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## Dedications

I would like to dedicate this thesis to my late beloved brother Phillip Ganye Maramba who untimely passed away on his birthday in 2011 after a road traffic accident. May his soul rest with eternal peace!

## Abstract

B chronic lymphocytic leukaemia (B-CLL) is a disease with an enormously heterogeneous prognosis. Due to lack of effective treatments and the heterogeneity in its course, B-CLL has remained largely incurable. In the last two decades a number of biological markers have been recognised as prognostic parameters to replace clinical staging, chief among them is ZAP-70 (a T-cell cytoplasmic tyrosine protein) albeit without a full understanding of the mechanism involved.

The aim of this thesis was to identify reliable prognostic factors that could be used in the management of B-CLL patients. Seventy-six B-CLL patients were studied over 30 months using a random stratified method of sampling, whereby adult B-CLL patients were recruited at different stages of the disease. Retrospective studies enabled some patients to be studied for up to 192 months. ZAP-70 expression was demonstrated in some B-CLL cells and was examined for possible phosphorylation following B-CLL cells stimulation, implying its involvement in cell signalling. Through the use of a novel method developed during the present study, B-CLL cells were stimulated via the SDF1-α-CD184 signalling pathway resulting in ZAP-70 phosphorylation. Its expression and phosphorylation was investigated alongside other prognostic factors and also correlated with B-CLL progression. More importantly, this study demonstrated that ZAP-70 positive cells are more responsive to signals derived from their surrounding environment, such as SDF-1α.

In conclusion ZAP-70, CD38, and CD23 expressions together with IgVH gene mutational status were confirmed as indicators of poor prognosis. Response to external stimuli by some B-CLL cells, resulting in ZAP-70 phosphorylation represents the most novel aspect of this thesis and demonstrates ZAP-70 involvement in B-CLL signalling. Additionally, raised NK: B-CLL and T: B-CLL cell ratios were identified as good modifiable variable predictors of time to treatment that could be used for monitoring and therapeutic purposes. Since B-CLL is an incurable disease, the identification of prognostic factors could help in deciding the best time for intervention in B-CLL patients.

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## List of Abbreviations

| | |
|---|---|
| ADCC | Antibody-dependent cellular cytotoxicity |
| ALL | Acute lymphoblastic leukaemia |
| AML | Acute myeloid leukaemia |
| APC | Allophycocyanin |
| ATM | Ataxia telangiectasia mutated |
| BCR | B cell receptor |
| BLNK | B-cell linker |
| BM | Bone marrow |
| BMT | Bone marrow transplant |
| BSAP | B-cell lineage specific activator protein |
| CADPR | Cyclin adenosine diphosphate-ribose |
| CXC | Chemokines that are classified depending on the spacing and number of their conserved cysteine residue |
| CCR | Chemokine receptor |
| CD | Cluster of differentiation |
| CDR | Complimentarity determining region |
| CHOP | Cyclophosphamide + Hydrodoxydaunorubicin + Oncovin + Predinisone |
| CLL | Chronic lymphocytic leukaemia |
| CML | Chronic myeloid leukaemia |
| CPD | Continuous Professional Development |
| CST | Cytometer setup and tracking |
| DBMS | Professional doctorate in biomedical science |
| DMSO | Dimethyl sulphoxide |

| | |
|---|---|
| DNA | Deoxyribonucleic acid |
| EBF | Early B-cell factor |
| EBRT | External beam radiotherapy |
| EBV | Epstein barr virus |
| EDTA | Ethylene-diamine-tetra-acetic acid |
| ELC | EBV-induced receptor ligand chemokine |
| ERK | Extracellular signal-regulated kinases |
| FACS | Fluorescence-activated cell sorting |
| FBC | Full blood counts |
| FCR | Fludarabine + Cyclophosphamide + Rituximab |
| FCS | Foetal calf serum |
| FDA | Food and drug administration |
| FITC | Fluorescein isothiocyanate |
| FR | Fragment region |
| FMC7 | Flinders medical centre, Australia |
| FSC | Forward scatter channel |
| GPCR | Glycoprotein-coupled receptor |
| GR | Glucocorticoid receptors |
| GPI | Glycosylphosphatidylinositol |
| GVHD | Graft-versus-host disease |
| GVL | Graft-versus leukaemia |
| $H_2O_2$ | Hydrogen peroxide |
| HBSS | Hank’s balanced salt solution |
| HDMP | High dose methylprednisolone |
| HIV | Human immunodeficiency virus |

| | |
|---|---|
| HPC | Health professions council |
| HSCT | Haematopoietic stem cell transplant |
| IBMS | Institute of biomedical science |
| Ig | Immunoglobulin |
| IgVH | Immunoglobulin heavy-chain variable region |
| IL | Interleukin |
| IMGT | International **I**m**M**uno**G**ene**T**ics |
| ITAM | Immunoreceptor tyrosine-based activation motifs |
| ITIM | Immunoreceptor tyrosine inhibitory motifs |
| ITP | Immune thrombocytopenia |
| JNK | Jun N-terminal kinase |
| LDT | Lymphocyte doubling time |
| LN | Lymph node |
| MAPK | Mitogen-activated protein kinase |
| MBC | Monoclonal B Cells |
| MDM2 | Mouse/ murine double minute 2 |
| MDS | Myelodysplastic syndromes |
| MFI | Median fluorescence intensity |
| MRD | Minimal residual disease |
| MRNA | Messenger RNA |
| mIg | Membrane immunoglobulin |
| MIP-3β | Macrophage inflammatory protein 3β |
| MNC | Mononuclear cell |
| MWM | Molecular weight marker |
| NFAT | Nuclear factor of activated T-cell |

| | |
|---|---|
| NHS | Nation health service |
| NICE | National institute of clinical excellence |
| NK | Natural killer |
| NRTI | Nucleoside analogue that work as reverse transcriptase inhibitors |
| OS | Overall survival |
| P53 | Tumour protein 53 |
| PCR | Polymerase chain reaction |
| PE | Phycoerythrin |
| Per CP | Peridicin chlorophyll-protein |
| PFS | Progression free survival |
| PLCγ2 | Phospholipase- Cγ2 |
| PMT | Photomultiplier tube |
| PRBCA | Pure red blood cell aplasia |
| PTK | Protein tyrosine kinase |
| PTP | Protein tyrosine phosphatase |
| RA | Refractory anaemia |
| RAG | Recombination activating genes |
| RIC | Reduced intensity conditioning |
| RNA | Ribonucleic acid |
| RPMI | Roswell park memorial institute |
| SCF | Stem cell factor |
| SDF-1α | Stromal cell-derived factor 1α |
| SH2 | Src homology 2 |
| SHM | Somatic hyper-mutations |

| | |
|---|---|
| SHIP | Src homology inositol phosphate |
| SHP1 | Src homology containing protein 1 |
| SLC | Secondary lymphoid tissue chemokine |
| SLP-76 | Src homology 2 domain containing leukocyte protein of 76kDa |
| SSC | Side scatter channel |
| ST | Survival times |
| Syk | Spleen tyrosine kinase |
| TAPA-1 | Target of anti-proliferative antibody–1 (CD81) |
| TCR | T-Cell receptor |
| TD | Thymus dependent |
| TdT | Terminal deoxyribonucleotidyl transferase |
| TEM | Transendothelial migration |
| TFS | Treatment free survival |
| TK | Tyrosine kinase |
| TP53 | p53 gene |
| TT | Time to treatment |
| VCAM-1 | Vascular cell adhesion molecule -1 |
| VLA-4 | Very late antigen -4 |
| XIAP | X-linked inactivator of apoptosis |
| WBC | White blood cells |
| ZAP-70 | Zeta associated protein of 70 kDa |

## PUBLICATIONS

Maramba A., North J., Lowdell MW and Donaldson C. IgM ligation triggers Zeta associated protein of 70 kDa (ZAP-70) tyrosine 292 and 319 phosphorylation in B-Chronic Lymphocytic Leukaemia (B-CLL). *Poster presentation at the British Society for Haematology* **British Journal of Haematology** 2011; **153 (Suppl. 1):** 72-73.