The Role of Cytokines in governing the Expansion of the T315I mutation in Chronic Myeloid Leukaemia

Oi-Lin Lee

The Melissa White Laboratory

Cancer Theme, South Australian Heath and Medical Research Institute (SAHMRI)

Department of Haematology, SA Pathology

Centre for Personalised Cancer Medicine (CPCM), University of Adelaide

Cancer for Cancer Biology, University of Adelaide

Adelaide, South Australia

&

Faculty of Health Sciences

School of Medicine

Discipline of Paediatrics

The University of Adelaide

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Abbreviations

7-AAD	7-Aminoactinomycin D
ABCB1	ATP binding cassette, sub-family B1
ABCG2	ATP binding cassette, sub-family G2
ABL1	Abelson murine leukaemia virus human homologue 1 gene
ASK1	Apoptotic signal kinase
Akt	a serine threonine kinase (also known as protein kinase B)
ALL	Acute lymphoblastic leukaemia
Ann V	Annexin V
AP	Accelerated phase
ATP	Adenosine triphosphate
Bad	BCL-2 antagonist of cell death
Bak	Bcl-2 homologous antagonist killer
Bax	Bcl-2 associated X protein
BC	Blast crisis
Bcl-2	B cell lymphoma 2
Bcl-X_L	B-cell lymphoma-extra large
BCR	Breakpoint cluster region
BCR-ABL1	*BCR-ABL1* oncogene
BELA trial	Bosutinib efficacy and safety in CML
BH	Bcl-2 homology
Bim	BCL-2 interacting mediator of cell death
BM	Bone marrow
BMSC	Bone marrow stromal cells
bp	Nucleotide base pair

BP	Blastic phase
BSA	Bovine serum albumin
Btk	Bruton's tyrosine kinase
Ca^{2+}	Calcium ion
CaCl	Calcium choride
CBL	Casitas B-lineage lymphoma proto-oncogene
CCyR	Complete cytogenetic response
CD	Cluster of differentiation
cDNA	Complementary DNA
C/EBP-α	CCAAT enhancer-binding protein α
CFSE	Carboxyfluorescein diacetate succinimidyl ester
Chr	Chromosome
CHR	Complete haematological response
CML	Chronic myeloid leukaemia
CMR	Complete molecular response
CSL	Commonwealth Serum Laboratory
CO_2	Carbon dioxide
CP	Chronic phase
Crk	CT10 sarcoma oncogene cellular homologue
Crkl	CT10 regulator of kinase-like
CXCL12	C-X-C motif chemokine 12 (also known as Stromal cell-derived factor 1)
CXCR4	C-X-C motif chemokine receptor 4
DAG	Diacylglycerol
das	Dasatinib

DASISION	Dasatinib versus Imatinib Study in Treatment-Naïve CML Patients
DEPC	Diethyl pyrocarbonate
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphates
DTT	Dithiothreitol
ECF	Enhanced chemifluorescence substrate
EDTA	Ethylenediaminetetra-acetic acid
eGFP	Enhanced green fluorescent protein
EGFR	Epidermal growth factor receptor
ENESTnd	Evaluating Nilotinib Efficacy and Safety in Clinical Trials–Newly Diagnosed Patients
Erk	Extracellular signal related kinase
et al	*et alia*
ETV6-PDGFRβ	ETS translocation variant 6- Platelet derived growth factor receptor β gene
FACS	Fluorescent activated cell sorting
FBS	Foetal bovine serum
FDA	Federal Drug Authority
Fgr	Gardner-Rasheed feline sarcoma viral oncogene homologue
FGF	Fibroblast growth factor
FGFb	Fibroblast growth factor basic (also known as FGF-2)
FGFR	Fibroblast growth factor receptor
FIP1L1-PDGFRα	Fip 1 like 1- Platelet derived growth factor receptor α gene
FLT3-ITD	FMS-like tyrosine kinase 3- internal tandem domain

c-FMS	Cellular homologue of the feline sarcoma virus, v-FMS
FOXO	Forkhead O transcription factor
FOXO 3A	Forkhead O family of transcription factors 3A
g	Gravitational force (Relative centrifugation force)
Gab1	GRB2-associated-binding protein 1
Gab2	GRB2-associated binding protein 2
GAP	Guanosine triphosphatase-activating protein
G-CSF	Granulocyte colony stimulating factor
GDP	Guanosine diphosphate
GIST	Gastrointerstinal stromal tumours
GM-CSF	Granulocyte macrophage colony stimulating factor
Grb2	Growth factor receptor bound protein 2
GTP	Guanosine triphosphate
KD	Kinase domain
Hck	Haematopoietic cell kinase
HGF	Hepatocyte growth factor
hnRNP-E2	Heterogeneous nuclear ribonucleoprotein- E2
HSC	Haematopoietic stem cell
HSCT	Haematopoietic stem cell transplantation
IC50	Concentration of drug required to inhibit Bcr-Abl kinase activity by 50%
IFNα	Interferon-α
IκB	Inhibitor of NF-κB
IKKα	Inhibitor of nuclear factor kappa B kinase subunit α
IL-1α	Interleukin 1α

IL-1β	Interleukin 1β
IL-6	Interleukin 6
IL-8	Interleukin 8
im	Imatinib mesylate
IMDM	Iscove's modification of Dulbecco's medium
IP3	Inositol triphosphate
IRIS	International randomised study of interferon versus STI571
IS	International standard
Jak	Janus kinase
JNK	c-Jun N-terminal kinase
kb	Kilo base pairs
kDa	kilo Dalton
Lck	Lymphocyte-specific protein tyrosine kinase
LIF	Leukaemia inhibitory factor
LSC	Leukaemic stem cells
Lyn	Lck/Yes-related novel protein
mA	mili (10^{-3}) Ampere
MAPK	Mitogen activated protein kinase
M-bcr	Major breakpoint cluster region
m-bcr	minor breakpoint cluster region
MCyR	Major cytogenetic response
MCP-1	Monocyte chemo attractant protein 1
MDC	Macrophage derived chemokine
MDR1	Multiple drug resistant protein 1
MEK	MAP kinase/Erk kinase

MET	Mesenchymal epithelial transition factor
MFI	Mean fluorescent intensity
µ	micro (10^{-6})
$MgCl_2$	Magnesium chloride
MIP-1α	Macrophage inflammatory factor 1α
MIP-1β	Macrophage inflammatory factor 1β
ml	mili (10^{-3}) litre
mM	mili (10^{-3}) Molar
MMR	Major molecular response
mRNA	Messenger Ribonucleic acid
MSC	Mesenchymal stem cells
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
mTOR	Mammalian Target of Rapamycin
NaCl	Sodium chloride
NaN_3	Sodium azide
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
ng	nanogram (10^{-9} gram)
nil	Nilotinib
nM	nano (10^{-9}) Molar
NSCLC	Non-small cell lung cancer
OA	Organic cation transporter 1 activity
OCT-1	Organic cation transporter 1
PACE	Ponatinib Ph+ ALL and CML Evaluation
PBS	Phosphate buffered saline or Pharmaceutical Benefit Scheme

PCR	Polymerase chain reaction
PDGFRα	Platelet derived growth factor receptor α
PE	Phycoerythrin
pg	pico (10^{-12}) gram
Ph	Philadelphia
PI	Proliferation index
PI3K	Phosphotidylinositol-3 kinase
PIP2	Phosphatidylinositol-4,5-biphosphate
PKC	Protein kinase C
PLCγ	Phospholipase Cγ
P-loop	Phosphate binding loop
PS	Phosphotidylserine
PUMA	p53 up regulated modulator of apoptosis
PVDF	Polyvinylidene fluoride
r(IL-6)	Recombinant (IL-6)
Rac	Ras-like guanosine triphosphatase
Raf	Rapidly accelerated sarcoma protein kinase
Ras	Rat sarcoma
RET	Rearranged during transfection proto-oncogene
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rpm	Revolutions per minute
RPMI	Roswell Park Memorial Institute (media)
RQ-PCR	Real-time quantitative polymerase chain reaction
SA Pathology	South Australia Pathology Services

SAPK	Stress activated protein kinase
SCF	Stem cell factor
SDF-1	Stromal cell derived factor 1
SDM	Serum deprived media
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	Standard error of the mean
SFK	Src family kinase
SH	Src homology domains
SHC	Src homology 2 domain-containing-transforming protein C1
SKP2	S-phase kinase-associated protein 2
SOS	Son of Sevenless guanine nucleotide exchange factor
STI571	Imatinib
STAT	Signal transducer and activator of transcription family
TAE	Tris-Acetate-EDTA
TBS	Tris buffered saline
TBST	Tris buffered saline with 0.1% Tween20
TGF-α/β	Transforming growth factor α/β
TKI	Tyrosine kinase inhibitor
TNF-α	Tumour necrosis factor-α
V	Volts
VCAM	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor
WB	Western blot
WT	Wild-type

Abstract

Since the introduction of imatinib, the outlook for patients with chronic myeloid leukaemia (CML) has dramatically improved. However, approximately 30-40% of patients develop intolerance or resistance to the drug and cease therapy. Among those who develop resistance, the most common cause is the development of point mutations in the kinase domain (KD) of *BCR-ABL1* which impair drug binding and hence result in the loss of kinase inhibition. The T315I mutation (gatekeeper mutation) is of particular significance as it is one of the most common mutations and it is resistant to all 3 tyrosine kinase inhibitors (TKI) currently approved for therapeutic use in Australia.

There is evidence to suggest that KD mutations in *BCR-ABL1* may alter the biological activity of Bcr-Abl. Both *in vitro* and clinical studies have suggested that the T315I mutation results in greater oncogenic potential. Patients harbouring this mutation have poorer prognoses and a significantly higher rate of progression compared to other mutations. There is also ample evidence to suggest that secretion of cytokines and growth factors play a role in rendering *BCR-ABL1* positive cells resistant to TKI.

This thesis focuses on the role of cytokines in the resistance mechanism of cells with the T315I mutation and how this mechanism is achieved. K562-T315I cells were developed in our laboratory by exposing them to increasing levels of dasatinib over several months. HL60 cell lines were virally transduced with the *BCR-ABL1*^{p210} and *BCR-ABL1*^{T315I} constructs. Investigations have identified that several soluble factors are preferentially secreted by cells with the T315I mutation namely, FGF-2, IL-8, MCP-1 and G-CSF. Additionally, the supernatant of K562-T315I cells also contains higher concentrations of GM-CSF and IL-6. These studies also identified that FGF-2 was able to protect K562 naïve cells from TKI-induced cell death and suggests that this occurs via activation of the MAPK and STAT5 pathways. Thus, in addition to acquiring point mutations that result in the inability of TKIs to bind Bcr-Abl, the T315I mutation also results in overexpression of FGF-2 which can confer resistance to non-mutated cells.

Furthermore, in the presence of imatinib, dasatinib and nilotinib, K562-T315I cells proliferate and survive better than in the absence of a TKI. This is due to hyperactivation of the MAPK pathway whereas signalling of other pathways, JAK/STAT5 and PI3K/Akt are not increased.

This phenomenon was demonstrated especially with nilotinib but studies did not indicate a cytokine mediated effect through an autocrine hypersecretion by the K562-T315I cells. Nevertheless, this finding may be one of the reasons why the T315I mutation confers a worse outcome in patients with CML if they remain on imatinib, nilotinib and dasatinib treatment.

Thesis Declaration

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