Imatinib mesylate (Glivec®, Novartis Pharma) is a highly efficacious tyrosine kinase inhibitor, designed for treatment of chronic myeloid leukaemia (CML), by virtue of its ability to inhibit the oncogenic signalling of the BCR-ABL protein believed to be the causative abnormality of the disease. However, resistance is observed in a subset of CML patients, which could be due to mutations in BCR-ABL that prohibit binding of imatinib or overexpression of the BCR-ABL gene (Paterson et al., 2003). Alternatively, circulating serum proteins have been proposed as an alternative mechanism that reduces imatinib efficacy through non specific binding to the drug. In particular, it has been suggested that the protein α1-acid glycoprotein (AGP) can bind to imatinib in the plasma and hence decrease the free, and therefore active, concentration of the drug (Gambacorti-Passerini et al., 2000).

Until recently, opinion regarding the merits of AGP as a significant contributor to imatinib resistance and/or dose reduction has been divided (Gambacorti-Passerini et al., 2002; Jørgensen et al., 2002b; reviewed in Paterson et al., 2003). Initial evidence appears to support the hypothesis since: (i) AGP is found at higher concentrations in the plasma from CML patients at all stages of disease (Jørgensen et al., 2002a) compared to disease-free individuals thus theoretically increases the capacity to bind drugs; and (ii) the glycosylation of AGP in CML is altered (Jørgensen et al., 2002a; Le Coutre et al., 2002) which could conceivably alter the affinity for a particular drug e.g. increase the percentage bound. Although the drug binding site of AGP is peptide in nature, altered glycosylation may influence drug binding capacity; the size and surface location of the oligosaccharide chains of AGP influences binding by affecting the conformation of, and thus access to, the binding site. Our previous studies (Jørgensen et al., 2002a) have ruled out AGP as a mechanism of resistance at concentrations equivalent to the plasma concentration arising from the usual imatinib dose of 400 mg/day. However, recent studies have suggested an increased rate of complete cytogenetic response and complete molecular response in groups of CML patients receiving 600 and 800 mg imatinib daily, compared to the standard dose of 400 mg/day (Kantarjian et al., 2004). Although these results are preliminary and not yet predictive of long-term responses, the fact that the 800 mg/day dose of imatinib is being tentatively proposed as the new minimum dose recently proposed. We used the previously described intrinsic fluorescence technique for the study of AGP-drug interactions (Parikh et al., 2000). This is based on fluorescence quenching of the tryptophan residues present in the protein backbone of AGP upon binding of a drug. We studied the binding of normal AGP to imatinib at 5 μM and 10 μM. The fluorescence of 0.5 mg/mL AGP, dissolved in Dulbecco’s phosphate buffered saline, was measured at 280 nm excitation and 300-400 nm emission. Imatinib, dissolved in DMSO, was added to the AGP solution at a concentration of 5 μM and the fluorescence measurement taken again. The experiment was repeated three times for this concentration and the mean and standard deviation calculated for the results. The whole procedure was repeated for 10 μM imatinib.

The results of the fluorescence binding experiment are shown in Figure 1. The quenching of the AGP fluorescence spectrum by 5 μM imatinib was 3.91±1.03%, while for 10 μM imatinib the value was 11.60±4.68%. Our previous study revealed there was no quenching of the AGP fluorescence spectrum by 1 μM imatinib, which represents the maximal trough concentration of 400mg/day imatinib at steady state (Jørgensen et al., 2002a). In the present study we note that when the imatinib concentration was increased to 5 μM representing the maximum peak steady state concentration of imatinib following a dose of 400 mg/day, there was also negligible quenching of AGP peak fluorescence (3.91%), correlating with an insignificant level of interaction between AGP and imatinib at this concentration. Peng et al (2004) have shown that 800 mg/day imatinib can result in a maximal steady state plasma concentration of 7.49±2.90 μM of the drug, and therefore we studied 10 μM imatinib as a representation of the upper limit of this range. The addition

### Table 1. Maximum measured plasma concentrations (average ± SD) for imatinib at standard 400mg/day dose compared to increased imatinib doses.

<table>
<thead>
<tr>
<th>Study</th>
<th>Imatinib Dose/day (mg)</th>
<th>Steady State Cmax.(ng/mL)</th>
<th>Steady State C∞(μM)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peng et al (2004)</td>
<td>400</td>
<td>2596±786</td>
<td>5.26±1.59</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>3701±1433</td>
<td>7.49±2.90</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>6760</td>
<td>13.70</td>
</tr>
<tr>
<td>Druker et al (2001)</td>
<td>400</td>
<td>2300</td>
<td>4.80</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>7200</td>
<td>1.46</td>
</tr>
</tbody>
</table>

*Abbreviations: SD, standard deviation; C∞, maximal measured plasma concentration of imatinib; Cmax, maximal measured plasma concentration of imatinib following a dose of 400 mg/day; C∞, maximal measured plasma concentration of imatinib following a dose of 400 mg/day.
of 10 µM imatinib to a solution of 0.5 mg/mL AGP resulted in increased quenching of the AGP fluorescence, to a value of 11.60±4.68%. Although this represents an increase in the degree of binding between AGP and imatinib, this level of quenching is very low compared to drugs that do bind strongly to AGP, such as chlorpromazine, which may have a quenching value of up to 98% (Parikh et al, 2000). Furthermore, there was no statistical difference (p<0.05) between the quenching induced by 5 µM and 10 µM imatinib respectively.

In conclusion our present findings suggest that following increased imatinib dose, although the binding interaction between AGP and imatinib is increased, it is still at a very low level that would not represent significant binding, and hence a decrease in the free concentration of imatinib, in the plasma.

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