

# **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)

*patient 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

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**Jane Engler**

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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)

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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.

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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

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

<b>dNTPs</b>	Deoxynucleotide triphosphates
<b>DTT</b>	Dithiothreitol
<b>EDTA</b>	Ethylene diamine tetraacetate
<b>eGFP</b>	Enhanced green fluorescence protein
<b>FACS</b>	Fluorescence activated cell sorting
<b>FITC</b>	Fluorescein isothiocyanate
<b>FCS</b>	Foetal calf serum
<b>Hanks</b>	Hanks Balanced Salt Solution
<b>HSC</b>	Haematopoietic stem cell
<b>IC50</b>	50% inhibitory concentration
<b>IFN-<math>\alpha</math></b>	Interferon alpha
<b>IM</b>	Imatinib (STI571)
<b>IRIS</b>	International randomised study of interferon versus STI571
<b>IUR</b>	Intracellular uptake and retention
<b>kD</b>	Kilo Dalton
<b>L</b>	Litre
<b>M</b>	Molar
<b>mA</b>	Milli Amp ( $10^{-3}$ Amp)
<b>MACS</b>	Magnetically activated cell sorting
<b>MCR</b>	Major cytogenetic response
<b>MMR</b>	Major molecular response
<b>mM</b>	Milli Molar ( $10^{-3}$ Molar)
<b>MNC</b>	Mononuclear cells
<b>mRNA</b>	Messenger ribonucleic acid
<b><math>\mu</math>M</b>	Micro Molar ( $10^{-6}$ Molar)
<b><math>\mu</math>g</b>	Micro gram ( $10^{-6}$ gram)

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

<b>U</b>	Units
<b>UV</b>	Ultraviolet
<b>v/v</b>	Volume per volume
<b>WBC</b>	White blood cells
<b>WCC</b>	White cell count
<b>w/v</b>	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.