ELUCIDATING THE EPIGENETIC ROLES OF NOTCH1 IN REGULATING GATA3 AND C-MYC IN PERIPHERAL T CELL LYMPHOMAS

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by

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LIST OF SYMBOLS

α	alpha
γ	gamma
β	beta
^{0}C	Celsius
<	less than
>	more than
Δ	delta
μ	micro
μM	micromolar
nM	nanomolar
mM	millimolar
mL	milliliters
μL	microlitres

LIST OF ABBREVIATIONS

2-DGE	Two-dimensional gel electrophoresis
5-AZA	5-Azacytidine
AITL	Angioimmunoblastic T cell lymphoma
ALCL	Anaplastic large cell lymphoma
AML	Acute myeloid leukemia
APC	Antigen-presenting cells
ASCT	Autologous stem-cell transplantation
ATLL	Adult T-cell leukemia/lymphoma
b/HLH/LZ	basic helix-loop-helix leucine zipper
Bcl-6	B cell lymphoma 6
bHLH	basic-helix-loop-helix
BL	Burkitt lymphoma
BM	Bone marrow
BV	Brentuximab vedotin
CBC	Complete Blood Count
CDK	Cyclin-dependent kinase
cHL	Classical Hodgkin lymphoma
CLPs	Common lymphoid progenitors
CNS	Central nervous system
CT	Computed tomography
CTCL	Cutaneous T cell lymphoma
DL-1	Delta-like 1
DLBCL	Diffuse large B cell lymphomas
DN cells	Double negative cells
DNMT	DNA methyltransferase
DNMTi	DNA methyltransferase inhibitor
DP cells	Double positive cells
EATL	Enteropathy-associated T cell lymphoma
EBV	Epstein-Barr virus
ECOG	Eastern Cooperative Oncology Group Scale of Performance Status scores
EGF	Epidermal growth factor

ELP	Early lymphoid progenitors
ENKTL-NT	Extranodal natural killer/T-cell lymphoma , nasal type
FDA	Food and Drug Administration
FFPE	Formalin-fixed, paraffin-embedded
FL	Follicular lymphomas
FLT3	FMS-like tyrosine kinase 3
GDP	Gross domestic product
GEP	Gene expression profiling
GIT	Gastrointestinal tract
HATs	histone acetyltransferases
HD	Hodgkin’s disease
HDACi	Histone deacetylases inhibitor
HDACs	Histone deacetylases
HDI	Human Development Index
HSCs	Hematopoietic stem cells
HTLV-1	Human T cell leukemia virus type-I
ICOS	Inducible T cell co-stimulator
IFN-γ	Interferon gamma
IHC	Immunohistochemistry
IPI	International Prognostic Index
IXA	Ixazomib
JAK/STAT	Janus kinases/signal transducers and activators of transcription
LDHL	Lymphocyte-depleted HL
LMPP	Lymphoid primed multipotent progenitors
LRHL	Lymphocyte-rich HL
MCHL	Mixed cellularity HL
MCL	Mantle cell lymphoma
MDS	Myelodysplastic syndrome
MF	Mycosis fungoides
MHC	Major histocompatibility complex
MS-PCR	Methylation-specific-PCR
MZL	Marginal zone lymphoma
NFAT	Nuclear factor of activated T cell
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHL	non-Hodgkin lymphoma

NLPHL	Nodular lymphocyte-predominant Hodgkin lymphoma
NSHL	Nodular sclerosis HL
OPN	Osteopontin
OS	Overall survival
PA	Panobinostat
PI3K	Phosphatidylinositol 3-kinase
PTCL	Peripheral T cell lymphoma
PTCL-NOS	PTCL-not otherwise specified
PTCRA	pre-T cell antigen receptor alpha
r/r	relapsed/refractory
Rag1	Recombination activating gene 1
REAL	Revised European-American Classification of Lymphoid Neoplasm
RORγt	Retinoic acid-related orphan receptor gamma t
RT-qPCR	Quantitative real-time-PCR
SCT	Stem cell transplantation
SEER database	Surveillance, Epidemiology and End Results database
SLL	small lymphocytic lymphoma
SP cells	Single-positive cells
SS	Sézary syndrome
TAMs	Tumor-associated macrophages
TBI-Cy	Total-body irradiation with cyclophosphamide
Tc	T cytotoxic
TCR	T cell receptor
TFH	T-follicular helper cell
Th2	T helper 2
TME	Tumor microenvironment
TNF	Tumor necrosis factor
Tregs	regulatory T cells
TSA	Trichostatin A
TSSP	Thymus-specific serine protease
WHO	World Health Organization

LIST OF APPENDICES

MERUNGKAI PERANAN EPIGENETIK NOTCH1 DALAM MENGAWALATUR GATA3 DAN C-MYC PADA LIMFOMA SEL T PERIFERAL

ABSTRAK

Limfoma sel T periferal (PTCL) adalah kanser darah yang agresif yang mepunyai alternatif rawatan yang terhad dan prognosis yang lemah. Kajian ini meneroka pengawalaturan epigenetik gen utama (*NOTCH1*, *GATA3*, dan *c-Myc*) dalam PTCL untuk mengenal pasti sasaran terapeutik dan meningkatkan pemahaman tentang patogenesis penyakit ini. Perencat epigenetik seperti Trichostatin A (TSA), Panobinostat (PA), 5-Azacytidine (5-AZA), dan perencat proteasom, Ixazomib (IXA), diuji pada tiga titisan sel PTCL iaitu sel SU-DHL-1, Ki-JK, dan DL-40. Terlebih dahulu, ujian MTT digunakan bagi menentukan kesan kesitotoksikan melalui pengiraan pemekatan rencatan separuh maksimal (IC_{50}) dan indeks gabungan perencat (CBI). Sitometri aliran digunakan untuk menganalisis apoptosis dan dinamik kitaran sel. Tindak balas berantai polimerase kuantitatif masa nyata kuantitatif (RT-qPCR) menilai tahap pengekspresan *GATA3*, *c-Myc*, *NOTCH1*, dan subunit *HDAC1–11* dalam titisan-titisan sel PTCL selepas terawat dengan perencat-perencat tersebut. Ujian elektroforesis gel dua dimensi (2-DGE) dijalankan bagi mendedahkan perubahan corak proteom dalam titisan-titisan sel PTCL yang terawat. Profil pemetilan DNA dikenalpasti dalam kes PTCL dari spesimen tisu pesakit yang diawet formalin dan terbenam parafin (FFPE) dan titisan-titisan sel PTCL yang terawat menggunakan pemetilan spesifik PCR (MS-PCR). Ujian imunohistokimia (IHC) mengenalpasti pengekspresan protein NOTCH1, GATA3, dan c-Myc di dalam spesimen FFPE. Perencat-perencat menunjukkan tindak balas memuaskan dan kesan bererti terhadap

kesitotoksikan sel, dengan kesan bererti lebih tinggi ditunjukkan dari nilai IC_{50} yang lebih rendah dalam titisan sel SU-DHL-1 dan Ki-JK berbanding DL-40, yang menunjukkan kesan kesitotoksikan lebih rendah dengan nilai IC_{50} yang lebih tinggi, kecuali PA yang menunjukkan kesitotoksikan yang sederhana. Gabungan PA dan 5-AZA menunjukkan kesan bersinergi di dalam semua titisan sel. Semua perencat mencetus apoptosis yang bererti ($p < 0.05$) selepas terawat selama 48 jam, dan kitaran sel terhenti yang berubah pada fasa G0/G1 atau S di dalam subtip PTCL yang berbeza. Analisis pengekspresan gen menunjukkan pengawalaturan menurun *NOTCH1* yang konsisten dan bererti ($p < 0.05$) dalam semua rawatan, mencadangkan ia sebagai sasaran terapeutik yang berpotensi. *c-Myc* menunjukkan pengawalaturan menurun yang konsisten ($p < 0.05$) kecuali dalam beberapa SU-DHL-1 yang terawat. *GATA3* kebanyakannya mengalami pengawalaturan menaik, kecuali yang melibatkan titisan sel DL-40 dan hampir semua Ki-JK. Pengekspresan subunit *HDACs* menunjukkan corak berubah, dan analisis proteom menunjukkan perubahan bererti ($p < 0.05$) dalam corak pengekspresan protein dalam titisan sel PTCL terawat. Profil pemetilan DNA mendedahkan berlakunya pemetilan separa *GATA3* dan *NOTCH1* dalam garis sel PTCL yang terawat dan tidak terawat, manakala *c-Myc* pula kekal tiada pemetilan. Analisis kes pesakit PTCL (n = 42) menunjukkan profil pengekspresan protein yang jelas berbeza merentasi subtip kes PTCL yang bepotensi untuk sasaran terapi. Walau bagaimanapun, secara statistik, pengekspresan protein tidak menunjukkan kaitan bererti bersama ciri-ciri sosiodemografik, dan klinikopatologi ($p > 0.05$). Analisis pemetilan kes pesakit PTCL (n = 28) mengenalpasti pemetilan *NOTCH1*yang lebih tinggi (14.3%) berbanding gen-gen yang lain, mencadangkan potensi peranan *NOTCH1* dalam penyenyapan gen melalui pengubahsuaian epigenetik. Kajian ini menyediakan wawasan ke dalam pengawalaturan epigenetik PTCL dan potensi

sasaran terapi, khususnya apabila gabungan PA dan 5-AZA mempamerkan kesan bersinergi merentasi semua titisan sel, menonjolkan pendekatan terapi tersuai. Kesimpulannya, NOTCH1 dijumpai mempunyai kesan bererti terhadap c-Myc dan GATA3 dalam proses epigenetik dan progressi penyakit PTCL.

ELUCIDATING THE EPIGENETIC ROLES OF NOTCH1 IN REGULATING GATA3 AND C-MYC IN PERIPHERAL T CELL LYMPHOMAS

ABSTRACT

Peripheral T cell lymphomas (PTCLs) are aggressive haematological malignancies with limited treatment options and poor prognoses. This study explores the epigenetic regulation of key genes (*NOTCH1*, *GATA3*, and *c-Myc*) in PTCL to uncover therapeutic targets and enhance the understanding of disease pathogenesis. Epigenetic inhibitors, such as Trichostatin A (TSA), Panobinostat (PA), and 5-Azacytidine (5-AZA), and the proteasome inhibitor, Ixazomib (IXA), were examined on three PTCL cell lines (SU- DHL-1, Ki-JK, and DL-40). MTT assays determined cytotoxicity through the calculated half maximal inhibitory concentration (IC_{50}) and the combination drug index (CBI). Flow cytometry were employed to analyse apoptosis and cell cycle dynamics. Quantitative real-time polymerase chain reaction (RT-qPCR) evaluated *GATA3*, *c-Myc*, *NOTCH1*, and *HDAC1–11* expression levels in PTCL cell lines post-drug treatment. Two-dimensional gel electrophoresis (2-DGE) revealed proteome pattern alterations in treated PTCL cell lines. DNA methylation profiles were identified in PTCL cases from formalin-fixed paraffin embedded (FFPE) specimens and treated PTCL cell lines using methylation-specific PCR (MS-PCR). Immunohistochemistry (IHC) examined NOTCH1, GATA3, and c-Myc protein expression from the FFPE specimens. The inhibitors showed a favourable response and significant effect on cell cytotoxicity, with significantly higher cytotoxicity indicated by lower IC_{50} values in SU-DHL-1 and Ki- JK compared to DL-40, which exhibited lower drug cytotoxicity with higher IC_{50} values, except for PA treatment,

which showed moderate cytotoxicity. A combination of PA and 5-AZA exhibited a synergistic effect across all cell lines. All inhibitors induced significant ($p < 0.05$) cell apoptosis after 48 hours of treatment, with varying cell cycle arrest at the G0/G1 or S phases across different PTCL subtypes. Gene expression analysis demonstrated consistent and significant downregulation of *NOTCH1* ($p < 0.05$) in all treatments, suggesting it as a potential therapeutic target. *c-Myc* showed consistent downregulation ($p < 0.05$) except in a few treated SU-DHL-1. *GATA3* was mostly upregulated, with exceptions in a few treatments in DL-40 and nearly all Ki-JK. *HDACs* expression displayed variable patterns, and proteome analysis indicated significant ($p < 0.05$) changes in protein expression patterns in treated PTCL cell lines. DNA methylation profile revealed partial methylation of *GATA3* and *NOTCH1* in treated and untreated PTCL cell lines, while *c-Myc* remained unmethylated. Analysis of PTCL cases (n = 42) showed distinct protein expression profiles across PTCL subtypes, offering potential avenues for targeted therapy. However, protein expressions were not statistically associated with sociodemographic features, and clinicopathological characteristics ($p > 0.05$). Methylation analysis of PTCL cases (n = 28) identified higher *NOTCH1* methylation (14.3%) compared to other genes, suggesting *NOTCH1's* potential role in gene silencing through epigenetic modifications. This study provides insights into PTCL epigenetic regulation and potential therapeutic targets, particularly when a combination of PA and 5-AZA exhibited a synergistic effect across all cell lines, highlighting customised therapy approaches. In conclusion, NOTCH1 was found to have a significant effect on c-Myc and GATA3 in the epigenetic processes and disease progression of PTCL.

CHAPTER 1

INTRODUCTION

1.1 Introduction to the study

Peripheral T cell lymphoma (PTCL) is a heterogeneous group of mature T and NK tumours, consisting of approximately 29 different subtypes with various cellular morphologies and genetic mutations, according to the World Health Organization (WHO) classification (Swerdlow *et al.*, 2016). Because of their rarity and extensive molecular abnormalities, the pathophysiology of PTCLs is poorly understood (de Leval, 2020). A poorer prognosis in PTCL has been related to the expression of *NOTCH1*, *GATA3*, and *c-Myc*, which are also involved in downstream processes such as T cell proliferation, differentiation, and cell cycle progression (Manso *et al.*, 2016).

PTCL accounts for approximately 10-15% of all non-Hodgkin Lymphomas (NHLs) and exhibits more aggressive behaviour and poorer prognosis than most B cell NHLs (Satou *et al.*, 2019). A complete blood count, serum biochemistry analysis, radiological, and tissue biopsies are part of the clinical evaluation. Pathologists face a difficult challenge in the histological diagnosis of PTCL because of the extensive distinctions between PTCL in terms of cellular composition, morphological features, and immunophenotypes. Moreover, certain features of various diseases overlap significantly, and not all diseases are associated with specific mutations. Furthermore, the diagnosis of this disease is rare and challenging; therefore, many reactive T cell lymphoproliferations may be mistaken for cancer. Hence, it is crucial to incorporate pathological data into the assessment of the clinical scenario, as this step is essential in ensuring a comprehensive and accurate evaluation before arriving at a final diagnosis or treatment plan (de Leval, 2020).

PTCL pathogenesis mainly includes dysregulation of TCR signalling pathways, interactions with the non-neoplastic tumour microenvironment (TME), and neoplastic transformation due to viral and chronic inflammation. In addition, PTCL carcinogenesis includes genetic mutations and cytogenetic abnormalities, such as chromosomal translocations, insertions, and deletions leading to fusion proteins, constitutive activation, hyperactivation, and gene loss. The process by which neoplastic lymphocytes mediate these different biological behaviours is based on the reduction in tumour cell immunogenicity and the regulation of environmental signals. T-cell lymphomas evade immune recognition by suppressing responses to pro-apoptotic stimuli through oncogenic events. These include deregulated apoptotic signalling (Fas/FasL, TRAIL/TRAIL receptors), overexpression of anti-apoptotic proteins (FLIP, AIP), and upregulation of CD47 (Pizzi *et al.*, 2018).

Immune escape tactics rely primarily on modifying the TME to promote neoplastic cell proliferation. As a result, T cell lymphomas like PTCL generate soluble mediators such as cytokines and chemokines. Cytokine microenvironment plays an important role in lymphomagenesis. Mutation analysis is currently used in clinical trials of new drugs to assess various indicators of response to improve treatment decisions, such as CD30-directed antibody drugs, CD25, CCR4 tag, PI3 kinase inhibitors, JAK/STAT inhibitors, and ALK inhibitors. Furthermore, widespread epigenetic dysregulation affects processes, such as mutations in epigenetic regulators of *KDM6A*, *MLL2*, *TET2*, and DNMT3, which control genes in signalling pathways such as ZAP70, CHD8, APC, and TRAF3 (Zain, 2019). Gene expression studies on peripheral T cell lymphoma-not otherwise specified (PTCL-NOS) have identified two prognostic groups, each with a different putative cellular origin. GATA3 and its target genes (*CCR*, *IL18RA*, *CXCR7*, and *IK*) are highly expressed and are thought to originate from Th2-

like cells, with a poor prognosis. GATA3 is regarded as a master regulator of Th2 cell differentiation. In contrast, the expression of the transcription factors TBX21 and EOMES, as well as their target genes (*CXCR3*, *IL2RB*, *CCL3*, and *IFN*), is associated with a favourable outcome, and TBX21 is regarded as a master regulator of Th1 cell differentiation. A small subgroup of PTCLs that exhibit TBX21 and have poor prognosis show cytotoxic markers and specific cytokine transcripts, such as CXCR3 and CXCL12, and have been associated with $CD8^+$ cytotoxic cells (Piccaluga *et al.*, 2013; Iqbal *et al.*, 2019). To determine whether the GATA3-positive subset of tumours is enriched in Myc and proliferative gene signatures in comparison with other groups, researchers examined the potential relationship between the existence of c-Myc, GATA3, and high levels of Ki-67 expression in PTCL patients. The findings revealed no significant association between Ki-67 and GATA3 but significant positive associations between *c-Myc* expression and the existence of both Ki-67 and GATA3. In addition, a significant positive association between *c-Myc* expression and the prevalence of both Ki-67 and GATA3 was observed in the PTCL-NOS subset. Double-positive (DP) thymocytes are transformed into a pre-malignant state by forced *GATA3* expression during normal T cell development, which is emphasised by elevated *c-Myc* expression. This pre-malignant state then stimulates NOTCH1 signalling, which assists in the establishment of malignant transformation (van Hamburg *et al.*, 2008). GATA3 mRNA and protein levels inversely correlated with TBX21 levels. Additionally, a comparison of GATA3 and TBX21 revealed that GATA3 has a poorer overall survival rate than TBX21. The TBX21 subgroup had significantly higher IFNα/β/γ-regulated gene signatures, $CD8^+$ T cell profiles, and NF-kB pathway signatures than the GATA3 subgroup, which had slightly higher mTOR- and Myc-related gene signatures and significantly higher PI3 Kinase-induced gene signatures (Iqbal *et al.*, 2014). Moreover,

widespread epigenetic dysregulation affects these processes like histone and DNA methylation (Zain, 2019).

NOTCH1 signalling plays an important role in the normal development of T cells, involving GATA3 and c-Myc in downstream signalling (Al-Khreisat *et al.*, 2022). *NOTCH1* mutations and their interactions with *GATA3* and *c-Myc* have been widely studied in T lymphoblastic lymphoma (immature T cells) (Weng *et al.*, 2006; Dorfman *et al.*, 2017). Aberrant methylation of *NOTCH1*, *GATA3*, and *c-Myc* has also been studied and shown to affect normal T lymphocytes during the maturation process. However, there is still a lack of evidence regarding their involvement in PTCLs. Thus, in this study, the epigenetic relationship between NOTCH1 with GATA3 and c-Myc in PTCLs cell lines and patient samples were investigated. Real-time quantitative polymerase chain reaction (RT-qPCR) was used to investigate the expression of *GATA3*, *c-Myc*, and *NOTCH1* in PTCL cell lines (SU-DHL-1, Ki-JK, and DL-40) following treatment with epigenetic modulation drugs and proteasome-inhibitory drugs. Through two-dimensional gel electrophoresis (2-DGE), protein patterns in treated and untreated PTCL cell lines were determined. Using methylation-specific PCR (MS-PCR), the DNA methylation status of formalin-fixed, paraffin-embedded (FFPE) tissue from PTCL patients was determined. Immunohistochemistry (IHC) was performed to examine the protein expression of the selected genes (NOTCH1, GATA3, T-bet/TBX21, and c-Myc) in FFPE cases. The outcomes of this study will provide a platform for exploring aberrant genes in PTCL behaviour that could lead to alternative solutions in treatment, thus enhancing the overall survival rate through targeted therapy and a more strategised, cost-effective treatment.

1.2 Problem statements

PTCLs are typically more aggressive, and exhibit variable responses to drugs. Key factors in PTCL development, such as NOTCH1, GATA3, and c-Myc, remain poorly understood. Previous research has shown that GATA3 expression is linked to poor prognosis in PTCL-NOS, but its role in other PTCL types remains unexplored. The interaction of NOTCH1 with GATA3 and c-Myc in T cell development suggests that dysregulated signalling could contribute to PTCL development. However, the epigenetic mechanisms by which NOTCH1 regulates GATA3 and c-Myc expression in PTCLs are not fully understood.

1.3 Research questions

1. How do epigenetic modulating agents and proteasome-inhibitory drugs affect IC_{50} values, CBI, apoptosis induction, and cell cycle arrest in PTCL cell lines compared to untreated cells?
2. What are the changes in gene expression and proteome profiles of *NOTCH1*, *GATA3*, *c-Myc*, and *HDAC1-11* in PTCL cell lines following treatment with epigenetic modulating agents and proteasome-inhibitory drugs?
3. What is the role of the *NOTCH1* in regulating the expression of *GATA3* and *c-Myc* in PTCL cases, and how are these effects associated with changes in methylation profiles and protein expression in PTCL cases?
4. How do the protein expression levels of NOTCH1, GATA3, c-Myc, and T-bet/TBX21 correlate with DNA methylation profiles in PTCL cases?
5. What are the associations between the protein expression levels of NOTCH1, GATA3, c-Myc, and T-bet/TBX21 and sociodemographic and clinicopathological factors in PTCL patients?

1.4 Justification of the study

The rationale for this study lies in the need for our comprehension of the pathogenesis of PTCLs, a diverse and often aggressive group of malignancies for which limited information is available, especially when compared to their B cell counterparts. Investigating the roles of key genes like NOTCH1, GATA3, and c-Myc, as well as their interactions within PTCLs, holds the potential to unveil crucial insights into the development, progression, and response to treatment of these diseases. In Malaysia, NHL accounted for approximately 4% of all reported cancer cases in 2020. The treatment of NHL T cells varies depending on their specific types, but it predominantly involves intensive chemotherapy and a combination of multiple drugs due to the fast-growing nature of PTCLs, creating a substantial economic burden for the country (Satou *et al.*, 2019). Moreover, this study addresses a critical gap in our understanding of the epigenetic regulation of these genes in PTCLs. Gaining insights into the role of epigenetic modifications, such as methylation, in gene expression can provide valuable knowledge regarding the molecular mechanisms that underlie PTCL pathogenicity. This information is essential for the development of targeted therapies and the enhancement of clinical management. Furthermore, this research carries significant weight by delving into different PTCL subtypes, including PTCL-NOS, and evaluating the differential roles and effects of these key genes. By uncovering subtype-specific information, the study can potentially provide a more precise foundation for diagnosis, prognosis, and personalised treatment strategies.

1.5 Hypothesis

1. Epigenetic modulating agents and proteasome-inhibitory drugs will induce significant apoptosis and cell cycle arrest in PTCL cell lines, affecting the IC_{50} values and resulting in favourable CBI, compared to untreated cells.
2. Treatment with epigenetic modulating agents and proteasome-inhibitory drugs will significantly alter the gene expression and proteome profiles of *NOTCH1*, *GATA3*, *c-Myc*, and *HDAC1-11* in PTCL cell lines, resulting in distinct upregulation or downregulation patterns that differ from untreated cells.
3. The NOTCH1 plays a critical role in PTCL by modulating the expression of *GATA3* and *c-Myc*, with alterations in NOTCH1 leading to significant changes in methylation profiles and protein expression within PTCL cases.
4. In PTCL cases, the protein expression levels of NOTCH1, GATA3, c-Myc, and T-bet/TBX21 are correlated with distinct DNA methylation profiles indicating epigenetic regulation of these proteins.
5. There is a significant association between the protein expression levels of NOTCH1, GATA3, c-Myc, and T-bet/TBX21 in PTCL patients and various sociodemographic and clinicopathological factors, such as age, gender, tumor stage, and prognosis.

1.6 Objective of the study

1.6.1 General objective

To investigate the epigenetic impact of *NOTCH1* on the regulation of downstream signalling pathways involving *GATA3* and *c-Myc* in PTCL cell lines (SU-DHL-1, Ki-JK, and DL-40) treated with epigenetic modulating agents such as Trichostatin A (TSA), Panobinostat (PA), and 5-Azacytidine (5-AZA), along with a proteasome inhibitory drug, Ixazomib (IXA), and to elucidate the role of the *NOTCH1* in PTCL patients by investigating methylation profiles and protein expression in archived FFPE tissues, aiming to understand their implications for PTCL patient outcomes.

1.6.2 Specific objectives

1. To determine the IC_{50}, CBI, apoptosis induction and cell cycle arrest in PTCL cell lines treated with epigenetic modulating agents and proteasome-inhibitory drugs using MTT assay and flow cytometry.

2. To study the gene expression and proteome profile changes of *NOTCH1*, *GATA3*, *c-Myc*, and *HDAC1-11* in PTCL cell lines treated with epigenetic modulating agents and proteasome-inhibitory drugs using RT-qPCR and 2-DGE.

3. To investigate the specific role of the NOTCH1 in PTCL cases by assessing its impact on the regulation of *GATA3* and *c-Myc* expression and explore the associated changes in methylation profiles and protein expression in PTCL cases.

4. To identify the proteins expression of NOTCH1, GATA3, c-Myc, and T-bet/TBX21 and determine the DNA methylation profiles in PTCL cases using IHC and MS-PCR.

5. To determine the association of protein expression of NOTCH1, GATA3, c-Myc, and T-bet/TBX21 in PTCL patients with the sociodemographic and clinicopathological factors.

1.7 Research methodology flowchart

The research methodology flowchart is presented in Figure 1.1.

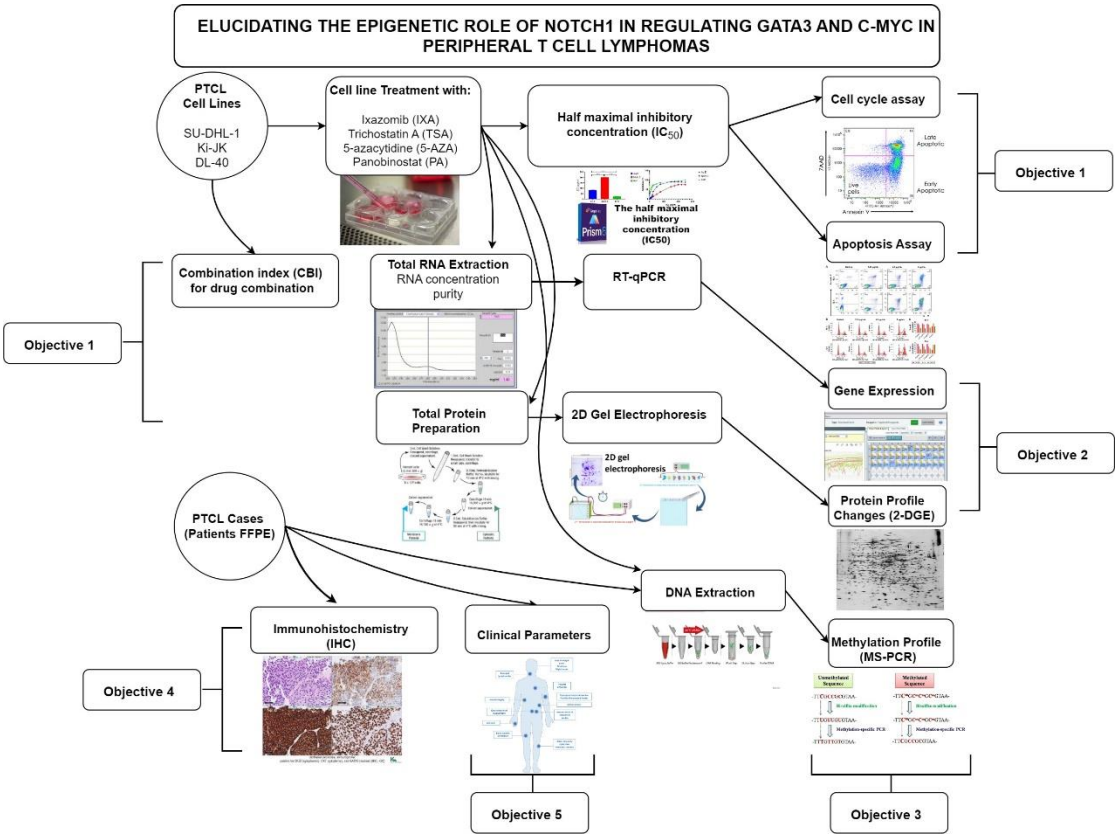

Figure 1.1 Research methodology flowchart.

CHAPTER 2

LITERATURE REVIEW

2.1 Lymphoma

Lymphoma is a heterogeneous group of malignant tumours of the haematopoietic system and is characterized by aberrant proliferation of mature lymphoid cells or their precursors (Oueslati *et al.*, 2020). Moreover, lymphoma has abnormal lymphocyte proliferation that affects lymphoid tissues, including lymph nodes, spleen, bone marrow (BM), blood, and other organs. In addition, lymphoma presents as a solid tumour of lymphoid cells (Swerdlow *et al.*, 2016). Lymphomas are classified into two main types: Hodgkin lymphoma (HL) and NHL, depending on the histopathological evidence of a biopsy taken from an enlarged lymph node (Swerdlow *et al.*, 2008).

Lymphoma can occur at any age; however, it has a bimodal presentation, with one peak in the early years of life and another after middle age (Jose *et al.*, 2005). Patients with lymphoma usually present with constitutional symptoms such as weight loss, fever, night sweats, and enlarged lymph nodes. Symptoms may also develop due to pressure effects of lymph nodes on surrounding structures or due to the involvement of extranodal sites such as the gastrointestinal tract (GIT), central nervous system (CNS), liver, or bone, thus leading to atypical presentations (O'Sullivan *et al.*, 2015). Several classifications have been developed for lymphomas. The current classification is that of the World Health Organization (WHO) and was based on the principles of the Revised European-American Classification of Lymphoid Neoplasm (REAL) in 1994 (Harris *et al.*, 1994).

The subtype of lymphoma is defined based on the cell of origin: B-cell lymphoma, T cell lymphoma, natural killer cell lymphoma (T/NK-NHL), and HL (Storck *et al.*, 2019). The two recent WHO classifications from 2008 and 2017 include and encompass morphology, immunophenotype, genetic, and clinical features in order to define "real" diseases (Swerdlow *et al.*, 2008; Arber *et al.*, 2016; Swerdlow *et al.*, 2016; Swerdlow *et al.*, 2017).

2.1.1 Classification of Lymphoma

Lymphomas entities are divided into three main categories: HLs, and T and NK-cell neoplasms, and B-cell neoplasms (Figure 2.1) (de Leval and Jaffe, 2020).

2017 WHO Classification of Mature Lymphomas

B-Cell Neoplasms

Predominantly disseminated
- Chronic lymphocytic leukemia/B-cell small lymphocytic lymphoma
- Monoclonal B-cell lymphocytosis,
- B-cell prolymphocytic leukemia
- Splenic marginal zone B-cell lymphoma
- Hairy cell leukemia
- Splenic B-cell lymphoma/leukemia, unclassifiable*
- Splenic diffuse red pulp small B-cell lymphoma*
- Hairy cell leukemia—variant*
- Lymphoplasmacytic lymphoma
- Waldenström macroglobulinemia
- Monoclonal gammopathy of unknown significance (MGUS) immunoglobulin M
- Mu heavy-chain disease
- Gamma heavy-chain disease
- Alpha heavy-chain disease
- MGUS immunoglobulin G/A^{+}
- Plasma cell myeloma
- Solitary plasmacytoma of bone
- Extraosseous plasmacytoma
- Monoclonal immunoglobulin deposition disease

Primary extranodal or nodal
- Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue
- Nodal marginal zone B-cell lymphoma
  Pediatric nodal marginal zone lymphoma*
- Follicular lymphoma
  In situ follicular neoplasia
  Duodenal-type follicular lymphoma
- Pediatric-type follicular lymphoma
- Large B-cell lymphoma with *IRF4* rearrangement*
- Primary cutaneous follicle center lymphoma
- Mantle cell lymphoma
  In situ mantle cell neoplasia
- DLBCL-NOS
  Germinal center B-cell type
  Activated B-cell type
- T-cell/histiocyte-rich large B-cell lymphoma
- Primary DLBCL of the central nervous system
- Primary cutaneous DLBCL, leg-type
- EBV^{+} DLBCL-NOS
- EBV^{+} mucocutaneous ulcer
- DLBCL associated with chronic inflammation
- Lymphomatoid granulomatosis
- Mediastinal (thymic) large B-cell lymphoma
- Intravascular large B-cell lymphoma
- ALK^{+} large B-cell lymphoma
- Plasmablastic lymphoma
- Primary effusion lymphoma
- HHV8^{+} DLBCL-NOS*
- Burkitt lymphoma
- Burkitt-like lymphoma with 11q aberration*
- Lymphomatoid granulomatosis
- High-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* gene rearrangements*
- High-grade B-cell lymphoma NOS*
- B-cell lymphoma unclassifiable with features intermediate between DLBCL and cHL

T and NK-cell Neoplasms

Predominantly disseminated
- T-cell prolymphocytic leukemia
- T-cell large granular lymphocytic leukemia
- Aggressive NK-cell leukemia
- Chronic lymphoproliferative disorder of NK cells*
- Systemic EBV-positive T-cell lymphoma of childhood*
- Chronic active EBV infection of T- and NK-cell type, systemic form
- Adult T-cell lymphoma/leukemia (HTLV-1^{+})

(Continued)

Primary extranodal
- Extranodal NK/T-cell lymphoma, nasal type
- EATL
- Monomorphic epitheliotropic intestinal T-cell lymphoma
- Indolent T-cell lymphoproliferative disorder of the gastro-intestinal tract*
- Hepatosplenic T-cell lymphoma
- Subcutaneous panniculitis-like T-cell lymphoma
- Breast implant–associated ALCL*
- Subcutaneous panniculitis-like T-cell lymphoma

Primary cutaneous
- Mycosis fungoides
- Sézary syndrome
- Primary cutaneous CD30^{+} lymphoproliferative disorders
  Lymphomatoid papulosis
  Primary cutaneous ALCL
- Primary cutaneous γδ T-cell lymphoma
- Provisional CD4^{+} or CD8^{+} entities*
- Hydroa vacciniforme–like lymphoproliferative disorder
- Severe mosquito bite allergy

Predominantly nodal
- PTCL-NOS
- Angioimmunoblastic T-cell lymphoma
- Follicular T-cell lymphoma
- Nodal PTCL with T follicular helper phenotype
- ALCL, ALK-positive
- ALCL, ALK-negative

Hodgkin lymphomas
- **Nodular lymphocyte predominance HL**
- classical HL

Nodular sclerosis cHL
Mixed cellularity cHL
Lymphocyte-rich cHL
Lymphocyte-depleted cHL

Immunodeficiency-associated lymphoproliferative disorders
Posttransplantation lymphoproliferative disorders (PTLD)
Nondestructive PTLD
  Plasmacytic hyperplasia
  Infectious mononucleosis PTLD
  Florid follicular hyperplasia PTLD
Polymorphic PTLD
Monomorphic PTLD
cHL PTLD
Other iatrogenic immunodeficiency-associated lymphoproliferative disorders

*Provisional entities.
Swerdlow et al.[22]

Figure 2.1 WHO classification of mature lymphoma. Adapted from de Leval and Jaffe, (2020).

2.1.1(a) Hodgkin lymphoma classification

HL has a number of distinctive characteristics that make it almost entirely unique among human cancers, including a young age of onset, the rarity of malignant cells, a high cure rate, even when the patient initially has advanced metastatic spread, and a heightened susceptibility to radiation therapy. HLs have been classified into two main types that are quite distinct from each other in terms of biology, morphology, and phenotype: classical Hodgkin lymphoma (cHL) and Hodgkin lymphoma with nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) (Swerdlow *et al.*, 2017; Shanbhag and Ambinder, 2018).

cHL is a monoclonal lymphoid neoplasm derived from B cells that accounts for 85% of all Hodgkin lymphomas. It is characterized by Reed-Sternberg (HRS) cells that are dispersed throughout a microenvironment made up of inflammatory cells, lymphocytes, and macrophages and that express the cell surface CD30 antigen (also known as tumour necrosis factor (TNF) receptor superfamily member 8) on their surfaces (Bray *et al.*, 2015; Swerdlow *et al.*, 2017; Wang *et al.*, 2019). Nodular sclerosis HL (NSHL), mixed cellularity HL (MCHL), lymphocyte-rich HL (LRHL), and lymphocyte-depleted HL (LDHL) are the four subtypes of cHL. However, in 5% of patients with cHL, a subtype cannot be determined, typically due to a lack of data for a thorough examination (Connors *et al.*, 2020; Moscona-Nissan *et al.*, 2023) (Figure 2.2).

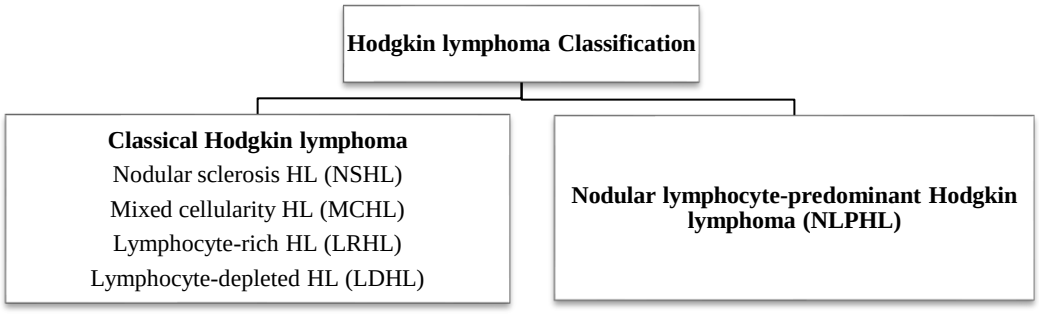

Figure 2.2 WHO classification of HL.

2.1.1(b) Non-Hodgkin lymphoma classification

NHL involves a heterogeneous group of over 40 lymphoproliferative malignancies with various behavioural patterns and treatment responses. NHL is much less predictable than HL, and prognosis depends on the histologic type, stage, and treatment (Abramson, 2020; Singh *et al.*, 2020b). NHL has also become more prevalent in recent years; this may be related to the rise in the number of people with immune systems that are suppressed, such as HIV patients and recipients of organ transplants, due to the use of immune-suppressing medications. Based on the cell of origin, NHL subtypes are classified as either B lymphocytes (which account for around 86% of NHL) or T/NK cells (which account for about 14%) (Swerdlow *et al.*, 2008; Arber *et al.*, 2016; Swerdlow *et al.*, 2016). NHL is classified into T/NK and B cell lymphoma.

T cell lymphomas form a heterogeneous group of lymphoproliferative disorders, comprising 10-15% of all lymphomas. The prevalence of these disorders varies based on geographic location (Somasundaram and Thye Lim, 2019). The majority of T cell lymphomas are aggressive in nature, with a few subtypes linked to a more indolent course. PTCL patients have a very poor prognosis because of their aggressive disease course and the lack of effective treatment. The 2017 updated WHO classification of tumours of hematopoietic and lymphoid tissues has resulted in the reclassification of some mature T cell lymphomas and the establishment of a few new provisional entities as a result of the accumulation of data from clinical and laboratory studies, particularly molecular genetic studies (Hsi, 2017). Based on the 2017 WHO classification of lymphoid malignancies, T- and NK-cell lymphomas can be classified into predominantly nodal, primary extranodal, primary cutaneous, and predominantly disseminated (Swerdlow *et al.*, 2017; de Leval and Jaffe, 2020) (Figure 2.3).

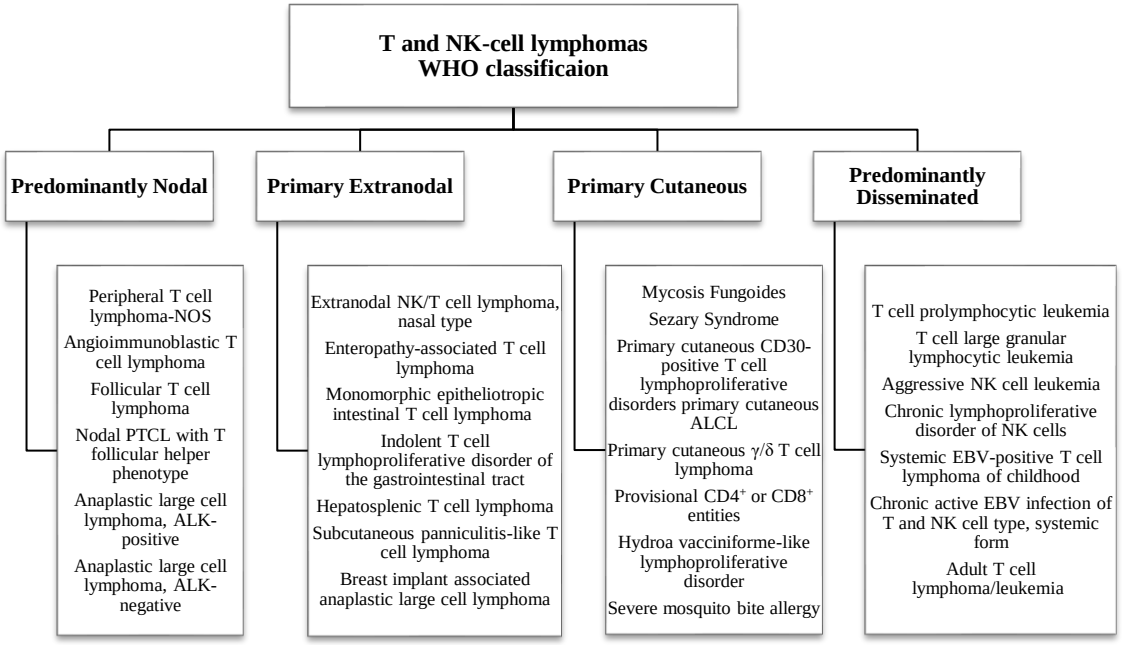

Figure 2.3 WHO classification of mature T/NK cell NHL.

Due to common genetic mutations like those found in *TET2*, *IDH2*, *DNMT3A*, *RHOA*, and *CD28*, as well as fusions like ITK-SYK and CTLA4-CD28, nodal PTCL, previously classified as PTCL-NOS, was reclassified under the angioimmunoblastic T cell lymphoma (AITL) classification because of the similar genetic landscape according to the latest classification (Swerdlow *et al.*, 2016; Swerdlow *et al.*, 2017; Matutes, 2018).

2.1.2 Epidemiology of Lymphoma

2.1.2(a) Hodgkin lymphoma epidemiology

In HL, the histological subtype, Epstein-Barr virus (EBV) tumors status, and demographics all affect the trimodal age-specific incidence pattern significantly. Furthermore, in populations of European ancestry, the average annual age-adjusted incidence of HL is between 2 and 3 per 100,000 people, which indicates a low overall incidence of the disease (Connors *et al.*, 2020).

In 2020, the global incidence and mortality rates of HL were 0.98 and 0.26 per 100,000, respectively. A higher incidence was observed in high-income countries, while a higher mortality rate was found in low-income countries. At the population level, incidence and mortality were associated with gross domestic product (GDP) per capita and the prevalence of smoking, obesity, and hypertension. Despite the decreasing mortality trend, there was an increasing incidence, especially among females, the younger population, and subjects from Asian countries (Huang *et al.*, 2022). In Malaysia, the updated cancer statistics report revealed that the incidence and mortality rates of HL in 2023 were 0.5 and 0.25, respectively (Ferlay *et al.*, 2024).

2.1.2(b) Non-Hodgkin lymphoma epidemiology

In 2020, there were an estimated 544,352 new cases of cancer, leading to 259,793 cancer-related deaths, with NHL ranked as the 12th most prevalent malignancy worldwide (Sung et al., 2021). Importantly, in Malaysia, NHL ranked as the 6th most prevalent cancer, with incidence and mortality rates of 4.2 and 4.1, respectively, in 2023 (Ferlay et al., 2024). Incidence rates of NHL ranged almost five times among WHO regions for both men and women. The highest age-standardized incidence rates (per 100,000) for both sexes were found in Australia and New Zealand (12.5) and North America (12), while the lowest rates (2.7), a 4.6-fold difference, were found in South-Central Asia. NHL rates were higher (9.3) in areas with a very high Human Development Index (HDI) than in areas with a medium (3.1) or low (4.5) HDI. For both sexes, the worldwide variation in NHL mortality rates is 2.5-fold. South-Central Asia had the lowest mortality rates (1.6), whereas Melanesia had the highest rates (4.0). While the 5-year relative survival rates increased from 47% in 1975 to 73.8% in 2018, the age-standardized NHL incidence rate (per 100,000) doubled from 1975 (11) to 2007 (21.3) and subsequently stabilized. These changes have significantly impacted age-

adjusted NHL mortality rates (per 100,000), which were 5.6 in 1975, peaked at 8.9 in 1997, and have been declining ever since, most recently reaching 5 in 2019. Other Western nations showed the same wide temporal trend patterns. NHL incidence rates have increased in Japan from 1993 to 2010 and in the Republic of Korea from 1999 to 2012. Assuming incidence rates of NHL in different countries or regions remain consistent and do not change, an estimated 779,000 new NHL cases are expected to be diagnosed globally in 2040, a 43% rise from the comparable 544,000 cases in 2020 (Sedeta *et al.*, 2022).

2.2 Peripheral T cell lymphoma

PTCLs are heterogenous groups of mature T cell NHL. PTCL specifically implicates T cells and results when T cells develop and grow abnormally. The term "peripheral T cell lymphoma" refers to PTCL's lymphatic system origin. The word "peripheral" in the context of PTCL describes the cancer as developing in lymphoid tissues outside of the BM, including the lymph nodes, spleen, GIT, and skin (Islam, 2018).

2.2.1 Development and regulation of T cells

T cells are pivotal in the immune response against infections and malignancies, with their differentiation into subsets such as CD4 T helper cells (Th1, Th2, Th17, Treg) and CD8 cytotoxic T cells (Tc) being essential for effective immunity (Zhang and Bevan, 2011). This differentiation involves several critical stages, beginning with the activation of the NOTCH signalling pathway in early T cell progenitors (ETPs) upon their entry into the thymus. In the first phase, NOTCH signalling drives the commitment to the T cell lineage, with transcription factors like GATA3 and Bcl11b playing key roles (Artavanis-Tsakonas *et al.*, 1999; Almotiri *et al.*, 2021). The second phase

involves TCR rearrangement and slow proliferation. During this phase, the expression of Bcl11b is crucial as it ensures that cells committed to the T cell lineage do not revert to other cell types. Meanwhile, E proteins regulate the expression of genes necessary for T cell development (Yui and Rothenberg, 2014; Hosokawa and Rothenberg, 2018). In the third phase of T cell development, T cells transition from NOTCH-dependent to NOTCH-independent signalling, marked by increased IL-7R expression and rapid proliferation (Maillard *et al.*, 2006). During this stage, T cells express CD4 or CD8 co-receptors, which define their future roles as helper or cytotoxic T cells, respectively. Transcription factors IKZF3 and RORγt play crucial roles in regulating gene expression and differentiation by directing the maturation and function of T cells (Janas *et al.*, 2010; Hosokawa and Rothenberg, 2018). T cell development is completed through thymic selection processes: positive selection in the thymic cortex ensures that T cells can recognize MHC molecules, and negative selection in the thymic medulla eliminates self-reactive T cells, preventing autoimmunity. These steps ensure that the T cells are both functional and self-tolerant (Murata *et al.*, 2007; Klein *et al.*, 2014).

2.2.2 Classification of peripheral T cell lymphomas

The 2017 WHO classification categorizes PTCL based on distinct features. This classification helps in distinguishing various PTCL subtypes, contributing to a more thorough understanding of this diverse lymphoma group.

2.2.2(a) Peripheral T cell lymphoma not otherwise specified

Lymphoproliferative disorder of the T cell lineage diagnosed as PTCL-NOS when the lymphoma lacks any distinctive features that fit into the subtypes. The NOS types, which account for between 60% and 70% of T cell lymphomas, cannot be further categorized based on morphology, phenotype, and conventional molecular research

(Swerdlow *et al.*, 2008). In PTCL-NOS, two unique molecular subgroups have been identified, each with a different clinical result and prognosis. High *GATA3* expression was linked to worse clinical outcomes, whereas *TBX21* expression, which was enriched through the IFNγ and NF-κB pathways, was linked to better survival (Iqbal *et al.*, 2014; Watatani *et al.*, 2019). These findings provide insights into a condition that previously had an exclusionary diagnosis, poor clinical outcomes, and no progress in its management. The majority of people with PTCL-NOS have an advanced stage (III/IV), and men account for two-thirds of the patient population. The mean age at presentation was around 60 years old. Achieving a favourable prognosis is heavily dependent on early diagnosis, given that the majority of patients are often diagnosed in advanced stages and the tumour tends to exhibit aggressive behaviour (Schatz *et al.*, 2015; Singh *et al.*, 2019a). PTCL-NOS frequently involves nodal or nodal/extranodal tissue. Among patients with PTCL-NOS, 29% had involvement of multiple extranodal sites, while 38% had only nodal disease, 49% had both nodal and extranodal disease, and 13% had extranodal disease alone. Extranodal sites commonly affected include the spleen, BM, liver, and skin, with each observed in at least 10% of patients (Weisenburger *et al.*, 2011).

2.2.2(b) Anaplastic large cell lymphoma

Anaplastic large cell lymphoma (ALCL) is a subset of $CD30^+$ mature T cell lymphomas that share similar immunophenotypic and morphological characteristics but differ in their clinical and genetic features (Swerdlow *et al.*, 2017). Approximately 15% of PTCLs worldwide are now ALCLs of all types (Vose *et al.*, 2008; Laurent *et al.*, 2017). The majority (50–70%) of patients present with ALCL have an advanced disease with peripheral, including mediastinal, and/or abdominal lymphadenopathy. A male to female ratio (M:F) around 1.2:3.0, and peak incidence of ALK-negative ALCL after 40

years old, while ALK-positive ALCL in the early three decades (Tsuyama *et al.*, 2017). In accordance with estimations, there are 0.25 cases of ALCL for every 100,000 people in the United States, accounting for 3 to 5% of all non-Hodgkin's lymphomas (Morton *et al.*, 2006; Adams *et al.*, 2016; Philippe *et al.*, 2021). The anaplastic lymphoma kinase (ALK)-negative ALCL subtype has been recognized as a distinct entity. The expression of *TNFRSF8*, *BATF3*, and *TMOD1* distinguish PTCL-NOS from ALK-negative ALCL (Agnelli *et al.*, 2012). The ALK gene rearrangements in PTCLs that result in oncogenic ALK fusion proteins and identify a particular WHO subtype are the best to characterize ALCL (Swerdlow *et al.*, 2008; Vose *et al.*, 2008). Patients with ALK-positive ALCLs had a better outcome than those with ALK-negative ALCLs or other nodal PTCLs, such as PTCL-NOS and AITL (Hapgood and Savage, 2015; Amador and Feldman, 2021; Montazersaheb *et al.*, 2021). ALK-negative ALCL has recently been identified to exhibit recurrent chromosomal rearrangements involving the *DUSP22-IRF4* gene on 6p25.3 (known as *DUSP22* rearrangements) and the *TP53* homolog *TP63* on 3q28. Retrospective research has indicated that ALCL cases with DUSP22 rearrangements are associated with favorable outcomes, particularly in ALK-negative instances. Poor outcomes and aggressive clinical behavior were linked to TP63 rearrangements that encode p63 fusion proteins (Parrilla Castellar *et al.*, 2014; Pedersen *et al.*, 2017a; Pedersen *et al.*, 2017b). Breast implant-associated ALCL is a rare type of ALCL and represents a novel variation in which the lymphomatous cells are restricted to the seroma fluid around the implant without capsular invasion. As a result, surgical removal of the implant and its capsule is frequently curative, and systemic therapy is seldom necessary (Somasundaram and Thye Lim, 2019).

2.2.2(c) Extranodal natural killer/T cell lymphoma, nasal type

An aggressive lymphoma that has a strong association with the EBV virus is extranodal natural killer/T cell lymphoma, nasal type (ENKTL-NT). Only 0.2%–0.4% of newly diagnosed NHLs of the ENKTL type occur in the United States and Europe, indicating a low incidence, while in Asia and in Central and South America, ENKTL accounts for 5%–15% of all newly diagnosed lymphomas (Tse and Kwong, 2013; Karkera *et al.*, 2016; van Doesum *et al.*, 2021). Original cells of ENKTL-NT are either NK- or γδ T-cells, both of these express CD56 (Haverkos *et al.*, 2016). NK cells account for around 85% of ENKTLs, whereas T cells account for 15% (de Mel *et al.*, 2018). In ENKTL, the nasopharynx is the most typical site of occurrence, but tonsils, sinuses, the Waldeyer ring, and the oropharynx are other frequent sites. At presentation, 70% to 90% of patients have stage I or stage II lymphoma (Yang *et al.*, 2015). A small percentage of patients with advanced NKTL may exhibit hemophagocytic syndrome, which can include high fevers, cytopenias, coagulopathy, abnormal liver function tests, and very high ferritin levels (Somasundaram and Thye Lim, 2019).

2.2.2(d) Adult T cell leukemia/lymphoma

Adult T cell leukemia/lymphoma (ATLL) is a rare and often aggressive (fast-growing) PTCL with a CD4 and CD25 phenotype caused by human T cell leukemia virus type-I (HTLV-1) (Ohshima, 2015). HTLV-1 primarily infects individuals through breastfeeding (Millen and Thoma-Kress, 2022) and sexual contact (Martel and Gotuzzo, 2022). Subsequently, it integrates its provirus into the host genome (Katsuya *et al.*, 2019). ATLL is categorized into four clinical subtypes based on clinical features and prognostic factors: acute, lymphoma, chronic, and smoldering. The smoldering type further divides into leukemic and non-leukemic, while the chronic type has favorable and unfavorable subcategories (Oliveira *et al.*, 2016; Ghobadi *et al.*, 2022). The acute,

lymphoma, and unfavorable chronic subtypes are considered aggressive, characterized by rapid growth and abnormalities in albumin, lactate dehydrogenase, or blood urea nitrogen levels. In contrast, the chronic and smoldering subtypes are less aggressive (Katsuya *et al.*, 2012; Yoshida *et al.*, 2014; Morichika *et al.*, 2019). The estimated annual prevalence of ATLL is 60 cases per 100,000 people, reaching its peak around the age of 70. The lifetime risk of developing ATLL has been approximated to be 3–5% (Ohshima, 2015).

2.2.2(e) Cutaneous T cell lymphoma

Cutaneous T cell lymphoma (CTCL) refers to heterogeneous T cell lymphomas that develop in the skin (Jawed *et al.*, 2014). The most prevalent subtypes are mycosis fungoides (MF) and Sézary syndrome (SS), constituting 60% to 80% and less than 10% of CTCL cases, respectively (Dai and Duvic, 2023). The incidence of these subtypes tends to increase with age. Despite mycosis fungoides being a generally slow-growing variant, Sézary syndrome is characterized by aggressiveness and typically carries a poor prognosis (Wilcox, 2017). Although CTCLs are primarily asymptomatic, cases with large cell transformation are commonly associated with an unfavorable (Pulitzer *et al.*, 2014).

2.2.2(f) Enteropathy-associated T cell lymphoma

Enteropathy-associated T cell lymphoma (EATL) is a rare NHL that accounts for around 5% of all GIT lymphomas and has an annual incidence rate of 0.5–1 per million (Verbeek *et al.*, 2008; Delabie *et al.*, 2011). The five-year survival rate for EATL is expected to be between 11 and 20% (Malamut *et al.*, 2013). EATL generally affects the small bowel and is more common in middle-aged males. With a varied interval of a few months to many decades between the diagnosis of celiac sprue and the

beginning of lymphoma, this lymphoma is significantly related to celiac sprue (Catassi *et al.*, 2005; van de Water *et al.*, 2010). Recurrent diarrhea, unexplained weight loss, stomach discomfort, fever, and night sweats are the most typical signs of EATL in individuals with previously well-controlled symptoms (Di Sabatino *et al.*, 2012). In EATL (Type 1), genetic markers such as chromosome 9q31.3 gain or 16q12.1 deletion are notably associated with celiac disease and the HLA-DQ2 haplotype. It's noteworthy that these lymphomas may occasionally exhibit large-cell or pleomorphic cytology and may express CD30. On the other hand, EATL (Type 2) is characterized by chromosomal 8q24 gains, and less frequently, 1q and 5q gains. EATL Type 2 is distinguished by monomorphic cytology and frequently expresses CD56. Importantly, it is less often linked to celiac disease and the HLA-DQ2 haplotype (Chott *et al.*, 1998; Deleeuw *et al.*, 2007).

2.2.3 Epidemiology of peripheral T cell lymphomas

A number of studies have shown epidemiologic variation present among various geographical populations. Age, gender, and ethnicity all have an impact on the prevalence of each subtype (Abouyabis *et al.*, 2008; Phan *et al.*, 2016; Bellei *et al.*, 2017). The incidence and mortality of PTCL varies geographically, with Asia usually experiencing higher rates than either Europe or North America. PTCL accounts for about 5%–10% of all lymphomas in Western countries (d'Amore *et al.*, 2015). According to the Surveillance, Epidemiology and End Results (SEER) database, PTCL incidence increased by 280% in the US between 1992 and 2005 across all racial, sex, and age categories. This could be essentially related to improved accuracy in diagnosis. Age-adjusted incidence rates were higher in black individuals and men compared to women (Adams *et al.*, 2016). PTCL incidence was 2.1 per 100 000 across all age categories (Shah *et al.*, 2019). According to estimates, there were 8,380 new cases of

PTCL in the USA during 2011–2012, accounting for 6.1% of all lymphoma cases, with an age-standardized incidence rate (ASIR) of 2.1 per 100,000 (Teras *et al.*, 2016). Additionally, 308 cases of PTCL represented 5.3% of all newly diagnosed cases of lymphoma in the UK population between 2004 and 2014 (Smith *et al.*, 2015). In Asia, PTCL accounts for 15% to 20% of lymphomas. Specifically, 18.5% of the 9,426 lymphoid neoplasm cases in Japan from 2007 to 2014 and 22.0% of the 836 cases of all malignant lymphomas in Korea from 2005 to 2006 were PTCL (Kim *et al.*, 2011; Muto *et al.*, 2018). Notably, Asians and individuals from Pacific island countries exhibited a higher prevalence of extranodal natural killer T/NK cell lymphoma, whereas Native Americans and Alaskans had the highest rates of PTCL (Abouyabis *et al.*, 2008).

Up to 2% of NHL cases can be defined by AITL; however, it accounts for one in every five PTCL cases annually. It is regarded as the second most typical PTCL subtype after PTCL-NOS. It has a ratio of 1:1 between males and females, is more prevalent in Europe than Asia or North America, and has a median age of 65 to 70 years in the USA and worldwide (Vose, 2008; Phan *et al.*, 2016; Lunning and Vose, 2017). The incidence of CTCL grew rapidly between 1973 and 1998, according to retrospective research, and has subsequently stabilized. In addition, men have a twice-higher incidence of CTCL than women. In urban regions, among patients with better incomes and educational levels, particularly among African Americans, it is widespread. Patients who had undergone organ transplants and people with HIV were more likely to have it (Wilkins *et al.*, 2006; Bradford *et al.*, 2009; Pomerantz *et al.*, 2010; Korgavkar *et al.*, 2013). The most common CTCL subtype, MF, accounts for around 50% of all CTCLs. Males with a diagnostic age between 55 and 60 have an incidence rate that is about twice as high. CTCL was shown to occur in Hispanic individuals at younger ages, in higher stages, and with an aggressive clinical course. SS

is more common among non-Hispanic whites and accounts for 3–5% of all CTCLs (Burg *et al.*, 2005; Criscione and Weinstock, 2007; Sun *et al.*, 2009; Wilson *et al.*, 2012; Scarisbrick *et al.*, 2014). The second most prevalent subtype after MF is large-cell cutaneous Ki-1 anaplastic lymphoma, which accounts for 25% to 30% of all CTCLs. Ki-1 anaplastic lymphoma It has a favorable prognosis and is more common among non-Hispanic whites; it may happen at any age, but the 60s have the highest prevalence (Bekkenk *et al.*, 2000; Willemze *et al.*, 2005; Bradford *et al.*, 2009).

2.2.4 Prognosis and staging of peripheral T cell lymphomas

Several studies have shown that extranodal involvement, advanced disease stage, bulky disease, high Ki67 index, and elevated lactate dehydrogenase (LDH) levels are associated with shorter OS in PTCL patients. Additionally, poor Eastern Cooperative Oncology Group (ECOG) Performance Status scores correlate with worse outcomes (Gallamini *et al.*, 2004; Went *et al.*, 2006; Weisenburger *et al.*, 2011). The International Prognostic Index (IPI) is the most widely used prognostic score for aggressive PTCL (Ellin *et al.*, 2017). Although the OS rates in each IPI category are generally lower in patients with most PTCLs compared to those with DLBCL, the IPI score remains inversely linked to survival in PTCL patients similarly to DLBCL patients (Shibata *et al.*, 2014). The IPI has been clinically validated in patients with PTCL. However, the absence of well-defined risk categories has led to the development of PTCL-specific prognostic indices (Luminari and Skrypets, 2021). The Intergruppo Italiano Linfomi, now known as Fondazione Italiana Linfomi (FIL), conducted a significant study involving 385 patients diagnosed and treated during the 1990s. They established the Prognostic Index for PTCL-unspecified (PIT), which identifying age, ECOG performance status, LDH level, and BM involvement as independent predictors of OS in PTCL-NOS patients (Gallamini *et al.*, 2004). The PIT was alter to include