The possibility that the CsA arm might “do worse” was not, as the letter alleges, “dismiss[ed]” by the design or analysis of the study. The stated principal objectives of the trial included comparison not only of induction response and survival but also of the toxicity of the 2 regimens. In addition, the study’s protocol required that the planned interim analyses include assessments of whether the study should be terminated early due to evidence against a clinically meaningful benefit from CsA, a much easier condition to satisfy than evidence of worse outcomes with CsA, which a 2-sided design would have required.

The assertion in the letter that designing the study to achieve an inherently one-sided objective “diminishes the power of the study” is rather misleading. For any given one-sided alternative hypothesis, that is, for any given magnitude of CsA benefit, a one-sided comparison has greater statistical power than the corresponding 2-sided comparison based on the same sample size and critical level. Therefore, employing a 2-sided test when a one-sided test is appropriate in fact “diminishes” statistical power. In other words (and we assume this is the authors’ intended point), a 2-sided design requires a larger sample size than a one-sided design in order to have equivalent statistical power to detect any given magnitude of CsA benefit. This is indeed true but is simply a consequence of the fact that the objective of the study determines its design (including sample size). Requiring a 2-sided study’s sample size when a one-sided design is appropriate prolongs the duration of the study and delays the availability of clinically important information.

The second issue raised in the letter concerns the role of stem cell transplantation. The analysis reported in the paper\(^1\) was performed to investigate a specific question: whether the apparently beneficial effects of CsA might be attributable to an increased likelihood of receiving stem cell transplantation in remission. The letter requests “more detailed analysis” of the characteristics of the transplants and patients who received them. It is ironic that the authors criticize the use of “secondary and subgroup analysis” in one paragraph and then call for a much more extreme form of such analysis in the following paragraph. Any attempt to attribute differences in outcomes to differences in events following randomization, which is of course not based on protocol-directed “intent-to-treat” comparisons, is subject to unquantifiable biases. Our analysis of the impact of transplantation is certainly subject to this criticism, and we were therefore careful to claim only that the results “suggest[ed] that the CsA effect was not attributable to transplantation in remission.”\(^1\)

The requested “more detailed analysis” is an attempt to identify these biases but is doomed to inconclusiveness. The decision as to whether or not a patient receives a transplant of a given type is the result of a complex balance of the expectations, experiences, resources, and preferences of the physicians, the patient, and even the patient’s family and friends. The decision process is highly individualized, quite possibly differing according to prognosis and/or between treatment arms, and is largely undocumented. Therefore the question, which addresses a non-protocol-directed decision (ie, “Who were the patients that received transplants and by what criteria was transplantation determined?”), cannot be answered with sufficient detail and confidence to ensure that the possibly large biases are all accounted for.

In addition, the authors make a strong argument against the very analysis they request, by emphasizing that “[w]hen dealing with such a small number of patients, . . . [small, uncontrolled effects] . . . may dramatically affect the results.” This is certainly true of any attempt to try to identify biases arising from the decision processes that led to transplantations in only 26 patients.

Finally, we note that the letter incorrectly implies that complete remission (CR) rate was the sole primary end point of the study. In fact, as described above, overall survival and toxicity were also primary end points of the study.

To the editor:

**Binding of imatinib by α1-acid glycoprotein**

In their recent report Jørgensen et al\(^1\) raised doubts on the ability of α1-acid glycoprotein (AGP) to bind and inhibit imatinib (STI571), as shown in our previous report.\(^2\) We would like to comment on this paper, on some methodological inaccuracies of their paper, and on additional in vivo data that in our opinion strongly indicate an important role for AGP in modulating imatinib bioavailability and pharmacokinetics (PK).

First, it is well known that chromatographically isolated AGP, the one used by Jørgensen et al, show less-efficient binding of drugs in general than chemically isolated AGP, the one used in our paper.\(^3\) It is surprising in this respect that Jørgensen et al never used as a control our preparation of AGP.

Second, in their paper the authors state that our AGP preparation, supplied by Sigma, “risks desialylation of the protein.”\(^1\) But the authors fail to acknowledge that such a phenomenon has been associated with a decrease (or with no change at all) in drug binding,\(^3,5\) and not with an increase in binding, as their data apparently suggest.

Third, the drug-binding assay shown is misleading. Quenching of AGP fluorescence requires detailed information on a given drug’s binding site to AGP, since several binding sites for drugs on AGP are known; this information was not provided for imatinib. In addition, quenching should be shown using progressively increasing concentrations of the drugs being studied and not, as done by Jørgensen et al, by comparing 2 different drugs, used at a single concentration, which differed in the 2 drugs studied (imatinib at 1 μM, chlorpromazine at 2.5 μM).

Fourth, in vitro experiments using unmanipulated AGP (in the form of sera containing different concentrations of AGP) performed by 2

References

independent groups\textsuperscript{5,6} show that the inhibition on imatinib activity is proportional to the content of AGP and can be blocked by the coincubation with erythromycin, a known binder of AGP.

Fifth, additional ex vivo experiments were performed using unseparated blood samples from patients on treatment with imatinib and clinically resistant to it. Although plasma levels of imatinib exceeded 3 \textmu M levels in these patients, Bcr/Abl was highly phosphorylated; short-term incubation (1 h) with erythromycin resulted in almost total (> 85\%) phosphorylation inhibition.\textsuperscript{7}

Sixth, in vivo studies in patients treated with imatinib show that there is a significant correlation between AGP levels and some PK data such as Cmax.\textsuperscript{8} In addition, the coadministration of imatinib and clindamycin, another antibiotic known to bind AGP, resulted in significantly reduced Cmax and AUC and in increased free fraction of imatinib; in particular, clindamycin induced within 5 minutes a fall in plasma imatinib concentrations ranging from 2-fold to 5-fold (Gambacorti-Passerini et al\textsuperscript{9} and Gambacorti-Passerini, April 9, 2002, manuscript submitted for publication).

For the above-mentioned reasons, the data from Jørgensen et al are difficult to evaluate and their in vivo relevance is questionable.

\textbf{Response:}

\textbf{Further observations on the debated ability of AGP to bind imatinib}

We thank Gambacorti-Passerini et al for their comments on our paper, which we note with interest. We are indeed encouraged by the debate provoked by the publication of our brief report\textsuperscript{1} and welcome objective discussion from scientific colleagues. But we feel that some of the points made by Gambacorti-Passerini et al potentially arise from a lack of appreciation of the methods utilized for the purification and characterization of glycoproteins, the importance of glycosylation as a secondary modification of proteins, and its implication for drug binding. Our responses to the specific points raised are as follows:

First, historically the majority of techniques for the isolation of human \alpha\textsubscript{1}-acid glycoprotein (AGP) were chromatographic procedures with strongly acidic buffer. Indeed, the commercial AGP product assayed by Gambacorti-Passerini et al\textsuperscript{2} was isolated according to the process of Hao and Wickerhauser,\textsuperscript{3} which is a combination diethyl-amino ethyl (DEAE)-Sephadex/carboxymethyl (CM)-cellulose chromatographic method at pH 4.7, not a chemical method as stated by Gambacorti-Passerini et al. Thus, both the commercial product and our AGP are chromatographically isolated. Review of the literature, including Kremer et al,\textsuperscript{4} indicates that acidic isolation methods will damage AGP oligosaccharide (principally by desialylation) and polypeptide components. Thus we are satisfied that, by avoiding harsh acidic conditions, our published purification method yields AGP without any structural degradation\textsuperscript{2} and thus gives a valid representation of the actual in vivo presentation of the glycoprotein. Furthermore, the fluorescence data (see the third point below) presented indicates that chlorpromazine, a known AGP binder, effectively binds to our isolated AGP, which is not in keeping with “less-efficient binding.”

As clearly presented in our paper, the main aim was to examine AGP in the CML setting, as it is well documented that the glycoprotein alters both quantitatively and qualitatively in disease. AGP in the CML setting, as it is well documented that the glycoprotein alters both quantitatively and qualitatively in disease. Thus, commercial AGP isolated from normal plasma would not satisfy this requirement. Nonetheless, the most logical approach was to isolate AGP from normal plasma as a control by the same method as for CML-derived AGP.

Second, glycosylation, in the form of oligosaccharide chains covalently bound to protein, is a significant presence on the surface of glycoproteins, such as AGP, and functions to affect the conformation of the underlying polypeptide largely owing to the huge hydrodynamic volume occupied relative to amino acids. Thus, the presence of a particular glycosylation pattern may influence the protein conformation and thus the degree of access to the drug-binding site. Any change in glycosylation, including the removal of sialic acid, may result in a conformational rearrangement that could conceivably increase, decrease, or leave unaltered the access to the drug-binding site. Our isolation method does not involve denaturing steps, such as preliminary acidic preparation and/or exposure to strongly acidic buffers during chromatography, and has been proven not to result in structural degradation. The quoted phrase was included to emphasize that the latter could not be used to explain our results. In other words, our observed lack of binding of imatinib to CML-derived AGP is not an artifact of processing, but rather reflects more truly the nature of the in vivo interaction. We do not read pretense of increased binding into our data.

Third, the fluorescence-quenching experiment, a method utilized to directly study AGP drug binding,\textsuperscript{6} was simply employed to demonstrate the retention of drug-binding potential by our purified
glycoprotein, which was amply shown with the known AGP-binder, chlorpromazine. An inability to bind the control substance, chlorpromazine, would have been indicative of loss of AGP structural integrity, which clearly had not been induced by our purification processing. As this assay revealed no interaction between CML-derived AGP and imatinib in the face of proven chlorpromazine binding, we feel we cannot comment further on the nature of the purported imatinib-binding site on AGP. It is not accepted that chlorpromazine and imatinib should be tested at identical concentrations providing clinically relevant concentrations are chosen. (A correlation was observed between quenching and chlorpromazine concentration up to 250 μM; data not shown).

Fourth, we would consider the evidence presented suggesting a stoichiometric interaction specifically between AGP and erythromycin in whole sera to be circumstantial and difficult to evaluate. For this reason, we deliberately isolated AGP from contaminating, nonspecific plasma-protein drug binders. Additionally, can 2 groups be described as independent when they have shared membership?

Regarding the fifth and sixth points, we feel unable to comment on unpublished manuscripts but look forward to scrutinizing the data once in print. We do, however, wish to reiterate the findings of Gorre et al., exponents of the “cell intrinsic” theory of resistance, which our data supports: (a) cells taken from relapsing patients exhibited reduced sensitivity to imatinib compared with pretreatment cells; (b) relapsing patients did not have significantly reduced imatinib plasma concentrations, despite a presumed concomitant increase in plasma AGP concentration with disease progression mirroring the findings in our cohort of patients; and (c) dose escalation has not proven successful in inducing remissions in relapsing, resistant patients (our observations, and Gorre et al.).

We trust that our responses will facilitate further evaluation of our data.

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References


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