CELL LINEAGE, CELL MATURITY AND BCR-ABL: FACTORS WHICH INFLUENCE IMATINIB UPTAKE IN CHRONIC MYELOID LEUKAEMIA

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candor dat viribus alas

(Sincerity gives wings to strength)

ipsa scientia potestas est

(Knowledge itself is power)

prefer et obdura; dolor hic tibi proderit olim

(Be patient and tough; some day this pain will be useful to you)

aut viam inveniam aut faciam

(I'll either find a way or make one)

per aspera ad astra

(Through adversities to the stars!)

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Jane Engler 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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PUBLICATIONS

Manuscripts

JR Engler, TP Hughes & DL White. OCT-1 as a determinant of response to anti-leukemic treatment. *Clinical Pharmacology and Therapeutics.* 2011. Feb 23; Epub ahead of print. (Impact Factor: 7.586)

JR Engler, AC Zannettino, C Bailey, J Rasko, TP Hughes & DL White. OCT-1 function varies with cell lineage but is not influenced by BCR-ABL. *Haematologica.* 2011. Feb;96(2):213-20. (Impact Factor: 6.416)

JR Engler, A Frede, V Saunders, AC Zannettino, DL White & TP Hughes. The poor response to imatinib observed in CML patients with low OCT-1 activity is not attributable to lower uptake of imatinib into their CD34+ cells. *Blood.* 2010. Oct;116(15):2776-8. (Impact Factor: 10.555)

JR Engler, A Frede, V Saunders, AC Zannettino, TP Hughes & DL White. Chronic myeloid leukemia CD34+ cells have reduced uptake of imatinib due to low OCT-1 activity. *Leukemia.* 2010. Apr;24(4):765-70. (Impact Factor: 8.296)

Conference Abstracts

JR Engler, C Bailey, J Rasko, AC Zannettino, DL White & TP Hughes. OCT-1 function varies with cell lineage but is not influenced by BCR-ABL. *New Directions in Leukaemia Research*, March 2010. Sunshine Coast, Australia. Poster Presentation.

JR Engler, A Frede, V Saunders, AC Zannettino, DL White & TP Hughes. OCT-1 activity in CML CD34+ cells is not predictive of molecular response to imatinib treatment in CP-CML patients, despite the strong predictive value of MNC OCT-1 activity. *American Society of Hematology Annual Meeting*, December 2009. New Orleans, USA. Poster Presentation.

JR Engler, A Frede, V Saunders, AC Zannettino, R D'Andrea, TP Hughes & DL White. CML CD34+ cells have reduced uptake of imatinib due to uniformly low OCT-1 activity, which can be increased with diclofenac treatment. *Centre for Stem Cell Research Annual Meeting*, November 2009. Adelaide, Australia. Poster Presentation.

JR Engler, A Frede, V Saunders, AC Zannettino, DL White & TP Hughes. OCT-1 activity in CML CD34+ cells is not predictive of molecular response to imatinib treatment in CP-CML patients. *Haematology Society of Australia and New Zealand Annual Meeting*, October 2009. Adelaide, Australia. Oral Presentation.

JR Engler, A Frede, AC Zannettino, DL White & TP Hughes. Reduced activity of the OCT-1 protein in primitive CML cells: A likely determinant of stem cell resistance in imatinib treated CML patients. *American Society of Haematology Annual Meeting*, December 2008. San Francisco, USA. Oral Presentation.

JR Engler, A Frede, S Quinn, AC Zannettino, TP Hughes & DL White. Reduced activity of the OCT-1 protein in primitive CML cells may be a key to stem cell resistance in imatinib treated CML patients. *New Directions in Leukaemia Research*, March 2008. Sunshine Coast, Australia. Poster Presentation.

SCHOLARSHIPS & AWARDS

Poster Prize, Centre for Stem Cell Research Annual Meeting. 2009.

For the abstract entitled “CML CD34+ cells have reduced uptake of imatinib due to uniformly low OCT-1 activity, which can be increased with diclofenac treatment”, Adelaide, November 2009.

Student Travel Scholarship, The Leukaemia Foundation of Australia. 2008.

Support for students to attend the New Directions in Leukaemia Research Conference. Awarded on the basis of submitted abstracts for work of exceptional novelty and significance. For the abstract entitled “Reduced activity of the OCT-1 protein in primitive CML cells may be a key to stem cell resistance in imatinib treated CML patients”, Sunshine Coast, March 2008.

PhD Scholarship, The Leukaemia Foundation of Australia. 2007-2010.

To provide support for the educational and professional development of researchers and other professionals undertaking a PhD. The award is to support basic, applied and translational research in Australia into the causes, treatment and care of people with leukaemia, lymphoma, myeloma and related blood disorders.

ABBREVIATIONS

ABL	Abelson kinase
ACD	Anticoagulent Citrate Dextrose Solution Formula A
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
AP	Accelerated phase
ATP	Adenosine triphosphate
BC	Blast crisis
BCR	Breakpoint cluster region
BM	Bone marrow
BSA	Bovine serum albumin
C	Celcius
CCR	Complete cytogenetic response
cDNA	Complementary deoxyribonucleic acid
CFSE	5-6-carboxyfluorescein diacetate, succinimidyl ester
CML	Chronic myeloid leukaemia
CMR	Complete molecular response
CP	Chronic phase
CPM	Counts per minute
Crkl	Crk-like protein
p-Crkl	Phosphorylated Crk-like protein
CV	Control vector
DEPC	Diethyl pyrocarbonate
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid

dNTPs	Deoxynucleotide triphosphates
DTT	Dithiothreitol
EDTA	Ethylene diamine tetraacetate
eGFP	Enhanced green fluorescence protein
FACS	Fluorescence activated cell sorting
FITC	Fluorescein isothiocyanate
FCS	Foetal calf serum
Hanks	Hanks Balanced Salt Solution
HSC	Haematopoietic stem cell
IC50	50% inhibitory concentration
IFN-α	Interferon alpha
IM	Imatinib (STI571)
IRIS	International randomised study of interferon versus STI571
IUR	Intracellular uptake and retention
kD	Kilo Dalton
L	Litre
M	Molar
mA	Milli Amp (10^{-3} Amp)
MACS	Magnetically activated cell sorting
MCR	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)

ng	Nano gram (10^{-9} gram)
Nil	Nilotinib (AMN107)
OA	OCT-1 activity
OCT-1	Organic cation transporter 1
PB	Peripheral blood
PBS	Phosphate Buffered Saline
PCR	Polymerase chain reaction
PE	Phycoerythrin
Ph	Philadelphia chromosome
PI	Propidium Iodide
PMA	Phorbol-12-myristate-13-acetate
PVDF	Polyvinylidene fluoride
RNA	Ribonucleic acid
RPM	Revolutions per minute
RPMI	Roswell Park Memorial Institute (media)
RT	Room temperature
RT-PCR	Reverse transcription polymerase chain reaction
RQ-PCR	Real time quantitative polymerase chain reaction
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SEM	Standard error of the mean
S/N	Supernatant
STI571	Signal transduction inhibitor 571 (imatinib)
TBS	Tris buffered saline
TBST	Tris buffered saline with 0.1% Tween20
TKI	Tyrosine kinase inhibitor

U	Units
UV	Ultraviolet
v/v	Volume per volume
WBC	White blood cells
WCC	White cell count
w/v	Weight per unit volume

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ABSTRACT

Despite the excellent responses observed in patients with chronic phase (CP) chronic myeloid leukaemia (CML) on imatinib therapy, approximately 25% display primary resistance or sub-optimal response. The organic cation transporter 1 (OCT-1) is the major active influx pump for imatinib in CML cells. The functional OCT-1 activity in mononuclear cells (MNC) is highly variable between patients and significantly correlates with a patient's molecular response to imatinib treatment and overall survival. Given the strong predictive value of OCT-1 activity, the present study was aimed at identifying factors responsible for the variation in OCT-1 activity seen in patients.

Pure populations of neutrophils, monocytes and lymphocytes were isolated from the peripheral blood of CML patients at diagnosis. The OCT-1 activity and OCT-1 mRNA expression was found to be the highest in the neutrophil population, followed by monocytes then lymphocytes. When the surface expression of the granulocytic antigens CD15 and CD16 were examined, a significant correlation was observed between MNC OCT-1 activity and the proportion of immature myeloid cells expressing CD15+16-. Interestingly, the neutrophil OCT-1 activity was found to be similar when recovered from CML patients at diagnosis, CML patients in cytogenetic remission and normal donors, implying that BCR-ABL expression is unlikely to influence OCT-1 activity. This hypothesis was confirmed in a cell line model, in which ectopic BCR-ABL expression was not found to directly affect OCT-1 expression or function, but stimulated myeloid differentiation which, in turn, led to increased OCT-1 activity. These data suggest that the predictive MNC OCT-1 activity is most strongly related to cell lineage, particularly the proportion of immature myeloid cells, but is not directly related to BCR-ABL.

CML early progenitor cells are less sensitive to imatinib induced apoptosis and are likely contributors to disease persistence. It was found that the OCT-1 activity and OCT-1 mRNA expression was significantly lower in primitive CD34+ cells compared with mature CD34- cells recovered from CML patients. These results indicate that low imatinib accumulation in primitive CML cells may be a critical determinant of long-term disease persistence. Studies to investigate whether the MNC OCT-1 activity provides a surrogate indicator of effective targeting of the more immature CD34+ cells failed to identify a relationship between high CD34+ OCT-1 activity and the achievement of major molecular response. This is despite the confirmation of previous findings that high MNC OCT-1 activity is significantly associated with the achievement of major molecular response to imatinib treatment. These important findings suggest that kinase inhibition in these mature cells, and not the CD34+ cells, may be the key determinant of response in CML.

In conclusion, the studies outlined in this thesis have identified cell lineage as a key contributor to MNC OCT-1 activity and hence response to imatinib treatment. While primitive CD34+ cells demonstrate low OCT-1 activity, which may contribute to their persistence despite imatinib therapy, the OCT-1 activity in these cells does not correlate with patient response to treatment. Therefore, direct targeting of this primitive population may not be essential for achievement of early and deep molecular responses.