

TABLE OF CONTENTS

Title	Page No.
LIST OF ABBREVIATIONS.....	i
LIST OF FIGURES.....	iii
LIST OF TABLES.....	vii
ABSTRACT.....	ix
1. INTRODUCTION.....	12
2. REVIEW OF LITERATURE.....	17
2.1 Head and neck squamous cell carcinomas (HNSCCs).....	17
2.1.1 General description.....	17
2.1.2 Epidemiology.....	18
2.2 Classification of HNSCC.....	19
2.2.1 Classification based on anatomy.....	19
2.2.2 Classification based on tumor staging.....	19
2.3 Major risk factors.....	21
2.3.1 Consumption of tobacco and alcohol.....	21
2.3.2 Infection by Human papillomavirus (HPV).....	23
2.3.3 Genetic determinants.....	24
2.4 Pro-oncogenic factors for HNSCC.....	24
2.4.1 Survival and proliferation.....	24
2.4.2 Stemness.....	25
2.4.3 EMT.....	26
2.4.4 Metastasis.....	27
2.5 Treatment.....	28
2.5.1 Surgery.....	28

2.5.2 Radiotherapy.....	28
2.5.3 Chemotherapy.....	29
2.5.4 Targeted therapy and immunotherapy.....	31
2.6 Toll-Like Receptors (TLRs).....	33
2.6.1 Expression of TLRs.....	34
2.6.2 Structure of TLRs.....	34
2.6.3 TLR ligands.....	35
2.6.4 TLR signaling pathways.....	37
2.7 Interleukin-1 receptor associated kinases (IRAKs).....	38
2.7.1 Members and structure of IRAK proteins.....	38
2.7.2 The Myddosome complex formation and downstream signaling.....	39
2.7.3 IRAK-2 and IRAK-3: Pseudokinases.....	40
2.8 Dysregulation in TLR signaling pathway.....	40
2.8.1 Role of TLRs in cancer.....	41
2.8.2 Role of IRAKs in cancer.....	42
2.8.3 Expression status and role of IRAKs in haematological malignancies..	42
2.8.4 Expression status and role of IRAKs in solid tumors.....	43
2.9 TLR based therapeutic approaches.....	45
2.9.1 TLR agonists.....	45
2.9.2 TLR antagonists.....	47
2.9.2.1 Small molecule inhibitors of IRAK-1 and IRAK-4.....	49
3. RATIONALE.....	52
Aim.....	54
Objectives.....	54
4. MATERIALS AND METHODS.....	55
4.1 Materials.....	55

4.1.1 Cell line.....	55
4.1.2 Antibodies and reagents.....	55
4.2 Methods.....	56
4.2.1 Drug sensitivity assay.....	56
4.2.1.1 Estimation of cytotoxic potential of drugs.....	56
4.2.1.2 Development of triple chemo-resistant cell line.....	57
4.2.1.3 Determination of the efficacy of combination treatment.....	58
4.2.2 mRNA-based expression analysis using quantitative PCR.....	59
4.2.3 Flow cytometry.....	62
4.2.3.1 Staining procedure for surface markers.....	62
4.2.3.2 Staining procedure for intracellular markers.....	62
4.2.3.3 Acquisition and analysis of flow cytometry results.....	63
4.2.4 Western blotting.....	63
4.2.4.1 Sample preparation and protein quantification.....	63
4.2.4.2 Gel electrophoresis, blotting and antibody-incubation.....	64
4.2.4.3 Visualization and analysis.....	64
4.2.5 ELISA.....	64
4.2.6 Statistical analysis.....	65
5. RESULTS.....	66
5.1 Expression profiling of TLRs 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 on HNSCC cell line.....	66
5.2 Evaluation of constitutive TLR signaling in HNSCC cell line.....	67
5.3 Evaluation of the impact of TLR signaling on the oncogenic properties of HNSCC cell line.....	69
5.3.1 Impact of TLR signaling on the viability of HNSCC cell line.....	69
5.3.2 Impact of TLR signaling on survival of HNSCC cell line.....	71
5.3.3 Impact of TLR signaling on the proliferative potential of HNSCC cell line.....	72

5.3.4 Impact of TLR signaling on the CSCs formation in HNSCC cell line.....	73
5.3.5 Impact of TLR signaling on EMT of HNSCC cell line.....	76
5.3.6 Impact of TLR signaling on Metastasis of HNSCC cell line.....	77
5.4 Evaluation of the role of TLR signaling in chemo-resistant HNSCC.....	78
5.4.1 Determination of IC ₅₀ of chemo-drugs on HNSCC cell line.....	78
5.4.2 Morphological characteristics of the triple-chemo-resistant HNSCC cell line.....	80
5.4.3 Validation of the acquired chemo-resistance of the triple-chemo-resistant HNSCC cell line.....	81
5.4.4 Evaluation of TLRs 1-10 expression on chemo-resistant HNSCC cell line.....	82
5.4.5 Evaluation of constitutive TLR signaling in chemo-resistant HNSCC cell line.....	83
5.4.6 Impact of inhibition of TLR signaling pathway on the viability of chemo-resistant HNSCC cell line.....	85
5.4.7 Impact of TLR signaling on the pro-oncogenic properties of chemo-resistant HNSCC cell line.....	86
5.4.7.1 Impact of TLR signaling on the survival of chemo-resistant HNSCC cell line.....	86
5.4.7.2 Impact of TLR signaling on the proliferative potential of chemo-resistant HNSCC cell line.....	88
5.4.7.3 Impact of TLR signaling on the CSCs formation of chemo-resistant HNSCC cell line.....	89
5.4.7.4 Impact of TLR signaling on EMT of chemo-resistant HNSCC cell line.....	92
5.4.7.5 Impact of TLR signaling on the metastasis of chemo-resistant HNSCC cell line.....	94
5.5 Evaluation of therapeutic potential of TLR signaling inhibitor as combination therapy with conventional chemo-drugs using HNSCC cell line.....	95

5.5.1 Effect of TLR signaling inhibitor-based combination therapy on cell viability of parent and chemo-resistant HNSCC cell lines.....	95
5.5.1.1 Effect of TLR signaling inhibitor and docetaxel-based combination therapy on the cell viability of parent and chemo-resistant HNSCC cell lines.....	96
5.5.1.2 Effect of TLR signaling inhibitor and cisplatin-based combination therapy on the cell viability of parent and chemo-resistant HNSCC cell lines.....	97
5.5.1.3 Effect of TLR signaling inhibitor and 5-FU-based combination therapy on the cell viability of parent and chemo-resistant HNSCC cell lines.....	98
5.5.2 Effect of TLR signaling inhibitor-based combination therapy on survival of chemo-resistant HNSCC cell line.....	99
5.5.3 Effect of TLR signaling inhibitor-based combination therapy on the proliferative potential of parent and chemo-resistant HNSCC cell lines.....	101
5.5.4 Effect of TLR signaling inhibitor-based combination therapy on the CSCs of chemo-resistant HNSCC cell lines.....	104
5.5.4.1 Effect of TLR signaling inhibitor-based combination therapy on the CD44 expression of parent and chemo-resistant HNSCC cell lines.....	104
5.5.4.2 Effect of TLR signaling inhibitor-based combination therapy on the Nanog expression of parent and chemo-resistant HNSCC cell lines.....	107
5.5.4.3 Effect of TLR signaling inhibitor-based combination therapy on the ALDH1 expression of chemo-resistant HNSCC cell line.....	110
5.5.5 Effect of TLR signaling inhibitor-based combination therapy on the EMT of chemo-resistant HNSCC cell line.....	111
5.5.6 Effect of TLR signaling inhibitor-based combination therapy on the metastasis of chemo-resistant HNSCC cell line.....	114
5.5.7 Effect of TLR signaling inhibitor-based combination therapy on the BST-2 expression of parent and chemo-resistant HNSCC cell lines.....	116
6. DISCUSSION.....	119

7. SUMMARY.....	127
8. REFERENCES.....	128
APPENDIX.....	154
PUBLICATIONS AND PRESENTATIONS.....	156