Regulation of Cell Survival in Acute Myeloid Leukaemia

Daniel Thomas
MBBS, BMedSc (Hons), FRACP, FRCPA

Thesis submitted for the degree of
Doctor of Philosophy

The School of Medicine
Faculty of Health Sciences
The University of Adelaide

Growth and Differentiation Laboratory
Centre for Cancer Biology
SA Pathology

December 2011

To Jesus Christ, the healer of the nations and to my mother, Stephanie and father, Philip who have given me all the love, support, encouragement, and opportunity to study leukaemia

How much better it is to get wisdom than gold!
And to get understanding is to be chosen above silver.
Proverbs 16:16

Contents

Abstract

Acute myeloid leukaemia (AML) is a clonal disease of myelopoiesis characterised by poor survival due to frequent relapse. The cytokines interleukin-3 (IL-3) and granulocyte macrophage-colony stimulating factor (GM-CSF) are soluble glycoproteins that bind cell surface receptors on haemopoietic cells to stimulate their biological effects including cell survival and proliferation. Cancers cells usurp cytokine signalling pathways via various mechanisms to evade apoptosis and deregulate proliferation, two fundamental hallmarks of the cancer phenotype. Within a given patient's leukaemia there are smaller sub-populations of cells that possess the unique ability to self-renew and initiate tumour formation, termed "leukaemia stem cells" (LSCs). LSCs possess robust survival properties, are resistant to chemotherapy and over-express the IL-3 receptor α chain, however therapies directed specifically against LSCs are currently lacking. This body of work examines the cytokine receptor signalling events transduced by the IL-3/GM-CSF receptor active in acute myeloid leukaemia blasts and LSCs with the aim of discovering novel avenues for therapy.

In this thesis four targets for AML therapy are investigated and developed:-

(1) Firstly, we demonstrate feasibility of LSC targeting as a therapeutic strategy by demonstrating efficacy in vivo and in vitro of a blocking monoclonal antibody 7G3 directed to the extracellular domain of the IL-3 receptor CD123 which is over-expressed on AML stem cells.

(2) Secondly, we show that in the majority of AML patients, a distinct serine in the IL-3 receptor beta chain is constitutively phosphorylated and is required for cytokine-mediated survival. In a search for druggable kinases that may be responsible for this phosphorylation we isolated phosphatidyl inositol 3-kinase with novel activity as a *protein kinase* and show that it is linked to cell survival.

(3) We demonstrate that osteopontin (*OPN*) is a functionally relevant gene regulated by the serine-survival pathway that mediates survival of $CD34^{+}CD38^{-}CD123^{+}$ leukemic stem/progenitors. Furthermore, increased expression of OPN is associated with poor prognosis in normal karyotype AML.

(4) Finally, we identified a small molecule kinase inhibitor PIK-75 that is capable of the dual targetting of both PI3K and CDK9 in AML in vivo and in vitro. It is suggested that the simultaneous targeting of different molecules converging on a cell survival pathway represents a novel therapeutic avenue.

Author Declaration

This work contains no material that has been accepted for the award of any other degree or diploma in any University or other tertiary institution to Daniel Thomas, 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 made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Thesis Program (ADTP), and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

I acknowledge that copyright of published works contained within this thesis (as listed below) resides with the copyright holders of those works.

Powell, JA, Thomas D, Barry, EF, Kok, C, McClure, BM, Tyskin, A, To, LB, Brown, A, Lewis, ID, Herbert, K, Goodall, GJ, Speed, TP, Asou, N, Jacob, B, Osato, M, Haylock, DN, Nilsson, SK, D'Andrea, RJ, Lopez, AF and Guthridge, MA. (2009) Expression profiling of a hemopoietic cell survival transcriptome implicates osteopontin as a functional prognostic factor in AML. *Blood*. **114**(23):4850-4870.
Copyright held by The American Society of Hematology.

Hercus TR, Thomas, D , Guthridge MA, , Ekert, PG, King-Scott, J, Parker, M and Lopez, AF. (2009) The GM-CSF receptor: linking its structure to cell signalling and its role in disease. *Blood*. **114**(7):1289-98.
Copyright held by The American Society of Hematology.

Liqing Jin, L, Lee, EM, Ramshaw, HS, Busfield, SJ, Peoppl, AG, Wilkinson, L, Guthridge, MA, Thomas, D, Barry, E, Boyd, A, Gearing, D, Vairo, G, Lopez, AF, Dick, J & Lock, RB. (2009) Monoclonal antibody-mediated targeting of CD123 (IL-3 receptor α chain) eliminates human acute myeloid leukemic stem cells. *Cell Stem Cell*; **5**(1):31-42.
Copyright held by Elsevier Inc.

Daniel Thomas

Joint-Author Contributions

Contributions to publications included in this thesis by individual co-authors are outlined below.

Expression profiling of a hemopoietic cell survival transcriptome implicates osteopontin as a functional prognostic factor in AML.
Blood. 2009 **114**(23):4850-4870.

Jason Powell

Designed and performed research, analyzed and interpreted data, and wrote the paper.

I, Jason Powell hereby certify that the above statement of contribution is correct

Signed Date: 9/12/11

Daniel Thomas

Performed research, analyzed and interpreted data and wrote the paper.

I, DANIEL THOMAS hereby certify that the above statement of contribution is correct

Signed: Date: 10/12/11

Emma Barry

Performed research.

I, EMMA BARRY hereby certify that the above statement of contribution is correct

Signed: Date: 10/12/11

Chung Kok

Performed statistical analysis.

I, Chung Kok hereby certify that the above statement of contribution is correct

Signed: Date: 13/12/11

Barbara McClure

Performed research.

I, … hereby certify that the above statement of contribution is correct

Signed:.. Date:..12/12/11

Anna Tyskin

Performed statistical analysis.

I, … . hereby certify that the above statement of contribution is correct

Signed:..Anna Tsykin..... Date:..10/12/11.....

Luen Bik To

Provided AML samples and edited manuscript.

I, ..LB To.. hereby certify that the above statement of contribution is correct

Signed Date:..13.12.11

Anna Brown

Performed research.

I, Anna Brown hereby certify that the above statement of contribution is correct

Signed:.. Date:..13/12/11

Ian Lewis

Provided AML samples.

I, I.D.Lewis hereby certify that the above statement of contribution is correct

Signed:.... Date:..13.12.11

Kirsten Herbert

Provided AML samples.

Kirsten Herbert

I, ... hereby certify that the above statement of contribution is correct

Signed:. Date:.....29/5/11

Gregory Goodall

Analyzed and interpreted data.

I, Greg Goodall hereby certify that the above statement of contribution is correct

Signed: Date:...9/12/11

Terence Speed

Performed statistical analysis.

I, Terence Paul Speed hereby certify that the above statement of contribution is correct

Signed: Date: May 28, 2011

Norio Asou

Provided AML samples.

I, NORIO ASOU. hereby certify that the above statement of contribution is correct

Signed: Date:..30/5/2011

Bindya Jacob

Performed research.

I, Bindya Jacob, hereby certify that the above statement of contribution is correct

Signed:.. Date: 07/06/2011...

Motomi Osato

Performed research and analyzed and interpreted data.

I, Motomi Osato hereby certify that the above statement of contribution is correct

Signed: Date: 31/5/2011

David Haylock

Performed research and analyzed and interpreted data.

I, David Haylock hereby certify that the above statement of contribution is correct

Signed: Date: 30th May 2011

Susan Nilsson

Performed research and analyzed and interpreted data.

I, Susie Nilsson hereby certify that the above statement of contribution is correct

Signed: Date:30.5.11

Richard D'Andrea

Analyzed and interpreted data.

I, RICHARD D'ANDREA hereby certify that the above statement of contribution is correct

Signed Date:12/12/2011

Angel Lopez

Analyzed and interpreted data.

I, ANGEL LOPEZ.... hereby certify that the above statement of contribution is correct

Signed: Date:12/12/11..

Mark Guthridge

Designed and performed research, analyzed and interpreted data, and wrote the paper

I, Mark Guthridge hereby certify that the above statement of contribution is correct

Signed:… …… Date:…8/12/2011……

The GM-CSF receptor: linking its structure to cell signalling and its role in disease.
Blood. 2009 **114**(7):1289-98.

Timothy Hercus

Wrote part of the manuscript and reviewed the complete manuscript; generated figures; submitted manuscript.

I, …Tim Hercus. hereby certify that the above statement of contribution is correct

Signed:. Date:…9/12/11..

Daniel Thomas

Wrote part of the manuscript and reviewed the complete manuscript.

I, DANIEL Thomas hereby certify that the above statement of contribution is correct

Signed:.. Date:…6/12/11……

Mark Guthridge

Wrote part of the manuscript and reviewed the complete manuscript.

I, Mark Guthridge hereby certify that the above statement of contribution is correct

Signed:… …… Date:…8/12/2011……

Paul Ekert

Wrote part of the manuscript and reviewed the complete manuscript.

I, DAN Ekert hereby certify that the above statement of contribution is correct

Signed:. Date:…12/12/11.

Jack King-Scott

Wrote part of the manuscript and reviewed the complete manuscript; generated figures.

I, Jack King-Scott hereby certify that the above statement of contribution is correct

Signed: Date: 1 June 2011

Michael Parker

Wrote part of the manuscript and reviewed the complete manuscript; generated figures.

I, Michael William Parker hereby certify that the above statement of contribution is correct

Signed; Date: 1 June 2011

Angel Lopez

Wrote part of the manuscript and reviewed the complete manuscript.

I, ANGEL LOPEZ, hereby certify that the above statement of contribution is correct

Signed:... Date:...12/12/11.

Monoclonal antibody-mediated targeting of CD123 (IL-3 receptor α chain) eliminates human acute myeloid leukemic stem cells.

Cell Stem Cell; 2009 **5**(1):31-42.

Liqing Jin

Designed experiments, performed research, analyzed and interpreted data, wrote paper

I, LIQING JIN hereby certify that the above statement of contribution is correct

Signed Date: May 12, 2011

Erwin Lee

Designed experiments, performed research, analyzed and interpreted data, wrote paper

I,Erwin Lee hereby certify that the above statement of contribution is correct

Signed: Date 30th May 2011

Hayley Ramshaw

Designed experiments, performed research, analyzed and interpreted data

I, Hayley Ramshaw hereby certify that the above statement of contribution is correct

Signed:. Date:12/12/2011

Samantha Busfield

Designed experiments, performed research, analyzed and interpreted data

I, Samantha Busfield hereby certify that the above statement of contribution is correct

Signed; Date:…30..May 2011

Armando Peoppl

Designed experiments, performed research, analyzed and interpreted data

I, Armando Peoppl hereby certify that the above statement of contribution is correct

Signed Date:…May..10. 2011

Lucy Wilkinson

Designed experiments, performed research, analyzed and interpreted data.

I, DANIEL THOMAS hereby certify that the above statement of contribution is correct on behalf of Lucy Wilkinson

Signed Date:…12/12/11..

Mark Guthridge

Revised and co-edited manuscript.

I, Mark Guthridge hereby certify that the above statement of contribution is correct

Signed:… …… Date:…8/12/2011……

Daniel Thomas

Designed experiments, performed research, analyzed and interpreted data, edited final manuscript, provided AML samples and clinical data.

I, ... hereby certify that the above statement of contribution is correct

Signed: Date: 12/12/2011

Emma Barry

I, EMMA BARRY. hereby certify that the above statement of contribution is correct

Signed: Date: 10/12/11

Andrew Boyd

Analyzed and interpreted data, provided AML samples.

I, IAN Thomas on behalf Andrew Boyd hereby certify that the above statement of contribution is correct

Signed: Date: 12/12/12

David Gearing

Analyzed and interpreted data, provided key reagents.

I, David Gearing hereby certify that the above statement of contribution is correct

Signed: Date 30/5/2011

Gino Vairo

Analyzed and interpreted data, provided key reagents.

I, Gino Vairo... hereby certify that the above statement of contribution is correct

Signed: Date: 30/5/11

Angel Lopez

Revised and co-edited manuscript.

I, ANGEL LOPEZ.... hereby certify that the above statement of contribution is correct

Signed: Date: 12/12/11...

John Dick

Designed experiments, analyzed and interpreted data, wrote paper

I, John Dick hereby certify that the above statement of contribution is correct

Signed Date: 05/30/2011

Richard Lock

Designed experiments, analyzed and interpreted data, wrote paper

I, Richard Lock, hereby certify that the above statement of contribution is correct

Signed: ..... Date:30/5/11...

Acknowledgements

I sincerely thank my supervisor Dr Mark Guthridge for his guidance, creativity and sheer doggedness and co-supervisor Professor Angel Lopez for inspiring in me a desire for excellence in science. I gratefully acknowledge the generosity and expertise of the fantastic team of scientists I have had the privilege of working with at the Centre for Cancer Biology, SA Pathology. I especially thank Jason Powell and Emma Barry for their friendship, technical assistance, teamwork and dedication to the projects presented in this thesis and Joy Mundy for diagnostic flow cytometry. Special thanks goes to Dr Emily Paterson and Dr Leila Belle for assistance in writing and presenting this thesis. I am grateful for Dr Paul Ekert (Walter & Eliza Hall) for insights and collaboration for the data presented in Chapter 4 and Chapter 7. I thank Dr Andrew Wei (Monash University) for his expertise and collaboration for the project presented in Chapter 7 and Dr Timothy Hercus for assistance with structural insights into GM-CSF receptor signalling and protein chemistry.

I wish also to thank my family Stephanie, Philip, Abbie and Zoe Thomas for their patience and encouragement and support over 4 years. I am grateful to Peter and Carla Magarey for inspiration and mentoring. I also thank Chris McPeake, Daniel Lohmeyer, Josiah Mann, Naomi Rattley and Aaron Scott for priceless friendship and support. I also wish to acknowledge my many patients at the Royal Adelaide Hospital who consented for research to be performed on their leukaemia cells, knowing the results would not directly benefit them.

List of Figures

Chapter 1

Chapter 3

Chapter 4

Chapter 6

Chapter 7

List of Tables

Chapter 4

Chapter 6

Abbreviations

4HT	4-hydroxy tamoxifen
AML	acute myeloid leukemia
ATP	adenine triphosphate
βc	GM-CSF and IL-3 receptor beta subunit
BM	bone marrow
Cdk9	cyclin-dependent kinase 9
CTD	carboxy terminal domain of RNA polymerase II
CI	confidence interval
CLL	chronic lymphocytic leukemia
CPM	counts per minute
CSL	Commonwealth Serum Laboratories
FAB	French-American-British
FACS	fluorescence-activated cell sorting
FDM	factor-dependent myeloid
GFP	green fluorescent protein
GM-CSF	granulocyte-macrophage colony stimulating factor
GMRα	granulocyte-macrophage colony stimulating factor receptor alpha chain
hour	h
IC50	half maximal inhibitory concentration
IL-3	interleukin-3
ITD	internal tandem duplication
LSPCs	leukemic stem and progenitor cells
mAb	monoclonal antibody
min	minute
MNCs	mononuclear cells
ND	not determined
NS	not significant
OPN	human osteopontin gene
pAb	polyclonal antibody
PtdSer	phosphatidylserine
PtdIns	phosphatidylinositol

PI	propidium iodide
PI3K	phosphoinositide 3-kinase
PIP	phosphatidylinositol phosphates
PH	pleckstrin homology
RAH	Royal Adelaide Hospital
SD	standard deviation
SEM	standard error of the mean
TKIs	tyrosine kinase inhibitors
WCC	white cell count
wt	wild-type