Investigating the effects of ABL kinase inhibitors on the signalling and function of normal leukocytes and leukemic cells

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Declaration

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Stephen Blake

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Abstract

Specifically targeting the oncogenic proteins that cause cancers is a rapidly growing field of laboratory and clinical research. The paradigm of this field is the tyrosine kinase inhibitor imatinib, which specifically targets chronic myeloid leukaemia (CML) by inhibiting the kinase activity of the BCR/ABL oncogene. Whilst highly successful for the treatment of CML, 10-20% of patients are resistant to imatinib and require an alternative treatment. Identification of the poor responding patients is important and many different methods have been used to determine patient prognosis. In this study, flow cytometric detection of protein phosphorylation was tested as a method to determine patient prognosis and stat5 was identified as a useful marker in CML. Although only a small group of patients was analysed, there appeared to be a correlation between the percentage of cells with detectable p-stat5 and cellular response to imatinib.

A need for alternative therapies for patients failing imatinib has led to the development of more potent second generation ABL kinase inhibitors. The currently approved second generation inhibitors nilotinib and dasatinib are respectively around 20 and 300 times more potent at inhibiting ABL than imatinib. While imatinib blocks ABL kinase activity, it also inhibits several other kinases including LCK, a Src-family kinase involved in T-cell development and activation. Imatinib has been shown to affect the function of normal T-cells *in vitro* and *in vivo*, likely through inhibition of LCK and thus, the impact of the more potent second generation ABL inhibitors on T-cell function was tested. Nilotinib was found to inhibit T-cell function *in vitro*, affecting T-cell proliferation activation and cytokine production approximately twice as potently as imatinib. Like imatinib, nilotinib also blocked LCK kinase activity with an IC50 of

550 nM, half the IC50 for imatinib that was approximately 1250 nM. Dasatinib has previously been shown to potently inhibit LCK with an IC50 of 1.1 nM, and accordingly was shown to strongly inhibit all T-cell functions tested *in vitro*. Dasatinib was also shown to inhibit natural killer cytotoxicity at a similar potency to T-cells, most likely through inhibition of Src-family kinases LCK and FYN.

While T-cell suppression is a potential side effect during cancer therapy, it suggests ABL kinase inhibitors may be a useful for treating diseases involving T-cells such as rheumatoid arthritis. To examine their potential to add to conventional T-cell suppressive regimes, T-cell proliferation in the presence of kinase inhibitors in combination with a standard immuno-suppressive drug cyclosporine. This was performed using a novel flow cytometry based method utilizing CFSE dye to detect cell proliferation. Using this method, both dasatinib and imatinib were shown to act in synergy with cyclosporine to block T-cell proliferation for most stimuli. However, following stimulation with anti-CD3/CD28 microbeads the interaction between dasatinib and cyclosporine was found to be strongly antagonistic. Overall this study has characterised the off-target effects of nilotinib and dasatinib on immune cell functions, and developed a new flow cyometric method which can be used to determine interactions between any drugs that inhibit cell proliferation.

Abbreviations

7AAD	7-aminoactinomycinD
ABL	Abelson kinase
AKI	ABL kinase inhibitor
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
AP	Accelerated phase
ATP	Adenosine triphosphate
BC	Blast crisis
BCR	Breakpoint cluster region
BM	Bone marrow
bp	Base pairs
BSA	Bovine serum albumin
C	Celcius
cDNA	Complementary deoxyribonucleic acid
CFSE	5-6-carboxyfluorescein diacetate, succinimidyl ester
CI	Combination index
CML	Chronic myeloid leukaemia
ConA	Concanavalin A
CP	Chronic phase
CPM	Counts per minute
CsA	Cyclosporine A
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetraacetate

ELISA	Enzyme linked immunosorbent assay
FACS	Fluorescence activated cell sorting
FITC	Fluorescein isothiocyanate
FCS	Foetal calf serum
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte monocyte colony stimulating factor
Grb2	Growth factor receptor bound Protein 2
HBSS	Hanks Balanced Salt Solution
HLA	Human leukocyte antigen
HRP	Horseradish peroxidise
IC50	50% inhibitory concentration
IFN-α	Interferon alpha
IFN-γ	Interferon gamma
IL-3	Interleukin-3
JAK	Janus Kinase
kD	Kilo Dalton
L	Litre
LCK	Leukocyte specific protein tyrosine kinase
LFU	Lytic formic unit
M	Molar
mA	Milli Amp (10^{-3} Amp)
MACS	Magnetically activated cell sorting
MAPK	Mitogen activated protein kinase
MFI	Mean fluorescence intensity
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)
ND	Not done
NK	Natural killer
NS	Non-stimulated
ng	Nano gram (10^{-9} gram)
PBMC	Peripheral blood mononuclear cell
PBA	PBS with BSA
PBS	Phosphate Buffered Saline
PBS-T	PBS with Tween 20®
PCR	Polymerase chain reaction
PE	Phycoerythrin
Pg	Pico gram (10^{-12} gram)
PFA	Paraformaldehyde
Ph	Philadelphia chromosome
PHA	Phytohaemagglutinin
Phe	Phenylalanine
PI	proliferation index
PI3-K	Phosphotidylinositol – 3-kinase
PKA	cAMP- dependent protein kinase
PVDF	Polyvinylidene Fluoride
RCF	Relative centrifugal force
RF	Responder frequency
RNA	Ribonucleic acid
RPM	Revolutions per minute

RPMI	Roswell Park Memorial Institute (media)
RO	Reverse osmosis
RQ-PCR	Reverse transcription quantitative polymerase chain reaction
RT	Room temperature
RT-PCR	Reverse transcription polymerase chain reaction
SD	Standard deviation
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Elctrophoresis
SFK	Src-family tyrosine kinase
TEMED	1,2-bis(dimethylamino)- ethane
TKI	Tyrosine kinase inhibitor
Tyr	Tyrosine
UV	Ultraviolet
v/v	Volume per volume
WCC	White cell count
WCF	White cell fluid
w/v	Weight per unit volume

Publications

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