Nilotinib Efflux and Resistance Development: The Effects of Combination and Concomitant Therapies on the Transport and Efficacy of Nilotinib

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Abstract

Chronic myeloid leukaemia (CML) is characterised by the presence of Bcr-Abl tyrosine kinase. Tyrosine kinase inhibitors (TKIs), such as imatinib, and more recently nilotinib and dasatinib, act by specifically binding to the Bcr-Abl kinase domain. The advent of TKIs resulted in significantly improved treatment outcomes for the majority of patients with CML. However, the focus is now customised treatment regimes employing drug combinations to reduce resistance development and maximise treatment outcomes. The present study investigated the interaction of nilotinib with efflux transporters and 1) assessed how concomitant administration of additional drugs may enhance the effects of nilotinib in patients and 2) how altered expression or inhibition of these transporters affected nilotinib transport and function. Secondly, *in vitro* cell line models of nilotinib resistance were generated in order to replicate modes of nilotinib resistance *in vivo*.

The reported relationship between nilotinib and efflux transporters ABCB1 and ABCG2 is conflicting and nilotinib has previously been reported to inhibit the function of OCT-1. Thus, in order to resolve conjecture, a novel approach was employed to determine the effect of ABCB1/ABCG2 inhibition on nilotinib-mediated Bcr-Abl kinase inhibition. Results demonstrated ABCB1-mediated nilotinib transport was concentration dependent: transport of nilotinib occurred at low concentrations whereas inhibition of both ABCB1 and ABCG2 occurred at high nilotinib concentrations. Additionally, data demonstrated nilotinib had no inhibitory effect on the functional activity of OCT-1 but may reduce intracellular imatinib concentrations by impairing passive influx.

Bcr-Abl dependent modes of resistance relating to kinase domain mutations and Bcr-Abl overexpression are well documented. The mechanisms underlying Lyn-mediated resistance however, require further investigation and Bcr-Abl-independent resistance is even more poorly understood. Accordingly, *in vitro* cell line models of nilotinib resistance were developed. ABCB1 overexpression was consistently

demonstrated as the initiator of nilotinib resistance in all cell lines, however, both Bcr-Abl dependent and Bcr-Abl independent resistance mechanisms were subsequently observed. These results suggest determination of ABCB1 expression levels at diagnosis and 3 months post-therapy, for example, may predict resistance in patients. Furthermore, this is the first reported nilotinib resistant, genuine Bcr-Abl independent cell line model and may provide insight into unexplained TKI resistance observed in patients.

Additionally, both nilotinib resistant cell lines demonstrated ABCC6 overexpression suggesting this transporter may play a role in nilotinib resistance *in vitro*. Further investigation in patient mononuclear cells confirmed nilotinib as a likely substrate of ABCC6. This is the first report of ABCC6 involvement in nilotinib transport and concomitant administration of ABCC6 inhibitors may present an attractive option to enhance TKI efficacy and prevent resistance.

Findings detailed in this thesis may assist in developing new therapeutic strategies using TKIs in combination with other medications in order to enhance the intracellular concentrations of TKI. Additionally, further insight into the modes of resistance to nilotinib, as well as the kinetics of resistance emergence, may assist in identifying patients at risk of developing resistance to TKIs. Finally, ABCB1/ABCC6 mRNA expression levels in *de novo* CML patients at diagnosis may present a novel technique for predicting response to nilotinib at 12 months.

Declaration

I, Laura Eadie, 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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Laura Eadie

23rd March 2013

Publications

Manuscripts

Eadie LN, Saunders VA, Hughes TP, White DL. 2013. Degree of kinase inhibition achieved *in vitro* by imatinib and nilotinib is decreased by high levels of ABCB1 but not ABCG2. *Leukemia and Lymphoma*, 54 (3): 569-578. Impact factor: 2.580.

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Conference Abstracts

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Abbreviations

µg – Microgram/s

µL – Microlitre/s

µM – Micromolar

7-AAD – 7-Aminoactinomycin D

^{14}C – Carbon-14 radioactive isotope

ABC – ATP Binding Cassette

ACD – Acid Citrate Dextrose Acid

ADME-Tox – Absorption-Distribution-Metabolism-Excretion-Toxicity

AGP – α1 acid glycoprotein

ALL – Acute Lymphoblastic Leukaemia

-AP – Alkaline Phosphatase Conjugated Antibody

AP – Accelerated Phase

APS – Ammonium Persulfate

Ara-C – Arabinofuranosyl Cytidine

ATCC – American Type Tissue Culture Collection

ATP – Adenosine Triphosphate

AZT – Azathioprine

BC – Blast Crisis

BCR-ABL – Breakpoint Cluster Region-Abelson (mRNA)

Bcr-Abl – Breakpoint Cluster Region-Abelson (protein)

BCRP – Breast Cancer Resistance Protein

BM – Bone Marrow

B-P – BODIPY-prazosin

BSA – Bovine Serum Albumin

CCyR – Complete Cytogenetic Remission

cDNA – Complementary DNA

CHR – Complete Haematological Response

CML – Chronic Myeloid Leukaemia

CNS – Central Nervous System

CP –Chronic Phase

CrkI – C1T10 regulator of kinase like

Ct – Cycle Threshold

DAS – Dasatinib

DEPC – Diethylpyrocarbonate

DMEM – Dulbecco's Modified Eagle's Medium

DMSO – Dimethyl Sulphoxide

DNA – Deoxyribonucleic Acid

EDTA – Ethylenediaminetetraacetic Acid

FACS – Fluorescence Activated Cell Sorting

FCS – Foetal Calf Serum

FDA – Food and Drug Administration

g – see rcf

GM-CSFR – Granulocyte Macrophage Colony-Stimulating Factor Receptor

h – Hour/s

HBSS – Hanks Balanced Salt Solution

IC50 – Inhibitory Concentration 50

IFN – Interferon

IM – Imatinib

IUR – Intracellular Uptake and Retention

kD – Kilo Daltons

KD – Kinase Domain

L – Litre/s

M - Molar

MDR – Multidrug Resistance Protein

MFI – Mean Fluorescence Intensity

mg – milligram/s

min – Minutes/s

mL – Millilitre/s

mM – Millimolar

MMR – Major Molecular Response

MNC/s – Mononuclear Cell/s

MQ – Milli-Q

mRNA – messenger RNA

MRP – Multidrug Resistance-Associated Protein

MSR – Membrane Spanning Region

MTX – Methotrexate

MW – Molecular Weight

ND – Not Determined

NEG – Negative Expression Levels

ng – Nanogram/s

NIL – Nilotinib

nM – Nanomolar

OCT-1 – Organic Cation Transporter 1

p- – Phosphorylated Form of Protein

PAGE – Polyacrylamide Gel Electrophoresis

PB – Peripheral Blood

PBMNC/s – Peripheral Blood Mononuclear Cell/s

PBS – Phosphate Buffered Saline

PDGFR – Platelet-Derived Growth Factor Receptor

PE – Phycoerythrin

P-gp – P-Glycoprotein

Ph – Philadelphia Chromosome

P_i – Inorganic Phosphate

PON – Ponatinib

PP – Pantoprazole

PPI/s – Proton Pump Inhibitor/s

PSC – PSC-833

p-value – Probability Value

PVDF – Polyvinylidene Difluoride

rcf – Relative Centrifugal Force

rho-123 – Rhodamine-123

RNA – Ribonucleic Acid

RO – Reverse Osmosis

RQ-PCR – Real Time Quantitative PCR

SD – Standard Deviation

SDS – Sodium Dodecyl Sulphate

sec – second/s

SEM – Standard Error of the Mean

SFK/s – Src Family Kinase/s

SH1/SH2/SH3 – Src Homology Region 1/2/3

S/N – Supernatant

Syk – Spleen Tyrosine Kinase

TBS – Tris Buffered Saline

TBST – Tris Buffered Saline +Tween®20

TKI/s – Tyrosine Kinase Inhibitor/s

TEA – Tetraethylammonium Bromide

TMD – Transmembrane Domain

U/mL – Units Per Millilitre

ver – Verapamil

WCF – White Cell Fluid

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