantial evidence that they may have a growth advantage even in the absence of this drug.

To resolve this issue, it will be important to determine whether mutants with only marginally increased in vitro resistance respond to dose escalation of imatinib in vivo. If not, this would clearly argue for resistance mechanisms other than impaired imatinib binding.

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