Defining CP-CML Patient Subsets Associated with Poor Imatinib Uptake and Response

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The introduction of tyrosine kinase inhibitor (TKI) therapy, specifically imatinib, has dramatically improved the treatment outcome for the majority of chronic phase chronic myeloid leukaemia (CP-CML) patients. Although most patients will achieve excellent clinical (haematological, cytogenetic and molecular) responses on imatinib, it is clear that a subset of patients will respond poorly, or fail imatinib therapy. Currently, up to 35% of patients treated with imatinib fit into this subset, displaying either primary or acquired resistance, leading to sub-optimal response or imatinib failure. The organic cation transport-1 (OCT-1) protein is the major active protein involved in imatinib transport. Measuring the function of OCT-1 in leukaemic mononuclear cells prior to imatinib therapy, expressed as OCT-1 activity (OA), has been demonstrated to be a strong prognostic indicator. Notably, low OA is strongly associated with patients at significant risk of poor molecular response, mutation development and leukaemic transformation during imatinib therapy. It is important to therefore determine what factors underlie the range of OA levels observed in CP-CML patients, and whether patients with very low OA and poor response to imatinib have different overall disease characteristics associated with alternative biological mechanisms.

The present study sought to 1) determine the variation in CP-CML patient immunophenotype at diagnosis, in relation to patient characteristics, including OA; 2) determine the gene expression patterns associated with OA, and identify new biomarkers for CP-CML; and 3) determine the global DNA methylation profile of CP-CML, with particular focus on very low OA, and ascertain whether aberrant epigenetic programming may underlie poor imatinib response. Specific lineage differences were identified, with patients defined as very low OA associated with a decreased T-lymphocyte signature, compared to very high OA. Furthermore, an up-regulated histone gene signature associated with very low OA was
identified. Gene expression analysis also identified GFI1 as a novel biomarker for progression in CP-CML, as patients with low diagnostic GFI1 expression in their white cells were at significant risk of disease transformation to blast crisis (BC), even when receiving TKI therapy. Additionally, significant differences in global DNA methylation patterns were identified between CP-CML and normal individuals; CP-CML patients with very low OA, compared to all other patients; and CP-CML versus BC. Importantly, this is the first report of global DNA methylation analysis in CML and identifies that aberrant epigenetic programming may have a significantly greater role in CML than originally first thought.

In conclusion, the findings detailed in this thesis provide further insight into the heterogeneity of CP-CML, and to a lesser extent OA. Additionally, a greater understanding of the possible factors influencing OA determination is presented, along with a new biomarker (GFI1) for disease progression to blast crisis in de novo CP-CML. Finally, the global DNA methylation results may present novel targets and pathways responsible for poor TKI response that will assist in developing new therapeutic strategies for de novo CP-CML patients, involving combination therapy to enhance patient outcome.
DECLARATION

I, Dale B. Watkins, 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Dale B. Watkins

1st December 2013
PUBLICATIONS

Manuscripts


* denotes equal first-authors.


Conference Abstracts


Watkins DB, Kok CH, Hughes TP, D’Andrea RJ, White DL. Global DNA methylation analysis identifies key pathway differences between poor (Low OCT-1 Activity) and standard risk CP-


SCHOLARSHIP AND AWARDS

PhD Scholarship; The Leukaemia Foundation of Australia, 2010 – 2013
Support for the educational and professional development of researchers and other professionals undertaking a PhD. The award is to support research in Australia into the causes, treatment and care of people with leukaemia, lymphoma, myeloma and related blood disorders, and is awarded on the merits of the applicant and project proposal.

Dawes Top-Up Scholarship; RAH/IMVS Research Committee, 2011 – 2013
Top-up scholarships are awarded to applicants in receipt of a major external scholarship based on merit and research proposal.

SAHMRI (Poster) Prize; The University of Adelaide, Faculty of Health Sciences Postgraduate Research Conference, 2013
For the abstract entitled “Global DNA methylation profiling in CP-CML, with emphasis on a poor-risk subset of CP-CML and during disease progression to Blast Crisis”, Adelaide, SA; August 2013.

Non-Member Travel Grant, HAA, 2012
School of Medicine Poster Prize; The University of Adelaide, Faculty of Health Sciences

Postgraduate Research Conference, 2012

For the abstract entitled “Global DNA methylation profiling in a poor-risk subset of CML”, Adelaide, SA; August 2012.

Leukaemia Foundation Poster Prize; New Directions in Leukaemia Research (NDLR) Conference, 2012

For the abstract entitled “Identification of differential lineage involvement and subsequent development of a predictive classifier for poor risk chronic-phase CML patients”, Sunshine Coast, QLD; March 2012.

Australian Postgraduate Award, Australia Government, 2010

Unable to accept due to acceptance of the LFA primary PhD scholarship.
ABBREVIATIONS

µ-bcr – Micro Breakpoint Cluster Region

µg – Microgram/s

µL – Microlitre/s

µM – Micromolar

$^{[14]}$C – Carbon-14 Radioactive Isotope

450K – Illumina Infinium® HumanMethylation450 BeadChip

ABC – ATP-Binding Cassette

ABL1 – Abelson Leukaemia Virus Proto-Oncogene Homolog 1

ADP – Adenosine Diphosphate

AGRF – Australian Genome Research Facility

ALL – Acute Lymphoblastic Leukaemia

AML – Acute Myeloid Leukaemia

AP – Accelerated Phase

Ara-C – Arabinofuranosyl Cytidine (Cytarabine)

ATCC – American Type Tissue Culture Collection

ATP – Adenosine Triphosphate

BC – Blast Crisis

BCR – Breakpoint Cluster Region

BCR-ABL1 – Fusion Gene

BCR-ABL1 – Fusion mRNA

Bcr-Abl1 – Fusion Protein

BH-FDR - Benjamini-Hochberg adjusted FDR

BM – Bone Marrow

BMA – Bayesian Model Averaging
bp – Base Pair

BSA – Bovine Serum Albumin

C – Celcius

CCyR – Complete Cytogenetic Response (absence of Ph-positive cells as measured by classical karyotyping or fluorescence in-situ hybridisation)

cDNA – Complementary DNA

CGI – CpG Island

ChIP – Chromatin Immunoprecipitation

CHR – Complete Haematological Response (sustained and significant reduction in WBCs to a normal range)

CMA – Classification for Microarrays

CML – Chronic Myeloid Leukaemia

CMR – Complete Molecular Response (BCR-ABL1 mRNA levels negative in 2 consecutive assays)

CP – Chronic Phase

CpG – Cytosine-phosphate-Guanine

Crkl – C1T10 regulator of kinase like

Ct – Cycle Threshold

CTL – Cytotoxic T-Lymphocyte

DD – Dimerization Domain

DEPC – Diethylpyrocarbonate

DMSO – Dimethyl Sulphoxide

DNA – Deoxyribonucleic Acid

dNTP - Deoxyribonucleotide

EDTA – Ethylenediaminetetraacetic Acid
EFS – Event-Free Survival
ELN – European Leukemia-Net
EMR – Early Molecular Response (3 month BCR-ABL1 mRNA levels < 10%)
ERK – Extracellular Signal-regulated Kinase
EUTOS – European Treatment and Outcome Study
FACS – Fluorescence Activated Cell Sorting
FC – Fold Change
FCS – Foetal Calf Serum
FDA – Food and Drug Administration, United States of America
FDR – False Discovery Rate
g – also known as rcf (Relative Centrifugal Force)
GDP – Guanidine Diphosphate
GSEA – Gene-Set Enrichment Analysis
GTP – Guanidine Triphosphate
h – Hour/s
HBSS – Hanks Balanced Salt Solution
HLA – Human Leukocyte Antigen
HPC – Haematopoietic Progenitor Cell
HSC/s – Haematopoietic Stem Cell/s
HTqPCR – High-Throughput Quantitative PCR
IC50 – 50% Inhibitory Concentration
IFN-α – Interferon-α
IUR – Intracellular Uptake and Retention
kD – Kilo Dalton
KD – Kinase Domain
L – Litre/s
LIMMA – Linear Models for Microarray Data
LSC/s – Leukaemic Stem Cell/s
M – Molar
M-bcr – Major Breakpoint Cluster Region
m-bcr – Minor Breakpoint Cluster Region
MAP – Mitogen-Activated Protein
MCyR – Major Cytogenetic Response (> 35% Ph+ metaphases)
MDR – Multi-Drug Resistance
MDS – Multi-Dimensional Scaling
MeV – Multi-Experiment Viewer
MFI – Mean Fluorescence Intensity
mg – milligram/s
min – Minutes/s
mL – Millilitre/s
mM – Millimolar
MMR – Major Molecular Response (BCR-ABL1 mRNA levels < 0.1%)
MNC/s – Mononuclear Cell/s
MQ – Milli-Q
mRNA – Messenger RNA
MSigDB – Molecular Signatures Database
MW – Molecular Weight
ND – Not Determined
ng – Nanogram/s
NLS – Nuclear Localisation Signal
nM – Nanomolar
OA – OCT-1 Activity
OCT-1 – Organic Cation Transporter 1
OS – Overall Survival
p- – Phosphorylated Form of Protein
P-loop – Nucleotide Binding Loop
P-value – Probability Value
PAM – Prediction Analysis for Microarrays
PB – Peripheral Blood
PBS – Phosphate Buffered Saline
PFS – Progression-Free Survival
Ph – Philadelphia Chromosome
PI-3K – Phosphoinositide 3-Kinase
PMNC/s – Polymorphonuclear Cell/s
PVDF – Polyvinylidene Difluoride
QC – Quality Control
RIPA – Radioimmunoprecipitation Buffer
RMA – Robust Multi-Array Averaging
RNA – Ribonucleic Acid
RO – Reverse Osmosis
ROC – Receiver Operating Characteristic
RPMI – Roswell Park Memorial Institute (media)
RQ-PCR – Quantitative Reverse Transcription-Polymerase Chain Reaction
SD – Standard Deviation
SDS-PAGE – Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
sec – second/s

SEM – Standard Error of the Mean

SH – Src-Homology Region

SNP/s – Single Nucleotide Polymorphism/s

STAT – Signal Transducer and Activation of Transcription

SWAN – Subset-Quantile Within Array Normalisation

TBS – Tris Buffered Saline

TBST – Tris Buffered Saline +Tween®20

TF – Transcription factor

TFS – Transformation-Free Survival

TGA – Therapeutic Goods Administration, Australia

TKI/s – Tyrosine Kinase Inhibitor/s

TLDA – TaqMan® Low Density Array

TMDs – Transmembrane Domains

TSS – Transcription Start Site

TWC/s – Total White Cell/s

U/mL – Units Per Millilitre

UTR – Untranslated Region

WBC/s – White Blood Cell/s

WCC – White Cell Count

WCF – White Cell Fluid
CLINICAL TRIALS REFERRED TO IN THIS THESIS

ALLG TIDEL II (CML9)

A Phase II study in adult patients with newly-diagnosed chronic phase, chronic myeloid leukaemia of initial intensified imatinib therapy, and sequential dose-escalation followed by treatment with nilotinib in suboptimal responders to determine the rate and duration of major molecular response.

Official Title: Australasian Leukaemia and Lymphoma Group (ALLG) Therapeutic Intensification in DE-novo Leukaemia (TIDEL) II trial

Trial ID: ACTRN12607000325404 (http://www.ANZCTR.org.au)

ENESTxtnd

A Phase III study in adult patients to further investigate the safety and efficacy of nilotinib in newly diagnosed chronic myeloid leukaemia patients in the chronic phase.

Official Title: Extending Molecular Responses With Nilotinib in Newly Diagnosed Chronic Myeloid Leukemia (CML) Patients in Chronic Phase (ENESTxtnd) trial

Trial ID: NCT01254188 (http://www.clinicaltrials.gov)

IRIS

A prospective, multi-centre, open-label, randomised Phase III study in adult patients with newly diagnosed chronic-phase chronic myeloid leukaemia to compare the effectiveness of imatinib (STI571) with that of Interferon-α plus low-dose Cytarabine (Ara-C).

Official Title: International Randomised Study of Interferon and STI571 (IRIS) trial

Trial ID: NCT00006343 (http://www.clinicaltrials.gov)
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