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

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DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Deborah Harland (nee White)
8th Day of April 2008

ACKNOWLEDGEMENTS

I recently had cause to interview prospective PhD students. One of these students asked me what I felt were "critical components" of a successful PhD. My answer "Firstly, you need to have a real passion for your topic. Secondly, you need to have a supervisor who shares this enthusiasm, and finally you need to have a strong support network of family and friends". I was sincerely lucky, I had all three of these things, and more….

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To my darling boys. As I write this, my 17 year old son Daniel is sitting his final Year 12 exams. My 11 year old son Matthew is reeling from living in a household where his brother and mother are incessantly staring at computers, and my husband Robert is seriously wondering what he said "yes" to! I thank you all for thinking this is "special". For tolerating my less than gracious moods at times, and for forgiving me when I just couldn't be there. I never would have got this far without your unconditional love and encouragement and for this I sincerely thank, and love you all. The great news is, as a family we have made it! A little battle weary maybe, but nothing a bit of time and TLC won't cure!

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"Don't wait for a light to appear at the end of the tunnel; stride down there and light the bloody thing yourself!"

Sara Henderson

"Real success is finding your lifework in the work that you love."

David McCullough

"It is the mark of an educated mind to be able to entertain a thought without accepting it."

Aristotle

"The outcome of any serious research can only be to make two questions grow where only one grew before. "

Thorstein Veblen

"If you think you can, you can. And if you think you can't, you're right."

Mary Kay Ash

GLOSSARY

AKI	ABL kinase inhibitor
ALL	acute lymphoblastic leukaemia
AMN107	second generation kinase inhibitor - nilotinib
AP	accelerated phase
ATP	adenosine triphosphate
BC	blast crisis
BCR	breakpoint cluster region
bp	base pairs
C	celcius
CCR	complete cytogenetic response
CCyR	complete cytogenetic response
cDNA	complementary deoxyribonucleic acid
CE	clonal evolution
CHR	complete haematologic response
CML	chronic myeloid leukaemia
COS-1 cells	Transformed African Green Monkey Kidney Fibroblast Cells
CP	chronic phase
CV	coefficient of variation
DEPC	diethyl pyrocarbonate
DMSO	dimethyl sulphoxide
DNA	Deoxyribonucleic acid
dNTPs	deoxynucleotide triphosphates
DSB	double strand break
DTT	dithiothreitol
EDTA	ethylene diamine tetraacetate
FISH	fluorescent in situ hybridisation
Grb2	Growth factor receptor bound Protein 2

HBSS	Hanks Balanced Salt Solution
HLA	human leucocyte antigen
IFN-AraC	interferon-α plus low dose cytarabine
IMDM	Iscove's modification of Dulbecco's medium.
IPTG	Isopropyl-β-thio-galactoside
IRIS	International randomised study of interferon versus STI571
JAK	Janus Kinase
kD	kilo Dalton
L	litre
M	molar
M-bcr	major breakpoint cluster region
m-bcr	minor breakpoint cluster region
MCR	major cytogenetic response
MCyR	major cytogenetic response
MMR	major molecular response
mM	milli Molar (10^{-3} Molar)
MNC	Mononuclear cells
mRNA	messenger ribonucleic acid
µM	micro Molar (10^{-6} Molar)
µg	micro gram (10^{-6} gram)
ND	not detected
Ng	nano gram (10^{-9} gram)
PBS	Phosphate Buffered Saline
PCR	polymerase chain reaction
PCyR	partial cytogenetic response
PFS	progression free survival
Pg	pico gram (10^{-12} gram)
Ph	Philadelphia chromosome
Phe	Phenylalanine

PHR	partial haematologic response
PI3-K	Phosphotidylinositol – 3-kinase
PKA	cAMP- dependent protein kinase
PKC	Protein kinase C
P-loop	phosphate binding loop
QC	quality control
RNA	ribonucleic acid
RPMI	Roswell Park Memorial Institute (media)
RT-PCR	reverse transcription polymerase chain reaction
SD	standard deviation
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Elctrophoresis
SH	Src homology
STI571	signal transduction inhibitor 571 (imatinib)
TKI	Tyrosine kinase inhibitor
Tyr	Tyrosine
U	units
UV	ultraviolet
v/v	volume per volume
WCC	white cell count
w/v	Weight per unit volume
y	year

Clinical Trials Referred to in this Thesis

TIDEL
(CML6)

A Phase II study in adult patients with newly-diagnosed chronic myeloid leukaemia of initial intensified Glivec® therapy, and sequential combination therapy for non-responders.

The study involved an initial single arm of intensified dose imatinib (600mg), followed by a branching pattern of therapeutic options (acceleration to 800mg or combination of 600mg + AraC), determined by each patient's response.

TOPS
(CSTI571K2301)

A randomized open-label study of 400mg versus 800 mg Gleevec/Glivec (imatinib mesylate) in patients with newly diagnosed, previously untreated chronic myeloid leukaemic in chronic phase (CP-CML) using molecular endpoints.

Patients were randomized 2:1 to the 800mg or 400mg treatments arms. Randomization into each arm was stratified by the Sokal score at entry into the study.

I am very grateful to the principal investigators and clinical trial coordinators within Australia and New Zealand who have provided clinical data, and samples from these Trials for the studies discussed in this Thesis.

Abstract

The natural history of CML has been transformed in recent years by the introduction of Glivec™ (imatinib mesylate), an ABL kinase inhibitor, which provides the new treatment paradigm for chronic phase CML. While the majority of patients with CP-CML respond very well to imatinib, there are approximately 15% of patients who fail to respond, or respond suboptimally. While the major cause of secondary imatinib resistance can be attributable to kinase domain mutations, the underlying cause of primary resistance is yet to be elucidated.

Utilizing the phosphorylation of the adaptor protein Crkl, an immediate downstream partner of BCR-ABL, as a surrogate measure of BCR-ABL kinase activity, a large interpatient variation in the degree of imatinib induced kinase inhibition achieved in-vitro, was observed in previously untreated CP-CML patients. The observed in-vitro sensitivity was a good predictor of molecular response in patients treated with 600mg imatinib as front line therapy. Furthermore, analysis of the in-vivo reduction in p-Crkl mediated measured in blood cells in response to imatinib over the first 28 days of therapy, revealed that patients with higher % reductions respond significantly better over a two year period, than those with lower % reductions.

Using 14-C labelled imatinib, it was demonstrated that this intrinsic sensitivity correlated to the amount of drug which was retained within the target haemopoietic cell, and furthermore, that a critical determinant of the active influx of imatinib, was the functional activity of the human organic cation transporter -1 (OCT-1), as determined by a prazosin (potent inhibitor of OCT-1) inhibition assay. Patients with high OCT-1 Activity had superior molecular responses when compared to those with low OCT-1 Activity, but in those patients who could tolerate increased imatinib dose, these inferior responses could be largely overcome. In contrast, Nilotinib, a more potent second generation tyrosine kinase inhibitor, is not dependent on OCT-1 for influx, making it a possible treatment choice for patients with low OCT-1 Activity.

Both imatinib and nilotinib interact with the efflux transporters ABCB1, and ABCG2. In combination studies imatinib results in a significantly increased intracellular concentration of nilotinib, most likely through interaction with these efflux transporters. Furthermore, commonly used therapies such as proton pump inhibitors also interact with ABCB1 and ABCG2, and demonstrable changes in

intracellular drug concentrations were observed in-vitro with concomitant administration of these agents and imatinib or nilotinib at clinically relevant concentrations.

In conclusion, these data demonstrate that the degree of kinase inhibition mediated in-vitro and in-vivo by imatinib, is a critical determinant of subsequent molecular response. This intrinsic sensitivity to imatinib induced kinase inhibition is related to the activity of the OCT-1 protein. This protein is not involved in the transport of nilotinib, suggesting it as a possible treatment alternative in those patients with low OCT-1 Activity.