

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	vii
ABSTRACT	xi
ABBREVIATIONS	xiii
LIST OF FIGURES	xxiii
LIST OF TABLES	xxvii
CHAPTER 1	1
INTRODUCTION	1
1.1 ACUTE LYMPHOBLASTIC LEUKEMIA.....	1
1.1.1 Hematopoiesis and ALL.....	2
1.1.1.1 Transcription factors (TFs)	4
1.1.1.2 Cytokines and Stromal cells.....	6
1.1.1.3 Pathways controlling normal and leukemic lymphoid development	7
1.1.1.3.1 Wnt signalling in hematopoiesis and ALL.....	8
1.1.1.3.2 Notch signalling in haematopoiesis and ALL.....	10
1.1.1.3.3 Hedgehog signalling in haematopoiesis and ALL	11
1.1.1.3.4 Other signalling mechanisms involved in leukemogenesis and ALL	12
1.1.2 Differential genes expression patterns associated with ALL and relapse	14
1.1.3 Other risk factors associated with ALL.....	17
1.1.4 Treatment strategies for ALL and assessment of minimal residual disease to monitor relapsed ALL.....	20
1.2 CENTRAL NERVOUS SYSTEM (CNS) LEUKEMIA	21
1.2.1 Biomarkers and its importance in cancer	25
1.2.2 Molecules identified as biomarkers for CNS leukemia.....	26
1.2.2.1 Limitations of molecules identified as CNS leukemia markers	28
1.2.3 Is the CNS infiltration of lymphocytes is a normal event?.....	29
1.2.3.1 CNS infiltration of leukocytes as a part of immune surveillance	29
1.2.3.2 Mechanism of immune cell trafficking to CNS	30
1.2.4 Methods for the identification of molecular interaction at protein level	33
1.2.5 Proteomics as a tool for biomarker discovery	37
1.2.5.1 Applicability of body fluids as clinical samples for proteomics based biomarker discovery.....	39
1.2.5.1.1 Cerebrospinal fluid as a source of biomarker.....	41
1.3 REVIEW OF LITERATURE	42
1.4 THESIS SCOPE	43
1.5 RESEARCH QUESTIONS	44

1.6 HYPOTHESIS	45
1.7 OBJECTIVES OF THE STUDY	45
1.8 THESIS OUTLINE	45
CHAPTER 2	47
MATERIALS AND METHODS	47
2.1 REAGENTS AND CHEMICALS	47
2.2 METHODS	48
2.2.1 Cell culture and cell lysate preparation	48
2.2.2 Phenol:Chloroform:Isoamyl alcohol precipitation (49.5:49.5:1)	48
2.2.3 Acetone precipitation	48
2.2.4 Methanol + 100mM ammonium acetate (Mac) precipitation.....	48
2.2.5 Ethanol precipitation	49
2.2.6 Concentration of sample using amicon ultra-4 centrifugal filter unit 10 kDa cut concentrator (centricon).....	49
2.2.7 Protein estimation.....	49
2.2.8 DNA quantification	49
2.2.9 Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE).....	49
2.2.10 CSF samples from ALL patients	50
2.2.11 CSF Sample processing and biotinylation.....	50
2.2.12 Profiling of lymphoblastic proteins by 2D-PAGE and western blotting.....	50
2.2.13 Far-western analysis of CSF samples from ALL patients	51
2.2.14 Quantification of CSF reactive spots by Image J software	51
2.2.15 Mass spectrometric analysis of CSF reactive lymphoblastic proteins.....	51
2.2.16 Cloning of mass spectrometrically identified proteins to bacterial expression vector	52
2.2.17 Gene expression standardization in a small scale in BL21	52
2.2.18 Large scale expression of PFDN5 α /CIP29/ECH1/PRDX6 in bacteria and purification by Ni- agarose bead.....	52
2.2.19 CSF reactivity to purified PFDN5 α /CIP29/ECH1/PRDX6 on the blot.....	53
2.2.20 Quantification of CSF reactivity to PFDN5 α by ELISA	53
2.2.21 Statistical analysis	53
CHAPTER 3	54
RESULTS AND DISCUSSIONS	54
3.1 STANDARDISATION OF TWO DIMENSIONAL PROFILING OF LYMPHOBLASTIC PROTEINS FOR BIOMARKER IDENTIFICATION	54
3.1.1 Introduction	54
3.1.2 Two-Dimensional Electrophoresis (2-DE) of JM-1 lysate.....	55
3.1.3 MAc versus Acetone precipitation versus PCI alcohol extraction of proteins	57
3.1.4 Ethanol precipitation versus unprocessed lysate 2-D versus centricon	60

3.2 FAR-WESTERN CLINICAL PROTEOMICS APPROACH FOR THE DISCOVERY OF NOVEL MOLECULES OF CLINICAL AND PATHOGENIC SIGNIFICANCE IN CNS LEUKEMIA.....	66
3.2.1 Introduction	66
3.2.2 Validation of biotinylation of CSF proteins	68
3.2.3 Identification of lymphoblastic proteins reactive to biotinylated CSF from ALL patients at different time points	69
3.2.4 CSF reactivity to molecules reportedly involved in tumor formation	78
3.3 VALIDATION OF CSF REACTIVITY TOWARDS MASS SPECTROMETRICALLY IDENTIFIED PROTEINS	81
3.3.1 Introduction	81
3.3.2 Cloning and expression of PFDN5 α /CIP29/ECH1/PRDX6 in bacterial system.....	82
3.3.3 Comparison of CSF reactivity to purified proteins to reactivity on 2D blot	85
3.3.4 ELISA quantification of CSF reactivity to pure PFDN5 α protein at different clinical conditions	87
CHAPTER 4.....	100
CONCLUSIONS AND FUTURE PERSPECTIVES.....	100
CHAPTER 5.....	107
REFERENCES.....	107
ANNEXURE.....	131