TKI resistance in CML cell lines: Investigating resistance pathways

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Declaration

I, Carine Tang, certify that 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.

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Carine Tang

1st September 2011

Acknowledgements

Romans 13:7 *Render therefore to all their dues: tribute to whom tribute is due; custom to whom custom; fear to whom fear; honour to whom honour.*

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Psalms 18:30 *As for God, His way is perfect: the word of the LORD is tried: He is a buckler to all those that trust in Him.*

Glossary

ABCB1/ABCG2	ATP binding cassette (ABC) transporter proteins B1 and G2
ALL	Acute lymphoblastic leukaemia
AP	Accelerated phase
APS	Ammonium persulfate
ATP	Adenosine triphosphate
BC	Blast crisis
BCR	Breakpoint cluster region
BCR-ABL	Breakpoint cluster region-Ableson kinase fusion transcript/protein
Bcr-Abl	Breakpoint cluster region-Ableson kinase fusion gene
C	Celcius
CCR	Complete cytogenetic response
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CHO	Chinese hamster ovary
CHR	Complete haematologic response
CML	Chronic myeloid leukaemia
CP	Chronic phase
Crkl	CT10 regulator of kinase-like
DABCO	1,4-diazabicyclo[2.2.2]octane
DAS	Dasatinib
ddNTP	Dideoxynucleotide triphosphate
DEPC	Diethyl pyrocarbonate
DMSO	Dimethyl sulphoxide
dmin	Double minutes
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphates
DTT	Dithiothreitol
FACS	Fluorescence activated cell sorting
FISH	Fluorescent *in situ* hybridisation

GUSB	Beta-glucuronidase
HBSS	Hanks Balanced Salt Solution
Hck	Haemopoietic cell kinase
HCl	Hydrochloric acid
HSR	Homogenously staining region
IC50	Inhibitory concentration 50%
IgG	Immunoglobulin G
IM	Imatinib mesylate
JAK	Janus kinase
KD	Kinase domain
kD	Kilo Dalton
KDR	Kinase insert domain protein receptor
L	Litre
Lyn	V-yes-1 Yamaguchi sarcoma viral related oncogene homolog
M	Molar (Moles per litre)
Mbq	Mega Becquerel (10^{6} Becquerel)
MCR	Major cytogenetic response
MDR	Multidrug resistance
µCi	Micro Curie (10^{-6} Curie)
µg	Micro gram (10^{-6} gram)
µM	Micro molar (10^{-6} Molar)
MMR	Major molecular response
mM	Milli molar (10^{-3} Molar)
mRNA	Messenger ribonucleic acid
NHEJ	Non-homologous end-joining
NIL	Nilotinib
nM	Nano molar (10^{-9} Molar)
OCT-1	Organic cation transporter 1
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
Ph	Philadelphia chromosome

PI3-K	Phosphatidylinositol – 3-kinase
P-loop	Phosphate binding loop
PVDF	Polyvinylidene Fluoride
P-value	Probability value
RANKL	Receptor activator of nuclear factor kappa-b ligand
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
RPMI	Roswell Park Memorial Institute (-1640 medium)
RT	Room temperature
RQ-PCR	Real-time quantitative polymerase chain reaction
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	Standard error of the mean
SH	Src Homology domain
SN	Supernatant
Src	Sarcoma
SSC	Saline-Sodium Citrate buffer
TBS	Tris buffered saline
TBST	Tris buffered saline with 0.1% Tween20
TKI	Tyrosine kinase inhibitor
Tris	Tris(hydroxymethyl)aminomethane
UV	Ultra violet

Abstract

Chronic myeloid leukaemia (CML) is characterised by the presence of the Philadelphia chromosome which harbours the Bcr-Abl oncogene. BCR-ABL is a constitutively active tyrosine kinase that can be inhibited by rationally designed tyrosine kinase inhibitors (TKIs) such as imatinib, nilotinib and dasatinib. Although TKI therapy is an effective treatment for many patients, resistance can arise. There are currently four identified resistance mechanisms. These are 1) overexpression of drug-efflux proteins (ABCB1 and ABCG2), 2) BCR-ABL kinase domain (KD) mutations, 3) increased BCR-ABL expression and 4) BCR-ABL independent mechanisms such as Lyn kinase expression. In this study the interplay between these four recognised modes of TKI resistance is investigated.

Imatinib- and dasatinib-resistant cell lines were established and used to investigate TKI resistance *in vitro*. Viability and IC50 assays were used to demonstrate TKI sensitivity/resistance. Flow cytometry was used to screen for ABCB1 and ABCG2 cell surface expression, while conventional sequencing and the MassARRAY method were used to determine the mutation status of the BCR-ABL KD. Fluorescence *in situ* hybridisation (FISH) and quantitative DNA PCR were used to investigate Bcr-Abl DNA copy number, and RQ-PCR was used to investigate expression levels of BCR-ABL and Lyn mRNA.

These studies revealed that IM-resistant K562 cell lines exhibited increased BCR-ABL expression at the onset of resistance. Interestingly, these cell lines had increased viability and IC50s for IM and NIL, while the DAS IC50s were variable. Further investigation revealed Lyn overexpression in the cell line which was more sensitive to DAS. The development of a DAS-resistant K562 culture resulted in the emergence of the T315I mutation. Studies of the intermediate stages of resistance of this DAS-resistant cell line revealed that increased BCR-ABL expression occurred gradually, preceding the emergence of the mutation, at which time the BCR-ABL expression decreased and plateaued. Thus, it appears that increased BCR-ABL expression may be the initial mechanism of resistance, followed by the emergence of a KD mutation which has a clear selective advantage. This phenomenon was observed a further four times (in a DAS-resistant K562 Dox culture, and in three IM-resistant KU812 cultures) each time with the emergence of different KD mutations. Different KD mutations resulted in differential resistance to the three TKIs used in this study.

In contrast, three IM-resistant K562 Dox cell lines were not found to have any KD mutations, nor BCR-ABL overexpression. Instead, the primary cause of resistance in these lines appeared to be an increase in ABCB1 expression. The addition of PSC833 (an ABCB1 inhibitor) decreased the IM, NIL, and DAS IC50s for all three resistant lines to the level of the naïve control. This indicated that ABCB1 expression, facilitating active efflux of the drugs, is the primary mechanism of resistance in these lines.

This study demonstrates that KD emergence is a stochastic event, as the same mutation did not always occur twice when exposed to the same TKI. However, increased ABCB1 expression was more likely to arise recurrently in the predisposed K562 Dox cell line. Notably, different TKIs elicited different resistant mechanisms, and all but one (the Lyn overexpressing K562 cell line) were BCR-ABL dependent. Furthermore, all resistant cell lines showed cross-resistance (at least to some extent) to the three TKIs tested, suggesting that currently available TKIs share the same susceptibilities to drug resistance.