Haemopoiesis, Leukaemia & Imatinib: c-fms, a Novel Target for Small Molecule Inhibitor Therapy

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TABLE OF CONTENTS

DECLARATION

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.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Andrea Dewar

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tougher without you by my side. You are an extremely wise person, and your perspective has always provided me with guidance and the inspiration to keep going with a smile on my face. And as a well known song sums it up, “I want to thank you for giving me the best day of my life…. to be with you is having the best day of my life”.

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“Obstacles cannot crush me;
every obstacle yields to stern resolve”

Leonardo da Vinci

“Don’t wait for a light to appear at the end of the tunnel;
stride down there and light the bloody thing yourself!”

Sara Henderson

ABSTRACT

Understanding the factors that regulate the growth and differentiation of haemopoietic stem cells (HSC) remains a major challenge. In this study, the proliferation and differentiation of $CD34^+$ cells from normal donors and chronic myeloid leukaemia (CML) patients was compared. The proliferation and entry of CML cells into the cell cycle was decreased relative to cells from normal donors, and greater heterogeneity in the phenotype of CML cells at the initiation of culture was observed. Analysis of phenotype concomitant with cell division also demonstrated that the differentiation of normal $CD34^+$ cells was consistent between donors, while marked variability was observed in the differentiation of $CD34^+$ cells from CML patients. This included expression of CD13, CD33, CD38 and HLA-DR, which were linked to cell division in normal but not CML cells.

The tyrosine kinase inhibitor, imatinib, is a novel drug displaying promising results in the treatment of CML by specifically inhibiting the growth of leukaemic cells. To examine whether myelosuppression observed in patients treated with imatinib may arise from inhibition of normal haemopoiesis, imatinib was added to colony assays established using cells from normal bone marrow. Suppression of monocyte/macrophage growth, but not that of eosinophils or neutrophils, was observed at therapeutic concentrations of imatinib. Inhibition of monocytic differentiation to macrophages was also observed and was associated with decreased functional capacity such as altered antigen uptake, production of proinflammatory cytokines and stimulation of responder cells.

The specific suppression of monocyte/macrophage differentiation and function was not due to blockade of tyrosine kinases known to be inhibited by imatinib and was consistent with an inhibition of the M-CSF/c-fms signalling pathway. This hypothesis was tested using a cell line that was dependent on M-CSF for growth and survival. Cell proliferation and phosphorylation of c-fms were inhibited at an IC_{50} of 1.9μM and 1.4μM imatinib respectively and this was not attributable to decreased c-fms expression. These important findings therefore identify c-fms as a further target of imatinib, and suggest that imatinib should be considered for treatment of diseases where c-fms is implicated. This includes breast and ovarian cancer and inflammatory conditions such as rheumatoid arthritis. Potential side effects resulting from imatinib treatment must also be considered.

ABBREVIATIONS

μg	Micro gram
μm	Micro metre
μM	Micro molar
4HGF	Four haemopoietic growth factors (IL-3, IL-6, G-CSF, GM-CSF)
5HGF	Five haemopoietic growth factors (IL-3, IL-6, G-CSF, GM-CSF, SCF)
ATP	Adenosine triphosphate
BM	Bone marrow
BMMNC	Bone marrow mononuclear cell
BSA	Bovine serum albumin
BSA	Bovine serum albumin
CD	Cluster of differentiation
CFSE	Carboxyfluorescein diacetate succinimidyl ester
CM	Cultured monocyte
CML	Chronic myeloid leukaemia
CSF	Colony stimulating factor
DMEM	Dulbecco's modification of Eagle's medium
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetra acetic acid
ELISA	Enzyme linked immunosorbent assay
EPO	Erythropoietin
FACS	Fluorescence activated cell sorting
FCS	Foetal calf serum
FITC	Fluorescein isothiocyanate
g	Gram
G-CSF	Granulocyte colony stimulating factor
GIST	Gastrointestinal stromal tumour
GM-CSF	Granulocyte-macrophage colony stimulating factor

HBSS	Hank’s balanced salt solution
HLA	Human leukocyte antigen
HSC	Haemopoietic stem cell
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IMDM	Iscove’s modification of Dulbecco’s medium
LPS	Lipopolysaccharide
M	Molar
MACS	Magnetic activated cell sorting
M-CSF	Macrophage colony stimulating factor
MFI	Mean fluorescence intensity
m	Murine
mg	Milli-gram
mL	Millilitre
MLR	Mixed lymphocyte reaction
mM	Milli molar
MNC	Mononuclear cell
PB	Peripheral blood
PBS	Phosphate buffered saline
PDGF	Platelet derived growth factor
PE	Phycoerythrin
Ph chromosome	Philadelphia chromosome
PI	Proliferation index
Rh	Recombinant human
RT	Room temperature
SCF	Stem cell factor
SDM	Serum deprived medium
TNF	Tumour necrosis factor
TPO	Thrombopoietin
v/v	Volume per volume
w/v	Weight per volume

PUBLICATIONS

1. Imatinib Inhibits the Tyrosine Kinase Activity of the Macrophage Colony Stimulating Factor Receptor c-fms at Clinically Relevant Concentrations. **AL Dewar**, AC Cambareri, AC Zannettino, BL Miller, KV Doherty, TP Hughes, AB Lyons. *Manuscript in preparation.*

2. Imatinib inhibits the functional capacity of cultured human monocytes. **AL Dewar**, KV Doherty, TP Hughes, AB Lyons. *Journal of Immunology & Cell Biology* (2004). *In Press.*

3. Imatinib inhibits the *in vitro* development of the monocyte/macrophage lineage from normal human bone marrow progenitors. **AL Dewar**, RM Domaschenz, KV Doherty, TP Hughes, AB Lyons. *Leukemia* (2003). 17(9):1713-1721.

4. Acquisition of immune function during the development of the Langerhans cell network in neonatal mice. **AL Dewar**, KV Doherty, GM. Woods, AB. Lyons, HK Muller. *Immunology* (2001). 103: 61-69.

5. Prevention of autoimmunity by induction of cutaneous tolerance. GM Woods, YP Chen, **AL Dewar**, KV Doherty, BH Toh, HK Muller. *Cell Immunol* (2001). 207(1):1-5.

THESIS AMENDMENTS

Corrections Relevant to Entire Thesis

- Error bars represent the SEM, and were derived from triplicate data points unless otherwise stated.
- Human genes should be written in capitals and italics. Proteins encoded by these genes should be written with the first letter in capital case, and should not be preceded by the "c-" prefix.

Chapter 1: Introduction

- Page 11, paragraph 1: the final sentence should be deleted as it does not follow.
- Page 14, paragraph 3: RAS is part of the MAPK pathway. The MAPK pathway is therefore not a further example of signal transduction pathways activated following ligand binding to the GM-CSF, IL-3 and IL-5 receptors.
- Page 26: The word "invariably" should be changed to read "usually", as there are many cases where CML patents remain in chronic phase for longer than 5 years.
- Page 28, first paragraph, final sentence: *Heistercamp et al (1990)* reference is incorrect. The reference should be *Daley et al (1990) Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome; Science. Feb 16; 247(4944): 824-30.*
- Page 31, 2nd paragraph: Reference to Deininger *et al* (1997) in statements that imatinib inhibits the growth of CML cells with minimal effects on normal haemopoiesis were omitted.
- Figure 1.1: LT-HSC is a "long-term" HSC. ST-HSC is a "short-term" HSC.

Chapter 2: Materials and Methods

- Page 46, 2.3.1: The final sentence in paragraph one should read: "Media *were* prewarmed…".
- Page 47, 2.3.1.1 & page 71, 2.5.5: The dilutions of IL-3 and GM-CSF that provided maximal cell growth were determined by titration using a factor dependent cell line.
- Page 71. 2.5.5: Use of the term selected means that the cells were selected for the ability to grow in M-CSF.

Chapter 3: Results Chapter 1

- Viability was determined following calculation of the percentage of cells with decreased FS/SS values.
- Surface markers were chosen which were expressed on specific myeloid lineages.
- Table 3.2: CML values were deemed significantly different from normal controls when p values calculated using ANOVA were less than 0.05.
- The data in Figures 3.13-16 are derived from the same experiment as Figures 3.5-3.8.
- Page 99, paragraph 1: "proliferative potential" should read "proliferation".

Chapter 4: Results Chapter 2

- Figure 4.2: The data was normalised relative to the 5HGF control, not the 4HGF control.
- Figure 4.9A (and elsewhere): Contrary to the statement in the legend, the marker tool was set on the 95th percentile of the fluorescence for the negative control.
- Figure 4.10: The morphology of the cells is not that of promyelocytes. The cells display an atypical morphology (excentric nucleus and a large, clear, vacuolated cytoplasm), however tristaining suggests the cells are immature myeloid cells.

Chapter 5: Results Chapter 3

Page 129, 5.2.3.2: The opsonin used was derived from purified rabbit polyclonal IgG antibodies that are specific for the zymosan particles. Phagocytosis of opsonsised zymosan particles was mediated by Fc and/or complement receptors.

Chapter 6: Results Chapter 4

Figures 6.6 and 6.7: "…infected with human c-fms…" should read "infected with a retrovirus encoding human FMS…".

Chapter 7: General Discussion

Page 152, paragraph 2: Abl is an intracellular tyrosine kinase, not a receptor tyrosine kinase.