Factors which impact on the response of CML patients to ABL kinase inhibitor therapy: A study of imatinib and nilotinib.

Deborah Lee Harland
(nee White)

The Melissa White Laboratory
Hanson Institute and
Institute of Medical & Veterinary Science
&
The Faculty of Health Science
Department of Medicine
The University of Adelaide
South Australia

Thesis submitted to the University of Adelaide
in candidature for the degree of Doctor of Philosophy
April 2008

Chapter 7:

The effect of proton pump inhibitors on the intracellular accumulation of imatinib and nilotinib

7.1 Introduction

Imatinib is generally a well tolerated therapy. The most frequent side effects reported in phase II trials, were mild nausea, vomiting, oedema and muscle cramps (Kantarjian *et al.* 2002a; Sawyers *et al.* 2002; Talpaz *et al.* 2002). Most events were of mild to moderate grade, but drug was discontinued for adverse events in 2% of patients in chronic phase, 3% in accelerated phase and 5% in blast crisis. In phase I trials, nilotinib was also reasonably well-tolerated with primary toxicities related to the haematologic, gastrointestinal and dermatologic systems (Kantarjian *et al.* 2006).

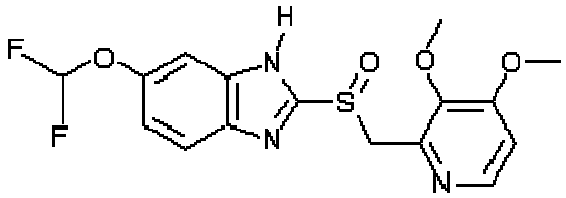
Proton pump inhibitors (PPI) including pantoprazole (Somac™), esomeprazole (Nexium™) (Figure 7.1) and others, are widely prescribed in the greater community, but also for patients on imatinib therapy to control the documented gastric side effects. Proton pump inhibitors block the activity of H+/K+ ATPase, and reduce the production of gastric acid, thus reducing the frequency and side effects of stomach and duodenal ulcers, gastroesophageal reflux disease and oesophagitis (Lindberg *et al.* 1986; Stedman & Barclay 2000). Both pantoprazole and esomeprazole are metabolised in the liver via the Cytochrome P450 system, in particular by the isoenzymes CXP2C19 and CYP3A4. Imatinib and nilotinib are also metablised via the Cytochrome P450 system. While there is a potential for interaction between PPI and these therapies, their prescription, while on concomitant imatinib or nilotinib is not restricted.

In addition to interactions at the level of metabolism, PPI have also been reported to interact with the drug efflux transporters, ABCB1 and ABCG2. In this context, PPI have been found to be both inhibitors and substrates of ABCB1 (Luciani *et al.* 2004; Pauli-Magnus *et al.* 2001), and ABCG2 (Breedveld *et al.* 2006; Breedveld *et al.* 2005) (Balayssac *et al.* 2005).

The evidence presented in this thesis (Chapters 4, 5 and 6) and from others(Dai *et al.* 2003; Gurney *et al.* 2007; Hegedus *et al.* 2002; Illmer *et al.* 2004; Mahon *et al.* 2003; Ozvegy-Laczka *et al.* 2004b; White *et al.* 2006) (Houghton *et al.* 2004) (Burger *et al.* 2004) (Nakanishi *et al.* 2006), suggests that imatinib and nilotinib themselves interact with both ABCB1 and ABCG2. These findings provide impetus for further investigation of the possible interactions which may occur, when PPI and these drugs are used in combination. Furthermore, reports of adverse cutaneous reactions, arising during concomitant imatinib and PPI (lansoprazole) therapy, (Severino *et al.* 2005) not present when either agent was used in singleton, suggests that drug interactions occur, and may result in symptomatic presentations. In addition to adverse events currently attributable to concomitant imatinib: PPI therapy,

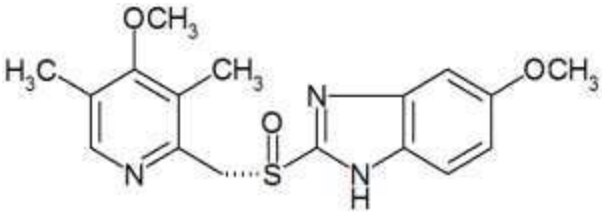
it may be that some sub-optimal responses, or toxicities can be attributable to this interaction. Since PPI's and imatinib, are substrates and inhibitors for ABCB1 and ABCG2, and are also metabolised by Cytochrome 450, drug interactions could potentially occur either during metabolism, or at the cellular level.

To investigate interactions relevant to the leukaemic cell, the intracellular uptake and retention of both imatinib and nilotinib were assessed, in the presence or absence of PPI, and the degree of change monitored.



Pantoprazole

5-(difluoromethoxy)- 2-[(3,4-dimethoxypyridin-2-yl) methylsulfinyl]- 3H-benzoimidazole



Esomeprazole

(S)-5-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)
methylsulfinyl]-3H-benzoimidazole

Figure 7.1 Chemical structure and chemical name for Pantoprazole and Esomeprazole

7.2 Summary and research contribution

The research detailed in this chapter, demonstrates drug interactions which occur at the cellular level, when proton pump inhibitor therapy is used in combination with imatinib or nilotinib. This is the first report into this interaction at the cellular level, and provides insight into a possible mechanism for drug interaction.

Conference Presentations.

White Deborah L, Verity A Saunders, et al. (2007). The intracellular concentrations of imatinib and nilotinib, can be substantially altered by interaction with other drugs: Studies with proton pump inhibitors. European Haematology Society, Vienna. (White Deborah L *et al.* 2007)

White, D. L., Saunders AV, et al. (2007). Proton pump inhibitors interact with transport of both imatinib and nilotinib. AACR, Los Angeles. (White *et al.* 2007b)

.

7.3 Assay development

7.3.1 Intracellular Uptake and Retention (IUR) Assay

The IUR assay was performed essentially as previously described ((White *et al.* 2006) Chapter 4, 5, 6 and Appendix I Materials and Methods).

Pantoprazole, esomeprazole and prazosin (purchased from Royal Adelaide Hospital Pharmacy) were made up in sterile water as per manufacturer's instructions. Stock solution was at 10mM, 20mM and 100mM respectively. The ABCB1 inhibitor PSC833 and the ABCG2 inhibitor Ko143, were used as previously described.

For initial combination studies, drugs were added simultaneously using the schema in Table 1. For subsequent experiments, the same schema was applied, but PPI's were used at lower concentrations.

Results for each arm of the experiment were displayed as ng/200,000 cells and calculated as follows:-

% drug incorporation = cpm cells/cpm total (cells + supernatant)

ng/200,000 cells = (% drug incorporation)/100 x amount of drug added in ng.

To assess the effect of PPI on the IUR for either imatinib or niltoinib results were displayed as percentage of the control arm, where the control was the test sample with no added combination drug. Taking the addition of 200 µM pantoprazole added to 1µM [^{14}C]-imatinib as an example

	[^{14}C]-imatinib (ng/200,000 cells)		**Calculation**	**% Change**
	Control	**+200µM pantoprazole**		
CML1	**20**	**10**	**([20-10)/20)x 100**	**50%**
CML3	**30**	**20**	**([30-20)/30)x 100**	**33%**

A t-test was used to test for significance of any observed change.

7.3.2 Patient samples and cell lines

Assays were performed in triplicate, in 10 newly diagnosed CML patients, in chronic phase, prior to the commencement of therapy, and in K562 and K562 DOX cells (previously described).

7.3.3 Cell viability

Cell viability was assessed at the beginning and at the completion of assays to assess the effect of the drug combination on cell integrity. This was performed using Trypan Blue exclusion.

Table 7.1 Schema for the addition of drugs imatinib/nilotinib and PPI to assess the effect of the combination.

This approach provides an IUR readout for the assays in singleton:

IUR of imatinib [^{14}C]-imatinib @ 1µM

IUR of imatinib [^{14}C]-imatinib @ 2µM

IUR of nilotinib [^{14}C]- nilotinib @1µM

IUR of nilotinib [^{14}C]- nilotinib @ 2µM

And in combination

IUR of imatinib [^{14}C]-imatinib @ 1µM + 100, 200, and 400µM PPI

IUR of imatinib [^{14}C]-imatinib @ 2µM + 100, 200, and 400µM PPI

IUR of nilotinib [^{14}C]- nilotinib @ 1µM + 100, 200, and 400µM PPI

IUR of nilotinib [^{14}C]- nilotinib @ 2µM + 100, 200, and 400µM PPI

Pantop - pantoprazole

Eso – esomeprazole

	1 µM /2µM	Pantoprazole			Esomeprazole		
	Imatinib or Nilotinib	100µM	200µM	400µM	100µM	200µM	400µM
No Drug - cells only							
Imatinib/nilotinib	√						
IM/NIL + 100 µM Pantop	√	√					
IM/NIL + 200 µM Pantop	√		√				
IM/NIL + 400 µM Pantop	√			√			
IM/NIL + 100 µM Eso	√				√		
IM/NIL + 200 µM Eso	√					√	
IM/NIL + 400 µM Eso	√						√

7.4 Results

7.4.1 The effect of pantoprazole on the IUR of imatinib

The median imatinib IUR at 1µM for the 10 patients analysed was 13.4ng/200,000 cells within a range of 8 to 18ng. The addition of pantoprazole at 100, 200 and 400µM resulted in a universal decrease in the IUR. (10.9, 9.9 and 8.8 ng respectively). This decrease occurred in 9 of the 10 patients at all pantoprazole concentrations (Figure 7.2).The median imatinib IUR at 2µM was 24.6ng/200,000 cells within a range of 17 to 46ng. The addition of pantoprazole and 100, 200 and 400µM resulted in a universal decrease in the IUR. (20.3, 18.9 and 16.2 ng respectively) (Figure 7.2).

The average decrease in the 1µM imatinib IUR with the addition of 100µM pantoprazole was 19.5%, at 200µM was 23.6% and at 400µM was 30.5%. This decrease was statistically significant at all pantoprazole doses (*p=0.031, 0.0006 and <0.001* respectively) (Figure 7.3 and 7.10). The average decrease in the 2µM imatinib IUR was less than that observed in the 1µM assays, with the addition of 100µM pantoprazole reducing the IUR by 14.5%, at 200µM 21.5% and at 400µM 29.2%. This decrease was only statistically significant at 400µM pantoprazole (*p=0.201, 0.076 and 0.01* respectively) (Figure 7.3 and 7.10).

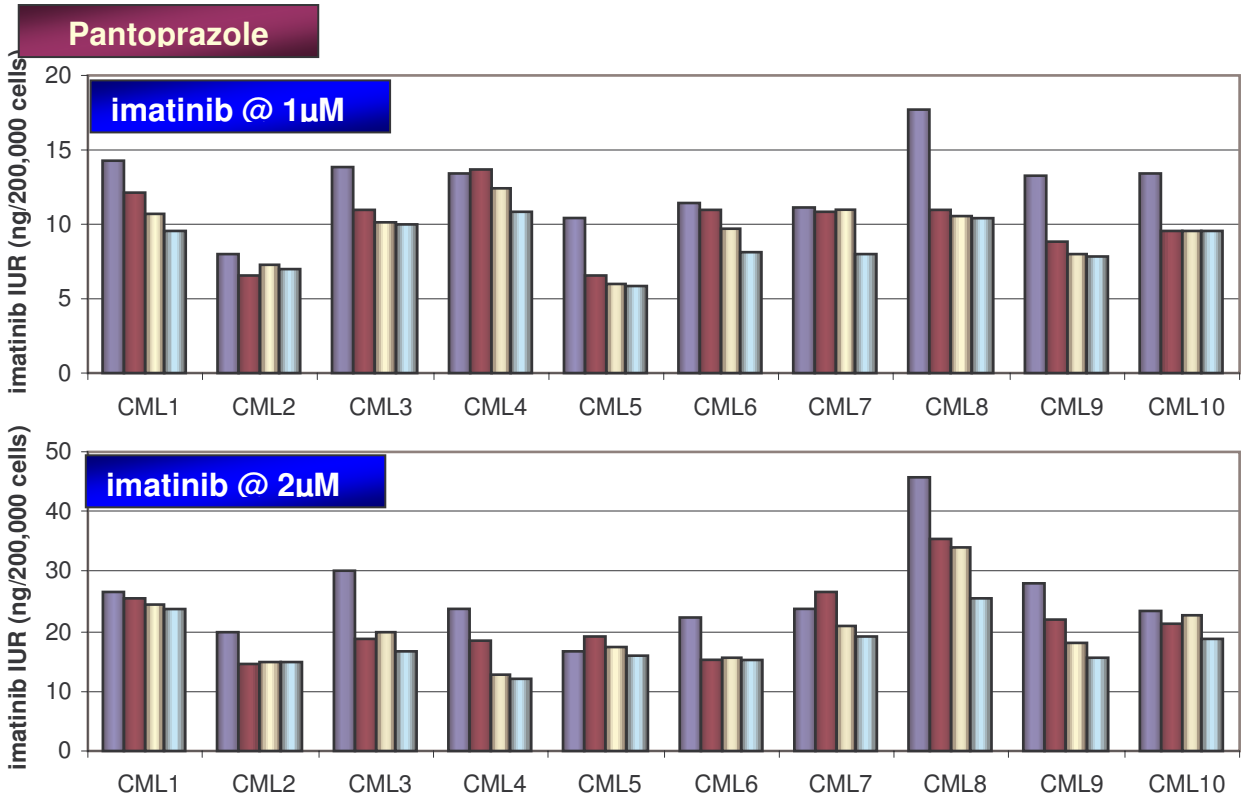


Figure 7.2 The effect of the addition of pantoprazole on the intracellular concentration of imatinib

All assays were performed in triplicate. Assays were only accepted, if triplicate results were concordant within 10%, if not, assays were repeated. Pantoprazole was added to the imatinib IUR at concentrations ranging from 100 to 400µM. At both 1µM and 2µM imatinib there was a reduction in the intracellular concentrations of imatinib in the presence of pantoprazole.

Control

+100µM pantoprazole

+200µM pantoprazole

+400µM pantoprazole

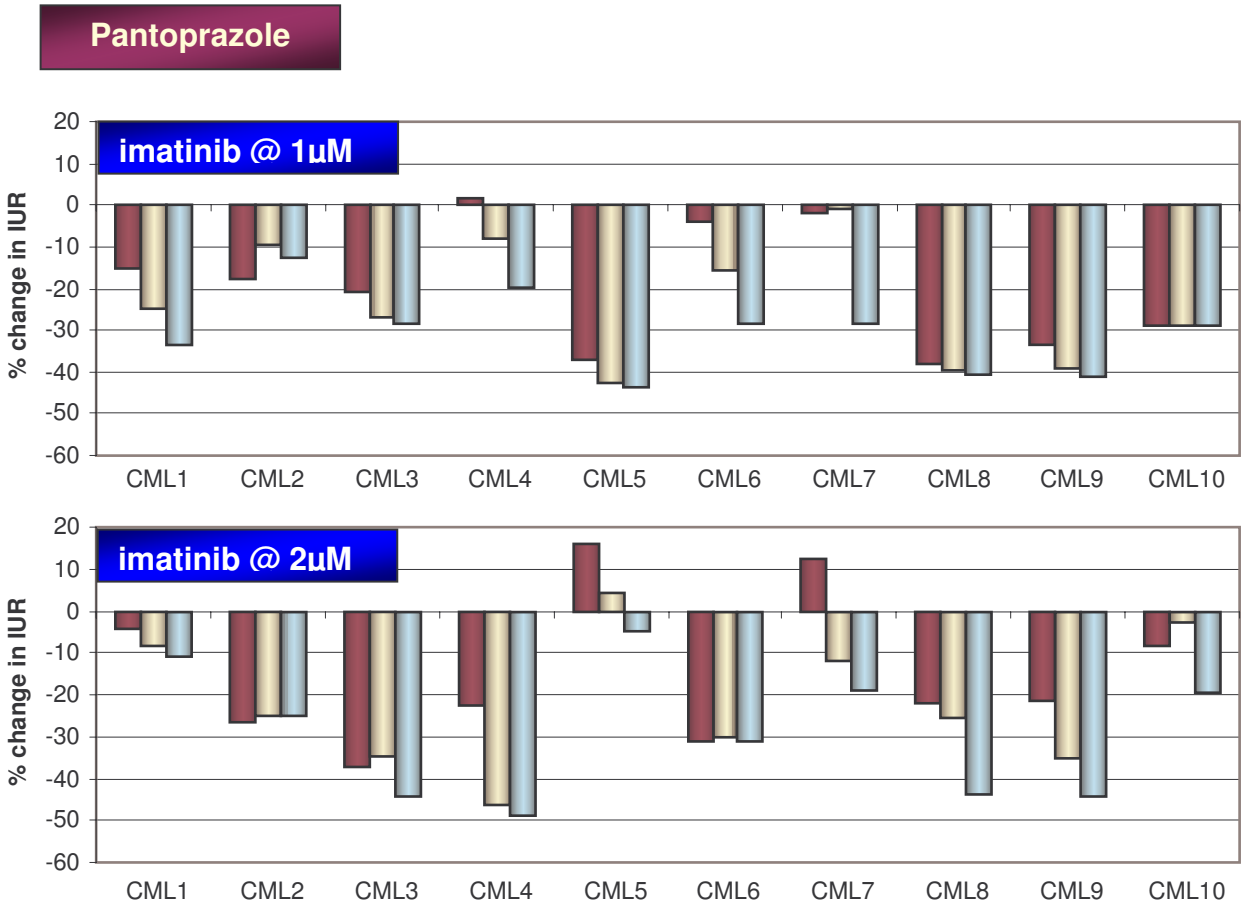


Figure 7.3 The percentage change in the intracellular concentration for imatinib in the presence of pantoprazole

At 1µM imatinib there was a decrease in the IUR of imatinib, in the presence of pantoprazole. This decrease occurred in 90% of patients at all pantoprazole concentrations tested.

At 2µM this decrease occurred in 80% of patients at all concentrations tested.

+100µM pantoprazole

+200µM pantoprazole

+400µM pantoprazole

7.4.2 The effect of esomeprazole on the IUR of imatinib

The addition of esomeprazole at 100, 200 and 400μM to the IUR assay in 9 patients, reduced the median IUR for imatinib at 1μM from 13.3ng/200,000 cells to 10.2, 10.6 and 9.2ng respectively (*p=0.075, 0.045, and 0.019)(*Figure 7.4).The addition of esomeprazole (at 100, 200 and 400μM) to the 2μM imatinib IUR, reduced the median IUR from 24ng/200,000 cells to 21.7, 19.8, 18.6 ng respectively (Figure 7.5). The average % decrease was 7.2% at 100μM, 10.6% at 200μM and 16.8% at 400 μM. This reduction reached statistical significance with the addition of 400μM esomeprazole only (*p=0.287, 0.111 and 0.027*) (Figure 7.5 and 7.10).

7.4.3 The effect of pantoprazole on the IUR of nilotinib

In contrast to the findings with imatinib, the addition of pantoprazole to the nilotinib IUR assay resulted in an increased intracellular concentration of nilotinib. The median IUR for nilotinib at 1μM was 13.0ng/200,000 cells within a range of 9.9 to 19.7 ng. The addition of 100, 200 and 400μM pantoprazole increased the IUR to 16.4ng, 16.6 and 17.4ng respectively. At 2μM the median IUR was 30.4ng within a range of 22 to 46ng/200,000 cells. The addition of pantoprazole increased the IUR to 56.8, 66.2 and 74.7 ng respectively (Figure 7.6).

The average % increase in the 1μM IUR was 25% for 100μM, 34% for 200μM and 28% for 400μM (Figure 7.7). This increase only achieved statistical significance at 200μM pantoprazole (*p= 0.054, 0.02 and 0.066*), though numbers for 100 and 400μM were approaching significance (Figure 7.10). In contrast, the addition of pantoprazole to the 2μM nilotinib IUR resulted in statistically significant increases at all concentrations of pantoprazole added. The average increase with 100μM pantoprazole was 76%, at 200μM, 99% and at 400μM a119.6% increase was observed (*p=0.02, <0.001 and <0.001* respectively) (Figure 7.7 and 7.10).

7.4.4 The effect of esomeprazole on the IUR of nilotinib

Like pantoprazole, and in contrast to the effect on imatinib, the addition of esomeprazole resulted in a universal increase in the IUR of nilotinib at both 1 and 2μM. With the addition of 100μM the IUR for nilotinib rose to 15ng, then with sequential additions to 17.3 (at 200μM) and 16ng/200,000 cells (at 400μM). At 2μM nilotinib, 100μM pantoprazole increased the IUR to 37.3ng, 200uM to 51.6 and 400μM to 60.7 ng/200,000 cells (Figure 7.8). This increase occurred in all patients at all concentrations.

The average % increases at 1µM nilotinib were 19%, 32.4% and 31.7% for 100, 200 and 400µM esomeprazole respectively (*p= 0.065, <0.001 and 0.001*). At 2µM nilotinib the IUR increased 50%. 117% and 156% with the sequential addition of esomeprazole (*p=0.01, 0.005 and 0.003* respectively) Figure 7.9 and 7.10.

7.4.5 Comparison of the effects of pantoprazole and esomeprazole on the intracellular concentration of both imatinib and nilotinib.

Interestingly, while esomeprazole and pantoprazole effected the intracellular concentration of imatinib and nilotinib, the effects were opposing. The intracellular concentration of imatinib decreased, in a statistically significant manner, with the addition of pantoprazole (at all concentrations tested in the 1µM IUR, and at 400µM in the 2µM IUR). In contrast, nilotinib increased significantly (at all concentrations tested in the 2µM IUR, and at 200µM in the 1µM IUR). Similarly, as shown in figure 7.10, the addition of esomeprazole resulted in decreases in the IUR of imatinib, and universal increases in the intracellular concentration of nilotinib.

There was no statistical difference between the effect mediated by pantoprazole or esomeprazole on the intracellular concentration of nilotinib. However, pantoprazole resulted in greater reductions in the IUR for imatinib, which was statistically significant when 400µM of both agents were added to the 1µM IUR for imatinib.

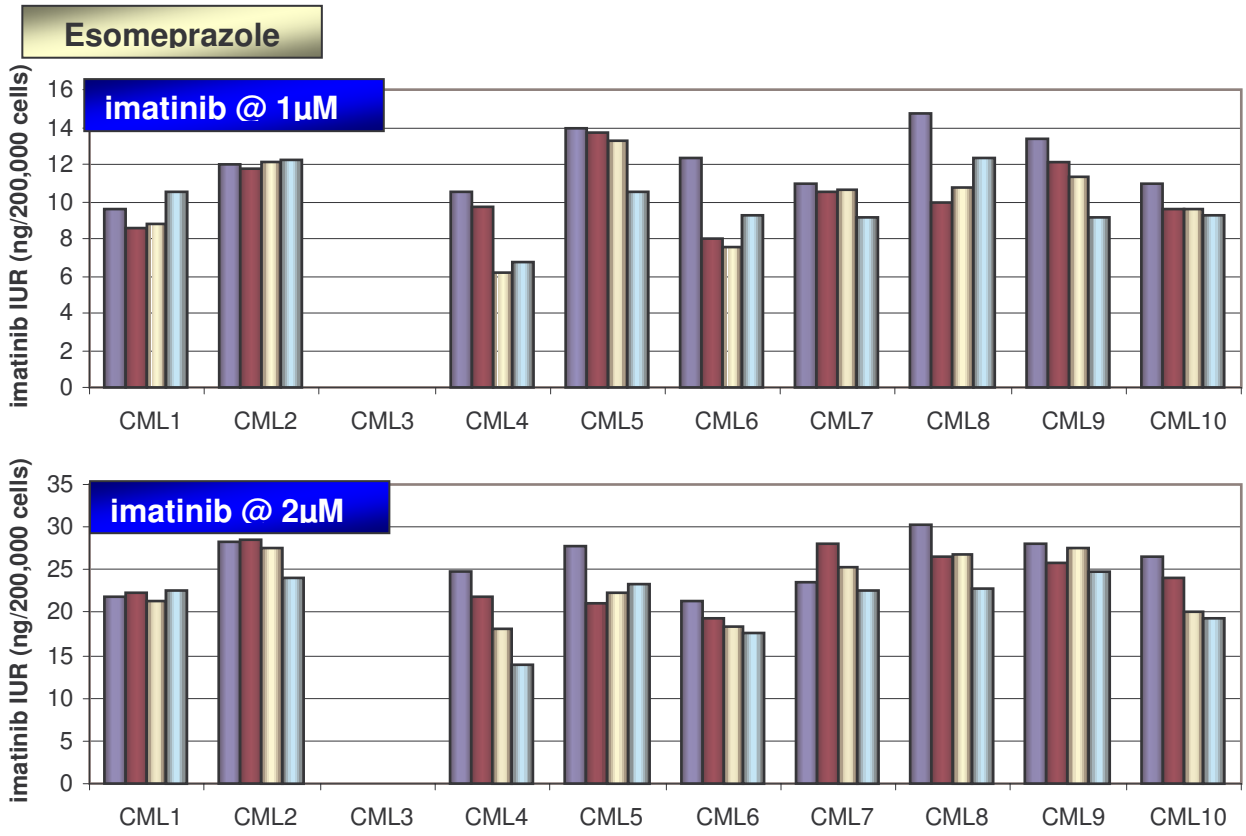


Figure 7.4 The effect of esomeprazole on the intracellular concentration of imatinib

All assays were performed in triplicate. Assays were only accepted, if triplicate results were concordant within 10%, if not, assays were repeated. Like pantoprazole, esomeprazole was added to the imatinib IUR at concentrations ranging from 100 to 400µM. At both 1µM and 2µM imatinib there was a reduction in the intracellular concentrations of imatinib in the presence of esomeprazole.

Control

+100µM esomeprazole

+200µM esomeprazole

+400µM esomeprazole

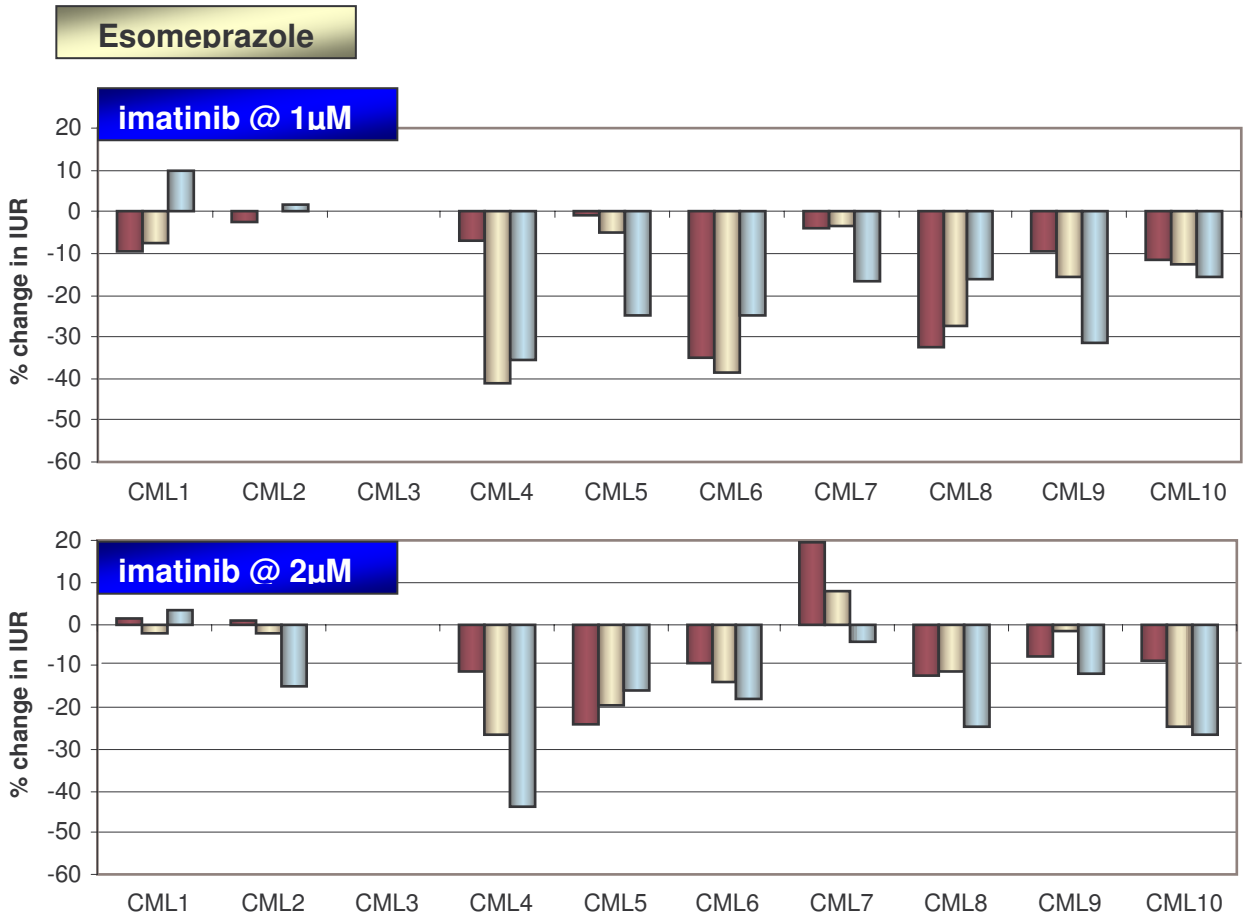


Figure 7.5 The percentage change in the intracellular concentration of imatinib in the presence of esomeprazole

The addition of esomeprazole to the imatinib IUR reduced the intracellular concentration of imatinib by up to 40% at both 1 and 2µM imatinib..

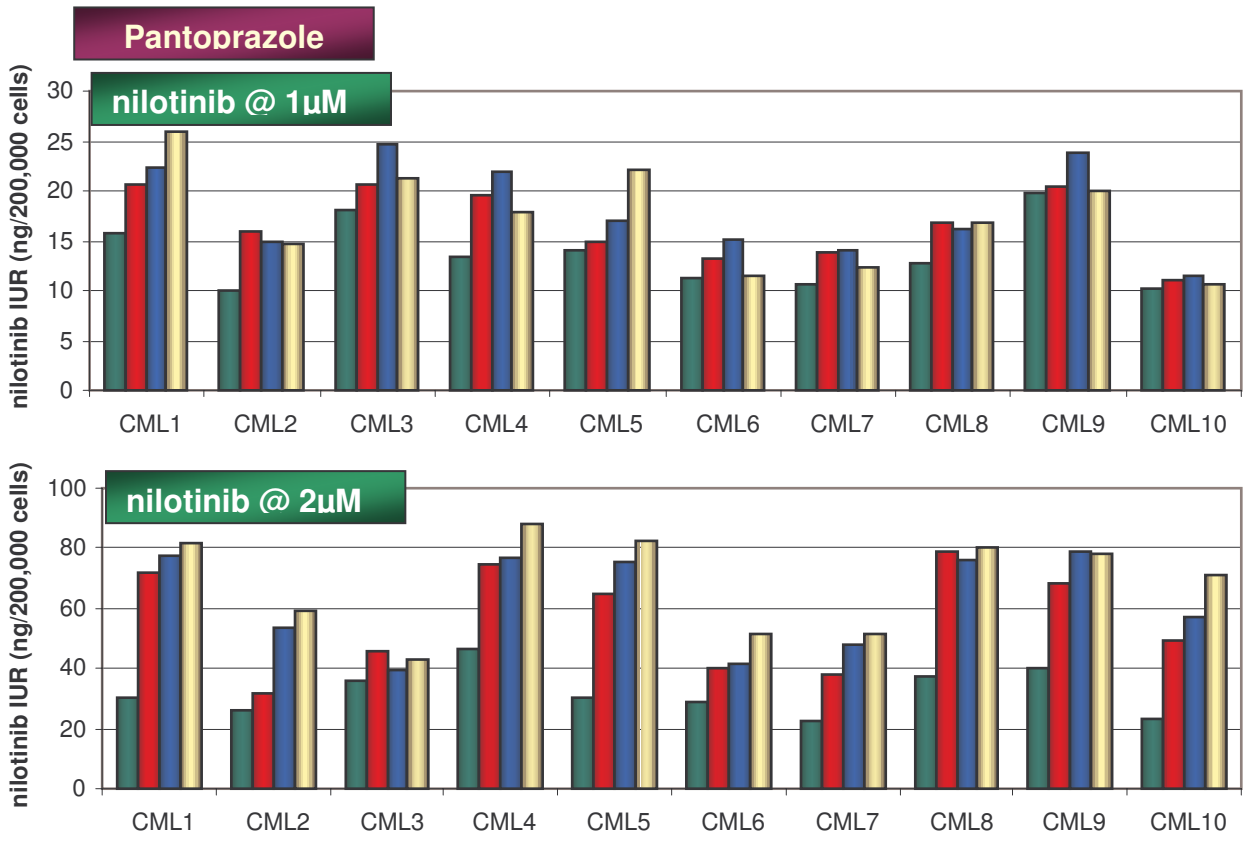


Figure 7.6 The effect of pantoprazole on the intracellular concentration of nilotinib

All assays were performed in triplicate. Assays were only accepted, if triplicate results were concordant within 10%, if not, assays were repeated. In contrast to the findings with imatinib, pantoprazole, when added to the nilotinib IUR at concentrations ranging from 100 to 400µM resulted in a increase in the IUR for nilotinib, in all patients, at both 1µM and 2µM nilotinib.

Control

+100µM pantoprazole

+200µM pantoprazole

+400µM pantoprazole

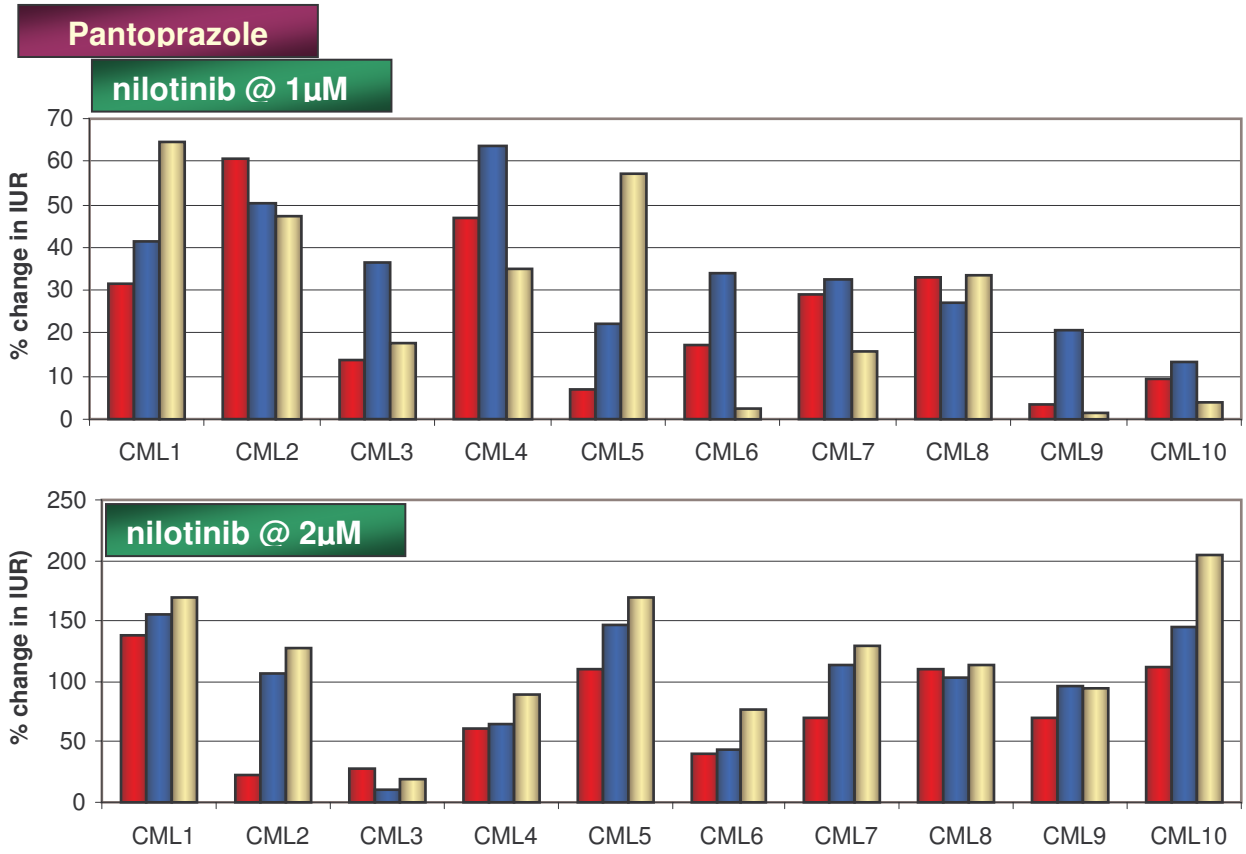


Figure 7.7 The percentage change, from no imatinib control, in the intracellular concentration of nilotinib, when pantoprazole was added

The addition of pantoprazole resulted in a increase in the IUR for nilotinib in all patients. Here the data from Figure 7.6 is displayed as the % change from control (no PPI). As is displayed, the addition of pantoprazole resulted in increases in nilotinib of up to 60% at 1µM nilotinib, and greater than 150% at 2µM nilotinib.

+100µM pantoprazole

+200µM pantoprazole

+400µM pantoprazole

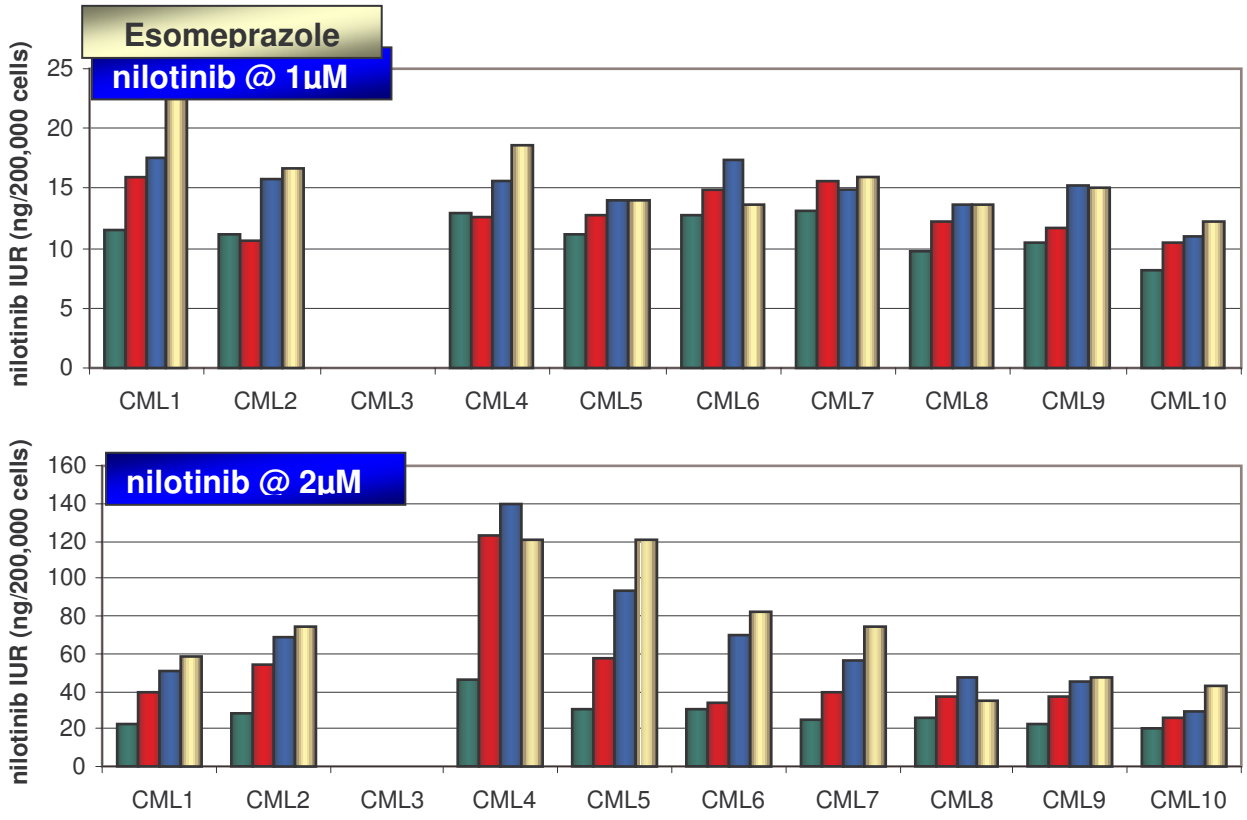


Figure 7.8 The effect of esomeprazole on the intracellular concentration of nilotinib

All assays were performed in triplicate. Assays were only accepted, if triplicate results were concordant within 10%, if not, assays were repeated. In keeping with the findings for pantoprazole, esomeprazole, when added to the nilotinib IUR at concentrations ranging from 100 to 400µM resulted in an increase in the IUR at both 1µM and 2µM nilotinib.

Control

+100µM esomeprazole

+200µM esomeprazole

+400µM esomeprazole

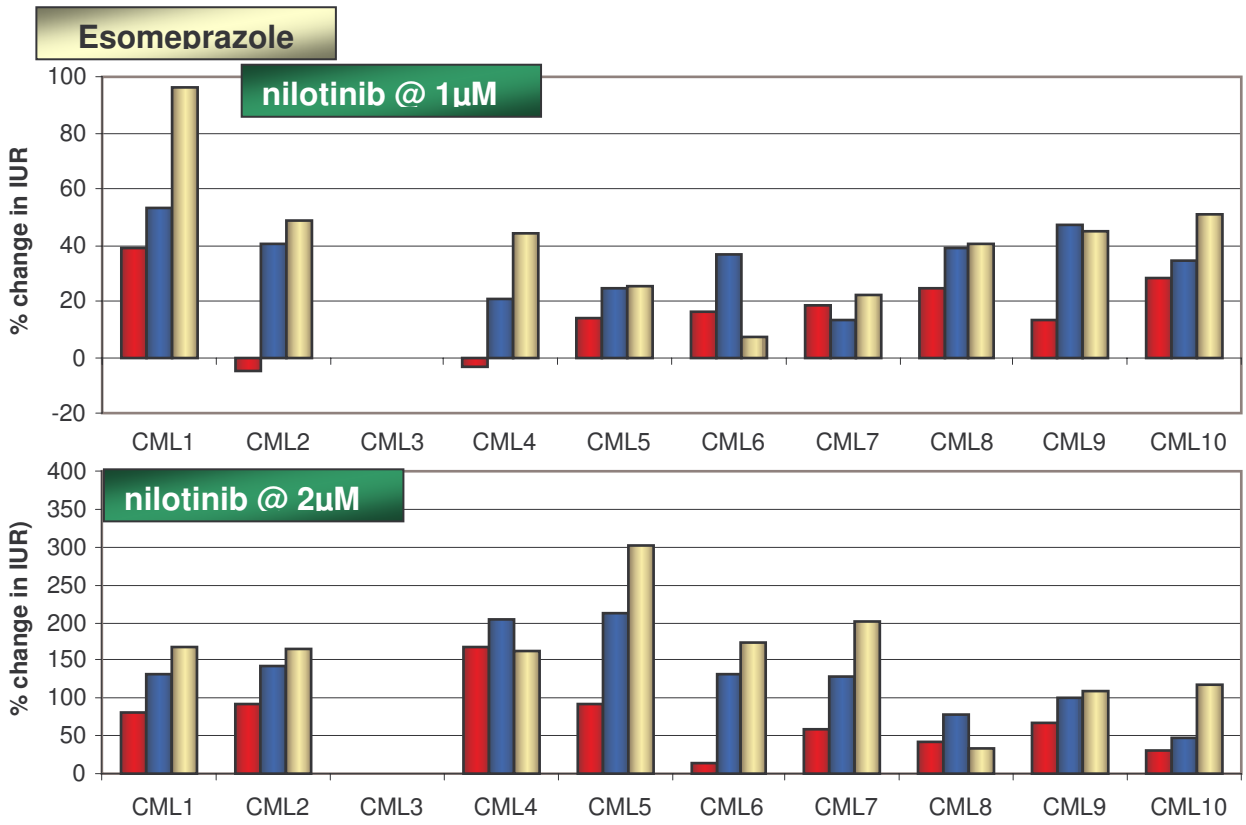


Figure 7.9 The percentage change, from no esomeprazole control, in the intracellular concentration of nilotinib, when esomeprazole is added

As observed for pantoprazole, while the extent of the increase in IUR of nilotinib did vary between patients, the effect was universal to all patients. This occurred regardless of whether nilotinib was studied at 1 or 2µM.

+100µM esomeprazole

+200µM esomeprazole

+400µM esomeprazole

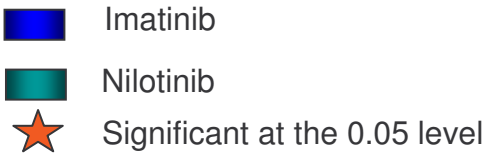
Figure 7.10 The overall effect of both pantoprazole and esomeprazole on the IUR of both imatinib and nilotinib

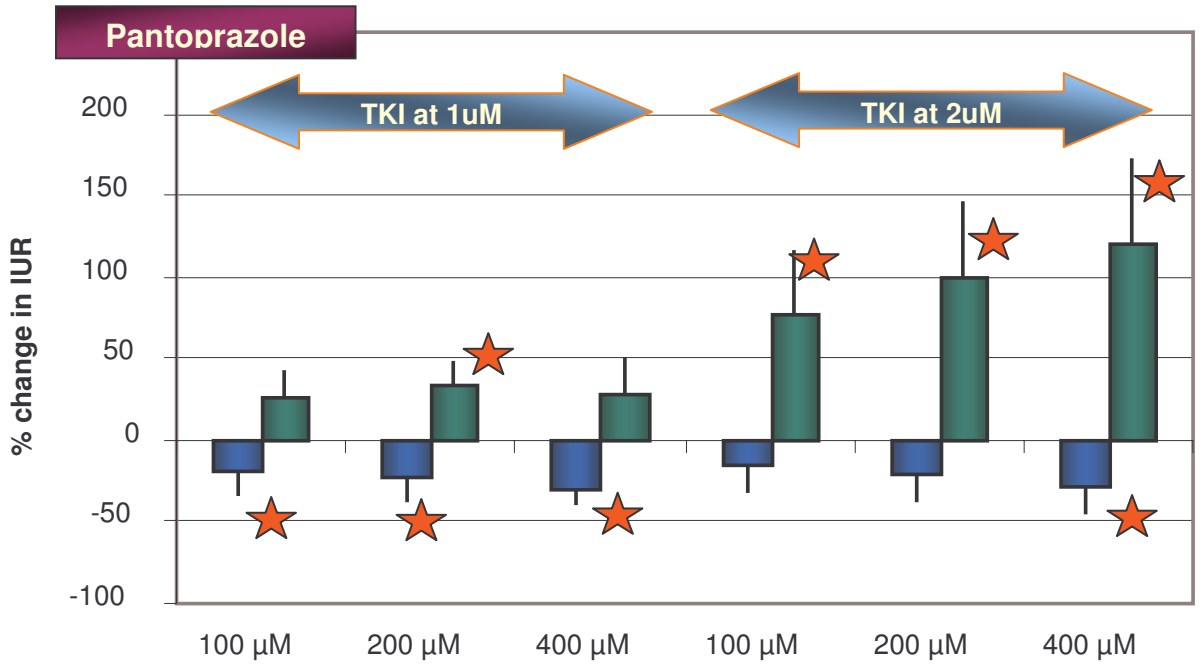
The percent changes are the change mediated by the addition of PPI, at the stated concentrations, from the standard IUR assay in the absence of added PPI.

Two hundred μM pantoprazole results in a statistically significant increase in the 1μM IUR for nilotinib. In the 2μM IUR all doses of pantoprazole result in statistically significant increases. In contrast, all doses of pantoprazole (100, 200 and 400μM) result in statsitically significant decreases in the IUR for imatinib at 1μM, but only the addition of 400μM pantoprazole results in a statistically significant change in the 2μM IUR.

The addition of both 200 and 400μM Esomeprazole resulted in a statistically significant increase in the 1μM nilotinib IUR. All concentrations of esomeprazole (100, 200 and 400μM) resulted in statistically significant increases in the 2μM nilotinib IUR. In contrast, the addition of esomeprazole at 200 and 400μM resulted in a statistically significant decrease in the 1μM IUR, but only 400μM esomeprazole mediated this response in the 2 μM nilotinib IUR.

These data show that both pantoprazole and esomeprazole effect the intracellular concentration of imatinib and nilotinib, however this effect is different for both kinase inhibitors, and more significant in the nilotinib setting.




Pantoprazole
TKI at 1uM
TKI at 2uM
% change in IUR
200
150
100
50
0
-50
-100
100 µM
200 µM
400 µM
100 µM
200 µM
400 µM

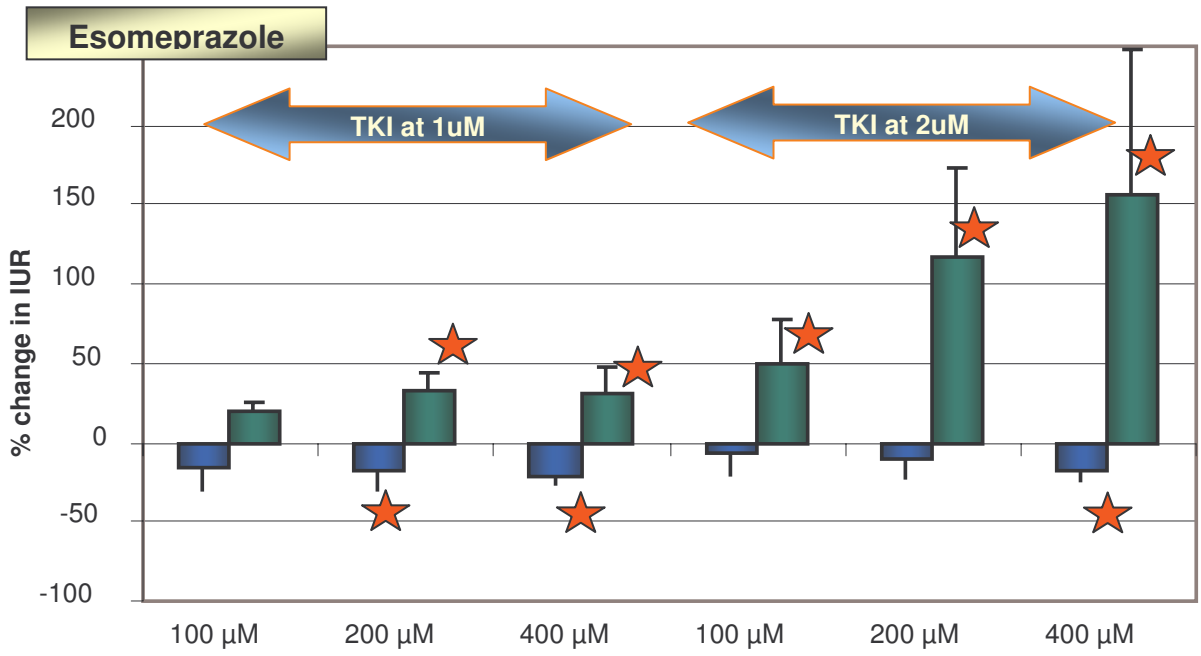
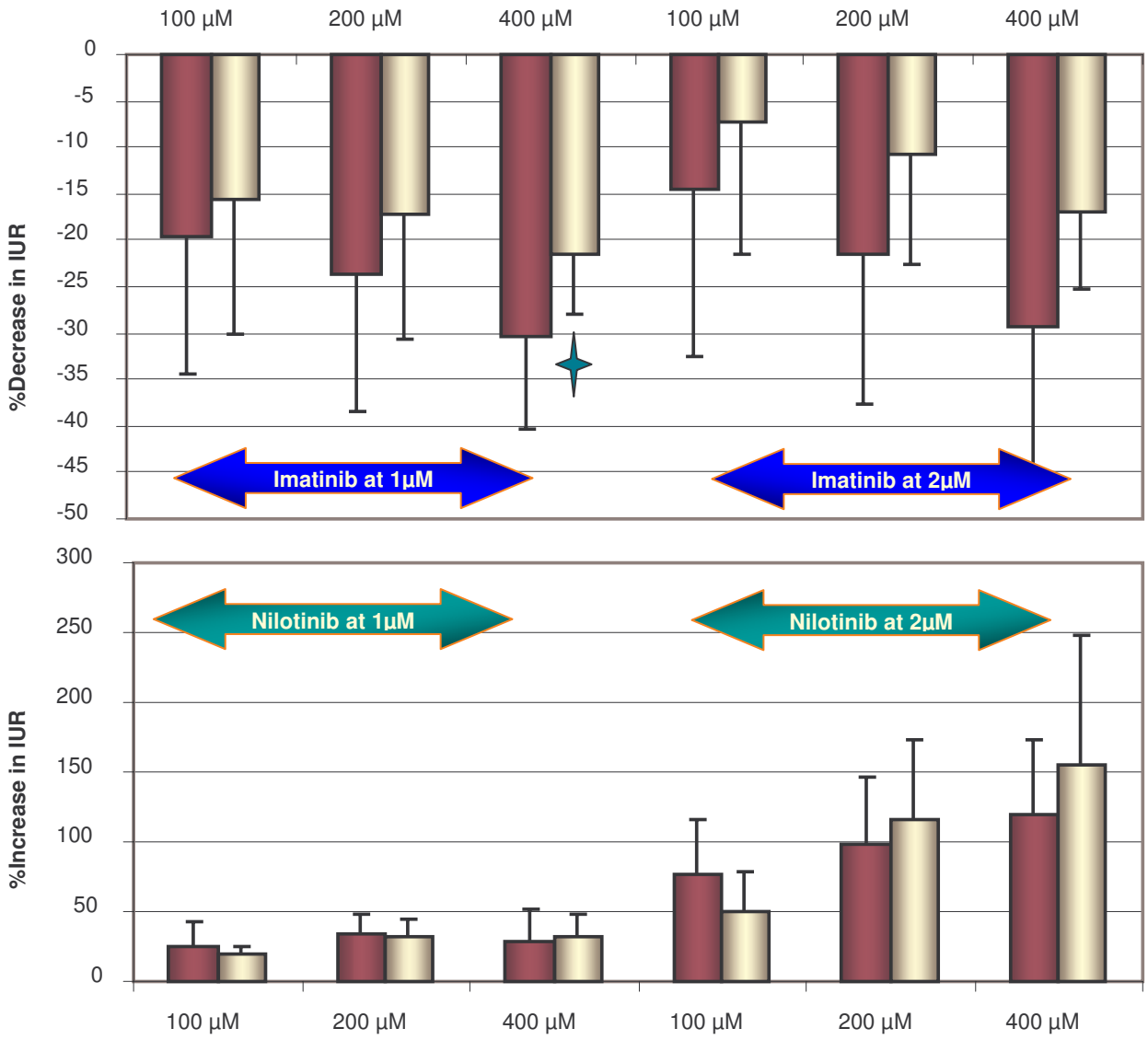

Esomeprazole
TKI at 1uM
TKI at 2uM
% change in IUR
200
150
100
50
0
-50
-100
100 µM
200 µM
400 µM
100 µM
200 µM
400 µM

Figure 7.11 Comparison of the effect on the intracellular concentrations of nilotinib and imatinib with the addition of pantoprazole and esomeprazole (PPI)

The effect of PPI is different for both drugs. The intracellular concentration of imatinib is reduced by the addition of PPI, but conversely the intracellular concentration of nilotinib is increased. While only significant at one time point, there is a trend toward pantoprazole being more effective on imatinib than esomeprazole. There is no significant difference between the effect of the two drugs on nilotinib IUR.


100 µM
200 µM
400 µM
100 µM
200 µM
400 µM
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
%Decrease in IUR
Imatinib at 1µM
Imatinib at 2µM
300
250
200
150
100
50
0
%Increase in IUR
Nilotinib at 1µM
Nilotinib at 2µM
100 µM
200 µM
400 µM
100 µM
200 µM
400 µM

7.4.6 Effect of PPI on the IC50 for nilotinib and imatinib.

Pantoprazole and esomeprazole were both added at 200µM to the IC50 assays for imatinib and nilotinib, in cell lines and in patient samples. There was no measurable effect of esomeprazole on the $IC50^{imatinib}$ in K562 cells (Figure 7.12). In contrast the addition of esomeprazole to the K562 $IC50^{nilotinib}$ resulted in a 50% reduction in the $IC50^{nilotinib}$ from 500nM to 250nM (Figure 7.12). In the ABCB1 over expressing cell line K562DOX, the $IC50^{nilotinib}$ was reduced by 58% with and 62% in the presence of 200µM esomeprazole (Figure 7.13). In contrast there was a 25% increase (from 8 to 10µM) in the $IC50^{imatinib}$ for these cells when 200µM pantoprazole was added, but an 18% decrease when 200µM esomeprazole was added (from 8 to 6.5µM) (Figure 7.14). Similarly, in patient material the $IC50^{nilotinib}$ decreased by 40% (from 91 to 54nM) in the presence of 200µM pantoprazole, but increased by 15% (from 1.3µM to 1.5µM) when pantoprazole was added to the $IC50^{imatinib}$ (Figure 7.15).

7.4.7 The effect of low dose PPI on the IUR of imatinib and nilotinib.

The concentrations of pantoprazole and esomeprazole chosen for the first experiments were selected based on publications documenting the interactions of PPI with efflux pumps such as ABCB1 and ABCG2 in in-vitro experiments (Breedveld *et al.* 2005) (Breedveld *et al.* 2006). However, with such a marked change observed for the majority of patients, it was important to assess whether these finding would be clinically relevant. The concentrations of imatinib and nilotinib used in this study are clinically achievable on standard dosing regimens. However, In order to assess clinical relevance, PPI's were added to the IUR, as previously, but at more clinical relevant concentrations (ie at 5, 10 and 25µM).

When pantoprazole was added at low doses (5, 10 or 25µM) it resulted in a reduction of the intracellular concentration of imatinib. However, this failed to reach statistical significance at any dose (Table 7.2). In contrast, the addition of pantoprazole at low dose resulted in an increase in the intracellular concentration of nilotinib, which was statistically significant at 25µM in both the 1µM and 2µM nilotinib IUR.

7.4.8 Effect of low dose pantoprazole on the IC50 for imatinib and nilotinib.

The $IC50^{imatinib}$ and $IC50^{nilotinib}$ were assessed in K562 and K562 DOX cells in the presence of 25µM pantoprazole. In K562 cells the $IC50^{nilotinib}$ was reduced from 500 to 400nM (20% reduction) in the presence of 25µM pantoprazole. The $IC50^{imatinib}$ in these cells remained constant (Figure 7.16). While the $IC50^{imatinib}$ also remained constant in K562 DOX cells in the presence and absence of pantoprazole, the $IC50^{nilotinib}$ reduced by 58%, from 600nM to 250nM. This indicates the increased intracellular concentration of nilotinib, in the presence of pantoprazole, translates to a significantly reduced

$IC50^{nilotinib}$. This effect is greater in K562DOX (ABCB1 over expressing) cells than in K562, again suggesting involvement of ABCB1. The small, and statistically insignificant change observed in the imatinib IUR when low dose pantoprazole is added, translates to no measurable effect on the $IC50^{imatinib}$ (Figure 7.16).

7.4.9 The affect of PPI on the uptake of imatinib.

In 7 patients, the intracellular concentration of imatinib was assessed in the presence and absence of high dose PPI (100 to 400µM), with and without the addition of the OCT-1 inhibitor prazosin (Figure 7.17 A). There was a significant reduction in the IUR for imatinib with the addition of PPI at 400µM (*p=0.04*). The addition of prazosin reduced the IUR in the control experiments though this reduction did not reach statistical significance. (*p>0.05*). The uptake of imatinib inhibited by prazosin (Total IUR less the IUR + prazosin) was significantly different from the Total IUR (*p<0.001*). With the addition of PPI a small percentage increase (<20%) in IUR was noted in the cells exposed simultaneously with prazosin (*p>0.05*) (figure 7.17 B). However, when the prazosin inhibitable IUR, was assessed a significant difference was observed with the addition of increasing concentrations of PPI (Figure 7.17 B) This suggests that PPI may be affecting the uptake and retention of imatinib which is inhibitable by prazosin, and infers that an interaction between OCT-1 and PPI may exist. As the interaction between imatinib and PPI was not significant at lower PPI concentrations, and because of a scarcity of available cells on these patients, these assays were not undertaken at lower, clinically relevant PPI concentrations.

7.4.10 Cell viability

The viability was assessed at the beginning and end of the 2 hour culture in selected assays. No observed difference was noted in viability, with viabilities remaining above 98%.

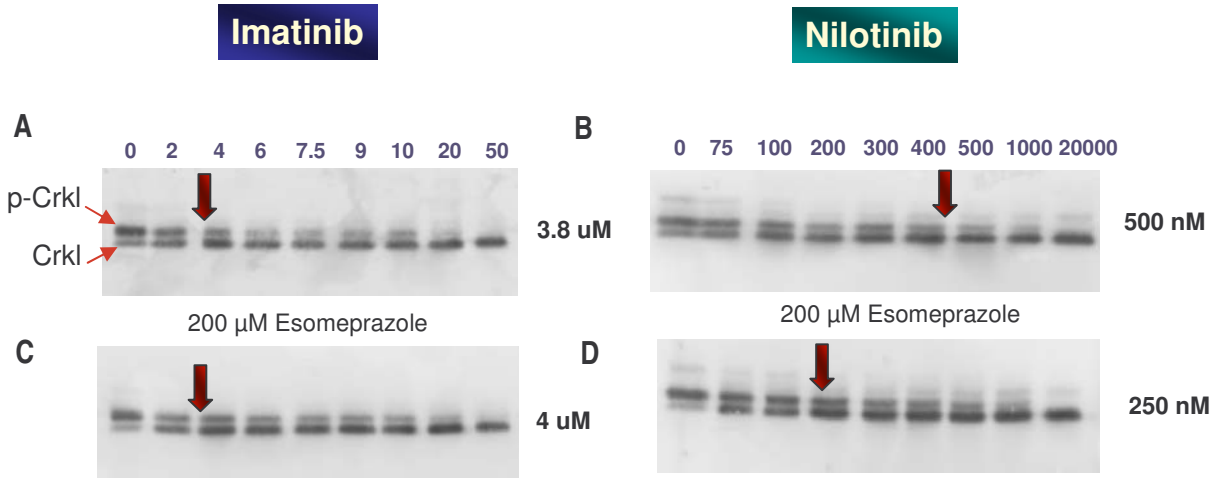


Figure 7.12 Effect of Esomeprazole at 200µM on the IC50^{imatinib} and IC50^{nilotinib} in K562 cells.

The IC50^{imatinib} and IC50^{nilotinib} was assessed in K562 cells in the presence (Panel A and B) and absence (Panel C and D) of 200µM esomeprazole. There was no effect of 200µM esomeprazole on the IC50^{imatinib} for K562 cells (IC50^{imatinib} = 3.8nM and 4nM). However the addition of 200µM esomeprazole reduced the IC50^{nilotinib} in this cell line by 50 %, from 500nM to 250nM.

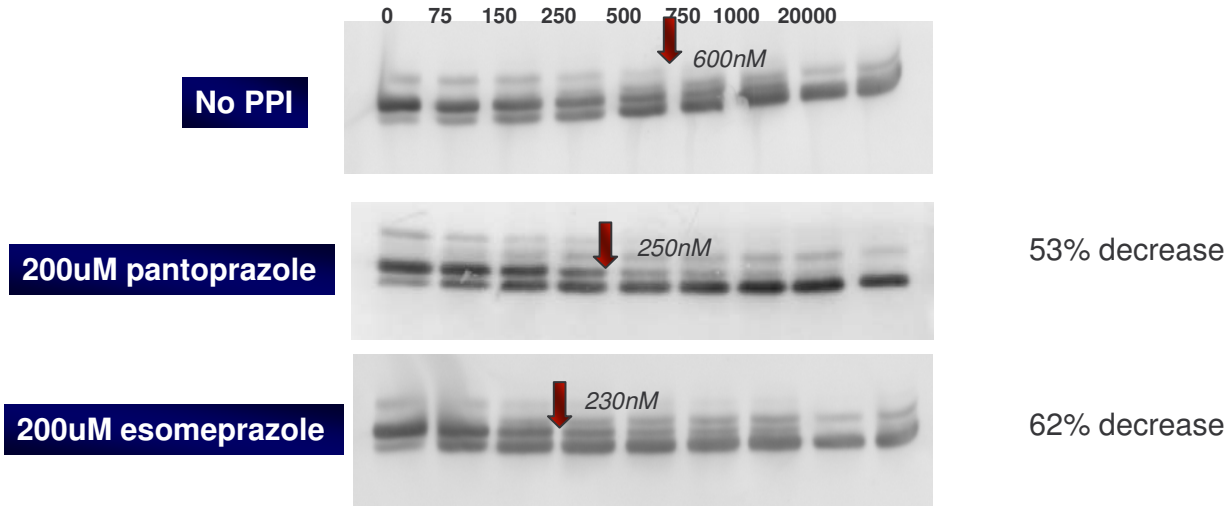


Figure 7.13 Effect of esomeprazole and pantoprazole at 200µM on the $IC50^{nilotinib}$ in K562DOXcells.

As demonstrated in the IUR assay the addition of PPI increase the intracellular concentration of nilotinib. These western blots demonstrate that this increase in intracellular concentration of nilotinib, translates to decreases in the $IC50^{nilotinib}$ of 53 and 62% respectively.

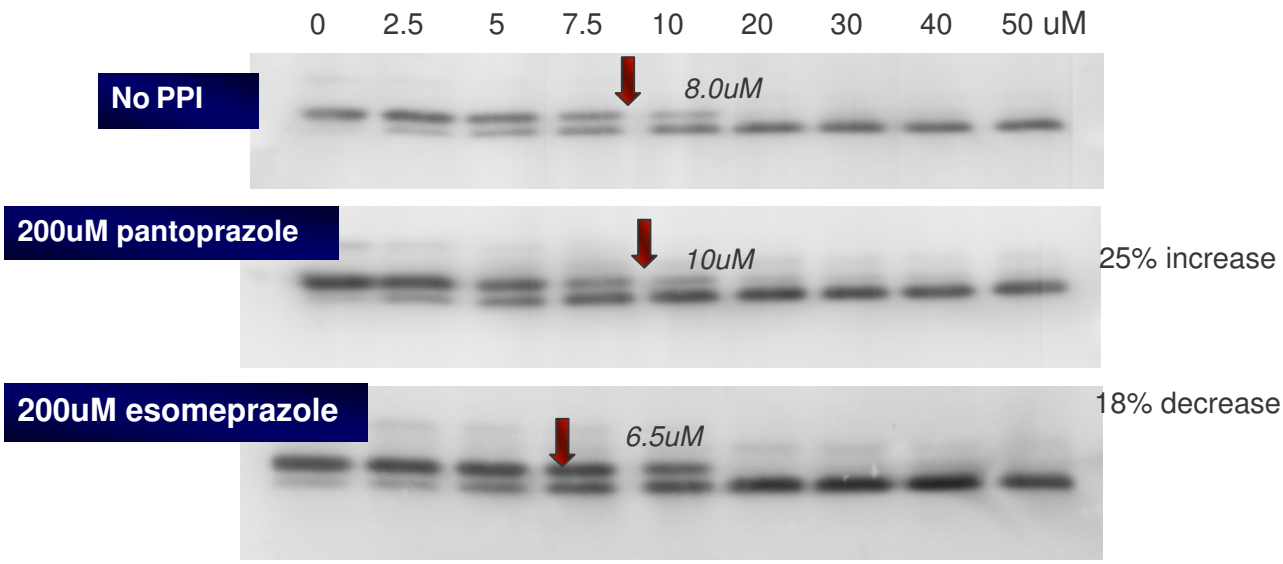


Figure 7.14 Effect of esomeprazole and pantoprazole at 200µM on the $IC50^{imatinib}$ in K562DOXcells.

Both esomeprazole and pantoprazole decrease the intracellular concentration of imatinib. As shown in these western blots, this decrease did not result in consistently higher $IC50^{imatinib}$ levels, as may have been anticipated in the scenario of decreased intracellular drug concentration.

Table 7.2 The effect of low dose pantoprazole on the intracellular concentration of imatinib and nilotinib.

Data is expressed as % change from control. Control is the standard IUR in the absence of pantoprazole.

The addition of pantoprazole, at clinically relevant concentrations, between 5 and 25μM resulted in no significant change in the IUR of imatinib (Table A).

However, doses of 25μM pantoprazole significantly increased the IUR for nilotinib,

These data suggest that the effect of PPI on the intracellular concentration of nilotinib, occurs at clinically relevant concentrations, and as such may be of clinical relevance.

% change in imatinib IUR (average)	1 µM imatinib or nilotinib			2 µM imatinib or nilotinib		
	5µM Pantoprazole	10µM Pantoprazole	25µM Pantoprazole	5µM Pantoprazole	10µM Pantoprazole	25µM Pantoprazole
imatinib	4%	-2.3%	-8.5%	-9.6%	-7.4%	-12.5%
p value	*0.902*	*0.77*	*0.584*	*0.705*	*0.749*	*0.623*

	5µM Pantoprazole	10µM Pantoprazole	25µM Pantoprazole	5µM Pantoprazole	10µM Pantoprazole	25µM Pantoprazole
nilotinib	-2.0%	5.4%	15.23%	12%	38.8%	67.03%
p value	*0.448*	*0.131*	*0.01*	*0.257*	*0.159*	*0.027*

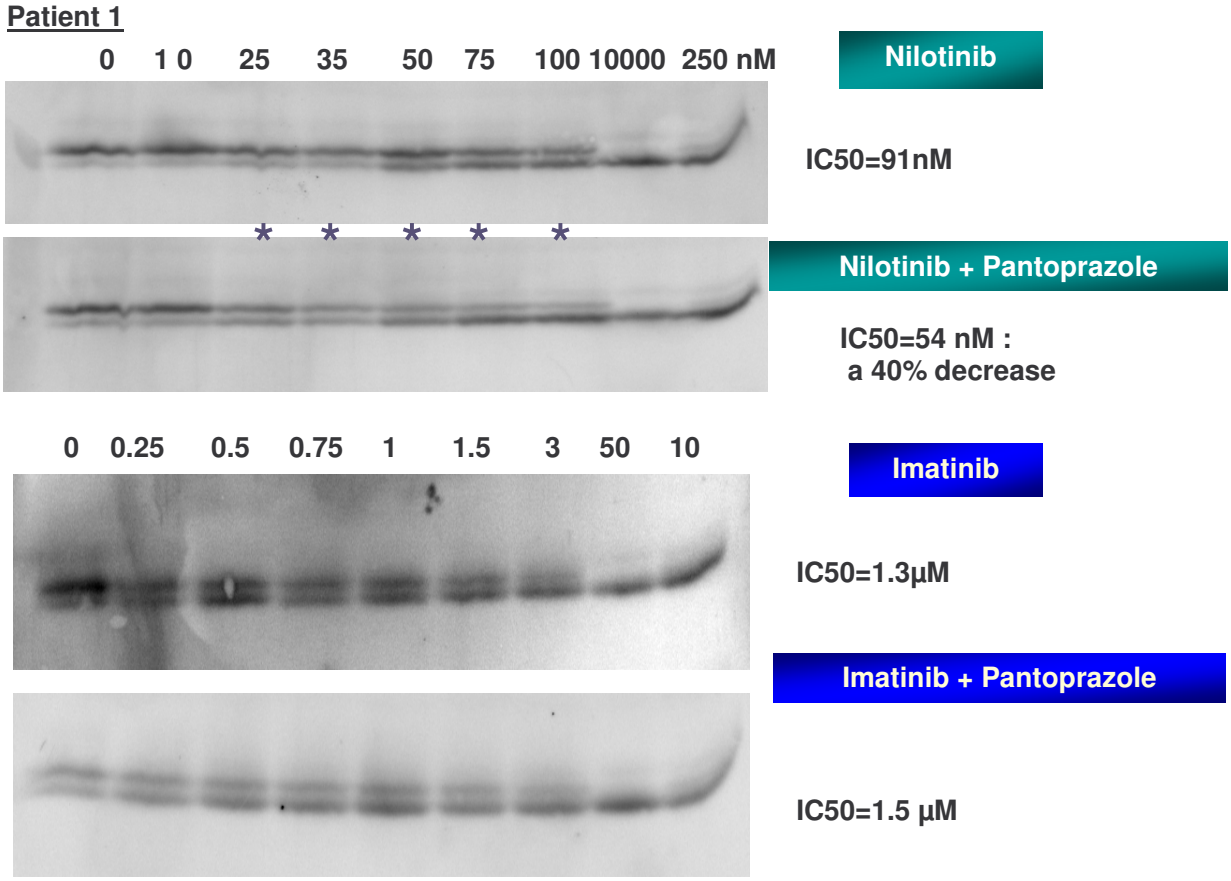


Figure 7.15 Effect of pantoprazole at 200µM on the IC50 for imatinib and nilotinib in patient cells.

In keeping with the findings in cell lines, the addition of 200µM pantoprazole resulted in a 40% decrease in $IC50^{nilotinib}$ in patient's cells. There was no significant change in the $IC50^{imatinib}$ with the addition of pantoprazole.

*indicates difference in %p-Crkl.

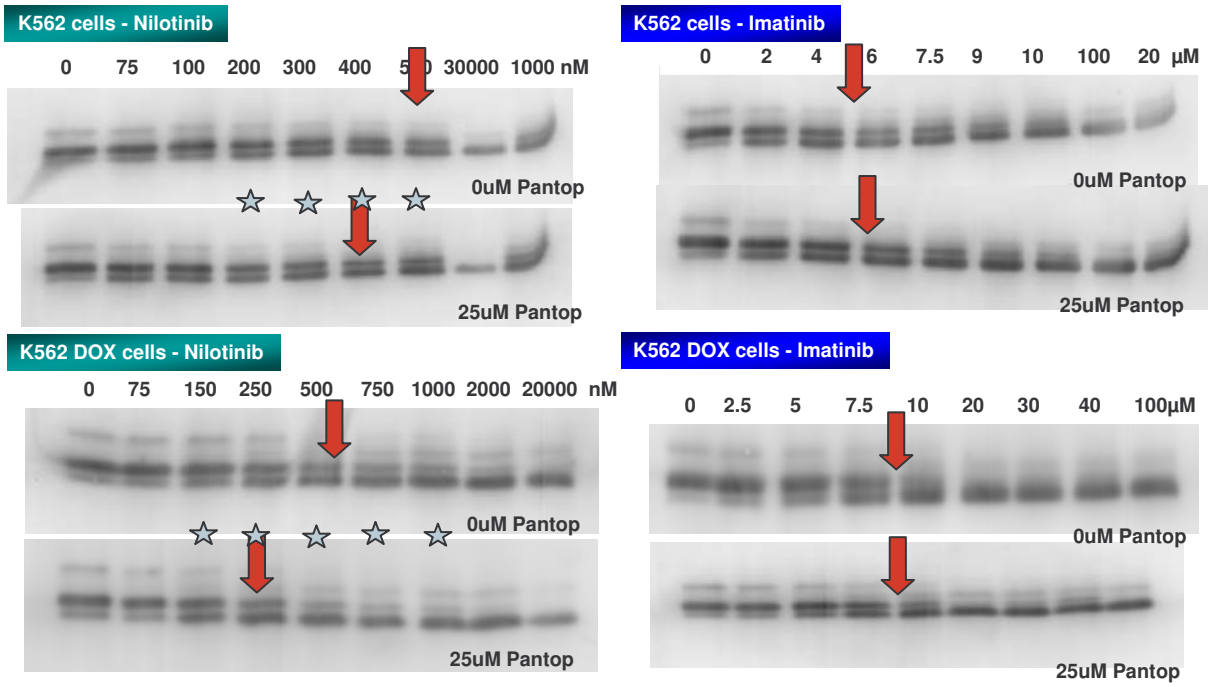


Figure 7.16 Effect of low dose pantoprazole (25µM) on the IC50 for imatinib and nilotinib in K562 and K562DOX cells.

Similar to the findings with high PPI doses, the addition of 25µM pantoprazole results in a decrease in the $IC50^{nilotinib}$ in K562 and K562-DOX cells. However there was no significant difference observed in the $IC50^{imatinib}$ for either cell line in the presence of 25µM pantoprazole.

Figure 7.17 Effect of pantoprazole and esomeprazole on the intracellular concentration of imatinib assessed as total, prazosin not inhibited and prazosin inhibit able uptake.

Graph A

In 7 patients the reduction in the IUR for imatinib was assessed with respect to the effect of PPI on imatinib uptake. There was a universal decrease in imatinib IUR as shown previously, but of interest the uptake of imatinib which is inhibitable with prazosin was significantly decreased. There was no effect on the uptake not inhibited by prazosin (indicated here with the X axis heading Prazosin). This indicates that the addition of PPI, at these concentrations, may be also affecting the uptake of imatinib.

Graph B

The % change is calculated from the no PPI control and measured as a % change from each baseline parameter ie: The % change in Prazosin is the % change from baseline Prazosin and the % change in prazosin inhibitable is the % change from baseline prazosin inhibitable.

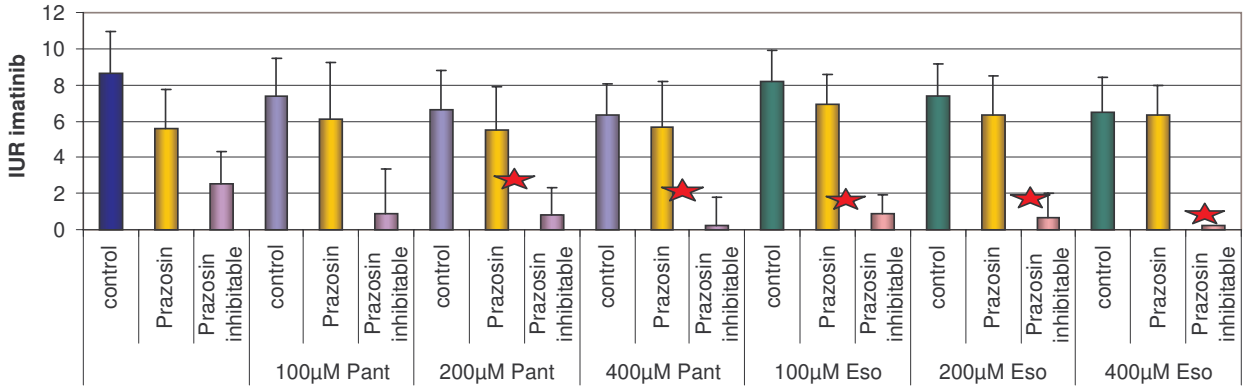
This serves to reiterate the data above and reinforces the observed difference in prazosin inhibitable uptake, and demonstrates the small increase in the imatinib uptake which is not inhibited by prazosin.

★ - indicates significant difference at the 0.05 level.

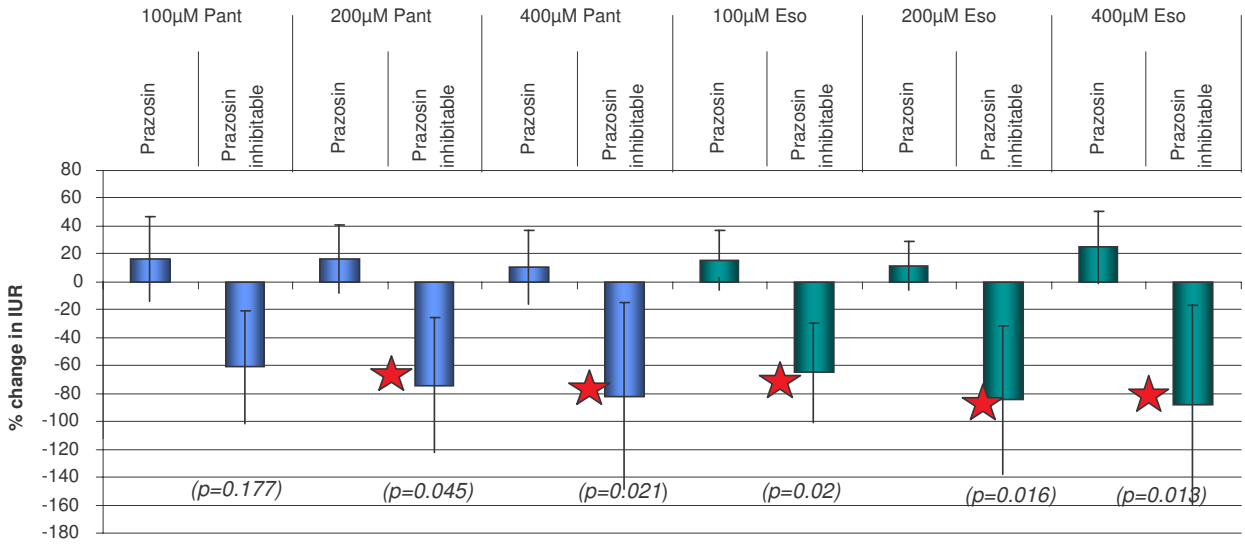
Prazosin – indicates the IUR not affected by the addition of Prazosin

Prazosin inhibitable – indicates the uptake of imatinib which has been inhibited by the addition of prazosin.

A


IUR imatinib
12
10
8
6
4
2
0
control
Prazosin
Prazosin inhibitable
100µM Pant
200µM Pant
400µM Pant
100µM Eso
200µM Eso
400µM Eso

B


100µM Pant
200µM Pant
400µM Pant
100µM Eso
200µM Eso
400µM Eso
Prazosin
Prazosin inhibitable
% change in IUR
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
(p=0.177)
(p=0.045)
(p=0.021)
(p=0.02)
(p=0.016)
(p=0.013)

7.5 Summary

Imatinib has proven to be a very successful therapy for CP CML. This therapeutic success, and low toxicity profile, has to date abrogated the need for extensive investigations into the effect of concomitant therapies. While some therapies which interact with the Cytochrome P450 metabolising isoforms, utilized by imatinib and nilotinib, are contraindicated, their use is not prohibited as a concomitant therapy to either drug. To date, no studies have investigated the clinical effect of concomitant drugs which may interact with intracellular drug transporters in the setting of either imatinib or nilotinib.

In Chapter 6, the concomitant use of imatinib and nilotinib was investigated, and a drug interaction at the target cell level was identified. The evidence presented suggests that this interaction was most likely mediated via the efflux transporters, ABCB1 and ABCG2. This interaction resulted in significantly increased intracellular levels of nilotinib in the presence of imatinib, and suggested that the synergistic to additive effects observed by Weisberg et al (Weisberg *et al.* 2007) were mediated by increased cellular level of nilotinib, but not imatinib. In that study, the effect of nilotinib reduced the intracellular concentration of imatinib, though not significantly.

In the current study, the effect of proton pump inhibitors, namely pantoprazole and esomeprazole, on the intracellular concentrations of imatinib and nilotinib were studied. This study was limited to these two PPI, because of their availability in liquid formulation amenable to in-vitro testing. Proton pump inhibitors have previously been demonstrated to interact with ABCB1 and ABCG2 (Luciani *et al.* 2004; Pauli-Magnus *et al.* 2001), (Breedveld *et al.* 2006; Breedveld *et al.* 2005), as have imatinib and nilotinib (Dai *et al.* 2003; Gurney *et al.* 2007; Hegedus *et al.* 2002; Illmer *et al.* 2004; Mahon *et al.* 2003; Ozvegy-Laczka *et al.* 2004b; White *et al.* 2006). In this Chapter, a significant increase in the IUR for nilotinib, in the presence of both pantoprazole and esomeprazole was demonstrated. This increase was statistically significant at all concentrations above 25µM. Pantoprazole and esomeprazole are equipotent in mediating this effect. Furthermore, this increase translated to a significant reduction in the $IC50^{nilotinib}$ in both cell lines and patient material. In one patient this resulted in a 40% reduction in the $IC50^{nilotinib}$. The largest reduction in $IC50^{nilotinib}$ was observed in the ABCB1 over expressing cell line K562DOX. Importantly, this reduction occurred in these cells with PPI levels of 25µM and above.

These findings implicate ABCB1 in this interaction, though because of the absence of an ABCG2 over expressing, BCR-ABL positive cell line, the additional involvement of ABCG2 cannot be excluded. In

contrast to the findings with concomitant imatinib administration, the effect of TKI's was more marked at higher concentrations of nilotinib (ie in 2μM nilotinib arm), though the *p* values in the 1μM arm may be indicative that in a larger data series, statistical significance may be reached. In line with the model suggested in Chapter 6, it would appear likely that PPI are acting as inhibitors of efflux pumps, thus allowing the intracellular accumulation of nilotinib. The finding of equivalent efficacy suggests that PPI bind more avidly to efflux proteins, and are not displaced by competition with high concentrations of nilotinib. While the intracellular uptake mechanism for PPI remain to be determined, some involvement of facilitated influx of nilotinib, although unlikely.

In contrast to nilotinib, PPI result in a decrease in the IUR for imatinib, which in contrast to the findings in Chapter 6, did reach statistical significance at PPI doses of 100μM and above. In contrast to the effect noted above, but in keeping with the effect noted in Chapter 6, this finding was more pronounced at 1μM imatinib than at 2μM. There was also an apparent difference between the two PPI. Pantoprazole appeared more effective in reducing the IUR for imatinib at all concentrations tested, though the difference only achieved statistical significance at 400μM PPI, in the 1μM imatinib IUR. In line with this, the addition of pantoprazole to the $IC50^{imatinib}$ assay resulted in a 25% increase in IC50. In contrast the addition of esomeprazole resulted in an 18% decrease. There was no significant effect of PPI on the $IC50^{imatinib}$ observed in patient cells, and no effect of low dose (25μM) pantoprazole in either $IC50^{imatinib}$ or IUR. These findings suggest that either PPI are interacting with the influx of imatinib, thus reducing the IUR, or that imatinib is being bound/and or transported preferentially by ABCB1 or ABCG2 or both, in a concentration dependent manner. Speculatively, at low concentrations of imatinib PPI, (in particular pantoprazole) facilitate imatinib transport. At higher concentrations, imatinib binds to efflux pumps and acts as primarily as an inhibitor. Titrations of higher concentrations of PPI, overcome the inhibitory effect of imatinib binding, and thus facilitate the transport of imatinib (ie 400μM in 2μM imatinib IUR)

The alternate hypothesis that PPI may be interacting with the uptake of imatinib was investigated in a small series at high PPI concentrations (100 to 400μM). Interestingly, the addition of PPI significantly reduced the uptake of imatinib which is inhibited by prazosin, the OCT-1 mediated uptake. The reasons for this are unclear. Conceivably this could occur by competitive binding for the OCT-1 protein between PPI and imatinib. However as radiolabelled PPI are not currently available, this is difficult to investigate. Alternatively PPI may affect a downstream modulator or effector of the OCT-1 protein. Again, without adequate reagents this would be difficult to investigate. Furthermore, the effect on imatinib intracellular concentration, mediated by PPI, does not appear to be significant at low, or

clinically relevant PPI concentrations. Therefore, in the clinical setting this interaction may not mediate any significant problem, and thus further investigations, if possible, may be of academic interest only.

While this Chapter has focussed on the interaction of imatinib and nilotinib with Proton Pump Inhibitors, it is important to note, that the interactions may be relevant to many other classes of drug. Many clinical drugs have been implicated as substrates or inhibitors of pGP (ABCB1). In the setting of concomitant medications it is also important to note, that those drugs listed as substrates, may also act as inhibitors where competitive substrate binding occurs.

As stated, the variations observed in this Chapter can most likely be attributable to changes mediated by drug interactions with ABCB1, ABCG2 or other efflux pumps and OCT-1, as discussed, and can in some cases be seen as mediating significant interactions at clinically relevant concentrations.

7.6 Overall Comment and significance of this work

The data presented here adds further to the evidence of the importance of the interaction of imatinib and nilotinib with intracellular drug transporters. In this instance, a significant interactions of PPI with TKI has been demonstrated, which in the case of nilotinib may be of significant clinical relevance. Importantly, PPIs may increase intracellular nilotinib levels, leading to toxicity which may not be suggested by the plasma levels of this drug. However the positive clinical effects of these findings may be a more uniform exposure to nilotinib by the blockade of variable effects of efflux transporters by PPI, which may in turn lead to more sustained kinase inhibition. Furthermore, these findings may be potential exploited to achieve better drug penetration to areas such as CNS and testes.

Chapter 8

The Functional Activity of the human Organic Cation Transporter – 1 (hOCT-1) Protein

8.1 Introduction

As discussed previously in this thesis, and described in the literature (White *et al.* 2006) (White *et al.* 2005c) (Thomas *et al.* 2004) (Wang *et al.* 2007) (Crossman. L *et al.* 2005) the primary active influx mechanism for imatinib is via the human Organic Cation Transporter–1 (OCT-1). The OCT-1 protein is a member of the largest superfamily of transporters, the solute carrier family (Koepsell & Endou 2004) which transport in an electrogenic fashion a variety of organic cations including drugs, toxins and other xenobiotics. The transporter is predicted to have 12 transmembrane domains, and binding pockets with partially overlapping interaction domains for different substrates and inhibitors (Koepsell *et al.* 2003). Polymorphisms that affect function have been reported (Kerb *et al.* 2002) (Shu *et al.* 2003). Post transcriptional regulation of OCT-1 by phosphorylation status (Ciarimboli *et al.* 2005; Ciarimboli & Schlatter 2005) and such compounds as (protein kinase A (PKA), Src-like p56 and Calmodulin (CaM) have also been demonstrated.

Crossman et al (Crossman. L *et al.* 2005) demonstrated in a small patient cohort, that the level of OCT-1 mRNA was significantly lower in cytogenetic non-responders, than in cytogenetic responders. This data was recently supported by a larger data series (Wang *et al.* 2007). However, these finding were from various diagnostic subgroups (early to late CP) and the majority of patients (78% in the Wang paper) had been treated with either interferon or hydroxyurea, with several patients demonstrating progressive disease. This data requires validation in a large series of de-novo, chronic phase, previously untreated patients.

Both quantitative and qualitative changes in OCT-1 may impact on imatinib uptake. Hence a functional assay of imatinib uptake into patient's cells may provide the most clinically predictive assay of OCT-1 activity. To determine the functional level of the OCT-1 protein, prazosin, a potent inhibitor of OCT-1 (IC50 -1.8uM (Hayer-Zillgen *et al.* 2002)) was used to inhibit OCT-1 from translocating imatinib into the cell. The rationale was that the difference in intracellular uptake and retention (IUR) of imatinib determined using [^{14}C]-labelled imatinib, in the presence and absence of prazosin would provide an estimate of the functional activity of the OCT-1 protein with respect to imatinib transport. This difference was then termed, OCT-1 Activity. In this Chapter, OCT-1 Activity has been compared with the IUR of imatinib and the $IC50^{imatinib}$. Furthermore linking this data to a clinical trial has enabled assessment of the predictive value of OCT-1 Activity with respect to molecular response, and also compare outcomes with differing dosage regimens.

These studies provide valuable information on the role of the OCT-1 protein, in achieving an adequate intracellular concentration of imatinib and give further insight into the variable responses seen in some CML patients treated with imatinib. Importantly, these studies can potentially be used to develop individualized patient treatment strategies, aimed at delivering early and maximal molecular responses.

8.2 Summary and research contribution

The research detailed in this chapter, the attached manuscript and conference proceedings, increase the understanding of the importance of the OCT-1 protein and its activity in imatinib response. The research in this chapter describes the development of the assay and addresses correlations with previous findings.

Publications

White, D. L., V. A. Saunders, et al. (2007). "Most CML patients who have a suboptimal response to imatinib have low OCT-1 activity: higher doses of imatinib may overcome the negative impact of low OCT-1 activity." Blood **110**(12): 4064-4072. (White *et al.* 2007d)

Conference Presentations.

White, D., V. Saunders, et al. (2006). "Molecular Response to Imatinib Is Dependent on Dose in CML Patients with Low OCT-1 Influx Activity. Patients with High Activity May Respond Equally Well to Standard or Increased Dose Imatinib." ASH Annual Meeting Abstracts 108(11): 738-.(White Deborah *et al.* November 2006)

8.3 Assay development

8.3.1 Intracellular Uptake and Retention (IUR) Assay

This assay was performed in 132 patients, as previously described. The IUR at 2 µM was performed in the presence and absence of 100µM prazosin to inhibit the function of OCT-1, and thus assess the OCT-1 Activity of each patient's cells. As a comparison, Procainamide, also an OCT-1 inhibitor, was included in the 2µM imatinib arm in 59 of the 132 patients.

The OCT-1 Activity is defined as:-
Total IUR of imatinib – IUR of imarinib in the presence of Prazosin or Procainamide (ng/200,000 cells).
For example: If the total IUR was 23ng/200,000 cells, and the IUR with prazosin was 13ng then the OCT-1 Activity was 23-13, therefore 10 ng/200,000 cells.

8.3.2 $IC50^{imatinib}$ assay

The $IC50^{imatinib}$ assay was performed as previously described (Chapters 4, 5 and Appendix I Materials and Methods).

8.3.3 OCT-1 mRNA levels.

RNA was extracted from a minimum of $5x10^6$ cells using TRIzol Reagent (Invitrogen, Carlsbad, CA). cDNA was synthesised using Random Hexamers (GeneWorks Pty. Ltd., Hindmarsh, Australia) and Superscript™ II Reverse Transcriptase (Invitrogen). Primers were designed using Primer Express® software v2.0 (Applied Biosystems, Foster City, CA), sequences were as follows:-

hOCT-1 Forward CTG AGC TGT ACC CCA CAT TCG:

hOCT-1 Reverse CCA ACA CCG CAA ACA AAA TGA

(Sigma-Aldrich Pty. Ltd., Castle Hill, Australia).

Amplification was performed using RT^2 Real-time™ SYBR Green/ROX PCR Master Mix (SuperArray Bioscience Corporation) on a RotaGene® real time PCR machine (Corbett Biosciences).

8.4 Results

8.4.1 OCT-1 Activity

The addition of prazosin, a potent inhibitor of OCT-1, to the IUR assay impairs the active transport of imatinib by the OCT-1 protein. The OCT-1 Activity, which provides a measure of the actual activity of the OCT-1 protein in the transport of imatinib, was calculated as the difference in IUR in the absence (total IUR) and presence of prazosin. In 59 patients, inhibition of OCT-1 was also confirmed using an alternate OCT-1 inhibitor, Procainamide. The correlation in OCT-1 Activities between matched samples using the two inhibitors was r= 0.945 *p<0.001* (Figure 8.1). Furthermore the Chi square analysis, when OCT-1 Activities were dichotomised about the median for each inhibitor, was found to be 31.007 *p<0.001*.

Examination of 132 patients enrolled to both trials, revealed a wide variation in OCT-1 Activity (median 8.2: Range 0 to 31.2ng/200,000 cells). In replicate assays of 5 patients the IUR values with prazosin were equal or lower than the values without prazosin. These patients were scored as having negligible (0ng/200,000 cells) OCT-1 Activity.

8.4.2 The Intracellular uptake and retention of imatinib (IUR) and the $IC50^{imatinib}$.

The IUR and $IC50^{imatinib}$ for this cohort were extensively examined and discussed in Chapter 4. As demonstrated, there was a very strong correlation between these two parameters (R=-0.342;*p=0.00056*) Refer figures 4.2 and 4.3.

8.4.3 OCT-1 Activity and $IC50^{imatinib}$

In 99 patients where both $IC50^{imatinib}$ and OCT-1 Activity were measured, there was a significant correlation between the $IC50^{imatinib}$ and the OCT-1 Activity (r=-.238, *p=0.019*) (Figure 8.2A). In addition, grouping $IC50^{imatinib}$ into low and high cohorts according to the median value revealed a significantly greater OCT-1 Activity in the low $IC50^{imatinib}$ group when compared to the high group (*p=0.008*)(Figure 8.2B), suggesting that low $IC50^{imatinib}$ and therefore drug effect is related to intracellular uptake.

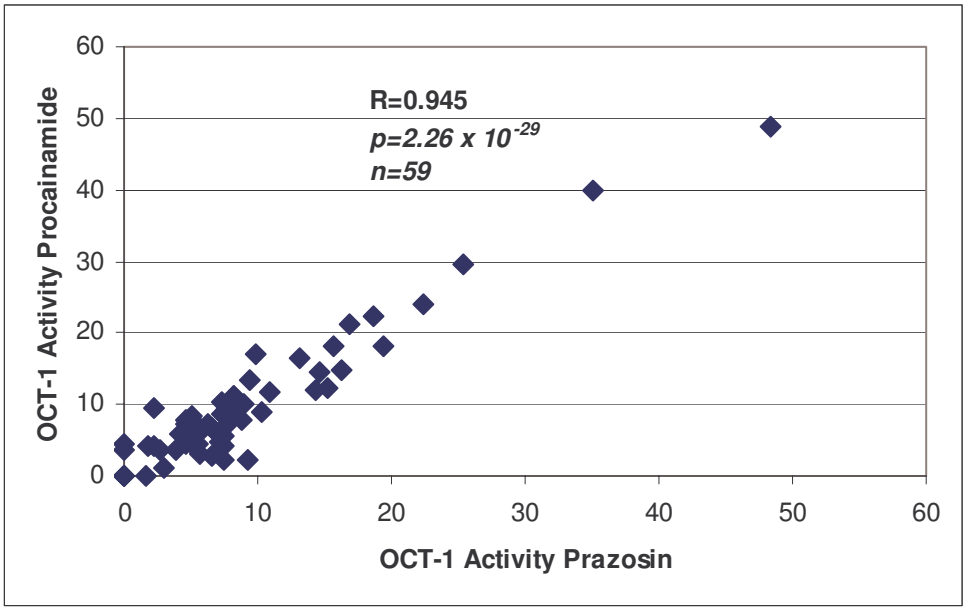


Figure 8.1 Comparison between the OCT-1 Activities defined by the OCT-1 inhibitors prazosin and procainamide.

The OCT-1 Activity was assessed in 59 patients using a second OCT-1 inhibitor Proacainamide (inhibits OCT-1 and OCT-2). As can be seen there was excellent correlation between the OCT-1 Activity as a result of Prazosin (OCT-1 and OCT-3 inhibitor) inhibition and Procainamide inhibition.

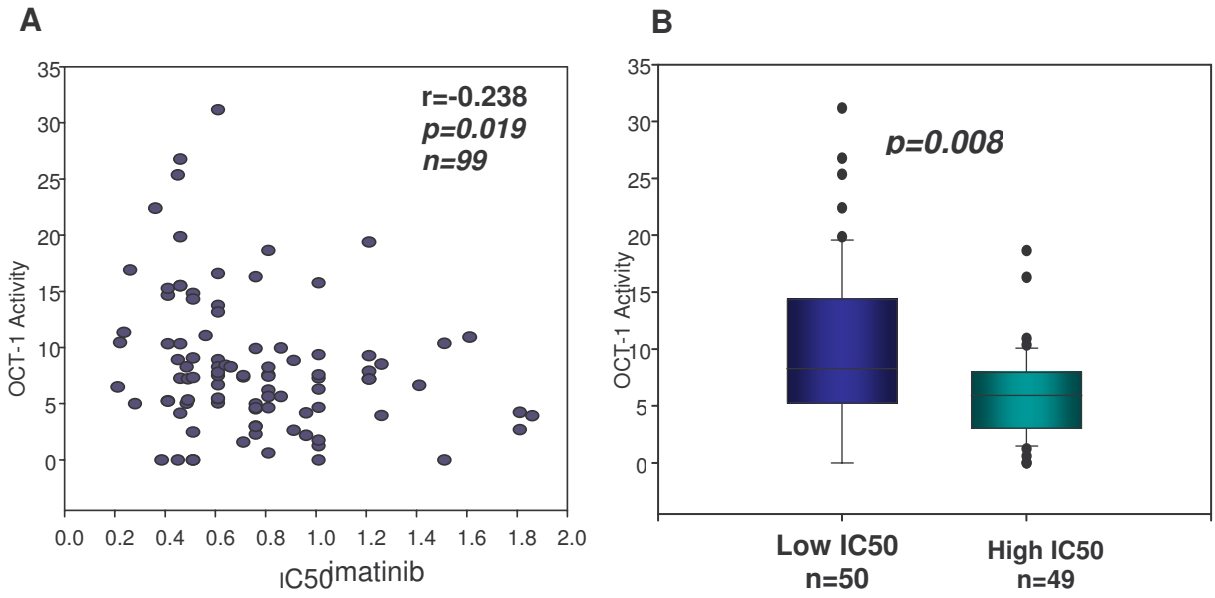


Figure 8.2 Correlation analysis reveals a link between the IC50^{imatinib} and the OCT-1 Activity for imatinib

A. There was a correlation between the IC50^{imatinib} and the OCT-1 Activity for imatinib in 99 CP CML patients who had both parameters measured. Furthermore, as shown in B, patients with a low IC50^{imatinib} have a significantly higher OCT-1 Activity, than patients with a high IC50^{imatinib}.

8.4.4 OCT-1 Activity and Sokal Prognostic Score.

The OCT-1 Activity does not correlate with the Sokal Prognostic Score (Sokal *et al.* 1984)(r=0.019, *p=0.890*) (Figure 8.3), and there is no significant difference between the median Sokal scores of the low and high OCT-1 Activity groups (low OCT-1 Activity 0.96; high OCT-1 Activity 0.975 *p=0.712*).

8.4.5 OCT-1 Activity, molecular response and the effect of actual dose received.

The OCT-1 Activity was compared to molecular response over the first 24 months of imatinib therapy in 56 patients enrolled to the TIDEL trial, and for whom samples were available for this analysis**.** Patients were grouped into low and high OCT-1 Activity based on the median Activity (7.2 ng/200,000 cells) for this cohort. As shown in Table 8.1. Patients with high OCT-1 Activity (n=27) achieved significantly higher molecular response over the time course than patients with low OCT-1 Activity (n=29)(*p=0.005* at 24 months).

However, because of tolerability issues, not all patients received 600mg of imatinib consistently over the first 12 months of therapy. To assess the effect of varying dose patients were further sub-grouped into those patients who received an average daily dose (ADD) of 600mg (n=33) over the first 12 months of imatinib therapy, and those who received an ADD of less than 600mg per day (n=23 median ADD 523mg). Four patients with ADD <400mg were included in this cohort.

Assessing only those patients with high OCT-1 Activity there is no significant difference in molecular response between those patients who received <600mg versus those patients who received 600mg or more over the first 12 months (*p=0.449* at 24 months)(Table 8.1). In contrast, in the cohort of patients with low OCT-1 Activity there is a significant dose effect, with patients receiving 600mg achieving significantly better molecular responses than those who fail to receive 600mg (*p=0.005* at 24 months)(Table 8.1).

Kaplan Meier Analysis revealed an 85% probability of achieving MMR by 24 months in patients with high OCT-1 Activity (median time to MMR 9 months) versus only 45% of patients with low OCT-1 Activity (median time 24 months) *p=0.009* (Figure 8.4). Analysing only those patients who received <600mg ADD over the first 12 months reveals a significant effect of dose between low and high OCT-1 Activity patients. Of those patients with high OCT-1 Activity (n=12) 83% achieve MMR compared with only 18% of patients with a low OCT-1 Activity (n=11;*p=0.022*). Among the 33 patients receiving an ADD of 600 mg, there was no significant difference according to OCT-1 Activity (*p=0.110*).

Performance of a two way analysis of variance, with the dependent variable being molecular response at 24 months, and dichotomising OCT-1 Activity into low and high based on median values, and ADD to 12 months into 600mg or not, revealed the effect of different levels of dose to 12 months, depends on the level of OCT-1 Activity (*p=0.018*). This demonstrates a significant interaction between OCT-1 Activity and dose in the determination of 24 month molecular response.

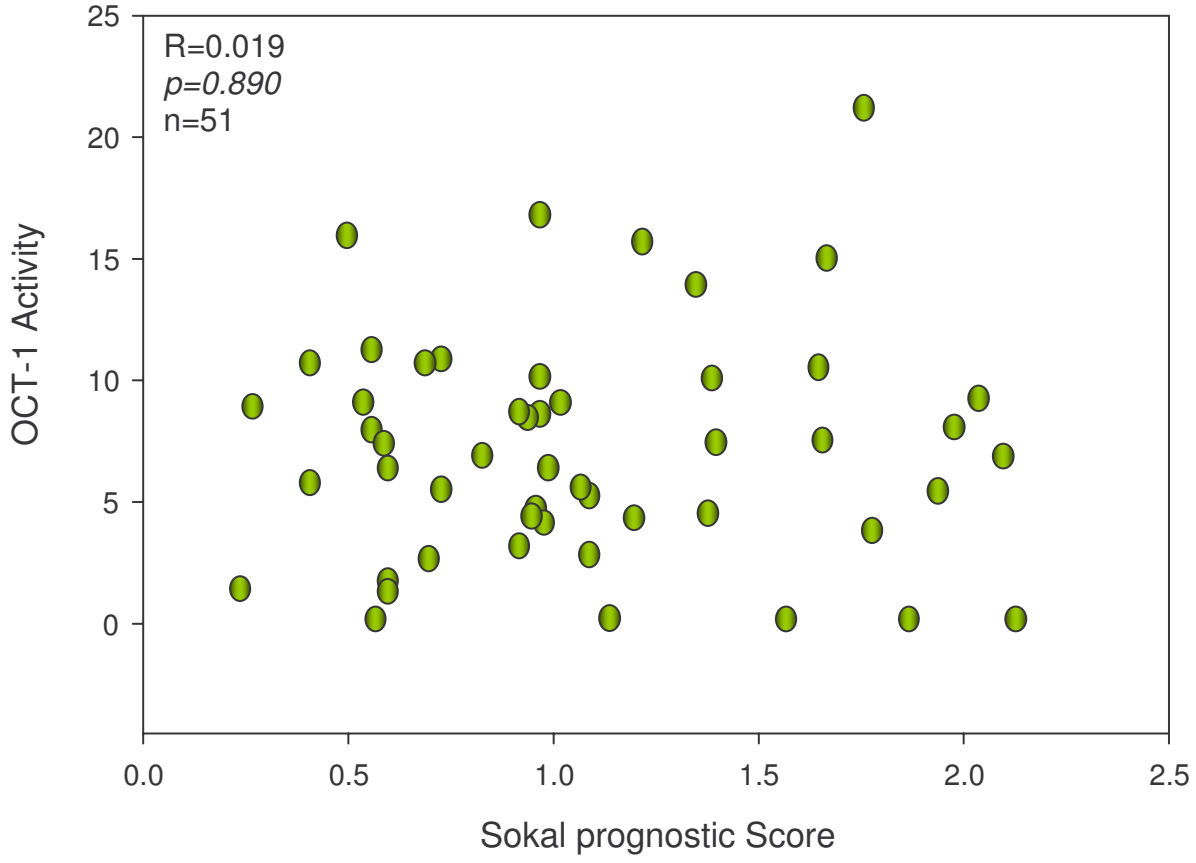


Figure 8.3 X-Y plot, demonstrating the lack of correlation between the OCT-1 Activity for imatinib and the Sokal Prognostic Score.

In 51 patients enrolled to TIDEL where the Sokal Score and the OCT-1 Activities were available, analysis revealed there was no correlation between the two parameters.

Note: the Sokal Score was not provided as a number, rather as a category (low, medium or high) for TOPS patients, and hence this data could not be included.

Table 8.1 Assessing the impact of OCT-1 Activity on molecular response in 56 patients enrolled to the TIDEL trial

(Note: the molecular response is to date not available in patients enrolled to the TOPS trial)

Patients with high OCT-1 Activity achieve superior molecular response at 12, 18 and 24 months when compared to those patients with low OCT-1 Activity. To address the effect of actual dose received patients were grouped into those with low, and those with high OCT-1 Activities, and then further sub grouped into patients receiving <600mg per day, and those receiving 600mg or more. In the groups of patients with low OCT-1 Activity dose was a significant factor in the achievement of molecular response, with those receiving ≥ 600mg achieving significantly better molecular responses? However, in the group of patients with high OCT-1 Activity dose was not a significant factor, with both groups achieving similar molecular outcomes.

	Average Molecular Response (log reduction in BCR-ABL from standardised baseline) at 6 monthly Intervals		
OCT-1 Activity	12	18	24
low (n=29)	2.6	2.6	2.8
high (n=27)	3.1	3.9	3.9
p-value	***0.032***	***0.006***	***0.005***
Low OCT-1 Activity			
<600mg (n=11)	2.1	2.3	2.4
≥600mg (n=18)	2.8	3.2	3.4
p- value	***0.121***	***0.023***	***0.005***
High OCT-1 Activity			
<600mg (n=12)	2.9	3.3	3.5
≥600mg (n=15)	2.9	3.9	3.9
p- value	***0.789***	***0.625***	***0.449***

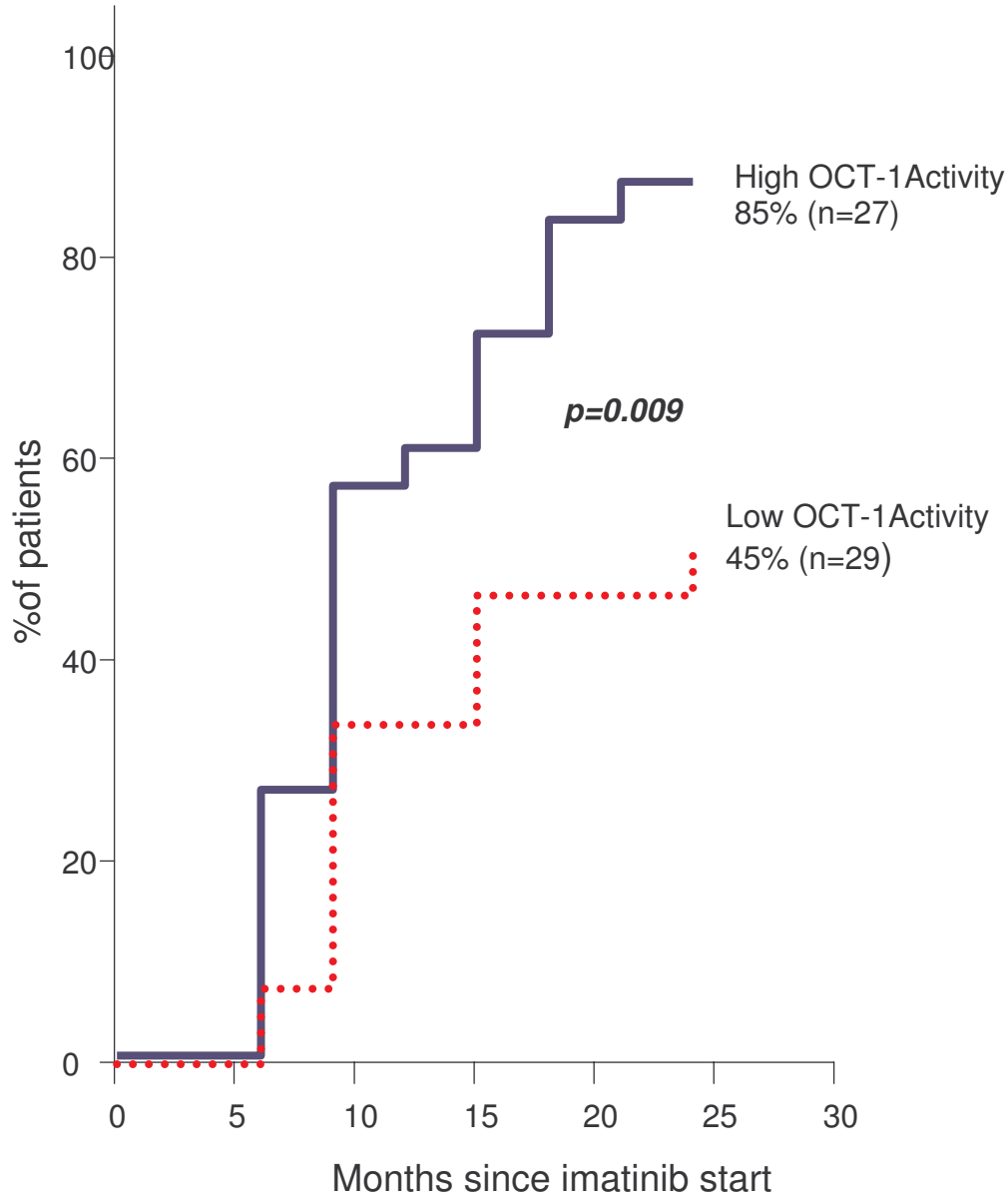


Figure 8.4 Kaplan Meier analysis showing the % of patients achieving a major molecular response (MMR) based on low and high OCT-1 Activity groups.

A significantly greater proportion of patients with high OCT-1 Activity achieve a MMR by 24 months, when compared to patients with low OCT-1 Activity.

8.4.6 Effect of dose increase

In the TIDEL trial, dose increases from the initial 600mg to 800mg per day were mandated if a 4 log reduction in BCR-ABL from the standardized baseline was not achieved by 12 months. In our patient cohort, 46 patients were scheduled to receive dose increases. In this analysis, a dose increase was considered to have occurred if the patient received 800mg ADD for at least one month.

For reasons of toxicity only 29/46 (63%) patients in this cohort successfully dose increased to 800mg per day. The primary reason for inability to increase dose was previous toxicity/tolerance issues, occurring in 13 of those 17 patients who did not dose escalate. Of the remaining 4 patients, 2 dose escalated for one month, but did not reach 600mg ADD. It is not known why the remaining 2 patients failed to escalate.

Dose increase occurred by 14 months in all patients who were able to tolerate dose increase. To assess the impact of dose escalation, patients were grouped into low and high OCT-1 Activity groups as previously described, then were further subdivided into those who received increased dose and those who remained on 600mg or less as shown in Figure 8.5. In the high OCT-1 Activity cohort, there was no significant difference in molecular response between the patients who dose increased and the patients who failed to dose increase ($p>0.05$ at all time points). In contrast, patients with a low OCT-1 Activity perform equally well as those patients with high Activity when they are dose increased. However, those who failed to dose increase in the setting of a low OCT-1 Activity had significantly inferior molecular responses at 24 months (Figure 8.5).

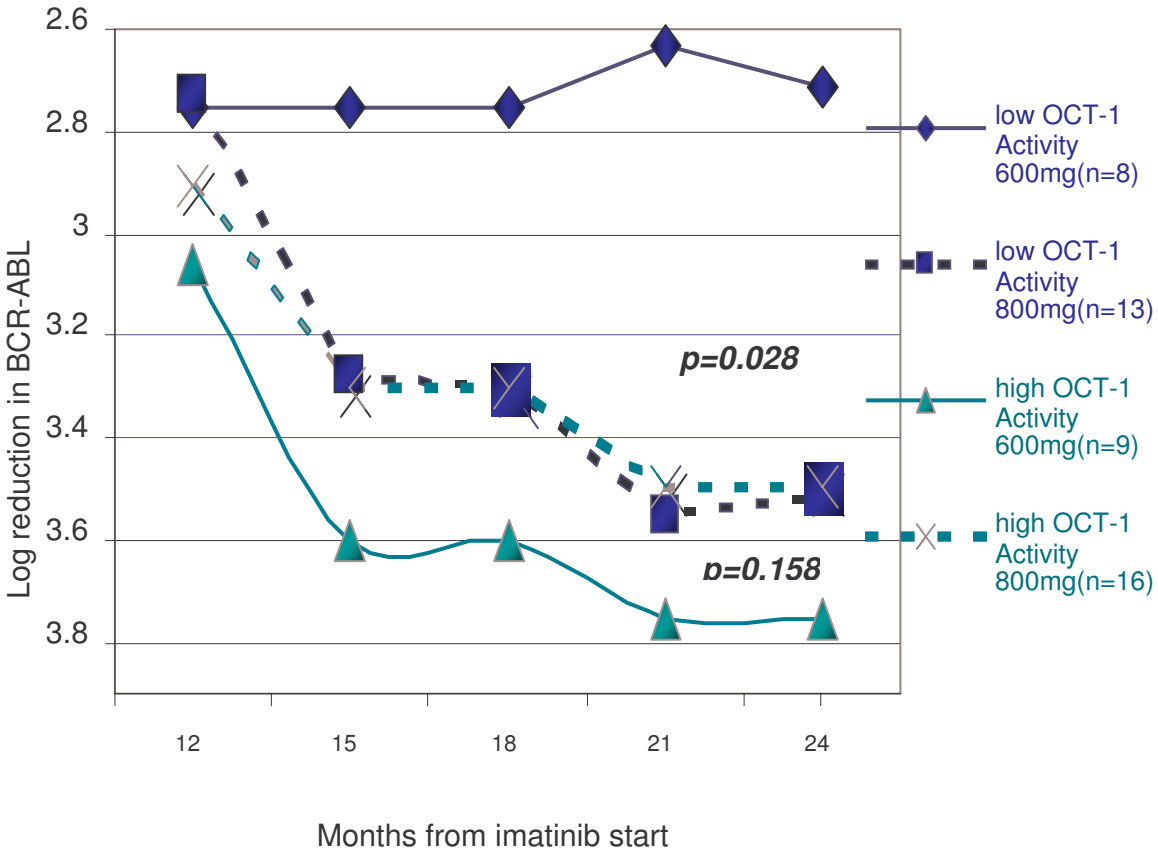


Figure 8.5 The variable effect of dose increase, on molecular response after 12 months, in patients grouped as having either high or low OCT-1 Activity.

Patients with low OCT-1 Activity, who failed to dose increase, did not demonstrate an increase in molecular response over the second 12 months of imatinib therapy. In contrast, those that did dose increase showed a significant increase in molecular response (*p=0.028*). In patients with high OCT-1 Activity there was no significant difference in the molecular response achieved over the second 12 months based on dose increase, or maintenance of 600mg ADD (*p=0.158).*

The median OCT-1 Activity in patients who failed to achieve MMR by 18 months is significantly lower than OCT-1 Activity in patients who did achieve MMR (Figure 8.6). These differences in OCT-1 activity between molecular response groups is most pronounced in patients who were not able to dose escalate at 12 months (Figure 8.6c). In patients who dose increased at 12 months there is no significant difference between the 3 groups (Figure 8.6d).

8.4.7 OCT-1 Activity, Suboptimal response and imatinib failure

Suboptimal response has been defined as failure to achieve a major cytogenetic response by 6 months, a complete cytogenetic response by 12 months or a MMR by 18 months (Baccarani *et al.* 2006). As good correlation between molecular and cytogenetic response has been demonstrated previously (Branford *et al.* 1999) we assessed the suboptimal response as failure to achieve 1 log reduction in BCR-ABL by 6 months, a 2 log reduction by 12 months and a 3 log reduction (MMR) by 18 months. Log Rank Survival Analysis, as reported in Table 8.2, reveals there is no significant difference in the frequency of suboptimal response at any time point when patients receive 600mg ADD. However there is a significant difference at all time points when patients receive reduced dose, demonstrating patients with low OCT-1 Activity who receive reduced dosing (median ADD for this cohort 511mg) are at substantial risk of suboptimal response to imatinib.

Assessing the failure to achieve a 2 log reduction by 18 months (imatinib failure (Baccarani *et al.* 2006)) reveals also that a significantly lower proportion of patients with low OCT-1 Activity who receive reduced dose achieve a 2 log reduction by 18 months (low OCT-1 Activity 36% vs high OCT-1 Activity 8%, *p-0.04*).

8.4.8 OCT-1 mRNA and OCT-1 Activity

The level of OCT-1 mRNA was measured relative to BCR mRNA in 93 patients. The mean %OCT-1/BCR mRNA was 0.835 within a range 0.02 to 3.5%. Measures of OCT-1 mRNA were correlated with OCT-1 Activity (*p=0.002*, r= 0.378 Pearson Product Moment)(Figure 8.7). Dividing patients into low and high OCT-1 Activity about the median for this cohort of 7.9 reveals a significant difference between the two groups. The median %OCT-1 mRNA for patients with low OCT-1 Activity (n=47) was 0.367 compared to 0.635 (n=46) for patients with high OCT-1 Activity (*p=0.016*)(Figure 8.7).

Interestingly, in the 77 patients where IC50 and OCT-1 mRNA analyses were available, there was no correlation (r=0.162; *p=0.171*) between the two parameters (Figure 8.8). Further grouping the patients

into low and high $IC50^{imatinib}$ and assessing the level of OCT-1 mRNA expression revealed no significant difference between the two groups (low $IC50^{imatinib}$ n=42, median 0.586; high $IC50^{imatinib}$ n=35 median 0.546*; p=0.570*) (Figure 8.8).

Limiting analysis to those patients where 24 month molecular follow-up is available (TIDEL patients), a significant difference in the molecular response between the two groups (low and high OCT-1 mRNA) could not be demonstrated (Table 8.3). Also, in contrast to OCT-1 Activity there was no significant difference between the two groups with respect to dose (600mg or lower), and dose escalation (to 800mg) (Table 8.3) Unlike OCT-1 Activity, mRNA analysis did not reveal a group of patients with suboptimal response or at risk for imatinib failure (*p>0.05* Table 8.4)

Figure 8.6 The OCT-1 Activity compared to molecular responses (log reduction in BCR-ABL) at 18 months.

Response criteria assessed are suboptimal response (failure to achieve MMR by 18 months), and optimal responses (achievement of 3 to 4 log reduction in BCR-ABL, and >4 log reduction by 18 months). Cohorts are divided as follows:-

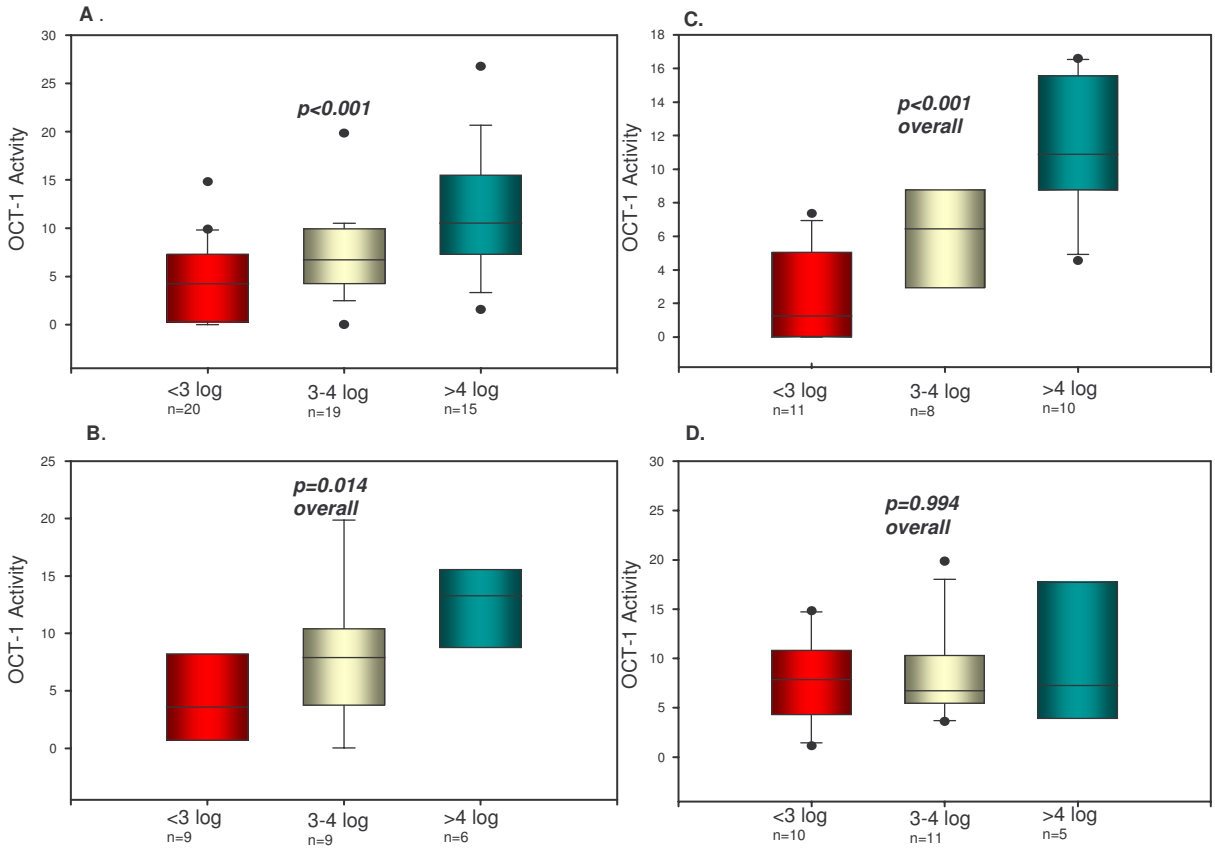
A All patients irrespective of dose received

B Patients receiving <600mg ADD over the first 12 months

C Patients who failed to dose escalate to 800mg ADD after 12 months

D Patients who successfully dose escalated to 800mg ADD after 12 months of therapy

These data demonstrate that in patients who dose escalate (above 600mg), the impact of the OCT-1 activity is reduced. This finding suggests that dose can overcome the effects of a low OCT-1 Activity.


A .
OCT-1 Activity
p<0.001
<3 log
n=20
3-4 log
n=19
>4 log
n=15
C.
OCT-1 Activity
p<0.001
overall
<3 log
n=11
3-4 log
n=8
>4 log
n=10
B.
OCT-1 Activity
p=0.014
overall
<3 log
n=9
3-4 log
n=9
>4 log
n=6
D.
OCT-1 Activity
p=0.994
overall
<3 log
n=10
3-4 log
n=11
>4 log
n=5

	% of patients failing to achieve		
	1 log reduction in BCR-ABL by 6 months	**2 log reduction in BCR-ABL by 12 months**	**3 log reduction in BCR-ABL by 18 months**
	Patients receiving ≥ 600mg ADD		
high OCT-1 Activity (n=15)	7%	13%	20%
low OCT-1 Activity (n=18)	0%	0%	44%
P value	0.669	0.560	0.133
	Patients receiving < 600mg ADD		
high OCT-1 Activity (n=12)	0%	8%	17%
low OCT-1 Activity (n=11)	27%	45%	82%
P value	0.005	0.021	0.022

Table 8.2 Percentage of patients failing to achieve previously defined (Baccarani *et al.* 2006) sub-optimal response criteria.

There is no significant difference in the frequency of suboptimal response at any time point when patients receive 600mg ADD, however there is a significant difference at all time points when patients receive reduced dose.

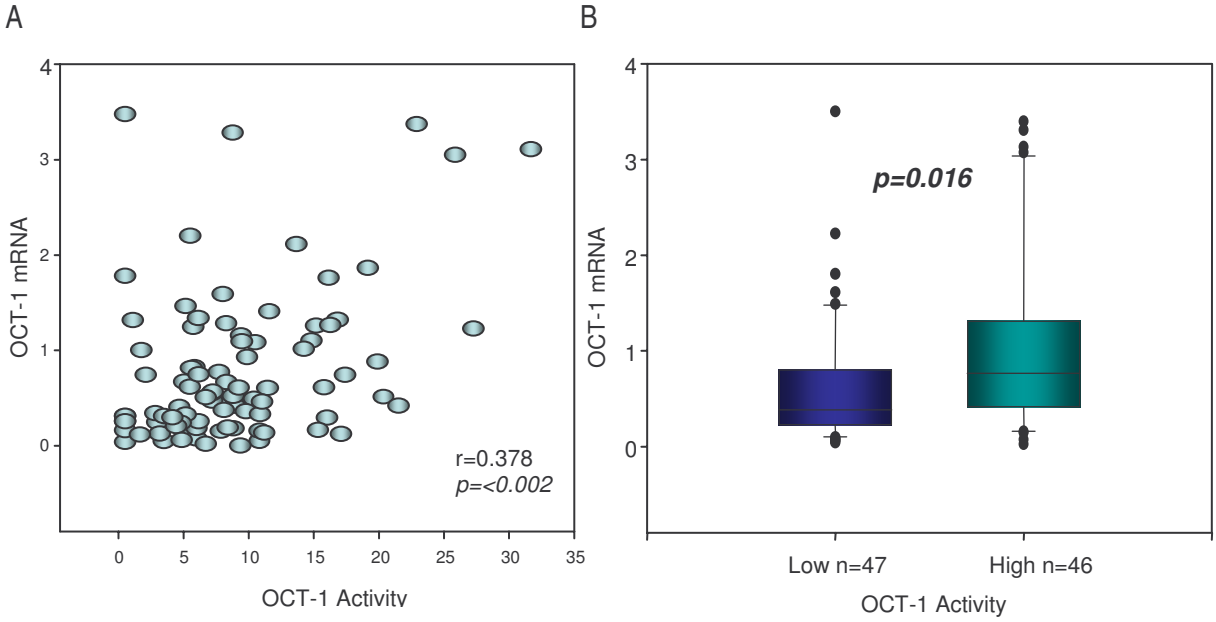


Figure 8.7 The OCT-1 Activity is compared to the level of OCT-1 mRNA in 93 CML patients

Graph A shows the correlation between the OCT-1 Activity and OCT-1 mRNA ($p<0.002$).

In Graph B patients were divided into low and high OCT-1 Activity cohorts about the median. Representative box plot reveal that patients with a low OCT-1 Activity have lower levels of OCT-1 mRNA, than patients with high OCT-1 Activity ($p=0.016$)

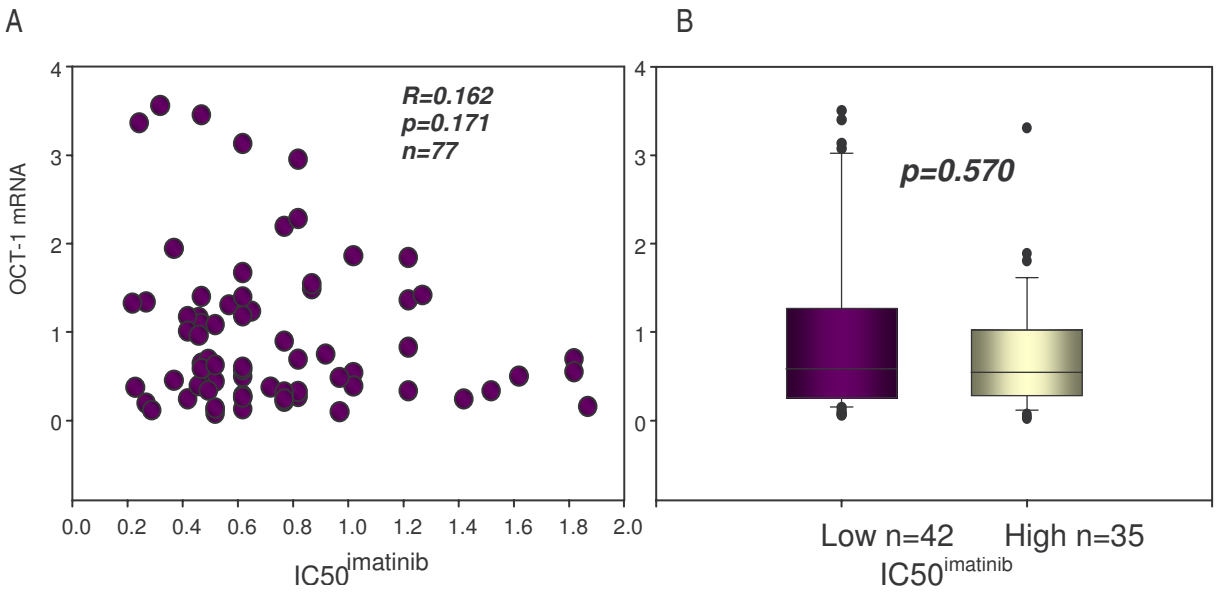


Figure 8.8 There was no demonstrable correlation between the IC50^{imatinib} and the level of OCT-1 mRNA.

As shown in Graph A there was no correlation between the IC50^{imatinib} and the level of OCT-1 mRNA (p=0.171). Furthermore, grouping patients into low and high IC50^{imatinib} groups revealed no significant difference in the level of mRNA between the two groups (Graph B) (p=0.570).

	Average Molecular Response (log reduction in BCR-ABL from standardised baseline) at 6 monthly Intervals		
mRNA	**12**	**18**	**24**
low (n=22)	2.9	3.0	3.3
high (n=21)	2.9	3.9	3.9
p-value	*0.693*	*0.09*	*0.125*
Low OCT-1 mRNA			
<600mg (n=10)	2.8	2.9	2.9
≥600mg (n=12)	2.8	3.1	3.3
p- value	*0.971*	*0.485*	*0.402*
High OCT-1 mRNA			
<600mg (n=8)	2.5	3.6	3.7
≥600mg (n=13)	2.9	3.9	3.9
p- value	*0.337*	*0.856*	*0.8*

Table 8.3 Assessing the impact of the level of OCT-1 mRNA on molecular response

There is no significant difference in the level of molecular response achieved when patients are divided into low and high OCT1 mRNA groups. In contrast to the findings with OCT-1 Activity there was no significant effect of dose on either low or high OCT-1 mRNA groups.

	% of patients failing to achieve		
	1 log reduction in BCR-ABL by 6 months	2 log reduction in BCR-ABL by 12 months	3 log reduction in BCR-ABL by 18 months
	Patients receiving ≥ 600mg ADD		
low OCT-1 mRNA (n=14)	7%	21%	43%
high OCT-1 mRNA (n=17)	6%	12%	35%
p value	0.662	0.824	0.726
	Patients receiving < 600mg ADD		
low OCT-1 mRNA (n=15)	13%	13%	50%
high OCT-1 mRNA (n=15)	0%	14%	29%
p value	0.069	0.23	0.444

Table 8.4 Percentage of patients failing to achieve previously defined (Baccarani *et al.* 2006) sub-optimal response criteria, based on the level of OCT-1 mRNA.

In contrast to the findings with OCT-1 Activity there is no significant difference in the frequency of sub-optimal response based on OCT-1 mRNA levels, regardless of dose.

8.4.9 BCR-ABL kinase domain mutations and OCT-1 Activity

Five patients within our cohort of TIDEL patients developed BCR-ABL kinase domain mutations. 4/5 patients had low OCT-1 Activity. 3 were removed from study due to disease progression prior to 12 months, the other being removed at 21 months. The one patient with high OCT-1 Activity who developed a mutation remained on study for 30 months. Of the 5 patients 3 had mutations within the P-loop (ATP binding domain of ABL), and 1 patient had multiple mutations.

Given that 4 of these 5 patients had low OCT-1 Activity the primary analysis of the predictive value of OCT-1 Activity in molecular response was repeated after removing these patients from the analysis. This was to exclude the possibility that poor response in the low OCT-1 group was due to a higher risk of acquired resistance, rather than an inferior primary response to imatinib. There remained a significant difference between low (mean log-reduction in BCR-ABL of 3.0; n=25) and high (mean log-reduction in BCR-ABL of 3.9; n=27) OCT-1 Activity groups in this modified dataset (*p=0.02* at 24 months). In addition, there remained a significant dose related difference within the low OCT-1 Activity cohort (<600mg log reduction 2.5; >600 log reduction 3.4; *p=0.019*).

8.4.10 Patients with negligible OCT-1 Activity

There were 5 patients who, in replicate assays demonstrated IUR values without Prazosin equal or lower than the values with Prazosin (negligible;0ng/200,000 cells) OCT-1 Activity) all failed to achieve MMR by both 18 and 24 months. 3/5 were intolerant of 600mg imatinib, and all patients could not dose escalate at 12 months. 2/5 patients had ABL kinase domain mutations (detected at 3 and 9 months respectively). 3/5 patients had low OCT-1 mRNA levels, 2 of these were the patients with BCR-ABL kinase mutations.

8.5 Summary

As discussed previously, the IUR of imatinib correlates with the $IC50^{imatinib}$, and inhibition of OCT-1 with Prazosin removes the difference in IUR between the high and low $IC50^{imatinib}$ cohorts. This current study sought to examine the functional activity of the OCT-1 protein, and explore the relationship between the amount of imatinib actively transported by OCT-1 and the molecular response of patients enrolled to the TIDEL trial. The rationale for this approach was that OCT-1, like most drug transporters is a complex protein, and while mRNA levels may be important (Thomas *et al.* 2004) (Wang *et al.* 2007) post-transcriptional regulation, protein level and membrane localisation (Koepsell *et al.* 2003) (Cetinkaya *et al.* 2003) (Ciarimboli *et al.* 2005) (Hayer, Bonisch & Bruss 1999) are likely key determinants of functional activity. The combined effects of these variables can be most sensitively examined in an assay which provides a functional readout of activity.

In this current study, patients with a low $IC50^{imatinib}$ were shown to have significantly greater OCT-1 Activity than patients with high $IC50^{imatinib}$. Comparing the OCT-1 Activity with molecular response in all patients enrolled to the TIDEL trial revealed that patients with high OCT-1 Activity achieve significantly greater molecular responses over 24 months of imatinib treatment than patients with low OCT-1 Activity. While the specified dose was 600mg in this patient cohort, because of tolerability issues not all patients averaged 600mg over the first 12 months. Separating patients into those averaging 600mg, and those failing to do so revealed patients with a high OCT-1 Activity achieved good molecular responses regardless of dose. In contrast, the molecular response of those patients with low OCT-1 Activity was highly dose dependent, with patients receiving 600mg achieving significantly better molecular response by 24 months. These findings also held true when patients with BCR-ABL kinase domain mutations who ended study early were removed from analysis. This suggests that the OCT-1 Activity is important in determining primary response to imatinib, and that the significant difference observed between the two groups stratified on the basis of OCT-1 Activity is not attributable to a small cohort of patients with secondary resistance. Furthermore, analysis of average daily dose received in the low and high OCT-1 Activity cohorts reveals no significant difference. This indicates that the significant difference in molecular response observed between groups is not simply a result of dose variation.

The TIDEL trial mandated dose increases, if patients failed to reach various molecular milestones. In this cohort, 29 patients increased dose to 800mg per day by 14 months, as a result of failure to achieve a 4 log reduction in BCR-ABL by 12 months. Assessing the effect of dose increase at 12

months and beyond revealed no significant effect in the high OCT-1 Activity group. In contrast, patients with low OCT-1 Activity who dose increased, achieved comparable molecular responses from 12 to 24 months as those in the high OCT-1 Activity group, and this response was significantly superior when compared to response in those patients who failed to dose increase. These data indicate that dose is a critical determinant of long term molecular response in the low OCT-1 Activity cohort, but patients with a high OCT-1 Activity generally perform well regardless of dose.

Importantly, a group of patients with low OCT-1 Activity who are at higher risk for suboptimal or failed imatinib response if they do not receive the trial dose of 600mg per day, have been identified. The data presented here clearly demonstrates that dose is an important factor for overcoming suboptimal response in low OCT-1 Activity patients. These findings indicate a potential role for increased imatinib dose up front, in those patients found to have low OCT-1 Activity prior to imatinib start. Intolerance to higher dose imatinib in these patients may be a trigger to switch to an ABL kinase inhibitor that is not dependent on OCT-1 for its uptake into cells. We have previously shown that nilotinib is not dependent on OCT-1 for its intracellular uptake (White *et al.* 2005a).

One possible determinant of OCT-1 Activity is the level of OCT-1 mRNA present. Using RQ-PCR a correlation was demonstrated between the level of OCT-1 mRNA and OCT-1 Activity, and there was a significant difference in the level of mRNA between the low and high OCT-1 Activity cohorts. Despite this correlation, it is of interest that the level of OCT-1 mRNA is not itself a strong predictor of molecular response in this de-novo cohort, and the same sensitivity to dose is not demonstrable in the low OCT-1 mRNA cohort. Furthermore, the level of OCT-1 mRNA did not identify a group of patients at risk for sub-optimal response or primary imatinib failure. These data suggest that while there is a correlation between OCT-1 Activity and OCT-1 mRNA, the latter is not sufficient, as a single factor, to account for the observed differences between the two OCT-1 Activity groups. Other studies have demonstrated a significant link between OCT-1 mRNA levels and imatinib response (Wang *et al.* 2007) (Crossman. L *et al.* 2005). In the study by Crossman (Crossman. L *et al.* 2005), a significant difference was observed between mRNA levels in cytogenetic responders (CyR) and non-responders (CyNR). This current study differs from that of Crossman et al in that their study patients were assessed after an extensive period of prior therapy, whereas the TIDEL patient cohort was comprised of previously untreated, de-novo patients. Furthermore, in this current study 86%(49) of patients achieved CCyR by 12 months of therapy compared to 50%(15) in the Crossman cohort (Crossman. L *et al.* 2005).

In the recent paper of Wang at al (Wang *et al.* 2007) 70 patients were examined, but only 16 of these patients were previously untreated. High baseline OCT-1 mRNA was found to be a predictor for superior progression free and overall survival at 5 years. While this study suggests that OCT-1 expression studies are of prognostic value in late chronic phase patients, this may not necessarily apply to de-novo patients. Many of the patients studied in the Wang paper had developed resistance to interferon or had disease progression. These variables might impact on OCT-1 mRNA expression.

In this current study five patients developed BCR-ABL kinase domain mutations. Four of these patients had low OCT-1 Activity and were removed from study prematurely, in comparison to the one patient with high OCT-1 Activity who remained on drug for 30 months. While it is tempting to speculate about a link between OCT-1 Activity and Abl kinase domain mutation development, particularly in the setting of dose sensitivity and superior molecular responses, the current data set remains too small.

Recent studies have suggested that the trough plasma level of imatinib may be a predictor of cytogenetic and molecular response (Picard *et al.* 2007). However the critical determinant of the efficacy of imatinib therapy is the degree of kinase inhibition achieved, which is in turn determined by the intracellular concentration, rather than the plasma concentration, of imatinib. The intracellular concentration of imatinib is determined by the plasma concentration as well as the efficiency of uptake of the drug into the leukemic cells. The OCT-1 Activity assay directly measures the efficiency of imatinib uptake. Whether a particular plasma imatinib level represents a satisfactory level or not may depend on the OCT-1 Activity in that particular patient. Thus a combination of plasma imatinib level and OCT-1 Activity may allow individualised optimization of imatinib dose.

It is interesting to note that even under “optimal” circumstances – patients who averaged 600 mg of imatinib and had high OCT-1 Activity; there were still approximately 20% who failed to achieve MMR by 18 months. We speculate that there are likely to be other causes for suboptimal response in these cases, perhaps not related to the adequacy of BCR-ABL kinase inhibition. However, we have demonstrated that lower than average doses of imatinib in patients with low OCT-1 Activity account for the majority of cases of suboptimal response in this patient series.

8.6 Overall Comment and significance of this work

In this Chapter the differential function of the OCT-1 protein has been explored and found to be a significant determinant of molecular response in chronic phase CML patients treated with imatinib. Significantly, the combined effect of low dose and low OCT-1 Activity accounts for the majority of suboptimal responses observed. Importantly, using this functional assay to determine OCT-1 Activity at the time of diagnosis may identify patients likely to respond well to standard dose imatinib, and those who would be most likely to benefit from a higher dose of imatinib.

Chapter 9

Summary

Chronic Myeloid Leukaemia (CML) is a myeloproliferative disorder, resulting from neoplastic transformation of the haemopoietic stem cell compartment (Fialkow, Jacobson & Papayannopoulou 1977). The cytogenetic hallmark of the disease is the Philadelphia chromosome, which results from a balanced translocation between chromosomes 9 and 22 (t(9;22)(q34;q11) (Nowell and Hungerford 1960) (Rowley 1973) (Caspersson *et al.* 1970). This translocation results in the juxtaposition of the c-abl oncogene on chromosome 9, with the Major breakpoint region (M-bcr) on chromosome 22 (Groffen, Heisterkamp & Stam 1986) (Groffen *et al.* 1984) (Heisterkamp *et al.* 1982) (de Klein *et al.* 1982). The resultant BCR-ABL fusion gene translates to either a 210kD or 190kD protein (Shtivelman *et al.* 1985), with constituitively active tyrosine kinase activity, which results in phosphorylation on tyrosine residues, and aberrant activation of downstream effector pathways (Daley & Baltimore 1988) (Daley *et al.* 1990). The downstream effects of BCR-ABL, which result in uncontrolled proliferation, decreased propensity to programmed cell death, and genomic instability, have been detailed in Chapter One. Confirmation that BCR-ABL is causative in the pathogenesis of CML has been gleaned from various mouse models(Daley *et al.* 1990; Laneuville *et al.* 1992) (Daley 1993) (Daley *et al.* 1991; Heisterkamp *et al.* 1990).

The natural history of the disease has been transformed in recent years by the introduction of Glivec™ (imatinib mesylate), which has rapidly been embraced as the new treatment paradigm for chronic phase CML. The results of Phase I, II and III clinical trials (Kantarjian *et al.* 2002a; Sawyers *et al.* 2002; Talpaz *et al.* 2002). are reported in Chapter One, and demonstrate that 86% of patients enrolled to the Phase III IRIS trial achieve a complete cytogenetic response by 5 years (Simonsson & On Behalf of the 2005) (Druker *et al.* 2006). Despite this remarkable success, there remains a group of patients, comprising approximately 15% of patients treated in chronic phase, in whom imatinib is ineffective (primary resistance), and a second group who respond initially, but then lose response (secondary resistance). The emergence of heterogeneous mutations within the ABL kinase domain are most frequently implicated in the occurrence of secondary resistance (Gorre *et al.* 2001).(Branford *et al.* 2002; Branford *et al.* 2003b; Hochhaus *et al.* 2002; Kreill *et al.* 2003; Shah *et al.* 2002). In addition, duplication of BCR-ABL and additional chromosomal abnormalities have also been implicated(Gorre *et al.* 2001; Hochhaus *et al.* 2002).

However, the underlying cause of primary imatinib resistance is less well defined. Imatinib targets the ATP binding pocket of the BCR-ABL oncoprotein. Since BCR-ABL is known to be causative of CML, and the primary genomic abnormality in patients diagnosed in chronic phase, it is somewhat of a

conundrum that a variability of responses between patients is observed, in CP CML patients. This variability of response can range from those patients who demonstrate delayed response, to those with no significant evidence of efficacy. It was this clinical observation that led to the investigations into the intrinsic sensitivity of patients to imatinib induced kinase inhibition, as detailed in Chapter Two. In this Chapter utilizing the phosphorylation of the adaptor protein Crkl, an immediate downstream partner of BCR-ABL, it was demonstrated that there was a large degree of interpatient variation in the degree of imatinib induced kinase inhibition achieved in-vitro. This study was performed on newly diagnosed, chronic phase CML patients, prior to the initiation of treatment with 600mg per day imatinib, in the TIDEL trial. Investigations revealed that patients with good intrinsic sensitivity to imatinib induced kinase inhibition (low $IC50^{imatinib}$) achieved superior molecular responses following 12 months of imatinib therapy, than those patients with poor intrinsic sensitivity (high $IC50^{imatinib}$). This was the first report to identify that the degree of kinase inhibition achieved in-vitro, prior to imatinib start, was an important predictor of subsequent molecular outcome. Furthermore, this study demonstrated that the degree of kinase inhibition achieved in most patients is partial. Importantly, a group of patients predisposed to subsequent sub-optimal response, could be delineated prior to imatinib start with the IC50imatinib assay. These findings were made in the context of a clinical trial where 600mg per day was mandated. Subsequent validation of this finding in standard (400mg per day) or high dose (800mg per day) imatinib may lead to the utilization of the IC50imatinib assay as a new prognostic test for CML patients prior to imatinib therapy. The validity of this test with the second generation kinase inhibitors, nilotinib and dasatinib, remains to be tested in future clinical trials.

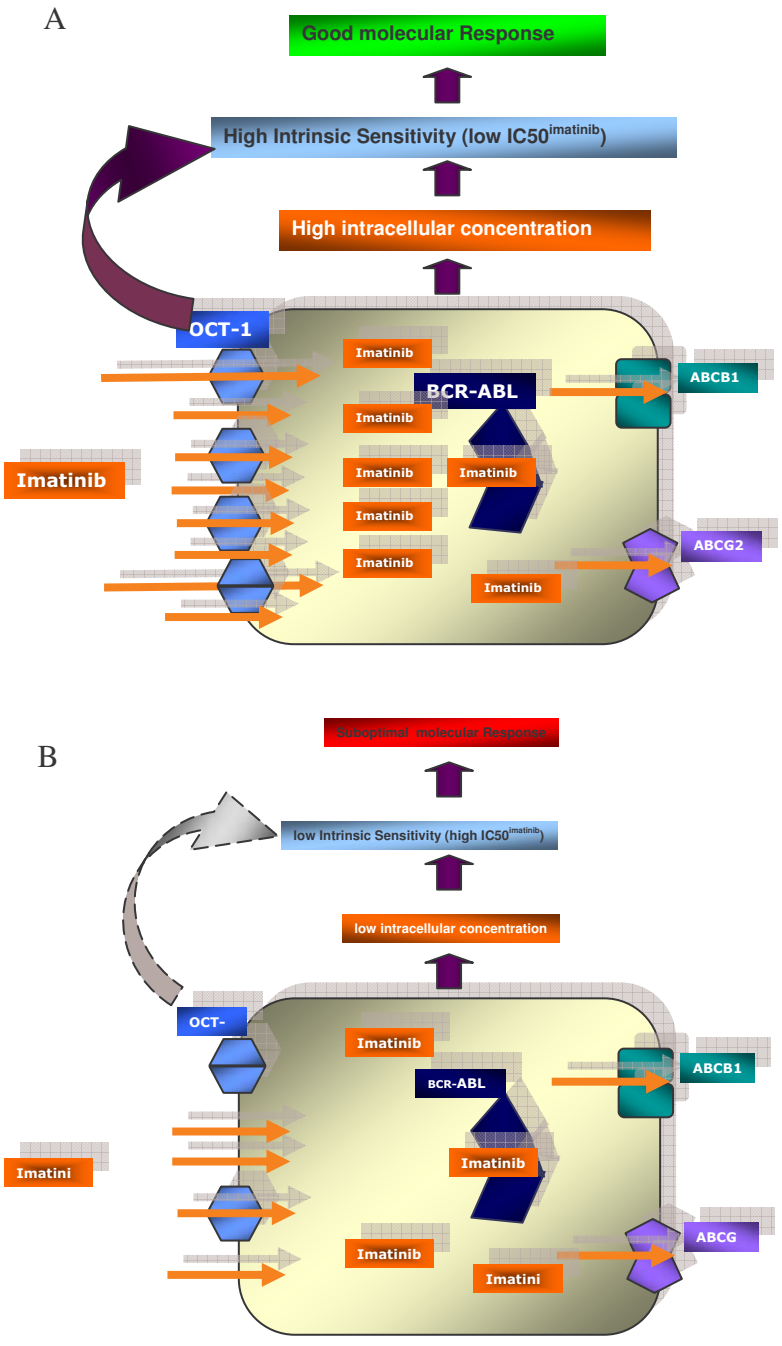
Since imatinib targets BCR-ABL, a logical approach to address the efficacy of the drug in patients, is to assess the degree of BCR-ABL kinase inhibition which occurs in the early imatinib treatment phase. In Chapter Three, the degree of in-vivo kinase inhibition achieved in peripheral blood cells over the first 28 days was assessed, using inhibition of p-Crkl as a marker of imatinib induced kinase inhibition. In this study, analogous to the findings in Chapter Two, a wide variation in the degree of kinase inhibition achieved in-vivo, was observed between patients. Patients who achieve 50% kinase inhibition by day 28 achieve significantly superior molecular responses over the first 2 years of imatinib therapy, than those patients who failed to do so.

Figure 9.1. Intracellular concentration, OCT-1 Activity and their effect on the intrinsic sensitivity to imatinib induced kinase inhibition.

Panel A demonstrates the scenario of good molecular response arising from high intracellular imatinib concentration, mediated through good OCT-1 Activity, culminating in high in-vitro sensitivity.

Panel B demonstrates the converse effect. Low intracellular drug concentration, mediated through poor OCT-1 Activity , resulting in a high $IC50^{imatinib}$ and sub optimal molecular response.

While this diagram implies that the reduced OCT-1 Activity may be a result of quantitative differences in the OCT-1 protein only, equally this scenario may also arise if the functional activity of the OCT-1 protein is reduced, irrespective of the amount of protein present.


A
Good molecular Response
High Intrinsic Sensitivity (low IC50imatinib)
High intracellular concentration
OCT-1
Imatinib
BCR-ABL
ABCB1
ABCG2
Imatinib
B
Suboptimal molecular Response
low Intrinsic Sensitivity (high IC50imatinib)
low intracellular concentration
OCT-
Imatinib
BCR-ABL
ABCB1
ABCG
Imatini

Indeed, 100% of patients who achieved 50% kinase inhibition achieved a major molecular response. Furthermore, those patients who failed to achieve 50% kinase inhibition were more likely to exhibit primary imatinib failure or suboptimal response.

The level of in-vivo kinase inhibition achieved results from a summation of the effects of intrinsic sensitivity ($IC50^{imatinib}$), actual dose received and pharmacokinetic factors, such a GI absorption and hepatic metabolism. The findings in this Chapter suggest that the degree of kinase inhibition achieved is the main determinant of the extent of molecular response achieved with imatinib therapy, in this cohort of patients. Importantly, failure to achieve MMR is due predominantly to inadequate kinase inhibition. Further to the findings in Chapter Two, the results here are consistent with the concept that BCR-ABL kinase inhibition remains partial in most patients, and that the optimal level of kinase inhibition is not often achieved on imatinib monotherapy.

Intrinsic sensitivity ($IC50^{imatinib}$) of patients to imatinib induced kinase inhibition was demonstrated in-vitro (Chapter Two), and the predictive power of the $IC50^{imatinib}$ detailed. The contribution intrinsic sensitivity makes to in-vivo kinase inhibition has also been confirmed (Chapter Three). The question as to the underlying cause of this intrinsic sensitivity remained however to be answered. In Chapter 4 utilising radiolabelled imatinib [^{14}C], it was demonstrated that the amount of imatinib which entered and was retained by the cell over a two hour period (the same period of time used for the $IC50^{imatinib}$ determination) correlated strongly with the $IC50^{imatinib}$. Furthermore, the human organic cation transporter one (OCT-1) was verified as the major active facilitator of imatinib entry into target CML cells. While efflux pumps ABCB1 and ABCG2 interact with imatinib, their contribution to the intracellular concentration of imatinib, in the newly diagnosed CML setting would appear minimal in most cases.

OCT-1, like most drug transporters, is a complex protein whose functional expression is a result of the level of mRNA(Wang *et al.* 2007) (Crossman. L *et al.* 2005)., the degree of post transcriptional regulation mediated, the actual protein level and the efficacy of membrane localisation (Wang *et al.* 2007) (Crossman. L *et al.* 2005). (Koepsell *et al.* 2003) (Cetinkaya *et al.* 2003) (Ciarimboli *et al.* 2005) (Hayer *et al.* 1999). Previous studies have demonstrated that the level of OCT-1 mRNA is predictive of cytogenetic outcome in imatinib treated CML patients(Crossman. L *et al.* 2005; Wang *et al.* 2007). In Chapter Eight, the levels of OCT-1 mRNA, while correlating with the intracellular concentration of

imatinib achieved, was a poor predictor of molecular response. This discrepancy likely arises from the differing patient cohorts examined, as discussed in detail in this Chapter.

In this Chapter a functional assay of the OCT-1 protein was developed. Applying this assay to patients enrolled to the TIDEL and TOPS trials, demonstrated that there was a strong link between the OCT-1 Activity and the $IC50^{imatinib}$, with patients with low $IC50^{imatinib}$ demonstrating a significantly higher OCT-1 Activity, than patients with high $IC50^{imatinib}$. Furthermore the effect of OCT-1 Activity was closely linked to the actual dose of imatinib received in patients with low OCT-1 Activity. Indeed, patients with low OCT-1 Activity who received lower than average dosing over the first 12 months of therapy, account for the majority of cases of suboptimal response observed in this patient series. In contrast, the majority of patients with high OCT-1 Activity achieved good molecular outcome regardless of dose received (Figure 9.1). The failure of 15-20% of patients in this group to achieve MMR by 18 months, could possibly be a result of other factors, not related to the adequacy of BCR-ABL kinase inhibition.

Nilotinib, a second generation ABL kinase inhibitor, is up to 20 times more potent than imatinib in in-vitro autophosphorylation experiments(Manley *et al.* 2004; Weisberg *et al.* 2005). The development of nilotinib arose from the rational assessment of the crystallographic structure of the molecular interaction between imatinib and the ABL kinase domain. As discussed in Chapter 5, the $IC50^{nilotinib}$ was significantly lower than the $IC50^{imatinib}$, and despite the structural similarities between the two drugs, there was no correlation between the IC50's. Furthermore there was no correlation between the IUR for nilotinib and the $IC50^{nilotinib}$. In keeping with this finding, performance of drug uptake studies, in the presence of known inhibitors of OCT transporters, indicate that nilotinib, unlike imatinib, is not transported by the OCT-1 protein. These results suggest that selection of nilotinib as front line therapy, based on an in-vitro assessment of poor OCT-1 mediated imatinib uptake, may provide a more rational approach for the selection of the most appropriate tyrosine kinase inhibitor in the future.

Despite their different uptake mechanisms both imatinib and nilotinib, previous studies(Dai *et al.* 2003; Gurney *et al.* 2007; Hegedus *et al.* 2002; Illmer *et al.* 2004; Mahon *et al.* 2003; Ozvegy-Laczka *et al.* 2004b; White *et al.* 2006) (Houghton *et al.* 2004) (Burger *et al.* 2004) (Nakanishi *et al.* 2006), and those detailed in Chapters Four and Five, show that these drugs interact with the ABC transporters ABCB1 and ABCG2, to varying degrees. While the expression of these transporters is low in chronic phase CML patients prior to imatinib exposure, previous studies have demonstrated that imatinib exposure can result in drug induced upregulation of both ABCB1 and ABCG2 (Burger *et al.* 2005). In a

recent paper of Weisberg (Weisberg *et al.* 2007) additive and in some cases, synergistic effects were observed when imatinib was combined with nilotinib, in in-vitro and in-vivo models. In Chapter Six this finding was investigated further, and using radiolabelled imatinib and nilotinib, it was demonstrated that the additive effects are probably the result of an increase in the intracellular concentration of nilotinib, but not imatinib. Since this is an in-vitro assay, the most likely point of interaction is via transport proteins, as interactions at the level of metabolism will not be captured using this approach. The evidence presented here suggests that in the presence of nilotinib, imatinib acts as an inhibitor of ABCB1, or ABCG2 or both, facilitating intracellular accumulation of nilotinib observed. In keeping with the findings of Weisberg (Weisberg *et al.* 2007), this interaction was concentration dependent.

Other commonly used drug classes are known to be inhibitors of, or transported by, the ABC transporters ABCB1 and ABCG2. Proton pump inhibitors (PPI), such as pantoprazole and esomeprazole, have been reported to be both inhibitors and substrates for these transporters. Proton pump inhibitors are used widely in general medicine, but also commonly in imatinib treated patients, for control of gastric related side effects. In view of the interactions observed in drug combination studies presented in Chapter Six, the effect of PPI on the intracellular drug concentration of imatinib and nilotinib was investigated. As presented in Chapter 7, and in keeping with the findings of Chapter Six, inhibition of ABCB1 and/or ABCG2 by PPI resulted in a significant increase in the intracellular concentration of nilotinib. Significantly, this increase occurred at clinically relevant concentrations. This finding confirms the findings in the previous Chapters and suggests that nilotinib is a substrate for the ABC transporters analysed.

In contrast, the intracellular concentration of imatinib was significantly reduced in the presence of high concentrations of PPI. While this reduction was only statistically significant at high PPI concentrations, a decrease was also observed at loer, clinically relevant concentrations. Conceivably this observations could be the result of imatinib being effluxed in the presence of PPI in a concentration dependent manner. Alternatively, as the evidence presented for high dose PPI suggests, this class of drug may act to reduce the OCT-1 Activity. The exact mechanism for this has yet to be elucidated, primarily because of the absence of appropriate reagents. Speculatively, the interaction may be a direct one, with PPI interacting directly with the OCT-1 protein. Alternatively, PPI may interact with a downstream modulator or effector of the OCT-1 protein.

Chapters Six and Seven, indicate that imatinib and nilotinib display the ability to interact with other drugs, and with each other. The weight of evidence suggests that the majority of this interaction is not through direct drug:drug interactions but rather is mediated through the ABC efflux transporters ABCB1 and ABCG2 (Figure 9.2). However there is also some evidence that the OCT-1 Activity may be modulated in the presence of some concomitant medications, such as PPI. While the data presented here is related to imatinib:nilotinib combinations, and also to the interaction of PPI with both TKI's, the findings may be relevant to the many classes of drugs known to interact with ABCB1 and ABCG2 transporters.

Speculatively, increases in the intracellular concentration of nilotinib, in the presence of imatinib, or other ABC inhibitors, may result in off target toxicity, not identified by the plasma levels of the ABL kinase inhibitors. However, as demonstrated earlier in this thesis, the degree of kinase inhibition occurring in-vitro and in-vivo in imatinib treated patients, is a key determinant of subsequent molecular response. Therefore, the positive clinical effects of these findings may be a more uniform exposure to nilotinib by the blockade of variable effects of efflux transporters by PPI, which may in turn lead to more sustained kinase inhibition. Furthermore these findings may be potential exploited to achieve better drug penetration to areas such as CNS and testes.

Taken in its entirety, the data presented in this thesis has demonstrated that the degree of kinase inhibition mediated through exposure to imatinib, both in-vitro and in-vivo, is a significant predictor of subsequent molecular response, in previously untreated, chronic phase CML patients. A major determinant of this intrinsic sensitivity is the amount of imatinib which is accumulated within the target leukaemic cell, and this is controlled by the functional activity of the human organic cation transporter one (OCT-1). Furthermore, evidence is presented to demonstrate that imatinib interacts with the ABC efflux transporters ABCB1 and ABCG2, but that the influence of these transporters is not significant in the majority of CP CML patients. In contrast, to imatinib, intrinsic sensitivity to the second generation kinase inhibitor, nilotinib, does not appear related to the intracellular concentration of the drug achieved. Furthermore, nilotinib is not dependent on OCT-1 for uptake into haematopoietic cells. Like imatinib, nilotinib also interacts with ABCB1 and ABCG2. Strikingly, and in contrast to the findings with imatinib, modulation of these proteins using known inhibitors, results in significantly increased nilotinib intracellular accumulation, which translates to increased in-vitro sensitivity. The impact of these finding with nilotinib is yet to be tested in the clinical setting, in CP CML patients.

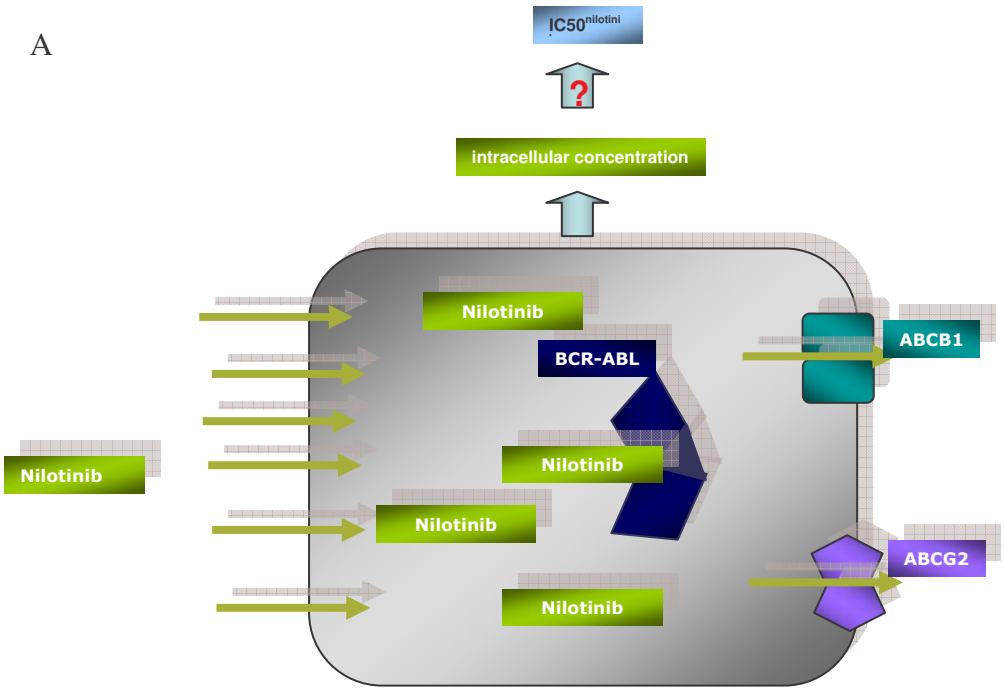
In conclusion the findings with imatinib, when substantiated in standard and high dose settings, may allow for the rational selection of the most appropriate front line kinase inhibitor, based on intrinsic sensitivity and OCT-1 Activity, for all CP CML patients. In the clinical setting, exploitation of the findings described with nilotinib may lead to enhanced drug delivery, and better drug penetration, into previously problematic areas such as the CNS, where ABC transporters provide a protective and resistant barrier to efficient exogenous drug delivery.

Figure 9.2 Nilotinib transport mechanism.

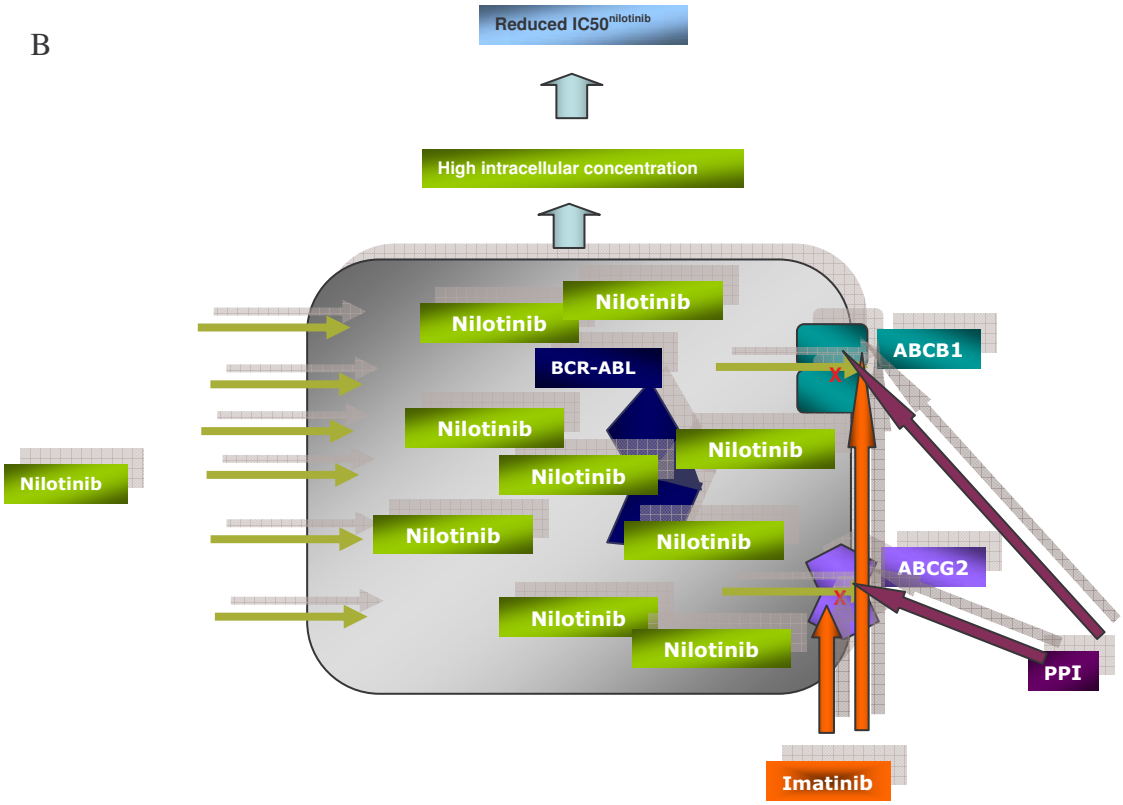
As depicted in panel A, the $IC50^{nilotinib}$ appears to not be related to the intracellular concentration of nilotinib achieved. The uptake of the drug based on the evidence in this thesis is not OCT-1 mediated, and appears largely to be passive. Nilotinib, like imatinib interacts with both ABCB1 and ABCG2.

In Panel B, the intracellular concentration of nilotinib is increased significantly in the presence of ABCB1 and ABCG2 inhibitors such as proton pump inhibitors (PPI) and imatinib. This results in an overall decrease in the $IC50^{nilotinib}$.

A


IC50nilotini
?
intracellular concentration
Nilotinib
BCR-ABL
ABCB1
Nilotinib
Nilotinib
Nilotinib
ABCG2
Nilotinib

B


Reduced IC50nilotinib
High intracellular concentration
Nilotinib
Nilotinib
Nilotinib
BCR-ABL
ABCB1
Nilotinib
Nilotinib
Nilotinib
Nilotinib
Nilotinib
Nilotinib
ABCG2
Nilotinib
Nilotinib
PPI
Imatinib

Appendix I:

Materials and Methods

Table I.1 Suppliers of commonly used reagents

Reagent	Company/ Supplier	Catalogue number
0.1 M DTT	Initrogen	18064-014
Acrylamide Solution 40% bis-acrylamide, 37.5:1 ratio	Bio Rad	161-0148
Alkaline-phosphatase-conjugated anti-rabbit immunoglobulin	SantaCruz	SC2007
Anti-Crkl antibody C20Ab	Santa Cruz	SC-319
Chloroform	Merck	100776B
DEPC water	MP Biomedicals inc.	821739
dNTP set (N = A, C, G, T)	Amersham	27-2035-02
ECF substrate *Attaphos*	Amersham Biosciences	RPN5785
Ethanol	Merck	6-10107-2511
Foetal Bovine Serum (FBS)	JRH Biosciences	12103-500ml
Glycogen	Roche	901393
Hanks Balanced Salt Solution (HBSS) Ca^{++} and Mg^{++} free.	SAFC Biosciences USA	#55021C-500ML
HEPES 1M	SAFC Biosciences	59205C-100ml
Isopropanol	Merck	6-10224.2511
L Glutamine 200mM	SAFC Biosciences	59202C-100ml
Lymphoprep	Axis Shield. Oslo Norway	# 1114547
Membrane blocking agent	Amersham Biosciences	RPN 2125V
MICROSCINT-20 scintillation fluid	PerkinElmer	#6013621
Monoclonal antibody for Flow Anti-human Bcrp1/ABCG2-PE	R&D Systems	# FAB995P
Monoclonal antibody for Flow P-glycoprotein (MDR-1) PE	Immunotech : France	2370
Negative Control Antibody for Flow, Mouse IgG1 FITC-conjugated	Dako Cytomation Denmark	x0927
Negative Control Antibody for Flow, Mouse IgG1 RPE-conjugated	Dako Cytomation Denmark	# x0928
Negative Control Antibody for Flow,	Dako Cytomation Denmark	X0951

Mouse IgG2b RPE-conjugated		
Penicillin 5000U/ml Streptomycin 5000µg/ml	Sigma Biosciences	P4458
Prazosin (Prazosin Hydrochloride, MW=419.9)	Sigma	P7791-(250MG)
Procainamide (Procainamide Hydrochloride, MW=271.79)	Sigma	P9391-100MG)
Progesterone	Sigma	P-6149
Protein Markers Benchmark	Invitrogen	10748-010
PVDF Membrane	Amersham Biosciences	PRN 303F
Random Hexamer Primer (100µg stock)	Geneworks	RP-6
RPMI Medium w/o L-Glutamine. Ca^{++} and Mg^{++} free.	SAFC Biosciences USA	51502C-500ML
See Blue Plus 2 Standard	Invitrogen	LC5925
SuperScript II Reverse Transcriptase	Invitrogen	18064-014
SYBR Green Supermix (BIO-RAD)	Bio Rad	PA-012-24
Trizol reagent	Invitrogen	10296-028
Trypan Blue Solution	Sigma	T8154

I.2 Commonly used Reagents

1.2.1 Cell culture media

For all cell lines used in this study

RPMI 1640 Medium	500 ml
To this the following was added	
1% L-Glutamine(200mM)	5ml
Pen 5000 U/mL / Streptomycin Sulphate 5000 µg/mL	5 ml
10% Foetal Bovine Serum (FBS)	50 ml

Store at 4°C, and preheat to 37°C in a waterbath prior to use.

1.2.2 Imatinib mesylate, MW = 589.72

Imatinib mesylate (imatinib; Glivec; formerly STI-571) was provided by Novartis (Switzerland). Stock solutions of this compound were prepared at 10 mM with distilled water, sterile filtered and stored at -70ºC.

1.2.3 Nilotinib, MW = 529.5

Nilotinib (formerly AMN107) was provided by Novartis (Switzerland). Stock solutions were prepared at 10mM in DMSO and stored at 4ºC.

1.2.4 50% Mixture of hot and cold imatinib, 100 µM – for 1 mL :

14C-Imatinib,1695.72 µM	29.5 µl
10mM imatinib	5 µl
RPMI medium	966 µl

1.2.5 50% Mixture of hot and cold nilotinib, 10 µM – for 1 mL :

14C-Nilotinib, 1882 µM	2.66 µl
1mM nilotinib	5 µl
RPMI medium	992.3 µl

1.2.6 Flow Cytometry Fixative (FACS Fixative)

PBS 500mL

40% w/v Formaldehyde 5.0mL

D-glucose 10.0g

NaN_3 0.1g

The formaldehyde, azide and glucose were added to the PBS, and the solution stored indefinitely at 4°C.

I.2.7 Freeze Mix

70% Hanks BSS

20% FBS

10% DMSO

This is made up fresh for each batch of samples to be cryopreserved.

1.2.8 Thaw Solution

Hanks BSS	500mls
To this add	
FBS	25 mls (ie 5% FBS)
ACD	25mls (5%)
HEPES 1M	5mls

The solution was stored at 4°C, and heated to 37°C in a water bath prior to use.

1.2.9 White Cell Fluid

Acetic acid 2.0mL

Milli-Q® Water 98mL

Methyl Violet Few crystals

Acetic acid was added slowly to Milli-Q® water. The methyl violet (Gurr®, BDH) was then added, and the solution mixed well to ensure that the crystals completely dissolved. The solution was filtered using a 0.2µm bottle top filter, and stored indefinitely at room temperature.

1.2.10 Hanks Balanced Salt Solution (HBSS)

Ca^{++} and Mg^{++} free.

Add 0.05mM Hepes prior to use.

JRH Biosciences USA Cat #55021-500mls

1.2.11 Laemmli's Buffer

50 mmol/L Tris-HCl, pH 6.8,

10% glycerol

2% SDS

5% β-mercaptoethanol

0.1% bromophenol blue

1 mM NaVanadate

10 mM NaFluoride

1.2.12 1 x TBS

20 mmol/L Tris-HCl, pH 7.5

150 mmol/L NaCl

1.2.13 1 x TBST

20 mmol/L Tris, pH 7.5

150 mmol/L NaCl

0.1% Tween20

1.2.14 dNTP set (N = A, C, G, T)

25mM stock = 40µl of each dNTP

Working stock: 5mM = 20 µl of 25mM stock in 80 µl DEPC water

1.2.15 Random Hexamer Primer (100µg stock)

Working stock: 250ng/µl = 100µg in 0.4 ml DEPC water

1.2.16 Prazosin - inhibits OCT-1

Used at 100 µM from 10mM stock

10mM stock = 4.2 mg / mL methanol

1.2.17 PSC833 – inhibits ABCB1/MDR

(kindly provided by Novartis)

Used at 10 µM from 8.23mM stock

8.23mM stock = 10 mg/mL = 10mg / 500 µl 9:1 Ethanol:Tween20, + 500 µl water

1.2.18 Procainamide – inhibits OCT-1 and OCT-2

Used at 100µM from 1M stock

1M stock = 271.79 mg / mL water

1.2.19 Ko143 – inhibits ABCG2/BCRP1

(kindly provided by Dr John Allen)

Used at 0.5 µM from 1mM stock in sterile water

I.3 General techniques.

I.3.1 Lymphoprep isolation of mononuclear cells

40-60 mls of Peripheral blood from patients was CML, was collected into Lithium Heparin tubes. All blood was collected with informed consent in accordance with the Declaration of Helsinki. A white cell count was performed using white cell fluid and a maximum of 10^8 cells were placed into a 50mL polypropylene conical tube (Falcon), (maximum of 25 mls of blood). The blood volume was brought to 35mls using HBSS and underlaid with 15ml of lymphoprep . Tubes were then centrifuged at 1400rpm (400g) for 30 mins. The interface containing the mononuclear cells was then transferred to another 50mls centrifuge tube and cells were washed once in HBSS. Cells were then counted using white cell fluid.

I.3.2 Cryopreservation of cells

Cells were cryopreserved in a solution of 70% Hanks BSS, 20% FBS and 10% cryoprotectant, dimethylsulphoxide (10% (v/v) final concentration) (DMSO)[Freeze Mix]. Cells were pelletted at the desired concentration and Freeze Mix was added to the cells drop-wise with constant mixing. The cell suspension was transferred quickly to cryoampoules (Nalgene®) and the samples stored in liquid nitrogen (-196°C). The final concentration of cells was a maximum of $2.0x10^7$/mL.

I.3.3 Thawing of cells

Cells were removed from liquid nitrogen and thawed rapidly in a 37°C water bath. The thawed cell suspension was transferred quickly to a 50mL polypropylene conical tube (Falcon), and approximately 15mL of thaw solution (warmed to 37°C) was added dropwise with constant mixing. The sample volume was then increased to 30mL with thaw solution and the cells pelleted by centrifugation at 420g for 5 minutes in a Heraeus multifuge 3S-R bench top centrifuge. The supernatant was aspirated and the cells washed a further twice with HHF to remove any residual DMSO.

I.3.4 Cell counts and viability

Cell concentration was determined by diluting the cell suspension in white cell fluid (WCF), while cell viability was assessed by diluting samples with 0.4% trypan blue solution. Ten microlitres (10µL) of these suspensions were transferred to a haemocytometer counting chamber (Neubauer Improved, Assistant, Germany) and cell concentration and viability calculated accordingly.

1.3.5 Maintenance of cell lines

All appropriate tissue culture techniques were performed in a Class two "biohazard" laminar flow hood (Gelman Sciences).

Non-adherent cell lines were maintained at a cell density between $1.0x10^5$ and $1.0x10^6$/mL in $25cm^2$, $75cm^2$ or $150cm^2$ tissue culture flasks (Greiner). Media (RPMI + 10% FBS as per above *Commonly used reagents*) was prewarmed to 37°C prior to use. Cultures were incubated in a $37^oC/5\%CO_2$ incubator. Cell cultures were checked every second day for contamination, counted and replated at the above concentrations

I.4 Specialised Techniques

I.4.1 IC50 Assay (In-vitro kinase inhibition assay)

Preface notes:

Peripheral blood (PB) samples must be obtained prior to the commencement of Glivec therapy, or from patients in cytogenetic and molecular relapse. Mononuclear cells (MNCs) are harvested from PB by Lymphoprep density gradient centrifugation. Experiments must be performed on fresh cells, either on the day of isolation or after overnight storage at 4ºC in HBSS medium supplemented with 5% FBS (Foetal Bovine Serum).

$4x10^6$ MNCs were cultured at $2x10^5$ cells/ml in RPMI 1640 medium supplemented with 10% Foetal Bovine Serum (FBS) in T/C flasks for 2 hours (37ºC, 5% CO_2, 100% humidity) with a range of concentrations of imatinib (See Table I.2). In addition a no imatinib/nilotinib control was set up as a 100% p-Crkl measure. For this $4x10^6$ MNCs are incubated in the absence of imatinib/nilotinib

After 2 hours, the cells were washed once with cold PBS and lysed in Laemmli's buffer at 10^6 cells per 10µl buffer, by boiling in a 100ºC heat block for 12 minutes. Cell lysates were then clarified by microfugation for 5 minutes at 13000rpm and stored at -20ºC until gel is to be run. 20µl of protein lysate (corresponding to $2x10^6$ cells) is then resolved on a SDS 10% polyacrylamide gel. Protein markers, as listed in reagents, were loaded on either side of the gel (ie: two markers per gel).

When electrophoresis was complete then protein was electrophoretically transferred to PVDF membrane. The membrane was then blocked for 1 hour at room temperature with 2.5% membrane blocking agent (Amersham Biosciences) in 1xTBS. The membrane was then probed 2 hours at room temperature with anti-Crkl antibody (Santa Cruz) in 2.5% blocking solution. Following this, the membrane was rinsed twice in 1 x TBST buffer, and then washed for 3x5 min with 1xTBST buffer.

The membrane was then Incubated for 1 hour at room temperature with alkaline-phosphatase-conjugated anti-rabbit immunoglobulin (SantaCruz) in 2.5% blocking solution., then rinsed twice in 1x TBST buffer and washed 3x5 minutes in 1xTBST buffer and then 2x5 minutes in 1xTBS buffer.

Bound antibodies were detected with ECF substrate (AmershamBiosciences) by FluorImager analysis (Molecular Dynamics). Phosphorylated and non-phophorylated Crkl bands were then quantified using ImageQuant software and the % of phosphorylated Crkl (p-Crkl) determined as a % of the total Crkl.

The IC50 was the calculated by plotting a curve of %p-Crkl against the dose of imatinib after normalising the 0uM imatinib lane to 100%.

	µM inhibitor	[stock]	vol (20 mL)	DMSO
IMATINIB				
B1	0	---	---	100 µl
B2	0.25	1 mM	5 µl	100 µl
B3	0.5	1	10 µl	100 µl
B4	0.75	1	15 µl	100 µl
B5	1	1	20 µl	100 µl
B6	1.5	1	30 µl	100 µl
B7	3	10 mM	6 µl	100 µl
B8	10	10	20 µl	100 µl
B9	50	10	100 µl	100 µl
AMN107 (Nilotinib)				
C1	0	---	---	100 µl
C2	0.005	10 µM	10 µl	90
C3	0.01	10 µM	20 µl	80
C4*	0.025	100 µM	5 µl	95
C5	0.05	100 µM	10 µl	90
C6	0.1	100 µM	20 µl	80
C7	0.25	1 mM	5 µl	95
C8	0.5	1 mM	10 µl	90
C9	50	10 mM	100 µl	---

Table I.2 **Imatinib /AMN107(nilotinib) Titrations**
For 4 x 10^6 cells per flask

The coloured regions indicate the most common range of results.

I.4.2 In-vivo kinase inhibition assay.

Peripheral blood (PB) samples were collected at baseline (pre –imatinib therapy) then weekly intervals for the first 28 days. Mononuclear cells (MNCs) were harvested from PB by Lymphoprep density gradient centrifugation.

2 to 5 x 10^6 MNC were dispensed into a 1.5ml eppendorf tube, and pelleted by microfugation for 5 mins at 6500rpm and all media was removed..MNC were lysed in Laemmli's buffer at 10^6 cells per 10µl buffer, by boiling in a 100ºC heat block for 12 minutes. Cell lysates were clarified by microfugation for 5 minutes at 13000rpm and stored at -20ºC until the gel was run. 20µl of protein lysate (corresponding to 2x10^6 cells) was resolved on an SDS 10% polyacrylamide gel, and then protein was electrophoretically transferred to PVDF membrane.

The membrane was then blocked for 1 hour at room temperature with 2.5% membrane blocking agent (Amersham Biosciences) in 1xTBS. The membrane was then probed 2 hours at room temperature with anti-Crkl antibody (Santa Cruz) in 2.5% blocking solution. Following this, the membrane was rinsed twice in 1 x TBST buffer, and then washed for 3x5 min with 1xTBST buffer.

The membrane was then Incubated for 1 hour at room temperature with alkaline-phosphatase-conjugated anti-rabbit immunoglobulin (SantaCruz) in 2.5% blocking solution., then rinsed twice in 1x TBST buffer and washed 3x5 minutes in 1xTBST buffer and then 2x5 minutes in 1xTBS buffer.

Bound antibodies were detected with ECF substrate (AmershamBiosciences) by FluorImager analysis (Molecular Dynamics). Phosphorylated and non-phophorylated Crkl bands were then quantified using ImageQuant softwaren and the % of phosphorylated Crkl (p-Crkl) determined as a % of the total Crkl.

% of phosphorylated Crkl (p-Crkl) as a % of the total Crkl was then calculated for each timepoint

I.4.3 Imatinib/nilotinib IUR assay

All assay points were performed in triplicate. 200,000 thawed, viable MNC were exposed to 14-C labelled imatinib or nilotinib (50% mixture as listed in reagents), in the presence or absence of influx / efflux inhibitors as required. Total test volume: 2 mls with RPMI +10% FBS. Cells and reagents were incubated for 2 hours at 37ºC/5% CO_2 . Tubes were then centrifuged at 6800rpm for 5 mins, then pulse spun to 13000rpm, for 30 secs.

A 20 µl aliquot of supernatant (S/N) from each tube was then mixed with 100 µl Microscint-20 in 96-well flat bottomed plate. The plate was then covered with adhesive plastic cover-seal.
The remaining supernatant was then aspirated from the test tubes, so as not to disturb the cell pellet. Fifty µl of Microscint was then added to the cell pellet, to lyse cells . The tube was then vortexed well, and pulse spun for 15 secs at 13000rpm. The lysed cells twere then transferred o a second 96 well plate, and the plate covered with adhesive plastic seal. All plates were then counted on the Top Count scintillation counter.

The ng/200,000 cells of 14-C labeled imatinib/nilotinib per cell was then calculated using formulae below

Conc in ng/200,000 cells:-

$$\frac{\text{cpm cells} - \text{background}}{\{(\text{cpm cells} - \text{background}) + (\text{cpm supernatant} - \text{background})\}} \times \text{conc in ng of 14C-TKI added}$$

I.4.4 Immunophenotyping for ABCB1 and ABCG2 expression

A known number of cells were transferred to 5mL round bottom polystyrene tubes (Falcon) and suspended in a minimum volume (~200µL) of HBSS. Appropriate antibodies (see Table I3) were added to tubes at a concentration of 10µL/106 cells, unless otherwise stated, and incubated for a period of 45 minutes on ice in the dark. Cells were then washed twice prior to the addition of a secondary antibody conjugate, or prior to fixation in FACS fix. Fixed samples were stored at 4°C in the dark until analysis was performed. The fluorescence intensity of cell suspensions was examined using an Epics®-XL-MCL flow cytometer and CytomicsTM RXP Analysis Version 1.0 Software (Beckman Coulter). Control tubes stained with an isotype-matched control were included in all experiments, and were used to define the cut-off point for positive/negative staining. Flow cytometric analysis was performed using an Epics®-XL-MCL flow cytometer (Beckman Coulter). Cell populations were analysed on the basis of their forward and side light scattering properties (indicative of cell size and granularity respectively) and/or the fluorescence intensity of various fluorochromes.

Table I.3: Commonly used antibody volumes

Antibody	Antibody Vol.*
IgG1 FITC	5µl
IgG2b PE	5µl
ABCG2 PE	20µl
MDR-1 PE	20µl

* Antibody volumes apply to a x 10_5 cells for isotype control and 1 x 10^6 test cells

I.4.5 mRNA isolation

Target cells were lysed in Trizol reagent (1 ml per 5-10 x 10^6), and incubated at room temperature for 5 minutes. 0.2 ml of chloroform per 1 ml of Trizol (1/5 volume) was then added and tubes were then vigorously shaken for 15 seconds. Tubes were then incubated at temperature for 2-3 minutes. Following this the tubes were centriged at 12,000xg for 15 minutes at 4°C.

The aqueous phase (top layer) was then transferred to a a new Rnase/DNase free 1.5ml tube. RNA was then precipitated by the addition of 1 µl glycogen (20 µg) and isopropanol (0.5 ml per 1 ml of Trizol). The samples were then gently mixed, and incubated at room temperature for 10 minutes. RNA was pelleted by spin centrifuging at 12,000xg for 10 minutes at 4°C. Following removal of the supernatant, the RNA pellet was gently washed in 75% ethanol (1ml per tube) and tubes were centrifuged at 7,500xg for 5 minutes at 4°C. Supernatant was then removed and tubes again centrifuges briefly to remove any excess ethanol. The RNA pellet was briefly dried, then dissolved in 20µl DEPC water (depending on size of pellet , more or less DEPC water can be used) at 55°C for 10 minutes. Samples were immediately placed on ice and store at -70°C
RNA was quantitated using NanoDrop Spectrophotometer

I.4.6 cDNA synthesis

1µg of template RNA was added to 1µl of random hexamers (250ng/µl) and total volume taken to 11µl with DEPC water. The mixture was heated to 70°C for 10 minutes and chilled briefly at 4°C. The premix was prepared as per Table I.4

Reagent/concentration:	Volume:
5x First strand buffer	4 μl
0.1 M DTT	2 μl
5 mM dNTPs	2 μl
Superscript II	1 μl

Note above is sufficient for one test sample only.

Table I.4 First Strand Synthesis Reagent Mix

The premix is then added to the above tube and contents gently mixed, and placed Mastercycler. Incubation conditions are shown as follows

25°C for 10 minute

42°C for 50 minutes

70 for 10 minutes

Chill samples at 4°C

At the completion of this cycle, 20 μl DEPC water is then added to each tube, and the cDNA is stored at - 20°C

I.4.7 PCR for OCT-1 mRNA Expression

Prepare cDNA as per I.4.6.

Primers are listed in Table I.5

Place reagents in tubes following the schema (Table I.6). Prepare standard by adding l μl of RankL standard cDNA to RANKL tubes and 2 μl of unknown/ cell line control cDNA samples to allocated tubes. Lids were then added to all tubes, and they were placed into the 72 well rotor. (Rotor Gene) Profile settings on the rotor-gene are as follows (Table I.7)

Primer name	Sequence 5' to 3'
Genes of interest	
hOCT-1	CTG AGC TGT ACC CCA CAT TCG
hOCT-1 R	CCA ACA CCG CAA ACA AAA TGA
Standard	
hRANKL-D For	TCA GCC TTT TGC TCA TCT CAC TAT
hRANKL-G rev	CCA CCC CCG ATC ATG GT
House-keeping genes	
BCR r	CCT GCG ATG GCG TTC AC
BCR f	CCT TCG ACG TCA ATA ACA AGG AT

Table I.5 Primers are diluted to a working stock of 100 ng/μl in DEPC wate**r**

Reagent	x 1 (µl)
SYBR Green	5
Primer Forward	0.5
Primer Reverse	0.5
Water	2
Total:	8

Table I.6 Preparation of PCR Master mix.

Cycle	Cycle point
Hold @ 50°C, 2 min 0 secs	
Hold 2 @ 95°C, 15 min 0 secs	
Cycling (48 repeats)	Step 1 @ 95°C, hold 15 secs
	Step 2 @ 60°C, hold 26 secs
	Step 3 @ 72°C, hold 10 secs, acquiring to Cycling A([Sybr][1][5])
Hold 3 @ 72°C, 0min 30 secs	
Melt (72°C - 99°C, hold secs on the 1st step, hold 5 secs on next steps, Melt A ([Green][1][1])	

Table I.7 Profile Settings for Rotor Gene.

I.5 Computer Software Required.

Excel – Microsoft Office Suite - Essential

Image Quant (Molecular Dynamics) – Essential

Sigma STAT . SPSS Incorporated – Essential

I.6 Laboratory Equipment Required

Heat block – capable of maintaining 100ºC

Vertical Electrophoresis tank

Electrophoretic Transfer Tank

Power packs capable of running and transferring gels

Rocking Platform

Fluor Imager (Molecular Dynamics)

Biological Safety Cabinet with Vacuum Facility

Rotor-gene 3000 real time PCR thermal cycler (Corbett Research)

NanoDrop Spectrophotometer

Vortex mixer

Appendix II:

References

Appendix II- References

Aboudola S., Murugesan G., Szpurka H., Ramsingh G., Zhao X., Prescott N., Tubbs R.R., Maciejewski J.P. & Hsi E.D. (2007) Bone marrow phospho-STAT5 expression in non-CML chronic myeloproliferative disorders correlates with JAK2 V617F mutation and provides evidence of in vivo JAK2 activation. Am J Surg Pathol 31, 233-239.

AG N.P. Gleevec Prescribing Information.

Allan N.C., Richards S.M. & Shepherd P.C. (1995) UK Medical Research Council randomised, multicentre trial of interferon-alpha n1 for chronic myeloid leukaemia: improved survival irrespective of cytogenetic response. The UK Medical Research Council's Working Parties for Therapeutic Trials in Adult Leukaemia. Lancet 345, 1392-1397.

Allen J.D., Van Dort S.C., Buitelaar M., van Tellingen O. & Schinkel A.H. (2003) Mouse breast cancer resistance protein (Bcrp1/Abcg2) mediates etoposide resistance and transport, but etoposide oral availability is limited primarily by P-glycoprotein. Cancer Res 63, 1339-1344.

Allen J.D., van Loevezijn A., Lakhai J.M., van der Valk M., van Tellingen O., Reid G., Schellens J.H., Koomen G.J. & Schinkel A.H. (2002) Potent and specific inhibition of the breast cancer resistance protein multidrug transporter in vitro and in mouse intestine by a novel analogue of fumitremorgin C. Mol Cancer Ther 1, 417-425.

Anafi M., Gazit A., Zehavi A., Ben-Neriah Y. & Levitzki A. (1993) Tyrphostin-induced inhibition of p210bcr-abl tyrosine kinase activity induces K562 to differentiate. Blood 82, 3524-3529.

Andreu E.J., Lledo E., Poch E., Ivorra C., Albero M.P., Martinez-Climent J.A., Montiel-Duarte C., Rifon J., Perez-Calvo J., Arbona C., Prosper F. & Perez-Roger

I. (2005) BCR-ABL induces the expression of Skp2 through the PI3K pathway to promote p27Kip1 degradation and proliferation of chronic myelogenous leukemia cells. Cancer Res 65, 3264-3272.

Baccarani M., Saglio G., Goldman J., Hochhaus A., Simonsson B., Appelbaum F., Apperley J., Cervantes F., Cortes J., Deininger M., Gratwohl A., Guilhot F., Horowitz M., Hughes T., Kantarjian H., Larson R., Niederwieser D., Silver R. & Hehlmann R. (2006) Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood 108, 1809-1820.

Balayssac D., Authier N., Cayre A. & Coudore F. (2005) Does inhibition of P-glycoprotein lead to drug-drug interactions? Toxicol Lett 156, 319-329.

Bedi A., Zehnbauer B.A., Barber J.P., Sharkis S.J. & Jones R.J. (1994) Inhibition of apoptosis by BCR-ABL in chronic myeloid leukemia. Blood 83, 2038-2044.

Bennett J.H. (1845) Case of hypertrophy of the spleen and liver in which death took place from suppuration of the blood. Edinburgh Medical and Surgical Journal 64, 413-423.

Blasdel C., Egorin M.J., Lagattuta T.F., Druker B.J. & Deininger M.W. (2007) Therapeutic drug monitoring in CML patients on imatinib. Blood 110, 1699-1701.

Bocchia M., Korontsvit T., Xu Q., Mackinnon S., Yang S.Y., Sette A. & Scheinberg D.A. (1996) Specific human cellular immunity to bcr-abl oncogene-derived peptides. Blood 87, 3587-3592.

Bossemeyer D. (1994) The glycine-rich sequence of protein kinases: a multifunctional element. Trends Biochem Sci 19, 201-205.

Bradeen H.A., Eide C.A., O'Hare T., Johnson K.J., Willis S.G., Lee F.Y., Druker B.J. & Deininger M.W. (2006) Comparison of imatinib mesylate, dasatinib (BMS-354825), and nilotinib (AMN107) in an N-ethyl-N-nitrosourea (ENU)-based mutagenesis screen: high efficacy of drug combinations. Blood 108, 2332-2338.

Branford S., Hughes T.P. & Rudzki Z. (1999) Monitoring chronic myeloid leukaemia therapy by real-time quantitative PCR in blood is a reliable alternative to bone marrow cytogenetics. Br J Haematol 107, 587-599.

Branford S., Rudzki Z., Harper A., Grigg A., Taylor K., Durrant S., Arthur C., Browett P., Schwarer A.P., Ma D., Seymour J.F., Bradstock K., Joske D., Lynch K., Gathmann I. & Hughes T.P. (2003a) Imatinib produces significantly superior molecular responses compared to interferon alfa plus cytarabine in patients with newly diagnosed chronic myeloid leukemia in chronic phase. Leukemia 17, 2401-2409.

Branford S., Rudzki Z., Parkinson I., Grigg A., Taylor K., Seymour J.F., Durrant S., Browett P., Schwarer A.P., Arthur C., Catalano J., Leahy M.F., Filshie R., Bradstock K., Herrmann R., Joske D., Lynch K. & Hughes T. (2004) Real-time quantitative PCR analysis can be used as a primary screen to identify patients with CML treated with imatinib who have BCR-ABL kinase domain mutations. Blood 104, 2926-2932.

Branford S., Rudzki Z., Walsh S., Grigg A., Arthur C., Taylor K., Herrmann R., Lynch K.P. & Hughes T.P. (2002) High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. Blood 99, 3472-3475.

Branford S., Rudzki Z., Walsh S., Parkinson I., Grigg A., Szer J., Taylor K., Herrmann R., Seymour J.F., Arthur C., Joske D., Lynch K. & Hughes T. (2003b) Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. Blood 102, 276-283.

Breedveld P., Beijnen J.H. & Schellens J.H. (2006) Use of P-glycoprotein and BCRP inhibitors to improve oral bioavailability and CNS penetration of anticancer drugs. Trends Pharmacol Sci 27, 17-24.

Breedveld P., Pluim D., Cipriani G., Wielinga P., van Tellingen O., Schinkel A.H. & Schellens J.H. (2005) The effect of Bcrp1 (Abcg2) on the in vivo pharmacokinetics and brain penetration of imatinib mesylate (Gleevec): implications for the use of breast cancer resistance protein and P-glycoprotein inhibitors to enable the brain penetration of imatinib in patients. Cancer Res 65, 2577-2582.

Brendel C., Scharenberg C., Dohse M., Robey R.W., Bates S.E., Shukla S., Ambudkar S.V., Wang Y., Wennemuth G., Burchert A., Boudriot U. & Neubauer A. (2007a) Imatinib mesylate and nilotinib (AMN107) exhibit high-affinity interaction with ABCG2 on primitive hematopoietic stem cells. Leukemia 21, 1267-1275.

Brendel C., Scharenberg C., Dohse M., Robey R.W., Bates S.E., Shukla S., Ambudkar S.V., Wang Y., Wennemuth G., Burchert A., Boudriot U. & Neubauer A. (2007b) Imatinib mesylate and nilotinib (AMN107) exhibit high-affinity interaction with ABCG2 on primitive hematopoietic stem cells. Leukemia 21, 1267-1275.

Buchdunger E., Zimmermann J., Mett H., Meyer T., Muller M., Druker B.J. & Lydon N.B. (1996) Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. Cancer Res 56, 100-104.

Burger H. & Nooter K. (2004) Pharmacokinetic resistance to imatinib mesylate: role of the ABC drug pumps ABCG2 (BCRP) and ABCB1 (MDR1) in the oral bioavailability of imatinib. Cell Cycle 3, 1502-1505.

Burger H., van Tol H., Boersma A.W., Brok M., Wiemer E.A., Stoter G. & Nooter K. (2004) Imatinib mesylate (STI571) is a substrate for the breast cancer resistance protein (BCRP)/ABCG2 drug pump. Blood 104, 2940-2942.

Burger H., van Tol H., Brok M., Wiemer E.A., de Bruijn E.A., Guetens G., de Boeck G., Sparreboom A., Verweij J. & Nooter K. (2005) Chronic Imatinib Mesylate Exposure

Leads to Reduced Intracellular Drug Accumulation by Induction of the ABCG2 (BCRP) and ABCB1 (MDR1) Drug Transport Pumps. Cancer Biol Ther 4, 747-752.

Burgess M.R., Skaggs B.J., Shah N.P., Lee F.Y. & Sawyers C.L. (2005) Comparative analysis of two clinically active BCR-ABL kinase inhibitors reveals the role of conformation-specific binding in resistance. Proc Natl Acad Sci U S A 102, 3395-3400.

Butturini A., Keating A., Goldman J. & Gale R.P. (1990) Autotransplants in chronic myelogenous leukaemia: strategies and results. Lancet 335, 1255-1258.

Campbell L.J., Patsouris C., Rayeroux K.C., Somana K., Januszewicz E.H. & Szer J. (2002) BCR/ABL amplification in chronic myelocytic leukemia blast crisis following imatinib mesylate administration. Cancer Genet Cytogenet 139, 30-33

Carella A.M., Lerma E., Corsetti M.T., Dejana A., Basta P., Vassallo F., Abate M., Soracco M., Benvenuto F., Figari O., Podesta M., Piaggio G., Ferrara R., Sessarego M., Parodi C., Pizzuti M., Rubagotti A., Occhini D. & Frassoni F. (1999) Autografting with philadelphia chromosome-negative mobilized hematopoietic progenitor cells in chronic myelogenous leukemia. Blood 93, 1534-1539.

Caspersson T., Gahrton G., Lindsten J. & Zech L. (1970) Identification of the Philadelphia chromosome as a number 22 by quinacrine mustard fluorescence analysis. Exp Cell Res 63, 238-240.

Catlett-Falcone R., Dalton W.S. & Jove R. (1999) STAT proteins as novel targets for cancer therapy. Signal transducer an activator of transcription. Curr Opin Oncol 11, 490-496.

Cervantes F., Hernandez-Boluda J.C., Steegmann J.L., Conde E., Alvarez-Larran A., Lopez-Jimenez J., Osorio S., Villalon L., Camos M., Garcia-Conde J. & Odriozola J. (2003) Imatinib mesylate therapy of chronic phase chronic myeloid leukemia resistant or intolerant to interferon: results and prognostic factors for response and progression-free survival in 150 patients. Haematologica 88, 1117-1122.

Cetinkaya I., Ciarimboli G., Yalcinkaya G., Mehrens T., Velic A., Hirsch J.R., Gorboulev V., Koepsell H. & Schlatter E. (2003) Regulation of human organic cation transporter hOCT2 by PKA, PI3K, and calmodulin-dependent kinases. Am J Physiol Renal Physiol 284, F293-302.

Choudhury A., Gajewski J.L., Liang J.C., Popat U., Claxton D.F., Kliche K.O., Andreeff M. & Champlin R.E. (1997) Use of leukemic dendritic cells for the generation of antileukemic cellular cytotoxicity against Philadelphia chromosome-positive chronic myelogenous leukemia. Blood 89, 1133-1142.

Ciarimboli G., Koepsell H., Iordanova M., Gorboulev V., Durner B., Lang D., Edemir B., Schroter R., Van Le T. & Schlatter E. (2005) Individual PKC-phosphorylation sites in organic cation transporter 1 determine substrate selectivity and transport regulation. J Am Soc Nephrol 16, 1562-1570.

Ciarimboli G. & Schlatter E. (2005) Regulation of organic cation transport. Pflugers Arch 449, 423-441.

Clarkson B., Strife A., Wisniewski D., Lambek C.L. & Liu C. (2003) Chronic myelogenous leukemia as a paradigm of early cancer and possible curative strategies. Leukemia 17, 1211-1262.

Clift R.A. & Storb R. (1996) Marrow transplantation for CML: the Seattle experience. Bone Marrow Transplant 17 Suppl 3, S1-3.

Cohen M.H., Williams G., Johnson J.R., Duan J., Gobburu J., Rahman A., Benson K., Leighton J., Kim S.K., Wood R., Rothmann M., Chen G., U K.M., Staten A.M. & Pazdur R. (2002) Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia. Clin Cancer Res 8, 935-942.

Corbin A.S., La Rosee P., Stoffregen E.P., Druker B.J. & Deininger M.W. (2003) Several Bcr-Abl kinase domain mutants associated with imatinib mesylate resistance remain sensitive to imatinib. Blood 101, 4611-4614.

Cortes J. (2004) Natural history and staging of chronic myelogenous leukemia. Hematol Oncol Clin North Am 18, 569-584, viii.

Cortes J., Rousselot P., Kim D.-W., Ritchie E., Hamerschlak N., Coutre S., Hochhaus A., Guilhot F., Saglio G., Apperley J., Ottmann O., Shah N., Erben P., Branford S., Agarwal P., Gollerkeri A. & Baccarani M. (2007) Dasatinib induces complete hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in blast crisis. Blood 109, 3207-3213.

Cortes J., Talpaz M., O'Brien S., Giles F., Beth Rios M., Shan J., Faderl S., Garcia-Manero G., Ferrajoli A., Wierda W. & Kantarjian H. (2003) Effects of age on prognosis with imatinib mesylate therapy for patients with Philadelphia chromosome-positive chronic myelogenous leukemia. Cancer 98, 1105-1113.

Cortez D., Kadlec L. & Pendergast A.M. (1995) Structural and signaling requirements for BCR-ABL-mediated transformation and inhibition of apoptosis. Mol Cell Biol 15, 5531-5541.

Cowan-Jacob S.W., Guez V., Fendrich G., Griffin J.D., Fabbro D., Furet P., Liebetanz J., Mestan J. & Manley P.W. (2004) Imatinib (STI571) resistance in chronic myelogenous leukemia: molecular basis of the underlying mechanisms and potential strategies for treatment. Mini Rev Med Chem 4, 285-299.

Craigie D. (1845) Case of disease of the spleen in which death took place as a consequence of the presence of purulent matter in the blood. Edinburgh Medical and Surgical Journal 64, 400-413.

Crossman. L, Druker B & Deininger M (2005) hOCT 1 and resistance to imatinib. Blood 106, 1133-1134.

Dai H., Marbach P., Lemaire M., Hayes M. & Elmquist W.F. (2003) Distribution of STI-571 to the brain is limited by P-glycoprotein-mediated efflux. J Pharmacol Exp Ther 304, 1085-1092.

Daley G.Q. (1993) Animal models of BCR/ABL-induced leukemias. Leuk Lymphoma 11 Suppl 1, 57-60.

Daley G.Q. & Baltimore D. (1988) Transformation of an interleukin 3-dependent hematopoietic cell line by the chronic myelogenous leukemia-specific P210bcr/abl protein. Proc Natl Acad Sci U S A 85, 9312-9316.

Daley G.Q., Van Etten R.A. & Baltimore D. (1990) Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science 247, 824-830.

Daley G.Q., Van Etten R.A. & Baltimore D. (1991) Blast crisis in a murine model of chronic myelogenous leukemia. Proc Natl Acad Sci U S A 88, 11335-11338.

de Fabritis P., Amadori S., Petti M.C., Mancini M., Montefusco E., Picardi A., Geiser T., Campbell K., Calabretta B. & Mandelli F. (1995) In vitro purging with BCR-ABL antisense autologous bone marrow transplantation. Leukemia 9, 662-664.

de Groot R.P., Raaijmakers J.A., Lammers J.W. & Koenderman L. (2000) STAT5-Dependent CyclinD1 and Bcl-xL expression in Bcr-Abl-transformed cells. Mol Cell Biol Res Commun 3, 299-305.

de Jong R., ten Hoeve J., Heisterkamp N. & Groffen J. (1997) Tyrosine 207 in CRKL is the BCR/ABL phosphorylation site. Oncogene 14, 507-513.

de Klein A., van Kessel A.G., Grosveld G., Bartram C.R., Hagemeijer A., Bootsma D., Spurr N.K., Heisterkamp N., Groffen J. & Stephenson J.R. (1982) A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature 300, 765-767.

Dewald G.W. (2002) Interphase FISH studies of chronic myeloid leukemia. Methods Mol Biol 204, 311-342.

Dewar A.L., Cambareri A.C., Zannettino A.C., Miller B.L., Doherty K.V., Hughes T.P. & Lyons A.B. (2005) Macrophage colony-stimulating factor receptor c-fms is a novel target of imatinib. Blood 105, 3127-3132.

Druker B.J., Guilhot F., O'Brien S.G., Gathmann I., Kantarjian H., Gattermann N., Deininger M.W., Silver R.T., Goldman J.M., Stone R.M., Cervantes F., Hochhaus A., Powell B.L., Gabrilove J.L., Rousselot P., Reiffers J., Cornelissen J.J., Hughes T., Agis H., Fischer T., Verhoef G., Shepherd J., Saglio G., Gratwohl A., Nielsen J.L., Radich J.P., Simonsson B., Taylor K., Baccarani M., So C., Letvak L. & Larson R.A. (2006) Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med 355, 2408-2417.

Druker B.J., Talpaz M., Resta D.J., Peng B., Buchdunger E., Ford J.M., Lydon N.B., Kantarjian H., Capdeville R., Ohno-Jones S. & Sawyers C.L. (2001) Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 344, 1031-1037.

Druker B.J., Tamura S., Buchdunger E., Ohno S., Segal G.M., Fanning S., Zimmermann J. & Lydon N.B. (1996) Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 2, 561-566.

Eaves C., Jiang X., Eisterer W., Chalandon Y., Porada G., Zanjani E. & Eaves A. (2003) New models to investigate mechanisms of disease genesis from primitive BCR-ABL(+) hematopoietic cells. Ann N Y Acad Sci 996, 1-9.

El-Zimaity M.M., Kantarjian H., Talpaz M., O'Brien S., Giles F., Garcia-Manero G., Verstovsek S., Thomas D., Ferrajoli A., Hayes K., Nebiyou Bekele B., Zhou X., Rios M.B., Glassman A.B. & Cortes J.E. (2004) Results of imatinib mesylate therapy in chronic myelogenous leukaemia with variant Philadelphia chromosome. Br J Haematol 125, 187-195.

Ferrao P.T., Frost M.J., Siah S.P. & Ashman L.K. (2003) Overexpression of P-glycoprotein in K562 cells does not confer resistance to the growth inhibitory effects of imatinib (STI571) in vitro. Blood 102, 4499-4503.

Fialkow P.J., Jacobson R.J. & Papayannopoulou T. (1977) Chronic myelocytic leukemia: clonal origin in a stem cell common to the granulocyte, erythrocyte, platelet and monocyte/macrophage. Am J Med 63, 125-130.

Fiedler W., Serve H., Dohner H., Schwittay M., Ottmann O.G., O'Farrell A.M., Bello C.L., Allred R., Manning W.C., Cherrington J.M., Louie S.G., Hong W., Brega N.M., Massimini G., Scigalla P., Berdel W.E. & Hossfeld D.K. (2005) A phase 1 study of SU11248 in the treatment of patients with refractory or resistant acute myeloid leukemia (AML) or not amenable to conventional therapy for the disease. Blood 105, 986-993.

Gambacorti-Passerini C., Barni R., le Coutre P., Zucchetti M., Cabrita G., Cleris L., Rossi F., Gianazza E., Brueggen J., Cozens R., Pioltelli P., Pogliani E., Corneo G., Formelli F. & D'Incalci M. (2000) Role of alpha1 acid glycoprotein in the in vivo resistance of human BCR-ABL(+) leukemic cells to the abl inhibitor STI571. J Natl Cancer Inst 92, 1641-1650.

Gambacorti-Passerini C., le Coutre P., Zucchetti M. & D'Incalci M. (2002) Binding of imatinib by alpha(1)-acid glycoprotein. Blood 100, 367-368; author reply 368-369.

Gambacorti-Passerini C., Zucchetti M., Russo D., Frapolli R., Verga M., Bungaro S., Tornaghi L., Rossi F., Pioltelli P., Pogliani E., Alberti D., Corneo G. & D'Incalci M. (2003) Alpha1 acid glycoprotein binds to imatinib (STI571) and substantially alters its pharmacokinetics in chronic myeloid leukemia patients. Clin Cancer Res 9, 625-632.

Gewirtz A.M. (1994) Treatment of chronic myelogenous leukemia (CML) with c-myb antisense oligodeoxynucleotides. Bone Marrow Transplant 14 Suppl 3, S57-61.

Goldman J.M. (1996) Autografting for CML--overview and perspectives. Bone Marrow Transplant 17 Suppl 3, S71-74.

Goldman J.M., Apperley J.F., Jones L., Marcus R., Goolden A.W., Batchelor R., Hale G., Waldmann H., Reid C.D., Hows J. & et al. (1986) Bone marrow transplantation for patients with chronic myeloid leukemia. N Engl J Med 314, 202-207.

Goldman J.M. & Melo J.V. (2003) Chronic myeloid leukemia--advances in biology and new approaches to treatment. N Engl J Med 349, 1451-1464.

Gong S., Lu X., Xu Y., Swiderski C.F., Jordan C.T. & Moscow J.A. (2002) Identification of OCT6 as a novel organic cation transporter preferentially expressed in hematopoietic cells and leukemias. Exp Hematol 30, 1162-1169.

Gorboulev V., Ulzheimer J.C., Akhoundova A., Ulzheimer-Teuber I., Karbach U., Quester S., Baumann C., Lang F., Busch A.E. & Koepsell H. (1997) Cloning and characterization of two human polyspecific organic cation transporters. DNA Cell Biol 16, 871-881.

Gordon M.Y., Dowding C.R., Riley G.P., Goldman J.M. & Greaves M.F. (1987) Altered adhesive interactions with marrow stroma of haematopoietic progenitor cells in chronic myeloid leukaemia. Nature 328, 342-344.

Gorre M.E., Mohammed M., Ellwood K., Hsu N., Paquette R., Rao P.N. & Sawyers C.L. (2001) Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 293, 876-880.

Goto T., Nishikori M., Arlin Z., Gee T., Kempin S., Burchenal J., Strife A., Wisniewski D., Lambek C., Little C., Jhanwar S., Chaganti R. & Clarkson B. (1982) Growth characteristics of leukemic and normal hematopoietic cells in Ph' + chronic myelogenous leukemia and effects of intensive treatment. Blood 59, 793-808.

Gotoh A., Miyazawa K., Ohyashiki K. & Toyama K. (1994) Potential molecules implicated in downstream signaling pathways of p185BCR-ABL in Ph+ ALL involve

GTPase-activating protein, phospholipase C-gamma 1, and phosphatidylinositol 3'-kinase. Leukemia 8, 115-120.

Groffen J., Heisterkamp N. & Stam K. (1986) Oncogene activation by chromosomal translocation in chronic myelocytic leukemia. Cold Spring Harb Symp Quant Biol 51 Pt 2, 911-921.

Groffen J., Stephenson J.R., Heisterkamp N., de Klein A., Bartram C.R. & Grosveld G. (1984) Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell 36, 93-99.

Guilhot F., Apperley J., Kim D.-W., Bullorsky E.O., Baccarani M., Roboz G.J., Amadori S., de Souza C.A., Lipton J.H., Hochhaus A., Heim D., Larson R.A., Branford S., Muller M.C., Agarwal P., Gollerkeri A. & Talpaz M. (2007) Dasatinib induces significant hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in accelerated phase. Blood 109, 4143-4150.

Gurney H., Wong M., Balleine R.L., Rivory L.P., McLachlan A.J., Hoskins J.M., Wilcken N., Clarke C.L., Mann G.J., Collins M., Delforce S.E., Lynch K. & Schran H. (2007) Imatinib disposition and ABCB1 (MDR1, P-glycoprotein) genotype. Clin Pharmacol Ther 82, 33-40.

Hahn E.A., Glendenning G.A., Sorensen M.V., Hudgens S.A., Druker B.J., Guilhot F., Larson R.A., O'Brien S.G., Dobrez D.G., Hensley M.L. & Cella D. (2003) Quality of life in patients with newly diagnosed chronic phase chronic myeloid leukemia on imatinib versus interferon alfa plus low-dose cytarabine: results from the IRIS Study. J Clin Oncol 21, 2138-2146.

Hasford J., Pfirrmann M., Hehlmann R., Allan N.C., Baccarani M., Kluin-Nelemans J.C., Alimena G., Steegmann J.L. & Ansari H. (1998) A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa. Writing Committee for the Collaborative CML Prognostic Factors Project Group. J Natl Cancer Inst 90, 850-858.

Hasford J., Pfirrmann M., Hehlmann R., Baccarani M., Guilhot F., Mahon F.X., Kluin-Nelemans H.C., Ohnishi K., Thaler J. & Steegmann J.L. (2003) Prognosis and prognostic factors for patients with chronic myeloid leukemia: nontransplant therapy. Semin Hematol 40, 4-12.

Hayer-Zillgen M., Bruss M. & Bonisch H. (2002) Expression and pharmacological profile of the human organic cation transporters hOCT1, hOCT2 and hOCT3. Br J Pharmacol 136, 829-836.

Hayer M., Bonisch H. & Bruss M. (1999) Molecular cloning, functional characterization and genomic organization of four alternatively spliced isoforms of the human organic cation transporter 1 (hOCT1/SLC22A1). Ann Hum Genet 63, 473-482.

Heaney C., Kolibaba K., Bhat A., Oda T., Ohno S., Fanning S. & Druker B.J. (1997) Direct binding of CRKL to BCR-ABL is not required for BCR-ABL transformation. Blood 89, 297-306.

Hegedus T., Orfi L., Seprodi A., Varadi A., Sarkadi B. & Keri G. (2002) Interaction of tyrosine kinase inhibitors with the human multidrug transporter proteins, MDR1 and MRP1. Biochim Biophys Acta 1587, 318-325.

Hehlmann R., Heimpel H., Hasford J., Kolb H.J., Pralle H., Hossfeld D.K., Queisser W., Loffler H., Hochhaus A., Heinze B. & et al. (1994) Randomized comparison of interferon-alpha with busulfan and hydroxyurea in chronic myelogenous leukemia. The German CML Study Group. Blood 84, 4064-4077.

Heid C.A., Stevens J., Livak K.J. & Williams P.M. (1996) Real time quantitative PCR. Genome Res 6, 986-994.

Heisterkamp N., Groffen J., Stephenson J.R., Spurr N.K., Goodfellow P.N., Solomon E., Carritt B. & Bodmer W.F. (1982) Chromosomal localization of human cellular homologues of two viral oncogenes. Nature 299, 747-749.

Heisterkamp N., Jenster G., ten Hoeve J., Zovich D., Pattengale P.K. & Groffen J. (1990) Acute leukaemia in bcr/abl transgenic mice. Nature 344, 251-253.

Herens C., Tassin F., Lemaire V., Beguin Y., Collard E., Lampertz S., Croisiau C., Lecomte M., De Prijk B., Longree L. & Koulischer L. (2000) Deletion of the 5'-ABL region: a recurrent anomaly detected by fluorescence in situ hybridization in about 10% of Philadelphia-positive chronic myeloid leukaemia patients. Br J Haematol 110, 214-216.

Higgins C.F. (2007) Multiple molecular mechanisms for multidrug resistance transporters. Nature 446, 749-757.

Hochhaus A., Kantarjian H.M., Baccarani M., Lipton J.H., Apperley J.F., Druker B.J., Facon T., Goldberg S.L., Cervantes F., Niederwieser D., Silver R.T., Stone R.M., Hughes T.P., Muller M.C., Ezzeddine R., Countouriotis A.M. & Shah N.P. (2007) Dasatinib induces notable hematologic and cytogenetic responses in chronic-phase chronic myeloid leukemia after failure of imatinib therapy. Blood 109, 2303-2309.

Hochhaus A., Kreil S., Corbin A., La Rosee P., Lahaye T., Berger U., Cross N.C., Linkesch W., Druker B.J., Hehlmann R., Gambacorti- Passerini C., Corneo G. & D'Incalci M. (2001) Roots of clinical resistance to STI-571 cancer therapy. Science 293, 2163.

Hochhaus A., Kreil S., Corbin A.S., La Rosee P., Muller M.C., Lahaye T., Hanfstein B., Schoch C., Cross N.C., Berger U., Gschaidmeier H., Druker B.J. & Hehlmann R. (2002) Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. Leukemia 16, 2190-2196.

Hochhaus A., Lin F., Reiter A., Skladny H., van Rhee F., Shepherd P.C., Allan N.C., Hehlmann R., Goldman J.M. & Cross N.C. (1995) Variable numbers of BCR-

ABL transcripts persist in CML patients who achieve complete cytogenetic remission with interferon-alpha. Br J Haematol 91, 126-131.

Holyoake T.L., Jiang X., Jorgensen H.G., Graham S., Alcorn M.J., Laird C., Eaves A.C. & Eaves C.J. (2001) Primitive quiescent leukemic cells from patients with chronic myeloid leukemia spontaneously initiate factor-independent growth in vitro in association with up-regulation of expression of interleukin-3. Blood 97, 720-728.

Horita M., Andreu E.J., Benito A., Arbona C., Sanz C., Benet I., Prosper F. & Fernandez-Luna J.L. (2000) Blockade of the Bcr-Abl kinase activity induces apoptosis of chronic myelogenous leukemia cells by suppressing signal transducer and activator of transcription 5-dependent expression of Bcl-xL. J Exp Med 191, 977-984.

Houghton P.J., Germain G.S., Harwood F.C., Schuetz J.D., Stewart C.F., Buchdunger E. & Traxler P. (2004) Imatinib mesylate is a potent inhibitor of the ABCG2 (BCRP) transporter and reverses resistance to topotecan and SN-38 in vitro. Cancer Res 64, 2333-2337.

Hoyle C., Bangs C.D., Chang P., Kamel O., Mehta B. & Negrin R.S. (1998) Expansion of Philadelphia chromosome-negative CD3(+)CD56(+) cytotoxic cells from chronic myeloid leukemia patients: in vitro and in vivo efficacy in severe combined immunodeficiency disease mice. Blood 92, 3318-3327.

Hughes T. & Branford S. (2003) Molecular monitoring of chronic myeloid leukemia. Semin Hematol 40, 62-68.

Hughes T. & Branford S. (2006) Molecular monitoring of BCR-ABL as a guide to clinical management in chronic myeloid leukaemia. Blood Rev 20, 29-41.

Hughes T.P., Kaeda J., Branford S., Rudzki Z., Hochhaus A., Hensley M.L., Gathmann I., Bolton A.E., van Hoomissen I.C., Goldman J.M. & Radich J.P. (2003) Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. N Engl J Med 349, 1423-1432.

Huntly B.J., Bench A. & Green A.R. (2003a) Double jeopardy from a single translocation: deletions of the derivative chromosome 9 in chronic myeloid leukemia. Blood 102, 1160-1168.

Huntly B.J., Bench A.J., Delabesse E., Reid A.G., Li J., Scott M.A., Campbell L., Byrne J., Pinto E., Brizard A., Niedermeiser D., Nacheva E.P., Guilhot F., Deininger M. & Green A.R. (2002) Derivative chromosome 9 deletions in chronic myeloid leukemia: poor prognosis is not associated with loss of ABL-BCR expression, elevated BCR-ABL levels, or karyotypic instability. Blood 99, 4547-4553.

Huntly B.J., Guilhot F., Reid A.G., Vassiliou G., Hennig E., Franke C., Byrne J., Brizard A., Niederwieser D., Freeman-Edward J., Cuthbert G., Bown N., Clark R.E., Nacheva E.P., Green A.R. & Deininger M.W. (2003b) Imatinib improves but may not fully reverse the poor prognosis of patients with CML with derivative chromosome 9 deletions. Blood 102, 2205-2212.

Huntly B.J., Reid A.G., Bench A.J., Campbell L.J., Telford N., Shepherd P., Szer J., Prince H.M., Turner P., Grace C., Nacheva E.P. & Green A.R. (2001) Deletions of the derivative chromosome 9 occur at the time of the Philadelphia translocation and provide a powerful and independent prognostic indicator in chronic myeloid leukemia. Blood 98, 1732-1738.

Hurley R.W., McCarthy J.B. & Verfaillie C.M. (1995) Direct adhesion to bone marrow stroma via fibronectin receptors inhibits hematopoietic progenitor proliferation. J Clin Invest 96, 511-519.

Ilaria R.L., Jr. & Van Etten R.A. (1996) P210 and P190(BCR/ABL) induce the tyrosine phosphorylation and DNA binding activity of multiple specific STAT family members. J Biol Chem 271, 31704-31710.

Illmer T., Schaich M., Platzbecker U., Freiberg-Richter J., Oelschlagel U., von Bonin M., Pursche S., Bergemann T., Ehninger G. & Schleyer E. (2004) P-glycoprotein-mediated drug efflux is a resistance mechanism of chronic myelogenous leukemia cells to treatment with imatinib mesylate. Leukemia 18, 401-408.

Ishikawa I., Kato C., Harigae H., Sugawara T., Tomiya Y., Yamada M., Ishizawa K., Kameoka J., Miyamura K. & Sasaki T. (2006) Dose modification of imatinib by monitoring the level of BCR-ABL transcript in chronic myelogenous leukemia. Tohoku J Exp Med 210, 355-363.

Jabbour E., Cortes J., Kantarjian H.M., Giralt S., Jones D., Jones R., Giles F., Andersson B.S., Champlin R. & de Lima M. (2006) Allogeneic stem cell transplantation for patients with chronic myeloid leukemia and acute lymphocytic leukemia after Bcr-Abl kinase mutation-related imatinib failure. Blood 108, 1421-1423.

Jacobberger J.W., Sramkoski R.M., Frisa P.S., Ye P.P., Gottlieb M.A., Hedley D.W., Shankey T.V., Smith B.L., Paniagua M. & Goolsby C.L. (2003) Immunoreactivity of Stat5 phosphorylated on tyrosine as a cell-based measure of Bcr/Abl kinase activity. Cytometry A 54, 75-88.

Jiang Y., Zhao R.C. & Verfaillie C.M. (2000) Abnormal integrin-mediated regulation of chronic myelogenous leukemia CD34+ cell proliferation: BCR/ABL up-regulates the cyclin-dependent kinase inhibitor, p27Kip, which is relocated to the cell cytoplasm and incapable of regulating cdk2 activity. Proc Natl Acad Sci U S A 97, 10538-10543.

Jonker J.W. & Schinkel A.H. (2004) Pharmacological and physiological functions of the polyspecific organic cation transporters: OCT1, 2, and 3 (SLC22A1-3). J Pharmacol Exp Ther 308, 2-9.

Jordanides N.E., Jorgensen H.G., Holyoake T.L. & Mountford J.C. (2006) Functional ABCG2 is overexpressed on primary CML CD34+ cells and is inhibited by imatinib mesylate. Blood 108, 1370-1373.

Jorgensen H.G., Elliott M.A., Allan E.K., Carr C.E., Holyoake T.L. & Smith K.D. (2002) Alpha1-acid glycoprotein expressed in the plasma of chronic myeloid leukemia patients does not mediate significant in vitro resistance to STI571. Blood 99, 713-715.

Kantarjian H. & Cortes J. (2004) Testing the prognostic model of Marin et al in an independent chronic myelogenous leukemia study group. Leukemia 18, 650.

Kantarjian H., Giles F., Wunderle L., Bhalla K., O'Brien S., Wassmann B., Tanaka C., Manley P., Rae P., Mietlowski W., Bochinski K., Hochhaus A., Griffin J.D., Hoelzer D., Albitar M., Dugan M., Cortes J., Alland L. & Ottmann O.G. (2006) Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. N Engl J Med 354, 2542-2551.

Kantarjian H., Pasquini R., Hamerschlak N., Rousselot P., Holowiecki J., Jootar S., Robak T., Khoroshko N., Masszi T., Skotnicki A., Hellmann A., Zaritsky A., Golenkov A., Radich J., Hughes T., Countouriotis A. & Shah N. (2007) Dasatinib or high-dose imatinib for chronic-phase chronic myeloid leukemia after failure of first-line imatinib: a randomized phase 2 trial. Blood 109, 5143-5150.

Kantarjian H., Sawyers C., Hochhaus A., Guilhot F., Schiffer C., Gambacorti-Passerini C., Niederwieser D., Resta D., Capdeville R., Zoellner U., Talpaz M., Druker B., Goldman J., O'Brien S.G., Russell N., Fischer T., Ottmann O., Cony-Makhoul P., Facon T., Stone R., Miller C., Tallman M., Brown R., Schuster M., Loughran T., Gratwohl A., Mandelli F., Saglio G., Lazzarino M., Russo D., Baccarani M. & Morra E. (2002a) Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N Engl J Med 346, 645-652.

Kantarjian H.M., Talpaz M., Andersson B., Khouri I., Giralt S., Rios M.B., Champlin R., Hester J. & Deisseroth A.B. (1994) High doses of cyclophosphamide, etoposide and total body irradiation followed by autologous stem cell transplantation in the management of patients with chronic myelogenous leukemia. Bone Marrow Transplant 14, 57-61.

Kantarjian H.M., Talpaz M., O'Brien S., Smith T.L., Giles F.J., Faderl S., Thomas D.A., Garcia-Manero G., Issa J.P., Andreeff M., Kornblau S.M., Koller C., Beran M.,

Keating M., Rios M.B., Shan J., Resta D., Capdeville R., Hayes K., Albitar M., Freireich E.J. & Cortes J.E. (2002b) Imatinib mesylate for Philadelphia chromosome-positive, chronic-phase myeloid leukemia after failure of interferon-alpha: follow-up results. Clin Cancer Res 8, 2177-2187.

Kaur G., Gazit A., Levitzki A., Stowe E., Cooney D.A. & Sausville E.A. (1994) Tyrphostin induced growth inhibition: correlation with effect on p210bcr-abl autokinase activity in K562 chronic myelogenous leukemia. Anticancer Drugs 5, 213-222.

Kerb R., Brinkmann U., Chatskaia N., Gorbunov D., Gorboulev V., Mornhinweg E., Keil A., Eichelbaum M. & Koepsell H. (2002) Identification of genetic variations of the human organic cation transporter hOCT1 and their functional consequences. Pharmacogenetics 12, 591-595.

Kimura H., Takeda M., Narikawa S., Enomoto A., Ichida K. & Endou H. (2002) Human organic anion transporters and human organic cation transporters mediate renal transport of prostaglandins. J Pharmacol Exp Ther 301, 293-298.

Kloke O., Niederle N., Opalka B., Hawig I., Seeber S. & Becher R. (1996) Prognostic impact of interferon alpha-induced cytogenetic remission in chronic myelogenous leukaemia: long-term follow-up. Eur J Haematol 56, 78-81.

Koepsell H. & Endou H. (2004) The SLC22 drug transporter family. Pflugers Arch 447, 666-676.

Koepsell H., Schmitt B.M. & Gorboulev V. (2003) Organic cation transporters. Rev Physiol Biochem Pharmacol 150, 36-90.

Kreill S., Mueller M., Hanfstein B., La Rosee P., Lahaye T., Helmann R. & Hochhaus A. (2003) Management and clinical outcome of CML patients after imatinib resistance associated with ABL kinase domain mutations. Blood 102, 71a.

Kumar S., Mishra N., Raina D., Saxena S. & Kufe D. (2003) Abrogation of the cell death response to oxidative stress by the c-Abl tyrosine kinase inhibitor STI571. Mol Pharmacol 63, 276-282.

Kurzrock R., Gutterman J.U. & Talpaz M. (1988) The molecular genetics of Philadelphia chromosome-positive leukemias. N Engl J Med 319, 990-998.

Laneuville P. (1995) Abl tyrosine protein kinase. Semin Immunol 7, 255-266.

Laneuville P., Sun G., Timm M. & Vekemans M. (1992) Clonal evolution in a myeloid cell line transformed to interleukin-3 independent growth by retroviral transduction and expression of p210bcr/abl. Blood 80, 1788-1797.

le Coutre P., Kreuzer K.A., Na I.K., Lupberger J., Holdhoff M., Appelt C., Schwarz M., Muller C., Gambacorti-Passerini C., Platzbecker U., Bonnet R., Ehninger G. & Schmidt C.A. (2002) Determination of alpha-1 acid glycoprotein in patients with Ph+ chronic myeloid leukemia during the first 13 weeks of therapy with STI571. Blood Cells Mol Dis 28, 75-85.

le Coutre P., Tassi E., Varella-Garcia M., Barni R., Mologni L., Cabrita G., Marchesi E., Supino R. & Gambacorti-Passerini C. (2000) Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification. Blood 95, 1758-1766.

Lee M.S., Kantarjian H., Talpaz M., Freireich E.J., Deisseroth A., Trujillo J.M. & Stass S.A. (1992) Detection of minimal residual disease by polymerase chain reaction in Philadelphia chromosome-positive chronic myelogenous leukemia following interferon therapy. Blood 79, 1920-1923.

Lindberg P., Nordberg P., Alminger T., Brandstrom A. & Wallmark B. (1986) The mechanism of action of the gastric acid secretion inhibitor omeprazole. J Med Chem 29, 1327-1329.

Loncarevic I.F., Romer J., Starke H., Heller A., Bleck C., Ziegler M., Fiedler W., Liehr T., Clement J.H. & Claussen U. (2002) Heterogenic molecular basis for loss of

ABL1-BCR transcription: deletions in der(9)t(9;22) and variants of standard t(9;22) in BCR-ABL1-positive chronic myeloid leukemia. Genes Chromosomes Cancer 34, 193-200.

Luciani F., Spada M., De Milito A., Molinari A., Rivoltini L., Montinaro A., Marra M., Lugini L., Logozzi M., Lozupone F., Federici C., Iessi E., Parmiani G., Arancia G., Belardelli F. & Fais S. (2004) Effect of proton pump inhibitor pretreatment on resistance of solid tumors to cytotoxic drugs. J Natl Cancer Inst 96, 1702-1713.

Lyseng-Williamson K. & Jarvis B. (2001) Imatinib. Drugs 61, 1765-1774; discussion 1775-1766.

Ma G., Lu D., Wu Y., Liu J. & Arlinghaus R.B. (1997) Bcr phosphorylated on tyrosine 177 binds Grb2. Oncogene 14, 2367-2372.

Mahon F.X., Belloc F., Lagarde V., Chollet C., Moreau-Gaudry F., Reiffers J., Goldman J.M. & Melo J.V. (2003) MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. Blood 101, 2368-2373.

Mahon F.X., Picard S., Marit G., Robinson P. & Molimard M. (2007) Use of therapeutic drug monitoring in CML patients on imatinib. Blood 110, 1701.

Mancini M., Brusa G., Zuffa E., Corrado P., Martinelli G., Grafone T., Barbieri E. & Santucci M.A. (2007) Persistent Cdk2 inactivation drives growth arrest of BCR-ABL-expressing cells in response to dual inhibitor of SRC and ABL kinases SKI606. Leuk Res 31, 979-987.

Mandanas R.A., Leibowitz D.S., Gharehbaghi K., Tauchi T., Burgess G.S., Miyazawa K., Jayaram H.N. & Boswell H.S. (1993) Role of p21 RAS in p210 bcr-abl transformation of murine myeloid cells. Blood 82, 1838-1847.

Manley P.W., Breitenstein W., Bruggen J., Cowan-Jacob S.W., Furet P., Mestan J. & Meyer T. (2004) Urea derivatives of STI571 as inhibitors of Bcr-Abl and PDGFR kinases. Bioorg Med Chem Lett 14, 5793-5797.

Manley P.W., Cowan-Jacob S.W. & Mestan J. (2005) Advances in the structural biology, design and clinical development of Bcr-Abl kinase inhibitors for the treatment of chronic myeloid leukaemia. Biochim Biophys Acta.

Marin D., Marktel S., Bua M., Szydlo R.M., Franceschino A., Nathan I., Foot N., Crawley C., Na Nakorn T., Olavarria E., Lennard A., Neylon A., O'Brien S.G., Goldman J.M. & Apperley J.F. (2003) Prognostic factors for patients with chronic myeloid leukaemia in chronic phase treated with imatinib mesylate after failure of interferon alfa. Leukemia 17, 1448-1453.

Martin C., Berridge G., Higgins C.F., Mistry P., Charlton P. & Callaghan R. (2000) Communication between multiple drug binding sites on P-glycoprotein. Mol Pharmacol 58, 624-632.

Mayer B.J., Hamaguchi M. & Hanafusa H. (1988) A novel viral oncogene with structural similarity to phospholipase C. Nature 332, 272-275.

Mazzacurati L., Pattacini L., Brusa G., Mancini M., Benvenuti M., Barbieri E., Martinelli G., Baccarani M., Greenberger J.S. & Santucci M.A. (2004) Chk2 drives late G1/early S phase arrest of clonal myeloid progenitors expressing the p210 BCR-ABL tyrosine kinase in response to STI571. Hematol J 5, 168-177.

McGahon A., Bissonnette R., Schmitt M., Cotter K.M., Green D.R. & Cotter T.G. (1994) BCR-ABL maintains resistance of chronic myelogenous leukemia cells to apoptotic cell death. Blood 83, 1179-1187.

McGary E.C., Onn A., Mills L., Heimberger A., Eton O., Thomas G.W., Shtivelband M. & Bar-Eli M. (2004) Imatinib mesylate inhibits platelet-derived growth factor receptor phosphorylation of melanoma cells but does not affect tumorigenicity in vivo. J Invest Dermatol 122, 400-405.

McGary E.C., Weber K., Mills L., Doucet M., Lewis V., Lev D.C., Fidler I.J. & Bar-Eli M. (2002) Inhibition of platelet-derived growth factor-mediated proliferation of osteosarcoma cells by the novel tyrosine kinase inhibitor STI571. Clin Cancer Res 8, 3584-3591.

McGlave P.B., De Fabritiis P., Deisseroth A., Goldman J., Barnett M., Reiffers J., Simonsson B., Carella A. & Aeppli D. (1994) Autologous transplants for chronic myelogenous leukaemia: results from eight transplant groups. Lancet 343, 1486-1488.

McKenna S.L. & Cotter T.G. (1997) Functional aspects of apoptosis in hematopoiesis and consequences of failure. Adv Cancer Res 71, 121-164.

Melo J.V. (1996) The molecular biology of chronic myeloid leukaemia. Leukemia 10, 751-756.

Melo J.V. (2006) Imatinib and ABCG2: who controls whom? Blood 108, 1116-1117.

Mensink E., van de Locht A., Schattenberg A., Linders E., Schaap N., Geurts van Kessel A. & De Witte T. (1998) Quantitation of minimal residual disease in Philadelphia chromosome positive chronic myeloid leukaemia patients using real-time quantitative RT-PCR. Br J Haematol 102, 768-774.

Minderman H., Suvannasankha A., O'Loughlin K.L., Scheffer G.L., Scheper R.J., Robey R.W. & Baer M.R. (2002) Flow cytometric analysis of breast cancer resistance protein expression and function. Cytometry 48, 59-65.

Miura A. (1998) [Progress in laboratory medicine in chronic myeloid leukemia]. Rinsho Byori 46, 1226-1231.

Miura I., Takahashi N., Kobayashi Y., Saito K. & Miura A.B. (2000) Molecular cytogenetics of stem cells: target cells of chromosome aberrations as revealed by the

application of fluorescence in situ hybridization to fluorescence-activated cell sorting. Int J Hematol 72, 310-317.

Muller A.J., Pendergast A.M., Havlik M.H., Puil L., Pawson T. & Witte O.N. (1992) A limited set of SH2 domains binds BCR through a high-affinity phosphotyrosine-independent interaction. Mol Cell Biol 12, 5087-5093.

Nagar B., Bornmann W.G., Pellicena P., Schindler T., Veach D.R., Miller W.T., Clarkson B. & Kuriyan J. (2002) Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). Cancer Res 62, 4236-4243.

Nagar B., Hantschel O., Young M.A., Scheffzek K., Veach D., Bornmann W., Clarkson B., Superti-Furga G. & Kuriyan J. (2003) Structural basis for the autoinhibition of c-Abl tyrosine kinase. Cell 112, 859-871.

Nakanishi T., Shiozawa K., Hassel B.A. & Ross D.D. (2006) Complex interaction of BCRP/ABCG2 and imatinib in BCR-ABL-expressing cells: BCRP-mediated resistance to imatinib is attenuated by imatinib-induced reduction of BCRP expression. Blood 108, 678-684.

Nowell and Hungerford (1960) A minute chromosome in human chronic granulocytic leukemia. Science 132, 1497.

O'Brien S. (2007) Dasatinib in accelerated phase. Blood 109, 4110-.

O'Brien S.G., Guilhot F., Larson R.A., Gathmann I., Baccarani M., Cervantes F., Cornelissen J.J., Fischer T., Hochhaus A., Hughes T., Lechner K., Nielsen J.L., Rousselot P., Reiffers J., Saglio G., Shepherd J., Simonsson B., Gratwohl A., Goldman J.M., Kantarjian H., Taylor K., Verhoef G., Bolton A.E., Capdeville R. & Druker B.J. (2003) Imatinib compared with interferon

and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 348, 994-1004.

O'Hare T., Walters D.K., Stoffregen E.P., Jia T., Manley P.W., Mestan J., Cowan-Jacob S.W., Lee F.Y., Heinrich M.C., Deininger M.W. & Druker B.J. (2005) In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. Cancer Res 65, 4500-4505.

Oda T., Heaney C., Hagopian J.R., Okuda K., Griffin J.D. & Druker B.J. (1994) Crkl is the major tyrosine-phosphorylated protein in neutrophils from patients with chronic myelogenous leukemia. J Biol Chem 269, 22925-22928.

Okabe M., Uehara Y., Miyagishima T., Itaya T., Tanaka M., Kuni-Eda Y., Kurosawa M. & Miyazaki T. (1992) Effect of herbimycin A, an antagonist of tyrosine kinase, on bcr/abl oncoprotein-associated cell proliferations: abrogative effect on the transformation of murine hematopoietic cells by transfection of a retroviral vector expressing oncoprotein P210bcr/abl and preferential inhibition on Ph1-positive leukemia cell growth. Blood 80, 1330-1338.

Okuda K., Weisberg E., Gilliland D.G. & Griffin J.D. (2001) ARG tyrosine kinase activity is inhibited by STI571. Blood 97, 2440-2448.

Ottmann O., Dombret H., Martinelli G., Simonsson B., Guilhot F., Larson R.A., Rege-Cambrin G., Radich J., Hochhaus A., Apanovitch A.M., Gollerkeri A. & Coutre S. (2007) Dasatinib induces rapid hematologic and cytogenetic responses in adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia with resistance or intolerance to imatinib: interim results of a Phase II study. Blood, blood-2007-2002-073528.

Ozvegy-Laczka C C.J., Elkind NB, Sarkadi B (2005) Tyrosine kinase inhibitor resistance in cancer; role of multidrug transporters. Drug Resistance Updates 8, 15-26.

Ozvegy-Laczka C., Heged""s T., Varady G., Ujhelly O., Schuetz J.D., Varadi A., Keri G., Orfi L., Nemet K. & Sarkadi B. (2004a) High-Affinity Interaction of Tyrosine Kinase Inhibitors with the ABCG2 Multidrug Transporter. Mol Pharmacol 65, 1485-1495.

Ozvegy-Laczka C., Hegedus T., Varady G., Ujhelly O., Schuetz J.D., Varadi A., Keri G., Orfi L., Nemet K. & Sarkadi B. (2004b) High-affinity interaction of tyrosine kinase inhibitors with the ABCG2 multidrug transporter. Mol Pharmacol 65, 1485-1495.

Pauli-Magnus C., Rekersbrink S., Klotz U. & Fromm M.F. (2001) Interaction of omeprazole, lansoprazole and pantoprazole with P-glycoprotein. Naunyn Schmiedebergs Arch Pharmacol 364, 551-557.

Pendergast A.M., Quilliam L.A., Cripe L.D., Bassing C.H., Dai Z., Li N., Batzer A., Rabun K.M., Der C.J., Schlessinger J. & et al. (1993) BCR-ABL-induced oncogenesis is mediated by direct interaction with the SH2 domain of the GRB-2 adaptor protein. Cell 75, 175-185.

Peng B., Hayes M., Resta D., Racine-Poon A., Druker B.J., Talpaz M., Sawyers C.L., Rosamilia M., Ford J., Lloyd P. & Capdeville R. (2004) Pharmacokinetics and pharmacodynamics of imatinib in a phase I trial with chronic myeloid leukemia patients. J Clin Oncol 22, 935-942.

Petzer A.L., Gunsilius E., Hayes M., Stockhammer G., Duba H.C., Schneller F., Grunewald K., Poewe W. & Gastl G. (2002) Low concentrations of STI571 in the cerebrospinal fluid: a case report. Br J Haematol 117, 623-625.

Pfeifer H., Wassmann B., Hofmann W.K., Komor M., Scheuring U., Bruck P., Binckebanck A., Schleyer E., Gokbuget N., Wolff T., Lubbert M., Leimer L., Gschaidmeier H., Hoelzer D. & Ottmann O.G. (2003) Risk and prognosis of central

nervous system leukemia in patients with Philadelphia chromosome-positive acute leukemias treated with imatinib mesylate. Clin Cancer Res 9, 4674-4681.

Picard S., Titier K., Etienne G., Teilhet E., Ducint D., Bernard M.A., Lassalle R., Marit G., Reiffers J., Begaud B., Moore N., Molimard M. & Mahon F.X. (2007) Trough imatinib plasma levels are associated with both cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia. Blood 109, 3496-3499.

Potter A.M., Watmore A.E., Cooke P., Lilleyman J.S. & Sokol R.J. (1981) Significance of non-standard Philadelphia chromosomes in chronic granulocytic leukaemia. Br J Cancer 44, 51-54.

Preston D.L., Kusumi S., Tomonaga M., Izumi S., Ron E., Kuramoto A., Kamada N., Dohy H., Matsuo T., Matsui T. & et al. (1994) Cancer incidence in atomic bomb survivors. Part III. Leukemia, lymphoma and multiple myeloma, 1950-1987. Radiat Res 137, S68-97.

Puil L., Liu J., Gish G., Mbamalu G., Bowtell D., Pelicci P.G., Arlinghaus R. & Pawson T. (1994) Bcr-Abl oncoproteins bind directly to activators of the Ras signalling pathway. Embo J 13, 764-773.

Quintas-Cardama A., Kantarjian H., Jones D., Nicaise C., O'Brien S., Giles F., Talpaz M. & Cortes J. (2007) Dasatinib (BMS-354825) is active in Philadelphia chromosome-positive chronic myelogenous leukemia after imatinib and nilotinib (AMN107) therapy failure. Blood 109, 497-499.

Quintas-Cardama A., Kantarjian H., Talpaz M., O'Brien S., Garcia-Manero G., Verstovsek S., Rios M.B., Hayes K., Glassman A., Bekele B.N., Zhou X. & Cortes J. (2005) Imatinib mesylate therapy may overcome the poor prognostic significance of deletions of derivative chromosome 9 in patients with chronic myelogenous leukemia. Blood 105, 2281-2286.

Raitano A.B., Halpern J.R., Hambuch T.M. & Sawyers C.L. (1995) The Bcr-Abl leukemia oncogene activates Jun kinase and requires Jun for transformation. Proc Natl Acad Sci U S A 92, 11746-11750.

Reid A.G., Huntly B.J., Grace C., Green A.R. & Nacheva E.P. (2003) Survival implications of molecular heterogeneity in variant Philadelphia-positive chronic myeloid leukaemia. Br J Haematol 121, 419-427.

Reiffers J., Goldman J., Meloni G., Cahn J.Y., Faberes C. & Apperley J. (1994a) Autologous transplantation in chronic myelogenous leukemia: European results: Chronic Leukemia Working Party of the EBMT. Bone Marrow Transplant 14 Suppl 3, S51-54.

Reiffers J., Goldman J., Meloni G., Cahn J.Y. & Gratwohl A. (1994b) Autologous stem cell transplantation in chronic myelogenous leukemia: a retrospective analysis of the European Group for Bone Marrow Transplantation. Chronic Leukemia Working Party of the EBMT. Bone Marrow Transplant 14, 407-410.

Reiffers J., Montastruc M., Marit G., Cony-Makhoul P., Faberes C., Bilhou-Nabera C., Gharbi M.J., Bernard P., Vezon G. & Broustet A. (1993) Autologous blood stem cell transplantation followed by recombinant alpha interferon as treatment for patients with high-risk chronic myelogenous leukemia. A report of 32 cases. Leuk Lymphoma 11 Suppl 1, 297-299.

Rowley J.D. (1973) Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 243, 290-293.

Sattler M. & Salgia R. (1998) Role of the adapter protein CRKL in signal transduction of normal hematopoietic and BCR/ABL-transformed cells. Leukemia 12, 637-644.

Sattler M., Salgia R., Okuda K., Uemura N., Durstin M.A., Pisick E., Xu G., Li J.L., Prasad K.V. & Griffin J.D. (1996) The proto-oncogene product p120CBL and the

adaptor proteins CRKL and c-CRK link c-ABL, p190BCR/ABL and p210BCR/ABL to the phosphatidylinositol-3' kinase pathway. Oncogene 12, 839-846.

Savage D.G., Szydlo R.M. & Goldman J.M. (1997) Clinical features at diagnosis in 430 patients with chronic myeloid leukaemia seen at a referral centre over a 16-year period. Br J Haematol 96, 111-116.

Sawyers C.L. (1999) Chronic myeloid leukemia. N Engl J Med 340, 1330-1340.

Sawyers C.L., Callahan W. & Witte O.N. (1992) Dominant negative MYC blocks transformation by ABL oncogenes. Cell 70, 901-910.

Sawyers C.L., Hochhaus A., Feldman E., Goldman J.M., Miller C.B., Ottmann O.G., Schiffer C.A., Talpaz M., Guilhot F., Deininger M.W., Fischer T., O'Brien S.G., Stone R.M., Gambacorti-Passerini C.B., Russell N.H., Reiffers J.J., Shea T.C., Chapuis B., Coutre S., Tura S., Morra E., Larson R.A., Saven A., Peschel C., Gratwohl A., Mandelli F., Ben-Am M., Gathmann I., Capdeville R., Paquette R.L. & Druker B.J. (2002) Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. Blood 99, 3530-3539.

Schindler T., Bornmann W., Pellicena P., Miller W.T., Clarkson B. & Kuriyan J. (2000) Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. Science 289, 1938-1942.

Schmidli H., Peng B., Riviere G.J., Capdeville R., Hensley M., Gathmann I., Bolton A.E. & Racine-Poon A. (2005) Population pharmacokinetics of imatinib mesylate in patients with chronic-phase chronic myeloid leukaemia: results of a phase III study. Br J Clin Pharmacol 60, 35-44.

Schoch C., Schnittger S., Bursch S., Gerstner D., Hochhaus A., Berger U., Hehlmann R., Hiddemann W. & Haferlach T. (2002) Comparison of chromosome banding analysis, interphase- and hypermetaphase-FISH, qualitative and quantitative

PCR for diagnosis and for follow-up in chronic myeloid leukemia: a study on 350 cases. Leukemia 16, 53-59.

Schultheis B., Szydlo R., Mahon F.X., Apperley J.F. & Melo J.V. (2005) Analysis of total phosphotyrosine levels in CD34+ cells from CML patients to predict the response to imatinib mesylate treatment. Blood 105, 4893-4894.

Selleri C. & Maciejewski J.P. (2000) The role of FAS-mediated apoptosis in chronic myelogenous leukemia. Leuk Lymphoma 37, 283-297.

Selleri C., Maciejewski J.P., Pane F., Luciano L., Raiola A.M., Mostarda I., Salvatore F. & Rotoli B. (1998) Fas-mediated modulation of Bcr/Abl in chronic myelogenous leukemia results in differential effects on apoptosis. Blood 92, 981-989.

Selleri C., Sato T., Del Vecchio L., Luciano L., Barrett A.J., Rotoli B., Young N.S. & Maciejewski J.P. (1997) Involvement of Fas-mediated apoptosis in the inhibitory effects of interferon-alpha in chronic myelogenous leukemia. Blood 89, 957-964.

Senechal K., Heaney C., Druker B. & Sawyers C.L. (1998) Structural requirements for function of the Crkl adapter protein in fibroblasts and hematopoietic cells. Mol Cell Biol 18, 5082-5090.

Severino G., Chillotti C., De Lisa R., Del Zompo M. & Ardau R. (2005) Adverse reactions during imatinib and lansoprazole treatment in gastrointestinal stromal tumors. Ann Pharmacother 39, 162-164.

Shah N.P., Lee F.Y., Luo R., Jiang Y., Donker M. & Akin C. (2006) Dasatinib (BMS-354825) inhibits KITD816V, an imatinib-resistant activating mutation that triggers neoplastic growth in most patients with systemic mastocytosis. Blood 108, 286-291.

Shah N.P., Nicoll J.M., Nagar B., Gorre M.E., Paquette R.L., Kuriyan J. & Sawyers C.L. (2002) Multiple BCR-ABL kinase domain mutations confer polyclonal resistance

to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell 2, 117-125.

Shah N.P., Tran C., Lee F.Y., Chen P., Norris D. & Sawyers C.L. (2004) Overriding imatinib resistance with a novel ABL kinase inhibitor. Science 305, 399-401.

Shapiro A.B. & Ling V. (1997) Positively cooperative sites for drug transport by P-glycoprotein with distinct drug specificities. Eur J Biochem 250, 130-137.

Shapiro A.B. & Ling V. (1998) The mechanism of ATP-dependent multidrug transport by P-glycoprotein. Acta Physiol Scand Suppl 643, 227-234.

Shtivelman E., Lifshitz B., Gale R.P. & Canaani E. (1985) Fused transcript of abl and bcr genes in chronic myelogenous leukaemia. Nature 315, 550-554.

Shu Y., Leabman M.K., Feng B., Mangravite L.M., Huang C.C., Stryke D., Kawamoto M., Johns S.J., DeYoung J., Carlson E., Ferrin T.E., Herskowitz I. & Giacomini K.M. (2003) Evolutionary conservation predicts function of variants of the human organic cation transporter, OCT1. Proc Natl Acad Sci U S A 100, 5902-5907.

Shuai K., Halpern J., ten Hoeve J., Rao X. & Sawyers C.L. (1996) Constitutive activation of STAT5 by the BCR-ABL oncogene in chronic myelogenous leukemia. Oncogene 13, 247-254.

Simonsson B. & On Behalf of the I.S.G. (2005) Beneficial Effects of Cytogenetic and Molecular Response on Long-Term Outcome in Patients with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Treated with Imatinib (IM): Update from the IRIS Study. Blood (ASH Annual Meeting Abstracts) 106, 166-.

Sinclair P.B., Nacheva E.P., Leversha M., Telford N., Chang J., Reid A., Bench A., Champion K., Huntly B. & Green A.R. (2000) Large deletions at the t(9;22) breakpoint are common and may identify a poor-prognosis subgroup of patients with chronic myeloid leukemia. Blood 95, 738-743.

Skorski T. (2002) BCR/ABL regulates response to DNA damage: the role in resistance to genotoxic treatment and in genomic instability. Oncogene 21, 8591-8604.

Skorski T., Bellacosa A., Nieborowska-Skorska M., Majewski M., Martinez R., Choi J.K., Trotta R., Wlodarski P., Perrotti D., Chan T.O., Wasik M.A., Tsichlis P.N. & Calabretta B. (1997) Transformation of hematopoietic cells by BCR/ABL requires activation of a PI-3k/Akt-dependent pathway. Embo J 16, 6151-6161.

Sokal J.E., Cox E.B., Baccarani M., Tura S., Gomez G.A., Robertson J.E., Tso C.Y., Braun T.J., Clarkson B.D., Cervantes F. & et al. (1984) Prognostic discrimination in "good-risk" chronic granulocytic leukemia. Blood 63, 789-799.

Specchia G., Albano F., Anelli L., Storlazzi C.T., Zagaria A., Liso A., Pannunzio A., Pastore D., Mestice A., Greco G., Liso V. & Rocchi M. (2004) Derivative chromosome 9 deletions in chronic myeloid leukemia are associated with loss of tumor suppressor genes. Leuk Lymphoma 45, 689-694.

Stedman C.A. & Barclay M.L. (2000) Review article: comparison of the pharmacokinetics, acid suppression and efficacy of proton pump inhibitors. Aliment Pharmacol Ther 14, 963-978.

Takahashi N., Miura I., Saitoh K. & Miura A.B. (1998) Lineage involvement of stem cells bearing the philadelphia chromosome in chronic myeloid leukemia in the chronic phase as shown by a combination of fluorescence-activated cell sorting and fluorescence in situ hybridization. Blood 92, 4758-4763.

Takayama N., Sato N., O'Brien S.G., Ikeda Y. & Okamoto S. (2002) Imatinib mesylate has limited activity against the central nervous system involvement of Philadelphia chromosome-positive acute lymphoblastic leukaemia due to poor penetration into cerebrospinal fluid. Br J Haematol 119, 106-108.

Talpaz M., Shah N.P., Kantarjian H., Donato N., Nicoll J., Paquette R., Cortes J., O'Brien S., Nicaise C., Bleickardt E., Blackwood-Chirchir M.A., Iyer V., Chen T.T., Huang F., Decillis A.P. & Sawyers C.L. (2006) Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. N Engl J Med 354, 2531-2541.

Talpaz M., Silver R.T., Druker B.J., Goldman J.M., Gambacorti-Passerini C., Guilhot F., Schiffer C.A., Fischer T., Deininger M.W., Lennard A.L., Hochhaus A., Ottmann O.G., Gratwohl A., Baccarani M., Stone R., Tura S., Mahon F.X., Fernandes-Reese S., Gathmann I., Capdeville R., Kantarjian H.M. & Sawyers C.L. (2002) Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. Blood 99, 1928-1937.

ten Hoeve J., Arlinghaus R.B., Guo J.Q., Heisterkamp N. & Groffen J. (1994) Tyrosine phosphorylation of CRKL in Philadelphia+ leukemia. Blood 84, 1731-1736.

ten Hoeve J., Morris C., Heisterkamp N. & Groffen J. (1993) Isolation and chromosomal localization of CRKL, a human crk-like gene. Oncogene 8, 2469-2474.

Teofili L., Martini M., Cenci T., Petrucci G., Torti L., Storti S., Guidi F., Leone G. & Larocca L.M. (2007) Different STAT-3 and STAT-5 phosphorylation discriminates among Ph-negative chronic myeloproliferative diseases and is independent of the V617F JAK-2 mutation. Blood 110, 354-359.

Thomas J., Wang L., Clark R.E. & Pirmohamed M. (2004) Active transport of imatinib into and out of cells: implications for drug resistance. Blood 104, 3739-3745.

Tidefelt U., Liliemark J., Gruber A., Liliemark E., Sundman-Engberg B., Juliusson G., Stenke L., Elmhorn-Rosenborg A., Mollgard L., Lehman S., Xu D., Covelli A., Gustavsson B. & Paul C. (2000) P-Glycoprotein inhibitor valspodar (PSC 833)

increases the intracellular concentrations of daunorubicin in vivo in patients with P-glycoprotein-positive acute myeloid leukemia. J Clin Oncol 18, 1837-1844.

Tipping A.J., Mahon F.X., Lagarde V., Goldman J.M. & Melo J.V. (2001) Restoration of sensitivity to STI571 in STI571-resistant chronic myeloid leukemia cells. Blood 98, 3864-3867.

Underhill-Day N., Pierce A., Thompson S.E., Xenaki D., Whetton A.D. & Owen-Lynch P.J. (2006) Role of the C-terminal actin binding domain in BCR/ABL-mediated survival and drug resistance. Br J Haematol 132, 774-783.

Uzunel M., Mattsson J., Brune M., Johansson J.E., Aschan J. & Ringden O. (2003) Kinetics of minimal residual disease and chimerism in patients with chronic myeloid leukemia after nonmyeloablative conditioning and allogeneic stem cell transplantation. Blood 101, 469-472.

van Rhee F., Szydlo R.M., Hermans J., Devergie A., Frassoni F., Arcese W., de Witte T., Kolb H.J., Niederwiser D., Jacobsen N., Gahrton G., Bandini G., Carreras E., Bacigalupo A., Michallet M., Ruutu T., Reiffers J., Goldman J.M., Apperley J. & Gratwohl A. (1997) Long-term results after allogeneic bone marrow transplantation for chronic myelogenous leukemia in chronic phase: a report from the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant 20, 553-560.

Verfaillie C.M., Bhatia R., Miller W., Mortari F., Roy V., Burger S., McCullough J., Stieglbauer K., Dewald G., Heimfeld S., Miller J.S. & McGlave P.B. (1996) BCR/ABL-negative primitive progenitors suitable for transplantation can be selected from the marrow of most early-chronic phase but not accelerated-phase chronic myelogenous leukemia patients. Blood 87, 4770-4779.

Verfaillie C.M., Hurley R., Lundell B.I., Zhao C. & Bhatia R. (1997) Integrin-mediated regulation of hematopoiesis: do BCR/ABL-induced defects in integrin

function underlie the abnormal circulation and proliferation of CML progenitors? Acta Haematol 97, 40-52.

Verstovsek S., Golemovic M., Kantarjian H., Manshouri T., Estrov Z., Manley P., Sun T., Arlinghaus R.B., Alland L., Dugan M., Cortes J., Giles F. & Beran M. (2005) AMN107, a novel aminopyrimidine inhibitor of p190 Bcr-Abl activation and of in vitro proliferation of Philadelphia-positive acute lymphoblastic leukemia cells. Cancer 104, 1230-1236.

von Bubnoff N., Schneller F., Peschel C. & Duyster J. (2002) BCR-ABL gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to STI571: a prospective study. Lancet 359, 487-491.

Von Bubnoff N., Veach D.R., Van Der Kuip H., Aulitzky W.E., Sanger J., Seipel P., Bornmann W.G., Peschel C., Clarkson B. & Duyster J. (2004) A cell-based screen for resistance of Bcr-Abl positive leukemia identifies the mutation pattern for PD166326, an alternative Abl kinase inhibitor. Blood.

Wang J.Y. & Ki S.W. (2001) Choosing between growth arrest and apoptosis through the retinoblastoma tumour suppressor protein, Abl and p73. Biochem Soc Trans 29, 666-673.

Wang L., Giannoudis A., Lane S., Williamson P., Pirmohamed M. & Clark R.E. (2007) Expression of the Uptake Drug Transporter hOCT1 is an Important Clinical Determinant of the Response to Imatinib in Chronic Myeloid Leukemia. Clin Pharmacol Ther.

Weisberg E., Catley L., Wright R.D., Moreno D., Banerji L., Ray A., Manley P.W., Mestan J., Fabbro D., Jiang J., Hall-Meyers E., Callahan L., DellaGatta J.L., Kung A.L. & Griffin J.D. (2007) Beneficial effects of combining nilotinib and imatinib in preclinical models of BCR-ABL+ leukemias. Blood 109, 2112-2120.

Weisberg E. & Griffin J.D. (2000) Mechanism of resistance to the ABL tyrosine kinase inhibitor STI571 in BCR/ABL-transformed hematopoietic cell lines. Blood 95, 3498-3505

Weisberg E., Manley P.W., Breitenstein W., Bruggen J., Cowan-Jacob S.W., Ray A., Huntly B., Fabbro D., Fendrich G., Hall-Meyers E., Kung A.L., Mestan J., Daley G.Q., Callahan L., Catley L., Cavazza C., Azam M., Neuberg D., Wright R.D., Gilliland D.G. & Griffin J.D. (2005) Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. Cancer Cell 7, 129-141.

White D., Saunders V., Grigg A., Arthur C., Filshie R., Leahy M.F., Lynch K., To L.B. & Hughes T. (2007a) Measurement of In vivo BCR-ABL kinase inhibition to monitor imatinib-induced target blockade and predict response in chronic myeloid leukemia. J Clin Oncol 25, 4445-4451.

White D., Saunders V., Lyons A.B., Branford S., Grigg A., To L.B. & Hughes T. (2005a) In vitro sensitivity to imatinib-induced inhibition of ABL kinase activity is predictive of molecular response in patients with de novo CML. Blood 106, 2520-2526.

White Deborah, Verity A. Saunders, Susan Branford, Bruce Lyons & Hughes T.P. (2004) The Combination of Intrinsic Sensitivity to Imatinib and Sokal Prognostic Score Is Strongly Predictive of Molecular Response in Newly Diagnosed CML Patients Treated with Imatinib. In: ASH, Vol. 104, pp. 1010, San Diego.

White Deborah, Verity Saunders, Phuong Dang, Amity Venables, Steph Zrim, John Reynolds, Steven Quinn, Andrew Zannettino & Hughes T. (November 2006) Molecular Response to Imatinib Is Dependent on Dose in CML Patients with Low OCT-1 Influx Activity. Patients with High Activity May Respond Equally Well to Standard or Increased Dose Imatinib. In: ASH, Vol. 208, pp. 738, Orlando.

White Deborah L, Verity A Saunders, Dang P., Lynch K., Manley P. & Hughes T.P. (2007) The intracellular concentrations of imatinib and nilotinib, can be substantially altered by interaction with other drugs: Studies with proton pump inhibitors. In: European Haematology Society, Vienna.

White D.L., Branford S., Saunders V.A., Lynch K., Grigg A., To L.B. & Hughes T.P. (2005b) In Imatinib Treated CML Patients with Low Baseline IC50, Early Molecular Response Is HighlyPredictive of Longer Term Molecular Outcome. In Patients with High IC50, Other Prognostic Indicators Are Needed. ASH Annual Meeting Abstracts 106, 3283-.

White D.L., Saunders AV, Quinn SR, PW M. & TP. H. (2007b) Proton pump inhibitors interact with transport of both imatinib and nilotinib. In: AACR, Los Angeles.

White D.L., Saunders V.A., Dang P., Engler J., Venables A., Zrim S., Zannettino A., Lynch K., Manley P.W. & Hughes T. (2007c) Most CML patients who have a suboptimal response to imatinib have low OCT-1 Activity. Higher doses of imatinib may overcome the negative impact of low OCT-1 Activity. Blood Blood First Edition Paper, prepublished online August 30, 2007; DOI 10.1182/blood-2007-06-093617.

White D.L., Saunders V.A., Dang P., Engler J., Venables A., Zrim S., Zannettino A., Lynch K., Manley P.W. & Hughes T. (2007d) Most CML patients who have a suboptimal response to imatinib have low OCT-1 activity: higher doses of imatinib may overcome the negative impact of low OCT-1 activity. Blood 110, 4064-4072.

White D.L., Saunders V.A., Dang P., Engler J., Zannettino A.C., Cambareri A.C., Quinn S.R., Manley P.W. & Hughes T.P. (2006) OCT-1-mediated influx is a key determinant of the intracellular uptake of imatinib but not nilotinib (AMN107): reduced OCT-1 activity is the cause of low in vitro sensitivity to imatinib. Blood 108, 697-704.

White D.L., Saunders V.A., Engler J., Dang P., Zannettino A.C.W., Cambareri A., Quinn S., Manley P. & Hughes T.P. (2005c) Low Baseline Intrinsic Sensitivity to Imatinib (High IC50) in CML Patients Is Due to Reduced Oct-1 Mediated Influx. Intrinsic Sensitivity to AMN107 Does Not Correlate with That of Imatinib and Uptake of AMN107 Is Not Oct-1 Mediated. ASH Annual Meeting Abstracts 106, 1081-.

White D.L., Saunders V.A., Quinn S.R., Manley P.W. & Hughes T.P. (2007e) Imatinib increases the intracellular concentration of nilotinib, which may explain the observed synergy between these drugs. Blood 109, 3609-361

Zhang L., Gorset W., Dresser M.J. & Giacomini K.M. (1999) The interaction of n-tetraalkylammonium compounds with a human organic cation transporter, hOCT1. J Pharmacol Exp Ther 288, 1192-1198.

Zong Y., Zhou S. & Sorrentino B.P. (2005) Loss of P-glycoprotein expression in hematopoietic stem cells does not improve responses to imatinib in a murine model of chronic myelogenous leukemia. Leukemia 19, 1590-1596.

Appendix III:

Publications arising from the Thesis

White D., Saunders V., Lyons A.B., Branford S., Grigg A., To L.B. & Hughes T. (2005a) In vitro sensitivity to imatinib-induced inhibition of ABL kinase activity is predictive of molecularresponse in patients with de novo CML. Blood 106, 2520-2526.

White D.L., Saunders V.A., Dang P., Engler J., Zannettino A.C., Cambareri A.C., Quinn S.R., Manley P.W. & Hughes T.P. (2006) OCT-1-mediated influx is a key determinant of the intracellular uptake of imatinib but not nilotinib (AMN107): reduced OCT-1 activity is the causeof low in vitro sensitivity to imatinib. Blood 108, 697-704

White D.L., Saunders V.A., Quinn S.R., Manley P.W. & Hughes T.P. (2007e) Imatinib increases the intracellular concentration of nilotinib, which may explain the observed synergybetween these drugs. Blood 109, 3609-3610.

White D., Saunders V., Grigg A., Arthur C., Filshie R., Leahy M.F., Lynch K., To L.B. & Hughes T. (2007a) Measurement of In vivo BCR-ABL kinase inhibition to monitor imatinib-induced target blockade and predict response in chronic myeloid leukemia. J Clin Oncol 25,4445-4451.

White D.L., Saunders V.A., Dang P., Engler J., Venables A., Zrim S., Zannettino A., Lynch K., Manley P.W. & Hughes T. (2007d) Most CML patients who have a suboptimal response to imatinib have low OCT-1 activity: higher doses of imatinib may overcome the negative impact of low OCT-1 activity. Blood 110, 4064-4072.

White, D., Saunders, V., Lyons, A.B., Branford, S., Grigg, A., To, L.B. & Hughes, T. (2005) In vitro sensitivity to imatinib-induced inhibition of ABL kinase activity is predictive of molecularresponse in patients with de novo CML.
Blood, vol. 106 (7), pp. 2520-2526

White, D.L., Saunders, V.A., Dang, P., Engler, J., Zannettino, A.C., Cambareri, A.C., Quinn, S.R., Manley, P.W. & Hughes, T.P. (2006) OCT-1-mediated influx is a key determinant of the intracellular uptake of imatinib but not nilotinib (AMN107): reduced OCT-1 activity is the cause of low in vitro sensitivity to imatinib.
Blood, vol. 108(2), pp. 697-704

White, D.L., Saunders, V.A., Quinn, S.R., Manley, P.W. & Hughes, T.P. (2007) Imatinib increases the intracellular concentration of nilotinib, which may explain the observed synergy between these drugs.
Blood, vol. 109 (8), pp. 3609-3610.

White, D., Saunders, V., Grigg, A., Arthur, C., Filshie, R., Leahy, M.F., Lynch, K., To, L.B. & Hughes, T. (2007) Measurement of In vivo BCR-ABL kinase inhibition to monitor imatinibinduced target blockade and predict response in chronic myeloid leukemia.
Journal of Clinical Oncology, vol. 25 (28), pp. 4445-4451

White, D.L., Saunders, V.A., Dang, P., Engler, J., Venables, A., Zrim, S., Zannettino, A., Lynch, K., Manley, P.W. & Hughes, T. (2007) Most CML patients who have a suboptimal response to imatinib have low OCT-1 activity: higher doses of imatinib may overcome the negative impact of low OCT-1 activity.
Blood, vol. 110 (12), pp. 4064-4072.