



Regulation of Cell Survival in Acute Myeloid Leukaemia

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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

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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 phosphatidylinositol 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 targeting 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.

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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.

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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.
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Abbreviations

4HT	4-hydroxy tamoxifen
AML	acute myeloid leukemia
ATP	adenine triphosphate
β c	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
GMR α	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
<i>OPN</i>	human osteopontin gene
pAb	polyclonal antibody
PtdSer	phosphatidylserine
PtdIns	phosphatidylinositol

Abbreviations

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