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In Vitro Investigation Of Intracellular Ponatinib Transport And Modeling Ponatinib Resistance In *BCR-ABL1*+ Cell Lines: Implications For Therapeutic Strategies

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Abstract

The use of tyrosine kinase inhibitors (TKIs), which target Bcr-Abl, has become the first-line treatment for chronic myeloid leukemia (CML). However, TKI resistance remains a major impediment to successful treatment of CML. The novel third generation pan-Bcr-Abl TKI ponatinib has demonstrated efficacy in overcoming single *BCR-ABL1* kinase domain (KD) mutation based resistance including *BCR-ABL1*^{T315I}, which inhibits the binding of all other available TKIs. While intracellular transport of the first and second generation TKIs have been studied, little is known about the complex interactions between ponatinib and drug transporters. Additionally, clinically significant mechanisms that may result in resistance to ponatinib remain to be elucidated. In this study, we investigated the interaction of ponatinib with drug transporters, and emerging modes of ponatinib resistance in previously TKI-naïve and dasatinib resistant *BCR-ABL1+* cell lines.

This study examined the role of ABCB1, ABCG2 and OCT transporters in ponatinib efflux and influx, as these transporters have been previously implicated in the transport of other TKIs. Results demonstrated neither ABCB1 ABCG2 nor OCT-1, play major roles in ponatinib transport. In addition, data revealed that ponatinib transport is not ATP/temperature dependent, and therefore is most likely to be passive.

To investigate potential resistance mechanism(s), ponatinib resistance was generated by exposure to increasing concentrations of ponatinib in *BCR-ABL1*+ cell-lines that either priorly treated with a TKI (dasatinib) or naïve to all TKIs. Two resistant cell lines, previously resistant to dasatinib and then treated with ponatinib, demonstrated the emergence of *BCR-ABL1* KD mutation(s). In one of these cell lines, the level of T315I increased from 44% to 66%, with *BCR-ABL1* mRNA expression also increasing. In the second cell line the compound mutation G250E/E255K developed. In contrast, the TKI (imatinib, nilotinib and dasatinib) naïve ponatinib resistant cell lines did not demonstrate *BCR-ABL1* KD mutations. Conversely, both of these resistant lines developed Bcr-Abl-independent resistance via Axl overexpression. Axl, a receptor tyrosine kinase, has previously been associated with TKI resistance. My studies are the first to report it in association with ponatinib resistance. In agreement with the observation that Axl overexpression causes ponatinib resistance, ponatinib sensitivity was restored following Axl inhibition or shRNA-mediated-knockdown of Axl.

In conclusion, the studies outlined in this thesis reveal that unlike other TKIs, ponatinib is not transported by ABCB1, ABCG2 or OCT-1 and therefore patients are unlikely to be susceptible to resistance caused by deregulation of these transporters. Moreover, the study also identified that in the setting of prior TKI-exposure, Bcr-Abl dependent mechanisms, such as *BCR-ABL1* KD mutations and *BCR-ABL1* mRNA overexpression, are likely to cause ponatinib resistance. However, in the TKI-naïve setting, Bcr-Abl-independent modes of resistance develop preferentially, and Axl

presents as a key mediator of this resistance. While further studies are required, particularly in reference to Axl expression in patients being treated with ponatinib, these data may suggest that combination therapeutic approached may be the most efficacious in the setting of up-front ponatinib use and subsequent development of resistance.

Abbreviations

µg – Microgram/s

µL – Microlitre/s

µM – Micromolar

7-AAD – 7-Aminoactinomycin D

^{14}C – Carbon-14 radioactive isotope

ABC – ATP Binding Cassette

ALL – Acute Lymphoblastic Leukaemia

AP – Accelerated Phase

ATCC – American Type Tissue Culture Collection

ATP – Adenosine Triphosphate

BC – Blast Crisis

BCR-ABL1 – Breakpoint Cluster Region-Abelson 1 (mRNA)

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

BSA – Bovine Serum Albumin

CCyR – Complete Cytogenetic Remission

cDNA – Complementary DNA

CHR – Complete Haematological Response

CML – Chronic Myeloid Leukaemia

CP – Chronic phase

CrkL – CT10 regulator of kinase-like Ct – Cycle Threshold

DAS – Dasatinib

DEPC – Diethylpyrocarbonate

DMSO – Dimethyl Sulphoxide

DNA – Deoxyribonucleic Acid

EDTA – Ethylenediaminetetraacetic Acid

FACS – Fluorescence Activated Cell Sorting

FCS – Foetal Calf Serum

FDA – Food and Drug Administration

GUSB Beta-glucuronidase

x g – see rcf

h – Hour/s

HBSS – Hanks Balanced Salt Solution

IC50 – Inhibitory Concentration 50

IFN – Interferon

IgG –Immunoglobulin G

IUR – Intracellular Uptake and Retention

kDa – 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

MW – Molecular Weight

ng – Nanogram/s

nM – Nanomolar

OCT-1 – Organic Cation Transporter 1

p – Phosphorylated Form of Protein

PAGE – Polyacrylamide Gel Electrophoresis

PB – Peripheral Blood PB

MNC/s – Peripheral Blood Mononuclear Cell/s

PBS – Phosphate Buffered Saline

PE – Phycoerythrin

P-gp – P-Glycoprotein

Ph – Philadelphia Chromosome

PON – Ponatinib

PSC – PSC-833

P value – Probability Value

PVDF – Polyvinylidene Difluoride

rcf – Relative Centrifugal Force

RNA – Ribonucleic Acid

RO – Reverse Osmosis

RQ-PCR – Real Time Quantitative PCR

SD – Standard Deviation

SDS – Sodium Dodecyl Sulphate

sec – second/s

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

TBS – Tris Buffered Saline

TBST – Tris Buffered Saline +Tween®20

TKI/s – Tyrosine Kinase Inhibitor/s

TEA – Tetraethylammonium Bromide

U/mL – Units Per Millilitre