



**UNIVERSITI PUTRA MALAYSIA**

***IMMUNOHISTOCHEMICAL EXPRESSION OF HEDGEHOG PROTEINS IN  
DIFFUSE LARGE B-CELL LYMPHOMA***

**SITI NUR LINA BINTI AZMAN**

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DIFFUSE LARGE B-CELL LYMPHOMA**

**By**

**SITI NUR LINA BINTI AZMAN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
Fulfilment of the Requirements for the Degree of Master of Science**

**August 2016**

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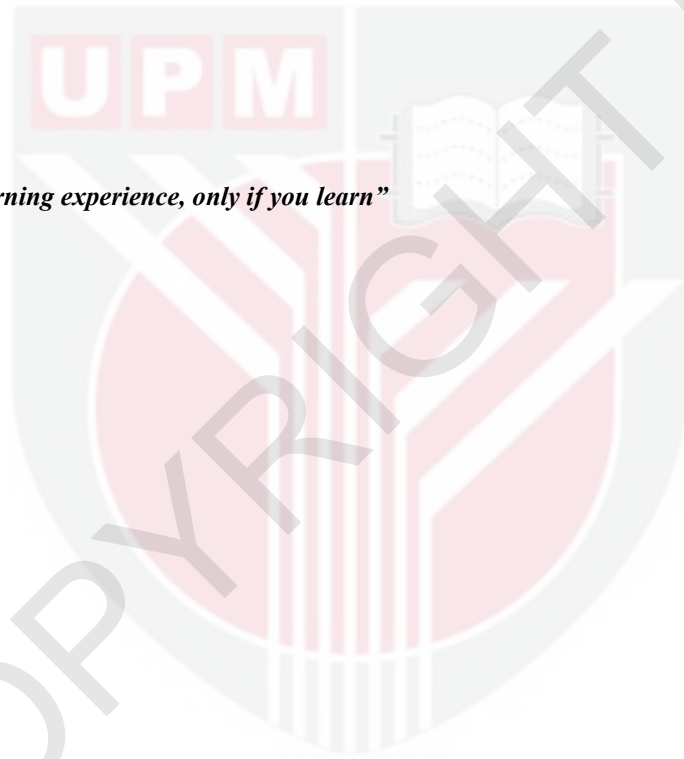
*Specially dedicated to*

*My parents, brothers, sisters and*

*Whom I love*

*For their invaluable love, endless support, understanding, encouragement and patients*

*“Life is a learning experience, only if you learn”*



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

## **IMMUNOHISTOCHEMICAL EXPRESSION OF HEDGEHOG PROTEINS IN DIFFUSE LARGE B-CELL LYMPHOMA**

By

**SITI NUR LINA BINTI AZMAN**

**August 2016**

**Chairman : Maizatun Atmadini Abdullah, PhD**  
**Faculty : Medicine and Health Sciences**

The Hedgehog (Hh) signalling pathway is a developmental signalling pathway involved in numerous developmental processes, including determination of cell fate, proliferation, patterning, survival and differentiation. In most adult tissues, this pathway remains silent and its aberrant activation has been documented in various types of malignancies including basal cell carcinoma, gastrointestinal, lung and prostate cancers. The aim of this study was to demonstrate the expression of Hh signalling pathway components in 107 DLBCL formalin fixed paraffin embedded (FFPE) tissues from Hospital Kuala Lumpur and determine their association with clinicopathological parameters. We assessed the expression of SHH (ligand), GLI1 (transcriptional effectors of SHH signalling), its receptor Patched (PTCH1) and signal transducer Smoothed (SMO) using immunohistochemistry. SHH was expressed in 42 (42.1%) cases, GLI1 in 61 (57%) cases, PTCH1 in 107 (100%) cases and SMO in 105 (98.1%) cases. Tumours display strong staining intensity for SMO and PTCH1 but low intensity for SHH and GLI1 when compared to normal tonsil tissues. Immunohistochemical staining results showed that SHH, GLI1 and SMO proteins were localised in the cytoplasm of tumour cells, whereas PTCH1 was mainly localised in their nucleus of tumour cells. Survival curves calculated using the Kaplan-Meier method also showed no significant association with sociodemographic distributions (age, gender, race, tumour involvement) and expression between HH signalling pathway proteins. To the best of our knowledge, there is no study investigating the correlation between SHH, PTCH1 and SMO. This study has shown that SHH expression was significantly associated with the expression of PTCH1 ( $P=0.030$ ) and PTCH1 was significantly associated with SMO ( $P=0.030$ ) expression. Our findings suggest that high expression of PTCH1 and SMO might be important in the pathogenesis of DLBCL. However, further studies are required to ascertain their roles in DLBCL.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Master Sains

## **EXPRESI PROTIN HEDGEHOG DALAM LIMFOMA SEL B BESAR DIFUS**

Oleh

**SITI NUR LINA BINTI AZMAN**

**Ogos 2016**

**Pengerusi : Maizatun Atmadini Abdullah, PhD**  
**Fakulti : Perubatan dan Sains Kesihatan**

Laluan Hedgehog (Hh) merupakan laluan signal yang terlibat dalam pelbagai proses pertumbuhan termasuk penentuan nasib sel, pembiakan sel, corak sel, perbezaan dan jangka hayat sel. Dalam kebanyakan tisu-tisu matang, laluan ini tidak aktif, namun pengaktifan laluan ini telah berlaku didalam beberapa jenis tumor termasuk sel basal karsinoma, tumor gastrousus, kanser paru-paru dan kanser prostat. Oleh itu, kajian ini bertujuan untuk mengenal pasti ekspresi protin Hh dalam 107 sampel terendam formalin dan terbenam-parafin daripada DLBCL di Hospital Kuala Lumpur dan hubung kaitnya dengan parameter demografi. Dalam kajian ini, kami menilai ekspresi protein SHH (ligand), GLI1 (faktor transkripsi), reseptor Patched (PTCH1) dan isyarat transduser, Smoothed (SMO) dengan menggunakan teknik imunohistokimia. Ekspresi protin SHH telah menunjukkan ekspresi yang positif dalam 42 (42.1%) kes, GLI1 sebanyak 61 (57%) kes, PTCH1 sebanyak 107 (100%) kes dan SMO sebanyak 105 (98.1%) kes. Tumor DLBCL memaparkan intensiti yang paling kuat bagi protin SMO dan PTCH1 tetapi berintensiti rendah bagi protin SHH dan GLI1 jika dibandingkan dengan tisu tonsil yang normal. Teknik pewarnaan imunohistokimia menunjukkan protin SHH, GLI1 dan SMO kebanyakannya terletak di sitoplasma sel kanser, manakala protin PTCH1 terletak di dalam nukleus sel tumor tersebut. Analisa jangka hayat menggunakan Kaplan Meir menunjukkan tiada sebarang signifikan dengan parameter demografi (umur, jantina, bangsa pesakit, lokasi tumor) dan ekspresi laluan signal diantara HH protins dalam DLBCL. Sepanjang pengetahuan kami, tiada kajian yang menyiasat hubungkait antara protein SHH, PTCH1 dan SMO. Dalam kajian ini, ekspresi SHH menunjukkan hubungan yang signifikan dengan ekspresi protin PTCH1 ( $P=0.030$ ), manakala protin PTCH1 telah menunjukkan ekspresi yang signifikan dengan ekspresi protin SMO ( $P=0.030$ ). Kesimpulan daripada kajian ini kebarangkalian menunjukkan bahawa ekspresi protin PTCH dan SMO yang tinggi adalah penting dalam patogenesis DLBCL. Walaubagaimanapun, kajian selanjutnya harus dilakukan untuk mengenal pasti peranan mereka dalam kanser sel DLBCL.

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I certify that a Thesis Examination Committee has met on 8 August 2016 to conduct the final examination of Siti Nur Lina binti Azman on her thesis entitled "Immunohistochemical Expression of Hedgehog Proteins in Diffuse Large B-Cell Lymphoma" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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## LIST OF ABBREVIATIONS

Ab	Antibody
ABC	Activated B-cell
Ag	Antigen
AILD	Angioimmunoblastic
ALCL	Anaplastic large cell lymphoma
AP	Alkaline phosphatase
BCC	Basal cell carcinoma
BcL2	B-cell CLL/Lymphoma 2
BCNS	Basal cell nevus syndrome
BL	Burkitt lymphoma
DLBCL	Diffuse large B cell lymphoma
DAB	Diaminobenzidine
Dhh	Desert Hedgehog
DPX	Di-N-Butyle Phthalate in Xylene
EBV	Epstein-Barr virus
FDC	Follicular dendritic cells
FFPE	Formalin fixed paraffin embedded
FL	Follicular lymphoma
Fu	Serine/threonine protein kinase Fused
GLI	Glioma-associated oncogene homolog
GCB	Germinal center B-cell
GEP	Gene expression profiling
GPCR	G-protein coupled receptor
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
H&E	Haematoxylin and eosin
HH	Hedgehog
HRP	Horseradish peroxidase
HL	Hodgkin lymphoma
IARC	International Agency for Research on Cancer
IHC	Immunohistochemistry
Ihh	Indian Hedgehog
LSAB	Labelled streptavidin-biotin
LL	Lymphoblastic lymphoma
MOH	Ministry of Health
MZ	Marginal zone
NCS	National Cancer Society Malaysia
NGCB	Non-germinal centre B-like
NHL	Non-Hodgkin lymphoma
NS	Nodular sclerosis subtype
NK	Natural killer lymphocyte
NLPHL	Nodular lymphocyte predominant Hodgkin lymphoma
NMRR	National Medical Research Registration
NOS	Not otherwise specified
OS	Overall survival
PAP	Peroxidase-antiperoxidase
PBS	Phosphate Buffered Saline
PL	Plasmablastic lymphoma
PTCH	Patched
RS	Reed-Sternberg

SD  
SHH  
SLL  
SL  
SMO  
VEGF  
WHO

Standard deviation  
Sonic hedgehog  
Small lymphoblastic lymphoma  
Subcutaneous lymphoma  
Smoothened  
Vascular endothelial growth factor  
World Health Organisation



## CHAPTER 1

### INTRODUCTION

#### 1.1 General Introduction

Cancer is a very complex disease involving numerous changes in the cell physiology that eventually leads to malignant tumours. It involves a process in which an unregulated division of abnormal cells spreads into nearby tissues or even in the blood. It is often shaped differently from healthy cells; they do not function properly, and they metastasize to other parts of the body. Tumours are clusters of cells that are capable of growing and dividing uncontrollably, and their growth is unregulated. Cancer is not just a disease but rather a group of diseases all of which cause cells in the body to change and grow out of control, invading normal tissues and organs and eventually spreading throughout the body. Cancers are classified according to their tissue of origin like breast cancer, lung cancer, prostate cancer, liver cancer renal cell carcinoma (kidney cancer), oral cancer and brain cancer. It can also be classified into tissue types, for example carcinoma sarcoma, lymphoma or cancers of mixed types which includes mixed mesodermal tumor, carcinosarcoma, adenosquamous carcinoma and teratocarcinoma.

There is no one single cause for cancer. According to Foster and Sarelius, 2015, the medical reviewer of University of Rochester Medical Center, believe that there are many factors that may contribute to cancer. The factors involved may be genetic, environmental, or biological characteristics of the individual for example age, gender and ethnicity. Diagnosis, treatment and prognosis for childhood cancers are different from adult cancers because childhood cancer is more responsive to therapy, and a child can tolerate more aggressive therapy (Foster & Sarelius, 2015). The overall five-years survival rate for common childhood cancers are acute lymphocytic leukemia (89%), acute myelogenous leukemia (65%), brain and other CNS (72%), wilms tumours (92%), hodgkin lymphoma (98%) and non-hodgkin lymphoma (89%). The overall five-years survival rate for common adult cancers are prostate (99%), melanoma of the skin (92%), breast (89%), urinary bladder (77%) colorectum (65) and lung and bronchus (17%) (American Cancer Society, 2015).

Cancer is one of the leading causes of death worldwide and the pattern continues to increase with time. These causes might have been due to avoidable risk factors that include tobacco exposure, smoking, poor diet, inadequate exercise and being overweight. Worldwide, one in eight deaths is due to cancer (American Cancer Society, 2008). When countries are grouped according to economic development, cancer is the leading cause of death in developing countries and the second-leading cause of deaths in emerging countries. According to the International Agency for Research on Cancer (IARC) Globocan of the World Health Organisation (WHO), there were 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide (Jacques Ferlay, Isabelle Soerjomataram, Rajesh Dikshit, 2012).

In Malaysia, the incidence of cancer has become the fourth leading cause of death and is expected to increase by year with an estimated of 30,000 cases annually. Cancer is one of the top ten causes of hospitalisation and the fifth cause of death in both Ministry of Health and private hospitals (Ministry of Health Malaysia, 2013). A newspaper

online article 'Rise in cancer deaths in Malaysia' 2014, stated that the incidence of cancer in Malaysia has increased from 32,000 new cases in 2008 to about 37,400 cases in 2012. This number, however, is expected to rise in the years to come if no actions such as living a healthy lifestyle, avoiding exposure to known cancer-causing substances and taking medicines or vaccines are taken (The Star Online, 2014). According to the National Cancer Registry of Malaysia, the most common cancer among Malaysian population is breast cancer, with one in 19 Malaysian developing breast cancer, one in 33 developing colorectal cancer, and one in 40 developing lung cancer followed by nasopharyngeal cancer, cervical cancer, lymphomas, leukaemia, ovarian cancer, stomach cancer and also liver cancer. Within different ethnic groups, cancer seems to be predominant among Chinese compared to Malays and Indians (Zainal & Nor Saleha, 2011). The National Cancer Society Malaysia (NCSM) estimated that in 2015, 90,000 to 100,000 Malaysians are living with cancer. Less than 10% of cancers occurred among children while over 50% in men and 35% in women aged 50 and above. However, it is estimated that a ratio of one in four Malaysian (1:4) will develop cancer at the age of 75 years old. It is also expected that by 2030, the global burden of cancer will increase to 21.4 million new cancer cases and 13.2 million deaths from cancer annually.

In 2010, the World Health Organization (WHO) recorded that lymphoma was the 21<sup>st</sup> top killer disease with 0.97% of the total death (World Health Organization, 2010). Almost one thousand patients are diagnosed every day with one million people worldwide living with lymphoma. According to the National Cancer Registry, 2007, lymphoma is the 6<sup>th</sup> commonest cancer among Malaysians. It is the sixth most common cancer in males and the eighth most common cancer in females (Zainal & Nor Saleha, 2011). There are two main subgroups of lymphoma, which are non-Hodgkin lymphoma (NHL) and Hodgkin's lymphoma (HL). HL is divided into four subtypes, and NHL is divided into more than 61 subtypes. The data collected at the GLOBOCAN, 2012, estimated that the incidence of HL patients is about 0.5% per 1,000,000 populations while for NHL is about 2.9% per 1,000,000 populations. In 2015, approximately about 9,050 new cases of HL and 4% case of NHL were expected to occur. There are approximately 1,150 deaths and 19,790 deaths from HL and NHL respectively (American Cancer Society, 2015). The most common subtype of NHL is diffuse large B-cell lymphoma (DLBCL) and are known to be the aggressive subtype. It develops when the body makes abnormal B-lymphocytes which is the lymphoma cells. B-cell lymphocytes are white blood cells that fight infection. Sometimes lymphoma cells begin in other parts of the body known as extranodal disease. The term diffuse refers to the fact that the cancer cells are spread around and not concentrated in one particular part of the node or in clusters within a part of the node. In other words the cancer cells don't clump together very well.

Although tumours differ based on their tissues of origin, all cancers share the fundamental features of propagated mutation to daughter cells. These propagated mutations generally lead to deregulated signalling pathways that gives a variety of clinical consequences and indications for new therapeutic involvement (Rajeev et al., 2011). Cancer cells aberrantly reactivate oncogenic developmental pathways such as Notch, Wnt and Hedgehog to provide growth and survival advantage to tumour cells. The Hedgehog pathway (Hh) protein family is a group of secreted signalling molecules for mammalian development and homeostasis of renewal adult tissues (McMahon, Ingham, & Tabin, 2003). It is normally activated during embryonic development

(Ingham, 2001) and promotes vascularization (Nagase, Nagase, & Machida, 2008). The basic components of Hh ligands refer to the three family members which include Sonic (Shh), Indian (Ihh) and Desert (Dhh) Hedgehog.

In adults, HH ligands are thought to be important for the renewal of hematopoietic stem cells (Varjosalo & Taipale, 2008). Ligands of the pathway are expressed in the hematopoietic niche and have been shown to be important for survival signals of leukemia, lymphoma and myeloma cancer stem cells by bone marrow imaging and transgenic E $\mu$ -Myc mice model, whereas inhibiting the pathway suppresses disease progression (Dierks et al., 2007, 2008; Zhao et al., 2009). The ligands Ihh and Shh promote survival of B-cell malignancies such as lymphoma (Lowrey et al., 2002; Stewart et al., 2002). Unlike solid tumours which produce HH ligand, Ihh and Shh are produced by surrounding non-malignant cells in the lymph nodes, bone marrow and spleen to permit the growth of lymphoma cells.

## **1.2 Problem Statement**

Despite treatment with conventional anthracyclin-based chemotherapy, approximately half of all DLBCL patients eventually die of this disease. Inappropriate activation of the HH signalling pathway has been found to occur in haematological neoplasms. This study is conducted to study the expression of HH signalling proteins expression (SHH, GLI1, PTCH1 and SMO) in DLBCL. This is important to unlock some possible diagnostic and treatment strategies in DLBCL.

## **1.3 Significance of Study**

The study will provide an overview on the expression of HH pathway proteins in DLBCL. Hence further in depth research can be performed to evaluate their functions in tumour progression and the possibilities of targeted therapeutic interventions.

## **1.4 Research Objective**

### **1.4.1 General Objective**

To evaluate the expression of Hh signalling pathway proteins in DLBCL

### **1.4.2 Specific Objectives**

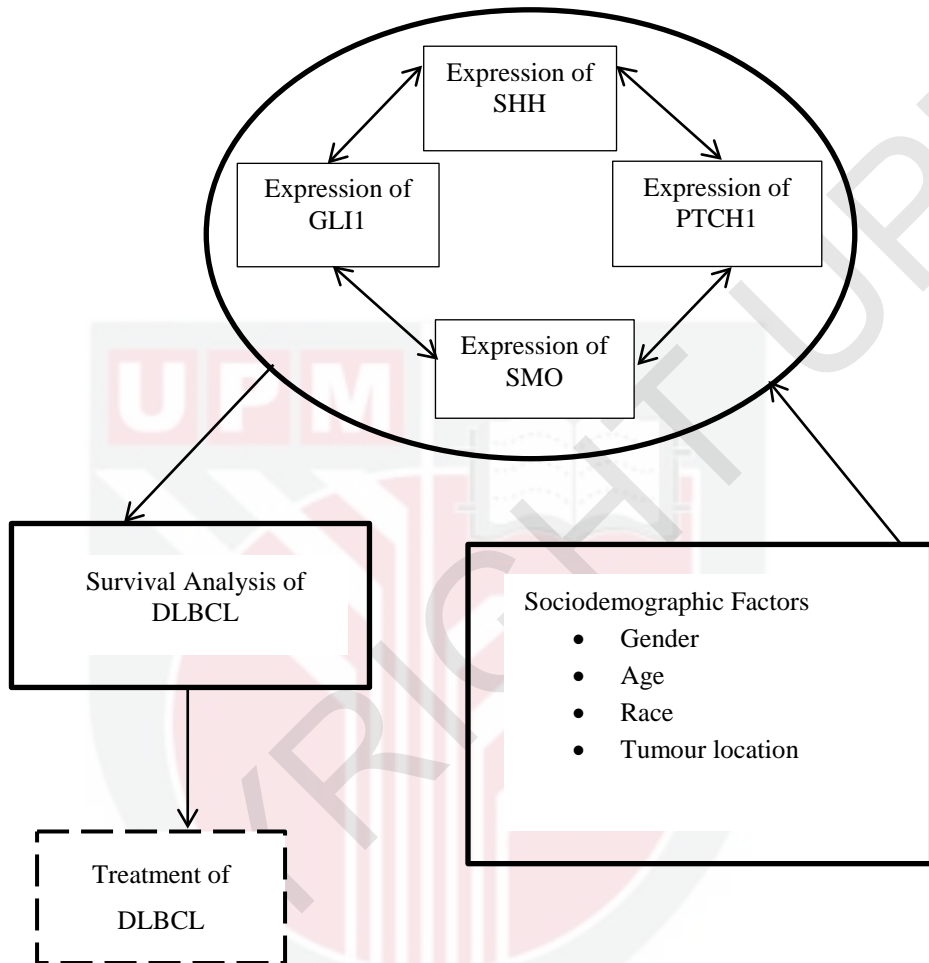
1. To determine the sociodemographic distribution of patients with DLBCL.
2. To analyze the immunohistochemical expression of four Hedgehog pathway proteins (SHH, GLI, PTCH1 and SMO) in DLBCL.
3. To determine the correlation of immunohistochemical expression between Hedgehog pathway proteins and sociodemographic factors in DLBCL.
4. To analyze the association between Hedgehog pathway proteins and sociodemographic factors with overall survival in DLBCL.



## 1.5 Hypothesis

1. There is high expression of Hedgehog pathway proteins in DLBCL.
2. There is significant association between the expression of four different HH pathway proteins (SHH, GLI1, PTCH1 and SMO) in DLBCL.
3. There is significant association between the expression of Hedgehog pathway proteins with sociodemographic factors and overall survival in DLBCL.



## 1.6 Conceptual Framework



-  Studied variables
-  Non-studied variable

## REFERENCES

- Adolphe, C., Hetherington, R., Ellis, T., & Wainwright, B. (2006). Patched1 Functions as a Gatekeeper by Promoting Cell Cycle Progression. *Cancer R*, 6(4), 2081–2088.
- Agarwal, N. K., Qu, C., Kunkulla, K., Liu, Y., & Vega, F. (2013). Transcriptional regulation of serine/threonine protein kinase (AKT) genes by glioma-associated oncogene homolog. *Journal of Biological Chemistry*, 288(21), 15390–15401.
- Alizadeh, a a, Eisen, M. B., Davis, R. E., Ma, C., Lossos, I. S., Rosenwald, a., Staudt, L. M. (2000). Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*, 403(6769), 503–11.
- American Cancer Society. (2014). Retrieved from <http://www.cancer.org/cancer/hodgkindisease/detailedguide/hodgkin-disease-key-statistics#>
- American Cancer Society. (2015). Retrieved from <http://www.cancer.org/>
- American Cancer Society. (2008). *Global Cancer Facts & Figures 2nd Edition*. Atlanta: American Cancer Society, (700), 1–57.
- American Cancer Society: Non-Hodgkin Lymphoma. (2014). Retrieved from <http://www.cancer.org/cancer/non-hodgkinlymphoma/detailedguide/non-hodgkin-lymphoma-risk-factors>
- Ayers, K. L., & Thérond, P. P. (2010). Evaluating Smoothed as a G-protein-coupled receptor for Hedgehog signalling. *Trends in Cell Biology*, 20(5), 287–298.
- Bacus, S., Flowers, J. L., Press, M. F., Bacus, J. W., & McCarty, K. S. (1988). The evaluation of estrogen receptor in primary breast carcinoma by computer-assisted image analysis. *American Journal of Clinical Pathology*, 90(3), 233–239.
- Bailey, J. M., Swanson, B. J., Hamada, T., Eggers, J. P., Singh, P. K., Caffery, T., Hollingsworth, M. a. (2008). Sonic Hedgehog Promotes Desmoplasia in Pancreatic Cancer Sonic Hedgehog Promotes Desmoplasia in Pancreatic Cancer, 14(19), 5995–6004.
- Barrans, S., Crouch, S., Smith, A., Turner, K., Owen, R., Patmore, R., Jack, A. (2010). Rearrangement of MYC Is Associated With Poor Prognosis in Patients With Diffuse Large B-Cell Lymphoma Treated in the Era of Rituximab Not known. *Journal of Clinical Oncology*, 28(20), 3360–3365.
- Barrans, S. L., Evans, P. A. S., Connor, S. J. M. O., Kendall, S. J., Owen, R. G., Haynes, A. P. Jack, A. S. (2003). The t(14;18) Is Associated with Germinal Center-derived Diffuse Large B-Cell Lymphoma and Is a Strong Predictor of Outcome 1. *Clinical Cancer Research*, 9, 2133–2139.
- Berman, D. M., Karhadkar, S. S., Hallahan, A. R., Pritchard, J. I., Eberhart, C. G., Watkins, D. N., Beachy, P. A. (2002). Medulloblastoma Growth Inhibition by Hedgehog Pathway Blockade. *Science*, 297(5586), 1559–1561.
- Berman, D. M., Karhadkar, S. S., Maitra, A., Montes De Oca, R., Gerstenblith, M. R., Briggs, K., Beachy, P. a. (2003). Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. *Nature*, 425(6960), 846–851.
- Blinder, V., & Fisher, S. G. (2008). The Role of Environmental Factors in the Etiology of Lymphoma, 2006(6), 306–316.
- Briscoe, J., & Thérond, P. P. (2013). The mechanisms of Hedgehog signalling and its roles in development and disease. *Nature Reviews. Molecular Cell Biology*, 14, 416–29.
- Chai, S. P., Peh, S. C., Kim, L. H., Lim, M. Y., & Gudum, H. R. (1999). The pattern of lymphoma in east Malaysian patients as experienced in the University Hospital, Kuala Lumpur. *The Malaysian Journal of Pathology*, 21(1), 45–50.



- Challis, D., Kelsall, R., Mc Ardle, J., McPherson, J., Phillips, G., & El Standish-White, S. (n.d.). Hodgkin disease (nodular scleritis). Retrieved 9 August 2016, from <https://secure.health.utas.edu.au/intranet/cds/pathprac/Files/Cases/Lymphatic/Case76/Case76.htm>
- Chan, J. K. (2001). The new World Health Organization classification of lymphomas: the past, the present and the future. *Hematological Oncology*, 19(4), 129–50.
- Chao, M. P. (2013). Treatment challenges in the management of relapsed or refractory non-Hodgkin ' s lymphoma – novel and emerging therapies. *Cancer Management and Research*, 5, 251–269.
- Choi, W. W. L., Weisenburger, D. D., Greiner, T. C., Piris, M. A., Banham, A. H., Delabie, J., Chan, W. C. (2009). A New Immunostain Algorithm Classifies Diffuse Large B-Cell Lymphoma into Molecular Subtypes with High Accuracy A New Immunostain Algorithm Classifies Diffuse Large B-Cell Lymphoma into Molecular Subtypes with High Accuracy. *Clinical Cancer Research*.
- Christiane Nusslein-Volhard, & Eric Wieschaus. (1980). Mutations affecting segment number and polarity in Drosophila. *European Molecular Biology Laboratory*, 287(5787), 795–801.
- Coiffier, B., Lepage, E., Briere, J., Herbrecht, R., Tilly, H., Bouabdallah, R., Gisselbrecht, C. (2002). CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med*, 346(4), 235–42.
- Connors, J. M. (2013). Non-Hodgkin lymphoma: the clinician's perspective - a view from the receiving end. *Modern Pathology*, 26, 111–118.
- Corbit, K. C., Aanstad, P., Singla, V., Norman, A. R., Stainier, D. Y. R., & Reiter, J. F. (2005). Vertebrate Smoothed functions at the primary cilium. *Nature*, 437, 1018–1021.
- Dahmane, N., Lee, J., Robins, P., Heller, P., & Altaba, A. R. i. (1997). Activation of the transcription factor Gli1 and the Sonic hedgehog signalling pathway in skin tumours. *Nature*, 389(6653), 876–881.
- Dierks, C., Beigi, R., Guo, G. R., Zirlik, K., Stegert, M. R., Manley, P., Warmuth, M. (2008). Expansion of Bcr-Abl-Positive Leukemic Stem Cells Is Dependent on Hedgehog Pathway Activation. *Cancer Cell*, 14(3), 238–249.
- Dierks, C., Grbic, J., Zirlik, K., Beigi, R., Englund, N. P., Guo, G.-R., Warmuth, M. (2007). Essential role of stromally induced hedgehog signaling in B-cell malignancies. *Nature Medicine*, 13(8), 944–951.
- Dierks, C., Grbic, J., Zirlik, K., Beigi, R., Englund, N. P., Rong, G., Warmuth, M. (2007). Essential role of stromally induced hedgehog signaling in B - cell malignancies. *Nature Medicine*, 13, 944–951.
- Echelard, Y., Epstein, D. J., St-Jacques, B., Shen, L., Mohler, J., McMahon, J. a., & McMahon, a. P. (1993). Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell*, 75(7), 1417–1430.
- Eltom, M. A., Jemal, A., Mbulaiteye, S. M., Devesa, S. S., & Biggar, R. J. (2002). Trends in Kaposi ' s Sarcoma and Non-Hodgkin ' s Lymphoma Incidence in the United States From 1973 Through 1998. *Journal of National Cancer Institute*, 94(16), 1204–1210.
- Engelhard, B. M., Huhn, D., Gerhartz, H. H., Meusers, P., Siegert, W., Bierwolf, S., Wilmanns, W. (1997). Subclassification of Diffuse Large B-Cell Lymphomas According to the Kiel Classification: Distinction of Centroblastic and Immunoblastic Lymphomas Is a Significant Prognostic Risk Factor. *Blood*, 89(7), 2291–2297.

- Evans, L. S., & Hancock, B. W. (2003). Non-Hodgkin lymphoma. *The Lancet*, 362(9378), 139–146.
- Feugier, P. (2005). Long-Term Results of the R-CHOP Study in the Treatment of Elderly Patients With Diffuse Large B-Cell Lymphoma: A Study by the Groupe d'Etude des Lymphomes de l'Adulte. *Journal of Clinical Oncology*, 23(18), 4117–4126.
- Feugier, P., Van Hoof, a, Sebban, C., Solal-Celigny, P., Bouabdallah, R., Fermé, C., Coiffier, B. (2005). Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: a study by the Groupe d'Etude des Lymphomes de l'Adulte. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, 23(18), 4117–26.
- Flowers, C. R., & Armitage, J. O. (2010). A decade of progress in lymphoma: advances and continuing challenges. *Clinical Lymphoma, Myeloma & Leukemia*, 10(6), 414–23.
- Foster, T. H., & Sarelius, I. H. (2015). Health Encyclopedia. Retrieved from <https://www.urmc.rochester.edu/Encyclopedia/Content.aspx?ContentTypeID=90&ContentID=P02719>
- Fritz, P., Wu, X., Tuzcek, H., Multhaupt, H., & Schwarzmann, P. (1995). Quantitation in immunohistochemistry. A research method or a diagnostic tool in surgical pathology? *Pathologica*, 87(3), 300-309. .
- Gailani, M. R., & Bale, A. E. (1997). Developmental Genes and Cancer: Role of Patched in Basal Cell Carcinoma of the Skin. *Journal of the National Cancer Institute*, 89, 1103–1109.
- Geng, L., & Wang, X. (2015). Epstein-Barr Virus-associated lymphoproliferative disorders: experimental and clinical developments. *International Journal of Clinical and Experimental Medicine*, 8(9), 14656–14671.
- Gonnissen, A., Isebaert, S., & Haustermans, K. (2013). Hedgehog Signaling in Prostate Cancer and Its Therapeutic Implication. *International Journal of Molecular Sciences*, 14(7), 13979–14007.
- Goodrich, L. V, Milenković, L., Higgins, K. M., & Scott, M. P. (1997). Altered neural cell fates and medulloblastoma in mouse patched mutants. *Science (New York, N.Y.)*, 277(5329), 1109–1113.
- Greaves, W. O., Kim, J. E., Singh, R. R., Drakos, E., Kunkalla, K., Sanchez-Espiridin, B., Vega, F. (2011). Glioma-associated oncogene homologue 3, a hedgehog transcription factor, is highly expressed in Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma. *Human Pathology*, 42(11), 1643–1652.
- Grulich, A. E., Vajdic, C. M., & Cozen, W. (2007). Altered immunity as a risk factor for non-Hodgkin lymphoma. *Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology*, 16(3), 405–8.
- Habermann, T. M., Weller, E. a., Morrison, V. a., Gascoyne, R. D., Cassileth, P. a., Cohn, J. B., Horning, S. J. (2006). Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. *Journal of Clinical Oncology*, 24(19), 3121–3127.
- Hahn, H., Wicking, C., Zaphiropoulos, P. G., Gailani, M. R., Shanley, S., Chidambaram, A., Bale, A. E. (1996). Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. *Cell*, 85(6).
- Hamed, S., Larue, H., Hovington, H., & Hamed, S. (2004). Accelerated Induction of Bladder Cancer in Patched Heterozygous Mutant Mice Advances in Brief Accelerated Induction of Bladder Cancer in Patched Heterozygous Mutant Mice. *Cancer Research*, (12), 1938–1942.

- Hans, C. P., Weisenburger, D. D., Greiner, T. C., Gascoyne, R. D., Delabie, J., Ott, G., Chan, W. C. (2004). Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Neoplasia*, 103(1), 275–282.
- Hao, L., Johnsen, R., Lauter, G., Baillie, D., & Bürglin, T. R. (2006). Comprehensive analysis of gene expression patterns of hedgehog -related genes. *BMC Genomics* 7(1),1-20.
- Harris, N. L., Jaffe, E. S., Stein, H., Banks, P. M., Chan, J. K., Cleary, M. L., Gatter, K. C. (1994). A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood*, 84(5), 1361–1392.
- Hartge, P., & Devesa, S. S. (1992). Quantification of the Impact of Known Risk Factors on Time Trends in Non-Hodgkin's Lymphoma Incidence. *Cancer Research* 52, 5566–5569.
- Hecke, D. Van. (2002). Routine Immunohistochemical Staining Today: Choices to Make, Challenges to Take. *Journal of Histotechnology*, 25(1), 45–54.
- Hegde, G. V, Peterson, K. J., Emanuel, K., Mittal, A. K., Joshi, A. D., Dickinson, J. D., Joshi, S. S. (2008). Hedgehog-induced survival of B-cell chronic lymphocytic leukemia cells in a stromal cell microenvironment: a potential new therapeutic target. *Mol Cancer Res*, 6(12), 1928–1936.
- Hodgkin. (1832). On some Morbid Appearances of the Absorbent Glands and Spleen. *Medico-Chirurgical Transactions*, 17, 68–114.
- Holford, T. R., Zheng, T., Mayne, S. T., & Mckay, L. A. (1992). Time Trends of Non-Hodgkin's Lymphoma: Are They Real? *Cancer Research*, (5) 19.
- Hunt, K. E., & Reichard, K. K. (2008). Diffuse large B-cell lymphoma. *Archives of Pathology & Laboratory Medicine*, 132, 118–124.
- Ingham, P. W. (2001). Hedgehog signaling in animal development: paradigms and principles. *Genes & Development*, 15(23), 3059–3087.
- Iqbal, J., Meyer, P. N., Smith, L. M., Johnson, N. A., Vose, J. M., Greiner, T. C., Gascoyne, R. D. (2011). BCL2 Predicts Survival in Germinal Center B-cell – like Diffuse Large B-cell Lymphoma Treated with CHOP-like Therapy and Rituximab, *Clinical Cancer Research*, 7785–77967
- Iqbal, J., Neppalli, V. T., Wright, G., Dave, B. J., Horsman, D. E., Rosenwald, A., Chan, W. C. (2006). BCL2 Expression Is a Prognostic Marker for the Activated B-Cell – Like Type of Diffuse Large B-Cell Lymphoma. *Journal of Clinical Oncology* 24(6), 961-968.
- Iqbal, J., Sanger, W. G., Horsman, D. E., Rosenwald, A., Pickering, D. L., Dave, B., Chan, W. C. (2004). BCL2 Translocation Defines a Unique Tumor Subset within the Germinal Center B-Cell-Like Diffuse Large B-Cell Lymphoma, *American Journal of Pathology*, 65(1), 159–166.
- Jacques Ferlay, Isabelle Soerjomataram, Rajesh Dikshit, S. E. (2012). Cancer incidence and mortality worldwide: sources, methods and major patterns in Globocan 2012. *Interantional Journal of Cancer*, Vol. 5.
- Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. (2001). Pathology and Genetics of Haematopoietic and and Lymphoid Tissues. Lyon, France: IARC Press Lymphoid Tissues. *World Health Organization Classification of Tumours.*, 171–175.
- Johnson, R. L., Rothman, a L., Xie, J., Goodrich, L. V, Bare, J. W., Bonifas, J. M., Scott, M. P. (1996). Human homolog of patched, a candidate gene for the basal cell nevus syndrome. *Science (New York, N.Y.)*, 272(5268), 1668–1671.
- Kappler, R., Bauer, R., Calzada-Wack, J., Rosemann, M., Hemmerlein, B., & Hahn, H.

- (2004). Profiling the molecular difference between Patched- and p53-dependent rhabdomyosarcoma. *Oncogene*, *23*, 8785–8795.
- Karhadkar, S. S., Steven Bova, G., Abdallah, N., Dhara, S., Gardner, D., Maitra, A., Beachy, P. A. (2004). Hedgehog signalling in prostate regeneration, neoplasia and metastasis. *Nature*, *431*(7009), 707–712. Retrieved from
- Kim, J. E., Singh, R. R., Cho-Vega, J. H., Drakos, E., Davuluri, Y., Khokhar, F. A., Vega, F. (2009). Sonic hedgehog signaling proteins and ATP-binding cassette G2 are aberrantly expressed in diffuse large B-Cell lymphoma. *Modern Pathology*, *22*(10), 1312–1320.
- Kim, N., Kim, J. E., Choung, H. K., Lee, M. J., & Khwarg, S. I. (2013). Expression of Shh and Wnt signaling pathway proteins in eyelid sebaceous gland carcinoma: Clinicopathologic study. *Investigative Ophthalmology and Visual Science*, *54*(1), 370–377.
- Kinzler, K. W., Bigner, S. H., Bigner, D. D., Trent, J. M., Law, M. L., O'Brien, S. J., Vogelstein, B. (1987). Identification of an amplified, highly expressed gene in a human glioma. *Science*, *236*(70), 70–73.
- Kobune, M., Kato, J., Kawano, Y., Sasaki, K., Uchida, H., Takada, K., Niitsu, Y. (2008). Adenoviral vector-mediated transfer of the Indian hedgehog gene modulates lymphomyelopoiesis in vivo. *Stem Cells*, *26*(2), 534–542.
- Kubo, M. (2004). Hedgehog Signaling Pathway is a New Therapeutic Target for Patients with Breast Cancer. *Cancer Research*, *64*(17), 6071–6074.
- Lal, A., Bhurgri, Y., Vaziri, I., Rizvi, N. B., Sadaf, A., Sartajuddin, S., Alidina, A. (2008). Extranodal non-Hodgkin's lymphomas--a retrospective review of clinicopathologic features and outcomes in comparison with nodal non-Hodgkin's lymphomas. *Asian Pacific Journal of Cancer Prevention: APJCP*, *9*(3), 453–8.
- Lindemann, R. K. (2008). Stroma-Initiated Hedgehog Signaling Takes Center Stage in B-Cell Lymphoma. *Cancer Research*, *68*(4), 961–964.
- Lister TA, Crowther D, Sutcliffe SB, et al. (1990). Report of a Committee Convened To Discuss the Evaluation and Staging of Patients with Hodgkin's Disease. *Journal of Clinical Oncology*, *8*(9), 1602–1603.
- Lowrey, J. a., Stewart, G. a., Lindey, S., Hoyne, G. F., Dallman, M. J., Howie, S. E. M., & Lamb, J. R. (2002). Sonic Hedgehog Promotes Cell Cycle Progression in Activated Peripheral CD4+ T Lymphocytes. *The Journal of Immunology*, *169*(4), 1869–1875.
- Lowry, L., & Linch, D. (2013). Non-Hodgkin's lymphoma. *Medicine*, *41*(5), 282–289.
- Lum, L., Zhang, C., Oh, S., Mann, R. K., Kessler, D. P. Von, Taipale, J., Beachy, P. A. (2003). Hedgehog Signal Transduction via Smoothed Association with a Cytoplasmic Complex Scaffolded by the Atypical Kinesin , Costal-2. *Molecular Cell*, *12*, 1261–1274.
- Macaron, N. C., Cohen, C., Chen, S. C., & Arbiser, J. L. (2005). gli-1 Oncogene is highly expressed in granulomatous skin disorders, including sarcoidosis, granuloma annulare, and necrobiosis lipoidica diabetorum. *Archives of Dermatology*, *141*(2), 259–62.
- Makinodan, E., & Marneros, A. G. (2012). Protein kinase A activation inhibits oncogenic Sonic hedgehog signalling and suppresses basal cell carcinoma of the skin. *Experimental Dermatology*, *21*(13), 847–852.
- Malaysia Demographics Profile 2014. (2014). Retrieved 2 November 2015, from [http://www.indexmundi.com/malaysia/demographics\\_profile.html](http://www.indexmundi.com/malaysia/demographics_profile.html)
- Mancer, K. (1990). The spectrum of lymphoma in malaysia: a histopathological study utilizing immunophenotyping. *Malaysian Journal of Pathology*, *12*(2), 77–88.
- Marasani, A. R., Anakonti, R., & Rudrapati, D. (2010). Hedgehog signaling pathway,

- The Embryo Project Encyclopedia* 1(4), 73–84.
- McClelland, R. A., Wilson, D., Leake, R., Finlay, P., & Nicholson, R. I. (1991). A multicentre study into the reliability of steroid receptor immunocytochemical assay quantification. British Quality Control Group. *European Journal of Cancer (Oxford, England: 1990)*, 27(6), 711–5.
- McMahon, A. P., Ingham, P. W., & Tabin, C. J. (2003). Developmental roles and clinical significance of hedgehog signaling. *Current Topics in Developmental Biology*, 53, 1–114.
- Meyer, N., & Penn, L. Z. (2008). Reflecting on 25 years with MYC. *Nature Reviews. Cancer*, 8(December), 976–990.
- Meyer, P. N., Fu, K., Greiner, T. C., Smith, L. M., Delabie, J., Gascoyne, R. D., Weisenburger, D. D. (2011). Immunohistochemical Methods for Predicting Cell of Origin and Survival in Patients With Diffuse Large B-Cell Lymphoma Treated With Rituximab. *Journal of Clinical Oncology*, 29(2), 200–207.
- Ministry of Health Malaysia. (2013). Malaysia Health Facts 2013. *Journal of Chemical Information and Modeling*, 53(9), 1689–1699.
- Monuki, E. S. (2007). The Morphogen Signaling Network in Forebrain Development and Holoprosencephaly. *Journal of Neuropathology & Experimental Neurology*, 66(7), 566–575.
- Mounier, N., Briere, J., Gisselbrecht, C., Emile, J., Lederlin, P., Sebban, C., Coiffier, B. (2003). Rituximab plus CHOP ( R-CHOP ) overcomes bcl-2 – associated resistance to chemotherapy in elderly patients with diffuse large B-cell lymphoma ( DLBCL ), 101, 4279–4284.
- Mozahab, Z., Aledavood, A., & Farzad, F. (2011). Distributions of major sub-types of lymphoid malignancies among adults in Mashhad, Iran. *Cancer Epidemiology*, 35(1), 26–29.
- Müller, a. M. S., Ihorst, G., Mertelsmann, R., & Engelhardt, M. (2005). Epidemiology of non-Hodgkin's lymphoma (NHL): Trends, geographic distribution, and etiology. *Annals of Hematology*, 84, 1–12.
- Nagase, T., Nagase, A. M., & Machida, A. M. (2008). Hedgehog signalling in vascular development. *Angiogenesis*, 11(1), 71–77.
- National Institute for Health and Care Excellence (NICE) Suspected cancer: recognition and referral. (2015). Retrieved from <https://www.nice.org.uk/guidance/NG12/chapter/1-Recommendations-organised-by-site-of-cancer#haematological-cancers>
- Nishimaki, H., Kasai, K., Kozaki, K., Takeo, T., Ikeda, H., Saga, S., Itoh, G. (2004). A role of activated Sonic hedgehog signaling for the cellular proliferation of oral squamous cell carcinoma cell line. *Biochemical and Biophysical Research Communications*, 314(2), 313–20. <http://doi.org/10.1016/j.bbrc.2003.12.097>
- Oakley, T. H. (2003). The eye as a replicating and diverging , modular developmental unit. *Elsevier*, 18(12), 623–627.
- Ok, C. Y., Li, L., Xu-Monette, Z., Visco, C., Tzankov, A., Manyam, G., Young, K. H. (2014). Prevalence and Clinical Implications of Epstein-Barr Virus Infection in de novo Diffuse Large B-Cell Lymphoma in Western Countries. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, 20(9), 1–34.
- Olive, K. P., Jacobetz, M. a, Davidson, C. J., McIntyre, D., Honess, D., Madhu, B., Iacobuzio-donahue, C. (2010). Chemotherapy in a Mouse Model of Pancreatic Cancer. *Cancer Research*, 324(5933), 1457–1461.

- Online, T. S. (2014, February 18). Rise in Cancer Deaths in Malaysia. *Community*. Retrieved from <http://www.thestar.com.my/news/community/2014/02/18/rise-in-cancer-deaths-in-msia-conference-and-expo-this-saturday-to-educate-public-on-how-to-deal-wit/>
- Ott, G., Rosenwald, A., Campo, E., Ott, G., Rosenwald, A., & Campo, E. (2013). Understanding MYC -driven aggressive B-cell lymphomas: pathogenesis and classification. *Blood*, *122*(24), 3884–3891.
- Peh, S. C., Kim, L. H., Thanaletchimy, N., Chai, S. P., & Poppema, S. (2000). Spectrum of malignant lymphomas in Klang Hospital, a public hospital in Malaysia. *The Malaysian Journal of Pathology*, *22*(1), 13–20.
- Peh, S. C., Shaminie, J., Jayasurya, P., & Hiew, J. (2003). Spectrum of Malignant Lymphoma in Queen Elizabeth Hospital, Sabah. *Medical Journal of Malaysia*, *58*(4), 546–555.
- Pfreundschuh, M., Trümper, L., Österborg, A., Pettengell, R., Trneny, M., Imrie, K., Loeffler, M. (2006). CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. *Lancet Oncology*, *7*(5), 379–391.
- Qu, C., Liu, Y., Kunkalla, K., Singh, R. R., Blonska, M., Lin, X., Vega, F. (2013). Trimeric G protein-CARMA1 axis links smoothed, the hedgehog receptor transducer, to NF- $\kappa$ B activation in diffuse large B-cell lymphoma. *Blood*, *121*(23), 4718–4728.
- Rajeev S Samant, L. a S. L. G. H. (2011). Hedgehog Signaling: Networking to Nurture a Promalignant Tumor Microenvironment. *Molecular Cancer Research*, *9*(9), 1165–1174.
- Ramos-Vara, J. A. (2005). Technical aspects of immunohistochemistry. *Vet Pathol*, *42*(4), 405–426.
- Reed, D. W. (1902). *On the pathological changes in Hodgkin's disease, with special reference to its relation to tuberculosis*. (J. H. H. Rep, Ed.). Retrieved from <http://books.google.com.my/books?id=Hn7DNAAACAAJ>
- Reifenberger, J., Wolter, M., Knobbe, C. B., Khler, B., Schnicke, A., Scharwchter, C., Reifenberger, G. (2005). Somatic mutations in the PTCH, SMOH, SUFUH and TP53 genes in sporadic basal cell carcinomas. *British Journal of Dermatology*, *152*(1), 43–51.
- Reifenberger, J., Wolter, M., & Weber, R. G. (1998). Missense mutations in SMOH in sporadic basal cell carcinomas of the skin and primitive neuroectodermal Tumors of the Central Nervous Syndrome. *Cancer Research*, *58*(May), 1798–1803.
- Roessler, E., Belloni, E., Gaudenz, K., Jay, P., Berta, P., Scherer, S. W., Muenke, M. (1996). Mutations in the human Sonic Hedgehog gene cause holoprosencephaly. *Nature Genetics*, *14*(3), 357–360.
- Roessler, E., & Muenke, M. (1998). Holoprosencephaly: A paradigm for the complex genetics of brain development. In *Journal of Inherited Metabolic Disease* (21) 481–497.
- Rosenwald, A., Wright, G., Chan, W. C., Connors, J. M., Campo, E., Fisher, R. I., Staudt, L. M. (2002). The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *The New England Journal of Medicine*, *346*(25), 1937–1947.
- Rubin, L. L., & de Sauvage, F. J. (2006). Targeting the Hedgehog pathway in cancer. *Nature Reviews Drug Discovery*, *5*(12), 1026–1033.

- Ruch, J. M., & Kim, E. J. (2013). Hedgehog signaling pathway and cancer therapeutics: Progress to date. *Drugs*, *73*, 613–623.
- Ruiz I Altaba A. (1999). Gli proteins encode context-dependent positive and negative functions: implications for development and disease. *Development*, *126*(14), 3205–3216.
- Ruiz-gómez, A., Molnar, C., Holguín, H., Mayor, F., & Celis, J. F. De. (2007). The cell biology of Smo signalling and its relationships with GPCRs. *Biochimica et Biophysica Acta*, *1768*, 901–912.
- Sacedón, R., Díez, B., Nuñez, V., Hernández-López, C., Gutierrez-Frías, C., Cejalvo, T., Varas, A. (2005). Sonic hedgehog is produced by follicular dendritic cells and protects germinal center B cells from apoptosis. *Journal of Immunology*, *174*(3), 1456–61.
- Salar, A., Fernández de Sevilla, A., Romagosa, V., Domingo-Claros, A., González-Barca, E., Pera, J., Grañena, A. (1998). Diffuse large B-cell lymphoma: is morphologic subdivision useful in clinical management? *European Journal of Haematology*, *60*(3), 202–8.
- Sanchez, P., Hernández, A. M., Stecca, B., Kahler, A. J., DeGueme, A. M., Barrett, A., Ruiz i Altaba, A. (2004). Inhibition of prostate cancer proliferation by interference with SONIC HEDGEHOG-GLI1 signaling. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(34), 12561–6.
- Sasaki, H., Hui, C., Nakafuku, M., & Kondoh, H. (1997). A binding site for Gli proteins is essential for HNF-3  $\beta$  floor plate enhancer activity in transgenics and can respond to Shh in vitro. *Development*, *1322*(124), 1313–1322.
- Savage, K. J., Johnson, N. A., Ben-neriah, S., Connors, J. M., Sehn, L. H., Farinha, P., Gascoyne, R. D. (2013). MYC gene rearrangements are associated with a poor prognosis in diffuse large B-cell lymphoma patients treated with R-CHOP chemotherapy MYC gene rearrangements are associated with a poor prognosis in diffuse large B-cell lymphoma patients treated with R-C. *Blood*, *3533*–3537.
- Scales, S. J., & de Sauvage, F. J. (2009). Mechanisms of Hedgehog pathway activation in cancer and implications for therapy. *Trends in Pharmacological Sciences*, *30*(6), 303–312.
- Shankland, K. R., Armitage, J. O., & Hancock, B. W. (2012). Non-Hodgkin lymphoma. *The Lancet*, *380*(9844), 848–857.
- Shiozawa, E., Yamochi-Onizuka, T., Takimoto, M., & Ota, H. (2007). The GCB subtype of diffuse large B-cell lymphoma is less frequent in Asian countries. *Leukemia Research*, *31*(11), 1579–1583.
- Shipp et al. (1993). A Predictive Model for Aggressive Non-Hodgkin's Lymphoma Society. All rights reserved. *The New England Journal of Medicine*, *329*(14), 987–994.
- Singh, R. R., Cho-Vega, J. H., Davuluri, Y., Ma, S., Kasbidi, F., Milito, C., ... Vega, F. (2009). Sonic Hedgehog Signaling Pathway Is Activated in ALK-Positive Anaplastic Large Cell Lymphoma. *Cancer Research*, *69*(6), 2550–2558.
- Singh, R. R., Kim, J. E., Davuluri, Y., Drakos, E., Cho-Vega, J. H., Amin, H. M., & Vega, F. (2010). Hedgehog signaling pathway is activated in diffuse large B-cell lymphoma and contributes to tumor cell survival and proliferation. *Leukemia*, *24*(5), 1025–1036.
- Singh, R. R., Kunkalla, K., Qu, C., Schlette, E., Neelapu, S. S., Samaniego, F., & Vega, F. (2011). ABCG2 is a direct transcriptional target of hedgehog signaling and involved in stroma-induced drug tolerance in diffuse large B-cell lymphoma. *Oncogene*, *30*(49), 4874–4886.

- Stecca, B., & Ruiz I Altaba, A. (2010). Context-dependent regulation of the GLI code in cancer by HEDGEHOG and non-HEDGEHOG signals. *Journal of Molecular Cell Biology*.
- Stefancikova, L., Moulis, M., Fabian, P., Vasova, I., Zedek, F., Ravcukova, B., Smardova, J. (2011). Prognostic impact of p53 aberrations for R-CHOP- treated patients with diffuse large B-cell lymphoma. *International Journal of Oncology*, 39(6), 1413–1420.
- Stewart, G. a, Lowrey, J. a, Wakelin, S. J., Fitch, P. M., Lindey, S., Dallman, M. J., Howie, S. E. M. (2002). Sonic hedgehog signaling modulates activation of and cytokine production by human peripheral CD4+ T cells. *Journal of Immunology (Baltimore, Md.: 1950)*, 169(10), 5451–7.
- Strachan, T., & Rees, J. L. (1999). Human molecular genetics 2. In *Cancer Genetics* (Vol. 8, pp. 1621–1630).
- Swerdlow, S. H., Campo, E., Harris, N. L., Jaffe, E. S., Pileri, S. A., Stein, H., Vardiman, J. W. (2008). *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2008. (Vol. 4th).
- Swerdlow, S. H., Campo, E., Pileri, S. A., Harris, N. L., Stein, H., Siebert, R., Jaffe, E. S. (2016). The 2016 revision of the World Health Organization (WHO) classification of lymphoid neoplasms. *Blood*, 127(20), blood–2016–01–643569.
- Syed, I. S., Pedram, A., & Farhat, W. A. (2016). Role of Sonic Hedgehog ( Shh ) Signaling in Bladder Cancer Stemness and Tumorigenesis. *Springer*, 17(2), 1–7.
- Taipale, J., Cooper, M. K., Maiti, T., & Beachy, P. A. (2002). Patched acts catalytically to suppress the activity of Smoothed J. *Nature*, 418(6900), 892–896.
- Tan, B., Goldschmidt, N., Iqbal, J., Ph, D., Vose, J., Bast, M., Ph, D. (2008). Stromal Gene Signatures in Large-B-Cell Lymphomas. *The New England Journal of Medicine*, 359(22), 2313–2323.
- Taylor, C. (2014). Introduction to Immunohistochemistry. In *Immunohistochemical Staining Methods Education Guide* (p. 2014).
- Taylor, M. D., Liu, L., Raffel, C., Hui, C., Mainprize, T. G., Zhang, X., Hogg, D. (2002). Mutations in SUFU predispose to medulloblastoma. *Nat Genet*, 31(3), 306–310.
- Taylor, M. D., Northcott, P. A., Korshunov, A., Remke, M., Cho, Y. J., Clifford, S. C., Pfister, S. M. (2012). Molecular subgroups of medulloblastoma: The current consensus. *Acta Neuropathologica*, 123(4), 465–472.
- Thabane, L. (2004). Sample size determination in clinical trials HRM-733 class notes, 42. Retrieved from <http://www.lehanathabane.com>
- Thayer, S. P., di Magliano, M. P., Heiser, P. W., Nielsen, C. M., Roberts, D. J., Lauwers, G. Y., Hebrok, M. (2003). Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature*, 425(6960), 851–6.
- Theunissen, J.-W., & de Sauvage, F. J. (2009). Paracrine Hedgehog signaling in cancer. *Cancer Research*, 69(15), 6007–6010.
- Toftgård, R. (2000). Hedgehog signalling in cancer. *Cellular and Molecular Life Science: CMLS*, 57(12), 1720–31. <http://doi.org/10.1007/PL00000654>
- Torre, L. A., Bray, F., Siegel, R. L., Ferlay, J., Lortet-tieulent, J., & Jemal, A. (2015). Global Cancer Statistics, 2012. *CA: A Cancer Journal of Clinicians.*, 65(2), 87–108.
- Tostar, U., Malm, C. J., Meis-Kindblom, J. M., Kindblom, L.-G., Toftgard, R., & Uden, A. B. (2006). Deregulation of the hedgehog signalling pathway: a possible role for the PTCH and SUFU genes in human rhabdomyoma and rhabdomyosarcoma development. *The Journal of Pathology*, 208(1), 17–25.



- Van't Veer, L. J., & De Jong, D. (2002). The microarray way to tailored cancer treatment. *Nat Med*, 8(1), 13–14.
- Varjosalo, M., & Taipale, J. (2008). Hedgehog: functions and mechanisms. *Genes & Development*, 22(18), 2454–2472.
- Watkins, D. N. (2003). Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. *Nature*, 422, 313–317.
- Watkins, D. N., & Peacock, C. D. (2004). Hedgehog signalling in foregut malignancy. In *Biochemical Pharmacology* (68) 1055–1060.
- Wilson, K. S., Sehn, L. H., Berry, B., Chhanabhai, M., Fitzgerald, C. A., Gill, K. K., Gascoyne, R. D. (2007). CHOP-R therapy overcomes the adverse prognostic influence of BCL-2 expression in diffuse large B-cell lymphoma, 48(6), 1102–1109.
- World Health Organization. Mortality Data-base 2010. (2010). Retrieved from <http://www.who.int/healthinfo/morttables/en/>.
- Xie, J., Murone, M., Luoh, S. M., Ryan, a, Gu, Q., Zhang, C., de Sauvage, F. J. (1998). Activating Smoothed mutations in sporadic basal-cell carcinoma. *Nature*, 391(January), 90–92.
- Yamauchi Amane, Fujita Shigeki, Ikeda Junichiro, Nakamichi Itsuko, Fukuhara Shiro, H. M. (2007). Diffuse large B-cell lymphoma in the young in Japan: A study by the Osaka Lymphoma Study Group. *American Journal of Hematology*, 82(9), 893–897.
- Yauch, R. L., Gould, S. E., Scales, S. J., Tang, T., Tian, H., Ahn, C. P., de Sauvage, F. J. (2008). A paracrine requirement for hedgehog signalling in cancer. *Nature*, 455(7211), 406–410.
- Young, K. H., Leroy, K., Møller, M. B., Colleoni, G. W. B., Sa, M., Haioun, C., Greiner, T. C. (2008). Structural profiles of TP53 gene mutations predict clinical outcome in diffuse large B-cell lymphoma: an international collaborative study. *Blood*, 112(8), 3088–3098.
- Young, K. H., Weisenburger, D. D., Dave, B. J., Smith, L., Sanger, W., Iqbal, J., Greiner, T. C. (2007). Mutations in the DNA-binding codons of TP53, which are associated with decreased expression of TRAIL receptor-2, predict for poor survival in diffuse large B-cell lymphoma. *Blood*, 110(13), 4396–4406.
- Yung, L., & Linch, D. (2003). Hodgkin's lymphoma. *Lancet (London, England)*, 361(9361), 943–51.
- Zainal, A. O., & Nor Saleha, I. T. (2011). *National Cancer Registry Report. Ministry of Health Malaysia*.
- Zainuddin, N., Berglund, M., Wanders, A., Ren, Z., Amini, R., Lindell, M., Enblad, G. (2009). TP53 mutations predict for poor survival in de novo diffuse large B-cell lymphoma of germinal center subtype. *Leukemia Research*, 33, 60–66. <http://doi.org/10.1016/j.leukres.2008.06.022>
- Zhao, C., Chen, A., Jamieson, C. H., Fereshteh, M., Abrahamsson, A., Blum, J., Reya, T. (2009). Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. *Nature*, 458(7239), 776–9.
- Zheng, C., Tao, J., Won, J., Villavicencio, E., Pfendler, K., Walterhouse, D., & Iannaccone, P. (1998). Characterization of the promoter region and genomic organization of GLI, a member of the Sonic hedgehog-Patched signaling pathway. *Gene*, 209(1-2), 1–11.