



**Faculty of Resource Science and Technology**

**A Meta-analysis of Gene Expression of Biomarkers and Construction of  
Multigene Prognosis Assessment Model in Nasopharyngeal Carcinoma**

**Sim Chor Chien**

**Master of Science  
2020**

# A Meta-analysis of Gene Expression of Biomarkers and Construction of Multigene Prognosis Assessment Model in Nasopharyngeal Carcinoma

Sim Chor Chien

A thesis submitted

In fulfilment of the requirements of the degree of Master of Science

(Medical Genetics)

Faculty of Resource Science and Technology  
UNIVERSITI MALAYSIA SARAWAK  
2020

## **DECLARATION**

I, Sim Chor Chien (17020107), from Faculty of Resource Science and Technology, hereby declare that the work entitled 'A Meta-analysis of Gene Expression of Biomarkers and Construction of Multigene Prognosis Assessment Model in Nasopharyngeal Carcinoma' is my original work. I have not copied from other students' work or from any other sources except where due references acknowledgement is made explicitly in the text, nor has any part been written for me by another person. The thesis has not been accepted for any degree and is not concurrently submitted in candidature for any other degree.



---

Sim Chor Chien

Matric No: 17020107

Date: 4 June 2020

## **ACKNOWLEDGEMENT**

First and foremost, I would like to express my sincere appreciation to my motivating supervisor, Prof. Dr Edmund Sim for his guidance, supervision and valuable suggestion and insightful comments that enable me to acquire better understanding. His helping hands and expertise always help me in producing better quality work. Thank you, Prof. E.

A special thanks to my co-supervisor, Dr Chong Yee Ling for giving me the direction in doing this research. Her professional guidance has provided me with intelligent assistance.

Besides, I would like to thank my dear colleagues, Ms Ng Kher Lee, Ms Felicia Kavita, Ms Cassandra Chee, Ms Yew Keh Li and Ms Cassandra Chong for the support and accompany. A special thanks also for my friend, Yee Siew who has provided me with much IT support throughout the research. Thank you for easing this stressful journey!

I place on record, my utmost gratitude to my dearest family for the unceasing love, encouragement, support and care. And thank you Stewart, for the constant prayers and unconditional support. My deepest heart-felt thanks to you all for accompanying me throughout this journey and sharing my stress and tears. Thank you with lots of love.

Last but not least, I would like to thank God the Almighty for giving me the strength, wisdom, faith and boundless grace to pursue this study. Thank You for leading me through ups and downs with Your out-strengthened arms. Thank you, Lord!

## **ABSTRACT**

Nasopharyngeal carcinoma (NPC) is a common cancer in Southeast Asia, including Malaysia. Overall survival rate of NPC patients is poor due to late diagnosis at the advanced stage. Thus, definitive prognostic biomarkers and multigene prognosis assessment models are crucial to better manage NPC. Cyclooxygenase-2 (COX-2) was identified as a significant prognostic biomarker via meta-analysis in head and neck (HNC) cancer, oral squamous cell carcinoma (OSCC) and breast cancer. Prognostic significance of COX-2 in NPC regarding lymph node metastasis has been done via meta-analysis. However, prognostic significance of COX-2 in NPC in term of overall survival (OS) rate analysed via meta-analysis remains unexplored. This study aims to evaluate COX-2 expression with OS and treatment response via meta-analysis by referring to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist. Results show that over expression of COX-2 predicts worse survival rate outcome for NPC (hazard ratio greater than one) but not treatment response (odd ratio less than one). Hazard ratio is a time dependent statistic measure while odd ratio is a time independent statistic measure. Hence, evaluation of COX-2 expression in NPC patients is important for management and treatment of NPC. Most existing NPC prognosis assessment models are constructed with small sample sizes and thus less relevant and applicable for medical practices. This study therefore aimed to construct a multigene NPC prognosis assessment model by pooling results of more significant meta-analysis studies on prognostic biomarkers of NPC via modification of PRISMA. Through this study, a multigene NPC prognosis assessment model consisting of ten prognostic biomarkers, including COX-2 done from this study is constructed. OS of NPC patients can be determined computationally as pooled hazard ratios (HRs) via this model by selecting the over expressed biomarker(s). The model can also predict the NPC prognosis assessment risk score of the

patient too. Through this model, patients can estimate the overall survival rate time effectively. To provide understanding and explanation to the multigene NPC prognosis assessment model generated computationally, pathways and interactions involved by all the prognostic biomarkers are studied to determine the physiological interactions among all the prognostic biomarkers incorporated. The information on the pathways and interactions are extracted from the literature and summarized as a molecular pathway network. The pathway network model explains the possible complex interplay among different prognostic biomarkers in the oncogenesis of NPC.

**Keywords:** Nasopharyngeal carcinoma, meta-analysis, cyclooxygenase-2, multigene NPC prognosis assessment model, NPC pathway network model.

***Meta-analisis Mengenai Ekspresi Gen Faktor Ramalan dan Penjanaan Model  
Penilaian Prognosis Pelbagai Gen dalam Karsinoma Nasofarinks***

**ABSTRAK**

*Karsinoma nasofarinks (NPC) ialah sejenis kanser yang lazim di Asia Tenggara, termasuk Malaysia. Kadar kelangsungan hidup pada pesakit NPC adalah rendah disebabkan oleh diagnosis lambat pada peringkat akhir. Oleh itu, faktor ramalan NPC yang muktamad dan modal penilaian prognosis pelbagai gen adalah penting untuk mengendali NPC dengan lebih baik. Cyclooxygenase-2 (COX-2) dikenali sebagai faktor ramalan yang penting melalui meta-analisis bagi kanser kepala dan leher, karsinoma sel skuamosa mulut dan kanser dada. Kepentingan COX-2 dalam prognosis NPC mengenai metastasis nodus limfa telah ditentukan melalui meta-analisis. Walaubagaimanapun, fungsi COX-2 sebagai faktor ramalan bagi menentukan kelangsungan hidup pada pesakit NPC belum lagi dikaji melalui meta-analisis. Matlamat kajian ini adalah untuk menilai hubungan antara ekspresi COX-2 dengan kadar kelangsungan hidup dan tindak balas rawatan melalui meta-analisis dengan rujukan 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)'. Keputusan menunjukkan bahawa ekspresi COX-2 yang tinggi menjangkakan kadar kelangsungan hidup yang rendah (nisbah bahaya melebihi satu) tetapi tidak berkaitan dengan tindak balas rawatan (nisbah ganjil kurang daripada satu). Nisbah bahaya merupakan pengiraan statistik bergantung pada masa sementara nisbah ganjil merupakan pengiraan statistik yang tidak bergantung pada masa. Jadi, kajian untuk menilai ekspresi COX-2 adalah penting untuk pengurusan dan rawatan NPC yang lebih cekap. Kebanyakan model penilaian prognosis NPC yang sedang ada dijana dengan saiz sampel yang kecil dan menyebabkan model tersebut kurang berkaitan dan berguna dalam bidang perubatan. Oleh itu, kajian tersebut bertujuan untuk menjana model penilaian prognosis NPC pelbagai gen*

yang berkomputasi dengan mengumpulkan keputusan meta-analisa mengenai faktor ramalan NPC yang lebih bermakna melalui pengubahsuaian PRISMA. Dengan kaedah tersebut, model penilaian prognosis NPC pelbagai gen yang terdiri daripada sepuluh faktor ramalan, termasuk COX-2 yang dikaji dalam kajian ini telah dijana. Kadar kelangsungan hidup bagi pesakit NPC dapat ditentukan secara berkomputasi sebagai nisbah bahaya keseluruhan (HRs) melalui model ini dengan memilih faktor ramalan yang diekspres lebih. Model tersebut juga dapat meramalkan angka risiko bagi penilaian prognosis NPC. Dengan menggunakan model ini, pesakit NPC dapat menjangka kadar hidup keseluruhan dalam masa yang singkat. Bagi memberikan pemahaman dan penjelasan mengenai model penilaian prognosis NPC pelbagai gen tersebut yang telah dijana secara berkomputasi, laluan dan interaksi antara faktor ramalan yang terlibat telah dikaji dan interaksi fisiologi telah dijelaskan. Maklumat mengenai laluan dan interaksi telah dikumpulkan dari kajian literatur dan diringkaskan dalam satu model rangkaian laluan NPC. Rangkaian tersebut menjelaskan interaksi kompleks antara faktor ramalan dalam onkogenesis NPC.

**Kata kunci:** Karsinoma nasofarinks, meta-analisis, cyclooxygenase-2, model penilaian NPC pelbagai gen, model rangkaian laluan NPC.

## TABLE OF CONTENTS

|                                                        | Page         |
|--------------------------------------------------------|--------------|
| <b>DECLARATION .....</b>                               | <b>i</b>     |
| <b>ACKNOWLEDGEMENT .....</b>                           | <b>ii</b>    |
| <b>ABSTRACT .....</b>                                  | <b>iii</b>   |
| <b>ABSTRAK .....</b>                                   | <b>v</b>     |
| <b>TABLE OF CONTENTS .....</b>                         | <b>vii</b>   |
| <b>LIST OF TABLES.....</b>                             | <b>xi</b>    |
| <b>LIST OF FIGURES.....</b>                            | <b>xii</b>   |
| <b>LIST OF ABBREVIATIONS .....</b>                     | <b>xv</b>    |
| <br><b>CHAPTER 1: INTRODUCTION .....</b>               | <br><b>1</b> |
| <br><b>CHAPTER 2: LITERATURE REVIEW .....</b>          | <br><b>5</b> |
| 2.1 Nasopharyngeal Carcinoma (NPC) .....               | 5            |
| 2.1.1 Anatomical position of NPC .....                 | 5            |
| 2.1.2 Histopatholgy of NPC .....                       | 5            |
| 2.1.3 Epidemiology of NPC .....                        | 7            |
| 2.1.4 Etiology of NPC .....                            | 10           |
| 2.1.4.1 EBV infection .....                            | 11           |
| 2.1.4.2 Dietary factor .....                           | 12           |
| 2.1.4.3 Environmental factors.....                     | 14           |
| 2.1.4.4 Gene abnormalities .....                       | 15           |
| 2.1.5 Clinical presentations, signs and symptoms ..... | 17           |
| 2.1.6 Treatments .....                                 | 18           |
| 2.1.7 Prognostic biomarkers .....                      | 20           |

|                                                                                     |    |
|-------------------------------------------------------------------------------------|----|
| 2.2 Meta-analysis.....                                                              | 22 |
| 2.2.1 Background of Meta-analysis.....                                              | 22 |
| 2.2.2 Principle of meta-anlaysis .....                                              | 23 |
| 2.3 Multigene NPC prognosis assessment model.....                                   | 32 |
| 2.3.1 Rationale of integration of individual studies into a model.....              | 32 |
| 2.3.2 Integration of prognostic biomarkers for NPC prognosis assessment model ..... | 35 |
| 2.4 Generate molecular pathway model .....                                          | 37 |
| 2.4.1 Prognostic biomarkers included .....                                          | 38 |
| 2.4.1.1 Cyclooxygenase-2 (COX-2).....                                               | 39 |
| 2.4.1.2 Latent membrane protein 1 (LMP1).....                                       | 40 |
| 2.4.1.3 Vascular endothelial growth factor (VEGF) .....                             | 41 |
| 2.4.1.4 Epidermal growth factor receptor (EGFR) .....                               | 42 |
| 2.4.1.5 Lactate dehydrogenase (LDH).....                                            | 43 |
| 2.4.1.6 C-reactive protein (CRP).....                                               | 44 |
| 2.4.1.7 Excision repair cross-complementation group 1 (ERCC1) .....                 | 45 |
| 2.4.1.8 Hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ ) .....                    | 46 |
| 2.4.1.9 Lysly oxidase (LOX) .....                                                   | 47 |
| 2.4.1.10 Matrix metalloproteinase 9 (MMP9) .....                                    | 48 |

|                                                                                                     |           |
|-----------------------------------------------------------------------------------------------------|-----------|
| <b>CHAPTER 3: META-ANALYSIS OF CYCLOOXYGENASE-2 (COX-2) IN NASOPHARYNGEAL CARCINOMA (NPC) .....</b> | <b>50</b> |
| 3.1 Introduction .....                                                                              | 50        |
| 3.2 Methods .....                                                                                   | 52        |
| 3.2.1 Literature search strategy.....                                                               | 52        |
| 3.2.2 Study criteria (inclusion and exclusion criteria).....                                        | 52        |
| 3.2.3 Method of data retrieves (data extraction).....                                               | 53        |
| 3.2.4 Statistical analysis .....                                                                    | 53        |

|                                                                         |    |
|-------------------------------------------------------------------------|----|
| 3.3 Results .....                                                       | 54 |
| 3.3.1 Eligible studies .....                                            | 54 |
| 3.3.2 Correlation between COX-2 expression and survival outcome .....   | 56 |
| 3.3.3 Correlation between COX-2 expression and treatment response ..... | 56 |
| 3.3.4 Assessment of publication bias.....                               | 60 |
| 3.3.5 Limitations.....                                                  | 60 |
| 3.4 Discussion.....                                                     | 61 |

## **CHAPTER 4: CONSTRUCTION OF MULTIGENE NASOPHARYNGEAL CARCINOMA (NPC) PROGNOSIS ASSESSMENT MODEL .....**

|                                                              |    |
|--------------------------------------------------------------|----|
| 4.1 Introduction .....                                       | 64 |
| 4.2 Methods .....                                            | 65 |
| 4.2.1 Literature search strategy.....                        | 66 |
| 4.2.2 Study criteria (inclusion and exclusion criteria)..... | 66 |
| 4.2.3 Method of data retrieves (data extraction).....        | 67 |
| 4.2.4 Statistical analysis .....                             | 67 |
| 4.3 Results and discussion.....                              | 71 |
| 4.3.1 Eligible studies .....                                 | 71 |
| 4.3.2 Statistical analysis .....                             | 74 |
| 4.3.3 Limitations.....                                       | 86 |

## **CHAPTER 5: CONSTRUCTION OF NASOPHARYNGEAL CARCINOMA (NPC) MOLECULAR NETWORK MODEL .....**

|                                 |    |
|---------------------------------|----|
| 5.1 Introduction .....          | 87 |
| 5.2 Methods .....               | 88 |
| 5.3 Result and discussion ..... | 88 |

|                                                               |            |
|---------------------------------------------------------------|------------|
| <b>CHAPTER 6: GENERAL DISCUSSION .....</b>                    | <b>97</b>  |
| <b>CHAPTER 7: CONCLUSION AND FUTURE RECOMMENDATIONS .....</b> | <b>104</b> |
| 7.1 Conclusion .....                                          | 104        |
| 7.2 Future recommendations .....                              | 105        |
| <b>REFERENCES .....</b>                                       | <b>106</b> |
| <b>APPENDICES.....</b>                                        | <b>157</b> |

## LIST OF TABLES

|                                                                                                                      | <b>Page</b> |
|----------------------------------------------------------------------------------------------------------------------|-------------|
| Table 2.1    The histopathological classification of NPC by WHO in 1991.                                             | 7           |
| Table 2.2    The number of new NPC cases at nasopharynx for different races<br>at all age group in Malaysia.         | 9           |
| Table 2.3    Different outcomes of the different prognostic biomarkers in<br>NPC via meta-analysis studies.          | 37          |
| Table 3.1    Characteristics of all identified studies for meta-analysis of<br>COX-2 in NPC.                         | 57          |
| Table 4.1    Assignment of NPC prognosis assessment risk score in relation<br>to Ln of HRs.                          | 67          |
| Table 4.2    The description for the designated cell and formula used in<br>Microsoft Excel.                         | 69          |
| Table 4.3    The description for the designated cell and formula used in<br>Microsoft Excel.                         | 70          |
| Table 4.4    Characteristics of selected studies for the development of<br>multigene NPC prognosis assessment model. | 73          |
| Table 4.5    The total possible combinations for over-expression of different<br>biomarkers.                         | 77          |
| Table 5.1    The inhibitors targeting different prognostic biomarkers in the<br>network model.                       | 95          |

## LIST OF FIGURES

|                                                                                                                                                           | <b>Page</b> |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| Figure 2.1 A diagram of the nasopharynx, presenting the common site of NPC occurrence, the Fossa of Rosenmuller.                                          | 6           |
| Figure 2.2 The diagram of the regions that depicted high incidence rate of NPC in China.                                                                  | 8           |
| Figure 2.3 A flow chart of study selection process in meta-analysis.                                                                                      | 25          |
| Figure 2.4 Flow diagram of establishing of hypothesis to testing of hypothesis.                                                                           | 34          |
| Figure 3.1 Selection of studies to obtain final studies for meta-analysis of COX-2 in NPC.                                                                | 55          |
| Figure 3.2 A forest plot depicting a meta-analysis estimating COX-2 expression with OS in NPC [hazard ratio (HR) estimated in random model].              | 58          |
| Figure 3.3 A forest plot depicting a meta-analysis estimating COX-2 expression with treatment response in NPC [odd ratio (OR) estimated in random model]. | 59          |
| Figure 3.4 A funnel plot to detect publication bias and systemic heterogeneity for meta-analysis of COX-2 in NPC.                                         | 60          |
| Figure 4.1 Flow chart of studies selection for the development of multigene NPC prognosis assessment model.                                               | 72          |
| Figure 4.2 Layout of the empty setup of multigene NPC prognosis assessment model in Microsoft Excel sheet.                                                | 75          |

|            |                                                                                                                                                                                                               |    |
|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Figure 4.3 | Layout of the empty results of NPC prognosis biomolecular profile in Microsoft Excel sheet.                                                                                                                   | 75 |
| Figure 4.4 | Output of multigene NPC prognosis assessment model when LMP1 is over expressed.                                                                                                                               | 79 |
| Figure 4.5 | Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene NPC prognosis assessment model when LMP1 is over expressed.                              | 79 |
| Figure 4.6 | Output of multigene NPC prognosis assessment model when LMP1, VEGF, EGFR, ERCC1 and MMP9 are over expressed.                                                                                                  | 82 |
| Figure 4.7 | Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene NPC prognosis assessment model when LMP1, VEGF, EGFR, ERCC1 and MMP9 are over expressed. | 82 |
| Figure 4.8 | Output of multigene NPC prognosis assessment model when all the biomarkers are over expressed.                                                                                                                | 84 |
| Figure 4.9 | Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene prognosis assessment model when all the biomarkers are over expressed.                   | 84 |
| Figure 5.1 | A molecular pathway involved by LMP1, CRP, COX-2, VEGF, MMP9 and EGFR in NPC oncogenesis (yellow boxes represented pathways and blue boxes represented prognostic biomarkers).                                | 90 |

|            |                                                                                                                                                                                                                                            |     |
|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Figure 5.2 | Molecular activity of COX-2 in NPC oncogenesis (blue box represented prognostic biomarker).                                                                                                                                                | 90  |
| Figure 5.3 | A molecular pathway network involved by HIF-1 $\alpha$ with LDH, VEGF and LOX in NPC oncogenesis (blue boxes represented prognostic biomarkers).                                                                                           | 92  |
| Figure 5.4 | A molecular pathway involved by ERCC1 after cisplatin-based chemotherapy that induced NER and caused anti-apoptosis in NPC (yellow box represented pathway and blue box represented prognostic biomarker).                                 | 92  |
| Figure 5.5 | The NPC molecular network model comprised of ten biomarkers involved in the generation of computational multigene NPC prognosis assessment model (yellow boxes represented pathways and blue boxes represented the prognostic biomarkers). | 94  |
| Figure 6.1 | The overall flow diagram of NPC prognosis study.                                                                                                                                                                                           | 103 |

## LIST OF ABBREVIATIONS

|                  |                                               |
|------------------|-----------------------------------------------|
| 5-FU             | 5-fluorouracil                                |
| ASR              | Age-adjusted rate                             |
| BAPN             | $\beta$ -aminopropionitrile                   |
| BRD7             | Bromodomain-containing protein 7              |
| $\chi^2$         | Chi-squared                                   |
| CCRT             | Concurrent chemoradiotherapy                  |
| CIs              | Confidence intervals                          |
| CRP              | C-reactive protein                            |
| CRT              | Chemoradiotherapy                             |
| CTAR 1 and 2     | C-terminal activating regions 1 and 2         |
| CXCL 5, 9 and 10 | CXC motif chemokine ligand 5, 9 and 10        |
| COX-2            | Cyclooxygenase-2                              |
| DNA              | Deoxyribonucleic acid                         |
| EBNA             | EBV-determined nuclear antigens               |
| EGF              | Epidermal growth factor                       |
| EGFR             | Epidermal growth factor receptor              |
| EBV              | Epstein-Barr virus                            |
| ERCC1            | Excision repair cross-complementation group 1 |
| ECM              | Extracellular matrix                          |
| FN1              | Fibronectin 1                                 |
| Gy               | Gray                                          |
| HRs              | Hazard ratios                                 |

|                |                                            |
|----------------|--------------------------------------------|
| HNSCC          | Head and neck squamous cell carcinoma      |
| HIF-1 $\alpha$ | Hypoxia-inducible factor-1 alpha           |
| IC             | Induction chemotherapy                     |
| ICRT           | Induction chemoradiotherapy,               |
| IHC            | Immunohistochemistry                       |
| IL-6           | Interleukin-6                              |
| ISH            | RNA-in situ hybridization                  |
| I <sup>2</sup> | I-squared                                  |
| LDH            | Lactate dehydrogenase                      |
| LTF            | Lactotransferrin                           |
| LMP1           | Latent membrane protein 1                  |
| logHR          | Log hazard ratio                           |
| LOH            | Loss of heterozygosity                     |
| LOXL1–4        | LOX-like proteins 1-4                      |
| MMP9           | Matrix metalloproteinase 9                 |
| MTA1           | Metastasis-associated protein 1            |
| MAP            | Mitogen-activated protein                  |
| NPC            | Nasopharyngeal carcinoma                   |
| NGX6           | Nasopharyngeal carcinoma associated gene 6 |
| Nor1           | Neuron-derived orphan receptor 1           |
| NSAIDs         | Non-steroidal anti-inflammatory drugs      |
| NR             | Not related                                |
| NF- $\kappa$ b | Nuclear factor $\kappa$ -B                 |
| OR             | Odd ratio                                  |

|                  |                                                                    |
|------------------|--------------------------------------------------------------------|
| OSCC             | Oral squamous cell carcinoma                                       |
| OS               | Overall survival                                                   |
| PI3K             | Phosphoinositol-3-kinase                                           |
| PRISMA           | Preferred Reporting Items for Systematic Reviews and Meta-Analyses |
| PD-L1/ PD-1      | Programmed cell death ligand-1/ Programmed cell death-1            |
| PTGS2            | Prostaglandin-endoperoxide synthase 2                              |
| PGE              | Prostaglandins                                                     |
| QUOROM           | Quality Of Reporting of Meta-analyses                              |
| RTCs             | Randomized controlled trials                                       |
| RR               | Risk ratio                                                         |
| RT               | Radiotherapy                                                       |
| RT-PCR           | Real-time polymerase chain reaction                                |
| SPLUNC1          | Short palate, lung, and nasal epithelium clone 1                   |
| SNP              | Single nucleotide polymorphism                                     |
| SMD              | Standardized mean difference                                       |
| TPZ              | Tirapazamine                                                       |
| TGF- $\alpha$    | Transforming growth factor alpha                                   |
| TSGs             | Tumor suppressor genes                                             |
| UBAP1            | Ubiquitin Associated Protein 1                                     |
| Var              | Variance                                                           |
| VEGF             | Vascular endothelial growth factor                                 |
| WHO              | World Health Organization                                          |
| Zn <sup>2+</sup> | Zinc                                                               |

## **CHAPTER 1**

### **INTRODUCTION**

Nasopharyngeal carcinoma (NPC) is the formation of malignancy in the tissue of the upper section of pharynx behind the nose (He et al., 2016), more precisely detected at the fossa of Rosenmüller (Lee, Laufer, Ove, Foote & Bonner, 2012). NPC is rare in the Western hemisphere but widely distributed in Southeast Asia countries and in China (Chang & Adami, 2006). In Peninsular Malaysia, NPC was utmost by the Chinese, followed by the Malays and the Indians (Prasad & Rampal, 1992). In Sarawak, the Bidayuh indigenous group showed the highest incident rate with age-adjusted rate (ASR) of 31.5, which was 50% higher than that in Hong Kong (Devi, Pisani, Tang & Parkin, 2004). Throughout Malaysia, it is reporting around 4000 new cases every year (Azizah, Nor-Saleha, Noor-Hashimah, Asmah & Mastulu, 2015). The concurrent chemoradiotherapy (CCRT) with or without neoadjuvant/adjuvant cisplatin-based chemotherapy are the current improved standard treatment protocol administrated to locally advanced NPC patients (Fendri et al., 2009; Wu et al., 2014). The major causes of death among NPC patient are due to local recurrence and distant metastasis, because a large number of patients reached advanced stage and/or metastasis at the time of diagnosis (You et al., 2016). Thus, prognosis is important to better increase the survival rate of NPC patients.

A number prognostic biomarkers such as excision repair cross-complementation group 1 (ERCC1) (Yang et al., 2019), latent membrane protein 1 (LMP1) (Chen et al., 2015), vascular endothelial growth factor (VEGF) (Wang et al., 2018), epidermal growth factor receptor (EGFR) (Sun et al., 2013), C-reactive protein (CRP) (Fang, Xu, Wu, Zhang, Li et

al., 2017), hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ ) (Xie et al., 2018), lactate dehydrogenase (LDH) (Zhai, Gu, Zhai, Wang & Zhang, 2017), lysyl oxidase (LOX) (Zhang, Wu, Ye & Xue, 2018) and matrix metalloproteinase 9 (MMP9) (Liao, Huang, Zhu, Li, Huang & He, 2016) were identified as significant prognostic indicators to determine overall survival (OS) of NPC patients. Comprehensive studies on these biomarkers through meta-analysis have been carried out to draw a more definitive conclusion for the above biomarkers too. Over expression of cyclooxygenase-2 (COX-2) was frequently identified as a prognostic biomarker in different cancers such as breast cancer (Jana et al., 2014), colorectal cancer (Peng, Zhou, Wang, Mou & Zhao, 2013) and pancreatic cancer (Juuti, Louhimo, Nordling, Ristimäki & Haglund, 2006). Studies of COX-2 as prognostic biomarker in NPC have also been done (Pan et al., 2008; Loong et al., 2009). However, the sample sizes of these studies are often small (n=35 and n=58) and the results are conflicting. Even though meta-analysis of COX-2 in NPC regarding lymph node metastasis has been carried out (Yang et al., 2017), a more conclusive meta-analysis of COX-2 in NPC as prognostic biomarker regarding survival outcome was not done before this. Thus, this study aims to determine the prognostic significance of COX-2 in NPC in term of survival outcome using the approach of meta-analysis.

There are a numbers of existing NPC prognosis assessment models such as prognostic nomogram integrating clinical characteristic and hematological/inflammatory biomarkers (Li et al., 2016; Li et al., 2018). Prognostic models to determine death and distant metastasis, disease progression and survival in non-endemic area of China were also reported (Zhang et al., 2013; Zang et al., 2016; Zhao et al., 2019). However, the sample sizes involved in the construction of all of these models were often relatively small. For example, study done by

Zhao and colleagues contained sample size of  $n=103$  only. The patients involved in each of the studies were solely from a particular area and thus, these models only represented and reflected the response on the isolated location of studies. The usage of these models was therefore restricted as these models possessed little/no medical uses in other regions. Additionally, these models focused on clinical characteristic and had little emphasis on the functional genetic prognostic biomarkers. Thus, this study also aims to develop a multigene NPC prognosis assessment model focusing on functional genetic biomarkers by pooling different individual NPC prognostic meta-analysis studies computationally via modification of meta-analysis approach.

Meta-analysis is employed in this study as it provides a more significant and precise result compared to single individual studies. Meta-analysis is a systematic, formal, and quantitative approach and study design used to identify, combine and synthesis the outcomes of previous relevant research. This approach is useful to draw conclusions about a body of studies (Haidich, 2010). The current approach used to conduct meta-analysis is by referring to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher, Liberati, Tetzlaff, Altman & The PRISMA Group, 2009) (refer to Appendix A, page 157-159). By using this method, conflicting results of different individual studies on COX-2 in NPC regarding the OS are pooled and analysed under the same platform to reflect a more significant and relevant output. By modifying PRISMA, different meta-analysis studies focusing on different prognostic factors in NPC are grouped into one platform. This is to integrate isolated aspects of different studies into a more dynamic system and this allows the generation of multigene NPC prognosis assessment model that is based on various functional genetic data. Instead of pooling individual studies that focus on the prognostic significance

of single biomarkers in NPC, pooling of meta-analysis studies of different prognostic biomarkers is therefore more significant and powerful. To our knowledge, this is the first time where modification of PRISMA is applied to generate multigene NPC prognosis assessment model containing different functional genetic biomarkers.

To further understand and explain the multigene NPC prognosis assessment model designed computationally based on data deposited in the databases, a molecular pathway network among the different biomarkers is generated to postulate the physiological interactions among the different biomarkers. This network can clearly display the connections between the different biomarkers in the process of NPC carcinogenesis and hence, explain the complex and dynamic interactions of the pooled biomarkers in the multigene NPC prognosis assessment model.

Herein, the objectives of this project are:

- i. To determine the prognostic significance of COX-2 in NPC in term of OS reflected by hazard ratios (HRs) via meta-analysis.
- ii. To generate multigene NPC prognosis assessment model focusing on functional genetic biomarkers by pooling different individual NPC prognostic meta-analysis studies via modification of meta-analysis approach.
- iii. To explain the multigene NPC prognosis assessment model by generating a molecular pathway network among the different biomarkers.

## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **2.1 Nasopharyngeal Carcinoma (NPC)**

##### **2.1.1 Anatomical position of NPC**

Nasopharyngeal carcinoma (NPC) is characterized by the malignancy in the tissue of the upper section of pharynx behind the nose (He et al., 2016). NPC is a non-lymphomatous squamous cell carcinoma that particularly occurs in the mucosa epithelial lateral cell lining (including squamous, ciliated pseudostratified columnar and transitional epithelium) (Hong & Kuo, 2003) and the minor salivary glands of the nasopharynx (Sham et al., 1990; Effert et al., 1992; Zong, 2001; Lang, Gao, Guo, Zhao & Zhang, 2014; You et al., 2016). Refer to Figure 2.1, the precise and common site of tumour growth of NPC is the fossa of Rosenmüller, the posteromedial to the medial crura of the Eustachian tube, formed by the intersection of superior and posterior walls of nasopharynx (Sham et al., 1990; Jeyakumar, Brickman & Doerr, 2006; Lee et al., 2012).

##### **2.1.2 Histopathology of NPC**

According to World Health Organisation (WHO), NPC is histologically subclassified into three subtypes, namely the keratinizing differentiated squamous cell carcinoma (WHO I or Type I), nonkeratinizing carcinoma which can be sub-divided into nonkeratinizing differentiated (WHO II or Type II/IIa) and nonkeratinizing undifferentiated carcinoma (WHO III or Type III/IIb) and basaloid squamous cell carcinoma (Shanmugarantnam & Sobin, 1993; Barnes, Eveson, Reichart & Sidransky, 2005). Nonkeratinizing

undifferentiated (WHO III) is exceptionally high in Asia whereas keratinizing differentiated (WHO I) and nonkeratinizing differentiated carcinoma (WHO II) are utmost in western countries (Fandi, Altun, Azli, Armand & Cvitkovic, 1994). The nonkeratinizing undifferentiated subtype (WHO III) is found to be highly infected by Epstein-Barr virus (EBV). It is sensitive to chemotherapy and radiotherapy treatment, showing better tumour control rate. WHO III subtype that was predominantly found in Asian NPC appeared to show higher extent of distant metastasis compare to differentiated carcinoma subtypes (Thompson, 2007).

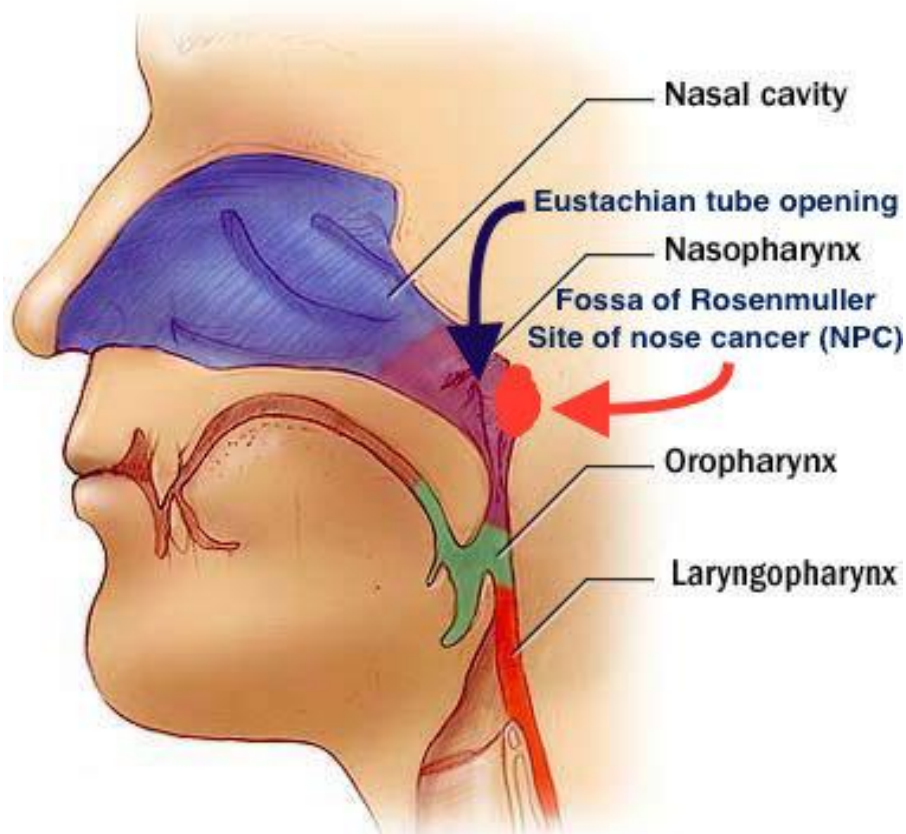


**Figure 2.1:** A diagram of the nasopharynx, presenting the common site of NPC occurrence, the Fossa of Rosenmuller (The Ear Nose and Throat Clinic, 2011). Adapted from <https://www.entspecialist.sg/doctors-corner/?p=98>

**Table 2.1:** The histopathological classification of NPC by WHO in 1991 (Barnes, Eveson, Shanmugaratnam & Sobin, 1993).

| WHO I                       | WHO II                      | WHO III                    |
|-----------------------------|-----------------------------|----------------------------|
| Type I                      | Type II/Ila                 | Type III/Ilb               |
| Keratinizing                | Nonkeratinizing             | Nonkeratinizing            |
| Differentiated              | Differentiated              | Undifferentiated           |
| Utmost in western countries | Utmost in western countries | Exceptionally high in Asia |

### 2.1.3 Epidemiology of NPC

Nasopharyngeal carcinoma (NPC) that is known as one of the leading cancers, has an incidence rate that varied regionally, showing unique geographical distribution (Ferlay et al., 2010). NPC comprised 0.7% of all cancers documented and 2% of all head and neck carcinomas (Union for International Cancer Control, 2014). Globally, its incidence rate increased from about 65,000 cases yearly (Ferlay et al., 2010) to approximately 86,000 cases yearly (MahdaviFar et al., 2016). NPC is identified as the 23<sup>rd</sup> most common cancer globally in 2018, with a total of 129,000 new NPC cases in that year (Bray, Ferlay, Soerjomataram, Siegel, Torre & Jemal, 2018). NPC is rare and uncommon outside endemic areas, such as in North America and Europe, the Western hemisphere. One is diagnosed with NPC among 100,000 population among the Caucasian individuals from North America and Europe annually (Fandi, Altun, Azli, Armand & Cvitkovic, 1994; Jeyakumar, Brickman & Doerr, 2006, Breda et al., 2010). However, NPC incidence rate is over 20 cases per 100,000 people in endemic regions including Southern China, Southeast Asia and the Middle East (Chang & Adami, 2006). The Arctic is included later into the list of NPC endemic regions (Wu, Li & Pan, 2018). The top five countries that show the highest incidence rate are China, Indonesia,

Vietnam, India and Malaysia, all five countries from the continent of Asia (Salehiniya, Mohammadian, Mohammadian-Hafshejani & Mahdavifar, 2018). In China alone, there were 68,272 new NPC cases reported (Mahdavifar, Ghoncheh, Mohammadian-Hafshejani, Khosravi & Salehiniya, 2016). Rate of NPC was high among the Chinese living in the Southern part, especially in Guangdong, Guangxi, Hunan and Fujian (National Cancer Control Office, 1979). Guangdong, Guangxi and Hunan remained the top three provinces in China that showed the highest NPC incidence rate, whereas Fujian ranked the sixth after Jiangxi and Hainan (Wei et al., 2017). Constant high incidence rate in Guangdong and Guangxi for almost 30 years exhibits the unique prevalence feature of NPC, that NPC is distributed in distinctive regional aggregation manner. Figure 2.2 shows the region with high NPC incidence rate. According to National Cancer Registry, NPC is the fourth most common cancer in Malaysia, after breast cancer, colorectal cancer and lung cancer (IARC, 2018).

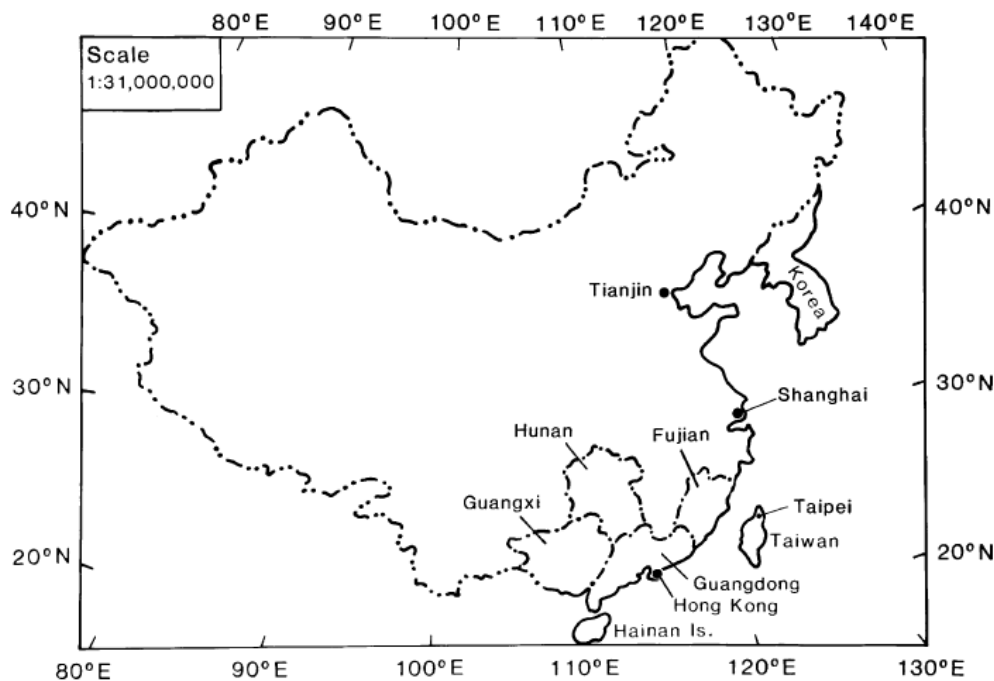


**Figure 2.2:** The diagram of the regions that depicted high incidence rate of NPC in China (Yu & Yuan, 2002). Adapted from <https://doi.org/10.1016/S1044579X02000858>

The Cantonese group reflected the highest incidence rate compare to Hakka, Hokkien and Chiu Chau people in the Southern regions of China (Yu, Ho, Ross & Henderson, 1981; Li, Yu & Henderson, 1985; Jia et al., 2010). NPC was found to be more prevalence and saturated among the Chinese than other community in the world such as the USA (Fandi, Altun, Azli, Armand & Cvitkovic, 1994; Wang, Zhang & Ma, 2013). In Singapore, NPC cases were utmost by Chinese (959 cases), followed by the Malays (88 cases) (National Registry of Diseases Office, 2017). In Penisular Malaysia, NPC was dominated by the Chinese, followed by the Malays and the Indians (Prasad & Rampal, 1992). The same trend was observed from year 2007-2011 as documented in Malaysian National Cancer Registry Report 2007-2011. The number of new NPC cases for different races in Malaysia was depicted in Table 2.2 (Azizah, Nor-Saleha, Noor-Hashimah, Asmah & Mastulu, 2015). In Sarawak, one of the states in Malaysia located on the island of Borneo, the Bidayuh indigenous group showed the highest incident rate with age-adjusted rate (ASR) of 31.5, which was 50% higher than that in Hong Kong (Devi et al., 2004). The high ASR in Bidayuh indigenous was found to be associated with high incidence of family history of NPC (Yeo, Beh, Tan & Pei-Jye, 2018). In Kuching, one of the cities in Sarawak, Chinese and Malays showed the second and third highest NPC incidence rate after Bidayuh indigenous (Sim, Tiong, Subramaniam, Ong & Teh, 2010).

**Table 2.2:** The number of new NPC cases at nasopharynx for different races at all age group in Malaysia (Azizah, Nor-Saleha, Noor-Hashimah, Asmah & Mastulu, 2015).

|        | Chinese | Malays | Indians | Others | Total |
|--------|---------|--------|---------|--------|-------|
| Male   | 2104    | 959    | 44      | 678    | 3785  |
| Female | 611     | 401    | 25      | 268    | 1305  |

Globally, NPC was the 18<sup>th</sup> and 22<sup>nd</sup> most common cancer in male and female respectively (Bray, Ferlay, Soerjomataram, Siegel, Torre & Jemal, 2018). Independent of race–ethnicity, rates of NPC were higher in men than women (Parkin et al., 1997). In Singapore, NPC was the ninth most common cancer among the male with a total of 1073 new cases from 2011-2015, accounting for 3.4% of all cancers in male. NPC was not the top ten most frequent cancer in Singapore female (National Registry of Diseases Office, 2017). A similar trend was observed in Hong Kong, with higher incidence rate in male than female, that was 20 to 30 per 100,000 and 15 to 20 per 100,000 in male and female respectively (Parkin, Whelan, Ferlay, Raymond & Young, 1997; Wei et al., 2010). There were 20,060 male and 7,519 females identified during the period 1983-2008 in Hong Kong (Xie, Yu, Tse, Mang & Yue, 2013). The trend was further supported by incidence rate in China, with the occurrence ratio of 2-3:1 for male:female (Chang & Adami, 2006). ASR per 100,000 in China for male and female were 4.3 and 1.7 respectively (Bray, Ferlay, Soerjomataram, Siegel, Torre & Jemal, 2018). In Malaysia, the total number of new male and female NPC cases were 3785 and 1305 (Azizah, Nor-Saleha, Noor-Hashimah, Asmah & Mastulu, 2015). ASR for both sexes, male and female were 6.3, 9.5 and 3.0 per 100,000 population respectively (Salehiniya, Mohammadian, Mohammadian-Hafshejani & Mahdavifar, 2018), reflecting a higher risk of NPC in male than female. However, in Sarawak, the female Bidayuh was reported to have higher risk of developing NPC than the male (Tan et al., 2018).

#### **2.1.4 Etiology of NPC**

Nasopharyngeal carcinoma (NPC) that has different incidence rate in different regions showing a geographical pattern is found to be closely related with a complex interaction of

viral (Epstein-Barr virus (EBV) infection), risky dietary (salted fish consumption), environmental (wood dust) and genetic (risky genotypes) factors that contribute to its carcinogenesis (Chan, 2010). The detailed molecular pathogenesis of NPC involves alteration of multiple genes in terms of expression and function, alteration of signalling pathways and alteration in the expression of oncogenes and tumour suppressor genes. Early stage of tumour development in NPC usually occurs due to invasion of NPC cell to surrounding tissue or metastasis to cervical lymph (Cho, 2007; Chou et al., 2008).

#### **2.1.4.1 EBV infection**

Nasopharyngeal carcinoma (NPC) is a virus-driven carcinoma (Young & Rickinson, 2004; Chen et al., 2014), predominantly caused by Epstein-Barr virus (EBV) infection. EBV is frequently found in Type IIb NPC than other subtypes (Thompson, 2007). This important primary etiology of NPC has been proven by molecular analyses and serological research (Wu et al., 2003; Chang & Adami, 2006).

EBV, as the name reflected, was discovered in 1964 by Epstein, Achong, and Barr from cultured cells of Burkitt's lymphoma tissue using electron microscope. EBV from the Herpes Virus family was then identified six years later in the cultured cells of NPC tissue (Bar-Sela, Kuten, Minkov, Gov-Ari & Ben-Izhak, 2004). EBV infection is subclinical at the primary stage but involved in different malignancies at the later stage (Chang & Adami, 2006). EBV that could be spread by saliva was the first identified human virus that involved in carcinogenesis. EBV causes latent infection of B lymphocytes after infecting and replicating in the stratified squamous epithelium of the nasopharynx (Thompson & Kurzrock, 2004;

Zhou et al., 2007). Incorporation of clonal viral DNA into the host genome via the infected stratified squamous epithelium of the nasopharynx activates NPC malignancy (Jeyakumar, Brickman & Doerr, 2006).

Elevated IgA class of EBV-associated antigens (Cohen, 2000) and consistent identification of clonal EBV genome in NPC has proven the association of EBV in NPC tumorigenesis (Zhou et al., 2007). Hence, EBV can be used as a diagnostic factor (Parkin, Bray, Ferlay & Pisani, 2005). EBV is also proposed as prognostic indicator as EBV is reported to correlate with treatment response and may predict disease recurrence (Lo et al., 1999; Lo et al., 2000; Lin et al., 2004). This can be done through evaluation of plasma EBV DNA level via real-time polymerase chain reaction (RT-PCR) in which the result obtained is useful in monitoring NPC progression (Zhang, Shu, Song, Li, Huang & Ma, 2016).

#### **2.1.4.2 Dietary factor**

Besides viral infection of Epstein-Barr virus (EBV), risk of nasopharyngeal carcinoma (NPC) is highly associated with non-viral exposure too. For example, diet with frequent intake of Cantonese-style salt-preserved fish was found to be associated with NPC in the endemic areas (Chang & Adami, 2006). This etiological factor was first identified by Ho in 1971, a radiation oncologist from Hong Kong (Ho, 1971). This finding was then further supported by other studies (Hildesheim & Levine, 1993; Klein, 2002; Chan, Teo & Johnson, 2002; Spano et al., 2003).

Studies in the communities of Tianjin (Ning, Yu, Wang & Henderson, 1990), Shanghai (Yuan et al., 2000), Guangxi (Yu, Mo, Chong, Yeh & Henderson, 1988; Zheng et al., 1994), Guangzhou (Ning et al., 1990), Hong Kong (Yu, Ho, Lai & Henderson, 1986) and Malaysia (Armstrong, Yu & Henderson, 1983; Armstrong et al., 1998; Yuan et al., 2000) showed that, frequent consumption of salted-fish was reported to increase NPC risk two to three folds. Experiments in rats have also proven the carcinogenic potential of salt-preserved fish. Salt-preserved fish caused nasal and nasopharyngeal tumour malignancy in rats in a dose-dependent manner (Armstrong et al., 1998; Shao et al., 1988; Yu et al., 1988; Sriamporn, Vatanasapt, Pisani, Yongchaiyudha & Rungpitarangsri, 1992; Zheng, Luo, Christensson & Drettner, 1994; Yuan et al., 2000).

Salt-preserved fish deteriorate due to inefficient salt preservation, thus causes accumulation of carcinogen by increasing the levels of nitrosamines (Ning et al., 1990; Zheng et al., 1994). N-nitrosodimethylamine is the predominant nitrosamine compound that accumulates due to partial food putrefaction during the preserving process of salt-preserved fish (Bartsch, Ohshima, Pignatelli & Calmels, 1992). Besides volatile nitrosamine and high content of nitroso compounds, bacterial mutagens, direct genotoxins, and EBV-activating substances are also found in salt-preserved fish (Armstrong et al., 1983; Yu et al., 1986; Shao et al., 1988; Poirier et al., 1989; Jeannel et al., 1990; Yuan et al., 2000). Apart from salt-preserved fish, compounds that cause carcinogenesis can also be found in different types of salted vegetables and roots/tuber, fermented beans and bean pastes among the Chinese in Guangxi (Yu et al., 1988), various fish/shrimps/seafood pastes in the population of Tianjin (Ning et al., 1990), salted soy bean and mustard green in Singapore Chinese and salted and fermented eggs in Malaysia community (Armstrong et al., 1998). However, Cantonese-style salted fish

still utmost other non-fish preserved food as carcinogen to NPC as reported by the epidemiological data (Poirier et al., 1989).

The exact underlying mechanisms of nitrosamine in NPC carcinogenesis have not been elucidated. It is proposed that high level of nitrate in preserved food reduced to nitrite in the saliva. Nitrite elevates the endogenous synthesis of genotoxic chemical carcinogen inducers as nitroso-compounds (Yi et al., 1993; Tang, Tang, Tian & Cao, 2012). N-nitrosamines are the most commonly found nitroso-compounds. It is activated via hydroxylation to form hydroxynitrosamine and it spontaneously removes aldehyde to form alkyldiazohydroxide, the ultimate carcinogen. It causes carcinogenesis through alkylation of DNA at nucleophilic sites of various cellular components (Tricker & Preussmann, 1991).

#### **2.1.4.3 Environmental factors**

Other factors that lead to the malignancy of nasopharyngeal carcinoma (NPC) include exposure to wood dust and formaldehyde. Inhalation of wood dust particles (5-10  $\mu\text{m}$ ) might trap and deposit in the nasopharynx, associated with increased risk of NPC (Armstrong et al., 2000). Wood dust causes tissue inflammation and injury, impair ciliary clearance and leads to mucostasis (Feron et al., 2001). Besides, wood dust also causes direct and indirect genotoxicity due to genotoxic agent from wood, such as chromium (Klein et al., 2001). Chlorophenol that is present in wood as preservatives also increases the risk of NPC in US wood workers as well (Mirabelli et al., 2000). Studies done in UK and US among furniture and plywood workers in relation to the risk of NPC agreed with the study done by Armstrong (Demers et al., 1995).

Formaldehyde is a chemical commonly found in adhesives and preservatives (Armstrong et al., 2000). Formaldehyde is identified as carcinogen in nasal cavity of rat and is classified as a carcinogen linked to NPC in human (IARC, 1995; Kwon, Kim, Song & Park, 2018). Blair showed the association of increased NPC risk among the industrial workers exposed to formaldehyde (Blair et al., 1986). Bioinformatic analysis of more than 30 studies via meta-analysis approach has revealed the relationship of NPC risk to the exposure of formaldehyde, in a dose-dependent manner (Blair, Saracci, Stewart, Hayes & Shy, 1990; Partanen, 1993). The underlying mechanism of formaldehyde in carcinogenesis is not clear. However, it is suggested that high exposure of formaldehyde forms DNA-protein crosslinks and genotoxicity in human nasal cell (Georgieva, Kimbell & Schlosser, 2003).

#### **2.1.4.4 Gene abnormalities**

The development and progression of nasopharyngeal carcinoma (NPC) might involve accumulation of various genetic alterations over a long period of time (Song et al., 2006). Activation of tumour genes and inactivation or suppression of tumour suppressor genes contributed to the development of NPC and they have been identified cytogenetically (Effert et al., 1992). Tumour suppressor genes that are found to be inactivated were such as short palate, lung, and nasal epithelium clone 1 (SPLUNC1), ubiquitin associated protein 1 (UBAP1), bromodomain-containing protein 7 (BRD7), neuron-derived orphan receptor 1 (Nor1), nasopharyngeal carcinoma associated gene 6 (NGX6), and lactotransferrin (LTF) (Zhang, Zhou & Xiao, 2008). Whereas, the up-regulated tumour causing genes in NPC are such as prostaglandin-endoperoxide synthase 2 (PTGS2), fibronectin 1 (FN1), CXC motif chemokine ligand 9 and 10 (CXCL9 and CXCL10) (Jiang, Feng, Dai, Li & Lu, 2017).

The major susceptible gene in NPC is p53 mutations that included single-point mutations, frame shifts, deletion, duplication, or complete loss of coding sequences (Effert et al., 1992). Among these chromosomal abnormalities, deletion on chromosome 3p is extremely important because 3p deletion is detected in almost 100% of small-cell lung cancer, renal cell carcinoma, and 95–100% of primary NPC biopsies, and even 75% of pre-cancerous lesions show loss of heterozygosity (LOH) on 3p. This, therefore implies that 3p deletion is an early and critical molecular event in the carcinogenesis and development of NPC and 3p should contain some important tumour suppressor genes (TSGs) closely related to NPC development (Zhou et al., 2009). Genetic factors as a role that contributes to NPC is supported by the high incidence of NPC in the Chinese group in Malaysia which highly associated with SPLUNC1 (Yew et al., 2011).

In the context of gene expression, aberrant expression of different genes contributes to NPC carcinogenesis. For example, over-expression of cyclooxygenase-2 (COX-2) converts arachidonic acid to prostaglandins (PGE) and causes inflammation and anti-apoptosis effect in NPC (William et al., 2000; Soo et al., 2005). More than 80% of NPC tumours show over-expression of latent membrane protein 1 (LMP1). LMP1 causes cell immortalisation and involved in NPC oncogenesis (Ding et al., 2005). High expression level of hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ ) induces the over-expression of lactate dehydrogenase (LDH), vascular endothelial growth factor (VEGF) and lysyl oxidase (LOX). High level of VEGF and LOX consequently promotes angiogenesis in NPC (Cox & Erler, 2011; Armstrong et al., 2012; Cox et al., 2013). Over-expression of matrix metalloproteinase 9 (MMP9) degrades extracellular matrix (ECM), accelerating NPC tumour growth and promoting NPC progression, invasion and metastasis (Liotta et al., 1980; Horikawa et al., 2000). High

expression level of C-reactive protein (CRP) creates chronic inflammatory microenvironment that favours metastasis and angiogenesis in NPC (Allin & Nordestgaard, 2011). Alteration in expression of functional gene products contributes to NPC oncogenesis.

### **2.1.5 Clinical presentations, signs and symptoms**

Nasopharyngeal carcinoma (NPC) is usually misdiagnosed due to the relative inaccessible anatomy location of nasopharynx and the late presentation of non-specific clinical signs and symptoms. Most of the NPC tumours remain undiagnosed until they have metastasized to the cervical lymph nodes and exhibit a symptom known as cervical lymphadenopathy (Krishna, James, Kattoor & Balaram, 2004; Jeyakumar, Brickman & Doerr, 2006). Lymph node biopsy is often done to properly diagnose NPC after identifying the cervical lymph node (Brennan, 2006).

Other early presenting clinical symptoms of NPC are such as difficult breathing (Chan et al., 2002; Spano et al., 2003), stuffy nose, nasal regurgitation, unilateral nasal obstruction, nasal discharge with blood stain/nose bleeding (Vokes, Liebowitz & Weichselbaum, 1997), diplopia (double vision), headache (Lo, To & Huang, 2004) and impair head and neck nerves (Chan et al., 2017). Ear pain, otitis media and unilateral hearing loss are some other common and frequent symptoms because the NPC tumour growth is proximate to the eustachian tubes (Brennan, 2006; Thompson, 2007).

Due to the silent anatomical site of nasopharynx, NPC shows higher rate of metastasis compared to other head and neck cancer (Thompson, 2007). The tumours grow unnoticed

and invaded regional area causing neurologic symptoms (Spano et al., 2003). Therefore, large number of newly-diagnosed NPC patients (more than 70%) are diagnosed at the advanced stage (clinical stage III and IV) (Razak et al., 2010). Metastasis in advanced NPC can spread to bone, lung, mediastinum and sometimes to the liver (Brennan, 2006).

However, due to the late diagnosis of NPC at the advanced stage, the treatment results for NPC are not satisfactory. Therefore, it is crucial to improve treatment results by early diagnosis and appropriate management. This can be achieved through developing a primary NPC screening protocol which can contribute to the early diagnosis and hence improve the treatment outcome (Wei & Sham, 2005).

#### **2.1.6 Treatments**

Treatments for nasopharyngeal carcinoma (NPC) are through implication of surgery, radiotherapy treatment and chemotherapy treatment. Surgery as treatment for NPC is less preferred. This is mainly due to the anatomical structural location of nasopharynx, causing difficulties in obtaining sufficient exposure and surgical margin. Surgical resection is done for persistent lymph node or recurrent lymph node present in the neck (Wilson, 1951; Fee, Gilmer & Goffinet, 1988; Fee, Roberson & Goffinet, 1991; Hsu, Ko, Sheen & Chang, 1997). This surgery approach is very invasive and is restricted by the tumour size besides the location factor. Thus, transnasal and endoscopic approach, the less invasive techniques have been successfully applied on selected NPC patients (Chang, Hao, Tsang & Ueng, 2004; Danesi, Zanoletti & Mazzoni, 2007; Hao et al., 2008).

NPC diagnosed at an early stage (I or II) is highly curable. However, most NPC cases are diagnosed at the advanced stage. The tumour site of NPC is hardly reachable and nonresectable. Herein, radiotherapy treatment remains the primary and mainstay treatment modality of NPC with the 70 Gray (Gy) radiotherapy regime targeting the primary tumour and affected lymph nodes (Al-sarraf et al., 1998; Chan et al., 2005; Baujat et al., 2006). The radiation fields enclose retropharyngeal lymphatic drainage pathway, adjacent skull base besides nasopharynx (Lee, Sham, Poon & Ho, 1989). Radiotherapy treatment do not prevent the occurrence of distant metastasis even though it can control primary tumour growth in NPC patients (Pick, Maurer & McWilliams, 1974; Deutsch, Mercado & Parsons, 1978; Jenkin et al., 1981).

Despite the radio sensitivity of NPC, treatment results for advanced NPC is not satisfactory. Advanced stage of NPC patients still suffer from poor survival rate (Teo et al., 1996; Geara et al., 1997, Yeh, Tang, Lui, Huang & Huang, 2005; Rusthoven et al., 2017). According to studies done, treatment outcome for NPC patients was significantly improved with addition of chemotherapy to radiotherapy treatment (Blanchard et al., 2015). Meta-analysis done on individual study design has shown the improved 5-year survival rate among the NPC patients receiving chemotherapy treatment in addition of radiotherapy therapy compared to radiotherapy treatment alone (Pignon, Bourhis, Domenge & Designe, 2000; Blanchard et al., 2015).

The concurrent chemoradiotherapy (CCRT) with or without neoadjuvant/adjuvant cisplatin-based chemotherapy are the current improved standard treatment protocol administrated to locally advanced NPC patients (Al-sarraf et al., 1998; Chan et al., 2005; Fendri et al., 2009;

Wu et al., 2014; Prawira et al., 2017). Inhibitory effect of cisplatin on the tumour cell recover from radiation damage and minor myelosuppression has made cisplatin the first-line chemotherapeutic regimen to combat advanced metastatic NPC (Lin et al., 2003; Guigay, 2008; Chen et al., 2012). Formation of DNA adduct by cisplatin restricts DNA replication and transcription (Martin, Hamilton & Schilder, 2008).

However, relapse and metastasis still occur in approximately 20%-50% of patients, the major factor causing mortality in NPC patients (Hughes et al., 2000). Therefore, there is a great need to identify prognostic factors that correspond to the actual clinical outcome to better design or determine molecular targeted therapy for NPC patients to improve treatment outcome (Hughes et al., 2000; Dannenberg et al., 2001; Dirix, Nuyts & Van den Bogaert, 2006). This is however, yet to be done comprehensively.

#### **2.1.7 Prognostic biomarkers**

Studies recorded that nasopharyngeal carcinoma (NPC) patients in the same classification might depict different biological behaviour and prognosis (Chua, Nicholls, Sham & Au, 2004; Han et al., 2010). This finding suggests that other prognostic factors, such as ethnicity (Su & Wang, 2002) and a larger number of appropriate predictive biomolecular variable that corresponded to survival outcome (Shi et al., 2002) should be incorporated and implemented to better classified NPC patients, to maximise individualized and tailored treatment response and to minimise the adverse effects.

The three types of biomarkers used for cancer detection are screening, diagnostic and prognostic biomarkers. Biomarkers are recognized, measured, evaluated or monitored characteristics shown by alteration in the cellular, biochemical, molecular, genetic, pathogenic or pharmacologic process (Nair, Sandhu & Sharma, 2014; Biomarkers Definitions Working Group, 2001). NPC is often detected at the later stage. Therefore, identification of prognostic biomarkers is more crucial than diagnostic biomarkers to increase treatment outcome and overall survival of NPC. A clinically useful and promising prognostic biomarker can predict diseases outcome of patients in an independent and significant manner. A good biomarker is a marker that can be easily determined and can indicate therapeutic effect besides possess high specificity and reproducibility (Vlahou, 2013).

Examples of NPC prognostic biomarkers are excision repair cross-complementation group 1 (ERCC1) (Yang, Wei, Zhou, Wang & Hu, 2019), cyclooxygenase-2 (COX-2) (Yang et al., 2017), latent membrane protein 1 (LMP1) (Chen et al., 2015), vascular endothelial growth factor (VEGF) (Wang, Peng, Wang & Liu, 2018), epidermal growth factor receptor (EGFR) (Sun et al., 2013), C-reactive protein (CRP) (Fang, Xu, Wu, Zhang, Li et al., 2017), hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ ) (Xie, Liu, He & Yang, 2018), programmed cell death ligand-1 (PD-L1), programmed cell death-1 (PD-1) (Huang et al., 2019), lactate dehydrogenase (LDH) (Zhai, Gu, Zhai, Wang & Zhang, 2017) and matrix metalloproteinase 9 (MMP9) (Liao, Huang, Zhu, Li, Huang & He, 2016). All the biomarkers listed above contain meta-analysis studies regarding overall survival (OS) rate reflected as hazard ratios (HRs) except COX-2. Even though meta-analysis of COX-2 in NPC regarding lymph node metastasis reflected as odd ratios (ORs) has been carried out (Yang et al., 2017), a more

conclusive meta-analysis of COX-2 in NPC as prognostic biomarker regarding OS reflected by hazard ratios (HRs) is not done yet. Thus, this study aims to determine the prognostic significance of COX-2 in NPC in term of OS reflected by HRs via meta-analysis.

## **2.2 Meta-analysis**

### **2.2.1 Background of meta-analysis**

Biological information is generated in an increasing rate because more than one study is done to investigate the important medical questions. The studies are usually carried out at different regions by different research group. However, some of the research findings agree with another while some are diverse and conflicting. This phenomenon is undesirable, causing clinical decision-making unfavourable. To address this issue, meta-analysis is introduced to derive an evidence-based conclusion, useful in medical sciences (Guyatt et al., 1995; Sackett, Roseberg, Gray, Haynes & Richardson, 1996).

Meta-analysis was first defined by Glass in 1976 in social science study as "The statistical analysis of a wide ranging of analysis results from independent studies for the objective of assimilating the discoveries" (Glass, 1976). It is a systematic, formal, and quantitative approach and study design used to identify, combine and synthesis the outcomes of previous relevant research to draw conclusions about a body of studies (Haidich, 2010). Compared to the individual outcome that contribute to the pooled analysis, meta-analysis provides a more precise estimate of the effect of treatment (stronger evidence regarding an intervention), the effect of risk factor for disease and other outcomes. Due to the advancement of technology, meta-analysis which was not regularly used in the medical literature before 1970s emerged

and showed exponential growth over time. Meta-analysis is frequently applied to analyse results of randomized controlled trials (RCTs) (Thacker, 1988; Petiti, 1994). Moreover, it appears to be the most frequently cited form of clinical research nowadays (Patsopoulos, Analatos & Ioannidis, 2005).

### **2.2.2 Principles of meta-analysis**

Previously, meta-analysis was reported according to QUOROM (QUality Of Reporting of Meta-analyses). In year 2009, it was replaced by the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-analyses) (refer to Appendix A, page 157-159) as a useful ungraded guide to report studies on systematic reviews and meta-analyses (Moher et al., 1999; Moher, Liberati, Tetzlaff & Altman, 2009; Liberati et al., 2009). The changes in the guidelines aimed to improve the consistency throughout the systematic review report. Meta-analysis involved literature search, determination of inclusion and exclusion criteria, extraction of study characteristics, statistical analysis, determination of heterogeneity and biasness in meta-analysis (Johansen & Thomsen, 2016).

Bias in meta-analysis occurs if relevant studies are not included. Thus, to minimize the risk of bias and to increase the significance of the results obtained, all the potential studies are retrieved. Literature search using electronic databases is employed to identify published papers and abstracts. Examples of major electronic databases are such as PubMed, Science Direct, Scopus, and Google Scholar (Haidich, 2010). According to PRISMA statement, a full literature search in electronic databases is done in at least one major platform (Liberati et al., 2009). PudMed is the recommended database to start literature searching, a rather

comprehensive database useful for nucleating search concepts. Relevant papers, books, abstract and conference proceedings are hand searched in the database to ensure a comprehensive searching. Literature search is further improved with crosschecking of references and citations in review papers (Haidich, 2010).

Several thousand of potentially eligible studies might be retrieved via electronic literature search. Therefore, eligibility of each study is assessed by the inclusion and exclusion criteria in relation to the research question by attaching to PRISMA statement. The inclusion and exclusion criteria are ideally determined at the early stage of the development of study protocol. Criteria that might be incorporated are such as the number of treatment or exposure groups, types of gene expression, number of patients, types of study design and other relevant information. Eligible studies are chosen for meta-analysis based on the inclusion and exclusion criteria (Haidich, 2010).

There are no universal and standardized inclusion and exclusion criteria for studies selection in meta-analysis. However, the rationale and reasons for all criteria are to be clearly provided and recorded. Meta-analysis could be done in topics of various fields. Thus, universal criteria are not applicable. The process of study selection is summarized and displayed in a flow chart. The chart comprises the number of studies included and excluded in each stage. There are generally four stages, which are identification, screening, determine eligibility of potential studies and final inclusion of selected studies (Haidich, 2010). Figure 2.3 depicts an example of the number of studies identified, included and excluded during screening, and the reasons for exclusions before identifying the final selected studies.

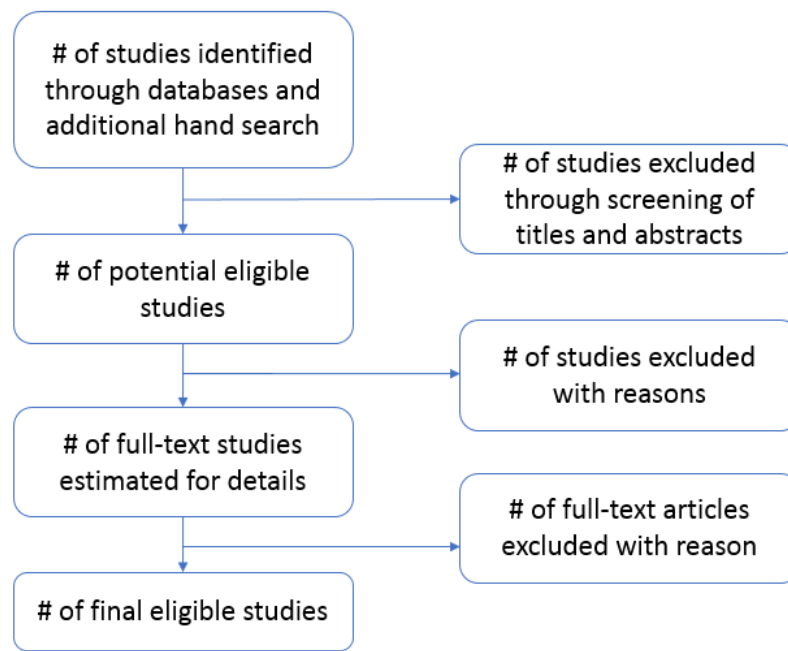


**Figure 2.3:** A flow chart of study selection process in meta-analysis.

The main characteristics of the included studies are tabulated. The relevance and applicability of the included studies could therefore be assessed by reader or other researcher. Relevant information with respect to the defined research question are such as the authors of the studies, the year of publication, the study size, the follow-up period, the method to determine gene expression and type of treatments (Fagerland, 2015).

Risk ratio, hazard ratio, odd ratio and standardized mean difference (SMD) are common measures of effect. To estimate the common effect, weighting of studies is included by determining the inverse of the variance. This is important because overall estimate effect is usually affected less by the smaller studies compare to larger studies. Hence, the value of evidence of any study could be determined by study weighting (Egger, Smith & Phillips, 1997; Haidich, 2010).

Before conducting a meta-analysis, one of the two main categories of statistical model for meta-analysis, fixed effects models or random effects models, is chosen to determine the common effect. The two models differ from one another by the way variability between studies is addressed and treated.

In a fixed effects model, existence of variation observed between studies is assumed to occur due to random variation. This model considers only sole source of variation and existence of one true effect within the study. The effect contributes by each study to the common effect is assumed to be the same. The model is therefore homogenous, having the same study population, the same subject selection criteria and the same way of treatment application (Stangl & Berry, 2000). Example of fixed-effect model is Mantel-Haenzel model (Fidler & Nagelkerke, 2013).

In a random-effects model, distribution of effects is assumed to exist and thus, variation and heterogeneity between studies are observed. To determine the weighting of studies in this model, inverse of the variance and inverse of the heterogeneity parameters are obtained. This approach is more appropriate and conservative as it has wider confidence interval compares to fixed effect model that only determines the weighting of the study from the inverse of the variance (Haidich, 2010). Fixed effect model is less appropriate and less preferable when statistical heterogeneity exist in the results of studies in the meta-analysis. The DerSimonian and Laird method is the random effects model that is most commonly applied. Random effect model is more regularly conducted now due to the computing power of newly improved software (Dersimonian & Laird, 1986).

For research study focusing on overall survival analysis that implies hazard ratios (HRs), pooled HR is determined via the formula proposed by Parmar as shown below (Parmar, Torri & Stewart, 1998).

$$\ln(HR_i) = \frac{\sum_{t=1}^T \frac{\ln(HR_i(t))}{\text{var}[\ln(HR_i(t))]} }{\sum_{t=1}^T \frac{1}{\text{var}[\ln(HR_i(t))]}}$$

K is the number of total studies included and  $i = 1, \dots, K$  studies. Pooled  $\ln(HR)$  is determined via the following formula when estimate of log hazard ratio,  $\ln(HR_i)$  and its variance,  $\text{var}[\ln(HR_i)]$ , are available. The overall log hazard ratio is a weighted average of the individual  $\ln(HR_i)$ , with the weights inversely proportional to the variance of the  $\ln(HR_i)$  of each studies. Variance (var) is obtained by the following formula:

$$\text{Var}[\ln(HR_i)] = [(\ln(\text{Upper CI}) - \ln(\text{Lower CI})) / (2 * 1.96)]^2$$

1.96 was the z- score for 95% CI for the HRs.

Pooled HR is obtained by getting the exponential of pooled  $\ln(HR)$ . Pooled lower and upper confident intervals (CIs) (Lower CI and Upper CI) are established via the formula stated below:

$$\text{Lower/Upper CI} = e^{\ln(HR) \pm \sqrt{\text{overall Var}[\ln(HR)]}}$$

After statistical analysis is done, information of individual studies, effect size and the corresponding 95% confidence intervals (CIs), amount of study heterogeneity and the pooled

result (estimated common effect) are summarized and presented in a forest plot. It is an indispensable tool that gave a visual idea of the findings all in one figure. Common effect across all studies is shown at the bottom row, represented graphically as diamonds shape, where the confidence interval of the common effect is depicted by the widths of the diamonds (Dersimonian & Laird, 1986).

Significant statistical heterogeneity happens in the process of meta-analysis due to the differences in methodology used and differences in results assessments. Although heterogeneity do not suggest a definite variation from true effect, it infers that studies are estimating different effect. Heterogeneity involves diverse methodology might indicate different degree of bias in the studies (Haidich, 2010). Substantive or clinical implications of the heterogeneity is often focused by researcher and thus, the researcher commonly considered heterogeneity as I-squared ( $I^2$ ) (Borenstein, Higgins, Hedges & Rothstein, 2017).

Before  $I^2$  statistic (also called the index of heterogeneity), variation among studies is determined via Cochran's Q statistic, a sum of the squared deviations of each study's estimate from the overall estimate, weighted by the study's impact on the overall estimate. Cochran's Q statistic is a chi-squared ( $\chi^2$ ) test of heterogeneity with  $k-1$  degrees of freedom (df) (equals to the number of studies minus one). However, it is found that, if the number of studies is small, Cochran's Q test is comparatively less significant to identify the heterogeneity. Consequently, hypothesis is tested with an  $\alpha$ -level of 0.10 (Cochran, 1954; Hardy & Thompson, 1998).

Heterogeneity and amount of dispersion (Higgins, Thompson, Deeks & Altman, 2003; Higgins & Thompson, 2002) of results among different studies are determined and quantified by using the inconsistency index  $I^2$ .  $I^2$  defines the total variation across studies in percentage based on the Q statistic. Applying the method described by Higgins and Thompson (Higgins et al., 2003), uncertainty intervals for  $I^2$  (dependent on Q and k) is obtained. The  $I^2$  statistic is calculated as:

$$I^2 = 100\% * (Q - df) / Q$$

Low heterogeneity is shown by a low  $I^2$  value indicating that the effect size is consistent and comparable across the studies. Whereas, greater amounts of inconsistency are reflected by a larger  $I^2$  indicating the varied effect size across studies (Borenstein et al., 2017). The level of precision of  $I^2$  value is indicated by uncertainty interval.  $I^2$  values are between 0 and 100% because negative values of  $I^2$  are put equal to zero.  $I^2$  values that are indicated with positive percentage enable simple detection of heterogeneity. A study is said to have high heterogeneity when the  $I^2$  value is greater than 75% (Fagerland, 2015).

$I^2$  value is independent and unaffected by effect size and the number of studies in the meta-analysis. Thus, even the number of studies is different, comparison among different analysis is still allowed by  $I^2$  values (Borenstein et al., 2017).  $I^2$  statistic is less affected by the number of studies incorporated compared with other methods used to determine the heterogeneity. Unlike Cochran's Q statistic,  $I^2$  statistic remains stable if only few studies are combined.  $I^2$  statistic reflects a logical and readily interpretable metric (Huedo-Medina, Sanchez-Meca, Marin-Martinez & Botella, 2006).

Determination of heterogeneity and identification of sources of variation of the selected studies in meta-analysis are important. Bias in meta-analysis arise due to several factors (Haidich, 2010). First factor being that, not all research findings are published. This scenario is known as publication bias. Some or even large numbers of studies are not published as some of the research are categorized as negative studies, reporting small studies with non-significant or “negative” results. This type of researching is usually having little or no commercial value. On the other hand, large studies with significant and positive results and great commercial values are often published. Unpublished research findings which are possibly different from published data could not be found through computer searching. This phenomenon leads to potential bias in meta-analysis studies (Haidich, 2010).

Another type of bias that might exist within meta-analysis study is reporting bias. This condition arises when the studies are not completely conducted due to protocol failure or treatment failure. Some studies have missing subjects which could actually yield important results. According to previous research done, intervention in medicine was found to be affected by missing studies (Chan, Hrobjartsson, Haahr, Gotzsche & Altman, 2004; Dwan et al., 2008). Thus, all potential studies are collected to ensure the most significant and appropriate meta-analysis.

Several efforts are done to minimize the potential bias existed from meta-analysis studies. For examples, searching of unpublished findings, investigating of graduate dissertations, crosschecking of the references and citations in review papers. These provide a more extensive and far-reaching search. It is almost impossible to find entirely every relevant

study on a subject even though the ideal objective of meta-analysis is to find and pooled all studies that meet the inclusion criteria (Haidich, 2010).

Publication bias is assessed systematically via several methods. Funnel plot is the most commonly used tool to identify publication bias in the form of missing studies, especially in small study design (Sterne et al., 2011). Funnel plot was a simple graphical scatterplot designed by Light and Pillemer and the details of the plot were discussed later by Egger and colleagues (Light & Piller, 1984; Egger, Smith & Phillips, 1997; Sterne & Egger, 2001). Funnel plot evaluates the potential bias of treatment effect estimated from individual research on the x-axis against study size/study precision on the y-axis. Small studies tend to show greater inconsistency and greater treatment effect. Studies with low precision and high precision are placed at the bottom and top of the plot respectively. X-axis is log transformed when the effect measure is in ratio form, such as risk ratio (RR) or hazard ratio (HR).

Small studies that show great inconsistency in effect estimate are placed at the bottom of the plot, while large studies that show small variability are placed at the top of the plot. Estimated common effect is plotted in the graphic as dotted vertical line. Funnel plot shows symmetrical inverted funnel shape with wide base and narrow top if no publication bias is observed. If the bias present is big, asymmetry is observed from the funnel plot. However, asymmetry of funnel plot might also due to clinical heterogeneity between different studies (such as difference in methodology used) besides the factor of publication bias (Haidich, 2010).

Meta-analysis studies on different NPC prognostic biomarkers are done based on the principle discussed in this section. In this study, the same principle is applied to conduct meta-analysis study of COX-2 in NPC regarding the overall survival (OS) reflected as hazard ratios (HRs) and treatment response reflect as odd ratios (ORs).

### **2.3 Multigene NPC prognosis assessment model**

Development of meaningful model is important in different medical aspects, especially in oncology. A model worth of medical practice allows early cancer detection by the oncologist. A model that incorporates the pathways enabled the identification of the most appropriate anti-cancer drugs and application of the drugs with the right dose at the right time (Assmus et al., 2006). However, the number of medically useful models to diagnose and monitor different kind of cancers are still lacking.

#### **2.3.1 Rationale of integration of individual studies into a model**

Due to the advancement in technology and the extensive exploration in medical research, biological data are being produced at an exponential rate (Guyatt et al., 1995). Large number of “wet” experiments with high throughout measurements regarding prognosis of various cancers are done. To handle this large quantity of data, the use of computer has been employed greatly. It is an ideal approach because it analyses and handles the surging amount of data in a time effective manner besides incorporates the findings into complex dynamic observed in nature. Understanding and organizing of data associated with biological

macromolecules by using computational technique is known as bioinformatics (Reichhardt, 1999).

However, large number of studies done focused on single gene, protein or metabolite molecule. The results from these different findings on the same topic are often inconsistent and conflicting. Meta-analysis on the massive data available in the database using advanced computational technique can solve the problem faced. This approach is known as “dry” experiments design. Example of “dry” experiments are such as systematic reviews and meta-analysis. Figure 2.4 shows the interactive cycle from establishing of hypothesis to testing of hypothesis, involving “wet” and “dry” experiments. This interactive process is still adopted but expanded where large-scale and genome-wide experimental methodology is employed. Data integration and model simulation are manageable via computational aid (Bunge, 1976).

Complex and dynamic interactions of molecules involved in the progression of complex diseases such as cancer are impossible to be fully understood from studies focusing on single parameter. Hence, the focus now have been shifted to understanding the association of those molecules that interact directly or indirectly in the form of pathway or complex network (Baranzini, 2009; Aitman et al., 2011). Although the results obtained from meta-analysis are more significant and relatively more reliable compare to individual studies, there are no interaction and correlation among the different studies and hence, lack of association among the different gene expression. The phenomenon is undesirable and the focus now has shifted from understanding, identifying and characterising of the biological system towards construction of dynamic interactions to reflect the functional activity (Assmus, Herwig, Cho & Wolkenhauer, 2006). Data obtained from systematic and quantitative studies, for example

from meta-analysis studies, is transformed into a comprehensive and high-level of structural organisation. In observed nature, biological system works in a complex dynamic. The dynamic changes by the interaction of the system components is the fundamental factor to the development and adaptation to disease (Assmus et al., 2006).

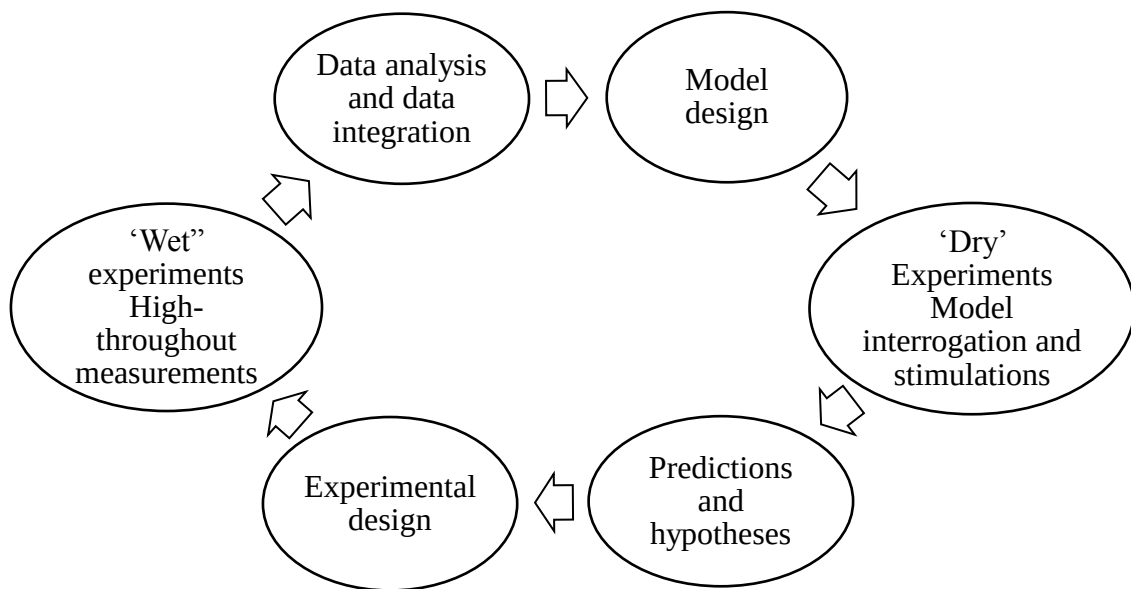


**Figure 2.4:** Flow diagram of establishing of hypothesis to testing of hypothesis (Bunge, 1967). Adapted from <https://www.jstor.org/stable/1170452>

Therefore, integrating and consolidating vast body of data and findings into a platform to understand the dynamic in the system enable better diagnosis, prognosis, prevention and optimising drug uses as treatment. Working beyond statistical analysis to reconstruct the data to provide a meaningful diagnostic/prognostic model in medical field is the current direction emphasized on. With the large amount of information available, systems-level models of gene or protein function is derived. Therefore, such a remodelling improves disease stratification, allows individualized and personalized treatment and development of

new drugs. This is done not to replace the findings from individual studies but to complement them (Assmus et al., 2006).

### **2.3.2 Integration of prognostic biomarkers for NPC prognosis assessment model**

Prognosis assessment models for different cancers are available. Examples are Gail model, the breast cancer risk prediction model (Gail, Brinton, Byar, Corle, Green, Schairer & Mulvihill, 1989; Gail & Benichou, 1994) and Bach model and Spize model (Bach et al., 2003; Spitz et al., 2007), the lung cancer risk prediction models. There are a numbers of existing NPC prognosis assessment models too such as prognostic nomogram integrating clinical characteristic and hematological biomarkers (Li et al., 2018), prognostic nomogram including inflammatory biomarkers (Li et al., 2016), prognostic nomogram determining distant metastasis in NPC (Zhao et al., 2019), prognostic model of death and distant metastasis in nonendemic area of China (Zang et al., 2016) and statistical prognostic score model to determine disease progression and survival of NPC patients (Zhang et al., 2013). However, most of these models are constructed with small sample sizes where the patients of each studies representing and reflecting the outcome only on the isolated location of studies. The usage of these models is restricted as most of the models are only relevant and significant at the areas of studies. Therefore, most of the models have no medical uses. Besides, these prognostic models stressed more on clinical characteristics and only emphasized none or a few prognostic biomarkers such as inflammatory biomarkers. The models listed do not incorporate functional genetic biomarkers as the main prognostic parameter. Thus, this study aims to develop multigene NPC prognosis assessment model

focusing on functional genetic biomarkers by pooling different individual NPC prognostic meta-analysis studies via modification of meta-analysis approach.

Meta-analysis for different prognostic biomarkers of nasopharyngeal carcinoma (NPC) has been done. However, little of these research findings are put into medical practice. Example of NPC prognostic biomarkers are excision repair cross-complementation group 1 (ERCC1) (Yang et al., 2019), cyclooxygenase-2 (COX-2) (Yang et al., 2017), latent membrane protein 1 (LMP1) (Chen et al., 2015), vascular endothelial growth factor (VEGF) (Wang et al., 2018), epidermal growth factor receptor (EGFR) (Sun et al., 2013), C-reactive protein (CRP) (Fang, Xu, Wu, Zhang, Li et al., 2017), hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ ) (Xie et al., 2018), lactate dehydrogenase (LDH) (Zhai, Gu, Zhai, Wang & Zhang, 2017), lysyl oxidase (LOX) (Zhang, Wu, Ye & Xue, 2018) and matrix metalloproteinase 9 (MMP9) (Liao, Huang, Zhu, Li, Huang & He, 2016). Except COX-2, all the biomarkers listed above contain meta-analysis studies regarding overall survival (OS) rate reflected as hazard ratios (HRs). Meta-analysis of COX-2 in NPC regarding lymph node metastasis reflected as odd ratios (ORs) has been carried out (Yang et al., 2017). However, a more conclusive meta-analysis of COX-2 in NPC as prognostic biomarker regarding OS reflected by hazard ratios (HRs) is not done yet. Table 2.3 shows the details of the listed biomarkers. Thus, this study aims to determine the prognostic significance of COX-2 in NPC in term of OS reflected by HRs via meta-analysis approach. Having done this, all the prognostic biomarkers can be pooled under one single platform via modification of PRISMA to generate a multigene NPC prognosis assessment model. Through this, the complex and dynamic interactions between different biomarkers are placed under the same platform, making the model more significant and relevant. Development of molecular pathway network model could further explain the

prognosis assessment model that is generated computationally. This helps to explain the physiological interactions between all biomarkers that are pooled into a model through computational approach in a molecular manner.

**Table 2.3:** Different clinical outcomes of the different prognostic biomarkers in NPC via meta-analysis studies.

| <b>Prognostic Biomarkers</b>                          | <b>Clinical Outcomes</b> | <b>Measure of effect</b> |
|-------------------------------------------------------|--------------------------|--------------------------|
| Cyclooxygenase-2 (COX-2)                              | LNM                      | OR                       |
| Latent Membrane Protein 1 (LMP1)                      | OS                       | HR                       |
| Vascular Endothelial Growth Factor (VEGF)             | OS                       | HR                       |
| Epidermal Growth Factor Receptor (EGFR)               | OS                       | HR                       |
| Serum Lactate Dehydrogenase (LDH)                     | OS                       | HR                       |
| C-Reactive Protein (CRP)                              | OS                       | HR                       |
| Excision Repair Cross-Complementation Group 1 (ERCC1) | OS                       | HR                       |
| Hypoxia-Inducible Factor-1 alpha (HIF-1 $\alpha$ )    | OS                       | HR                       |
| Lysyl Oxidase (LOX)                                   | OS                       | HR                       |
| Matrix Metalloproteinase 9 (MMP9)                     | OS                       | HR                       |

\*\*LNM = Lymph node metastasis, OS = Overall survival, OR = Odd ratio, HR = Hazard ratio

## 2.4 Generate molecular pathway model

Mechanism of the progression of complex diseases is studied and understood via the combination of genomics and/or proteomic data. Development of complex diseases such as cancer involves multifactorial biological pathways. Thus, the robustness of a model could be further explained by identifying the molecular interaction of all biomolecules in a pathway network model. Molecular network allows the discovery of risk factors that leads

to the occurrence of diseases and identification of individualized treatment (Zheng, Wei, Zhao & Ni, 2018). This network could clearly display the connections between the different biomarkers in the process of NPC carcinogenesis and hence, provides understanding to the complex and dynamic interactions of the pooled biomarkers in the prognosis assessment model.

Network models concerning the predictive molecular biomarkers on breast cancer is done by Toss and his colleagues in a review (Toss et al., 2017). Herein, by combining prognostic biomarkers involved in the development of model, the biomarkers are integrated into a network, to prove the dynamic and complex interactions among the different biomarkers contributing to the formation and progression of NPC. This is done via literature reviewing of previous research done on the respective biomarkers. Relevant data are extracted, analysed and combined into a molecular pathway network.

#### **2.4.1 Prognostic biomarkers included**

The final ten prognostic biomarkers incorporated in the multigene NPC prognosis assessment model are cyclooxygenase-2 (COX-2), latent membrane protein 1 (LMP1), vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), lactate dehydrogenase (LDH), C-reactive protein (CRP), excision repair cross-complementation group 1 (ERCC1), hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ ), lysyl oxidase (LOX) and matrix metalloproteinase 9 (MMP9).

#### **2.4.1.1 Cyclooxygenase-2 (COX-2)**

Interaction of development of malignancy with persistent inflammation has been studied and acknowledged since the 19<sup>th</sup> century (Mantovani, Allavena, Sica & Balkwill, 2008). Chronic and persistent inflammation is found to contribute to about 25% of cancers (Balkwill & Mantovani, 2012). Inflammation leads to cancer via two pathways, that are the intrinsic pathway and the extrinsic pathway. Expression of inflammation mediated programmes are activated via intrinsic pathway by the oncogenes and tumour suppressor genes. Whereas, carcinogenesis due to chronic inflammation occurs via extrinsic pathway (Grivennikov et al., 2010; Solinas, Marchesi, Garlanda, Mantovani & Allavena, 2010).

Over expression of cyclooxygenase (COX)-2 is found to cause inflammation in various cancers, including NPC. Over expression of COX-2 leads to NPC carcinogenesis through multiple steps (Soo et al., 2005). COX-2 is involved in the conversion of arachidonic acid to prostaglandins (PGE), mediating cell proliferation, angiogenesis and anti-apoptosis (Tsuji & Dubois, 1995; Tsuji et al., 1998; Williams et al., 2000).

To counter the effects caused by COX-2, COX-2 inhibitors have been introduced. Recent studies found that in a dose dependent manner, COX-2 inhibitors reduce the growth of NPC cell line, inhibit invasion and migration of NPC cells besides reducing cyclin D1 levels (Zhang, Qiu, Chen, Liang & Huang, 2014). Also, COX-2 inhibitors are able to suppress tumour and competent to enhance the radiotherapy outcome, making COX-2 inhibitors treatment promising (Tan & Putti, 2005). The most commonly used COX-2 inhibitor is Celecoxib.

#### **2.4.1.2 Latent membrane protein 1 (LMP1)**

NPC is commonly recognized as virus-driven malignancy (Young & Rickinson, 2004; Chen et al., 2014), predominated by Epstein-Barr virus (EBV) infection (Shanmugaratnam & Sobin, 1993; Khanna et al., 1998). NPC patients usually do not show any signs and symptoms at the early stage. Thus, EBV DNA in plasma is determined as screening parameter for asymptomatic patients (Chan et al., 2017). The tumorigenic potential of EBV in NPC is due to the two unique sets of latent genes. First set of latent genes are the latent membrane proteins (LMP) which includes LMP1, LMP2A, and LMP2B. Another set of latent genes are the EBV-determined nuclear antigens (EBNA), sub-divided into EBNA1 and EBNA2 (Brooks, Yao, Rickinson & Young, 1992).

About 80-90% of NPC tumours show over expressed LMP1. LMP1 that causes cell immortalisation is recognized as the principal oncogene of NPC (Wang, Liebowitz & Kieff, 1986). Structural characteristics of LMP1 allow the infection in the host. LMP1 contains six transmembrane domains that allow the association to host membrane. LMP1 also contains two signalling domains that activate several signalling pathways (Tulalamba & Janvilisri, 2012; Huang et al., 2018; Zhu et al., 2018). The two signalling domains are known as the C-terminal activating regions 1 and 2 (CTAR 1 and 2) with carboxy-terminal (Huen, Henderson, Carter & Rowe, 1995). These two regions could activate nuclear factor  $\kappa$ -B (NF- $\kappa$ B) pathway via binding to tumour necrosis factor receptor-associated factors (TRAFs) (Mainou, Everly & Raab-traub, 2005). Upregulation of NF- $\kappa$ B activates proliferative signals such as COX-2 and VEGF (Murono et al., 2001; Deng et al., 2003). Besides, LMP1-induced NF- $\kappa$ B activation ultimately promotes telomerase activation and cell immortalization (Ding et al., 2005). The two signalling domains could also activate mitogen-activated protein

(MAP) kinases, and phosphoinositol-3-kinase (PI3K) pathways (Mainou & Raab-traub, 2006). LMP1-positive cells mainly prevent apoptosis, enhance metastasis (Ozyar, Ayhan, Korcum & Atahan, 2004) and mediate disease progression (Liu, Peng, Chang & Hung, 2003). Studies show that, the two activation domains of LMP1 transactivated matrix metalloproteinase 9 (MMP9) at the promoter region too by up-regulating NF-kB and activator protein 1 (AP-1) (Takeshita et al., 1999). LMP1 inhibitor such as Simvastatin potentiates chemotherapy treatment by enhancing the sensitivity (Pioche-durieu et al., 2005; Mei et al., 2007).

#### **2.4.1.3 Vascular endothelial growth factor (VEGF)**

Tumour growth and metastasis in cancer are acted by angiogenesis. The degree of this fundamental process is greatly affected by the distribution of proangiogenic and antiangiogenic factors. One of the most commonly known angiogenic factors is vascular endothelial growth factor (VEGF) (Dvorak, Brown, Detmar & Dvorak, 1995). VEGF has other names such as vascular permeability factor (VPF) or vasculotropin. VEGF is a dimeric glycoprotein with at least six variants that functions at endothelial cells by increasing vascular permeability (Brown et al., 1997; Poltorak et al., 1997; Sugihara, Wadhwa, Kaul & Mitsui, 1998; Ferrara, Gerber & LeCouter, 2003). It is a potent mitogen that is highly induced and triggered by hypoxia (Forsythe et al., 1996). VEGF is one of the target gene of HIF-1 $\alpha$ , contributed to hypoxic tumour profile of NPC. Co-expression of VEGF and HIF-1 $\alpha$  shows distant metastasis, local recurrence and low survival rate in NPC patients (Hui et al., 2002; Hui et al., 2008; Lu et al., 2008).

Over expression of VEGF induced by HIF-1 $\alpha$  (Cox & Erler, 2011) is observed in various cancers such as breast cancer, gastric cancer and bladder cancer. The over expression of VEGF is demonstrated to show poor prognosis.

#### **2.4.1.4 Epidermal growth factor receptor (EGFR)**

Epidermal growth factor receptor (EGFR) is the receptor tyrosine kinases that is most often investigated. EGFR activates downstream signalling protein after activating the respective receptor via its extracellular domain. Natural ligands that commonly bound to EGFR are such as epidermal growth factor (EGF) and transforming growth factor alpha (TGF- $\alpha$ ) protein. Activation of signalling protein triggers cascading of various cellular process (Olayioye, Neve, Lane & Hynes, 2000). According to Human Protein Reference Database, there are around 177 molecules targeting about 200 targets in EGFR signalling pathway (Blagoev, Ong, Kratchmarova & Mann, 2004). Examples of pathways activated are such as PI3K/AKT/mTOR, JAK/ STAT, and Ras/Raf/MAPK pathways (Prenzel, Fischer, Streit, Hart & Ullrich, 2001).

Over expression of EGFR is commonly observed in various cancer, including NPC (Zheng, Hu, Chen & Christensson, 1994; Roychowdhury, Tseng, Fu, Weinburg & Weidner, 1996; Leong, Loh, Putti, Goh & Tan, 2004). The statistic shows moderate to high EGFR expression in 85% of Chinese NPC patients. Most NPC cell lines reveal high EGFR expression too. Over expression of EGFR involves in NPC carcinogenesis via tumour metastasis, tumour development, progression, recurrence, poor survival and therapeutic resistance (Ma et al.,

2003; Pan et al., 2008; Ma et al., 2010). Hence, EGFR is now widely studied as NPC target for therapy (Sung et al., 2005; Xiao et al., 2007).

#### **2.4.1.5 Lactate dehydrogenase (LDH)**

Expression level of lactate dehydrogenase (LDH) is important in determining the hematological malignancies and other solid tumour in term of the clinical significance (Wimazal et al., 2008; Armstrong, George & Halabi, 2012). Large tumour that leads to hypoxic microenvironment could be indicated through LDH expression level (Firth, Ebert & Ratcliffe, 1995). However, understanding of the exact pathway to this phenomenon is yet to be fully studied. For patients who undergo radiotherapy and chemotherapy treatment, the clinical outcomes could be determined through the oxygen level or the hypoxic level of the tumour. The higher the oxygenation status in the tumour, the better the clinical outcome upon treatment (Durand, 1991).

Besides clinical outcome, the LDH expression level is also closely associated with oncogenesis. The cellular LDH expression level is found to be regulated by Myc and PI3K/Akt/mTOR pathways (Kim & Dang, 2006). Activation of the pathways and the increment of the LDH level are demonstrated as poor prognostic factor in NPC (Yu et al., 2013). Activation of LDH that leads to tumour growth is ultimately related to cellular turn over and progressive inflammation (Armstrong et al., 2012). Oxamate is the promising therapeutic target used for NPC patients with over expressed LDH (Zhai, Yang, Wan, Zhu & Wu, 2013), arresting and downregulating the pathways (Wimazal et al., 2008).

#### **2.4.1.6 C-reactive protein (CRP)**

The C-reactive protein (CRP) gene contains two main regions, a region that encodes mature protein and a region that encodes signalling peptide. Both these regions are separated by only one intron, located at the short arm of chromosome 1. CRP could be detected in acute phase systemic inflammation, acting as non-specific biomarker. CRP also reacts to tissue damage and various infections. Expression of CRP is often used to predict survival in various cancers, such as breast cancer (Guo et al., 2015), lung cancer (Jing et al., 2015), ovarian cancer (Poole et al., 2013), hepatocellular carcinoma (Fang et al., 2017) and also NPC (Li et al., 2016).

In 1863, Virchow proposed the link between CRP and cancer. However, the network interaction of CRP in cancer was studied only since the past two decades (Tillett & Francis, 1930; Balkwill & Mantovani, 2001). The detailed and precise mechanism of CRP in oncogenesis on NPC is not clearly known. However, there are some hypothesis made regarding this matter. CRP functions as an important member of innate immune system. CRP could recognise non-self-pathogen, identify membrane component of injured cells and react to tumour necrosis (Wong et al., 2007; Mccarthy, 2015). The tumour microenvironment that are always in chronic inflammation state favoured the development and carcinogenesis of cancers, including NPC. Such a condition is conducive for distant metastasis, angiogenesis and deoxyribonucleic acid (DNA) damage (Allin & Nordestgaard, 2011). Excessive CRP is also produced due to activation of signalling pathways such as NF- $\kappa$ B1 by cytokines. Cytokines are inflammatory factors, having interleukin-6 (IL-6) and IL-8 as the common examples (Pine et al., 2011). Due to its roles and properties, CRP is chosen

as suitable inflammatory biomarker in clinical practice as a mean to stratify patients into different prognosis category.

#### **2.4.1.7 Excision repair cross-complementation group 1 (ERCC1)**

The excision repair cross-complementation group 1 protein (ERCC1) is a drug-resistance related protein that is involved in several mechanisms to repair damaged DNA (Murray, Vallee-Lucic, Rosenberg & Andersson, 2002; Olaussen et al., 2006; Gossage & Madhusudan, 2007; Martin, Hamilton & Schilder, 2008; Curtin, 2012). It is associated with the nucleotide excision repair (NER) pathway that repairs DNA damage by removing cisplatin-induced DNA adducts, identifying, resecting and repairing double-stranded breaks and inter-strand crosslinks (Ahmad et al., 2008), and ultimately halting cancer cells apoptosis (Reed, 2005; Olaussen et al., 2006). Hence, patients are resistance to cisplatin-based chemotherapy if ERCC1 expression is high and acquires better survival benefit if ERCC1 expression is low (Azuma et al., 2007; Gossage & Madhusudan, 2007; Li et al., 2014). ERCC1 has been found to be the rate limiting protein involved in the removal of platinum adducts from genomic DNA, thereby influencing cisplatin sensitivity and treatment outcome (Arora, Kothandapani, Tillison, Kalman-maltese & Patrick, 2010; Kirschner & Melton, 2010). Predictive and prognosis value of ERCC1 expression has been studied in several cancer types such as non-small cell lung cancer (Olaussen et al., 2006), gastric cancer (Leibel et al., 1991), oesophageal cancer (Ma, Hui & Chan, 2008), colorectal cancer (Kim et al., 2009), ovarian and head and neck tumours (Metzger et al., 1998; Olaussen et al., 2006; Azuma et al., 2007; Handra-luca et al., 2007; Wei et al., 2008; Kim et al., 2009; Steffensen, Waldstrom & Jakobsen, 2009). Studies in NPC reveal that the high expression of ERCC1 in

tumours correlates with low chemotherapy response and poor survival in patients treated with cisplatin-based induction chemotherapy (Huang et al., 2012). A comprehensive assessment of ERCC1 expression patterns in studies on NPC might provide insights into the identification of a definite biomarkers of prognostic value.

#### **2.4.1.8 Hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ )**

Hypoxia is a condition with deprivation and deficiency of oxygen supply. Hypoxia promotes tumour progression and thus, it is a common feature in many solid tumours. Hypoxia-inducible proteins are triggered and induced by hypoxia microenvironment, causing a series of cellular effects such as anti-apoptosis, adaptation to stress, promote metastasis, invasiveness, angiogenesis and poor prognosis. Hypoxia is also known to reduce the efficiency of radiotherapy and chemotherapy treatment. According to evidence from research done, all the adaptive responses of cells to the hypoxia conditions in cancers are mediated by hypoxia-inducible factor-1 (HIF-1)-dependent pathway (Ji, 2014).

HIF-1 is a helix-loop-helix transcription factor consisted of two subunits, which are HIF-1 $\alpha$  and HIF-1 $\beta$ . Both of these subunits exist as heterodimer. HIF-1 $\alpha$  regulates the biological activity via its expression and function (Rankin & Giaccia, 2008). HIF-1 $\alpha$  subunit involves in glucose uptake, metabolism and growth rate, besides playing the major functions of other hypoxia-inducible proteins (Hu, Liu & Huang, 2013). Over expression of HIF-1 $\alpha$  leads to malignancy and reflects a poor prognosis in various types of solid tumours. Worse prognosis in relation to high expression of HIF-1 $\alpha$  in NPC was reported by Kitagawa (Kitagawa et al., 2013). Moon reported that stabilisation of HIF-1 $\alpha$  transcription complex by metastasis-

associated protein 1 (MTA1) enhanced solid tumour progression and metastasis, supported by angiogenesis (Moon et al., 2006). Over expression of HIF-1 $\alpha$  up regulates the expression of TWIST, a transcription factor that mediates cancer metastasis, causing resistance to treatment and increase in mortality in malignancy (Yang et al., 2008). HIF-1 $\alpha$  signalling pathway is also demonstrated to be regulated by VEGF and COX-2, contributing to cancer progression (Maroni et al., 2011; Fu et al., 2012; Min et al., 2012). To reduce the progression of NPC, the expression of both HIF-1 $\alpha$  and VEGF could be controlled by using hypoxic inhibitor, tirapazamine (TPZ) (Hong et al., 2011).

#### **2.4.1.9 Lysyl oxidase (LOX)**

Lysyl oxidase (LOX) is a hypoxia-inducible protein (Cox et al., 2013). It contains a hypoxia responsive site at the promoter region, targeted by hypoxia-inducible factor (HIF) at the transcriptional level (Erler et al., 2006). Another principal hypoxia-inducible protein is HIF-1 $\alpha$ , responsible for management of responses due to hypoxia. Thus, upon hypoxia, HIF-1 $\alpha$  would be over expressed and consequently activates other hypoxia-inducible genes.

LOX is an extracellular copper-containing amine oxidase. It has four other protein family members namely LOX-like proteins (LOXL1-4) (Panchenko, Stetler-stevenson, Trubetskoy, Gacheru & Kagan, 1996). LOX functions in extracellular matrix, catalysing the covalent cross-linkage of collagen and elastin fibres extracellularly. LOX participates in tumour progression and development due to its function in enhancing cell migration and motility (Wilgus et al., 2011). Other intracellular activities such as cell adhesion and migration, gene

transcription and cell differentiation are also carried out by LOX, but intracellularly (Kagan & Trackman, 1991; Molnar et al., 2003; Kagan & Li, 2003; Albinger-hegyi et al., 2010).

Association between high LOX expression and low overall survival rate/high recurrence rate is observed in cancers cell lines, breast cancer, renal cancer, gastric cancer, prostatic cancer, head and neck cancer and NPC (Stassar et al., 2001; Lapointe et al., 2004; Erler et al., 2006; Payne, Hendrix & Kirschmann, 2007; Weise et al., 2008; Albinger-hegyi et al., 2010). Combined expression of LOX, HIF and VEGF promotes cancer cells to grow faster, leading to metastasis (Chung, Lee & Ferrara, 2010).  $\beta$ -aminopropionitrile (BAPN) is an irreversible inhibitor that could inhibit the catalytic activity of LOX, disturbed cell invasion and progression caused by hypoxia hence improving NPC overall survival (Tang, Trackman & Kagang, 1983; Xiao & Ge, 2012).

#### **2.4.1.10 Matrix metalloproteinase 9 (MMP9)**

Matrix metalloproteinases (MMPs) is a Zinc ( $\text{Zn}^{2+}$ ) dependent endopeptidase. MMPs are synthesized and released as zymogen belonging to gelatinase subgroup, as a monomer. MMPs basically exert the function in the extracellular matrix (ECM), managing the integrity and composition of the matrix (Liotta et al., 1980). Examples of MMPs are MMP2 and MMP9, belonging to gelatinase A and B respectively.

MMP9 involves in the degradation of collagen type IV and V molecules (the major component of extracellular matrix protein) and gelatin (the major component of basal membrane) (Liotta, 1986; Fridman, Toth, Chvyrkova, Meroueh & Mobashery, 2003; Angulo,

Ferruelo, Nunez, Fata & Gonzalez, 2011). Degradation of ECM by MMP9 enhances the invasion, progression and metastasis of tumour cell, accelerating tumour growth (Liotta, 1986; Himelstein, Canete, Bernhard, Dilks & Muschel, 1994). This phenomenon is observed in lung cancer (Tokuraku et al., 1995), colon cancer (Newell, Witty, Rodgers & Matrisian, 1994), pancreatic cancer (Tian et al., 2008), gastric cancer (Zhao, Wang, Ye & Tao, 2009), head and neck cancer (Yoshizaki et al., 1997; Charoenrat, Rhys-Evans & Eccles, 2001), breast cancer (Provatopoulou et al., 2009) and NPC (Horikawa, Yoshizaki, Sheen, Lee & Furukawa, 2000). High expression of MMP9 increases rate of lymph node metastasis in NPC (Horikawa, Yoshizaki, Sheen, Lee & Furukawa, 2000). In NPC, a positive correlation between LMP1 and MMP9 is observed. It is found that the increase of LMP1 increases the expression of MMP9 (Yoshizaki, Sato, Furukawa & Pagano, 1998). Thus, the results reflect the negative role of MMP9 in NPC oncogenesis, induced by LMP1. NPC patients with high MMP9 expression is found to have short survival time, an inverse relationship to overall survival rate.

All the prognostic biomarkers discussed above involve in the oncogenesis of NPC in a complex dynamic. Integration of all these biomarkers into one platform enables better understanding on the interactions and mechanism among the different biomarkers in the occurrence of NPC.

## **CHAPTER 3**

### **META-ANALYSIS OF CYCLOOXYGENASE-2 (COX-2) IN NASOPHARYNGEAL CARCINOMA (NPC)**

#### **3.1 Introduction**

Prognostic significance of the expression of COX-2 has been carried out on head and neck cancer (HNC) via meta-analysis (Yang et al., 2016). NPC is being considered in the family of HNC but there is no individual exclusive meta-analysis of COX-2 expression done on NPC to determine survival outcome. Although association of COX-2 and NPC has been investigated in different studies, the results were not conclusive and were varied among different studies. These were probably due to the small sample sizes used in the studies. Hence, this study aims to determine a more significant and conclusive relationship between COX-2 expression and the overall survival rate for NPC patients in a larger population.

Cyclooxygenase (COX) exists in two isoforms, namely COX-1 and COX-2. COX-1 is commonly expressed in various normal organ and tissue, taking part in maintaining the homeostasis of physiological function. On the other hand, COX-2 is a key enzyme involved in the conversion of arachidonic acid to prostaglandins (PGE). It is lowly expressed or not detectable in most normal tissues, but rapidly induced by mitogenic and inflammatory stimuli, such as pro-inflammatory cytokines, growth factors, carcinogens and tumour promoters (Dannenberg et al., 2001; Dannenberg & Subbaramaiah, 2003; Brown & DuBois, 2005; Greenhough et al., 2009; Simonsson et al., 2017). COX-2 is observed to be highly expressed in different malignant tumour types, through stimulating tumour cell proliferation, angiogenesis and cell invasiveness (Gupta et al., 2007). It promotes resistance to cell

apoptosis, suggesting its involvement in development of inflammation-induced tumour, and therefore affects the prognosis and survival of cancers (Bakhle, 2001; Greenhough et al., 2009; Huang, Chen, Xiao, Liu & Zhao, 2013; Peng, Zhou, Wang, Mou & Zhao, 2013; Xu, Wang & Shu, 2014; Li et al., 2015). Cancer progression is found to have a reversed effect by inhibiting the function of COX-2 using Cox inhibitor (Schellhorn, Haustein, Frank, Linnebacher & Hinz, 2005; Vosooghi & Amini, 2014; Pan et al., 2015; Ben-batalla et al., 2015). Effects of Cox inhibitor and non-steroidal anti-inflammatory drugs (NSAIDs) were shown by the reduced incidence and progression of tumour in cancer animal models. Hence, potential targets for therapy could be developed (Pan et al., 2008).

Numerous studies have been done to analyse the expression of COX-2 and to examine its prognostic effect in various cancers (Costa et al., 2002; Pan et al., 2008; Saba et al., 2009). Results showed that COX-2 expression might be useful in predicting the survival in patients with cancer, acting as a potential prognostic factor (Yang et al., 2016). COX-2 involves in the malignancies of NPC by participating in the formation of inflammatory microenvironment, promoting pathogenesis and progression of cancer (Grivennikov, Greten & Karin, 2010). COX-2 is identified to be involved in metastasis of cancer cells (Gupta et al., 2007) besides mediating lymph node formation (Fendri et al., 2007). It was reported that COX-2 promoted metastasis in NPC by associating interactions between cancer cells and myeloid-derived suppressor cell (Li et al., 2015). However, due to the relatively small sample sizes and different in the methodology in conducting the studies, the findings have not been consistent, and therefore advanced meta-analysis approach is employed to draw a more convincing conclusion.

Thus, a meta-analysis to determine the expression of COX-2 in patients diagnosed with NPC corresponding to survival outcome and treatment response is very much needed. Hence, this study aims to determine the prognostic significance of COX-2 in NPC in term of OS reflected by HRs via meta-analysis.

## **3.2 Methods**

### **3.2.1 Literature search strategy**

Electronic searches on PubMed (<https://www.ncbi.nlm.nih.gov/pubmed/>), Science Direct (<https://www.sciencedirect.com/>) and Scopus (<https://www.scopus.com/>) were conducted on 7<sup>th</sup> March 2019. The following combined keywords were used to retrieve related articles and abstracts: Nasopharyngeal Carcinoma (NPC) and Cyclooxygenase-2 (COX-2). Searching was confined to articles published within year 2005-2019 (15 years). Reference lists of relevant articles for additional publications were also manually searched to prevent the missing of important studies.

### **3.2.2 Study criteria (inclusion and exclusion criteria)**

Titles and abstracts of all the articles retrieved from literature search strategy were reviewed. Articles were included or excluded based on the inclusion and exclusion criteria stated below. Full texts of potential eligible studies were downloaded according to inclusion criteria for relevant data retrieve. To avoid duplication, only the newest article with biggest samples size was included when more than a trial was done within the same patient population.

In this meta-analysis, studies containing the following inclusion criteria were employed: (1) containing NPC patients, (2) evaluating the expression of COX-2, (3) including data related to hazard ratio (HR) and/or odd ratio (OR) with corresponding 95% confidence interval (CI), (4) reported in English and (5) done in human samples.

Studies that contained the following exclusion criteria were eliminated: (1) review articles or letters, (2) non-English articles, (3) duplicated data, (4) contained insufficient information to calculate the log hazard ratio (logHR), SE (logHR) (SE) and/or odd ratio (OR) for analysis, (5) meta-analysis articles and (6) single nucleotide polymorphism (SNP) related articles.

### **3.2.3 Method of data retrieves (data extraction)**

Data that were extracted after reviewing each eligible studies were as followed: first author's last name, publication year, region of publication, ethnicity, number of female patients, number of patients with high/low COX-2 expression, COX-2 expression assay (method and cut-off level), clinicopathological data (number of patients with different tumour stage), therapy regimen used and survival data (HR with its corresponding 95% CIs for OS and OR).

### **3.2.4 Statistical analysis**

The primary results of this meta-analysis were overall survival (OS) rate, which was expressed as hazard ratio (HR) with 95% confidence intervals (CIs). OS was a direct estimate of clinical benefit to a patient, defined as the time from random assignment to death (Department of Health and Human Services Food and Drug, 2011). LogHR and SE needed

in the meta-analysis were extracted if included in the studies. Otherwise, if logHR and SE were not shown directly, methods developed by Parmar was used to obtain the relative data (Parmar, Torri & Stewart, 1998). Relevant methods provided in the same studies were also used to determine the pooled HR, CI and weight.

Dealing with analysis of survival in patients, when the HR obtained was more than one ( $HR > 1$ ), it reflected a poor prognosis in COX-2 over-expression. Significance of the outcome was proven at p value  $< 0.05$ . Significant heterogeneity exists in pooled HRs at  $P < 0.10$  or  $I^2 > 50\%$ . Results for each individual study and the pooled result for included eligible studies were displayed as forest plot.

Odd ratio (OR) was evaluated to determine the impact of COX-2 expression to the treatment response. MedCalc (version 18.11) software was used to determine the OR with 95% CI. Forest plot of OR for included studies was also graphed. The same software was also used to determine the publication bias, presented by I-squared and p-value. Heterogeneity would be found significant when pooled ORs showed  $P < 0.10$  or  $I^2 > 50\%$ . A funnel plot displaying heterogeneity was then plotted to depict the results graphically.

### **3.3 Results**

#### **3.3.1 Eligible studies**

Through the initial search strategy, 46, 24 and 37 potential studies were obtained from Scopus, Science Direct and PubMed respectively, yielding a total of 107 studies. After reviewing the title and abstract, 53 irrelevant studies were excluded. Full texts of the

remaining 54 potentially relevant studies were downloaded for detailed review. With this, another 36 studies were excluded for the reason that nine were non-English articles and 27 were duplicates. Eleven of the remaining 18 articles have incomplete data for meta-analysis, leaving a final of seven eligible studies. A flow chart of this was shown in Figure 3.1.

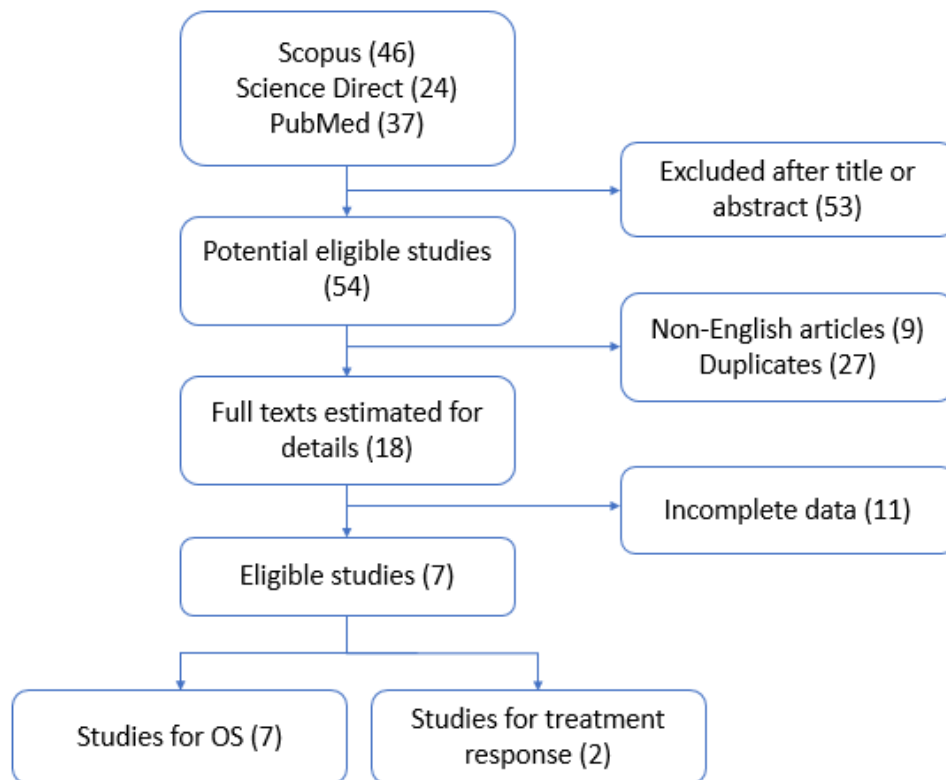


**Figure 3.1:** Selection of studies to obtain final studies for meta-analysis of COX-2 in NPC.

Clinical particulars of the patients of the seven eligible studies enrolled in this study were as shown in Table 3.1. These eligible studies were published from 2009 to 2018, which included a total of 495 patients, ranging from 38 to 148 per study. OS that reflected overall survival were found in seven studies, whereas OR that reflected treatment response was found in two of the seven studies.

### **3.3.2 Correlation between COX-2 expression and survival outcome**

Prognostic effect of COX-2 expression in predicting survival outcome was determined by conducting a meta-analysis of all seven studies for the hazard ratio (HR) of OS. The pooled (HR) (95% CI) of seven studies for OS was 2.02 [1.65, 2.47], ( $I^2 = 84\%$ ,  $P < 0.001$ ) in random model as shown in Figure 3.2. NPC patient groups with higher COX-2 expression showed higher hazard of death compared to the control groups as the HR obtained was greater than one. HR of 2.02 implied that at any time during the follow-up, the NPC patient groups with COX-2 over-expression have a 102% higher risk of death. OS was statistically significant because the 95% CI did not include one. However, significant heterogeneity was observed from the result obtained, implying the need to enhance the meta-analysis with more studies besides handling the result with caution.

### **3.3.3 Correlation between COX-2 expression and treatment response**

Prognostic effect of COX-2 expression in predicting treatment response was determined by conducting a meta-analysis of two of the seven studies for odd ratio (OR). The pooled OR (95% CI) was 0.98 [0.27, 3.49], ( $I^2 = 46.70\%$ ,  $P = 0.171$ ) in random model as shown in Figure 3.3. The OR of lesser than 1 suggested the treatment response in the high COX-2 group was better than that in the control group (low COX-2 expression group). However, the OR was not statistically significant because the 95% CI included one.

**Table 3.1:** Characteristics of all identified studies eligible for meta-analysis of COX-2 in NPC.

| First Author | Year | Region    | Ethnicity | Female (N, %) | High/Low Cox2 (N, %) | Cox2 Detection Method | Cut-off value | Stage of NPC Patients      | Chemotherapy Regimen    | Survival Outcome |
|--------------|------|-----------|-----------|---------------|----------------------|-----------------------|---------------|----------------------------|-------------------------|------------------|
| Li Y. J.     | 2018 | China     | Asian     | 28 (24.3)     | 53/62 (46.1/53.9)    | ISH                   | NR            | I & II (31), III & IV (84) | RT, CCRT (platinum)     | OS               |
| Xu L.        | 2013 | China     | Asian     | 93 (62.8)     | 107/41 (72.3/27.7)   | IHC                   | Score         | I & II (95), III & IV (53) | NR                      | OS               |
| Pan J.       | 2012 | China     | Asian     | 27 (24.3)     | 59/52 (53.2/46.8)    | IHC                   | NR            | I & II (30), III & IV (81) | RT, combined CRT (5-FU) | OS               |
| Kim T. J.    | 2011 | Korea     | Asian     | 19 (27.5)     | 43/26 (62.3/37.7)    | IHC                   | Score         | I & II (17), III & IV (52) | RT, ICRT, CCRT          | OS               |
| Huang T. L.  | 2010 | Taiwan    | Asian     | 61 (36)       | NR                   | IHC                   | H-score       | I & II (71), III & IV (99) | RT                      | OS               |
| Kim Y. J.    | 2010 | Korea     | Asian     | 8 (21)        | 31/7 (82/18)         | IHC                   | NR            | I & II (6), III & IV (32)  | Induction CT, RT, CCRT  | OS               |
| Loong S. L.  | 2009 | Singapore | Asian     | 9 (15.5)      | 24/34 (41.4/58.6)    | IHC                   | Score         | I & II (0), III & IV (58)  | RT, CRT                 | OS               |

\*\*ISH = RNA-in situ hybridization, IHC = Immunohistochemistry, RT = radiotherapy, CCRT = concurrent chemoradiotherapy, IC = induction chemotherapy, ICRT = induction chemoradiotherapy, CRT = chemoradiotherapy, 5-FU = 5-fluorouracil, NR = not related

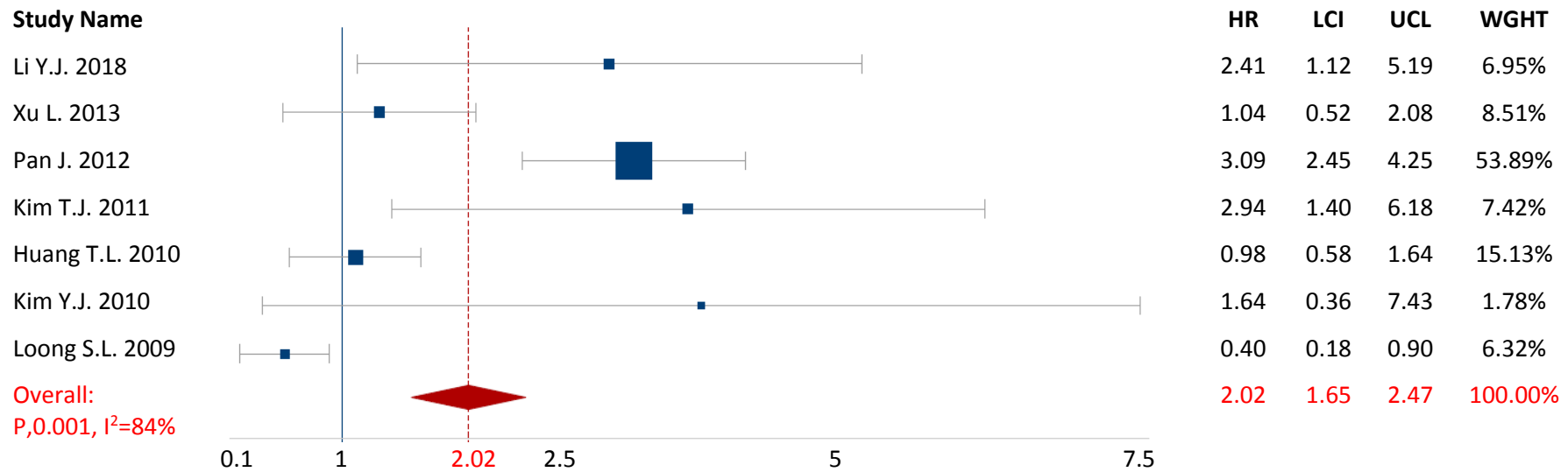


**Figure 3.2:** A forest plot depicting a meta-analysis estimating COX-2 expression with OS in NPC [hazard ratio (HR) estimated in random model].

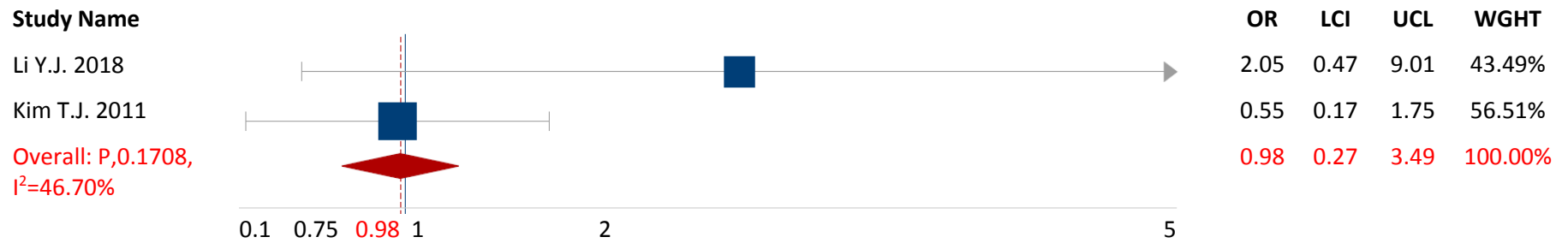


**Figure 3.3:** A forest plot depicting a meta-analysis estimating COX-2 expression with treatment response in NPC [odd ratio (OR) estimated in random model].

### 3.3.4 Assessment of publication bias

Publication bias is accepted when P-value  $>0.10$  and the studies are distributed equally on both sides within the funnel. A funnel plot (OR vs standard error) was plotted as shown in Figure 3.4. No publication bias was observed as the studies were symmetrically distributed and this suggested a significant finding that was not far from the actual situation.

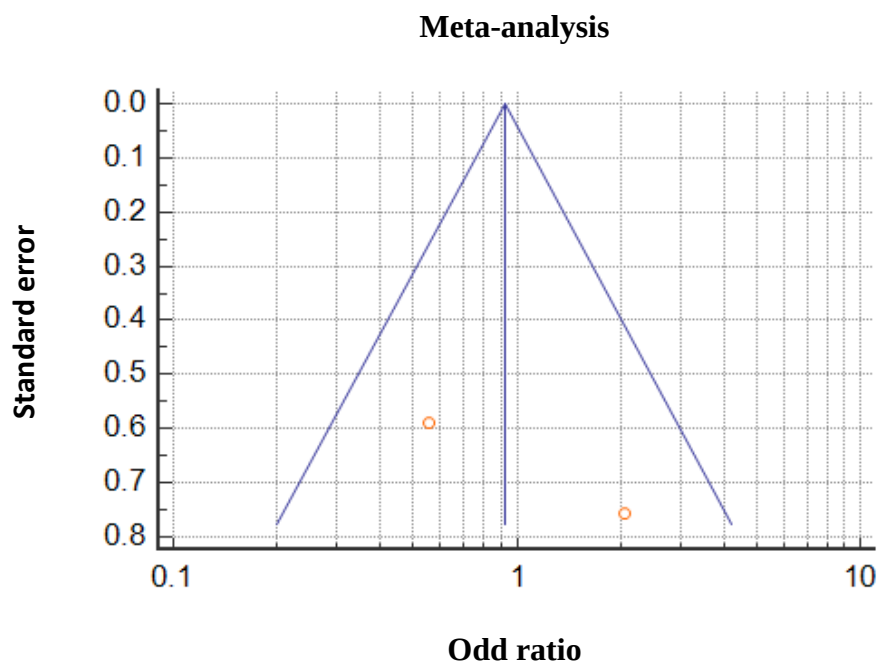


**Figure 3.4:** A funnel plot to detect publication bias and systemic heterogeneity for meta-analysis of COX-2 in NPC.

### 3.3.5 Limitations

Several limitations were taken into account in this meta-analysis. Firstly, a number of studies were excluded as they were published in non-English language or contained incomplete data. Non-English written articles were excluded as the initial search inclusion criteria, included only the English written articles. This was so to maintain the consistency of language used for data extraction and results reporting. Translating those articles into English was possible.

However, the chances of having translation different from the primary studies existed. This would again contribute to reporting bias. Nevertheless, such an exclusion might affect the pooled results to differ from true value. Also, meta-analysis that was done literature-based was less valid than individual patient data-based analysis. According PRISMA statement, only one major database is required for literature searching. In this project, three major electronic databases were used for literature searching. Some important studies might be deposited in other databases such as Web of Knowledge/Science or Google Scholar, causing possible publication bias.

### **3.4 Discussion**

COX-2 over expression has been demonstrated in various malignancies such as breast cancer (Costa et al., 2002), head and neck squamous cell carcinoma (HNSCC) (Saba et al., 2009), oral squamous cell carcinoma (OSCC) and nasopharyngeal carcinoma (NPC) (Pan et al., 2008). According to the previous studies, over expression of COX-2 shows poorer overall survival rate (OS) depicting the important role of COX-2 in carcinogenesis (Tan & Putti, 2005; Chou et al., 2008; Kim, Li, Cha, Zhang & Cha, 2012).

With the results obtained, it could be inferred that COX-2 is a good prognostic factor for NPC. The HR of OS is 2.02, higher than 2. As for treatment response, the pooled OR is 0.98, lesser than one, implying a 2% lesser risk in the high COX-2 expression group. However, this outcome is not conclusive as the confidence interval included one, causing it to be insignificant. The pooled results are supported by studies showing that COX-2 is associated with poor NPC prognosis (Kim, Wu, Park, Kim & Park, 2004; Xue et al., 2011). However,

Loong reported an opposite result, where weak COX-2 expression showed worse OS compared to over-expressed COX-2 (Loong et al., 2009). The different single studies that agree or conflict with our findings contained smaller sample sizes. Thus, our research output reinforces the findings with significant conclusion by pooling studies of large sample size.

In this study, COX-2 is recognized as a good prognostic factor in NPC. This result is consistent with other cancers. For example, COX-2 was identified as good prognostic factor in meta-analysis of oesophageal cancer, showing poor OS with HR of 1.60 [1.32–1.94] (Hu, Yang, Zhao & Huang, 2017). The same trend is observed in meta-analysis of COX-2 in breast cancer, having poor OS with HR of 1.51 [1.31-1.72] (Xu, Li, Zhang, Cui, Liu, Li & Jiang, 2017). Meta-analysis of COX-2 in ovarian cancer reported poor OS with HR of 1.48 [1.19-1.85], which further supported the significance of COX-2 as a prognostic biomarker for cancers (Sun, Zhang, Sun, Jia, Xu, Qiao & Jin, 2017).

Removal of NPC completely through surgical approach is one of the suggested treatments. However, due to the anatomy location of nasopharynx, surgery is nearly impossible due to the difficulties and complexity of procedure. Therefore, radiotherapy treatment is still adopted (Farias et al., 2003). To provide better treatment to NPC patients with reduced side effects, various attempts to use clinical and pathologic parameters have been implied for NPC prognosis (Perez et al., 1992). Such approaches aim to categorize patients with similar staging, severity, tumour size and treatment modality into the same group (Karademir et al., 2000). Therefore, identification of the prognostic function of COX-2 in NPC from this study helps to better categorize NPC patients. This could, therefore provide more suitable and individualized treatments to NPC patients according to different categories.

Development of NPC involved in complex mechanism driven by various factors. Recently, inducible enzyme COX-2 is found to play an important role in NPC carcinogenesis, angiogenesis, invasion, metastasis and inhibition of apoptosis. COX-2 is present in wide range of preneoplastic and malignant conditions (Pan et al., 2008). COX-2 is absent or undetectable in most normal tissue. It involves in the synthesis of prostaglandins present under the induction by inflammatory cytokines, tumour promoters, and growth factors (Turini & Dubois, 2002). COX-2 acts in association with up-regulation of VEGF and EGRF, and the expression is reported to show correlation with the stage of diseases (Williams, Tsujii, Reese, Dey & DuBois, 2000; Dannenberg et al., 2001; Soo et al., 2005; Pan et al., 2008).

Tumorigenic effects of COX-2 that involve in adverse prognostic features such as tumour growth, lymph node metastasis, inhibition of apoptosis and tumour angiogenesis are shown to be inhibited by selected COX-2 inhibitors, both in vitro and in animal models (Dannenberg et al., 2001; Costa et al., 2002; Ristimaki et al., 2002). Celecoxib with optimum dosage of 800 mg/day with concurrent radiotherapy (Xue et al., 2011) is the most well-known COX-2 inhibitor that shows anti-cancer role through antiproliferative, antiinvasive and antiangiogenic effects on an NPC cell line in a dose-dependent manner and besides its function in reducing cyclin D1 levels (Chou et al., 2008; Chen, Ran, Hong, Chen & You, 2010). The effects of inhibitor are also observed in NPC biopsy samples (Soo et al., 2005). Hence, to deal with NPC, celecoxib could be used as one of the targeted treatment regimens. Nevertheless, more studies on developing new drugs against COX-2 should be carried out, to further enhance the current existing regimen and therefore to provide better survival rate.

## **CHAPTER 4**

### **CONSTRUCTION OF MULTIGENE NASOPHARYNGEAL CARCINOMA (NPC) PROGNOSIS ASSESSMENT MODEL**

#### **4.1 Introduction**

Occurrence and progression of complex diseases such as cancer involve complex and dynamic interaction of molecules. The complexity is impossible to be fully understood from studies focusing on a single parameter. Thus, the current direction now has shifted from understanding of the functions/roles of molecules to assimilation of those molecules in a pathway, network or model (Baranzini, 2009; Aitman et al., 2011). Integration and consolidation of vast body of data and findings into a pathway/network/model to reflect the dynamic in the biological system has enabled better diagnosis, prognosis, prevention and optimisation of drug usage as treatment strategy (Assmus, Herwig, Cho & Wolkenhauer, 2006). Working beyond statistical analysis to reconstruct the data to provide a meaningful diagnostic/prognostic model in medical field is the current direction. Development of meaningful model is important in different medical aspects, especially in oncology (Assmus, Herwig, Cho & Wolkenhauer, 2006). However, the number of medically useful models to diagnose and monitor different kind of cancers are still lacking.

Gail model is the most applied breast cancer risk prediction model (Gail, Brinton, Byar, Corle, Green, Schairer & Mulvihill, 1989; Gail & Benichou, 1994) and Bach model and Spize model (Bach et al., 2003; Spitz et al., 2007) are the two common examples of the lung cancer risk prediction models. There are also a numbers of existing NPC prognosis assessment models, such as prognostic nomogram integrating clinical characteristic and

hematological biomarkers (Li et al., 2018) and statistical prognostic score model to determine disease progression and survival of NPC patients (Zhang et al., 2013). However, these models are built from small sample size where the patients of each studies representing and reflecting the outcome only on the isolated location of studies, causing the model to show limited medical uses. Such NPC prognostic models have little/no medical uses as the models are relevant and significant only at isolated location of studies, limiting the usage of the model at other region. Besides, these models focus more on clinical characteristics rather than emphasized on functional prognostic biomarkers. Thus, this study aims to develop a multigene NPC prognosis assessment model focusing on functional genetic biomarkers by pooling different individual NPC prognostic meta-analysis studies via modification of meta-analysis approach. Through this, a more reliable multigene NPC prognosis assessment model is derived due to pooling of a larger group of studies that are more significant and relevant besides reflecting the association and interaction among different biomarkers.

## **4.2 Methods**

To integrate the information from the databases for generation of a multigene NPC prognosis assessment model that is useful for prognosis assessment, we proposed a method that adopt guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement with relevant modifications. Literature search strategy, study selection criteria and data extraction were conducted as per described in PRISMA (refer to Appendix A, page 157-159). Data were statistically analysed and presented in the approach as shown in the following section.

#### **4.2.1 Literature search strategy**

Electronic searching was comprehensively and systematically done on 26<sup>th</sup> of May 2019 on PubMed (<https://www.ncbi.nlm.nih.gov/pubmed/>), Science Direct (<https://www.sciencedirect.com/>) and Scopus (<https://www.scopus.com/>). The following combined keywords were used as search terms to retrieve potentially related articles and abstracts: Nasopharyngeal Carcinoma (NPC) and meta-analysis. Searching was confined to articles published within year 2005-2019 (15 years). Moreover, we manually screened and reviewed the reference lists of relevant articles to identify additional potentially eligible publications to prevent missing of important studies and thus to minimize publication bias.

#### **4.2.2 Study criteria (inclusion and exclusion criteria)**

Titles and abstracts of all the articles retrieved from literature search strategy were reviewed. Articles were included or excluded based on the inclusion and exclusion criteria stated below. Full texts of potential eligible studies were downloaded according to inclusion criteria for relevant data retrieve.

In this study, studies of the following inclusion criteria were employed: (1) containing NPC patients, (2) involving meta-analysis of prognostic biomarkers, (3) including data related to hazard ratio (HR) with corresponding 95% confidence interval (CI), (4) done in human samples and (5) reported in English.

Studies containing the following exclusion criteria were excluded: (1) not NPC related, (2) are not meta-analysis, (3) focused on treatments, (4) articles were single nucleotide

polymorphism (SNP) related, (5) duplicated data, and (6) contained insufficient information to calculate the ln hazard ratio (lnHR).

#### 4.2.3 Method of data retrieves (data extraction)

Data that were extracted after reviewing each eligible study were as followed: first author's last name, publication year, types of prognostic biomarker examined in the studies, total number of patients included in the meta-analysis and hazard ratio (HR) with 95% confidence interval CI.

#### 4.2.4 Statistical analysis

With all HRs of OS collected for different NPC prognostic biomarkers, Ln(HRs) were generated by computing formulas using Microsoft Excel (refer to Table 4.2 and Table 4.3). NPC prognosis assessment scores were assigned for each of the Ln(HRs) as designated below, shown in Table 4.1. The higher the score, the higher the impact of the biomarkers contributed to poorer overall survival in NPC patients.

**Table 4.1:** Assignment of NPC prognosis assessment risk score in relation to Ln of HRs.

| <b>Ln(HR)</b>                   | <b>NPC Prognosis Assessment Risk Score</b> |
|---------------------------------|--------------------------------------------|
| $0.0 \leq \text{Ln HR} < 0.25$  | 1                                          |
| $0.25 \leq \text{Ln HR} < 0.50$ | 2                                          |
| $0.50 \leq \text{Ln HR} < 0.75$ | 3                                          |
| $0.75 \leq \text{Ln HR} < 1.00$ | 4                                          |

With all the compiled HRs of OS for different NPC biomarkers included, the pooled HR of a patient corresponding to all the over-expressed prognostic biomarkers were determined using the formula proposed by Parmar (Parmar, Torri, & Stewart, 1998).

$$\ln(HR_i) = \frac{\sum_{t=1}^T \frac{\ln(HR_i(t))}{\text{var}[\ln(HR_i(t))]} }{\sum_{t=1}^T \frac{1}{\text{var}[\ln(HR_i(t))]}}$$

K was the number of total studies included and  $i = 1, \dots, K$  studies. Pooled  $\ln(HR)$  was determined via the following formula when estimate of log hazard ratio,  $\ln(HR_i)$  and its variance,  $\text{var}[\ln(HR_i)]$ , were available. The overall log hazard ratio was a weighted average of the individual  $\ln(HR_i)$ , with the weights inversely proportional to the variance of the  $\ln(HR_i)$  of each studies. Variance (var) was obtained by the following formula:

$$\text{Var}[\ln(HR_i)] = [(\ln(\text{Upper CI}) - \ln(\text{Lower CI})) / (2 * 1.96)]^2$$

1.96 was the z-score for 95% CI for the HRs.

Based on this formula, pooled HR was obtained by getting the exponential of pooled  $\ln(HR)$ . Pooled lower and upper confident intervals (CIs) were established via the formula stated below:

$$\text{Lower/Upper CI} = e^{\ln(HR) \pm \sqrt{\text{overall Var}[\ln(HR)]}}$$

A formulated table was designed in Microsoft Excel to obtain pooled HR and NPC prognosis assessment score for different patients with over expression of different prognostic

biomarkers. The details of the table construction were summarized in Table 4.2 and Table 4.3.

**Table 4.2:** The description for the designated cell and formula used in Microsoft Excel.

| Column | Description                                                                                                                 | Formula                                                                                                              |
|--------|-----------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| C6-15  | Prognostic biomarkers                                                                                                       | Directly adopted from eligible studies                                                                               |
| D6-15  | HRs of prognostic biomarkers                                                                                                | Directly adopted from eligible studies                                                                               |
| E6-15  | Lower limit of 95% CIs for HRs of prognostic biomarkers                                                                     | Directly adopted from eligible studies                                                                               |
| F6-15  | Upper limit of 95% CIs for HRs of prognostic biomarkers                                                                     | Directly adopted from eligible studies                                                                               |
| H6-15  | Ln of upper limit minus Ln of lower limit of 95% CIs for HRs                                                                | Formula<br>=(LN(F6)-LN(E6))<br>Extend for row 6 to 15                                                                |
| I6-15  | Variance                                                                                                                    | Formula<br>=(H6/3.92)^2<br>Extend for row 6 to 15<br>3.92 is obtained from two times of 1.96 (the z-score of 95% CI) |
| J6-15  | Inverse of variance                                                                                                         | Formula<br>= 1/ I6<br>Extend for row 6 to 15                                                                         |
| L6-15  | Ln HR                                                                                                                       | Formula<br>=LN(D6)<br>Extend for row 6 to 15                                                                         |
| M6-15  | Ln HR over variance                                                                                                         | Formula<br>= L6/I6<br>Extend for row 6 to 15                                                                         |
| N6-15  | If prognostic biomarker in C6 is over expressed, A6 will be ticked, N will equal to M. If it is not over expressed, N = 0.  | Formula<br>=IF(A6=TRUE,M6,)<br>Extend for row 6 to 15                                                                |
| O6-15  | If prognostic biomarker in C6 is over expressed, A6 will be ticked, it will equal to J. If it is not over expressed, O = 0. | Formula<br>=IF(A6=TRUE,J6,)<br>Extend for row 6 to 15                                                                |
| Q6-15  | NPC prognosis assessment risk score                                                                                         | Assigned as discuss in Table 4.1                                                                                     |

**Table 4.2** continued

|       |                                                                                                                            |                                                     |
|-------|----------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------|
| R6-15 | If prognostic biomarker in C6 is over expressed, A6 will be ticked, R will equal to Q. If it is not over expressed, Q = 0. | Formula<br>=IF(A6=TRUE,Q,<br>Extend for row 6 to 15 |
|-------|----------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------|

**Table 4.3:** The description for the designated cell and formula used in Microsoft Excel.

| Cell | Description                                                                           | Formula                              |
|------|---------------------------------------------------------------------------------------|--------------------------------------|
| N16  | Sum of Ln HR over variance for over expressed prognostic biomarkers                   | Formula<br>=SUM(N6:N15)              |
| O16  | Sum of inverse of variance for over expressed prognostic biomarkers                   | Formula<br>=SUM(O6:O15)              |
| R16  | Sum of NPC prognosis assessment score for over expressed prognostic biomarkers        | Formula<br>=SUM(R6:R15)              |
| B18  | Ln of pooled HR for over expressed prognostic biomarkers                              | Formula<br>=N16/O16                  |
| B19  | Exponential of Ln of pooled HR for over expressed prognostic biomarkers               | Formula<br>=EXP(B18)                 |
| E18  | Ln of lower limit of CI for HR of over expressed prognostic biomarkers                | Formula<br>=B18-(1.96*(SQRT(O16^1))) |
| E19  | Exponential of Ln of lower limit of CI for HR of over expressed prognostic biomarkers | Formula<br>=EXP(E18)                 |
| H18  | Ln of upper limit of CI for HR of over expressed prognostic biomarkers                | Formula<br>=B18+(1.96*(SQRT(O16^1))) |
| H19  | Exponential of Ln of upper limit of CI for HR of over expressed prognostic biomarkers | Formula<br>=EXP(H18)                 |
| K19  | Total NPC prognosis assessment score                                                  | Formula<br>=R16                      |

The constructed multigene NPC prognosis assessment model shows its function after the expression of included prognostic biomarkers of NPC patients are determined via immunohistochemistry (IHC) using respective antibodies according to the standard protocol. After determining the over expressed prognostic biomarkers in the NPC patients via IHC, the boxes that corresponding to the over expressed biomarkers are selected in the respective column in the model. A total NPC prognosis assessment score of a patient is determined via

the summing up of the risk score corresponding to the Ln HR of the over expressed prognostic biomarkers. The higher the score, the more prognostic biomarkers with worse prognostic effect are found to be upregulated in that NPC patients. Thus, the more complex and advanced the NPC staging was. Besides, this allows the computational calculation of pooled HR with lower and upper CIs corresponding to the over expressed prognostic biomarkers in different NPC individual. The pooled HRs show the overall survival of the NPC patients. Some simulated trials are discussed in the results and discussions section in this chapter.

## **4.3 Results and discussion**

### **4.3.1 Eligible studies**

The total number of studies from the initial search was 844, comprising 189 studies in Scopus, 483 studies in Science Direct and 172 studies in PubMed. Following review of the titles and abstracts, 716 irrelevant studies were excluded which were not NPC and not meta-analysis related. The remaining 128 potentially relevant studies were selected for full-text review. A further 112 studies were excluded for the following reasons: 11 were single nucleotide polymorphism (SNP) studies, 59 were studies concerning types of treatment for NPC patients and 42 were duplicates. The remaining 16 studies were screened in details before data extraction. Seven studies were excluded from the 16 studies due to incomplete data (in six studies) and insignificant result (in one study where the CI of HR included one). Another study from our own finding (Chapter 3) was incorporated, generating a finally ten eligible studies for the development of biomolecular profile. A flow chart of this selection was shown in Figure 4.1.

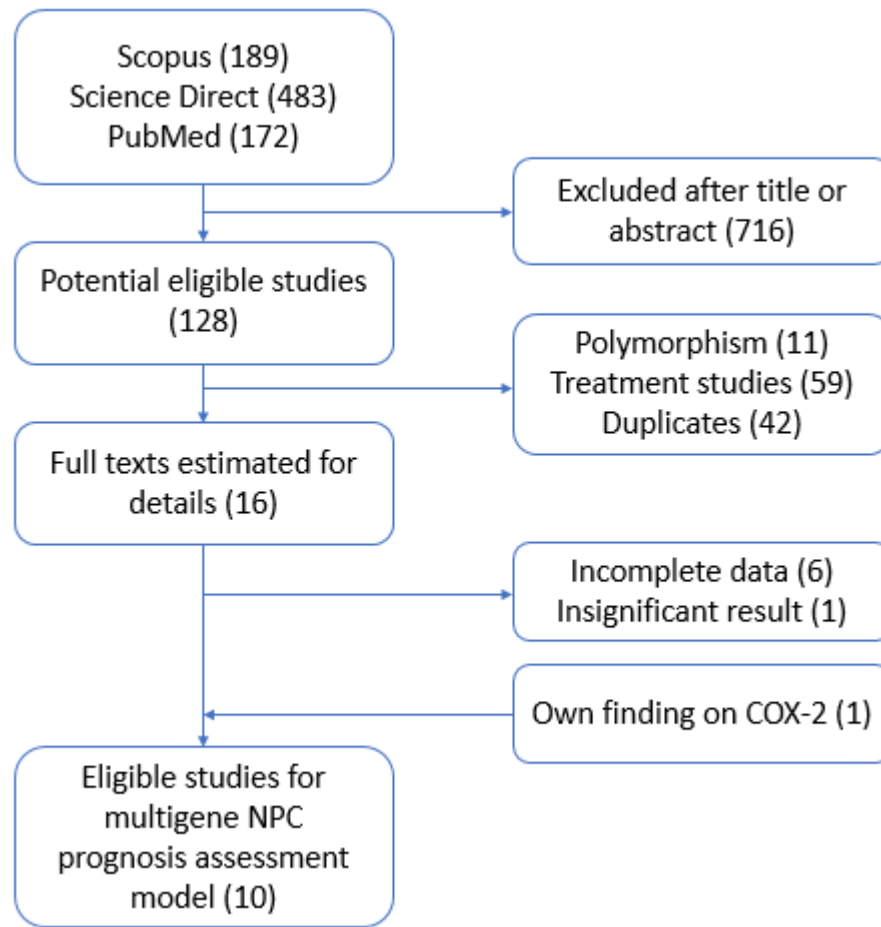


**Figure 4.1:** Flow chart of studies selection for the development of multigene NPC prognosis assessment model (Ma et al., 2015).

Finally, ten eligible studies containing pooled hazard ratios (HRs) regarding prognostic biomarkers in NPC are selected for the construction of multigene NPC prognosis assessment model. These were published from 2003 to 2019, and involved a total of 33,033 patients (495 to 13,789 per study). The ten studies provided ten sets of OS data. The details of the studies and the clinical findings from these eligible studies were tabulated in Table 4.4.

**Table 4.4:** Characteristics of selected studies for the development of multigene NPC prognosis assessment model.

| First Authors | Year of Publication | No. of Patients | Prognostic Biomarkers                                 | Hazard ratio (HR) | 95% Confidence Interval (CIs) |       |
|---------------|---------------------|-----------------|-------------------------------------------------------|-------------------|-------------------------------|-------|
|               |                     |                 |                                                       |                   | Lower                         | Upper |
| Sim, C. C.    | 2020 Jan            | 495             | Cyclooxygenase-2 (COX-2)                              | 2.02              | 1.65                          | 2.47  |
| Chen, Y. P.   | 2015 Aug            | 3752            | Latent membrane protein 1 (LMP1)                      | 2.48              | 1.77                          | 3.47  |
| Wang, F.      | 2018 Oct            | 1345            | Vascular endothelial growth factor (VEGF)             | 2.07              | 1.32                          | 3.25  |
| Sun, W.       | 2013 Dec            | 1179            | Epidermal growth factor receptor (EGFR)               | 1.86              | 1.25                          | 2.77  |
| Zhai, C.      | 2017 Nov            | 13789           | Serum lactate dehydrogenase (LDH)                     | 1.86              | 1.66                          | 2.08  |
| Fang, Y.      | 2017 Sep            | 5215            | C-reactive protein (CRP)                              | 1.84              | 1.57                          | 2.17  |
| Yang, L.      | 2019 Apr            | 2921            | Excision Repair Cross-Complementation Group 1 (ERCC1) | 1.77              | 1.48                          | 2.12  |
| Xie, W. J.    | 2018 May            | 1476            | Hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ )    | 1.77              | 1.35                          | 2.32  |
| Zhang, C. M.  | 2018 Nov            | 2235            | Lysyl oxidase (LOX)                                   | 1.68              | 1.52                          | 1.87  |
| Liao, D.      | 2016 Feb            | 626             | Matrix Metalloproteinase 9 (MMP9)                     | 1.65              | 1.38                          | 1.95  |

#### 4.3.2 Statistical analysis

Based on the methodology proposed, a multigene NPC prognosis assessment model was constructed in Microsoft Excel as shown in Figure 4.2. It was an empty setup (no biomarker was selected) showing different prognostic biomarkers with respective hazard ratio (HRs) and lower/upper confidence intervals (CIs). All the other parameter required for the determination of pooled HR, lower/upper CI and total NPC risk assessment score were formulated accordingly. Upon selection of over expressed biomarker(s) at Column A corresponding to the different over expression pattern of biomarkers in different NPC patients, the pooled HR, lower/upper CI and total NPC prognosis assessment score of a patient were auto generated and depicted as shown in Figure 4.3. Ticking of Column A converted the FALSE into TRUE, and thus Column N, O and R showed associated values according to the formula set. The opposite was true. If Column A was not selected as the biomarker in Column C was not over expressed, Column N, O and R remained zero. Cell N16, O16 and R16 were the sum of the respective parameter which were used to obtain pooled HR, lower CI and upper CI for pooled HR and the total NPC prognostic assessment risk score for NPC patients corresponding to the over expressed prognostic biomarkers as shown in Figure 4.2. After determining the over-expressed biomarkers of a particular patient, the pooled HR, the lower CI, the upper CI and the total NPC assessment risk score were shown on cell B19, E19, H19 and K19 respectively as depicted in Figure 4.3.

|    | A                              | B          | C    | D     | E        | F        | G       | H        | I          | J     | K              | L | M | N               | O               | P | Q             | R              |
|----|--------------------------------|------------|------|-------|----------|----------|---------|----------|------------|-------|----------------|---|---|-----------------|-----------------|---|---------------|----------------|
| 1  |                                |            |      |       |          |          |         |          |            |       |                |   |   |                 |                 |   |               |                |
| 2  |                                |            |      |       |          |          |         |          |            |       |                |   |   |                 |                 |   |               |                |
| 3  |                                |            |      |       | Lower CI | Upper CI |         | LN(LCI)- |            |       |                |   |   | Formula =       | Formula =       |   | NPC Prognosis |                |
| 4  | Tick if yes                    | Biomarkers | HR   | (LCI) | (UCI)    |          | LN(UCI) | Variance | 1/Variance | LN HR | LN HR/Variance |   |   | IF(A6=True,M6,) | IF(A6=True,J6,) |   | Assessment    | Formula =      |
| 5  |                                |            |      |       |          |          |         |          |            |       |                |   |   |                 |                 |   | Score         | IF(A6=TRUE,Q6) |
| 6  | <input type="checkbox"/> FALSE | Cox-2      | 2.02 | 1.65  | 2.47     |          | 0.40    | 0.01     | 94.41      | 0.70  | 66.38          |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 7  | <input type="checkbox"/> FALSE | LMP1       | 2.48 | 1.77  | 3.47     |          | 0.67    | 0.03     | 33.91      | 0.91  | 30.80          |   |   | 0.00            | 0.00            |   | 4             | 0              |
| 8  | <input type="checkbox"/> FALSE | VEGF       | 2.07 | 1.32  | 3.25     |          | 0.90    | 0.05     | 18.93      | 0.73  | 13.77          |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 9  | <input type="checkbox"/> FALSE | EGFR       | 1.86 | 1.25  | 2.77     |          | 0.80    | 0.04     | 24.27      | 0.62  | 15.06          |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 10 | <input type="checkbox"/> FALSE | LDH        | 2.86 | 1.66  | 2.08     |          | 0.23    | 0.00     | 302.05     | 1.05  | 317.41         |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 11 | <input type="checkbox"/> FALSE | CRP        | 1.84 | 1.57  | 2.17     |          | 0.32    | 0.01     | 146.70     | 0.61  | 89.45          |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 12 | <input type="checkbox"/> FALSE | ERCC1      | 1.77 | 1.48  | 2.12     |          | 0.36    | 0.01     | 118.98     | 0.57  | 67.94          |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 13 | <input type="checkbox"/> FALSE | HIF-1a     | 1.77 | 1.35  | 2.32     |          | 0.54    | 0.02     | 52.41      | 0.57  | 29.93          |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 14 | <input type="checkbox"/> FALSE | LOX        | 1.68 | 1.52  | 1.87     |          | 0.21    | 0.00     | 357.83     | 0.52  | 185.64         |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 15 | <input type="checkbox"/> FALSE | MMP9       | 1.65 | 1.38  | 1.95     |          | 0.35    | 0.01     | 128.55     | 0.50  | 64.37          |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 16 |                                |            |      |       |          |          |         |          |            |       |                |   |   | 0.00            | 0.00            |   |               | 0              |

**Figure 4.2:** Layout of the empty setup of multigene NPC prognosis assessment model in Microsoft Excel sheet.

|    | A         | B       | C | D           | E       | F | G           | H       | I | J                                         | K |
|----|-----------|---------|---|-------------|---------|---|-------------|---------|---|-------------------------------------------|---|
| 16 |           |         |   |             |         |   |             |         |   |                                           |   |
| 17 |           |         |   |             |         |   |             |         |   |                                           |   |
| 18 | LN(HR)    | #DIV/0! |   | LN Lower CI | #DIV/0! |   | LN Upper CI | #DIV/0! |   |                                           |   |
| 19 | Pooled HR | #DIV/0! |   | Lower CI    | #DIV/0! |   | Upper CI    | #DIV/0! |   | Total NPC Prognosis Assessment Risk Score | 0 |

**Figure 4.3:** Layout of the empty results of multigene NPC prognosis assessment model in Microsoft Excel sheet.

Pooled HR greater than one predicts a worse prognosis compare to those with lower-expressed biomarkers. For example, a pooled HR of 2.53 indicates that a patient has 153% higher risk to die compare to those biomarkers are lowly expressed. The larger the pooled HR, the poorer the prognosis. The pooled HR is significant if the lower and upper CI did not include one.

With this prognosis assessment model, there are a total of 1023 possible combinations of different over expressed prognostic biomarkers available. So, there are 1023 different HRs with lower and upper CIs can be obtained. However, if none of the biomarker is over-expressed, the pooled HR is given as one (no effect). The sum of the possible combinations is as shown in Table 4.5 using the mathematical combination of k subjects from a set of n objects,  ${}^nC_k$  where  $n=10$  and  $k=1, \dots, 10$ . Combination in this calculation involves no repetition of biomarkers and the order of the combination of different biomarkers was also disregarded. Mathematical combination is incorporated as part of the prognosis assessment modelling due to its personalized-modifying application to different randomized cases for different NPC patients. For example, after testing on all the different biomarkers, the NPC patients might show only two over expressed prognostic biomarkers. They could be LMP1 and MMP9, they could be ERCC1 and CRP or other combinations. By applying mathematical combination, it is shown that there are 45 different possible combinations. Hence, this model is unique to determine HR for the conditions of different NPC patients.

**Table 4.5:** The total possible combinations for over-expression of different biomarkers.

| Combination of biomarker(s)<br>over expressed | Formula of<br>combination | Number of possible<br>combination(s) |
|-----------------------------------------------|---------------------------|--------------------------------------|
| 1                                             | $^{10}C_1$                | 10                                   |
| 2                                             | $^{10}C_2$                | 45                                   |
| 3                                             | $^{10}C_3$                | 120                                  |
| 4                                             | $^{10}C_4$                | 210                                  |
| 5                                             | $^{10}C_5$                | 252                                  |
| 6                                             | $^{10}C_6$                | 210                                  |
| 7                                             | $^{10}C_7$                | 120                                  |
| 8                                             | $^{10}C_8$                | 45                                   |
| 9                                             | $^{10}C_9$                | 10                                   |
| 10                                            | $^{10}C_{10}$             | 1                                    |
| Total Possible Combinations                   |                           | 1023                                 |

A few simulated trials are set to demonstrate the function and application of this model. The interpretation of the results obtained from the simulation are also discussed and explained to better understand the use of this model.

**Simulated trial 1:** When only one of the biomarkers, for example LMP1 is overexpressed in NPC patients after IHC testing on all ten different biomarkers, cell A7 in the multigene NPC prognosis assessment model as shown in Figure 4.2 is selected. The FALSE in this cell changes to TRUE, and cell N7 (log of hazard ratio over variance), O7 (inverse of variance) and R7 (NPC prognosis assessment risk score) are computed accordingly (refer to Table 4.2 for description). Simultaneously, cell N16, O16 and R16, which are the sum of the respective

parameter for column N, O and R are calculated (refer to Table 4.3 for description). R16 depicts the total NPC prognosis assessment risk score which is also shown in cell K19. N16 and O16 are used to determine pooled  $\ln(HR)$  as shown in cell B18 and pooled HR is determined by getting the exponential of the value in cell B18 as shown in B19. O16 and B18 are used to determine the log of lower/upper confidence intervals (CIs) in cell E18 and H18 respectively. Exponential of the value in cell E18 and H19 derives the lower/upper CIs in cell E19 and H19. All the formulation discussed above occurs simultaneously when cell A7 is selected. Figure 4.4 showed the multigene NPC prognosis assessment model when LMP1 is over expressed and Figure 4.5 showed the outcome of the NPC patients corresponding to over expression of LMP1.

Based on the results obtained, the pooled HR of the patient is 2.48 when LMP1 is over expressed. It implies that the NPC patient is 148% more likely to die compared to other NPC patients with lowly expressed LMP1. The significance of this results falls within the confidence intervals (CIs) of 1.77 to 3.47. These results are significant as the lower and upper CIs do not include one. The total NPC prognosis assessment risk score was 4 out of total score of 31. Small risk score indicated that a smaller number of biomarkers are involved in the NPC oncogenesis of that patient, and thus rendering the complexity of the cancer.

|    | A                                        | B          | C    | D     | E        | F        | G        | H          | I     | J              | K               | L               | M | N          | O         | P | Q             | R              |
|----|------------------------------------------|------------|------|-------|----------|----------|----------|------------|-------|----------------|-----------------|-----------------|---|------------|-----------|---|---------------|----------------|
| 1  |                                          |            |      |       |          |          |          |            |       |                |                 |                 |   |            |           |   |               |                |
| 2  |                                          |            |      |       |          |          |          |            |       |                |                 |                 |   |            |           |   |               |                |
| 3  |                                          |            |      |       | Lower CI | Upper CI | LN(LCI)- |            |       |                |                 |                 |   | Formula =  | Formula = |   | NPC Prognosis |                |
| 4  | Tick if yes                              | Biomarkers | HR   | (LCI) | (UCI)    | LN(UCI)  | Variance | 1/Variance | LN HR | LN HR/Variance | IF(A6=True,M6,) | IF(A6=True,J6,) |   | Assessment | Formula = |   | Score         | IF(A6=TRUE,Q6) |
| 5  |                                          |            |      |       |          |          |          |            |       |                |                 |                 |   |            |           |   |               |                |
| 6  | <input type="checkbox"/> FALSE           | COX-2      | 2.02 | 1.65  | 2.47     | 0.40     | 0.01     | 94.41      | 0.70  | 66.38          | 0.00            | 0.00            |   | 3          | 0         |   |               |                |
| 7  | <input checked="" type="checkbox"/> TRUE | LMP1       | 2.48 | 1.77  | 3.47     | 0.67     | 0.03     | 33.91      | 0.91  | 30.80          | 30.80           | 33.91           |   | 4          | 4         |   |               |                |
| 8  | <input type="checkbox"/> FALSE           | VEGF       | 2.07 | 1.32  | 3.25     | 0.90     | 0.05     | 18.93      | 0.73  | 13.77          | 0.00            | 0.00            |   | 3          | 0         |   |               |                |
| 9  | <input type="checkbox"/> FALSE           | EGFR       | 1.86 | 1.25  | 2.77     | 0.80     | 0.04     | 24.27      | 0.62  | 15.06          | 0.00            | 0.00            |   | 3          | 0         |   |               |                |
| 10 | <input type="checkbox"/> FALSE           | LDH        | 2.86 | 1.66  | 2.08     | 0.23     | 0.00     | 302.05     | 1.05  | 317.41         | 0.00            | 0.00            |   | 3          | 0         |   |               |                |
| 11 | <input type="checkbox"/> FALSE           | CRP        | 1.84 | 1.57  | 2.17     | 0.32     | 0.01     | 146.70     | 0.61  | 89.45          | 0.00            | 0.00            |   | 3          | 0         |   |               |                |
| 12 | <input type="checkbox"/> FALSE           | ERCC1      | 1.77 | 1.48  | 2.12     | 0.36     | 0.01     | 118.98     | 0.57  | 67.94          | 0.00            | 0.00            |   | 3          | 0         |   |               |                |
| 13 | <input type="checkbox"/> FALSE           | HIF-1a     | 1.77 | 1.35  | 2.32     | 0.54     | 0.02     | 52.41      | 0.57  | 29.93          | 0.00            | 0.00            |   | 3          | 0         |   |               |                |
| 14 | <input type="checkbox"/> FALSE           | LOX        | 1.68 | 1.52  | 1.87     | 0.21     | 0.00     | 357.83     | 0.52  | 185.64         | 0.00            | 0.00            |   | 3          | 0         |   |               |                |
| 15 | <input type="checkbox"/> FALSE           | MMP9       | 1.65 | 1.38  | 1.95     | 0.35     | 0.01     | 128.55     | 0.50  | 64.37          | 0.00            | 0.00            |   | 3          | 0         |   |               |                |
| 16 |                                          |            |      |       |          |          |          |            |       |                | 30.80           | 33.91           |   |            |           |   |               | 4              |

**Figure 4.4:** Output of multigene NPC prognosis assessment model when LMP1 is over expressed.

|    | A         | B    | C           | D    | E           | F    | G                                         | H | I | J | K |
|----|-----------|------|-------------|------|-------------|------|-------------------------------------------|---|---|---|---|
| 16 |           |      |             |      |             |      |                                           |   |   |   |   |
| 17 |           |      |             |      |             |      |                                           |   |   |   |   |
| 18 | LN(HR)    | 0.91 | LN Lower CI | 0.57 | LN Upper CI | 1.24 |                                           |   |   |   |   |
| 19 | Pooled HR | 2.48 | Lower CI    | 1.77 | Upper CI    | 3.47 | Total NPC Prognosis Assessment Risk Score |   |   |   | 4 |

**Figure 4.5:** Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene NPC prognosis assessment model when LMP1 is over expressed.

Determination of the expression of the ten listed prognostic biomarkers in NPC patients via IHC might show over expression of only one biomarker. One patient might show over expression of LMP1 only and the other might show over expression of COX-2 or MMP9 only. According to mathematical combination as shown in Table 4.5, there are ten different possible outputs for different NPC patients. This model allows the determination of pooled HR and NPC prognosis assessment risk score for any of the situations discussed above, corresponding to different over expressed biomarkers for different individual.

**Simulated trial 2:** When five of the biomarkers, for example LMP1, VEGF, EGFR, ERCC1 and MMP9 are overexpressed in NPC patients after IHC testing on all ten different biomarkers, cell A7, A8, A9, A12 and A15 in the multigene NPC prognosis assessment model (Figure 4.2) are selected. The FALSE in these cells changes to TRUE, and cell N7, N8, N9, N12 and N15 (log of hazard ratio over variance), O7, O8, O9, O12 and O15 (inverse of variance) and R7, R8, R9, R12 and R15 (NPC prognosis assessment risk score) are computed accordingly (refer to Table 4.2 for description). Simultaneously, cell N16, O16 and R16, which are the sum of the respective parameter for column N, O and R are calculated (refer to Table 4.3 for description). R16 depicts the total NPC prognosis assessment risk score which is also shown in cell K19. N16 and O16 are used to determine pooled  $\ln(HR)$  as shown in cell B18 and pooled HR is determined by getting the exponential of the value in cell B18 as shown in B19. O16 and B18 are used to determine the log of lower/upper confidence intervals (CIs) in cell E18 and H18 respectively. Exponential of E18 and H19 derives the lower/upper CIs in cell E19 and H19. All the formulation discussed above occurs simultaneously when cell A7, A8, A9, A12 and A15 are selected. Figure 4.6 shows the multigene NPC prognosis assessment model when LMP1, VEGF, EGFR, ERCC1 and

MMP9 are over expressed and Figure 4.7 shows the outcome of the NPC patients corresponding to over expression of LMP1, VEGF, EGFR, ERCC1 and MMP9.

Based on the results obtained, the pooled HR of the patient is 1.81 when LMP1, VEGF, EGFR, ERCC1 and MMP9 are over expressed. It implies that the NPC patient is 81% more likely to die compared to other NPC patients with lowly expressed LMP1, VEGF, EGFR, ERCC1 and MMP9. The significance of this results falls within the confidence intervals (CIs) of 1.62 to 2.01. These results are significant as the lower and upper CIs do not include one. The total NPC prognosis assessment risk score is 16 out of total score of 31. Intermediate risk score indicates that a larger number of biomarkers are involved in the NPC oncogenesis of that patient, and thus increasing the complexity of the cancer.

Expression of the ten listed prognostic biomarkers in NPC patients determined via IHC might show over expression of only five biomarkers. One patient might show over expression of LMP1, VEGF, EGFR, ERCC1 and MMP9. Another patient might show over expression of COX-2, LMP1, CRP, ERCC1 and MMP9. Based on mathematical combination, there are 252 different possible combinations that can be formed from ten different biomarkers as shown in Table 4.5. This model allows the determination of pooled HR and NPC prognosis assessment risk score for any of the 252 situations discussed above, corresponding to personal output (different over expressed biomarker) for different individual. The same principal could be applied to the combination of different number of different over expressed biomarkers.

|    | A                                        | B          | C    | D     | E        | F        | G        | H          | I     | J              | K | L | M | N               | O               | P | Q             | R              |
|----|------------------------------------------|------------|------|-------|----------|----------|----------|------------|-------|----------------|---|---|---|-----------------|-----------------|---|---------------|----------------|
| 1  |                                          |            |      |       |          |          |          |            |       |                |   |   |   |                 |                 |   |               |                |
| 2  |                                          |            |      |       |          |          |          |            |       |                |   |   |   |                 |                 |   |               |                |
| 3  |                                          |            |      |       | Lower CI | Upper CI | LN(LCI)- |            |       |                |   |   |   | Formula =       | Formula =       |   | NPC Prognosis |                |
| 4  | Tick if yes                              | Biomarkers | HR   | (LCI) | (UCI)    | LN(UCI)  | Variance | 1/Variance | LN HR | LN HR/Variance |   |   |   | IF(A6=True,M6,) | IF(A6=True,J6,) |   | Assessment    | Formula =      |
| 5  |                                          |            |      |       |          |          |          |            |       |                |   |   |   |                 |                 |   | Score         | IF(A6=TRUE,Q6) |
| 6  | <input type="checkbox"/> FALSE           | COX-2      | 2.02 | 1.65  | 2.47     | 0.40     | 0.01     | 94.41      | 0.70  | 66.38          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 7  | <input checked="" type="checkbox"/> TRUE | LMP1       | 2.48 | 1.77  | 3.47     | 0.67     | 0.03     | 33.91      | 0.91  | 30.80          |   |   |   | 30.80           | 33.91           |   | 4             | 4              |
| 8  | <input checked="" type="checkbox"/> TRUE | VEGF       | 2.07 | 1.32  | 3.25     | 0.90     | 0.05     | 18.93      | 0.73  | 13.77          |   |   |   | 13.77           | 18.93           |   | 3             | 3              |
| 9  | <input checked="" type="checkbox"/> TRUE | EGFR       | 1.86 | 1.25  | 2.77     | 0.80     | 0.04     | 24.27      | 0.62  | 15.06          |   |   |   | 15.06           | 24.27           |   | 3             | 3              |
| 10 | <input type="checkbox"/> FALSE           | LDH        | 2.86 | 1.66  | 2.08     | 0.23     | 0.00     | 302.05     | 1.05  | 317.41         |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 11 | <input type="checkbox"/> FALSE           | CRP        | 1.84 | 1.57  | 2.17     | 0.32     | 0.01     | 146.70     | 0.61  | 89.45          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 12 | <input checked="" type="checkbox"/> TRUE | ERCC1      | 1.77 | 1.48  | 2.12     | 0.36     | 0.01     | 118.98     | 0.57  | 67.94          |   |   |   | 67.94           | 118.98          |   | 3             | 3              |
| 13 | <input type="checkbox"/> FALSE           | HIF-1a     | 1.77 | 1.35  | 2.32     | 0.54     | 0.02     | 52.41      | 0.57  | 29.93          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 14 | <input type="checkbox"/> FALSE           | LOX        | 1.68 | 1.52  | 1.87     | 0.21     | 0.00     | 357.83     | 0.52  | 185.64         |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 15 | <input checked="" type="checkbox"/> TRUE | MMP9       | 1.65 | 1.38  | 1.95     | 0.35     | 0.01     | 128.55     | 0.50  | 64.37          |   |   |   | 64.37           | 128.55          |   | 3             | 3              |
| 16 |                                          |            |      |       |          |          |          |            |       |                |   |   |   | 191.94          | 324.63          |   |               | 16             |

**Figure 4.6:** Output of multigene NPC prognosis assessment model when LMP1, VEGF, EGFR, ERCC1 and MMP9 are over expressed.

|    | A         | B    | C | D           | E    | F | G           | H    | I | J                                         | K  |
|----|-----------|------|---|-------------|------|---|-------------|------|---|-------------------------------------------|----|
| 16 |           |      |   |             |      |   |             |      |   |                                           |    |
| 17 |           |      |   |             |      |   |             |      |   |                                           |    |
| 18 | LN(HR)    | 0.59 |   | LN Lower CI | 0.48 |   | LN Upper CI | 0.70 |   |                                           |    |
| 19 | Pooled HR | 1.81 |   | Lower CI    | 1.62 |   | Upper CI    | 2.01 |   | Total NPC Prognosis Assessment Risk Score | 16 |

**Figure 4.7:** Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene NPC prognosis assessment model when LMP1, VEGF, EGFR, ERCC1 and MMP9 are over expressed.

**Simulated trial 3:** When all of the ten biomarkers are overexpressed in NPC patients after IHC testing on all ten different biomarkers, cell A6-A15 in the multigene NPC prognosis assessment model as shown in Figure 4.2 are selected. The FALSE in these cells changes to TRUE, and cell N6- N15 (log of hazard ratio over variance), O6-O15 (inverse of variance) and R6-R15 (NPC prognosis assessment risk score) are computed accordingly (refer to Table 4.2 for description). Simultaneously, cell N16, O16 and R16, which are the sum of the respective parameter for column N, O and R are calculated (refer to Table 4.3 for description). R16 depicts the total NPC prognosis assessment risk score which is also shown in cell K19. N16 and O16 are used to determine pooled  $\ln(HR)$  as shown in cell B18 and pooled HR is determined by getting the exponential of cell B18 as shown in B19. O16 and B18 are used to determine the log of lower/upper confidence intervals (CIs) in cell E18 and H18 respectively. Exponential of the values in cell E18 and H19 derives the lower/upper CIs in cell E19 and H19. All the formulation discusses above occurred simultaneously when cell A6-A15 are selected. Figure 4.8 shows the multigene NPC prognosis assessment model when all the biomarkers are over expressed and Figure 4.9 shows the outcome of the NPC patients corresponding to over expression of all biomarkers.

Based on the results obtained, the pooled HR of the patient is 1.99 when all the biomarkers are over expressed. It implies that the NPC patient is 99% more likely to die compared to other NPC patients with low expression of all biomarkers. The significance of this results falls within the confidence intervals (CIs) of 1.89 to 2.10. These results are significant as the lower and upper CIs do not include one. The total NPC prognosis assessment risk score is 31 out of total score of 31. High risk score indicates that a larger number of biomarkers are involved in the NPC oncogenesis of that patient, and thus showing high cancer complexity.

|    | A                                   | B          | C      | D     | E        | F        | G        | H          | I      | J              | K      | L | M      | N               | O               | P | Q             | R              |
|----|-------------------------------------|------------|--------|-------|----------|----------|----------|------------|--------|----------------|--------|---|--------|-----------------|-----------------|---|---------------|----------------|
| 1  |                                     |            |        |       |          |          |          |            |        |                |        |   |        |                 |                 |   |               |                |
| 2  |                                     |            |        |       |          |          |          |            |        |                |        |   |        |                 |                 |   |               |                |
| 3  |                                     |            |        |       | Lower CI | Upper CI | LN(LCI)- |            |        |                |        |   |        | Formula =       | Formula =       |   | NPC Prognosis |                |
| 4  | Tick if yes                         | Biomarkers | HR     | (LCI) | (UCI)    | LN(UCI)  | Variance | 1/Variance | LN HR  | LN HR/Variance |        |   |        | IF(A6=True,M6,) | IF(A6=True,J6,) |   | Assessment    | Formula =      |
| 5  |                                     |            |        |       |          |          |          |            |        |                |        |   |        |                 |                 |   | Score         | IF(A6=TRUE,Q6) |
| 6  | <input checked="" type="checkbox"/> | TRUE       | COX-2  | 2.02  | 1.65     | 2.47     | 0.40     | 0.01       | 94.41  | 0.70           | 66.38  |   | 66.38  |                 | 94.41           |   | 3             | 3              |
| 7  | <input checked="" type="checkbox"/> | TRUE       | LMP1   | 2.48  | 1.77     | 3.47     | 0.67     | 0.03       | 33.91  | 0.91           | 30.80  |   | 30.80  |                 | 33.91           |   | 4             | 4              |
| 8  | <input checked="" type="checkbox"/> | TRUE       | VEGF   | 2.07  | 1.32     | 3.25     | 0.90     | 0.05       | 18.93  | 0.73           | 13.77  |   | 13.77  |                 | 18.93           |   | 3             | 3              |
| 9  | <input checked="" type="checkbox"/> | TRUE       | EGFR   | 1.86  | 1.25     | 2.77     | 0.80     | 0.04       | 24.27  | 0.62           | 15.06  |   | 15.06  |                 | 24.27           |   | 3             | 3              |
| 10 | <input checked="" type="checkbox"/> | TRUE       | LDH    | 2.86  | 1.66     | 2.08     | 0.23     | 0.00       | 302.05 | 1.05           | 317.41 |   | 317.41 |                 | 302.05          |   | 3             | 3              |
| 11 | <input checked="" type="checkbox"/> | TRUE       | CRP    | 1.84  | 1.57     | 2.17     | 0.32     | 0.01       | 146.70 | 0.61           | 89.45  |   | 89.45  |                 | 146.70          |   | 3             | 3              |
| 12 | <input checked="" type="checkbox"/> | TRUE       | ERCC1  | 1.77  | 1.48     | 2.12     | 0.36     | 0.01       | 118.98 | 0.57           | 67.94  |   | 67.94  |                 | 118.98          |   | 3             | 3              |
| 13 | <input checked="" type="checkbox"/> | TRUE       | HIF-1a | 1.77  | 1.35     | 2.32     | 0.54     | 0.02       | 52.41  | 0.57           | 29.93  |   | 29.93  |                 | 52.41           |   | 3             | 3              |
| 14 | <input checked="" type="checkbox"/> | TRUE       | LOX    | 1.68  | 1.52     | 1.87     | 0.21     | 0.00       | 357.83 | 0.52           | 185.64 |   | 185.64 |                 | 357.83          |   | 3             | 3              |
| 15 | <input checked="" type="checkbox"/> | TRUE       | MMP9   | 1.65  | 1.38     | 1.95     | 0.35     | 0.01       | 128.55 | 0.50           | 64.37  |   | 64.37  |                 | 128.55          |   | 3             | 3              |
| 16 |                                     |            |        |       |          |          |          |            |        |                |        |   | 880.74 |                 | 1278.03         |   |               | 31             |

**Figure 4.8:** Output of multigene NPC prognosis assessment model when all the biomarkers are over expressed.

|    | A         | B    | C           | D    | E           | F    | G                                         | H | I | J | K  |
|----|-----------|------|-------------|------|-------------|------|-------------------------------------------|---|---|---|----|
| 16 |           |      |             |      |             |      |                                           |   |   |   |    |
| 17 |           |      |             |      |             |      |                                           |   |   |   |    |
| 18 | LN(HR)    | 0.69 | LN Lower CI | 0.63 | LN Upper CI | 0.74 |                                           |   |   |   |    |
| 19 | Pooled HR | 1.99 | Lower CI    | 1.89 | Upper CI    | 2.10 | Total NPC Prognosis Assessment Risk Score |   |   |   | 31 |

**Figure 4.9:** Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene NPC prognosis assessment model when all the biomarkers are over expressed.

Expression of the ten listed prognostic biomarkers of NPC patients determined via IHC might show over expression of all biomarkers. Based on mathematical combination, there is only one possible combination that can be formed from these ten different biomarkers. The mathematical combination is as shown in Table 4.5. More examples of this is included in Appendix B (refer to page 160-169). Inclusion of more prognostic biomarkers enables the determination of OS more accurately.

As far as we know, this is the first study that combined different meta-analysis studies conducted on different prognosis biomarkers to generate a multigene NPC prognosis assessment model. Existing NPC prognosis model such as prognostic nomogram of clinical characteristic and hematological/inflammatory biomarkers of NPC focused mainly on the clinical characteristics. Besides, these existing models comprise only hematological/inflammatory biomarkers rather than different types of prognostic biomarkers. The sample size used to construct these existing models is relatively small hence affecting the significance of the outcome. On top of that, the sample was from a single location, making the model applicable and pertinent only at the respective area of study (Li et al., 2016; Li et al., 2018). Another example of existing NPC prognosis model was statistical prognostic score model to determine disease progression and survival of NPC patients (Zhang et al., 2013). However, this model included only a single biomarker, that was CXCL5 motif chemokine ligand 5 (CXCL5). The sample size used to generate this model was small (n= 290) and has similar disadvantage as the model proposed by Li. Thus, the new multigene NPC prognosis assessment model in our study is a better updated NPC prognosis assessment model. Our model focuses more on the functional part (gene expression) and includes a large number of NPC prognostic biomarkers. The data incorporated in this study is adopted from

meta-analysis studies of respective biomarkers, making the data more significant and valid compared to individual study. Data from meta-analysis studies that are done by including various studies from different region, including China, Singapore, Taiwan and Korea, increased the usage of the model by minimising the regional restriction.

A large total NPC assessment risk score showed that more prognostic biomarkers are over expressed. This indicates that the patient is of higher risk compare to those showing a small number. The higher the score indicates involvement of more biomarkers and thus further increase the complexity of NPC oncogenesis. Identification of the biomarkers allow the better selection of personalized treatment for the patient, thus increases overall survival. Identification of individualized treatment could be made possible by determining the interaction of different biomarkers in a molecular network model as reported in the next chapter.

#### **4.3.3 Limitations**

Several limitations were taken into account in this secondary meta-analysis. Firstly, a number of studies are excluded as they contained incomplete data. Such an exclusion might affect the pooled results to differ from true value. Also, meta-analysis that is done literature-based is less valid than individual patient data-based analysis. Therefore, a molecular network pathway that proved the physiological interactions among the different biomarkers is constructed and discussed in the next chapter to further explain the role of the biomarkers in NPC oncogenesis.

## **CHAPTER 5**

### **CONSTRUCTION OF NASOPHARYNGEAL CARCINOMA (NPC) MOLECULAR NETWORK MODEL**

#### **5.1 Introduction**

Development of complex diseases such as cancer involved multifactorial biological pathways. Combination and integration of genomics and/or proteomic data into a molecular network help to understand the mechanism that contribute to the progression of complex diseases. Molecular pathway network allow the discovery of risk factors that led to the occurrence of diseases and identification of individualized treatments (Zheng, Wei, Zhao & Ni, 2018).

In this study, the robustness of the multigene NPC prognosis assessment model that is generated computational need further clarification with development of NPC molecular pathway network model. This helps to explain the physiological interactions among all prognostic biomarkers incorporated in the model. The interlinks between different biomarkers involve in the carcinogenesis and progression of NPC are displayed to justify the complex and dynamic interactions of the pooled biomarkers in the prognosis assessment model. This is done via literature reviewing of previous research done on the respective biomarkers. Relevant data are extracted, analysed and combined into a molecular pathway network as discussed in the following section.

## 5.2 Methods

Based on the final ten prognostic biomarkers that were selected for the development of NPC biomolecular profile, literature search in the databases was done without restriction to determine the molecular reaction of individual biomarker and the interaction between different biomarkers that led to the formation of NPC. The relevant findings were discussed and the interactions and pathways were shown in flow diagrams (Figure 5.1 to Figure 5.4). Different pathways involved by different biomarkers were combined into a network (Figure 5.5). The types of inhibitor for the expression of each biomarker were also tabulated (Table 5.1) to determine the type of inhibitors used corresponding to the over expressed biomarkers.

## 5.3 Results and discussion

The detailed mechanism that leads to the progression and development of NPC is yet to be fully explored. Definitive NPC early diagnosis tool or biomarkers are not strongly established too. Besides, there were limited numbers of current NPC treatment available (Ng et al., 2005; Coghill et al., 2014). Hence, to understand the carcinogenesis of NPC comprehensively, research done to study the molecular mechanisms of NPC proliferation and progression need to be incorporated.

The final ten prognostic biomarkers included in the prognosis assessment model are cyclooxygenase-2 (COX-2), latent membrane protein 1 (LMP1), vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), lactate dehydrogenase (LDH), C-reactive protein (CRP), excision repair cross-complementation group 1 (ERCC1), hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ ), lysyl oxidase (LOX) and matrix

metalloproteinase 9 (MMP9). The detailed pathways involved by each biomarker are discussed below and NPC molecular network model is constructed.

LMP1 is the principal oncogene of NPC (Wang, Liebowitz & Kieff, 1986) and LMP1-positive cells enhance NPC metastasis (Ozyar, Ayhan, Korcum & Atahan, 2004). C-terminal activating regions 1 and 2 (CTAR 1 and 2) on LMP1 activates nuclear factor  $\kappa$ -B (NF- $\kappa$ B) pathway (Huen, Henderson, Carter & Rowe, 1995; Mainou, Everly & Raab-traub, 2005) which in turn activates COX-2, VEGF and CRP (Muroso et al., 2001; Deng et al., 2003; Pine et al., 2011). CRP is an inflammatory biomarker and over expression of CRP causes metastasis and angiogenesis (Fang et al., 2017). Activation of nuclear factor  $\kappa$ B (NF- $\kappa$ B) pathway also transactivates MMP9 and EGFR via activator protein 1 (AP-1) pathway (Yoshizaki, Sato, Furukawa & Pagano, 1998; Takeshita et al., 1999). High expression of MMP9 (an endopeptidase) and EGFR (a receptor tyrosine kinases) increase rate of metastasis in NPC (Horikawa et al., 2000; Zhang, Huang, Zhou & Lin, 2018). Simvastatin is an example of LMP1 inhibitor that potentiates chemotherapy treatment by enhancing the sensitivity of the treatment (Pioche-durieu et al., 2005; Mei et al., 2007). Activities of MMP9 and EGFR are inhibited by Doxycycline (via exogenous inhibition) and Cetuximab (via phosphorylation and thus suppresses EGFR production) respectively (Wu et al., 2016). The pathways discussed above are integrated and shown in Figure 5.1.

COX-2 is an inflammatory biomarker that causes NPC carcinogenesis through multiple steps (Soo et al., 2005). COX-2 is involved in the conversion of arachidonic acid to prostaglandins (PGE), mediating cell proliferation, metastasis, angiogenesis and anti-apoptosis (Figure 5.2) (Tsuji & Dubois, 1995; Tsuji et al., 1998; Williams et al., 2000). The most commonly used

COX-2 inhibitor is Celecoxib. Non-steroidal anti-inflammatory drugs (NSAIDs) could inhibit COX-2 but they could also inhibit Cox-1, an undesirable reaction. Thus, Celecoxib is used to suppress tumour growth and to enhance radiotherapy outcome (Tan & Putti, 2005).

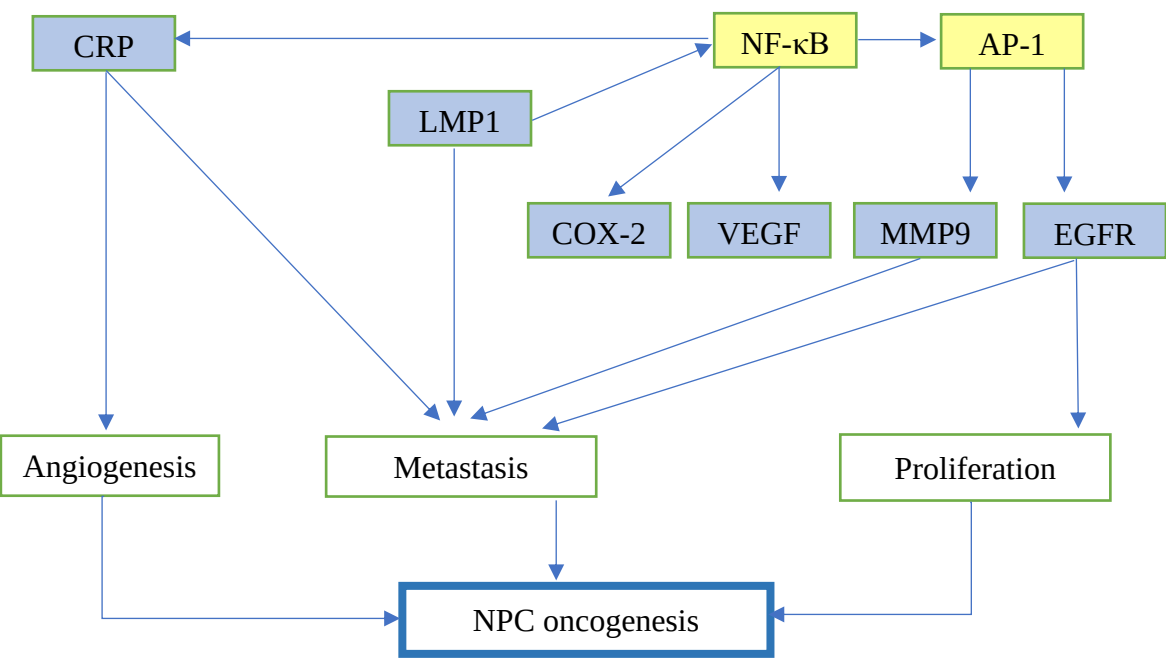


**Figure 5.1:** A molecular pathways involved by LMP1, CRP, COX-2, VEGF, MMP9 and EGFR in NPC oncogenesis (yellow boxes represented pathways and blue boxes represented prognostic biomarkers).

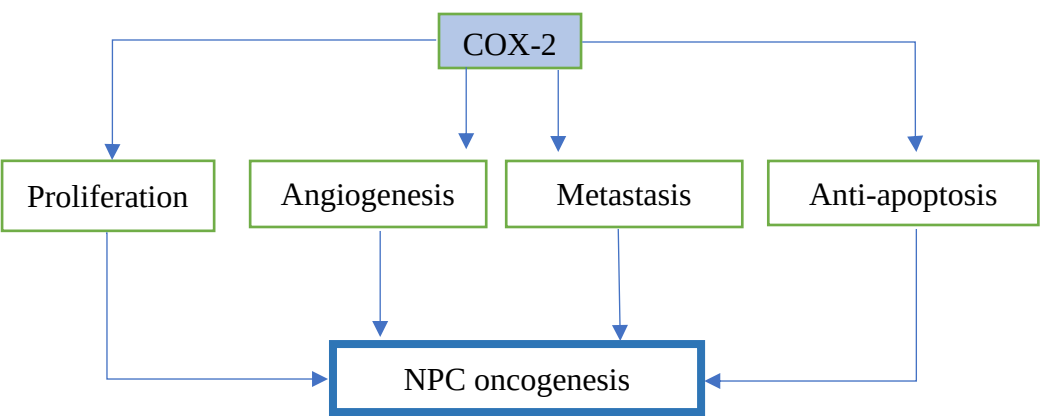


**Figure 5.2:** Molecular activity of COX-2 in NPC oncogenesis (blue box represented prognostic biomarker).

HIF-1 $\alpha$  is a hypoxic biomarker, triggered anti-apoptosis, promote metastasis and angiogenesis (Ji, 2014). Over expression of HIF-1 $\alpha$  induces the over expression of VEGF (Cox & Erler, 2011). VEGF is the most commonly known angiogenic factors (Dvorak, Brown, Detmar & Dvorak, 1995). Activation of VEGF leads to distant metastasis (Hui et al., 2002; Hui et al., 2008; Lu et al., 2008). Tirapazamine (TPZ) is the common hypoxic inhibitor used to control the expression of both HIF-1 $\alpha$  and VEGF via phosphorylation that in turn inhibits transcriptional activation of both biomarkers (Hong et al., 2011). Over expression of HIF-1 $\alpha$  activates LOX, a hypoxic biomarker (Cox et al., 2013). Combined expression of LOX, HIF and VEGF promotes cancer cells to grow faster, leading to metastasis (Chung, Lee & Ferrara, 2010).  $\beta$ -aminopropionitrile (BAPN) is an irreversible inhibitor that could inhibit the catalytic activity of LOX, halting metastasis, thus improving NPC overall survival (Tang, Trackman & Kagang, 1983; Xiao & Ge, 2012). Besides, high level of HIF-1 $\alpha$  upregulates LDH which in turn caused distant metastasis (Fantin, St-Pierre & Leder, 2006). Oxamate is used for NPC patients with over expressed LDH, acting through oxidative phosphorylation (Zhai, Yang, Wan, Zhu & Wu, 2013). A molecular pathway network involved by HIF-1 $\alpha$ , LDH, VEGF and LOX is summarized in Figure 5.3.

ERCC1 is involved in nucleotide excision repair (NER) pathway that repaired DNA damage and thus halting cancer cells apoptosis (Reed, 2005; Olaussen et al., 2006). Patients are resistance to cisplatin-based chemotherapy if ERCC1 expression is high. High expression of ERCC1 in tumours correlates with low chemotherapy response and poor survival in patients treated with cisplatin-based induction chemotherapy (Huang et al., 2012). Figure 5.4 shows the reaction of ERCC1 upon chemotherapy treatment with cisplatin which in turn activates NER and rendering NPC cells anti-apoptosis.

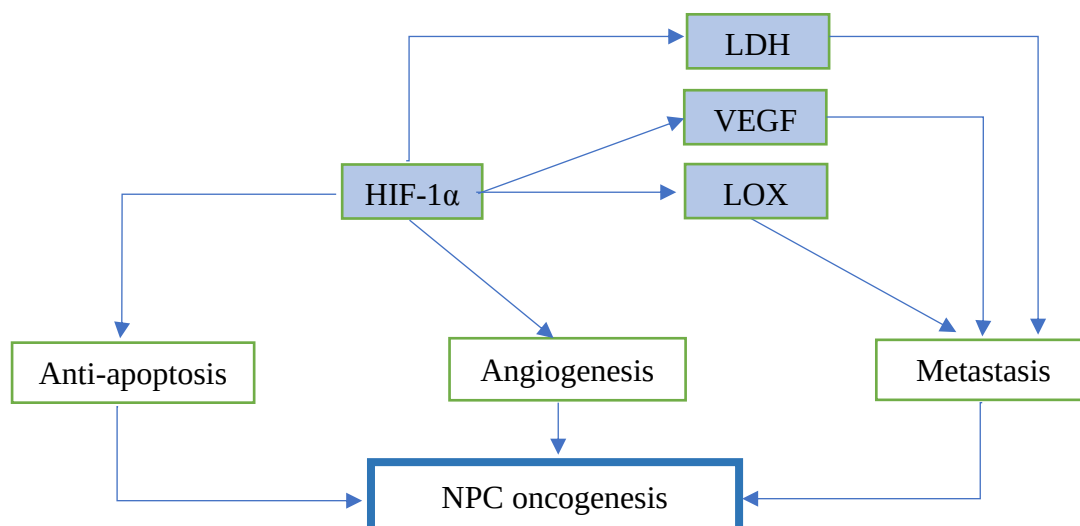


**Figure 5.3:** A molecular pathway network involved by HIF-1 $\alpha$  with LDH, VEGF and LOX in NPC oncogenesis (blue boxes represented prognostic biomarkers).

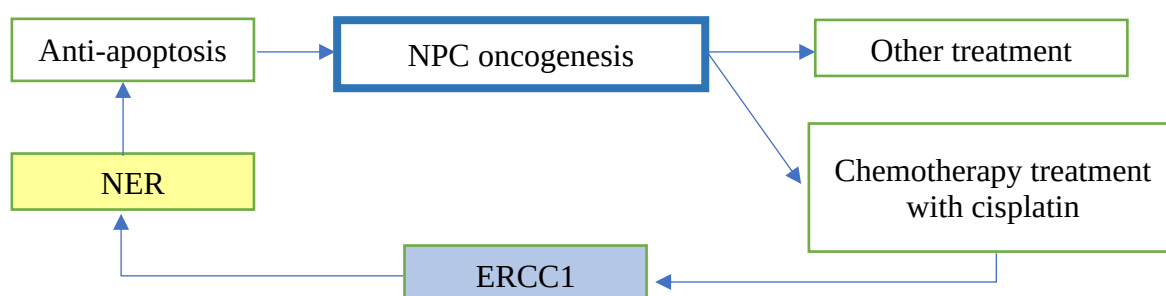


**Figure 5.4:** A molecular pathway involved by ERCC1 after cisplatin-based chemotherapy that induced NER and caused anti-apoptosis in NPC (yellow box represented pathway and blue box represented prognostic biomarker).

Based on the literature search, the interaction among all the selected biomarkers are summarized, integrated and displayed in a complex network model. COX-2 that is involved in NPC oncogenesis as per discussed in Figure 5.2 is also found in the molecular interaction of other biomarkers shown in Figure 5.1. Hence, Figure 5.2 is incorporated into Figure 5.1 to further enhance the molecular network model of NPC oncogenesis. The same is done for Figure 5.3 into Figure 5.1 where VEGF is common in these two pathways. Figure 5.4 is

consolidated into the network after integration of Figure 5.1, Figure 5.2 and Figure 5.3 as it is involved in the mechanism after NPC treatment. A final complex molecular pathways network of NPC is constructed as shown in Figure 5.5.

From Figure 5.5, the interactions among the ten prognostic biomarkers incorporated in the multigene NPC prognosis assessment model are clearly shown. This network model supported the reports in the literature, that LMP1 is a principal oncogene in NPC carcinogenesis (Wang, Liebowitz & Kieff, 1986). Expression of LMP1 initiates the activation of a large number of downstream biomarkers listed in the multigene NPC prognosis assessment model either directly or indirectly through various pathways. Based on this, NPC patients that show high LMP1 expression via IHC testing would probably show over expression of the other downstream biomarkers too. The OS of the patients can then be determined via multigene NPC prognosis assessment model as per discussed in Chapter 4.

Another biomarker that involves actively in NPC oncogenesis is HIF-1 $\alpha$ , a hypoxic biomarker that induces the activation of high number of other biomarkers, such as VEGF, LDH and LOX. HIF-1 $\alpha$  and LOX are both hypoxia biomarkers. Thus, NPC patient with upregulated HIF-1 $\alpha$  is expected to show over expression of LOX (and vice versa) under hypoxic condition too. COX-2 and CRP are both inflammatory biomarkers. A patient that shows over expression of either one of the biomarkers is expected to show over expression of the other biomarkers under inflammatory environment. Similarly, the overall survival of such a patient can be predicted via the constructed multigene NPC prognosis assessment model from our study.

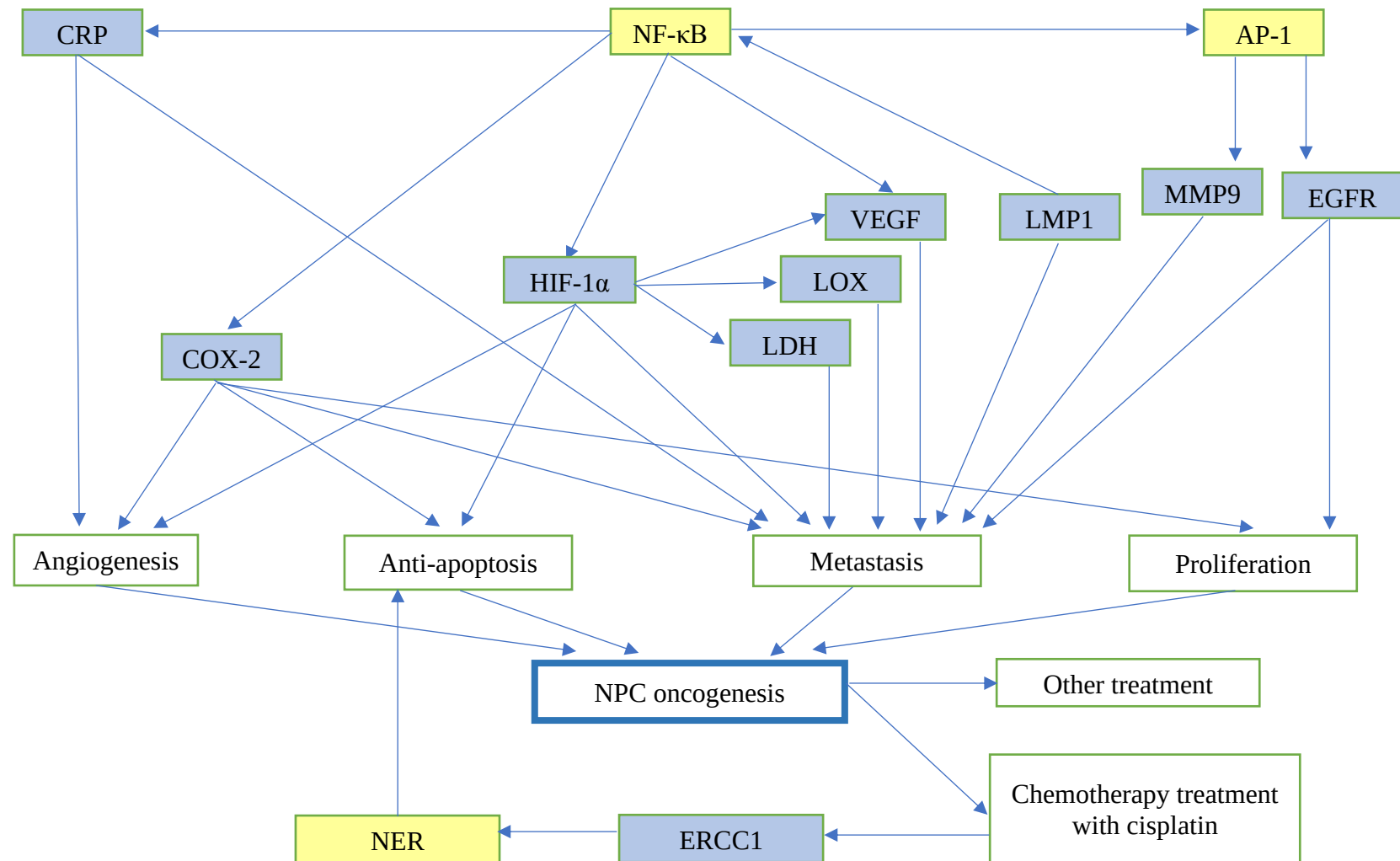


**Figure 5.5:** The NPC molecular network model comprised of ten biomarkers involved in the generation of multigene NPC prognosis assessment model (yellow boxes represented pathways and blue boxes represented the prognostic biomarkers).

Based on the network model (Figure 5.5), it is shown that most of the biomarkers contributed to NPC oncogenesis via metastasis. The types of inhibitors used to inhibit respective biomarkers in the network model were also summarized in Table 5.1. As concluded in Table 5.1, reactions all the biomarkers included in the network model can be inhibited by specific inhibitors except CRP and ERCC1. To date, there is no targeted therapy to inhibit over expression of these two prognostic biomarkers. Thus, studies on these can be done to further enhance the validity of the constructed network model.

**Table 5.1:** The inhibitors targeting different prognostic biomarkers in the network model.

| <b>Prognostic Biomarkers</b> | <b>Inhibitors</b>           |
|------------------------------|-----------------------------|
| COX-2                        | Celecoxib                   |
| LMP1                         | Simvastatin                 |
| VEGF                         | Tirapazamine                |
| EGFR                         | Cetuximab                   |
| LDH                          | Oxamate                     |
| CRP                          | ND                          |
| ERCC1                        | ND                          |
| HIF-1 $\alpha$               | Tirapazamine                |
| LOX                          | $\beta$ -aminopropionitrile |
| MMP9                         | Doxycycline                 |

\*\*ND = Not detected

There are a few existing NPC networks that show oncogenesis in NPC. For example, signalling pathways in the pathogenesis of NPC initiated by LMP1 (Tulalamba & Janvilisri, 2012) and miRNA signalling pathway in NPC (Fan et al., 2018). Pathway explained by Tulalamba and Janvilisri in 2012 showed mainly the involvement of LMP1 in the carcinogenesis of NPC by explaining the various molecular pathways that LMP1 took part rather than showing detailed interaction between LMP1 with other biomarkers. Another

model by Fan and colleagues in 2018 discussed only the interactions among different miRNA. These models although explained the mechanism of NPC oncogenesis, did not incorporate prognostic biomarkers as the key elements. Hence, as far as we know, the molecular pathway network constructed from our study is the first model that incorporated different prognostic biomarkers into one platform to show the different interactions that lead to NPC oncogenesis.

This network model provides a new insight toward the complex interplay of biomarkers that leads to the occurrence of complex NPC. This network not only supports the physiological interactions among the biomarkers incorporated in the risk model, it also reflects the feedback mechanism between the biomarkers. Over expression of a certain biomarkers cause the over expression of another biomarkers, contribute to NPC oncogenesis. Such a model also allows a faster and more accurate identification of panel biomarkers that activate the progression of cancer, and hence, enables the identification of the suitable drugs aiming at the target biomarkers to improve overall survival of NPC patients. The NPC molecular network model that is generated on molecular biology basis can function in complementary with multigene NPC prognosis assessment model that is generated on computational science basis, showing the multidisciplinary of this study.

## **CHAPTER 6**

### **GENERAL DISCUSSION**

Cyclooxygenase-2 (COX-2) is an inflammatory protein biomarker. Over expression of COX-2 is found to be involved in the carcinogenesis of various cancers, such as breast cancer (Costa et al., 2002), oral squamous cell carcinoma (OSCC) (Pan et al., 2008), head and neck squamous cell carcinoma (HNSCC) (Saba et al., 2009) and colorectal cancer (Peng, Zhou, Wang, Mou & Zhao, 2013). Studies to determine the prognostic significance of COX-2 have been done in nasopharyngeal carcinoma (NPC) too (Pan et al., 2009, Li, Luo, Xie, Li & Wang, 2018). These studies reported on the overall survival (OS) rate of NPC patients in terms of hazard ratios (HRs) in relation to the expression of COX-2. However, the sample size used in these studies are often small (n=35 and n=58) and the results are conflicting. A meta-analysis study to pool these studies into a single platform to draw a more relevant, significant and conclusive output is desirable. Hence, a meta-analysis to determine the prognostic significance of COX-2 in NPC in term of survival outcome (reported as HRs) is done in this study.

In our study, the pooled HR obtained after conducting a meta-analysis of seven studies is 2.02 [1.65, 2.47]. This HR value that is higher than one implies that COX-2 is a good prognostic biomarker in NPC patients, predicting worse overall survival rate. In other words, NPC patients with over expression of COX-2 have higher risk of death compared to those with low COX-2 expression. Through this meta-analysis, a more conclusive result is drawn from the conflicting results of the individual studies. Over expression of COX-2 also predicts worse survival rate in breast cancer and ovarian cancer (Xu, Li, Zhang, Cui, Liu, Li & Jiang,

2017; Sun, Zhang, Sun, Jia, Xu, Qiao & Jin, 2017), acting as a good prognostic biomarker that predicts worse survival outcome. COX-2 expression level shows an inverse relationship with overall survival rate of NPC patients. Having known that COX-2 is a prognostic biomarker in NPC, determination of COX-2 level helps to better predict the survival rate of the patients. Besides, suitable treatments can be given to the NPC patients to counter the effect of high COX-2 level, therefore to increase overall survival.

The finding in this study is obtained through meta-analysis of studies from secondary data deposited in the databases. The result of our finding can be further verified through the determination of COX-2 level via immunohistochemistry (IHC) in a larger patient sample size at various locations of studies. The expression of COX-2 in relation to the overall survival rate of the NPC patients can be obtained and can be analysed as primary data source to verify the reliability and accuracy of our finding (that is, high COX-2 expression predicts worse survival outcome in NPC patients) obtained through analysis of secondary data. The primary results of these studies verify not only the meta-analysis study done in our study, the results can also be incorporated into our meta-analysis study. This increases the sample sizes used in the meta-analysis, thus making the pooled HRs of COX-2 more significant and relevant.

Apart from meta-analysis of COX-2 in NPC done in our study, other meta-analysis studies on different biomarkers as to determine their prognostic significance in NPC have been done too. Examples are such as meta-analysis on excision repair cross-complementation group 1 (ERCC1) (Yang et al., 2019), latent membrane protein 1 (LMP1) (Chen et al., 2015),

vascular endothelial growth factor (VEGF) (Wang et al., 2018), epidermal growth factor receptor (EGFR) (Sun et al., 2013), C-reactive protein (CRP) (Fang, Xu, Wu, Zhang, Li et al., 2017), hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ ) (Xie et al., 2018), lactate dehydrogenase (LDH) (Zhai, Gu, Zhai, Wang & Zhang, 2017), lysyl oxidase (LOX) (Zhang, Wu, Ye & Xue, 2018) and matrix metalloproteinase 9 (MMP9) (Liao, Huang, Zhu, Li, Huang & He, 2016) in NPC. However, comprehensive study to explain the interactions between all these biomarkers in terms of prognosis assessment is not done. In this study, we generated a multigene NPC prognosis assessment model by pooling the relevant meta-analysis studies on different biomarkers into one platform via modification of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher, Liberati, Tetzlaff, Altman & The PRISMA Group, 2009). Meta-analysis of COX-2 in NPC from our own study is incorporated into the model as well (Sim & Sim, 2020).

With this multigene NPC prognosis assessment model, NPC patients can determine their overall survival. This is done by determining the expression of the prognostic biomarkers in the patients via IHC and the results of the test are incorporated into the model to compute HRs. The HRs generated corresponding to the number of prognostic biomarkers over expressed in different NPC patients. The HRs obtained are therefore specific for different NPC patients, reflecting personalized disease condition. Two NPC patients with five over expressed prognostic biomarkers might show different HRs value as the five over expressed biomarkers in two NPC patients might differ. The different HRs values show that the two NPC patients have two distinct survival outcomes due to the different interactions and pathogenesis involved by the different prognostic biomarkers in different NPC patients.

Thus, this model is suitable and applicable for different NPC patients, reporting personalized results.

Besides determining the survival outcome of NPC patients computationally, the multigene NPC prognosis assessment model that is generated in this study can also be used to stratify NPC patients according to the over expressed biomarkers. For example, when this model is used by NPC patients in different regions, the types of over expressed prognostic biomarkers for different NPC patients in different regions can be recorded and stored. The data collected can be used to generate a statistic that is useful to determine the types of prognostic biomarkers that are most frequently over expressed in NPC patients of different regions. The over expression of a certain NPC prognostic biomarkers at different regions might show relationship to the respective environment or regional life style (eating habit). Thus, studies to identify the activator that trigger the over expression of the prognostic biomarker in NPC patients at those areas can be done to better monitor the occurrence of NPC.

There are more NPC prognostic biomarkers than those included in our model. Biomarkers included are not chosen randomly but selected via specific inclusion and exclusion criteria (refer to Section 4.2.2, page 66-67). Individual studies on NPC prognostic biomarkers are not included in the model if meta-analysis on those studies is not done. Meta-analysis studies are chosen over individual studies reporting on the same biomarkers because the results are more significant and relevant. Therefore, if meta-analysis on those individual studies focusing on NPC prognostic biomarkers is done and incorporated into our multigene NPC prognosis assessment model, the model would be more significant and robust. When more

biomarkers are assimilated into the model, the pooled HRs that reflect the overall survival rate of NPC patients would be closer to the actual clinical outcome, ensuring a better medical practice of the model. Therefore, the model generated from our study can be updated from time to time by incorporating new meta-analysis studies on prognostic biomarkers of NPC, making the model better over time.

The multigene NPC prognosis assessment model discussed in this study is constructed computationally. To provide further understanding to the prognosis assessment model, the physiological interactions between all the prognostic biomarkers that are pooled in this model are explained in a molecular basis with an aid of NPC pathway network model. This pathway network model provides further explanation to the prognosis assessment model although it does not prove or validate the prognosis assessment model. Based on the pathway network model generated, it shows the interactions among all the prognostic biomarkers included in the prognosis assessment model. By referring to the pathway network model, LMP1 is identified as the principal prognostic biomarker that triggers and activates NF- $\kappa$ B pathway. Activation of NF- $\kappa$ B pathway in turn potentially initiates the activation of a number of downstream prognostic biomarkers, such as CRP, HIF-1 $\alpha$ , COX-2 and VEGF. Therefore, NPC patients with over expression of LMP1 tend to have over expression of the prognostic biomarkers mentioned above and the survival outcome can be determined via the prognosis assessment model in our study. Meta-analysis studies that focus on one prognostic biomarker in relation to overall survival, for example VEGF, can determine the survival outcome of the patient just by examining VEGF expression in that patient. However, determination of survival outcome in this manner is not as accurate because the expression of VEGF might be triggered by LMP1 and VEGF might also cause NPC carcinogenesis

through complex interactions with other prognostic biomarkers. However, all these possibilities are not considered in meta-analysis studies focusing on single prognostic biomarker. Therefore, assimilation of multiple genes into a single platform can better examine the survival outcome of NPC patient, getting a HR closer to the true clinical outcome.

The NPC network pathway generated in our study fulfills four out of six biological capabilities of the hallmarks of cancer. These biological competencies involve in complex and multistep development of human tumours. The hallmarks of cancer included in our model are angiogenesis, anti-apoptosis (resistance to cell death), metastasis and proliferation (proliferative signalling). The other two hallmarks that are not considered in our model are growth suppressors evasion and ability of replicative immortality (Hanahan & Weinberg, 2000). The other two hallmarks are not incorporated in the pathway network model as the prognostic biomarkers in the prognosis assessment model do not directly or indirectly involve in these two biological activities. A more comprehensive studies on NPC to develop a multigene NPC prognosis model that contain more prognostic biomarkers will enable generation of a more complex NPC pathway network model. Both of these models can incorporate more prognostic biomarkers, considering all the six hallmarks of cancer. By doing so, the multigene NPC prognosis assessment model and the NPC pathway network model would be more reliable and significant in the context of NPC prognosis. The general flow of this study is summarized in Figure 6.1.

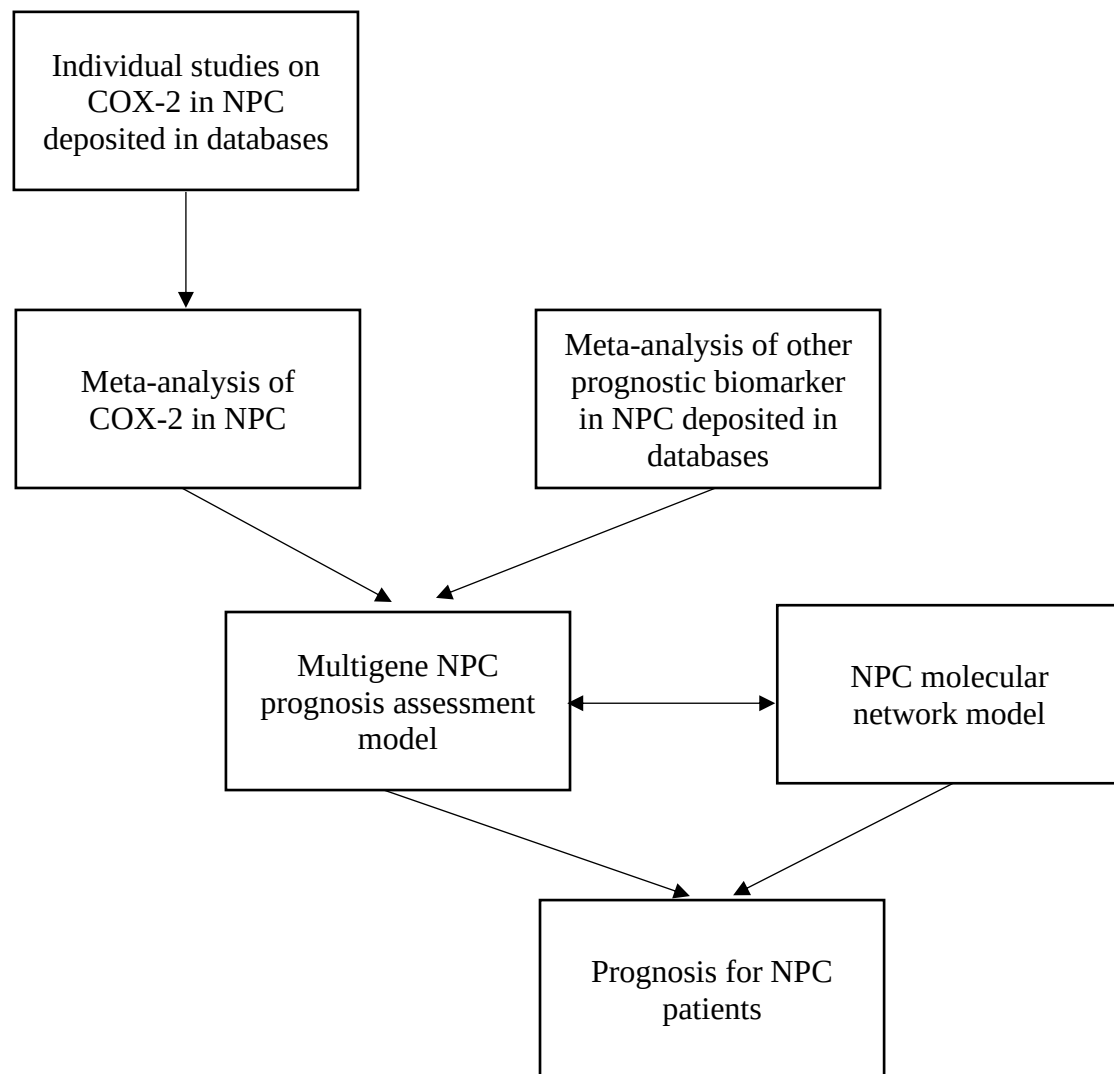


**Figure 6.1:** The overall flow diagram of NPC prognosis study.

## **CHAPTER 7**

### **CONCLUSION AND FUTURE RECOMMENDATIONS**

#### **7.1 Conclusion**

This study revealed the correlation of low survival rate among NPC patients with high COX-2 expression. COX-2 was computationally identified for the first time as a significant prognostic biomarker for NPC concerning overall survival outcome of NPC patients. This study also showed that there was no correlation between high COX-2 expression with treatment outcome of NPC patients (confidence intervals of odd ratio included one).

A multigene NPC prognosis assessment model was constructed with ten NPC prognostic biomarkers identified via modified PRISMA checklist. This model allowed prediction of overall survival with NPC prognostic assessment risk score of NPC patients corresponding to the over expression of different biomarkers in different NPC patients. This multigene NPC prognosis assessment model contained larger number of prognostic biomarkers compared to other existing models.

A molecular pathway network was generated to explain the physiological interaction of the ten prognostic biomarkers incorporated in the multigene NPC prognosis assessment model. Pathway involved by the over expressed biomarker could be identified from this network and therefore suitable treatments could be chosen and applied to different NPC patients with different biomarker expression patterns. This could hence, increase the overall survival of NPC patients.

## **7.2 Future recommendations**

Development of standard protocol for the evaluation of COX-2 expression could now be applied as a mean in the management and treatment for NPC patients. More clinical studies on COX-2 regarding treatment response could be done to determine its actual effect in NPC. The application of the multigene NPC prognosis assessment model should be tested on NPC patients. The data collected could be analysed and the patients could be stratified into different prognosis level corresponding to the different over-expressed biomarkers. This, in other word, is external validation of the multigene NPC prognosis assessment model. Both the prognosis assessment model and the molecular pathway network could be updated with any new relevant findings deposited in the databases. Cost is another consideration in term of application of both these models in medicals scenario. Research to design a cost-effective instrument/tool that can determine the expression of prognostic biomarkers time effectively is a possible future direction to further into.

## REFERENCES

- Ahmad, A., Robinson, A. R., Duensing, A., Drunen, E. V., Beverloo, H. B., Weisberg, D. B., ... Niedernhofer, L. J. (2008). ERCC1-XPF Endonuclease Facilitates DNA Double-Strand Break Repair. *Molecular and Cellular Biology*, 28(16), 5082-5092. <https://doi.org/10.1128/MCB.00293-08>
- Aitman, T. J., Boone, C., Churchill, G. A., Hengartner, M. O., Mackay, T. F., & Stemple, D. L. (2011). The Future of Model Organisms in Human Disease Research. *Nature Reviews Genetics*, 12(8), 575-582. <https://doi.org/10.1038/nrg3047>
- Al-sarraf, M., Leblanc, M., Giri, P. G., Fu, K. K., Cooper, J., Vuong, T., ... Ensley, J. F. (1998). Chemoradiotherapy Versus Radiotherapy in Patients with Advanced Nasopharyngeal Cancer: Phase III Randomized Intergroup Study 0099. *Journal of Clinical Oncology*, 16(4), 1310-1317. <http://doi: 10.1200/JCO.1998.16.4.1310>
- Albinger-hegyi, A., Stoeckli, S. J., Schmid, S., Storz, M., Iotzova, G., Probst-hensch, N. M., ... Hegyi, I. (2010). Lysyl Oxidase Expression is an Independent Marker of Prognosis and a Predictor of Lymph Node Metastasis in Oral and Oropharyngeal Squamous Cell Carcinoma (OSCC). *International Journal of Cancer*, 126(11), 2653-2662. <https://doi.org/10.1002/ijc.24948>
- Allin, K. H., & Nordestgaard, B. G. (2011). Elevated C-reactive Protein in the Diagnosis, Prognosis, and Cause of Cancer. *Critical Reviews in Clinical Laboratory Sciences*, 48(4), 155-170. <https://doi.org/10.3109/10408363.2011.599831>
- Angulo, J. C., Ferruelo, A., Nunez, C., Fata, F. R., & Gonzalez, J. (2011). Detection and Molecular Staging of Bladder Cancer Using Real-time RT-PCR for Gelatinases (MMP-2, MMP-9) and TIMP-2 in Peripheral Blood. *Actas Urologicas Espanolas (English Edition)*, 35(3), 127-136. [https://doi.org/10.1016/S2173-5786\(11\)70035-X](https://doi.org/10.1016/S2173-5786(11)70035-X)

- Armstrong, A. J., George, D. J., & Halabi, S. (2012). Serum Lactate Dehydrogenase Predicts for Overall Survival Benefit in Patients with Metastatic Renal Cell Carcinoma Treated with Inhibition of Mammalian Target of Rapamycin. *Journal of Clinical Oncology*, 30(27), 3402-3407. <https://doi.org/10.1200/JCO.2011.40.9631>
- Armstrong, R. W., Armstrong, M. J., Yu, M. C., & Henderson, B. E. (1983). Salted Fish and Inhalants as Risk Factors for Nasopharyngeal Carcinoma in Malaysian Chinese. *Cancer Research*, 43(6), 2967-2970.
- Armstrong, R. W., Imrey, P. B., Lye, M. S., Armstrong, M. J., Yu, M. C., & Sani, S. (1998). Nasopharyngeal Carcinoma in Malaysian Chinese: Salted Fish and Other Dietary Exposures. *International Journal of Cancer*, 77(2), 228-235. [https://doi.org/https://doi.org/10.1002/\(SICI\)1097-0215\(19980717\)77:2<228::AID-IJC11>3.0.CO;2-7](https://doi.org/https://doi.org/10.1002/(SICI)1097-0215(19980717)77:2<228::AID-IJC11>3.0.CO;2-7)
- Armstrong, R. W., Imrey, P. B., Lye, M. S., Armstrong, M. J., Yu, M. C., & Sani, S. (2000). Nasopharyngeal Carcinoma in Malaysian Chinese: Occupational Exposures to Particles, Formaldehyde and Heat. *International Journal of Epidemiology*, 29(6), 991-998. <http://doi:10.1093/ije/29.6.991>
- Arora, S., Kothandapani, A., Tillison, K., Kalman-maltese, V., & Patrick, S. M. (2010). Downregulation of XPF-ERCC1 Enhances Cisplatin Efficacy in Cancer Cells. *DNA Repair*, 9(7), 745-753. <https://doi.org/10.1016/j.dnarep.2010.03.010>
- Assmus, H. E., Herwig, R., Cho, K. H., & Wolkenhauer, O. (2006). Dynamics of Biological Systems: Role of Systems Biology in Medical Research. *Expert Review of Molecular Diagnostics*, 6(6), 891-902. <http://doi:10.1586/14737159.6.6.891>
- Azizah, Ab. M., Nor-Saleha, I. T., Noor-Hashimah, A., Asmah, Z. A., & Mastulu, W. (2015). Malaysian National Cancer Registry Report 2007-2011.

- Azuma, K., Komohara, Y., Sasada, T., Terazaki, Y., Ikeda, J., Hoshino, T., ... Aizawa, H. (2007). Excision Repair Cross-complementation Group 1 Predicts Progression-free and Overall Survival in Non-small Cell Lung Cancer Patients Treated with Platinum-based Chemotherapy. *Cancer Science*, 98(9), 1336-1343. <https://doi.org/10.1111/j.1349-7006.2007.00557.x>
- Bach, P. B., Kattan, M. W., Thornquist, M. D., Kris, M. G., Tate, R. C., Barnett, M. J., ... Begg, C. B. (2003). Variations in Lung Cancer Risk Among Smokers. *Journal of National Cancer Institute*, 95(6), 470-478.
- Bakhle, Y. S. (2001). COX-2 and Cancer: a New Approach to an Old Problem. *British Journal of Pharmacology*, 134(6), 1137-1150. <https://doi.org/10.1038/sj.bjp.0704365>
- Balkwill, F. R., & Mantovani, A. (2001). Inflammation and Cancer: Back to Virchow. *Lancet*, 357(9255), 539-545.
- Balkwill, F. R., & Mantovani, A. (2012). Cancer-related Inflammation: Common Themes and Therapeutic Opportunities. *Seminars in Cancer Biology*, 22(1), 33-40. <https://doi.org/10.1016/j.semcancer.2011.12.005>
- Baranzini, S. E. (2009). The Genetics of Autoimmune Diseases: a Networked Perspective. *Current Opinion in Immunology*, 21(6), 596-605. <https://doi.org/10.1016/j.coi.2009.09.014>
- Barnes, L., Eveson, J., Reichart, P., & Sidransky, D. (2005). *World Health Organization Classification of Tumors: Pathology and Genetics of Head and Neck Tumors*. Lyon: IARC Press.
- Bar-Sela, G., Kuten, A., Minkov, I., Gov-Ari, E., & Ben-Izhak, O. (2004). Prevalence and Relevance of EBV Latency in Nasopharyngeal Carcinoma in Israel. *Journal of Clinical Pathology*, 57(3), 290-293. <https://doi.org/10.1136/jcp.2003.013094>

- Bartsch, H., Ohshima, H., Pignatelli, B., & Calmels, S. (1992). Endogenously Formed N-nitroso Compounds and Nitrosating Agents in Human Cancer Etiology. *Pharmacogenetics*, 2(6):272-277.
- Baujat, B., Audry, H., Bourhis, J., Chan, A. T., Onat, H., Chua, D. T., ... Pignon, J. P. (2006). Chemotherapy in Locally Advanced Nasopharyngeal Carcinoma: an Individual Patient Data Meta-analysis of Eight Randomized Trials and 1753 Patients. *International Journal of Radiation Oncology Biology Physics*, 64(1), 47-56. <https://doi.org/10.1016/j.ijrobp.2005.06.037>
- Ben-batalla, I., Cubas-cordova, M., Udonta, F., Waizenegger, J. S., Janning, M., Sawall, S., ... Loges, S. (2015). Cyclooxygenase-2 Blockade can Improve Efficacy of VEGF-targeting Drugs. *Oncotarget*, 6(8), 6341-6358. [http://doi: 10.18632/oncotarget.3437](http://doi:10.18632/oncotarget.3437)
- Biomarkers Definitions Working Group. (2001). Biomarkers and Surrogate Endpoints: Preferred Definitions and Conceptual Framework. *Clinical Pharmacology and Therapeutics*, 69(3), 89-95. <https://doi.org/10.1067/mcp.2001.113989>
- Blagoev, B., Ong, S. E., Kratchmarova, I., & Mann, M. (2004). Temporal Analysis of Phosphotyrosine-dependent Signaling Networks by Quantitative Proteomics. *Nature Biotechnology*, 22(9), 1139-1145. <https://doi.org/10.1038/nbt1005>
- Blair, A., Saracci, R., Stewart, P. A., Hayes, R. B., & Shy, C. (1990). Epidemiologic Evidence on the Relationship Between Formaldehyde Exposure and Cancer. *Scandinavian Journal of Work, Environment & Health*, 16(6), 381-393. <https://doi.org/10.5271/sjweh.1767>
- Blair, A., Stewart, P., O'Berg, M., Gaffey, W., Walrath, J., Ward, J., ... Cubit, D. (1986). Mortality Among Industrial Workers Exposed to Formaldehyde. *Journal of The National Cancer Institute*, 76(6), 1071-1084. <http://doi:10.1093/jnci/76.6.1071>

- Blanchard, P., Lee, A., Marguet, S., Leclercq, J., Ng, W. T., Ma, J., ... Pignon, J. P. (2015). Chemotherapy and Radiotherapy in Nasopharyngeal Carcinoma: an Update of the MAC-NPC Meta-analysis. *The Lancet Oncology*, 16(6), 645-655. [http://doi:10.1016/s1470-2045\(15\)70126-9](http://doi:10.1016/s1470-2045(15)70126-9)
- Borenstein, M., Higgins, J. P. T., Hedges, L. V., & Rothstein, H. R. (2017). Basics of Meta-analysis: I2 is not an Absolute Measure of Heterogeneity. *Research Synthesis Methods*, 8(1), 5-18. <https://doi.org/10.1002/jrsm.1230>
- Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer Journal for Clinicians*, 68(6), 394-424. <http://doi: 10.3322/caac.21492>.
- Breda, E., Catarino, R. J. F., Azevedo, I., Lobao, M., Monteiro, E., & Medeiros, R. (2010). Epstein-Barr Virus Detection in Nasopharyngeal Carcinoma - Implications in a Low-risk Area. *Brazilian Journal of Otorhinolaryngology*, 76(3), 310-315. <https://doi.org/10.1590/S1808-86942010000300007>
- Brennan, B. (2006). Nasopharyngeal Carcinoma. *Orphanet Journal of Rare Diseases*, 1(23), 1-5. <https://doi.org/10.1186/1750-1172-1-23>
- Brooks, L., Yao, Q. Y., Rickinson, A. B., & Young, L. S. (1992). Epstein-Barr Virus Latent Gene Transcription in Nasopharyngeal Carcinoma Cells: Coexpression of EBNA1, LMP1, and LMP2 Transcripts. *Journal of Virology*, 66(5), 2689-2697.
- Brown, J. R., & DuBois, R. N. (2005). COX-2: a Molecular Target for Colorectal Cancer Prevention. *Journal of Clinical Oncology*, 23(12), 2840-2855. <https://doi.org/10.1200/JCO.2005.09.051>
- Brown, L. F., Detmar, M., Claffey, K., Nagy, J. A., Feng, D., Dvorak, A. M., & Dvorak, H.

- F. (1997). Vascular Permeability Factor/Vascular Endothelial Growth Factor: a Multifunctional Angiogenic Cytokine. *Experientia Supplementum*, 79, 233-269.
- Bunge, M. A. (1967). *Scientific Research*. Berlin, Germany: Springer.
- Chan, A. T. C. (2010). Nasopharyngeal Carcinoma. *Annals of Oncology*, 21(7), vii308-vii312. <https://doi.org/10.1093/annonc/mdf179>
- Chan, A. T. C., Teo, P. M. L., & Johnson, P. J. (2002). Nasopharyngeal Carcinoma. *Annals of Oncology*, 13(7), 1007-1015. <https://doi.org/10.1093/annonc/mdf179>
- Chan, A. T., Leung, S. F., Ngan, R. K., Teo, P. M., Lau, W. H., Kwan, W. H., ... Zee, B. C. (2005). Overall Survival After Concurrent Cisplatin-Radiotherapy Compared with Radiotherapy Alone in Locoregionally Advanced Nasopharyngeal Carcinoma. *Journal of the National Cancer Institute*, 97(7), 536-539. <https://doi.org/10.1093/jnci/dji084>
- Chan, A. W., Hrobjartsson, A., Haahr, M. T., Gøtzsche, P. C., & Altman, D. G. (2004). Empirical Evidence for Selective Reporting of Outcomes in Randomized Trials: Comparison of Protocols to Published Articles. *The Journal of the American Medical Association*, 291(20), 2457-2465. <http://doi: 10.1001/jama.291.20.2457>
- Chan, K. C., Woo, J. K., King, A., Zee, B. C., Lam, W. K., Chan, S. L., ... Hui, E. P. (2017). Analysis of Plasma Epstein-Barr Virus DNA to Screen for Nasopharyngeal Cancer. *The New England Journal of Medicine*, 377(6), 513-522. <https://doi.org/10.1056/NEJMoa1701717>
- Chang, E. T., & Adami, H. O. (2006). The Enigmatic Epidemiology of Nasopharyngeal Carcinoma. *Cancer Epidemiology Biomarkers and Prevention*, 15(10), 1765-1777. <https://doi.org/10.1158/1055-9965.EPI-06-0353>
- Chang, K. P., Hao, S. P., Tsang, N. M., & Ueng, S. H. (2004). Salvage Surgery for Locally Recurrent Nasopharyngeal Carcinoma: a 10-year Experience. *Otolaryngology Head and*

- Neck Surgery*, 131(4), 497-502. <https://doi.org/10.1016/j.otohns.2004.02.049>
- Charoenrat, P., Rhys-Evans, P. H., & Eccles, S. A. (2001). Expression of Matrix Metalloproteinases and Their Inhibitors Correlates with Invasion and Metastasis in Squamous Cell Carcinoma of the Head and Neck. *Arch Otolaryngol Head Neck Surgery*, 127(7), 813-820.
- Chen, C. J., Hsu, W. L., Yang, H. I., Lee, M. H., Chen, H. C., Chien, Y. C., & You, S. L. (2014). Epidemiology of Virus Infection and Human Cancer. *Recent Results Cancer Research*, 193, 11-32. <https://doi.org/10.1007/978-3-642-38965-8>
- Chen, C., Wang, F. H., Wang, Z. Q., An, X., Luo, H. Y., Zhang, L., ... Li, Y. H. (2012). Salvage Gemcitabine-vinorelbine Chemotherapy in Patients with Metastatic Nasopharyngeal Carcinoma Pretreated with Platinum-based Chemotherapy. *Oral Oncology*, 48(11), 1146-1151. <https://doi.org/10.1016/j.oraloncology.2012.05.021>
- Chen, J. Y., Ran, Y. G., Hong, C. Q., Chen, Z. J., & You, Y. J. (2010). Anti-cancer Effects of Celecoxib on Nasopharyngeal Carcinoma HNE-1 Cells Expressing COX-2 Oncoprotein. *Cytotechnology*, 62(5), 431-438. <https://doi.org/10.1007/s10616-010-9296-7>
- Chen, Y. P., Zhang, W. N., Chen, L., Tang, L. L., Mao, Y. P., Li, W. F., ... Ma, J. (2015). Effect of Latent Membrane Protein 1 Expression on Overall Survival in Epstein-Barr Virus-associated Cancers: a Literature-based Meta-analysis. *Oncotarget*, 6(30), 29311-29323. <http://doi:10.18632/oncotarget.4906>
- Cho, W. C. (2007). Nasopharyngeal Carcinoma: Molecular Biomarker Discovery and Progress. *Molecular Cancer*, 6, 1-9. <https://doi.org/10.1186/1476-4598-6-1>
- Chou, J., Lin, Y. C., Kim, J., You, L., Xu, Z. D., He, B., & Jablons, D. M. (2008). Nasopharyngeal Carcinoma - Reiew of the Molecular Mechanism of Tumorigenesis.

- Head Neck*, 30(7), 946-963. <https://doi.org/10.1002/hed.20833.NASOPHARYNGEAL>
- Chua, D. T., Nicholls, J. M., Sham, J. S., & Au, G. K. (2004). Prognostic Value of Epidermal Growth Factor Receptor Expression in Patients with Advanced Stage Nasopharyngeal Carcinoma Treated with Induction Chemotherapy and Radiotherapy. *International Journal of Radiation Oncology, Biology, Physics*, 59(1), 11-20. <https://doi.org/10.1016/j.ijrobp.2003.10.038>
- Chung, A. D., Lee, J., & Ferrara, N. (2010). Targeting the Tumour Vasculature: Insights from Physiological Angiogenesis. *Nature Review Cancer*, 10(7), 505-514.
- Cochran, W. G. (1954). The Combination of Estimates from Different Experiments. *Biometrics*, 10(1), 101-129. [http://doi: 10.2307/3001666](http://doi:10.2307/3001666)
- Coghill, A. E., Hsu, W. L., Pfeiffer, R. M., Juwana, H., Yu, K. J., Wang, C. P., ... Hildesheim, A. (2014). Epstein-Barr Virus Serology as a Potential Screening Marker for Nasopharyngeal Carcinoma Among High-risk Individuals from Multiplex Families in Taiwan. *Cancer Epidemiology Biomarkers and Prevention*, 23(7), 1213-1219. <https://doi.org/10.1158/1055-9965.EPI-13-1262.Epstein>
- Cohen, J. I. (2000). Epstein-Barr Virus Infection. *The New England Journal of Medicine*, 343, 481-492. [http://doi: 10.1056/NEJM200008173430707](http://doi:10.1056/NEJM200008173430707)
- Costa, C., Soares, R., Reis-Filho, J. S., Leitao, D., Amendoeira, I., & Schmitt, F. C. (2002). Cyclo-oxygenase 2 Expression is Associated with Angiogenesis and Lymph Node Metastasis in Human Breast Cancer. *Journal of Clinical Pathology*, 55(6), 429-434. <https://doi.org/10.1136/jcp.55.6.429>
- Cox, T. R., Bird, D., Baker, A. M., Barker, H. E., Ho, M. W., Lang, G., & Erler, J. T. (2013). LOX-Mediated Collagen Crosslinking is Responsible for Fibrosis-enhanced Metastasis. *Cancer Research*, 73(6), 1721-1732. <https://doi.org/10.1158/0008-5472.CAN-12->

2233.LOX-mediated

- Cox, T. R., & Erler, J. T. (2011). Remodeling and Homeostasis of the Extracellular Matrix: Implications for Fibrotic Diseases and Cancer. *Disease Models and Mechanism*, 4(3), 165-178. <https://doi.org/10.1242/dmm.004077>
- Curtin, N. J. (2012). DNA Repair Dysregulation from Cancer Driver to Therapeutic Target. *Nature Reviews Cancer*, 12(12), 801-817. <https://doi.org/10.1038/nrc3399>
- Danesi, G., Zanoletti, E., & Mazzoni, A. (2007). Salvage Surgery for Recurrent Nasopharyngeal Carcinoma. *Skull Base*, 17(3), 173-180. <https://doi.org/10.1055/s-2007-977470>.
- Dannenberg, A. J., Altorki, N. K., Boyle, J. O., Dang, C., Howe, L. R., Weksler, B. B., & Subbaramaiah, K. (2001). Cyclo-oxygenase 2: a Pharmacological Target for the Prevention of Cancer. *Lancet Oncology*, 2(9), 544-551. [https://doi.org/10.1016/S1470-2045\(01\)00488-0](https://doi.org/10.1016/S1470-2045(01)00488-0)
- Dannenberg, A. J., & Subbaramaiah, K. (2003). Targeting Cyclooxygenase-2 in Human Neoplasia: Rationale and Promise. *Cancer Cell*, 4(6), 431-436.
- Demers, P. A., Boffetta, P., Kogevinas, M., Blair, A., Miller, B. A., Robinson, C. F., ... Vainio, H. (1995). Pooled Reanalysis of Cancer Mortality Among Five Cohorts of Workers In Wood-related Industries. *Journal of Work, Environment & Health*, 21(3), 179-190. <https://doi.org/10.5271/sjweh.26>
- Deng, L., Yang, J., Zhao, X. R., Deng, X. Y., Zeng, L., Gu, H. H., ... Cao, Y. (2003). Cells in G2/M Phase Increased in Human Nasopharyngeal Carcinoma Cell Line by EBV-LMP1 Through Activation of NF- $\kappa$ B and AP-1. *Cell Research*, 13(3), 187-194. <http://doi: 10.1038/sj.cr.7290163>
- Department of Health and Human Services Food and Drug (2011). *Guidance for Industry:*

- Clinical Trial Endpoints for the Approval of Non-Small Cell Lung Cancer Drugs and Biologics*. Bethesda, United State. <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM259421.pdf>
- Dersimonian, R., & Laird, N. (1986). Meta-Analysis in Clinical Trials. *Controlled Clinical Trials*, 7(3), 177-188. [https://doi.org/10.1016/0197-2456\(86\)90046-2](https://doi.org/10.1016/0197-2456(86)90046-2)
- Deutsch, M., Mercado, R., & Parsons, J. A. (1978). Cancer of the Nasopharynx in Children. *Cancer*, 41(3), 1128-1133. [http://doi: 10.1002/1097-0142\(197803\)41:3%3C1128::aid-cncr2820410348%3E3.0.co;2-s](http://doi:10.1002/1097-0142(197803)41:3%3C1128::aid-cncr2820410348%3E3.0.co;2-s)
- Devi, B. C., Pisani, P., Tang, T. S., & Parkin, D. M. (2004). High Incidence of Nasopharyngeal Carcinoma in Native People of Sarawak, Borneo Island. *Cancer Epidemiology Biomarkers and Prevention*, 13(3), 482-486.
- Ding, L., Li, L. L., Yang, J., Tao, Y. G., Ye, M., Shi, Y., ... Cao, Y. (2005). Epstein-Barr Virus Encoded Latent Membrane Protein 1 Modulates Nuclear Translocation of Telomerase Reverse Transcriptase Protein by Activating Nuclear Factor-kappaB p65 in Human Nasopharyngeal Carcinoma Cells. *International Journal of Biochemistry and Cell Biology*, 37(9), 1881-1889. <https://doi.org/10.1016/j.biocel.2005.04.012>
- Dirix, P., Nuyts, S., & Van den Bogaert, W. (2006). Radiation-induced Xerostomia in Patients with Head and Neck Cancer: a Literature Review. *Cancer*, 107(11), 2525-2534. <https://doi.org/10.1002/cncr.22302>
- Durand, E. (1991). Keynote Address: the Influence of Microenvironmental Factors on the Activity of Radiation and Drugs. *International Journal of Radiation Oncology Biology Physics*, 20(2), 253-258.
- Dvorak, H. F., Brown, L. F., Detmar, M., & Dvorak, A. M. (1995). Vascular Permeability Factor/Vascular Endothelial Growth Factor, Microvascular Hyperpermeability and

- Angiogenesis. *American Journal of Pathology*, 146(5), 1029-1039.
- Dwan, K., Altman, D. G., Arnaiz, J. A., Bloom, J., Chan, A. W., Cronin, E., ... Williamson, P. R. (2008). Systematic Review of the Empirical Evidence of Study Publication Bias and Outcome Reporting Bias. *PLoS One*, 3(8), e3081-e3112. <https://doi.org/10.1371/journal.pone.0003081>
- Egger, M., Smith, G.D., Phillips, A.N. (1997). Meta-analysis: Principles and Procedures. *British Medical Journal*, 315, 1533-1537.
- Effert, P., McCoy, R., Abdel-Hamid, M., Flynn, K., Zhang, Q., Busson, P., ... Raab-Traub, N. (1992). Alterations of the p53 Gene in Nasopharyngeal Carcinoma. *Journal of Virology*, 66(6), 3768-3775.
- Erler, J. T., Bennewith, K. L., Nicolau, M., Dornhofer, N., Kong, C., Le, Q. T., ... Giaccia, A. J. (2006). Lysyl Oxidase is Essential for Hypoxia-induced Metastasis. *Nature*, 440(7088), 1222-1226. <https://doi.org/10.1038/nature04695>
- Fagerland, M. W. (2015). From Planning and Preparation to Grant Application and Publication. Research in the Medical and Biological Sciences (Second Edition). *Evidence-Based Medicine and Systematic Reviews* (pp431-461). Oslo, Norway: Elsevier Ltd. <https://doi.org/10.1016/B978-0-12-799943-2.00012-4>
- Fan, C., Tang, Y., Wang, J., Xiong, F., Guo, C., Wang, Y., ... Zeng, Z. (2018). The Emerging Role of Epstein-Barr virus Encoded microRNAs in Nasopharyngeal Carcinoma. *Journal of Cancer*, 9(16), 2852-2864. <http://doi:10.7150/jca.25460>
- Fandi, A., Altun, M., Azli, N., Armand, J.P., & Cvitkovic, E. (1994). Nasopharyngeal Cancer: Epidemiology, Staging, and Treatment. *Seminars in Oncology*, 21(3), 382-397.
- Fang, Y., Xu, C., Wu, P., Zhang, L. H., Li, D. W., Sun, J. H., ... Liao, Z. S. (2017). Prognostic Role of C-reactive Protein in Patients with Nasopharyngeal Carcinoma.

- Medicine*, 96(45), e8463-e8468. [http://doi: 10.1097/MD.00000000000008463](http://doi:10.1097/MD.00000000000008463)
- Fantin, V. R., St-Pierre, J., & Leder, P. (2006). Attenuation of LDH-A Expression Uncovers a Link Between Glycolysis, Mitochondrial Physiology and Tumor Maintenance. *Cancer Cell*, 9(6), 425-434. <http://doi:10.1016/j.ccr.2006.04.023>
- Farias, T. P., Dias, F. L., Lima, R. A., Klingerman, J., de Sa, G. M., Barbosa, M. M., & Goncalves, F. B. (2003). Prognostic Factors and Outcome for Nasopharyngeal Carcinoma. *Arch Otolaryngol Head Neck Surgery*, 129(7), 794-799. [http://doi: 10.1001/archotol.129.7.794](http://doi:10.1001/archotol.129.7.794)
- Fee, W. E., Gilmer, P. A., & Goffinet, D. R. (1988). Surgical Management of Recurrent Nasopharyngeal Carcinoma After Radiation Failure at the Primary Site. *Laryngoscope*, 98(11), 1220-1226. [http://doi: 10.1288/00005537-198811000-00013](http://doi:10.1288/00005537-198811000-00013)
- Fee, W. E., Roberson, J. B., & Goffinet, D. R. (1991). Long-term Survival After Surgical Resection for Recurrent Nasopharyngeal Cancer After Radiotherapy Failure. *Arch Otolaryngol Head Neck Surgery*, 117(11), 1233-1236. <http://doi:10.1001/archotol.1991.01870230049006>
- Fendri, A., Khabir, A., Hadhri-Guiga, B., Sellami-Boudawara, T., Ghorbel, A., Daoud, J., ... Mokdad-Gargouri, R. (2007). Overexpression of COX-2 and LMP1 are Correlated with Lymph Node in Tunisian NPC Patients. *Oral Oncology*, 44(7), 710-715. <https://doi.org/10.1016/j.oraloncology.2007.09.006>
- Fendri, A., Khabir, A., Mnejja, W., Sellami-Boudawara, T., Daoud, J., Frikha, M., ... Mokdad-Gargouri, R. (2009). PIK3CA Amplification is Predictive of Poor Prognosis in Tunisian Patients with Nasopharyngeal Carcinoma. *Cancer Science*, 100(11), 2034-2039. <https://doi.org/10.1111/j.1349-7006.2009.01292.x>
- Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C., & Parkin, D. M. (2010). Estimates

- of Worldwide Burden of Cancer in 2008: GLOBOCAN 2008. *International Journal of Cancer*, 127(12), 2893-2917. <https://doi.org/10.1002/ijc.25516>
- Feron, V. J., Arts, J. H., Kuper, C. F., Slootweg, P. J., & Wouterson, R. A. (2001). Health Risks Associated with Inhaled Nasal Toxicants. *Critical Reviews in Toxicology*, 31(3), 313-347.
- Ferrara, N., Gerber, H.P., & LeCouter, J. (2003). The Biology of VEGF and Its Receptors. *Nature Medicine*, 9(6), 669-676.
- Fidler, V., & Nagelkerke, N. (2013). The Mantel-Haenszel Procedure Revisited: Models and Generalizations. *PLoS One*, 8(3), 1-4. <https://doi.org/10.1371/journal.pone.0058327>
- Fridman, R., Toth, M., Chvyrkova, I., Meroueh, S. O., Mobashery, S. (2003). Cell Surface Association of Matrix Metalloproteinase-9 (Gelatinase B). *Cancer Metastasis Review*, 22(2-3), 153-166.
- Firth, J. D., Ebert, B. L., & Ratcliffe, P. J. (1995). Hypoxic Regulation of Lactate Dehydrogenase A. Interaction between Hypoxia-inducible Factor 1 and cAMP Response Elements. *The Journal of Biological Chemistry*, 270(36), 21021-21027. <http://doi: 10.1074/jbc.270.36.21021>
- Forsythe, J. A., Jiang, B. H., Iyer, N. V., Agani, F., Leung, S. W., Koos, R. D., & Semenza, G. L. (1996). Activation of Vascular Endothelial Growth Factor Gene Transcription by Hypoxia-inducible Factor 1. *Molecular and Cellular Biology*, 16(9), 4604-4613. <http://doi: 10.1128/mcb.16.9.4604>
- Fu, Y., Zheng, S., Zheng, Y., Huang, R., An, N., Liang, A., & Hu, C. (2012). Glioma Derived Isocitrate Dehydrogenase-2 Mutations Induced Up-regulation of HIF-1 $\alpha$  and  $\beta$ -catenin Signaling: Possible Impact on Glioma Cell Metastasis and Chemo-resistance. *International Journal of Biochemistry and Cell Biology*, 44(5), 770-775.

<https://doi.org/10.1016/j.biocel.2012.01.017>

- Gail, M. H., & Benichou, J. (1994). Validation Studies on a Model for Breast Cancer Risk. *Journal of the National Cancer Institute*, 86(8), 573-575.
- Gail, M. H., Brinton, L. A., Byar, D. P., Corle, D. K., Green, S. B., Schairer, C., & Mulvihill, J. J. (1989). Projecting Individualized Probabilities of Developing Breast Cancer for White Females Who are Being Examined Annually. *Journal of the National Cancer Institute*, 81(24), 1879-1886.
- Geara, F. B., Sanguineti, G., Tucker, S. L., Garden, A. S., Ang, K. K., Morrison, W. H., & Peters, L. J. (1997). Carcinoma of the Nasopharynx Treated by Radiotherapy Alone: Determinants of Distant Metastasis and Survival. *Radiotherapy and Oncology*, 43(1), 53-61. [https://doi.org/10.1016/S0167-8140\(97\)01914-2](https://doi.org/10.1016/S0167-8140(97)01914-2)
- Georgieva, A. V., Kimbell, J. S., & Schlosser, P. M. (2003). A Distributed-parameter Model for Formaldehyde Uptake and Disposition in the Rat Nasal Lining. *Inhalation Toxicology*, 15(14), 1435-1463. <http://doi: 10.1080/08958370390249085>
- Glass, G. V. (1976). Primary, Secondary and Meta-Analysis of Research. *Educational Researcher*, 5(10), 3-8. <http://doi: 10.2307/1174772>
- Gossage, L., & Madhusudan, S. (2007). Current Status of Excision Repair Cross Complementing-group 1 (ERCC1) in Cancer. *Cancer Treatment Reviews*, 33(6), 565-577. <https://doi.org/10.1016/j.ctrv.2007.07.001>
- Greenhough, A., Smartt, H. J., Moore, A. E., Roberts, H. R., Williams, A. C., Paraskeva, C., & Kaidi, A. (2009). The COX-2/PGE2 Pathway: Key Roles in the Hallmarks of Cancer and Adaptation to the Tumour Microenvironment. *Carcinogenesis*, 30(3), 377-386. <https://doi.org/10.1093/carcin/bgp014>
- Grivennikov, S. I., Greten, F. R., & Karin, M. (2010). Immunity, Inflammation and Cancer.

- Cell*, 140(6), 883-899. <https://doi.org/10.1016/j.cell.2010.01.025>.Immunity
- Guigay, J. (2008). Advances in Nasopharyngeal Carcinoma. *Current Opinion in Oncology*, 20(3), 264-269. <http://doi: 10.1097/CCO.0b013e3282fad846>.
- Guo, L., Liu, S., Zhang, S., Chen, Q., Zhang, M., Quan, P., ... Su, X. (2015). C-reactive Protein and Risk of Breast Cancer: A Systematic Review and Meta-analysis. *Scientific Report*, 5, 10508-10516. <https://doi.org/10.1038/srep10508>
- Gupta, G. P., Nguyen, D. X., Chiang, A. C., Bos, P. D., Kim, J. Y., Nadal, C., ... Massague, J. (2007). Mediators of Vascular Remodelling Co-opted for Sequential Steps in Lung Metastasis. *Nature*, 446(7137), 765-770. <https://doi.org/10.1038/nature05760>
- Guyatt, G. H., Sackett, D. L., Sinclair, J. C., Hayward, R., Cook, D. J., & Cook, R. J. (1995). Users' Guides to the Medical Literature. IX: a Method for Grading Health Care Recommendations. Evidence-Based Medicine Working Group. *The Journal of the American Medical Association*, 274(22), 1800-1804. <http://doi: 10.1001/jama.274.22.1800>
- Haidich, A. B. (2010). Meta-analysis in Medical Research. *Hippokratia*, 14(Supplement 1), 29-37.
- Han, L., Lin, S. J., Pan, J. J., Chen, C. B., Zhang, Y., Zhang, X. C., ... Chen, Q. S. (2010). Prognostic Factors of 305 Nasopharyngeal Carcinoma Patients Treated with Intensity-modulated Radiotherapy. *China Journal of Cancer*, 29(2), 145-150.
- Hanahan, D. & Weinberg, R. A. (2000). The Hallmarks of Cancer. *Cell*, 100(1), 57-70.
- Handra-luca, A., Hernandez, J., Mountzios, G., Taranchon, E., Lacau-st-guily, J., Soria, J. C., & Fouret, P. (2007). Excision Repair Cross Complementation Group1 Immunohistochemical Expression Predicts Objective Response and Cancer-Specific Survival in Patients Treated by Cisplatin-Based Induction Chemotherapy for Locally

- Advanced Head and Neck Squamous Cell Carcinoma. *Clinical Cancer Research*, 13(13), 3855-3860. <https://doi.org/10.1158/1078-0432.CCR-07-0252>
- Hao, S. P., Tsang, N. M., Chang, K. P., Hsu, Y. S., Chen, C. K., & Fang, K. H. (2008). Nasopharyngectomy for Recurrent Nasopharyngeal Carcinoma: a Review of 53 Patients and Prognostic Factors. *Acta Otolaryngologica*, 128(4), 473-481. <https://doi.org/10.1080/00016480701813806>
- Hardy, R. J., & Thompson, S. G. (1998). Detecting and Describing Heterogeneity in Meta-analysis. *Statistics in Medicine*, 17(8), 841-856.
- He, H. L., Lee, Y. E., Liang, P. I., Lee, S. W., Chen, T. J., Chan, T. C., ... Li, C. F. (2016). Overexpression of JAK2: a Predictor of Unfavorable Prognosis for Nasopharyngeal Carcinoma. *Future Oncology*, 12(16), 1887-1896. <https://doi.org/10.2217/fon-2016-0025>
- Higgins, J. P. T., & Thompson, S. G. (2002). Quantifying Heterogeneity in a Meta-analysis. *Statistics in Medicine*, 21(11), 1539-1558. <https://doi.org/10.1002/sim.1186>
- Higgins, J. P. T., Thompson, S. G., Deeks, J. J., & Altman, D. G. (2003). Measuring Inconsistency in Meta-analyses. *British Medical Journal*, 327(7414), 557-560. <http://doi:10.1136/bmj.327.7414.557>
- Hildesheim, A., & Levine, P. H. (1993). Etiology of Nasopharyngeal Carcinoma: a Review. *Epidemiologic Reviews*, 15(2), 466-485. <http://doi:10.1093/oxfordjournals.epirev.a036130>
- Himelstein, B. P., Canete, S. R., Bernhard, E. J., Dilks, D. W., & Muschel, R. J. (1994). Metalloproteinase in Tumor Progression: the Contribution of MMP-9. *Invasion Metastasis*, 14(1-6), 246-258.
- Ho, J. H. C. (1971). *Genetic and Environmental Factors in Nasopharyngeal Carcinoma*, in

- Recent Advances in Human Tumor Virology and Immunology*. Tokyo, Japan: University of Tokyo Press.
- Hong, B., Lui, V. W., Hui, E. P., Ng, M. H., Cheng, S. H., Sung, F. L., ... Chan, A. T. (2011). Hypoxia-targeting by Tirapazamine (TPZ) Induces Preferential Growth Inhibition of Nasopharyngeal Carcinoma Cells with Chk1/2 Activation. *Investigational New Drugs*, 29(3), 401-410. <https://doi.org/10.1007/s10637-009-9356-z>
- Hong M. H., & Guo, X. (2003). Nasopharyngeal Carcinoma. *Chinese Medical Science and Technology Press*, 11, 115-116.
- Horikawa, T., Yoshizaki, T., Sheen, T. S., Lee, S. Y., & Furukawa, M. (2000). Association of Latent Membrane Protein 1 and Matrix Metalloproteinase 9 with Metastasis in Nasopharyngeal Carcinoma. *Cancer*, 89(4), 715-723. [http://doi:10.1002/1097-0142\(20000815\)89:4%3C715::aid-cnrcr1%3E3.0.co;2-9](http://doi:10.1002/1097-0142(20000815)89:4%3C715::aid-cnrcr1%3E3.0.co;2-9)
- Hsu, M. M., Ko, J. Y., Sheen, T. S., & Chang, Y. L. (1997). Salvage Surgery for Recurrent Nasopharyngeal Carcinoma. *Arch Otolaryngol Head Neck Surgery*, 123(3), 305-309. <http://doi:10.1001/archotol.1997.01900030087011>
- Hu, Y., Liu, J., & Huang, H. (2013). Recent Agents Targeting HIF-1 a for Cancer Therapy. *Journal of Cellular Biochemistry*, 114(3), 498-509. <https://doi.org/10.1002/jcb.24390>
- Hu, Z., Yang, Y., Zhao, Y., & Huang, Y. (2017). The Prognostic Value of Cyclooxygenase-2 Expression in Patients with Esophageal Cancer: Evidence from a Meta-analysis. *Onco Targets and Therapy*, 10, 2893-2901. <https://doi.org/10.2147/OTT.S134599>
- Huang, M. L., Chen, Q., Xiao, J. P., Liu, C. H., & Zhao, X. M. (2013). Prognostic Significance of Cyclooxygenase-2 in Cervical Cancer: a Meta-analysis. *International Journal of Cancer*, 132(2), 363-373. <https://doi.org/10.1002/ijc.27686>
- Huang, P. Y., Li, Y., Mai, H. Q., Luo, R. Z., Cai, Y. C., & Zhang, L. (2012). Expression of

- ERCC1 Predicts Clinical Outcome in Locoregionally Advanced Nasopharyngeal Carcinoma Treated with Cisplatin-based Induction Chemotherapy. *Oral Oncology*, 48(10), 964-968. <https://doi.org/10.1016/j.oraloncology.2012.04.003>
- Huang, T. L., Li, C. F., Huang, H. Y., & Fang, F. M. (2010). Correlations Between Expression of Epidermal Growth Factor Receptor (EGFR), Phosphorylated EGFR, Cyclooxygenase-2 and Clinicopathological Variables and Treatment Outcomes in Nasopharyngeal Carcinomas. *Chang Gung Medical Journal*, 33(6), 619-627.
- Huang, Z., Du, M. Y., Zhu, H., Zhang, N., Lu, Z. W., Qian, L. X., ... Yin, L. (2018). MiRNA-34a Reversed TGF-B-Induced Epithelial-mesenchymal Transition via Suppression of SMAD4 in NPC Cells. *Biomedicine & Pharmacotherapy*, 106, 217-224. <https://doi.org/10.1016/j.biopha.2018.06.115>
- Huang, Z. L., Liu, S., Wang, G. N., Zheng, S. H., Ding, S. R., Tao, Y. L., ... Xia, Y. F. (2019). The Prognostic Significance of PD-L1 and PD-1 Expression in Patients with Nasopharyngeal Carcinoma: a Systematic Review and Meta-analysis. *Cancer Cell International*, 19(141), 1-12. <https://doi.org/10.1186/s12935-019-0863-5>
- Huedo-Medina, T. B., Sanchez-Meca, J., Marin-Martinez, F., & Botella, J. (2006). Assessing Heterogeneity in Meta-Analysis: Q Statistic or I2 Index? *Psychological Methods*, 11(2), 193-206. <https://doi.org/10.1037/1082-989X.11.2.193>
- Huen, D. S., Henderson, S. A., Carter, D. C., & Rowe, M. (1995). The Epstein-Barr Virus Latent Membrane Protein-1 (LMP1) Mediates Activation of NF-kappa B and Cell Surface Phenotype via Two Effector Regions in Its Carboxy-terminal Cytoplasmic Domain. *Oncogene*, 10(13), 549-560.
- Hughes, P. J., Scott, P. M. J., Kew, J., Cheung, D. M. C., Leung, S. F., Ahuja, A. T., & Van Hasselt, C. A. (2000). Dysphagia in Treated Nasopharyngeal Cancer. *Head and Neck*,

- 22(4), 393-397. [https://doi.org/10.1002/1097-0347\(200007\)22:4<393::AID-HED13>3.0.CO;2-2](https://doi.org/10.1002/1097-0347(200007)22:4<393::AID-HED13>3.0.CO;2-2)
- Hui, E. P., Chan, A. T., Pezzella, F., Turley, H., To, K. F., Poon, T. C., ... Harris, A. L. (2002). Coexpression of Hypoxia-inducible Factors 1 Alpha and 2 Alpha, Carbonic Anhydrase IX and Vascular Endothelial Growth Factor in Nasopharyngeal Carcinoma and Relationship to Survival. *Clinical Cancer Research*, 8(8), 2595-2604.
- Hui, E. P., Sung, F. L., Yu, B. K., Wong, C. S., Ma, B. B., Lin, X. R., ... Chan, A. T. (2008). Cancer Therapy: Clinical Plasma Osteopontin, Hypoxia and Response to Radiotherapy in Nasopharyngeal Cancer. *Clinical Cancer Research*, 14(21), 7080-7088. <https://doi.org/10.1158/1078-0432.CCR-08-0364>
- IARC (1995). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol 62. *Wood Dust and Formaldehyde*. Lyon: International Agency for Research on Cancer.
- IARC (2018). *Malaysia - The Global Cancer Observatory (Globocon)*. Lyon: International Agency for Research on Cancer. <http://gco.iarc.fr/today/data/factsheets/populations/935-asia-fact-sheets.pdf>
- Jana, D., Sarkar, D. K., Ganguly, S., Saha, S., Sa, G., Manna, A. K., ... Mandal, S. (2014). Role of Cyclooxygenase 2 (COX-2) in Prognosis of Breast Cancer. *Indian Journal of Surgical Oncology*, 5(1), 59-65. <http://doi:10.1007/s13193-014-0290-y>
- Jeannel, D., Hubert, A., De Vathaire, F., Ellouz, R., Camoun, M., Ben Salem, M., ... De-The, G. (1990). Diet, Living Conditions and Nasopharyngeal Carcinoma in Tunisia - a Case-control Study. *International Journal of Cancer*, 46(3), 421-425. <http://doi.org/10.1002/ijc.2910460316>
- Jenkin, R. D., Anderson, J. R., Jereb, B., Thompson, J. C., Pyesmany, A., Wara, W. M., & Hammond, D. (1981). Nasopharyngeal Carcinoma - a Retrospective Review of Patients

- Less Than Thirty Years of Age: a Report of Children's Cancer Study Group. *Cancer*, 47(2), 360-366.
- Jeyakumar A, Brickman TM, & Doerr T. (2006). Review of Nasopharyngeal Carcinoma. *Ear Nose Throat Journal*, 85(3): 168-184.
- Ji, R. C. (2014). Hypoxia and Lymphangiogenesis in Tumor Microenvironment and Metastasis. *Cancer Letters*, 346(1), 6-16. <https://doi.org/10.1016/j.canlet.2013.12.001>
- Jia, W. H., Luo, X. Y., Feng, B. J., Ruan, H. L., Bei, J. X., Liu, W. S., ... Zeng, Y. X. (2010). Traditional Cantonese Diet and Nasopharyngeal Carcinoma Risk: a Large-scale Case-control Study in Guangdong, China. *Bio Med Central Cancer*, 10, 446-452. <http://doi:10.1186/1471-2407-10-446>
- Jiang, X., Feng, L. C., Dai, B. Q., Li, L. P., & Lu, W. W. (2017). Identification of Key Genes Involved in Nasopharyngeal Carcinoma. *Brazilian Journal of Otorhinolaryngology*, 83(6), 670-676. <https://doi.org/10.1016/j.bjorl.2016.09.003>
- Jing, X., Huang, C., Zhou, H., Li, C., Fan, L., Chen, J., ... Qi, D. (2015). Association Between Serum C-reactive Protein Value and Prognosis of Patients with Non-small Cell Lung Cancer: a Meta-analysis. *International Journal of Clinical and Experimental Medicine*, 8(7), 10633-10639.
- Johansen, M. & Thomsen, S. F. (2016). Guidelines for Reporting Medical Research: a Critical Appraisal. *International Scholarly Research Notices*, 1-7. <https://doi.org/10.1155/2016/1346026>
- Juuti, A., Louhimo, J., Nordling, S., Ristimäki, A., & Haglund, C. (2006). Cyclooxygenase-2 expression correlates with poor prognosis in pancreatic cancer. *Journal of Clinical Pathology*, 59(4), 382-386. <http://doi:10.1136/jcp.2005.026831>
- Kagan, H. M., & Li, W. (2003). Lysyl Oxidase: Properties, Specificity and Biological Roles

- Inside and Outside of the Cell. *Journal of Cellular Biochemistry*, 88(4), 660-672.  
<https://doi.org/10.1002/jcb.10413>
- Kagan, H. M., & Trackman, P. C. (1991). Properties and Function of Lysyl Oxidase. *American Journal of Respiratory Cell and Molecular Biology*, 5(3), 206-210. <http://doi:10.1165/ajrcmb/5.3.206>
- Karademir, S., Sökmen, S., Terzi, C., Sağol, Ö., Özer, E., Astarcioglu, İ., ... Astarcioglu, I. (2000). Tumor Angiogenesis as a Prognostic Predictor in Pancreatic Cancer. *Journal of Hepato-Biliary-Pancreatic Surgery*, 7(5), 489-495.  
<https://doi.org/10.1007/s005340070020>
- Khanna, R., Busson, P., Burrows, S. R., Raffoux, C., Moss, D. J., Nicholls, J. M., & Cooper, L. (1998). Molecular Characterization of Antigen-processing Function in Nasopharyngeal Virus Carcinoma (NPC): Evidence for Efficient Presentation of Epstein-Barr Cytotoxic T-Cell Epitopes by NPC Cells' LCL T2 C15 C17 C18. *Cancer Research*, 58(2), 310-314.
- Kim, J. W., & Dang, C. V. (2006). Cancer's Molecular Sweet Tooth and the Warburg Effect. *Cancer Research*, 66(18), 8927-8931. <https://doi.org/10.1158/0008-5472.CAN-06-1501>
- Kim, K., Wu, H. G., Park, S. W., Kim, C. J., & Park, C. I. (2004). Expression of Cyclooxygenase (COX)-2 as a Prognostic Factor in Nasopharyngeal Cancer. *Cancer Research and Treatment*, 36(3), 187-191. <https://doi.org/10.4143/crt.2004.36.3.187>
- Kim, K. Y., Li, S. J., Cha, J. D., Zhang, X. L., & Cha, I. H. (2012). Significance of Molecular Markers in Survival Prediction of Oral Squamous Cell Carcinoma. *Head Neck*, 34(7), 929-936.
- Kim, S. H., Kwon, H. C., Oh, S. Y., Lee, D. M., Lee, S., Lee, J. H., ... Kim, H. J. (2009).

- Prognostic Value of ERCC1, Thymidylate Synthase, and Glutathione S-transferase pi for 5-FU/oxaliplatin Chemotherapy in Advanced Colorectal Cancer. *American Journal of Clinical Oncology*, 32(1), 38-41. <https://doi.org/10.1097/COC.0b013e31817be58e>
- Kim, T. J., Lee, Y. S., Kang, J. H., Kim, Y. S., & Kang, C. S. (2011). Prognostic Significance of Expression of VEGF and COX-2 in Nasopharyngeal Carcinoma and Its Association with Expression of C-erbB2 and EGFR. *Journal of Surgical Oncology*, 103(1), 46-52. <http://doi:10.1002/jso.21767>
- Kim, Y. J., Go, H., Wu, H. G., Jeon, Y. K., Park, S. W., & Lee, S. H. (2010). Immunohistochemical Study Identifying Prognostic Biomolecular Markers in Nasopharyngeal Carcinoma Treated by Radiotherapy. *Head & Neck*, 33(10), 1458-1466. <http://doi:10.1002/hed.21611>
- Kirschner, K., & Melton, D. W. (2010). Multiple Roles of the ERCC1-XPF Endonuclease in DNA Repair and Resistance to Anticancer Drugs. *Anticancer Research*, 30(9), 3223-3232.
- Kitagawa, N., Kondo, S., Wakisaka, N., Zen, Y., Nakanishi, Y., Tsuji, A., ... Yoshizaki, T. (2013). Expression of Seven-in-absentia Homologue 1 and Hypoxia-inducible Factor 1 Alpha: Novel Prognostic Factors of Nasopharyngeal Carcinoma. *Cancer Letters*, 331(1), 52-57. <https://doi.org/10.1016/j.canlet.2012.12.002>
- Klein, G. (2002). Nasopharyngeal carcinoma (NPC) is an Enigmatic Tumor. *Seminar in Cancer Biology*, 12(6), 415-418. <https://doi.org/10.1016/S1044>
- Klein, R. G., Schmezer, P., Amelung, F., Schroeder, H. G., Woeste, W., & Wolf, J. (2001). Carcinogenicity Assays of Wood Dust and Wood Additives in Rats Exposed by Long-term Inhalation. *International Archives of Occupational and Environmental Health*, 74(2), 109-118.

- Krishna, S. M., James, S., Kattoor, J., & Balaram, P. (2004). Serum EBV DNA as a Biomarker in Primary Nasopharyngeal Carcinoma of Indian Origin. *Japanese Journal of Clinical Oncology*, 34(6), 307-311. <http://doi: 10.1093/jjco/hyh055>
- Kwon, S. C., Kim, I., Song, J., & Park, J. (2018). Does Formaldehyde have a Causal Association with Nasopharyngeal Cancer and Leukaemia. *Annals of Occupational and Environmental Medicine*, 30(5), 1-13. <http://doi:10.1186/s40557-018-0218-z>
- Lang, J., Gao, L., Guo, Y., Zhao, C., & Zhang, C. (2014). Comprehensive Treatment of Squamous Cell Cancer of Head and Neck: Chinese Expert Consensus 2013. *Future Oncology*, 10(9), 1635-1648. <https://doi.org/10.2217/fon.14.44>
- Lapointe, J., Li, C., Higgins, J. P., VanDeRijn, M., Bair, E., Montgomery, K., ... Pollack, J. R. (2004). Gene Expression Profiling Identifies Clinically Relevant Subtypes of Prostate Cancer. *Proceedings of the National Academy of Sciences*, 101(3), 811-816. <http://doi: 10.1073/pnas.0304146101>
- Lee, A. W. M., Sham, J. S. T., Poon, Y. F., & Ho, J. H. C. (1989). Treatment of Stage I Nasopharyngeal Carcinoma: Analysis of the Patterns of Relapse and the Results of Withholding Elective Neck Irradiation. *International Journal of Radiation Oncology Biology Physics*, 17(6), 1183-1190.
- Lee, N., Laufer, M., Ove, R., Foote, R. L., & Bonner, J. A. (2012). Nasopharyngeal Carcinoma. *In Clinical Radiation Oncology*, 3, 619-638. <http://doi.org/10.1016/B978-1-4377-1637-5.00032-8>
- Leibel, S. A., Scott, C. B., Mohiuddin, M., Marcial, V. A., Coia, L. R., Davis, L. W., & Fuks, Z. (1991). The Effect of Local-regional Control on Distant Metastatic Dissemination in Carcinoma of the Head and Neck: Results of an Analysis from the RTOG Head and Neck Database. *International Journal of Radiation Oncology Biology Physics*, 21(3),

- 549-556. [http://doi: 10.1016/0360-3016\(91\)90669-u](http://doi:10.1016/0360-3016(91)90669-u)
- Leong, J. L., Loh, K. S., Putti, T. C., Goh, B. C., & Tan, L. K. (2004). Epidermal Growth Factor Receptor in Undifferentiated Carcinoma of the Nasopharynx. *Laryngoscope*, 114(1), 153-157. [http://doi: 10.1097/00005537-200401000-00029](http://doi:10.1097/00005537-200401000-00029)
- Li, C. C., Yu, M. C., & Henderson, B. E. (1985). Some Epidemiologic Observations of Nasopharyngeal Carcinoma in Guangdong, People's Republic of China. *National Cancer Institute Monograph*, 69, 49-52.
- Li, J., Chen, S., Peng, S., Liu, Y., Xing, S., He, X., & Chen, H. (2018). Prognostic Nomogram for Patients with Nasopharyngeal Carcinoma Incorporating Hematological Biomarkers and Clinical Characteristics. *International Journal of Biological Sciences*, 14(5), 549-556. <http://doi:10.7150/ijbs.24374>
- Li, J. P., Chen, S. L., Liu, X. M., He, X., Xing, S., Liu, Y. J., ... Liu, W. L. (2016). A Novel Inflammation-Based Stage (I Stage) Predicts Overall Survival of Patients with Nasopharyngeal Carcinoma. *International Journal of Molecular Sciences*, 17(11), 1-11. <https://doi.org/10.3390/ijms17111900>
- Li, S., Wu, J., Chen, Y., Tang, W., Peng, Q., Deng, Y., ... Zhao, J. (2014). ERCC1 Expression Levels Predict the Outcome of Platinum-based Chemotherapies in Advanced Bladder Cancer: a Meta-analysis. *Anticancer Drugs*, 25(1), 106-114. <https://doi.org/10.1097/CAD.0000000000000021>
- Li, X. H., Chang, H., Xu, B. Q., Tao, Y. L., Gao, J., Chen, C., ... Xia, Y. F. (2017). An Inflammatory Biomarker-based Nomogram to Predict Prognosis of Patients with Nasopharyngeal Carcinoma: an Analysis of a Prospective Study. *Cancer Medicine*, 6(1), 310-319. <http://doi:10.1002/cam4.947>
- Li, Y. J., Luo, Y., Xie, X. Q., Li, P., & Wang, F. (2018). The Prognostic Value of COX-2

- Expression on Circulating Tumour Cells in Nasopharyngeal Carcinoma: a Prospective Analysis. *Radiotherapy and Oncology*, 129(2), 396-402.  
<https://doi.org/10.1016/j.radonc.2018.07.022>
- Li, Z. L., Ye, S. B., OuYang, L. Y., Zhang, H., Zhang, X. S., Li, J., ... Zeng, Y. X. (2015). COX-2 Promotes Metastasis in Nasopharyngeal Carcinoma by Mediating Interactions Between Cancer Cells and Myeloid-derived Suppressor Cells. *Oncoimmunology*, 4(11), e1044712-e1044727. <https://doi.org/10.1080/2162402x.2015.1044712>
- Liao, D., Huang, H., Zhu, Z., Li, T., Huang, G. L., & He, Z. (2016). Prognostic Value of Matrix Metalloproteinase 9 in Nasopharyngeal Carcinoma: a Meta-analysis. *Minerva Medica*, 107(1), 54-61.
- Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P. A., ... Moher, D. (2009). The PRISMA Statement for Reporting Systematic Reviews and Meta-analyses of Studies that Evaluate Healthcare Interventions: Explanation and Elaboration. *Journal of Clinical Epidemiology*, 62, e1-34.  
<https://doi.org/10.1136/bmj.b2700>
- Light, R. J., & Pillemer, D. B. (1984). *Summing Up: The Science of Reviewing Research*. Cambridge, MA: Harvard University Press.
- Lin, J. C., Jan, J. S., Hsu, C. Y., Liang, W. M., Jiang, R. S., & Wang, W. Y. (2003). Phase III Study of Concurrent Chemoradiotherapy Versus Radiotherapy Alone for Advanced Nasopharyngeal Carcinoma: Positive Effect on Overall and Progression-Free Survival. *Journal of Clinical Oncology*, 21(4), 631-637.  
<https://doi.org/10.1200/JCO.2003.06.158>
- Lin, J. C., Wang, W. Y., Chen, K. Y., Wei, Y. H., Liang, W. M., & Jiang, R. S. (2004). Quantification of Plasma Epstein-Barr Virus DNA in Patients with Advanced

- Nasopharyngeal Carcinoma. *New England Journal of Medicine*, 10(350), 2461-2470.  
[http://doi: 10.1056/NEJMoa032260](http://doi:10.1056/NEJMoa032260)
- Liotta, L. A. (1986). Tumor Invasion and Metastases - Role of the Extracellular Matrix: Rhoads Memorial Award Lecture. *Cancer Research*, 46(1), 1-7.
- Liotta, L. A., Tryggvason, K., Garbisa, S., Hart, I. R., Foltz, C. M., & Shafie, S. (1980). Metastatic Potential Correlates with Enzymatic Degradation of Basement Membrane Collagen. *Nature*, 284, 67-68.
- Liu, L. T., Peng, J. P., Chang, H. C., & Hung, W. C. (2003). RECK is a Target of Epstein-Barr virus Latent Membrane Protein 1. *Oncogene*, 22(51), 8263-8270.  
<https://doi.org/10.1038/sj.onc.1207157>
- Lo, K. W., To, K. F., & Huang, D. P. (2004). Focus on Nasopharyngeal Carcinoma. *Cancer Cell*, 5(5), 423-428. [http://doi.org/10.1016/S1535-6108\(04\)00119-9](http://doi.org/10.1016/S1535-6108(04)00119-9)
- Lo, Y. . M. D., Leung, S. F., Chan, L. Y. S., Chan, A. T. C., Lo, K. W., Johnson, P. J., & Huang, D. P. (2000). Kinetics of Plasma Epstein-Barr Virus DNA during Radiation Therapy for Nasopharyngeal Carcinoma. *Cancer Research*, 60(9), 2351-2355.
- Lo, Y. M. D., Chan, L. Y. S., Lo, K. W., Leung, S. F., Zhang, J., Chan, A. T. C., ... Huang, D. P. (1999). Quantitative Analysis of Cell-free Epstein-Barr Virus DNA in Plasma of Patients with Nasopharyngeal Carcinoma. *Cancer Research*, 59(6), 1188-1191.
- Loong, S. L., Hwang, J. S., Li, H. H., Wee, J. T., Yap, S. P., Chua, M. L., ... Tan, T. W. (2009). Weak Expression of Cyclooxygenase-2 is Associated with Poorer Outcome in Endemic Nasopharyngeal Carcinoma: Analysis of Data from Randomized Trial Between Radiation Alone Versus Concurrent Chemo-Radiation (SQNP-01). *Radiation Oncology*, 4(23), 1-7. <https://doi.org/10.1186/1748-717X-4-23>
- Lu, X. G., Wang, X. S., Zhang, Y. S., Hu, C. S., Shen, C. Y., & Feng, Y. (2008). Hypoxia

- Inducible Factor-1  $\alpha$  and Vascular Endothelial Growth Factor Expression are Associated with a Poor Prognosis in Patients with Nasopharyngeal Carcinoma Receiving Radiotherapy with Carbogen and Nicotinamide. *Clinical Oncology*, 20(8), 606-612. <https://doi.org/10.1016/j.clon.2008.07.001>
- Ma, B. B., Hui, E. P., & Chan, A. T. (2008). Systemic Approach to Improving Treatment Outcome in Nasopharyngeal Carcinoma: Current and Future Directions. *Cancer Science*, 99(7), 1311-1318. <https://doi.org/10.1111/j.1349-7006.2008.00836.x>
- Ma, B. B., Lui, V. W., Poon, F. F., Wong, S. C., To, K. F., Wong, E., ... Cheng, S. H. (2010). Preclinical Activity of Gefitinib in Non-keratinizing Nasopharyngeal Carcinoma Cell Lines and Biomarkers of Response. *Investigational New Drugs*, 28(3), 326-333. <https://doi.org/10.1007/s10637-009-9316-7>
- Ma, B. B., Poon, T. C., To, K. F., Zee, B., Mo, F. K., Chan, C. M., ... Chan, A. T. (2003). Prognostic Significance of Tumor Angiogenesis, Ki 67, p53 Oncoprotein, Epidermal Growth Factor Receptor and HER2 Receptor Protein Expression in Undifferentiated Nasopharyngeal Carcinoma - a Prospective Study. *Head and Neck*, 25(10), 864-872. <https://doi.org/10.1002/hed.10307>
- Ma, X. L., Huang, J. W., Du, W., Zhuo, H. Y., Zhang, J., Shi, C. L., & Liu, L. (2015). ERCC1 Plays an Important Role in Predicting Survival Outcomes and Treatment Response for Patients with HNSCC: a Meta-analysis. *Oral Oncology*, 51(5), 483-492. <https://doi.org/10.1016/j.oraloncology.2015.02.094>
- MahdaviFar, N., Ghoncheh, M., Mohammadian-Hafshejani, A., Khosravi, B., & Salehiniya, H. (2016). Epidemiology and Inequality in the Incidence and Mortality of Nasopharynx Cancer in Asia. *Osong Public Health and Research Perspectives*, 7(6), 360-372. <https://doi.org/10.1016/j.phrp.2016.11.002>

- Mahdavifar, N., Towhidi, F., Makhsosi, B. R., Pakzad, R., Moini, A., Ahmadi, A., ... Salehiniya, H. (2016). Incidence and Mortality of Nasopharynx Cancer and Its Relationship With Human Development Index in the World in 2012. *World Journal of Oncology*, 7(5-6), 109-118. <http://doi:10.14740/wjon980w>
- Mainou, B. A., Everly, D. N., & Raab-traub, N. (2005). Epstein-Barr Virus Latent Membrane Protein 1 CTAR1 Mediates Rodent and Human Fibroblast Transformation Through Activation of PI3K. *Oncogene*, 24(46), 6917-6924. <https://doi.org/10.1038/sj.onc.1208846>
- Mainou, B. A., & Raab-traub, N. (2006). LMP1 Strain Variants: Biological and Molecular Properties. *Journal of Virology*, 80(13), 6458-6468. <https://doi.org/10.1128/JVI.00135-06>
- Mantovani, A., Allavena, P., Sica, A., & Balkwill, F. (2008). Cancer-related Inflammation. *Nature*, 454: 436-44.
- Maroni, P., Matteucci, E., Luzzati, A., Perrucchini, G., Bemdinelli, P., & Desiderio, M. A. (2011). Nuclear Co-localization and Functional Interaction of COX-2 and HIF-1: a Characterize Bone Metastasis of Human Breast Carcinoma. *Breast Cancer Research and Treatment*, 129(2), 433-450. <https://doi.org/10.1007/s10549-010-1240-1>
- Martin, L. P., Hamilton, T. C., & Schilder, R. J. (2008). Platinum Resistance: The Role of DNA Repair Pathways. *Clinical Cancer Research*, 14(5), 1291-1296. <https://doi.org/10.1158/1078-0432.CCR-07-2238>
- Mccarthy, N. (2015). Inflammation: An Innate Response. *Nature Reviews Cancer*, 15(4), 197-199. <https://doi.org/10.1038/nrc3927>
- Mei, Y. P., Zhou, J. M., Wang, Y., Huang, H., Deng, R., Feng, G. K., ... Zhu, X. F. (2007). Silencing of LMP1 Induces Cell Cycle Arrest and Enhances Chemosensitivity Through

- Inhibition of AKT Signaling Pathway in EBV-positive Nasopharyngeal Carcinoma Cells. *Cell Cycle*, 6(11), 1379-1385. <https://doi.org/10.4161/cc.6.11.4274>
- Metzger, R., Leichman, C. G., Danenberg, K. D., Danenberg, P. V., Lenz, H. J., Hayashi, K., ... Leichman, L. (1998). ERCC1 mRNA Levels Complement Thymidylate Synthase mRNA Levels in Predicting Response and Survival for Gastric Cancer Patients Receiving Combination Cisplatin and Fluorouracil Chemotherapy. *Journal of Clinical Oncology*, 16(1), 309-316.
- Min, Y., Ghose, S., Boelte, K., Li, J., Yang, L., & Charles, P. C. (2012). C/EBP- $\delta$  Regulates VEGF-C Autocrine Signaling in Lymphangiogenesis and Metastasis of Lung Cancer Through HIF-1 $\alpha$ . *Oncogene*, 30(49), 4901-4909. <https://doi.org/10.1038/onc.2011.187>.C/EBP-
- Mirabelli, M. C., Hoppin, J. A., Tolbert, P. E., Herrick, R. F., Gnepp, D. R., & Brann, E. A. (2000). Occupational Exposure to Chlorophenol and the Risk of Nasal and Nasopharyngeal Cancers Among U.S. Men Aged 30 to 60. *American Journal of Industrial Medicine*, 37(5), 532-541. [http://doi:10.1002/\(sici\)1097-0274](http://doi:10.1002/(sici)1097-0274)
- Moher, D., Cook, D. J., Eastwood, S., Olkin, I., Rennie, D., & Stroup, D. F. (1999). Improving the Quality of Reports of Meta-analyses of Randomised Controlled Trials: the QUOROM Statement. *Lancet*, 354(9193), 1896-1900. [http://doi:10.1016/s0140-6736\(99\)04149-5](http://doi:10.1016/s0140-6736(99)04149-5)
- Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Medicine*, 6(7), 1-7. <https://doi.org/10.1371/journal.pmed.1000097>
- Molnar, J., Fong, K. S., He, Q. P., Hayashi, K., Kim, Y., Fong, S. F., ... Csiszar, K. (2003). Structural and Functional Diversity of Lysyl Oxidase and the LOX-like Proteins.

- Biochemica et Biophysica Acta*, 1647(1-2), 220-224. [https://doi.org/10.1016/S1570-9639\(03\)00053-0](https://doi.org/10.1016/S1570-9639(03)00053-0)
- Moon, H. E., Cheon, H., Chun, K. H., Lee, S. K., Kim, Y. S., Jung, B. K., ... Lee, M. S. (2006). Metastasis-associated Protein 1 Enhances Angiogenesis by Stabilization of HIF-1 $\alpha$ . *Oncology Reports*, 16(4), 929-935.
- Murono, S., Inoue, H., Tanabe, T., Joab, I., Yoshizaki, T., Furukawa, M., & Pagano, J. S. (2001). Induction of Cyclooxygenase-2 by Epstein-Barr Virus Latent Membrane Protein 1 is Involved in Vascular Endothelial Growth Factor Production in Nasopharyngeal Carcinoma Cells. *Proceedings of the National Academy of Sciences*, 98(12), 6905-6910. <https://doi.org/10.1073/pnas.121016998>
- Murray, D., Vallee-Lucic, L., Rosenberg, E., & Andersson, B. (2002). Sensitivity of Nucleotide Excision Repair-Deficient Human Cells to Ionizing Radiation and Cyclophosphamide. *Anticancer Research*, 22(1A), 21-26.
- Nair, M., Sandhu, S. S., & Sharma, A. K. (2014). Prognostic and Predictive Biomarkers in Cancer. *Current Cancer Drug Targets*, 14(5), 477-504.
- National Cancer Control Office, Nanjing Institute of Geography (1979). *Atlas of Cancer Mortality in the People's Republic of China*. China Map Press: Shanghai.
- National Registry of Diseases Office (NRDO). (2017). Singapore Cancer Registry Annual Registry Report 2015.
- Newell, K. J., Witty, J. P., Rodgers, W. H., & Matrisian, L. M. (1994). Expression and Localization of Matrix-Degrading Metalloproteinases During Colorectal Tumorigenesis. *Molecular Carcinogenesis*, 10(4), 199-206.
- Ng, W. T., Yau, T. K., Yung, R. W., Sze, W. M., Tsang, A. H., Law, A. L., & Lee, A. W. (2005). Screening for Family Members of Patients with Nasopharyngeal Carcinoma.

- International Journal of Cancer*, 113(6), 998-1001. <https://doi.org/10.1002/ijc.20672>
- Ning, J. P., Yu, M. C., Wang, Q. S., & Henderson, B. E. (1990). Consumption of Salted Fish and Other Risk Factors for Nasopharyngeal Carcinoma (NPC) in Tianjin, a Low-Risk Region for NPC in the People's Republic of China. *Journal of The National Cancer Institute*, 82(4), 291-296. <http://doi: 10.1093/jnci/82.4.291>
- Olaussen, K. A., Dunant, A., Fouret, P., Brambilla, E., André, F., Haddad, V., ... Soria, J. C. (2006). DNA Repair by ERCC1 in Non-small-cell Lung Cancer and Cisplatin-based Adjuvant Chemotherapy. *The New England Journal of Medicine*, 355(10), 983-991. <http://doi: 10.1056/NEJMoa060570>
- Olayioye, M. A., Neve, R. M., Lane, H. A., & Hynes, N. E. (2000). The ErbB Signaling Network: Receptor Heterodimerization in Development and Cancer. *The European Molecular Biology Organization Journal*, 19(13), 3159-3167.
- Ozyar, E., Ayhan, A., Korcum, A., & Atahan, I. (2004). Prognostic Role of Epstein-Barr Virus Latent Membrane Protein-1 and Interleukin-10 Expression in Patients with Nasopharyngeal Carcinoma. *Cancer Investigation*, 22(4), 483-491.
- Pan, J., Kong, L., Lin, S., Chen, G., Chen, Q., & Lu, J. J. (2008). The Clinical Significance of Coexpression of Cyclooxygenase-2, Vascular Endothelial Growth Factors, and Epidermal Growth Factor Receptor in Nasopharyngeal Carcinoma. *Laryngoscope*, 118(11), 1970-1975. <https://doi.org/10.1097/MLG.0b013e3181805134>
- Pan, J., Tang, T., Xu, L., Lu, J. J., Lin, S., Qiu, S., ... Tham, I. W. K. (2012). Prognostic Significance of Expression of Cyclooxygenase-2, Vascular Endothelial Growth Factor and Epidermal Growth Factor Receptor in Nasopharyngeal Carcinoma. *Head & Neck*, 35(5), 1238-1247. <http://doi:10.1002/hed.23116>
- Pan, Y., Jiang, Y., Tan, L., Ravoori, M. K., Gagea, M., Kundra, V., ... Yang, P. Y. (2015).

- Deletion of Cyclooxygenase-2 Inhibits K-ras - Induced Lung Carcinogenesis. *Oncotarget*, 6(36), 38816-38826. <http://doi: 10.18632/oncotarget.5558>.
- Panchenko, M. V., Stetler-stevenson, W. G., Trubetskoy, O. V., Gacheru, S. N., & Kagan, H. M. (1996). Metalloproteinase Activity Secreted by Fibrogenic Cells in the Processing of Prolysyl Oxidase, Potential Role of Procollagen C-proteinase. *The Journal of Biological Chemistry*, 271(12), 7113-7119. <http://doi: 10.1074/jbc.271.12.7113>
- Parkin, D. M., Bray, F., Ferlay, J., & Pisani, P. (2005). Global Cancer Statistics 2002. *CA Cancer Journal of Clinicians*, 55(2), 74-108. <https://doi.org/doi.org/10.3322/canjclin.55.2.74>
- Parkin, D.M., Whelan, S.L., Ferlay, J., Raymond, L., & Young, J. (1997). Cancer Incidence in Five Continents. *IARC Scientific Publications IARC Press*, 7(143), i-xxxiv, 1-1240.
- Parmar, M. K., Torri, V., & Stewart, L. (1998a). Extracting Summary Statistics to Perform Meta-analyses of the Published Literature for Survival Endpoints. *Statistics in Medicine*, 17(24), 2815-2834.
- Partanen, T. (1993). Formaldehyde Exposure and Respiratory Cancer - a Meta-Analysis of the Epidemiologic Evidence. *Scandinavian Journal of Work, Environment & Health*, 19(1), 8-15. <https://doi.org/10.5271/sjweh.1500>
- Patsopoulos, N. A., Analatos, A. A., & Ioannidis, J. P. A. (2005). Relative Citation Impact of Various Study Designs in the Health Sciences. *The Journal of the American Medical Association*, 293(19), 2362-2366. <http://doi: 10.1001/jama.293.19.2362>
- Payne, S. L., Hendrix, M. J., & Kirschmann, D. A. (2007). Paradoxical Roles for Lysyl Oxidases in Cancer - a Prospect. *Journal of Cellular Biochemistry*, 101(6), 1338-1354. <https://doi.org/10.1002/jcb.21371>

- Peng, L., Zhou, Y., Wang, Y. N., Mou, H. B., & Zhao, Q. (2013). Prognostic Significance of COX-2 Immunohistochemical Expression in Colorectal Cancer: a Meta-Analysis of the Literature. *PLoS One*, 8(3), 1-9. <https://doi.org/10.1371/journal.pone.0058891>
- Perez, C. A., Devineni, V. R., Marcial-vega, V., Marks, J. E., Simpson, J. R., & Kucik, N. (1992). Carcinoma of The Nasopharynx: Factors Affecting Prognosis. *International Journal of Radiation Oncology, Biology, Physics*, 23, 271-280. [https://www.redjournal.org/article/0360-3016\(92\)90741-Y/pdf](https://www.redjournal.org/article/0360-3016(92)90741-Y/pdf)
- Petiti, D. (1994). *Meta-Analysis, Decision Analysis, and Cost Effectiveness Analysis*. New York, NY: Oxford University Press.
- Pick, T., Maurer, H. M., & McWilliams, N. B. (1974). Lymphoepithelioma in Childhood. *Journal of Pediatrics*, 84(1), 96-100. [https://doi.org/10.1016/S0022-3476\(74\)80560-3](https://doi.org/10.1016/S0022-3476(74)80560-3)
- Pignon, J. P., Bourhis, J., Domenge, C., & Designe, L. (2000). Chemotherapy Added to Locoregional Treatment for Head and Neck Squamous-Cell Carcinoma: Three Meta-Analyses of Updated Individual Data. MACH-NC Collaborative Group. Meta-Analysis of Chemotherapy on Head and Neck Cancer. *Lancet*, 355(9208), 949-955. [http://doi:https://doi.org/10.1016/S0140-6736\(00\)90011-4](http://doi:https://doi.org/10.1016/S0140-6736(00)90011-4)
- Pine, S. R., Mechanic, L. E., Enewold, L., Chaturvedi, A. K., Katki, H. A., Zheng, Y. L., ... Harris, C. C. (2011). Increased Levels of Circulating Interleukin 6, Interleukin 8, C-Reactive Protein, and Risk of Lung Cancer. *Journal of The National Cancer Institute*, 103(14), 1112-1122. <https://doi.org/10.1093/jnci/djr216>
- Pioche-durieu, C., Keryer, C., Souque, S., Bosq, J., Faigle, W., Loew, D., ... Busson, P. (2005). In Nasopharyngeal Carcinoma Cells, Epstein-Barr Virus LMP1 Interacts with Galectin 9 in Membrane Raft Elements Resistant to Simvastatin. *Journal of Virology*, 79(21), 13326-13337. <https://doi.org/10.1128/JVI.79.21.13326>

- Poirier, S., Bouvier, G., Malaveille, C., Ohshima, H., Shao, Y. M., Hubert, A., ... Bartsch, H. (1989). Volatile Nitrosamine Levels and Genotoxicity of Food Samples From High-Risk Areas for Nasopharyngeal Carcinoma Before and After Nitrosation. *International Journal of Cancer*, 44(6), 1088-1094. <http://doi.org/10.1002/ijc.2910440625>
- Poltorak, Z., Cohen, T., Sivan, R., Kandelis, Y., Spira, G., Vlodavsky, I., ... Neufeld, G. (1997). VEGF 145, a Secreted Vascular Endothelial Growth Factor Isoform That Binds to Extracellular Matrix. *The Journal of Biological Chemistry*, 272(11), 7151-7158. [http://doi: 10.1074/jbc.272.11.7151](http://doi:10.1074/jbc.272.11.7151)
- Prasad, U., & Rampal, L. (1992). Descriptive Epidemiology of Nasopharyngeal Carcinoma in Peninsular Malaysia. *Cancer Causes & Control*, 3(2), 179-182.
- Prawira, A., Oosting, S. F., Chen, T. W., Delos Santos, K. A., Saluja, R., Wang, L., ... Hansen, A. R. (2017). Systemic Therapies for Recurrent or Metastatic Nasopharyngeal Carcinoma: a Systematic Review. *British Journal of Cancer*, 117(12), 1743-1752. <https://doi.org/10.1038/bjc.2017.357>
- Prenzel, N., Fischer, O. M., Streit, S., Hart, S., & Ullrich, A. (2001). The Epidermal Growth Factor Receptor Family as a Central Element for Cellular Signal Transduction and Diversification. *Endocrine-Related Cancer*, 8(1), 11-31.
- Provatopoulou, X., Gounaris, A., Kalogera, E., Zagouri, F., Flessas, I., Goussetis, E., ... Zografos, G. (2009). Circulating Levels of Matrix Metalloproteinase-9 (MMP-9), Neutrophil Gelatinase-associated Lipocalin (NGAL) and Their Complex MMP-9/NGAL in Breast Cancer Disease. *Bio Med Central Cancer*, 9(930), 1-7. <https://doi.org/10.1186/1471-2407-9-390>
- Rankin, E. B., & Giaccia, A. J. (2008). The Role of Hypoxia-inducible Factors in Tumorigenesis. *Cell Death and Differentiation*, 15(4), 678-685.

<https://doi.org/10.1038/cdd.2008.21>

- Razak, A. R. A., Siu, L. L., Liu, F. F., Ito, E., O'Sullivan, B., & Chan, K. (2010). Nasopharyngeal Carcinoma: The Next Challenges. *European Journal of Cancer*, 46(11), 1967-1978. <https://doi.org/10.1016/j.ejca.2010.04.004>
- Reed, E. (2005). ERCC1 and Clinical Resistance to Platinum-Based Therapy. *Clinical Cancer Research*, 11(17), 6100-6103. <https://doi.org/10.1158/1078-0432.CCR-05-1083>
- Reichhardt, T. (1999). It's Sink or Swim as a Tidal Wave of Data Approaches. *Nature*, 399(6736), 517-520. <http://doi:10.1038/21044>
- Ristimäki, A., Sivula, A., Lundin, J., Lundin, M., Salminen, T., Haglund, C., ... Isola, J. (2002). Prognostic Significance of Elevated Cyclooxygenase-2 Expression in Breast Cancer. *Cancer Research*, 62(3), 632-635.
- Roychowdhury, D. F., Tseng, A., Fu, K. K., Weinburg, V., & Weidner, N. (1996). New Prognostic Factors in Nasopharyngeal Carcinoma: Tumor Angiogenesis and C-erbB2 Expression. *Cancer*, 77(8), 1419-1426. [http://doi:10.1002/\(SICI\)1097-0142\(19960415\)77:8%3C1419::AID-CNCR1%3E3.0.CO;2-7](http://doi:10.1002/(SICI)1097-0142(19960415)77:8%3C1419::AID-CNCR1%3E3.0.CO;2-7)
- Rusthoven, C. G., Lanning, R. M., Jones, B. L., Amini, A., Koshy, M., Sher, D. J., ... Karam, S. D. (2017). Metastatic Nasopharyngeal Carcinoma: Patterns of Care and Survival for Patients Receiving Chemotherapy with and without Local Radiotherapy. *Radiotherapy and Oncology*, 124(1), 139-146. <https://doi.org/10.1016/j.radonc.2017.03.019>
- Saba, N. F., Choi, M., Muller, S., Shin, H. J. C., Tighiouart, M., Papadimitrakopoulou, V. A., ... Shin, D. M. (2009). Role of Cyclooxygenase-2 in Tumor Progression and Survival of Head and Neck Squamous Cell Carcinoma. *Cancer Prevention Research*, 2(9), 823-829. <https://doi.org/10.1158/1940-6207.CAPR-09-0077>

- Sackett, D. L., Roseberg, W. M. C., Gray, J. A. M., Haynes, R. B., & Richardson, W. S. (1996). Evidence Based Medicine: What It is and What It isn't. *British Medical Journal*, 312(7023), 71-72.
- Salehiniya, H., Mohammadian, M., Mohammadian-Hafshejani, A. & Mahdavifar, N. (2018). Nasopharyngeal Cancer in The World: Epidemiology, Incidence, Mortality and Risk Factors. *World Cancer Research Journal*, 5(1), e1046-e1053.
- Schellhorn, M., Haustein, M., Frank, M., Linnebacher, M., & Hinz, B. (2005). Celecoxib Increases Lung Cancer Cell Lysis by Lymphokine-activated Killer Cells via Upregulation of ICAM-1. *Oncotarget*, 6(36), 39342-39356. [http://doi: 10.18632/oncotarget.5745](http://doi:10.18632/oncotarget.5745).
- Sham, J. S. T., Wei, W. I., Zong, Y. S., Choy, D., Guo, Y. Q., Luo, Y., ... Ng, M. H. (1990). Detection of Subclinical Nasopharyngeal Carcinoma by Fibreoptic Endoscopy and Multiple Biopsy. *The Lancet*, 17(335), 371-374. [http://doi: 10.1016/0140-6736\(90\)90206-k](http://doi:10.1016/0140-6736(90)90206-k)
- Shanmugaratnam, K., & Sobin, L. H. (1993). The World Health Organization Histological Classification of Tumours of the Upper Respiratory Tract and Ear: a Commentary on the Second Edition. *Cancer*, 71(8), 2689-2697. [http://doi: 10.1002/1097-0142\(19930415\)71:8%3C2689::aid-cnrcr2820710843%3E3.0.co;2-h](http://doi:10.1002/1097-0142(19930415)71:8%3C2689::aid-cnrcr2820710843%3E3.0.co;2-h)
- Shao, Y. M., Poirier, A., Ohshima, H., Malaveille, C., Zeng, Y., De-The, G., & Bartsch, H. (1988). Epstein-Barr Virus Activation in Raji Cells by Extracts of Preserved Food From High Risk Areas for Nasopharyngeal Carcinoma. *Carcinogenesis*, 9(8), 1455-1457. [http://doi: 10.1093/carcin/9.8.1455%0A\[Indexed for MEDLINE\]](http://doi:10.1093/carcin/9.8.1455%0A[Indexed for MEDLINE])
- Shi, W., Pataki, I., Macmillan, C., Pintilie, M., Payne, D., O'Sullivan, B., ... Liu, F. F. (2002). Molecular Pathology Parameters in Human Nasopharyngeal Carcinoma. *Cancer*, 94(7),

- 1997-2006. <https://doi.org/10.1002/cncr.10440>
- Sim, C. C., & Sim, E. U. H. (2020). Over-expression of Cyclo-oxygenase-2 Predicts Poor Survival of Patients with Nasopharyngeal Carcinoma: a Meta-Analysis. *The Journal of Laryngology & Otology*, 1-6. <http://doi:10.1017/S0022215120000614>
- Sim, E. U. H., Tiong, T. S., Subramaniam, S. K., Ong, T. A., & Teh, G. C. (2010). Relationship of Ethnicity, Age and Gender to Incidence of Bladder and Nasopharyngeal Cancers in Kuching, Sarawak. *The Sarawak Museum Journal*, LXVII(88), 25-38.
- Simonsson, M., Björner, S., Markkula, A., Nodin, B., Jirström, K., Rose, C., ... Jernström, H. (2017). The Prognostic Impact of COX-2 Expression in Breast Cancer Depends on Oral Contraceptive History, Preoperative NSAID Use and Tumor Size. *International Journal of Cancer*, 140(1), 163-175. <https://doi.org/10.1002/ijc.30432>
- Solinas, G., Marchesi, F., Garlanda, C., Mantovani, A., & Allavena, P. (2010). Inflammation-Mediated Promotion of Invasion and Metastasis. *Cancer Metastasis Reviews*, 29(2), 243-248. <https://doi.org/10.1007/s10555-010-9227-2>
- Song, L. B., Zeng, M. S., Liao, W. T., Zhang, L., Mo, H. Y., Liu, W. L., ... Zeng, Y. X. (2006). Bmi-1 Is a Novel Molecular Marker of Nasopharyngeal Carcinoma Progression and Immortalizes Primary Human Nasopharyngeal Epithelial Cells. *Cancer Research*, 66(12), 6225-6233. <https://doi.org/10.1158/0008-5472.CAN-06-0094>
- Soo, R., Putti, T., Tao, Q., Goh, B. C., Lee, K. H., Kwok-Seng, L., ... Hsieh, W. S. (2005). Overexpression of Cyclooxygenase-2 in Nasopharyngeal Carcinoma and Association with Epidermal Growth Factor Receptor Expression. *Archives of Otolaryngology - Head and Neck Surgery*, 131(2), 147-152. <https://doi.org/10.1001/archotol.131.2.147>
- Spano, J. P., Busson, P., Atlan, D., Bourhis, J., Pignon, J. P., Esteban, C., & Armand, J. P. (2003). Nasopharyngeal Carcinomas: an Update. *European Journal of Cancer*, 39(15),

2121-2135. [https://doi.org/10.1016/S0959-8049\(03\)00367-8](https://doi.org/10.1016/S0959-8049(03)00367-8)

- Spitz, M. R., Hong, W. K., Amos, C. I., Wu, X., Schabath, M. B., Dong, Q., ... Etzel, C. J. (2007). A Risk Model for Prediction of Lung Cancer. *Journal of National Cancer Institute*, 99(9), 715-726.
- Sriamporn, S., Vatanasapt, V., Pisani, P., Yongchaiyudha, S., & Rungpitarangsri, V. (1992). Environmental Risk Factors for Nasopharyngeal Carcinoma: a Case-Control Study in Northeastern Thailand. *Cancer Epidemiology Biomarkers and Prevention*, 1(5), 345-348.
- Stangl, D.K., & Berry, D.A. (2000). *Meta-analysis in Medicine and Health Policy*. Marcel Dekker, New York, NY.
- Stassar, M., Devitt, G., Brosius, M., Rinnab, L., Prang, J., Schradin, T., ... Zoller, M. (2001). Identification of Human Renal Cell Carcinoma Associated Genes by Suppression Subtractive Hybridization. *British Journal of Cancer*, 85(9), 1372-1382. <http://doi:10.1054/bjoc.2001.2074>
- Steffensen, K. D., Waldstrom, M., & Jakobsen, A. (2009). The Relationship of Platinum Resistance and ERCC1 Protein Expression in Epithelial Ovarian Cancer. *International Journal of Gynecological Cancer*, 19(5), 820-825. <https://doi.org/10.1111/IGC.0b013e3181a12e09>
- Sterne, J. A. C., & Egger, M. (2001). Funnel Plots for Detecting Bias in Meta-Analysis: Guidelines on Choice of Axis. *Journal of Clinical Epidemiology*, 54(10), 1046-1055.
- Sterne, J. A., Sutton, A. J., Ioannidis, J. P., Terrin, N., Jones, D. R., Lau, J., ... Higgins, J. P. (2011). Recommendations for Examining and Interpreting Funnel Plot Asymmetry in Meta-Analyses of Randomised Controlled Trials. *British Medical Journal*, 343, d4002-d4007. <https://doi.org/10.1136/bmj.d4002>

- Su, C. K., & Wang, C. C. (2002). Prognostic Value of Chinese Race in Nasopharyngeal Cancer. *International Journal of Radiation Oncology, Biology, Physics*, 54(3), 752-758. [http://doi: 10.1016/s0360-3016\(02\)02969-3](http://doi: 10.1016/s0360-3016(02)02969-3)
- Sugihara, T., Wadhwa, R., Kaul, S. C., & Mitsui, Y. (1998). A Novel Alternatively Spliced Form of Murine Vascular Endothelial Growth Factor, VEGF 115. *The Journal of Biological Chemistry*, 273(5), 3033-3038. <http://doi: 10.1074/jbc.273.5.3033>
- Sun, H., Zhang, X., Sun, D., Jia, X., Xu, L., Qiao, Y., & Jin, Y. (2017). COX-2 Expression in Ovarian Cancer: an Updated Meta-Analysis. *Oncotarget*, 8(50), 88152-88162. <http://doi:10.18632/oncotarget.21538>
- Sun, W., Long, G. X., Wang, J. F., Mei, Q., Liu, D. B., & Hu, G. Q. (2013). Prognostic Role of Epidermal Growth Factor Receptor in Nasopharyngeal Carcinoma: a Meta-Analysis. *Head and Neck*, 36(10), 1508-1516. <https://doi.org/10.1002/HED>
- Sung, F. L., Poon, T. C., Hui, E. P., Ma, B. B., Liong, E., To, K. F., ... Chan, A. T. (2005). Antitumor Effect and Enhancement of Cytotoxic Drug Activity by Cetuximab in Nasopharyngeal Carcinoma Cells. *In Vivo*, 19(1), 237-245.
- Takeshita, H., Yoshizaki, T., Miller, W. E., Sato, H., Furukawa, M., Pagano, J. S., & Raab-traub, N. (1999). Matrix Metalloproteinase 9 Expression is Induced by Epstein-Barr Virus Latent Membrane Protein 1 C-Terminal Activation Regions 1 and 2. *Journal of Virology*, 73(7), 5548-5555.
- Tan, C. K. Beh, S. P., Lee, R., Pei-Jye, V., Damoderam, S., Mohd-Naseri, N. I., Nagaswara, U., & Yeo, G. G. (2018). Gender Differences in Risk of Developing Nasopharyngeal Cancer (NPC) in One of the Highest NPC Ethnic in the World. *Annals of Oncology*, 29(Supplement 9), ix94-ix104. <http://doi:10.1093/annonc/mdy438>
- Tan, K. B., & Putti, T. C. (2005). Cyclooxygenase-2 Expression in Nasopharyngeal

- Carcinoma: Immunohistochemical Findings and Potential Implications. *Journal of Clinical Pathology*, 58(5), 535-538. <https://doi.org/10.1136/jcp.2004.021923>
- Tang, F., Tang, X., Tian, D., & Cao, Y. (2012). Chemical Carcinogenesis and Nasopharyngeal Carcinoma. *Carcinogenesis, Diagnosis, and Molecular Targeted Treatment for Nasopharyngeal Carcinoma*, 4, 61-82. <https://www.intechopen.com/books/carcinogenesis-diagnosis-and-molecular-targeted-treatment-for-nasopharyngeal-carcinoma/chemical-carcinogenesis-and-nasopharyngeal-carcinoma>. doi:10.5772/31185
- Tang, S. S., Trackman, P. C., & Kagang, H. M. (1983). Reaction of aortic Lysyl Oxidase with Beta-Aminopropionitrile. *The Journal of Biological Chemistry*, 258(7), 4331-4338.
- Teo, P., Yu, P., Lee, W. Y., Leung, S. F., Kwan, W. H., Yu, K. H., ... Johnson, P. J. (1996). Significant Prognosticators After Primary Radiotherapy in 903 Nondisseminated Nasopharyngeal Carcinoma Evaluated by Computer Tomography. *International Journal of Radiation Oncology, Biology, Physics*, 36(2), 291-304. [https://doi.org/10.1016/S0360-3016\(96\)00323-9](https://doi.org/10.1016/S0360-3016(96)00323-9)
- Thacker, S. B. (1988). Meta-analysis. A Quantitative Approach to Research Integration. *The Journal of the American Medical Association*, 259(11), 1865-1869. <http://doi:10.1001/jama.259.11.1685>
- The Ear Nose and Throat Clinic (2011). Head & Neck Cancer [Pictorial Illustration of Diagram Showing the Location where Nose Cancer Arises]. *A New Screening Tool for Nose Cancer*. <https://www.entspecialist.sg/doctors-corner/?p=98>
- Thompson, L. D. R. (2007). Update on Nasopharyngeal Carcinoma. *Head Neck Pathol*, 1(1), 81-86. <https://doi.org/10.1007/s12105-007-0012-7>

- Thompson, M. P., & Kurzrock, R. (2004). Epstein-Barr Virus and Cancer. *Clinical Cancer Research*, 10(3), 803-821. <http://doi: 10.1158/1078-0432.CCR-0670-3>
- Tian, M., Cui, Y. Z., Song, G. H., Zong, M. J., Zhou, X. Y., Chen, Y., & Han, J. X. (2008). Proteomic Analysis Identifies MMP-9, DJ-1 and A1BG as Overexpressed Proteins in Pancreatic Juice from Pancreatic Ductal Adenocarcinoma Patients. *BioMed Central Cancer*, 8(241), 1-11. <https://doi.org/10.1186/1471-2407-8-241>
- Tillett, W. S., & Francis, T. (1930). Serological Reactions in Pneumonia with a Non-protein Somatic Fraction of Pneumococcus. *Journal of Experimental Medicine*, 52(4), 561-571.
- Tokuraku, M., Sato, H., Murakami, S., Okada, Y., Watanabe, Y., & Seiki, M. (1995). Activation of the Precursor of Gelatinase A/72 kDa Type IV Collagenase/MMP-2 in Lung Carcinomas Correlates with the Expression of Membrane-Type Matrix Metalloproteinase (MT-MMP) and with Lymph Node Metastasis. *International Journal of Cancer*, 64(5), 355-359. <http://doi: 10.1002/ijc.2910640513>
- Toss, A., Venturelli, M., Peterle, C., Piacentini, F., Cascinu, S., & Cortesi, L. (2017). Molecular Biomarkers for Prediction of Targeted Therapy Response in Metastatic Breast Cancer: Trick or Treat . *International Journal of Molecular Sciences*, 18(1), 1-24. <https://doi.org/10.3390/ijms18010085>
- Tricker, A. R., & Preussmann, R. (1991). Carcinogenic N-nitrosamines in the Diet: Occurrence, Formation, Mechanisms and Carcinogenic Potential. *Mutation Research/Genetic Toxicology*, 259(3-4), 277-289. [http://doi:10.1016/0165-1218\(91\)90123-4](http://doi:10.1016/0165-1218(91)90123-4)
- Tsujii, M., & Dubois, N. (1995). Alterations in Cellular Adhesion and Apoptosis in Epithelial Cells Overexpressing Prostaglandin Endoperoxide Synthase 2. *Cell*, 83(3), 493-501. [http://doi: 10.1016/0092-8674\(95\)90127-2](http://doi: 10.1016/0092-8674(95)90127-2)

- Tsuji, M., Kawano, S., Tsuji, S., Sawaoka, H., Hori, M., & Dubois, R. N. (1998). Cyclooxygenase Regulates Angiogenesis Induced by Colon Cancer Cells. *Cell*, 93(5), 705-716. [http://doi: 10.1016/s0092-8674\(00\)81433-6](http://doi: 10.1016/s0092-8674(00)81433-6)
- Tulalamba, W., & Janvilisri, T. (2012). Nasopharyngeal Carcinoma Signaling Pathway: an Update on Molecular Biomarkers. *Hindawi*, 2012, 1-10. <https://doi.org/10.1155/2012/594681>
- Turini, M. E., & Dubois, R. N. (2002). Cyclooxygenase-2: a Therapeutic Target. *Annual Review of Medicine*, 53, 35-57. <http://doi: 10.1146/annurev.med.53.082901.103952>
- Union for International Cancer Control (UICC). (2014). Nasopharyngeal Carcinoma: Review of Cancer Medicines on the WHO List of Essential Medicines. [https://www.who.int/selection\\_medicines/committees/expert/20/applications/NasopharyngealCarcinoma.pdf?ua=1](https://www.who.int/selection_medicines/committees/expert/20/applications/NasopharyngealCarcinoma.pdf?ua=1)
- Vlahou, A. (2013). Network Views for Personalized Medicine. *Proteomics Clinical Application*, 7(5), 384-387. <https://doi.org/10.1002/prca.201200121>
- Vokes, E. E., Liebowitz, D. N., & Weichselbaum, R. R. (1997). Nasopharyngeal Carcinoma. *The Lancet*, 350(9084), 1087-1091. [http://doi.org/10.1016/S0140-6736\(97\)07269-3](http://doi.org/10.1016/S0140-6736(97)07269-3)
- Vosooghi, M., & Amini, M. (2014). The Discovery and Development of Cyclooxygenase-2 Inhibitors as Potential Anticancer Therapies. *Expert Opinion on Drug Discovery*, 9(3), 255-267. <http://doi: 10.1517/17460441.2014.883377>
- Wang, D., Liebowitz, D., & Kieff, E. (1986). An EBV Membrane Protein Expressed in Immortalized Lymphocytes Transforms Established Rodent Cells. *Cell*, 43(3), 831-840. [http://doi: 10.1016/0092-8674\(85\)90256-9](http://doi: 10.1016/0092-8674(85)90256-9)
- Wang, F., Peng, L., Wang, Y., & Liu, X. D. (2018). A Meta-Analysis of Vascular Endothelial Growth Factor for Nasopharyngeal Cancer Prognosis. *Frontiers in*

- Oncology*, 8(486), 1-9. <https://doi.org/10.3389/fonc.2018.00486>
- Wang, Y., Zhang, Y., & Ma, S. (2013). Racial Differences in Nasopharyngeal Carcinoma in the United States. *Cancer Epidemiology*, 37(6), 793-802. <http://doi:10.1016/j.canep.2013.08.008>
- Wei, J., Zou, Z., Qian, X., Ding, Y., Xie, L., Sanchez, J. J., ... Liu, B. (2008). ERCC1 mRNA Levels and Survival of Advanced Gastric Cancer Patients Treated with a Modified FOLFOX Regimen. *British Journal of Cancer*, 98(8), 1398-1402. <https://doi.org/10.1038/sj.bjc.6604317>
- Wei, K. R., Yu, Y. L., Yang, Y. Y., Ji, M. F., Yu, B. H., Liang, Z. H., & Reng, X. (2010). Mini-review: Epidemiological Trends of Nasopharyngeal Carcinoma in China. *Asian Pacific Journal of Cancer Prevention*, 11(1), 29-32.
- Wei, K. R., Zheng, R. S., Zhang, S. W., Liang, Z. H., Li, Z. M., & Chen, W. Q. (2017). Nasopharyngeal Carcinoma Incidence and Mortality in China, 2013. *Chinese Journal of Cancer*, 36(1), 90. <http://doi:10.1186/s40880-017-0257-9>
- Wei, W. I., & Sham, J. S. T. (2005). Nasopharyngeal Carcinoma. *The Lancet*, 365(9476), 2041-2054. [http://doi:10.1016/S0140-6736\(05\)66698-6](http://doi:10.1016/S0140-6736(05)66698-6)
- Weise, J. B., Rudolph, P., Heiser, A., Kruse, M. L., Hedderich, J., Cordes, C., ... Gorogh, T. (2008). LOXL4 is a Selectively Expressed Candidate Diagnostic Antigen in Head and Neck Cancer. *European Journal of Cancer*, 44(9), 1323-1331. <https://doi.org/10.1016/j.ejca.2008.03.026>
- Wilgus, M. L., Borczuk, A. C., Stoopler, M., Ginsburg, M., Gorenstein, L., Sonett, J. R., & Powell, C. A. (2011). Lysyl Oxidase: a Lung Adenocarcinoma Biomarker of Invasion and Survival. *Cancer*, 117(10), 2186-2191. <https://doi.org/10.1002/cncr.25768>
- Williams, C. S., Tsujii, M., Reese, J., Dey, S. K., & DuBois, R. N. (2000). Host

- Cyclooxygenase-2 Modulates Carcinoma Growth. *Journal of Clinical Investigation*, 105(11), 1589-1594. <https://doi.org/10.1172/jci9621>
- Wilson, C. P. (1951). The Approach to the Nasopharynx. *Proceedings of the Royal Society of Medicine*, 44(5), 353-358.
- Wimazal, F., Sperr, W. R., Kundi, M., Vales, A., Fonatsch, C., Schwarzingner, I., & Valent, P. (2008). Prognostic Significance of Serial Determinations of Lactate Dehydrogenase (LDH) in the Follow-up of Patients with Myelodysplastic Syndromes. *Annals of Oncology*, 19(5), 970-976. <https://doi.org/10.1093/annonc/mdm595>
- Wong, V. K., Malik, H. Z., Hamady, Z. Z., Al-Mulhtar, A., Gomez, D., Prasad, K. R., ... Lodge, J. P. (2007). C-reactive Protein as a Predictor of Prognosis Following Curative Resection for Colorectal Liver Metastases. *British Journal of Cancer*, 96(2), 222-225. <https://doi.org/10.1038/sj.bjc.6603558>
- Wu, F., Wang, R. S., Lu, H. M., Wei, B., Feng, G. S., Li, G. S., ... Hu, K. (2014). Concurrent Chemoradiotherapy in Locoregionally Advanced Nasopharyngeal Carcinoma: Treatment Outcomes of a Prospective Multicentric Clinical Study. *Radiotherapy and Oncology*, 112(1), 106-111. <https://doi.org/10.1016/j.radonc.2014.05.005>
- Wu, H. C., Lin, Y. J., Lee, J. J., Liu, Y. J., Liang, S. T., Peng, Y., ... Lin, C. (2003). Functional Analysis of EBV in Nasopharyngeal Carcinoma Cells. *Laboratory Investigation*, 83(6), 797-812. <https://doi.org/10.1097/01.LAB.0000074896.03561.FB>
- Wu, L., Li, C., & Pan, L. (2018). Nasopharyngeal Carcinoma: a Review of Current Updates. *Experimental and Therapeutic Medicine*, 15(4), 3687-3692. <http://doi:10.3892/etm.2018.5878>
- Wu, X., Huang, J., Liu, L., Li, H., Li, P., Zhang, J., & Xie, L. (2016). Cetuximab Concurrent with IMRT Versus Cisplatin Concurrent with IMRT in Locally Advanced

- Nasopharyngeal Carcinoma: a Retrospective Matched Case-Control Study. *Medicine*, 95(39), e4926-e4933. <http://doi:10.1097/MD.00000000000004926>
- Xiao, Q., & Ge, G. (2012). Lysyl Oxidase , Extracellular Matrix Remodeling and Cancer Metastasis. *Cancer Microenvironment*, 5(3), 261-273. <https://doi.org/10.1007/s12307-012-0105-z>
- Xiao, X., Wu, J., Zhu, X., Zhao, P., Zhou, J., Liu, Q. Q., ... Huang, W. (2007). Induction of Cell Cycle Arrest and Apoptosis in Human Nasopharyngeal Carcinoma Cells by ZD6474, an Inhibitor of VEGFR Tyrosine Kinase with Additional Activity Against EGFR Tyrosine Kinase. *International Journal of Cancer*, 121(9), 2095-2104. <https://doi.org/10.1002/ijc.22955>
- Xie, S. H., Yu, I. T., Tse, L. A., Mang, O. W., & Yue, L. (2013). Sex Difference in the Incidence of Nasopharyngeal Carcinoma in Hong Kong 1983-2008: Suggestion of a Potential Protective Role of Oestrogen. *European Journal of Cancer*, 49(1), 150-155. <http://doi:10.1016/j.ejca.2012.07.004>.
- Xie, W. J., Liu, L. H., He, H. X., & Yang, K. X. (2018). Prognostic Value of Hypoxia-inducible Factor-1 Alpha in Nasopharyngeal Carcinoma: a Meta-analysis. *The International Journal of Biological Markers*, 33(4), 447-454. <https://doi.org/10.1177/1724600818778756>
- Xu, F., Li, M., Zhang, C., Cui, J., Liu, J., Li, J., & Jiang, H. (2017). Clinicopathological and Prognostic Significance of COX-2 Immunohistochemical Expression in Breast Cancer: a Meta-Analysis. *Oncotarget*, 8(4), 6003-6012. <http://doi:10.18632/oncotarget.13990>
- Xu, K. M., Wang, L. F., & Shu, H. G. (2014). COX-2 Overexpression Increases Malignant Potential of Human Glioma Cells Through Id1. *Oncotarget*, 5(5), 1241-1252. <http://doi:10.18632/oncotarget.1370>

- Xu, L., Jiang, Y., Zheng, J., Xie, J., Li, J., Shi, L., & Fan, S. (2013). Aberrant Expression of  $\beta$ -catenin and E-cadherin is Correlated with Poor Prognosis of Nasopharyngeal Cancer. *Human Pathology*, 44(7), 1357-1364.
- Xue, W. P., Bai, S. M., Luo, M., Bi, Z. F., Liu, Y. M., & Wu, S. K. (2011). Phase I Clinical Trial of Nasopharyngeal Radiotherapy and Concurrent Celecoxib for Patients with Locoregionally Advanced Nasopharyngeal Carcinoma. *Oral Oncology*, 47(8), 753-757. <https://doi.org/10.1016/j.oraloncology.2011.06.002>
- Yang, B., Jia, L., Guo, Q. J., Ren, H., Hu, Y. P., & Xie, T. (2016). Clinicopathological and Prognostic Significance of Cyclooxygenase-2 Expression in Head and Neck Cancer: a Meta-analysis. *Oncotarget*, 7(30), 47265-47277. <https://doi.org/10.18632/oncotarget.10059>
- Yang, G., Deng, Q., Fan, W., Zhang, Z., Xu, P., Tang, S., ... Yu, M. (2017). Cyclooxygenase-2 Expression is Positively Associated with Lymph Node Metastasis in Nasopharyngeal Carcinoma. *PLoS ONE*, 12(3): e0173641-e0173659. <https://doi.org/10.1371/journal.pone.0173641>
- Yang, L., Wei, W. J., Zhou, L., Wang, J., & Hu, G. Y. (2019). High/positive Expression of ERCC1 Predicts Poor Treatment Response and Survival Prognosis in Nasopharyngeal Carcinoma: a Systematic Meta-Analysis from 21 Studies. *Medicine*, 98(21), 1-10. <https://doi.org/http://dx.doi.org/10.1097/MD.00000000000015641>
- Yang, M. H., Wu, M. Z., Chiou, S. H., Chen, P. M., Chang, S. Y., Liu, C. J., ... Wu, K. J. (2008). Direct Regulation of TWIST by HIF-1  $\alpha$  Promotes Metastasis. *Nature Cell Biology*, 10(3), 295-305. <https://doi.org/10.1038/ncb1691>
- Yeh, S. A., Tang, Y., Lui, C. C., Huang, Y. J., & Huang, E. Y. (2005). Treatment Outcomes and Late Complications of 849 Patients with Nasopharyngeal Carcinoma Treated with

- Radiotherapy Alone. *International Journal of Radiation Oncology Biology Physics*, 62(3), 672-679. <https://doi.org/10.1016/j.ijrobp.2004.11.002>
- Yeo, G. G., Beh, S. P., Tan, C. K., & Pei-Jye, V. (2018). Strong Family History of Nasopharyngeal Carcinoma (NPC) in Bidayuh, a Local Native Group in Sarawak, Malaysia. *Annals of Oncology*, 29(9), 1-6.
- Yew, P. Y., Mushiroda, T., Kiyotani, K., Govindasamy, G. K., Yap, L. F., Teo, S. H., ... Ng, C. C. (2011). Identification of a Functional Variant in SPLUNC1 Associated with Nasopharyngeal Carcinoma Susceptibility Among Malaysian Chinese. *Molecular Carcinogenesis*, 51, e74-e82. <https://doi.org/10.1002/mc.21857>
- Yoshizaki, T., Sato, H., Furukawa, M., & Pagano, J. S. (1998). The Expression of Matrix Metalloproteinase 9 is Enhanced by Epstein-Barr Virus Latent Membrane Protein 1. *Proceedings of the National Academy of Sciences*, 95(7), 3621-3626. <http://doi:10.1073/pnas.95.7.3621>
- Yoshizaki, T., Sato, H., Maruyama, Y., Muro, S., Furukawa, M., Park, C. S., ... Ph, D. (1997). Increased Expression of Membrane Type 1-Matrix Metalloproteinase in Head and Neck Carcinoma. *Cancer*, 79(1), 139-144. [http://doi:10.1002/\(sici\)1097-0142\(19970101\)79:1%3C139::aid-cnrc20%3E3.0.co;2-4](http://doi:10.1002/(sici)1097-0142(19970101)79:1%3C139::aid-cnrc20%3E3.0.co;2-4)
- You, B., Cao, X. L., Shao, X. Y., Ni, H. S., Shi, S., Shan, Y., ... You, Y. W. (2016). Clinical and Biological Significance of HAX-1 Overexpression in Nasopharyngeal Carcinoma. *Oncotarget*, 7(11), 12505–12524. <http://doi:10.18632/oncotarget.7274>.
- Young, L. S., & Rickinson, A. B. (2004). Epstein-Barr virus: 40 Years on. *Nature Reviews Cancer*, 4(10), 757-768. <https://doi.org/10.1038/nrc1452>
- Yu, M. C., Ho, J. H., Lai, S. H., & Henderson, B. E. (1986). Cantonese-style Salted Fish as a Cause of Nasopharyngeal Carcinoma: Report of a Case-Control Study in Hong Kong.

- Cancer Research*, 46(2), 956-961.
- Yu, M. C., Ho, J. H., Ross, R. K., & Henderson, B. E. (1981). Nasopharyngeal Carcinoma in Chinese -salted Fish or Inhaled Smoke? *Preventive Medicine*, 10(1), 15-24.
- Yu, M. C., Mo, C. C., Chong, W. X., Yeh, F. S., & Henderson, B. E. (1988). Preserved Foods and Nasopharyngeal Carcinoma: A Case-Control Study in Guangxi, China. *Cancer Research*, 48(7), 1954-1959.
- Yu, M. C., & Yuan, J. M. (2002). Epidemiology of Nasopharyngeal Carcinoma. *Seminars in Cancer Biology*, 12(6), 421-429. <http://doi:10.1016/s1044579x02000858>
- Yu, X., Zhen, Y., Yang, H., Wang, H., Zhou, Y., Wang, E., ... Fang, W. (2013). Loss of Connective Tissue Growth Factor as an Unfavorable Prognosis Factor Activates Mir-18b By PI3K/AKT/C-Jun and C-Myc and Promotes Cell Growth in Nasopharyngeal Carcinoma. *Cell Death and Disease*, 4, e634. <https://doi.org/10.1038/cddis.2013.153>
- Yuan, J. M., Wang, X. L., Xiang, Y. B., Gao, Y. T., Ross, R. K., & Yu, M. C. (2000). Preserved Foods in Relation to Risk of Nasopharyngeal Carcinoma in Shanghai, China. *International Journal of Cancer*, 85(3), 358-363. [https://doi.org/10.1002/\(SICI\)1097-0215\(20000201\)85:3%3C358::AID-IJC11%3E3.0.CO;2-E](https://doi.org/10.1002/(SICI)1097-0215(20000201)85:3%3C358::AID-IJC11%3E3.0.CO;2-E)
- Zang, J., Li, C., Zhao, L. N., Wang, J. H., Xu, M., Luo, S. Q., ... Shi, M. (2016). Prognostic Model of Death and Distant Metastasis for Nasopharyngeal Carcinoma Patients Receiving 3DCRT/IMRT in Nonendemic Area of China. *Medicine*, 95(21), e3794-e3801. <http://doi:10.1097/MD.00000000000003794>
- Zhai, C., Gu, K., Zhai, X., Wang, J., & Zhang, J. (2017). Prognostic Value of Serum Lactate Dehydrogenase in Patients with Nasopharyngeal Carcinoma: a Meta-Analysis. *Clinical Laboratory*, 63(11), 1777-1785.
- Zhai, X., Yang, Y., Wan, J., Zhu, R., & Wu, Y. (2013). Inhibition of LDH-A by Oxamate

- Induces G2/M arrest, Apoptosis and Increases Radiosensitivity in Nasopharyngeal Carcinoma Cells. *Oncology Reports*, 30(6), 2983-2991. <https://doi.org/10.3892/or.2013.2735>
- Zhang, C., Wu, J., Ye, J., & Xue, S. (2018). Association Between Expression of Lysyl Oxidase and Prognosis in Solid Tumor Patients: a Systematic Review and Meta-Analysis. *International Journal of Clinical Experimental Medicine*, 11(11), 11863-11875.
- Zhang, H., Xia, W., Lu, X., Sun, R., Wang, L., Zheng, L., ... Guo, X. (2013). A Novel Statistical Prognostic Score Model That Includes Serum CXCL5 Levels and Clinical Classification Predicts Risk of Disease Progression and Survival of Nasopharyngeal Carcinoma Patients. *PLoS One*, 8(2), e57830. <http://doi:10.1371/journal.pone.0057830>.
- Zhang, J., Shu, C., Song, Y., Li, Q., Huang, J., & Ma, X. (2016). Epstein-Barr Virus DNA Level as a novel Prognostic Factor in Nasopharyngeal Carcinoma: a Meta-Analysis. *Medicine*, 95(40), e5130. <http://doi:10.1097/MD.00000000000005130>
- Zhang, S., Huang, X., Zhou, L., & Lin, S. (2018). Efficacy of Concurrent Chemoradiotherapy Combined with Nimotuzumab for Low-Risk T4 Stage Nasopharyngeal Carcinoma. *Medicine*, 97(8), e12503-e12508. <http://doi:10.1097/MD.00000000000012503>
- Zhang, S. X., Qiu, Q. H., Chen, C. B., Liang, C. H., & Huang, B. (2014). Celecoxib Enhances Radiosensitivity via Induction of G2-M Phase Arrest and Apoptosis in Nasopharyngeal Carcinoma. *Cell Physiology and Biochemistry*, 33(5), 1484-1497. <https://doi.org/10.1159/000358713>
- Zhang, W. L., Zhou, Y. H., & Xiao, L. (2008). Biomarkers of Nasopharyngeal Carcinoma. *Progress in Biochemistry and Biophysics*, 35(1), 7-13.

- Zhao, L., Lin, Q., Gu, J., Zhang, H., Chen, H., & Lin, Q. (2019). A Nomogram for the Prediction of Prognosis in Patients with Distant Metastases of Nasopharyngeal Carcinoma. *Frontiers in Oncology*, 9(240), 1-9. <http://doi: 10.3389/fonc.2019.00240>
- Zhao, Z. S., Wang, Y. Y., Ye, Z. Y., & Tao, H. Q. (2009). Prognostic Value of Tumor-Related Molecular Expression in Gastric Carcinoma. *Pathology Oncology Research*, 15(4), 589-596. <https://doi.org/10.1007/s12253-009-9158-9>
- Zheng, F., Wei, L., Zhao, L., & Ni, F. C. (2018). Pathway Network Analysis of Complex Diseases Based on Multiple Biological Networks. *Hindawi*, 2018, 1-12. <https://doi.org/10.1155/2018/5670210>
- Zheng, X., Hu, L., Chen, F., & Christensson, B. (1994). Papers Expression of Ki67 Antigen, Epidermal Growth Factor Receptor and Epstein-Barr Virus-encoded Latent Membrane Protein in Nasopharyngeal Carcinoma. *European Journal of Cancer*, 30(5), 290-295.
- Zheng, X., Luo, Y., Christensson, B., & Drettner, B. (1994). Induction of Nasal and Nasopharyngeal Tumours in Sprague-Dawley Rats Fed with Chinese Salted Fish. *Acta Otolaryngol*, 114(1), 98-104.
- Zheng, Y. M., Tuppin, P., Hubert, A., Jeannel, D., Pan, Y. J., Zeng, Y., & De-The, G. (1994). Environmental and Dietary Risk Factors for Nasopharyngeal Carcinoma: Case-Control Study in Zangwu County, Guangxi, China. *British Journal of Cancer*, 69(3), 508-514. <http://doi: 10.1038/bjc.1994.92>
- Zhou, W., Feng, X. L., Li, H., Wang, L., Zhu, B., Liu, W. D., ... Ren, C. P. (2009). Inactivation of LARS2, Located at the Commonly Deleted Region 3p21.3, by Both Epigenetic And Genetic Mechanisms In Nasopharyngeal Carcinoma. *Acta Biochimica et Biophysica Sinica*, 41(1), 54-62. <https://doi.org/10.1093/abbs/gmn006>
- Zhou, X. F., Cui, J., Macias, V., Kajdacsy-Balla, A. A., Ye, H., Wang, J. G., & Rao, P. N.

- (2007). The Progress on Genetic Analysis of Nasopharyngeal Carcinoma. *Comparative and Functional Genomics*, 2007, 57513. <https://doi.org/10.1155/2007/57513>
- Zhu, H. M., Jiang, X. S., Li, H. Z., Qian, L. X., Du, M. Y., Lu, Z. W., ... Yin, L. (2018). miR-184 Inhibits Tumor Invasion, Migration and Metastasis in Nasopharyngeal Carcinoma by Targeting Notch2. *Cell Physiology and Biochemistry*, 49(4), 1564-1576. <https://doi.org/10.1159/000493458>
- Zong, Y. S. H. (2001). Pathological Diagnosing Study of Nasopharyngeal Carcinoma. *Shi Yong Zhong Liu Xue Za Zhi*, 16,1-3.

## APPENDICES

### Appendix A: PRISMA Statement

| Section/<br>Topic                  | #  | Checklist item                                                                                                                                                                                                                                                                                              | Reported on<br>page # |
|------------------------------------|----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| <b>TITLE</b>                       |    |                                                                                                                                                                                                                                                                                                             |                       |
| Title                              | 1  | Identify the report as a systematic review, meta-analysis, or both.                                                                                                                                                                                                                                         |                       |
| <b>ABSTRACT</b>                    |    |                                                                                                                                                                                                                                                                                                             |                       |
| Structured summary                 | 2  | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. |                       |
| <b>METHODS</b>                     |    |                                                                                                                                                                                                                                                                                                             |                       |
| Protocol and registration          | 5  | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.                                                                                                                               |                       |
| Eligibility criteria               | 6  | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.                                                                                                      |                       |
| Information sources                | 7  | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.                                                                                                                                  |                       |
| Search                             | 8  | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.                                                                                                                                                                               |                       |
| Study selection                    | 9  | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).                                                                                                                                                   |                       |
| Data collection process            | 10 | Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.                                                                                                                                  |                       |
| Data items                         | 11 | List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.                                                                                                                                                                       |                       |
| Risk of bias in individual studies | 12 | Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome                                                                                                                                                            |                       |

**Appendix A** continued

|                               |    |                                                                                                                                                                                                          |  |
|-------------------------------|----|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
|                               |    | level), and how this information is to be used in any data synthesis.                                                                                                                                    |  |
| Summary measures              | 13 | State the principal summary measures (e.g., risk ratio, difference in means).                                                                                                                            |  |
| Synthesis of results          | 14 | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$ ) for each meta-analysis.                                                |  |
|                               |    |                                                                                                                                                                                                          |  |
| Risk of bias across studies   | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).                                                             |  |
| Additional analyses           | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.                                                         |  |
| <b>RESULTS</b>                |    |                                                                                                                                                                                                          |  |
| Study selection               | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.                                          |  |
| Study characteristics         | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.                                                             |  |
| Risk of bias within studies   | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).                                                                                                |  |
| Results of individual studies | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. |  |
| Synthesis of results          | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency.                                                                                                  |  |
| Risk of bias across studies   | 22 | Present results of any assessment of risk of bias across studies (see Item 15).                                                                                                                          |  |
| Additional analysis           | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).                                                                                    |  |
| <b>DISCUSSION</b>             |    |                                                                                                                                                                                                          |  |
| Summary of evidence           | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).                     |  |
| Limitations                   | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g.,                                                                                                          |  |

**Appendix A** continued

|                |    |                                                                                                                                            |  |
|----------------|----|--------------------------------------------------------------------------------------------------------------------------------------------|--|
|                |    | incomplete retrieval of identified research, reporting bias).                                                                              |  |
| Conclusions    | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research.                    |  |
| <b>FUNDING</b> |    |                                                                                                                                            |  |
| Funding        | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. |  |

(Moher, Liberati, Tetzlaff, Altman, & The PRISMA Group, 2009)

## Appendix B: Examples of Outputs Using Multigene NPC Prognosis Assessment Model

|    | A                                        | B          | C    | D     | E        | F        | G        | H          | I     | J              | K | L | M | N               | O               | P | Q             | R              |
|----|------------------------------------------|------------|------|-------|----------|----------|----------|------------|-------|----------------|---|---|---|-----------------|-----------------|---|---------------|----------------|
| 1  |                                          |            |      |       |          |          |          |            |       |                |   |   |   |                 |                 |   |               |                |
| 2  |                                          |            |      |       |          |          |          |            |       |                |   |   |   |                 |                 |   |               |                |
| 3  |                                          |            |      |       | Lower CI | Upper CI | LN(LCI)- |            |       |                |   |   |   | Formula =       | Formula =       |   | NPC Prognosis |                |
| 4  | Tick if yes                              | Biomarkers | HR   | (LCI) | (UCI)    | LN(UCI)  | Variance | 1/Variance | LN HR | LN HR/Variance |   |   |   | IF(A6=True,M6,) | IF(A6=True,J6,) |   | Assessment    | Formula =      |
| 5  |                                          |            |      |       |          |          |          |            |       |                |   |   |   |                 |                 |   | Score         | IF(A6=TRUE,Q6) |
| 6  | <input type="checkbox"/> FALSE           | COX-2      | 2.02 | 1.65  | 2.47     | 0.40     | 0.01     | 94.41      | 0.70  | 66.38          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 7  | <input type="checkbox"/> FALSE           | LMP1       | 2.48 | 1.77  | 3.47     | 0.67     | 0.03     | 33.91      | 0.91  | 30.80          |   |   |   | 0.00            | 0.00            |   | 4             | 0              |
| 8  | <input type="checkbox"/> FALSE           | VEGF       | 2.07 | 1.32  | 3.25     | 0.90     | 0.05     | 18.93      | 0.73  | 13.77          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 9  | <input checked="" type="checkbox"/> TRUE | EGFR       | 1.86 | 1.25  | 2.77     | 0.80     | 0.04     | 24.27      | 0.62  | 15.06          |   |   |   | 15.06           | 24.27           |   | 3             | 3              |
| 10 | <input type="checkbox"/> FALSE           | LDH        | 2.86 | 1.66  | 2.08     | 0.23     | 0.00     | 302.05     | 1.05  | 317.41         |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 11 | <input type="checkbox"/> FALSE           | CRP        | 1.84 | 1.57  | 2.17     | 0.32     | 0.01     | 146.70     | 0.61  | 89.45          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 12 | <input type="checkbox"/> FALSE           | ERCC1      | 1.77 | 1.48  | 2.12     | 0.36     | 0.01     | 118.98     | 0.57  | 67.94          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 13 | <input type="checkbox"/> FALSE           | HIF-1a     | 1.77 | 1.35  | 2.32     | 0.54     | 0.02     | 52.41      | 0.57  | 29.93          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 14 | <input type="checkbox"/> FALSE           | LOX        | 1.68 | 1.52  | 1.87     | 0.21     | 0.00     | 357.83     | 0.52  | 185.64         |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 15 | <input type="checkbox"/> FALSE           | MMP9       | 1.65 | 1.38  | 1.95     | 0.35     | 0.01     | 128.55     | 0.50  | 64.37          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 16 |                                          |            |      |       |          |          |          |            |       |                |   |   |   | 15.06           | 24.27           |   |               | 3              |

**Figure B.1.1:** Output of multigene NPC prognosis assessment model when one biomarker was over expressed.

|    | A         | B    | C | D           | E    | F | G           | H    | I | J                                         | K |
|----|-----------|------|---|-------------|------|---|-------------|------|---|-------------------------------------------|---|
| 16 |           |      |   |             |      |   |             |      |   |                                           |   |
| 17 |           |      |   |             |      |   |             |      |   |                                           |   |
| 18 | LN(HR)    | 0.62 |   | LN Lower CI | 0.22 |   | LN Upper CI | 1.02 |   |                                           |   |
| 19 | Pooled HR | 1.86 |   | Lower CI    | 1.25 |   | Upper CI    | 2.77 |   | Total NPC Prognosis Assessment Risk Score | 3 |

**Figure B.1.2:** Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene NPC prognosis assessment model when one biomarker was over expressed.

## Appendix B continued

|    | A                                        | B          | C    | D        | E        | F        | G        | H          | I     | J              | K | L | M | N               | O               | P | Q             | R              |
|----|------------------------------------------|------------|------|----------|----------|----------|----------|------------|-------|----------------|---|---|---|-----------------|-----------------|---|---------------|----------------|
| 1  |                                          |            |      |          |          |          |          |            |       |                |   |   |   |                 |                 |   |               |                |
| 2  |                                          |            |      |          |          |          |          |            |       |                |   |   |   |                 |                 |   |               |                |
| 3  |                                          |            |      | Lower CI | Upper CI | LN(LCI)- |          |            |       |                |   |   |   | Formula =       | Formula =       |   | NPC Prognosis |                |
| 4  | Tick if yes                              | Biomarkers | HR   | (LCI)    | (UCI)    | LN(UCI)  | Variance | 1/Variance | LN HR | LN HR/Variance |   |   |   | IF(A6=True,M6,) | IF(A6=True,J6,) |   | Assessment    | Formula =      |
| 5  |                                          |            |      |          |          |          |          |            |       |                |   |   |   |                 |                 |   | Score         | IF(A6=TRUE,Q6) |
| 6  | <input type="checkbox"/> FALSE           | COX-2      | 2.02 | 1.65     | 2.47     | 0.40     | 0.01     | 94.41      | 0.70  | 66.38          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 7  | <input type="checkbox"/> FALSE           | LMP1       | 2.48 | 1.77     | 3.47     | 0.67     | 0.03     | 33.91      | 0.91  | 30.80          |   |   |   | 0.00            | 0.00            |   | 4             | 0              |
| 8  | <input type="checkbox"/> FALSE           | VEGF       | 2.07 | 1.32     | 3.25     | 0.90     | 0.05     | 18.93      | 0.73  | 13.77          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 9  | <input checked="" type="checkbox"/> TRUE | EGFR       | 1.86 | 1.25     | 2.77     | 0.80     | 0.04     | 24.27      | 0.62  | 15.06          |   |   |   | 15.06           | 24.27           |   | 3             | 3              |
| 10 | <input type="checkbox"/> FALSE           | LDH        | 2.86 | 1.66     | 2.08     | 0.23     | 0.00     | 302.05     | 1.05  | 317.41         |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 11 | <input type="checkbox"/> FALSE           | CRP        | 1.84 | 1.57     | 2.17     | 0.32     | 0.01     | 146.70     | 0.61  | 89.45          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 12 | <input type="checkbox"/> FALSE           | ERCC1      | 1.77 | 1.48     | 2.12     | 0.36     | 0.01     | 118.98     | 0.57  | 67.94          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 13 | <input checked="" type="checkbox"/> TRUE | HIF-1a     | 1.77 | 1.35     | 2.32     | 0.54     | 0.02     | 52.41      | 0.57  | 29.93          |   |   |   | 29.93           | 52.41           |   | 3             | 3              |
| 14 | <input type="checkbox"/> FALSE           | LOX        | 1.68 | 1.52     | 1.87     | 0.21     | 0.00     | 357.83     | 0.52  | 185.64         |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 15 | <input type="checkbox"/> FALSE           | MMP9       | 1.65 | 1.38     | 1.95     | 0.35     | 0.01     | 128.55     | 0.50  | 64.37          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 16 |                                          |            |      |          |          |          |          |            |       |                |   |   |   | 44.99           | 76.68           |   |               | 6              |

**Figure B.2.1:** Output of multigene NPC prognosis assessment model when two biomarkers were over expressed.

|    | A         | B    | C           | D    | E           | F    | G                                         | H | I | J | K |
|----|-----------|------|-------------|------|-------------|------|-------------------------------------------|---|---|---|---|
| 16 |           |      |             |      |             |      |                                           |   |   |   |   |
| 17 |           |      |             |      |             |      |                                           |   |   |   |   |
| 18 | LN(HR)    | 0.59 | LN Lower CI | 0.36 | LN Upper CI | 0.81 |                                           |   |   |   |   |
| 19 | Pooled HR | 1.80 | Lower CI    | 1.44 | Upper CI    | 2.25 | Total NPC Prognosis Assessment Risk Score |   |   |   | 6 |

**Figure B.2.2:** Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene NPC prognosis assessment model when two biomarkers were over expressed.

## Appendix B continued

|    | A                                        | B          | C    | D        | E        | F        | G        | H          | I     | J              | K               | L               | M | N         | O         | P | Q             | R              |
|----|------------------------------------------|------------|------|----------|----------|----------|----------|------------|-------|----------------|-----------------|-----------------|---|-----------|-----------|---|---------------|----------------|
| 1  |                                          |            |      |          |          |          |          |            |       |                |                 |                 |   |           |           |   |               |                |
| 2  |                                          |            |      |          |          |          |          |            |       |                |                 |                 |   |           |           |   |               |                |
| 3  |                                          |            |      | Lower CI | Upper CI | LN(LCI)- |          |            |       |                |                 |                 |   | Formula = | Formula = |   | NPC Prognosis |                |
| 4  | Tick if yes                              | Biomarkers | HR   | (LCI)    | (UCI)    | LN(UCI)  | Variance | 1/Variance | LN HR | LN HR/Variance | IF(A6=True,M6,) | IF(A6=True,J6,) |   |           |           |   | Assessment    | Formula =      |
| 5  |                                          |            |      |          |          |          |          |            |       |                |                 |                 |   |           |           |   | Score         | IF(A6=TRUE,Q6) |
| 6  | <input type="checkbox"/> FALSE           | COX-2      | 2.02 | 1.65     | 2.47     | 0.40     | 0.01     | 94.41      | 0.70  | 66.38          | 0.00            | 0.00            |   |           |           |   | 3             | 0              |
| 7  | <input type="checkbox"/> FALSE           | LMP1       | 2.48 | 1.77     | 3.47     | 0.67     | 0.03     | 33.91      | 0.91  | 30.80          | 0.00            | 0.00            |   |           |           |   | 4             | 0              |
| 8  | <input type="checkbox"/> FALSE           | VEGF       | 2.07 | 1.32     | 3.25     | 0.90     | 0.05     | 18.93      | 0.73  | 13.77          | 0.00            | 0.00            |   |           |           |   | 3             | 0              |
| 9  | <input checked="" type="checkbox"/> TRUE | EGFR       | 1.86 | 1.25     | 2.77     | 0.80     | 0.04     | 24.27      | 0.62  | 15.06          | 15.06           | 24.27           |   |           |           |   | 3             | 3              |
| 10 | <input type="checkbox"/> FALSE           | LDH        | 2.86 | 1.66     | 2.08     | 0.23     | 0.00     | 302.05     | 1.05  | 317.41         | 0.00            | 0.00            |   |           |           |   | 3             | 0              |
| 11 | <input type="checkbox"/> FALSE           | CRP        | 1.84 | 1.57     | 2.17     | 0.32     | 0.01     | 146.70     | 0.61  | 89.45          | 0.00            | 0.00            |   |           |           |   | 3             | 0              |
| 12 | <input type="checkbox"/> FALSE           | ERCC1      | 1.77 | 1.48     | 2.12     | 0.36     | 0.01     | 118.98     | 0.57  | 67.94          | 0.00            | 0.00            |   |           |           |   | 3             | 0              |
| 13 | <input checked="" type="checkbox"/> TRUE | HIF-1a     | 1.77 | 1.35     | 2.32     | 0.54     | 0.02     | 52.41      | 0.57  | 29.93          | 29.93           | 52.41           |   |           |           |   | 3             | 3              |
| 14 | <input type="checkbox"/> FALSE           | LOX        | 1.68 | 1.52     | 1.87     | 0.21     | 0.00     | 357.83     | 0.52  | 185.64         | 0.00            | 0.00            |   |           |           |   | 3             | 0              |
| 15 | <input checked="" type="checkbox"/> TRUE | MMP9       | 1.65 | 1.38     | 1.95     | 0.35     | 0.01     | 128.55     | 0.50  | 64.37          | 64.37           | 128.55          |   |           |           |   | 3             | 3              |
| 16 |                                          |            |      |          |          |          |          |            |       |                | 109.36          | 205.23          |   |           |           |   |               | 9              |

**Figure B.3.1:** Output of multigene NPC prognosis assessment model when three biomarkers were over expressed.

|    | A         | B    | C | D           | E    | F | G           | H    | I | J                                         | K |
|----|-----------|------|---|-------------|------|---|-------------|------|---|-------------------------------------------|---|
| 16 |           |      |   |             |      |   |             |      |   |                                           |   |
| 17 |           |      |   |             |      |   |             |      |   |                                           |   |
| 18 | LN(HR)    | 0.53 |   | LN Lower CI | 0.40 |   | LN Upper CI | 0.67 |   |                                           |   |
| 19 | Pooled HR | 1.70 |   | Lower CI    | 1.49 |   | Upper CI    | 1.95 |   | Total NPC Prognosis Assessment Risk Score | 9 |

**Figure B.3.2:** Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene NPC prognosis assessment model when three biomarkers were over expressed.

## Appendix B continued

|    | A                                   | B          | C      | D        | E        | F        | G        | H          | I      | J              | K      | L | M      | N               | O               | P | Q             | R              |
|----|-------------------------------------|------------|--------|----------|----------|----------|----------|------------|--------|----------------|--------|---|--------|-----------------|-----------------|---|---------------|----------------|
| 1  |                                     |            |        |          |          |          |          |            |        |                |        |   |        |                 |                 |   |               |                |
| 2  |                                     |            |        |          |          |          |          |            |        |                |        |   |        |                 |                 |   | NPC Prognosis |                |
| 3  |                                     |            |        | Lower CI | Upper CI | LN(LCI)- |          |            |        |                |        |   |        | Formula =       | Formula =       |   |               | Assessment     |
| 4  | Tick if yes                         | Biomarkers | HR     | (LCI)    | (UCI)    | LN(UCI)  | Variance | 1/Variance | LN HR  | LN HR/Variance |        |   |        | IF(A6=True,M6,) | IF(A6=True,J6,) |   | Score         | IF(A6=TRUE,Q6) |
| 5  |                                     |            |        |          |          |          |          |            |        |                |        |   |        |                 |                 |   |               |                |
| 6  | <input checked="" type="checkbox"/> | TRUE       | COX-2  | 2.02     | 1.65     | 2.47     | 0.40     | 0.01       | 94.41  | 0.70           | 66.38  |   | 66.38  | 94.41           |                 | 3 | 3             |                |
| 7  | <input type="checkbox"/>            | FALSE      | LMP1   | 2.48     | 1.77     | 3.47     | 0.67     | 0.03       | 33.91  | 0.91           | 30.80  |   | 0.00   | 0.00            |                 | 4 | 0             |                |
| 8  | <input type="checkbox"/>            | FALSE      | VEGF   | 2.07     | 1.32     | 3.25     | 0.90     | 0.05       | 18.93  | 0.73           | 13.77  |   | 0.00   | 0.00            |                 | 3 | 0             |                |
| 9  | <input checked="" type="checkbox"/> | TRUE       | EGFR   | 1.86     | 1.25     | 2.77     | 0.80     | 0.04       | 24.27  | 0.62           | 15.06  |   | 15.06  | 24.27           |                 | 3 | 3             |                |
| 10 | <input type="checkbox"/>            | FALSE      | LDH    | 2.86     | 1.66     | 2.08     | 0.23     | 0.00       | 302.05 | 1.05           | 317.41 |   | 0.00   | 0.00            |                 | 3 | 0             |                |
| 11 | <input type="checkbox"/>            | FALSE      | CRP    | 1.84     | 1.57     | 2.17     | 0.32     | 0.01       | 146.70 | 0.61           | 89.45  |   | 0.00   | 0.00            |                 | 3 | 0             |                |
| 12 | <input type="checkbox"/>            | FALSE      | ERCC1  | 1.77     | 1.48     | 2.12     | 0.36     | 0.01       | 118.98 | 0.57           | 67.94  |   | 0.00   | 0.00            |                 | 3 | 0             |                |
| 13 | <input checked="" type="checkbox"/> | TRUE       | HIF-1a | 1.77     | 1.35     | 2.32     | 0.54     | 0.02       | 52.41  | 0.57           | 29.93  |   | 29.93  | 52.41           |                 | 3 | 3             |                |
| 14 | <input type="checkbox"/>            | FALSE      | LOX    | 1.68     | 1.52     | 1.87     | 0.21     | 0.00       | 357.83 | 0.52           | 185.64 |   | 0.00   | 0.00            |                 | 3 | 0             |                |
| 15 | <input checked="" type="checkbox"/> | TRUE       | MMP9   | 1.65     | 1.38     | 1.95     | 0.35     | 0.01       | 128.55 | 0.50           | 64.37  |   | 64.37  | 128.55          |                 | 3 | 3             |                |
| 16 |                                     |            |        |          |          |          |          |            |        |                |        |   | 175.74 | 299.64          |                 |   | 12            |                |

**Figure B.4.1:** Output of multigene NPC prognosis assessment model when four biomarkers were over expressed.

|    | A         | B    | C           | D    | E           | F    | G                                         | H | I | J | K  |
|----|-----------|------|-------------|------|-------------|------|-------------------------------------------|---|---|---|----|
| 16 |           |      |             |      |             |      |                                           |   |   |   |    |
| 17 |           |      |             |      |             |      |                                           |   |   |   |    |
| 18 | LN(HR)    | 0.59 | LN Lower CI | 0.47 | LN Upper CI | 0.70 |                                           |   |   |   |    |
| 19 | Pooled HR | 1.80 | Lower CI    | 1.61 | Upper CI    | 2.01 | Total NPC Prognosis Assessment Risk Score |   |   |   | 12 |

**Figure B.4.2:** Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene NPC prognosis assessment model when four biomarkers were over expressed.

## Appendix B continued

|    | A                                        | B          | C    | D        | E        | F        | G        | H          | I     | J              | K | L | M | N               | O               | P | Q             | R              |
|----|------------------------------------------|------------|------|----------|----------|----------|----------|------------|-------|----------------|---|---|---|-----------------|-----------------|---|---------------|----------------|
| 1  |                                          |            |      |          |          |          |          |            |       |                |   |   |   |                 |                 |   |               |                |
| 2  |                                          |            |      |          |          |          |          |            |       |                |   |   |   |                 |                 |   |               |                |
| 3  |                                          |            |      | Lower CI | Upper CI | LN(LCI)- |          |            |       |                |   |   |   | Formula =       | Formula =       |   | NPC Prognosis |                |
| 4  | Tick if yes                              | Biomarkers | HR   | (LCI)    | (UCI)    | LN(UCI)  | Variance | 1/Variance | LN HR | LN HR/Variance |   |   |   | IF(A6=True,M6,) | IF(A6=True,J6,) |   | Assessment    | Formula =      |
| 5  |                                          |            |      |          |          |          |          |            |       |                |   |   |   |                 |                 |   | Score         | IF(A6=TRUE,Q6) |
| 6  | <input checked="" type="checkbox"/> TRUE | COX-2      | 2.02 | 1.65     | 2.47     | 0.40     | 0.01     | 94.41      | 0.70  | 66.38          |   |   |   | 66.38           | 94.41           |   | 3             | 3              |
| 7  | <input type="checkbox"/> FALSE           | LMP1       | 2.48 | 1.77     | 3.47     | 0.67     | 0.03     | 33.91      | 0.91  | 30.80          |   |   |   | 0.00            | 0.00            |   | 4             | 0              |
| 8  | <input checked="" type="checkbox"/> TRUE | VEGF       | 2.07 | 1.32     | 3.25     | 0.90     | 0.05     | 18.93      | 0.73  | 13.77          |   |   |   | 13.77           | 18.93           |   | 3             | 3              |
| 9  | <input checked="" type="checkbox"/> TRUE | EGFR       | 1.86 | 1.25     | 2.77     | 0.80     | 0.04     | 24.27      | 0.62  | 15.06          |   |   |   | 15.06           | 24.27           |   | 3             | 3              |
| 10 | <input type="checkbox"/> FALSE           | LDH        | 2.86 | 1.66     | 2.08     | 0.23     | 0.00     | 302.05     | 1.05  | 317.41         |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 11 | <input type="checkbox"/> FALSE           | CRP        | 1.84 | 1.57     | 2.17     | 0.32     | 0.01     | 146.70     | 0.61  | 89.45          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 12 | <input type="checkbox"/> FALSE           | ERCC1      | 1.77 | 1.48     | 2.12     | 0.36     | 0.01     | 118.98     | 0.57  | 67.94          |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 13 | <input checked="" type="checkbox"/> TRUE | HIF-1a     | 1.77 | 1.35     | 2.32     | 0.54     | 0.02     | 52.41      | 0.57  | 29.93          |   |   |   | 29.93           | 52.41           |   | 3             | 3              |
| 14 | <input type="checkbox"/> FALSE           | LOX        | 1.68 | 1.52     | 1.87     | 0.21     | 0.00     | 357.83     | 0.52  | 185.64         |   |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 15 | <input checked="" type="checkbox"/> TRUE | MMP9       | 1.65 | 1.38     | 1.95     | 0.35     | 0.01     | 128.55     | 0.50  | 64.37          |   |   |   | 64.37           | 128.55          |   | 3             | 3              |
| 16 |                                          |            |      |          |          |          |          |            |       |                |   |   |   | 189.51          | 318.56          |   |               | 15             |

**Figure B.5.1:** Output of multigene NPC prognosis assessment model when five biomarkers were over expressed.

|    | A         | B    | C           | D    | E           | F    | G                                         | H | I | J | K  |
|----|-----------|------|-------------|------|-------------|------|-------------------------------------------|---|---|---|----|
| 16 |           |      |             |      |             |      |                                           |   |   |   |    |
| 17 |           |      |             |      |             |      |                                           |   |   |   |    |
| 18 | LN(HR)    | 0.59 | LN Lower CI | 0.49 | LN Upper CI | 0.70 |                                           |   |   |   |    |
| 19 | Pooled HR | 1.81 | Lower CI    | 1.62 | Upper CI    | 2.02 | Total NPC Prognosis Assessment Risk Score |   |   |   | 15 |

**Figure B.5.2:** Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene NPC prognosis assessment model when five biomarkers were over expressed.

## Appendix B continued

|    | A                                   | B          | C      | D        | E        | F        | G        | H          | I      | J              | K      | L | M | N               | O               | P | Q             | R              |
|----|-------------------------------------|------------|--------|----------|----------|----------|----------|------------|--------|----------------|--------|---|---|-----------------|-----------------|---|---------------|----------------|
| 1  |                                     |            |        |          |          |          |          |            |        |                |        |   |   |                 |                 |   |               |                |
| 2  |                                     |            |        |          |          |          |          |            |        |                |        |   |   |                 |                 |   |               |                |
| 3  |                                     |            |        | Lower CI | Upper CI | LN(LCI)- |          |            |        |                |        |   |   | Formula =       | Formula =       |   | NPC Prognosis |                |
| 4  | Tick if yes                         | Biomarkers | HR     | (LCI)    | (UCI)    | LN(UCI)  | Variance | 1/Variance | LN HR  | LN HR/Variance |        |   |   | IF(A6=True,M6,) | IF(A6=True,J6,) |   | Assessment    | Formula =      |
| 5  |                                     |            |        |          |          |          |          |            |        |                |        |   |   |                 |                 |   | Score         | IF(A6=TRUE,Q6) |
| 6  | <input checked="" type="checkbox"/> | TRUE       | COX-2  | 2.02     | 1.65     | 2.47     | 0.40     | 0.01       | 94.41  | 0.70           | 66.38  |   |   | 66.38           | 94.41           |   | 3             | 3              |
| 7  | <input type="checkbox"/>            | FALSE      | LMP1   | 2.48     | 1.77     | 3.47     | 0.67     | 0.03       | 33.91  | 0.91           | 30.80  |   |   | 0.00            | 0.00            |   | 4             | 0              |
| 8  | <input checked="" type="checkbox"/> | TRUE       | VEGF   | 2.07     | 1.32     | 3.25     | 0.90     | 0.05       | 18.93  | 0.73           | 13.77  |   |   | 13.77           | 18.93           |   | 3             | 3              |
| 9  | <input checked="" type="checkbox"/> | TRUE       | EGFR   | 1.86     | 1.25     | 2.77     | 0.80     | 0.04       | 24.27  | 0.62           | 15.06  |   |   | 15.06           | 24.27           |   | 3             | 3              |
| 10 | <input type="checkbox"/>            | FALSE      | LDH    | 2.86     | 1.66     | 2.08     | 0.23     | 0.00       | 302.05 | 1.05           | 317.41 |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 11 | <input checked="" type="checkbox"/> | TRUE       | CRP    | 1.84     | 1.57     | 2.17     | 0.32     | 0.01       | 146.70 | 0.61           | 89.45  |   |   | 89.45           | 146.70          |   | 3             | 3              |
| 12 | <input type="checkbox"/>            | FALSE      | ERCC1  | 1.77     | 1.48     | 2.12     | 0.36     | 0.01       | 118.98 | 0.57           | 67.94  |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 13 | <input checked="" type="checkbox"/> | TRUE       | HIF-1a | 1.77     | 1.35     | 2.32     | 0.54     | 0.02       | 52.41  | 0.57           | 29.93  |   |   | 29.93           | 52.41           |   | 3             | 3              |
| 14 | <input type="checkbox"/>            | FALSE      | LOX    | 1.68     | 1.52     | 1.87     | 0.21     | 0.00       | 357.83 | 0.52           | 185.64 |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 15 | <input checked="" type="checkbox"/> | TRUE       | MMP9   | 1.65     | 1.38     | 1.95     | 0.35     | 0.01       | 128.55 | 0.50           | 64.37  |   |   | 64.37           | 128.55          |   | 3             | 3              |
| 16 |                                     |            |        |          |          |          |          |            |        |                |        |   |   | 278.96          | 465.26          |   |               | 18             |

**Figure B.6.1:** Output of multigene NPC prognosis assessment model when six biomarkers were over expressed.

|    | A         | B    | C | D           | E    | F | G           | H    | I | J                                         | K  |
|----|-----------|------|---|-------------|------|---|-------------|------|---|-------------------------------------------|----|
| 16 |           |      |   |             |      |   |             |      |   |                                           |    |
| 17 |           |      |   |             |      |   |             |      |   |                                           |    |
| 18 | LN(HR)    | 0.60 |   | LN Lower CI | 0.51 |   | LN Upper CI | 0.69 |   |                                           |    |
| 19 | Pooled HR | 1.82 |   | Lower CI    | 1.66 |   | Upper CI    | 1.99 |   | Total NPC Prognosis Assessment Risk Score | 18 |

**Figure B.6.2:** Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene NPC prognosis assessment model when six biomarkers were over expressed.

## Appendix B continued

|    | A                                   | B          | C      | D        | E        | F        | G        | H          | I      | J              | K      | L | M      | N               | O               | P | Q             | R              |
|----|-------------------------------------|------------|--------|----------|----------|----------|----------|------------|--------|----------------|--------|---|--------|-----------------|-----------------|---|---------------|----------------|
| 1  |                                     |            |        |          |          |          |          |            |        |                |        |   |        |                 |                 |   |               |                |
| 2  |                                     |            |        |          |          |          |          |            |        |                |        |   |        |                 |                 |   |               |                |
| 3  |                                     |            |        | Lower CI | Upper CI | LN(LCI)- |          |            |        |                |        |   |        | Formula =       | Formula =       |   | NPC Prognosis |                |
| 4  | Tick if yes                         | Biomarkers | HR     | (LCI)    | (UCI)    | LN(UCI)  | Variance | 1/Variance | LN HR  | LN HR/Variance |        |   |        | IF(A6=True,M6,) | IF(A6=True,J6,) |   | Assessment    | Formula =      |
| 5  |                                     |            |        |          |          |          |          |            |        |                |        |   |        |                 |                 |   | Score         | IF(A6=TRUE,Q6) |
| 6  | <input checked="" type="checkbox"/> | TRUE       | COX-2  | 2.02     | 1.65     | 2.47     | 0.40     | 0.01       | 94.41  | 0.70           | 66.38  |   | 66.38  | 66.38           | 94.41           |   | 3             | 3              |
| 7  | <input checked="" type="checkbox"/> | TRUE       | LMP1   | 2.48     | 1.77     | 3.47     | 0.67     | 0.03       | 33.91  | 0.91           | 30.80  |   | 30.80  | 30.80           | 33.91           |   | 4             | 4              |
| 8  | <input checked="" type="checkbox"/> | TRUE       | VEGF   | 2.07     | 1.32     | 3.25     | 0.90     | 0.05       | 18.93  | 0.73           | 13.77  |   | 13.77  | 18.93           | 18.93           |   | 3             | 3              |
| 9  | <input checked="" type="checkbox"/> | TRUE       | EGFR   | 1.86     | 1.25     | 2.77     | 0.80     | 0.04       | 24.27  | 0.62           | 15.06  |   | 15.06  | 15.06           | 24.27           |   | 3             | 3              |
| 10 | <input type="checkbox"/>            | FALSE      | LDH    | 2.86     | 1.66     | 2.08     | 0.23     | 0.00       | 302.05 | 1.05           | 317.41 |   | 0.00   | 0.00            |                 |   | 3             | 0              |
| 11 | <input checked="" type="checkbox"/> | TRUE       | CRP    | 1.84     | 1.57     | 2.17     | 0.32     | 0.01       | 146.70 | 0.61           | 89.45  |   | 89.45  | 146.70          |                 |   | 3             | 3              |
| 12 | <input type="checkbox"/>            | FALSE      | ERCC1  | 1.77     | 1.48     | 2.12     | 0.36     | 0.01       | 118.98 | 0.57           | 67.94  |   | 0.00   | 0.00            |                 |   | 3             | 0              |
| 13 | <input checked="" type="checkbox"/> | TRUE       | HIF-1a | 1.77     | 1.35     | 2.32     | 0.54     | 0.02       | 52.41  | 0.57           | 29.93  |   | 29.93  | 52.41           |                 |   | 3             | 3              |
| 14 | <input type="checkbox"/>            | FALSE      | LOX    | 1.68     | 1.52     | 1.87     | 0.21     | 0.00       | 357.83 | 0.52           | 185.64 |   | 0.00   | 0.00            |                 |   | 3             | 0              |
| 15 | <input checked="" type="checkbox"/> | TRUE       | MMP9   | 1.65     | 1.38     | 1.95     | 0.35     | 0.01       | 128.55 | 0.50           | 64.37  |   | 64.37  | 128.55          |                 |   | 3             | 3              |
| 16 |                                     |            |        |          |          |          |          |            |        |                |        |   | 309.76 | 499.17          |                 |   |               | 22             |

**Figure B.7.1:** Output of multigene NPC prognosis assessment model when seven biomarkers were over expressed.

|    | A         | B    | C | D           | E    | F | G           | H    | I | J                                         | K  |
|----|-----------|------|---|-------------|------|---|-------------|------|---|-------------------------------------------|----|
| 16 |           |      |   |             |      |   |             |      |   |                                           |    |
| 17 |           |      |   |             |      |   |             |      |   |                                           |    |
| 18 | LN(HR)    | 0.62 |   | LN Lower CI | 0.53 |   | LN Upper CI | 0.71 |   |                                           |    |
| 19 | Pooled HR | 1.86 |   | Lower CI    | 1.70 |   | Upper CI    | 2.03 |   | Total NPC Prognosis Assessment Risk Score | 22 |

**Figure B.7.2:** Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene NPC prognosis assessment model when seven biomarkers were over expressed.

## Appendix B continued

|    | A                                   | B          | C      | D        | E        | F        | G        | H          | I      | J              | K      | L | M | N               | O               | P | Q             | R              |
|----|-------------------------------------|------------|--------|----------|----------|----------|----------|------------|--------|----------------|--------|---|---|-----------------|-----------------|---|---------------|----------------|
| 1  |                                     |            |        |          |          |          |          |            |        |                |        |   |   |                 |                 |   |               |                |
| 2  |                                     |            |        |          |          |          |          |            |        |                |        |   |   |                 |                 |   |               |                |
| 3  |                                     |            |        | Lower CI | Upper CI | LN(LCI)- |          |            |        |                |        |   |   | Formula =       | Formula =       |   | NPC Prognosis |                |
| 4  | Tick if yes                         | Biomarkers | HR     | (LCI)    | (UCI)    | LN(UCI)  | Variance | 1/Variance | LN HR  | LN HR/Variance |        |   |   | IF(A6=True,M6,) | IF(A6=True,J6,) |   | Assessment    | Formula =      |
| 5  |                                     |            |        |          |          |          |          |            |        |                |        |   |   |                 |                 |   | Score         | IF(A6=TRUE,Q6) |
| 6  | <input checked="" type="checkbox"/> | TRUE       | COX-2  | 2.02     | 1.65     | 2.47     | 0.40     | 0.01       | 94.41  | 0.70           | 66.38  |   |   | 66.38           | 94.41           |   | 3             | 3              |
| 7  | <input checked="" type="checkbox"/> | TRUE       | LMP1   | 2.48     | 1.77     | 3.47     | 0.67     | 0.03       | 33.91  | 0.91           | 30.80  |   |   | 30.80           | 33.91           |   | 4             | 4              |
| 8  | <input checked="" type="checkbox"/> | TRUE       | VEGF   | 2.07     | 1.32     | 3.25     | 0.90     | 0.05       | 18.93  | 0.73           | 13.77  |   |   | 13.77           | 18.93           |   | 3             | 3              |
| 9  | <input checked="" type="checkbox"/> | TRUE       | EGFR   | 1.86     | 1.25     | 2.77     | 0.80     | 0.04       | 24.27  | 0.62           | 15.06  |   |   | 15.06           | 24.27           |   | 3             | 3              |
| 10 | <input checked="" type="checkbox"/> | TRUE       | LDH    | 2.86     | 1.66     | 2.08     | 0.23     | 0.00       | 302.05 | 1.05           | 317.41 |   |   | 317.41          | 302.05          |   | 3             | 3              |
| 11 | <input checked="" type="checkbox"/> | TRUE       | CRP    | 1.84     | 1.57     | 2.17     | 0.32     | 0.01       | 146.70 | 0.61           | 89.45  |   |   | 89.45           | 146.70          |   | 3             | 3              |
| 12 | <input type="checkbox"/>            | FALSE      | ERCC1  | 1.77     | 1.48     | 2.12     | 0.36     | 0.01       | 118.98 | 0.57           | 67.94  |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 13 | <input checked="" type="checkbox"/> | TRUE       | HIF-1a | 1.77     | 1.35     | 2.32     | 0.54     | 0.02       | 52.41  | 0.57           | 29.93  |   |   | 29.93           | 52.41           |   | 3             | 3              |
| 14 | <input type="checkbox"/>            | FALSE      | LOX    | 1.68     | 1.52     | 1.87     | 0.21     | 0.00       | 357.83 | 0.52           | 185.64 |   |   | 0.00            | 0.00            |   | 3             | 0              |
| 15 | <input checked="" type="checkbox"/> | TRUE       | MMP9   | 1.65     | 1.38     | 1.95     | 0.35     | 0.01       | 128.55 | 0.50           | 64.37  |   |   | 64.37           | 128.55          |   | 3             | 3              |
| 16 |                                     |            |        |          |          |          |          |            |        |                |        |   |   | 627.16          | 801.22          |   |               | 25             |

**Figure B.8.1:** Output of multigene NPC prognosis assessment model when eight biomarkers were over expressed.

|    | A         | B    | C           | D    | E           | F    | G                                         | H | I | J | K  |
|----|-----------|------|-------------|------|-------------|------|-------------------------------------------|---|---|---|----|
| 16 |           |      |             |      |             |      |                                           |   |   |   |    |
| 17 |           |      |             |      |             |      |                                           |   |   |   |    |
| 18 | LN(HR)    | 0.78 | LN Lower CI | 0.71 | LN Upper CI | 0.85 |                                           |   |   |   |    |
| 19 | Pooled HR | 2.19 | Lower CI    | 2.04 | Upper CI    | 2.34 | Total NPC Prognosis Assessment Risk Score |   |   |   | 25 |

**Figure B.8.2:** Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene NPC prognosis assessment model when eight biomarkers were over expressed.

## Appendix B continued

|    | A                                   | B          | C      | D        | E        | F        | G        | H          | I      | J              | K      | L | M      | N               | O               | P | Q             | R              |
|----|-------------------------------------|------------|--------|----------|----------|----------|----------|------------|--------|----------------|--------|---|--------|-----------------|-----------------|---|---------------|----------------|
| 1  |                                     |            |        |          |          |          |          |            |        |                |        |   |        |                 |                 |   |               |                |
| 2  |                                     |            |        |          |          |          |          |            |        |                |        |   |        |                 |                 |   |               |                |
| 3  |                                     |            |        | Lower CI | Upper CI | LN(LCI)- |          |            |        |                |        |   |        | Formula =       | Formula =       |   | NPC Prognosis |                |
| 4  | Tick if yes                         | Biomarkers | HR     | (LCI)    | (UCI)    | LN(UCI)  | Variance | 1/Variance | LN HR  | LN HR/Variance |        |   |        | IF(A6=True,M6,) | IF(A6=True,J6,) |   | Assessment    | Formula =      |
| 5  |                                     |            |        |          |          |          |          |            |        |                |        |   |        |                 |                 |   | Score         | IF(A6=TRUE,Q6) |
| 6  | <input checked="" type="checkbox"/> | TRUE       | COX-2  | 2.02     | 1.65     | 2.47     | 0.40     | 0.01       | 94.41  | 0.70           | 66.38  |   | 66.38  | 66.38           | 94.41           |   | 3             | 3              |
| 7  | <input checked="" type="checkbox"/> | TRUE       | LMP1   | 2.48     | 1.77     | 3.47     | 0.67     | 0.03       | 33.91  | 0.91           | 30.80  |   | 30.80  | 30.80           | 33.91           |   | 4             | 4              |
| 8  | <input checked="" type="checkbox"/> | TRUE       | VEGF   | 2.07     | 1.32     | 3.25     | 0.90     | 0.05       | 18.93  | 0.73           | 13.77  |   | 13.77  | 13.77           | 18.93           |   | 3             | 3              |
| 9  | <input checked="" type="checkbox"/> | TRUE       | EGFR   | 1.86     | 1.25     | 2.77     | 0.80     | 0.04       | 24.27  | 0.62           | 15.06  |   | 15.06  | 15.06           | 24.27           |   | 3             | 3              |
| 10 | <input checked="" type="checkbox"/> | TRUE       | LDH    | 2.86     | 1.66     | 2.08     | 0.23     | 0.00       | 302.05 | 1.05           | 317.41 |   | 317.41 | 302.05          |                 |   | 3             | 3              |
| 11 | <input checked="" type="checkbox"/> | TRUE       | CRP    | 1.84     | 1.57     | 2.17     | 0.32     | 0.01       | 146.70 | 0.61           | 89.45  |   | 89.45  | 146.70          |                 |   | 3             | 3              |
| 12 | <input checked="" type="checkbox"/> | TRUE       | ERCC1  | 1.77     | 1.48     | 2.12     | 0.36     | 0.01       | 118.98 | 0.57           | 67.94  |   | 67.94  | 118.98          |                 |   | 3             | 3              |
| 13 | <input checked="" type="checkbox"/> | TRUE       | HIF-1a | 1.77     | 1.35     | 2.32     | 0.54     | 0.02       | 52.41  | 0.57           | 29.93  |   | 29.93  | 52.41           |                 |   | 3             | 3              |
| 14 | <input type="checkbox"/>            | FALSE      | LOX    | 1.68     | 1.52     | 1.87     | 0.21     | 0.00       | 357.83 | 0.52           | 185.64 |   | 0.00   | 0.00            |                 |   | 3             | 0              |
| 15 | <input checked="" type="checkbox"/> | TRUE       | MMP9   | 1.65     | 1.38     | 1.95     | 0.35     | 0.01       | 128.55 | 0.50           | 64.37  |   | 64.37  | 128.55          |                 |   | 3             | 3              |
| 16 |                                     |            |        |          |          |          |          |            |        |                |        |   | 695.10 | 920.20          |                 |   |               | 28             |

**Figure B.9.1:** Output of multigene NPC prognosis assessment model when nine biomarkers were over expressed.

|    | A         | B    | C | D           | E    | F | G           | H    | I | J                                         | K  |
|----|-----------|------|---|-------------|------|---|-------------|------|---|-------------------------------------------|----|
| 16 |           |      |   |             |      |   |             |      |   |                                           |    |
| 17 |           |      |   |             |      |   |             |      |   |                                           |    |
| 18 | LN(HR)    | 0.76 |   | LN Lower CI | 0.69 |   | LN Upper CI | 0.82 |   |                                           |    |
| 19 | Pooled HR | 2.13 |   | Lower CI    | 2.00 |   | Upper CI    | 2.27 |   | Total NPC Prognosis Assessment Risk Score | 28 |

**Figure B.9.2:** Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene NPC prognosis assessment model when nine biomarkers were over expressed.

## Appendix B continued

|    | A                                   | B          | C      | D        | E        | F        | G        | H          | I      | J              | K      | L | M      | N               | O               | P | Q             | R              |
|----|-------------------------------------|------------|--------|----------|----------|----------|----------|------------|--------|----------------|--------|---|--------|-----------------|-----------------|---|---------------|----------------|
| 1  |                                     |            |        |          |          |          |          |            |        |                |        |   |        |                 |                 |   |               |                |
| 2  |                                     |            |        |          |          |          |          |            |        |                |        |   |        |                 |                 |   |               |                |
| 3  |                                     |            |        | Lower CI | Upper CI | LN(LCI)- |          |            |        |                |        |   |        | Formula =       | Formula =       |   | NPC Prognosis |                |
| 4  | Tick if yes                         | Biomarkers | HR     | (LCI)    | (UCI)    | LN(UCI)  | Variance | 1/Variance | LN HR  | LN HR/Variance |        |   |        | IF(A6=True,M6,) | IF(A6=True,J6,) |   | Assessment    | Formula =      |
| 5  |                                     |            |        |          |          |          |          |            |        |                |        |   |        |                 |                 |   | Score         | IF(A6=TRUE,Q6) |
| 6  | <input checked="" type="checkbox"/> | TRUE       | COX-2  | 2.02     | 1.65     | 2.47     | 0.40     | 0.01       | 94.41  | 0.70           | 66.38  |   | 66.38  | 66.38           | 94.41           |   | 3             | 3              |
| 7  | <input checked="" type="checkbox"/> | TRUE       | LMP1   | 2.48     | 1.77     | 3.47     | 0.67     | 0.03       | 33.91  | 0.91           | 30.80  |   | 30.80  | 30.80           | 33.91           |   | 4             | 4              |
| 8  | <input checked="" type="checkbox"/> | TRUE       | VEGF   | 2.07     | 1.32     | 3.25     | 0.90     | 0.05       | 18.93  | 0.73           | 13.77  |   | 13.77  | 13.77           | 18.93           |   | 3             | 3              |
| 9  | <input checked="" type="checkbox"/> | TRUE       | EGFR   | 1.86     | 1.25     | 2.77     | 0.80     | 0.04       | 24.27  | 0.62           | 15.06  |   | 15.06  | 15.06           | 24.27           |   | 3             | 3              |
| 10 | <input checked="" type="checkbox"/> | TRUE       | LDH    | 2.86     | 1.66     | 2.08     | 0.23     | 0.00       | 302.05 | 1.05           | 317.41 |   | 317.41 | 302.05          |                 |   | 3             | 3              |
| 11 | <input checked="" type="checkbox"/> | TRUE       | CRP    | 1.84     | 1.57     | 2.17     | 0.32     | 0.01       | 146.70 | 0.61           | 89.45  |   | 89.45  | 146.70          |                 |   | 3             | 3              |
| 12 | <input checked="" type="checkbox"/> | TRUE       | ERCC1  | 1.77     | 1.48     | 2.12     | 0.36     | 0.01       | 118.98 | 0.57           | 67.94  |   | 67.94  | 118.98          |                 |   | 3             | 3              |
| 13 | <input checked="" type="checkbox"/> | TRUE       | HIF-1a | 1.77     | 1.35     | 2.32     | 0.54     | 0.02       | 52.41  | 0.57           | 29.93  |   | 29.93  | 52.41           |                 |   | 3             | 3              |
| 14 | <input checked="" type="checkbox"/> | TRUE       | LOX    | 1.68     | 1.52     | 1.87     | 0.21     | 0.00       | 357.83 | 0.52           | 185.64 |   | 185.64 | 357.83          |                 |   | 3             | 3              |
| 15 | <input checked="" type="checkbox"/> | TRUE       | MMP9   | 1.65     | 1.38     | 1.95     | 0.35     | 0.01       | 128.55 | 0.50           | 64.37  |   | 64.37  | 128.55          |                 |   | 3             | 3              |
| 16 |                                     |            |        |          |          |          |          |            |        |                |        |   |        | 880.74          | 1278.03         |   |               | 31             |

**Figure B.10.1:** Output of multigene NPC prognosis assessment model when all biomarkers were over expressed.

|    | A         | B    | C | D           | E    | F | G           | H    | I | J                                         | K  |
|----|-----------|------|---|-------------|------|---|-------------|------|---|-------------------------------------------|----|
| 16 |           |      |   |             |      |   |             |      |   |                                           |    |
| 17 |           |      |   |             |      |   |             |      |   |                                           |    |
| 18 | LN(HR)    | 0.69 |   | LN Lower CI | 0.63 |   | LN Upper CI | 0.74 |   |                                           |    |
| 19 | Pooled HR | 1.99 |   | Lower CI    | 1.89 |   | Upper CI    | 2.10 |   | Total NPC Prognosis Assessment Risk Score | 31 |

**Figure B.10.2:** Pooled hazard ratio, lower and upper confidence intervals and total NPC prognosis assessment risk score in multigene NPC prognosis assessment model when all biomarkers were over expressed.