Primary Nodal EBV+ T/NK-Cell Lymphoma

Singapore Lymphoma Scientific Symposium
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EBV+ nodal T/NK-Cell Lymphoma

Definition:
EBV+ lymphoma of cytotoxic T- or NK-cell lineage, presenting primarily with nodal disease in adults

Terminology

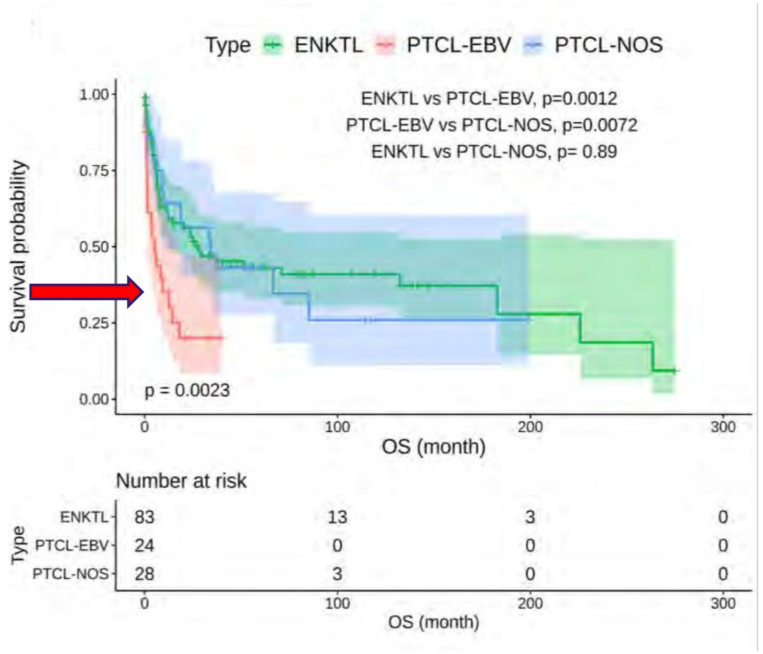
- ICC 2022: Primary nodal EBV-positive T/NK-cell lymphoma
- WHO (5th ed): EBV-positive nodal T- and NK-cell lymphoma
  - Acceptable:
    - nodal EBV+ cytotoxic T-cell lymphoma;
    - nodal peripheral T-cell lymphoma, EBV-positive
  - Not recommended: peripheral T-cell lymphoma NOS, EBV+

Clinical features

- Older adults (median 61-64 years)
- Eastern Asia >> Western population, M > F
- Underlying immune deficiency/conditions:
  - Viral infections (HIV, Hep B or C), DM, autoimmune conditions, prior AITL
- All cases presented with lymphadenopathy. No nasal disease.
- Extranodal sites can be involved
  - Liver and BM (24-60%), spleen
  - Other sites: skin, pleural effusion, lacrimal gland
  - Rarely upper aerodigestive tract (eg. Waldeyer ring)

Diagnostic overlap with ENKTL

- Advanced stage (>85%), high/intermediate high IPI (>60%)
- B symptoms (>70%)
- Median OS: 2.5 – 8.0 mths

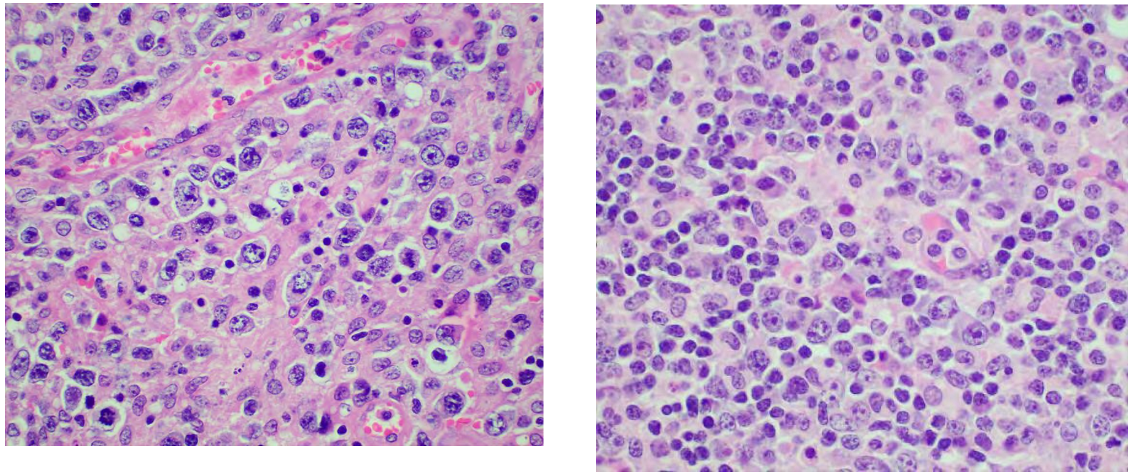


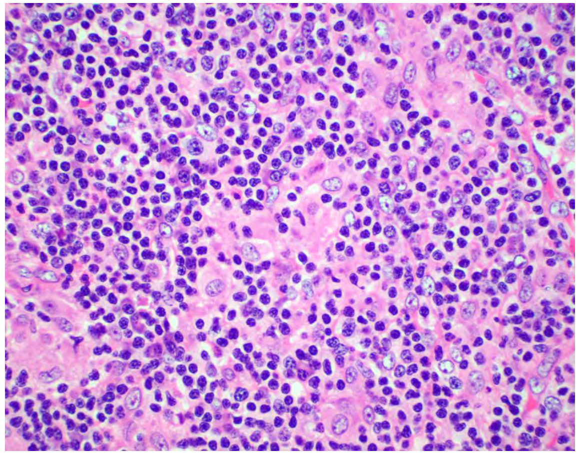
Type	0	100	200	300
ENKTL	83	13	3	0
PTCL-EBV	24	0	0	0
PTCL-NOS	28	3	0	0

Wai CMM. Haematologica. 2022 Aug 1;107(8):1864-1879

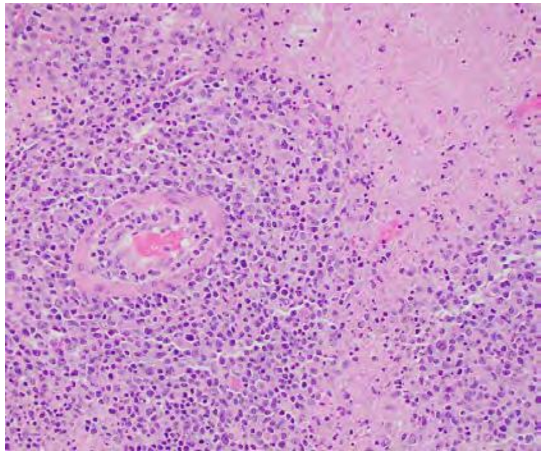
Histology

- Diffuse infiltrate
- Tumor cells:
  - medium to large lymphoid cells
  - Pleomorphic, mixed
  - Small cells (Lymphoepithelioid)
- Lacks prominent coagulative necrosis or angiocentric growth

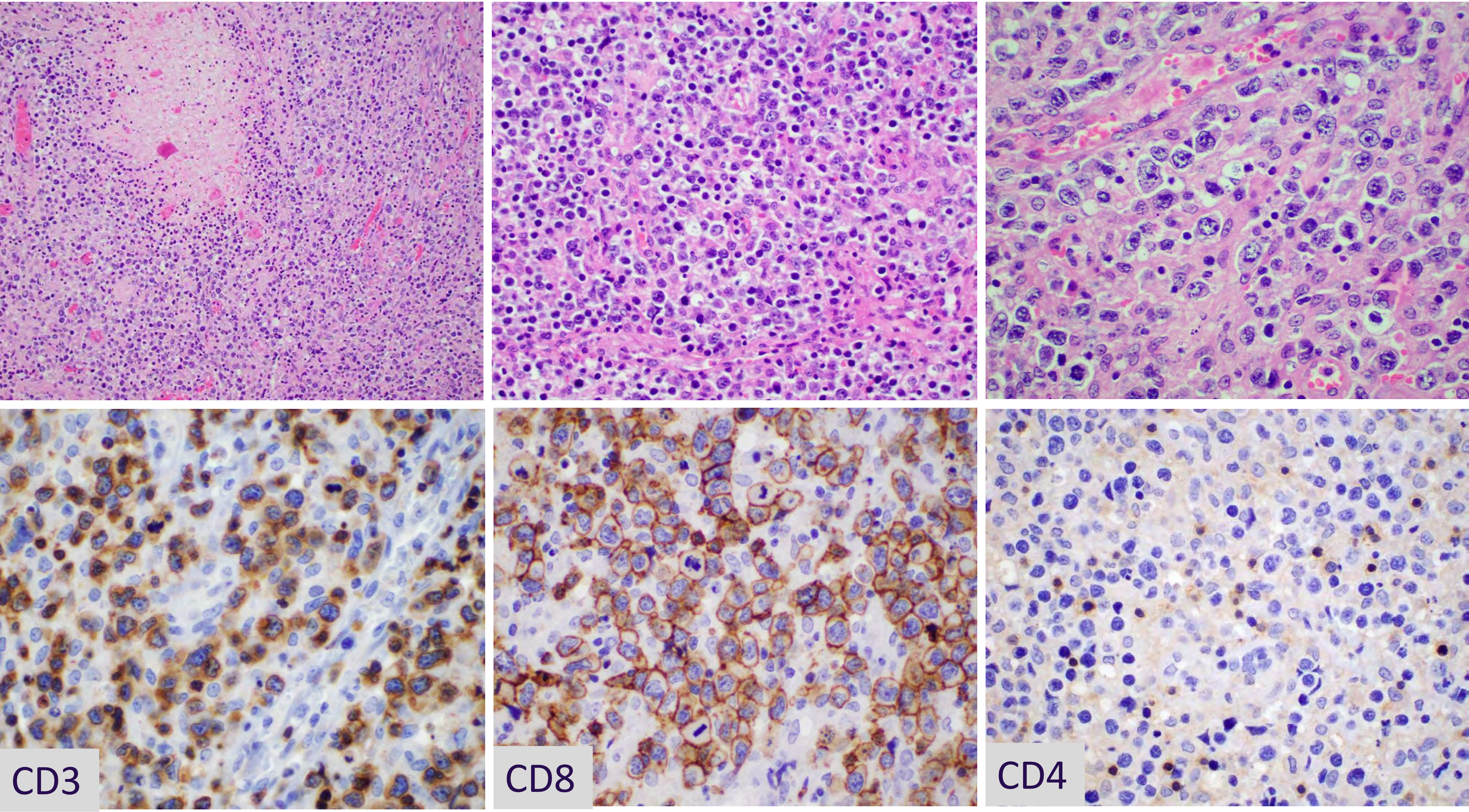


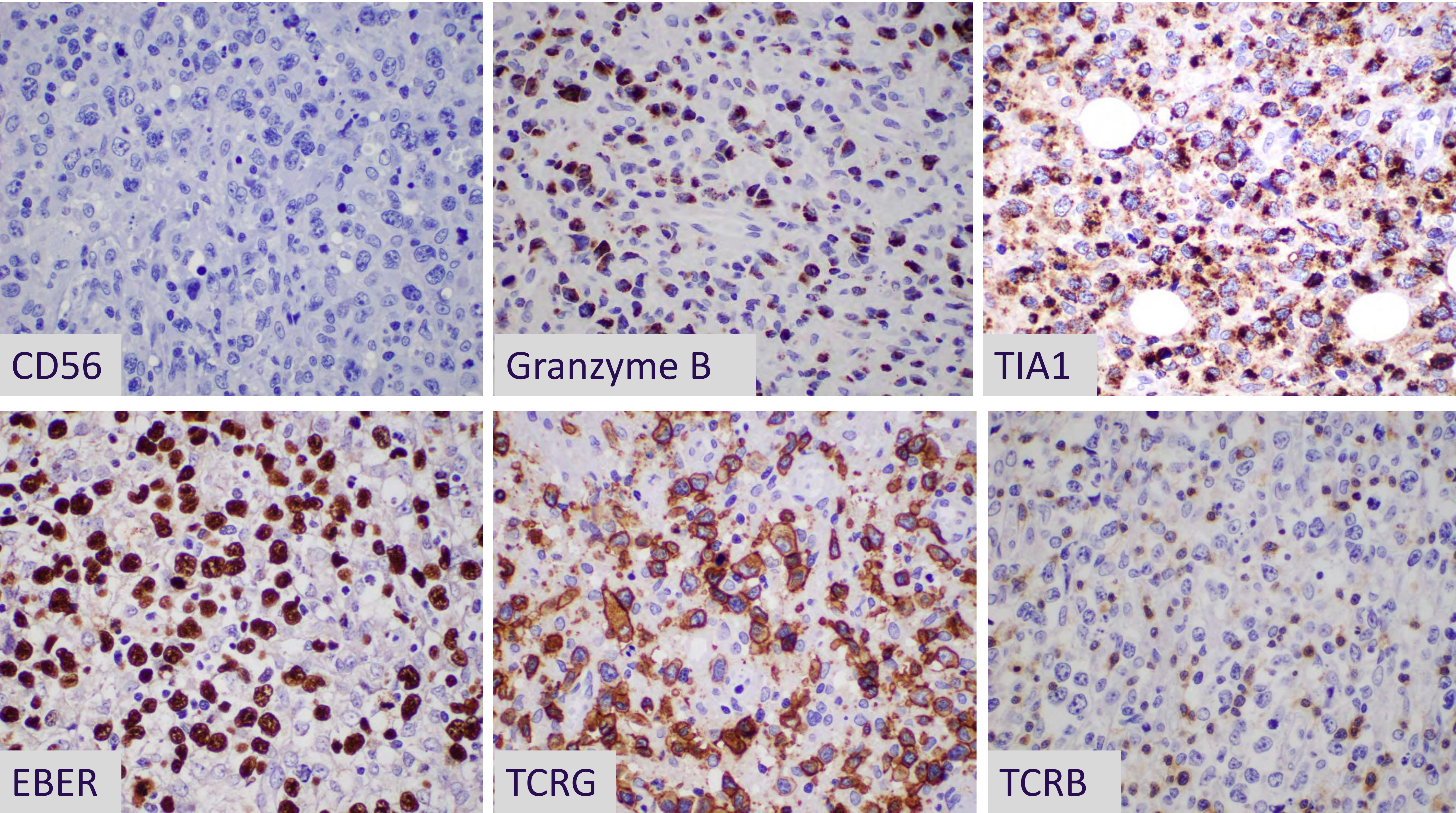


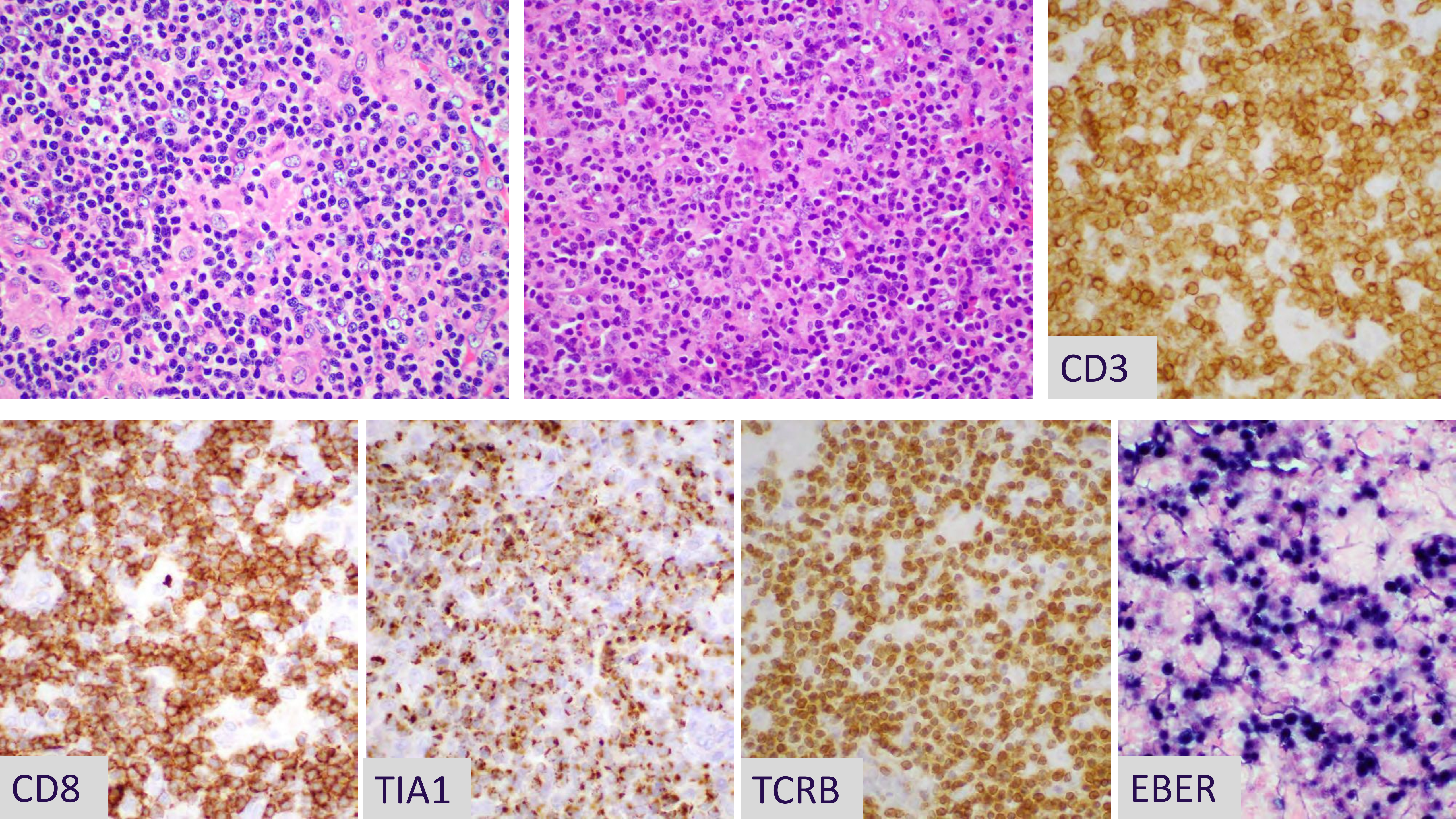
Lymphoepithelioid lymphoma



Foci of necrosis

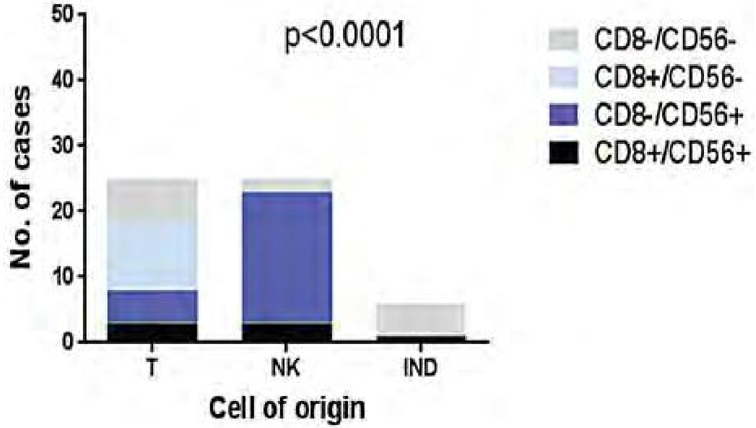

CD3
CD8
CD4

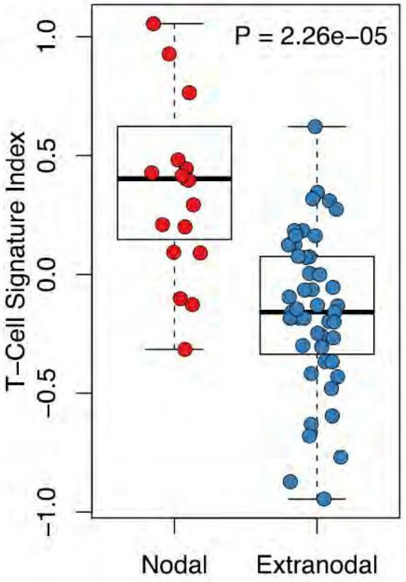

CD56
Granzyme B
TIA1
EBER
TCRG
TCRB


CD3
CD8
TIA1
TCRB
EBER

nodal-TNKL -------- Immunophenotype

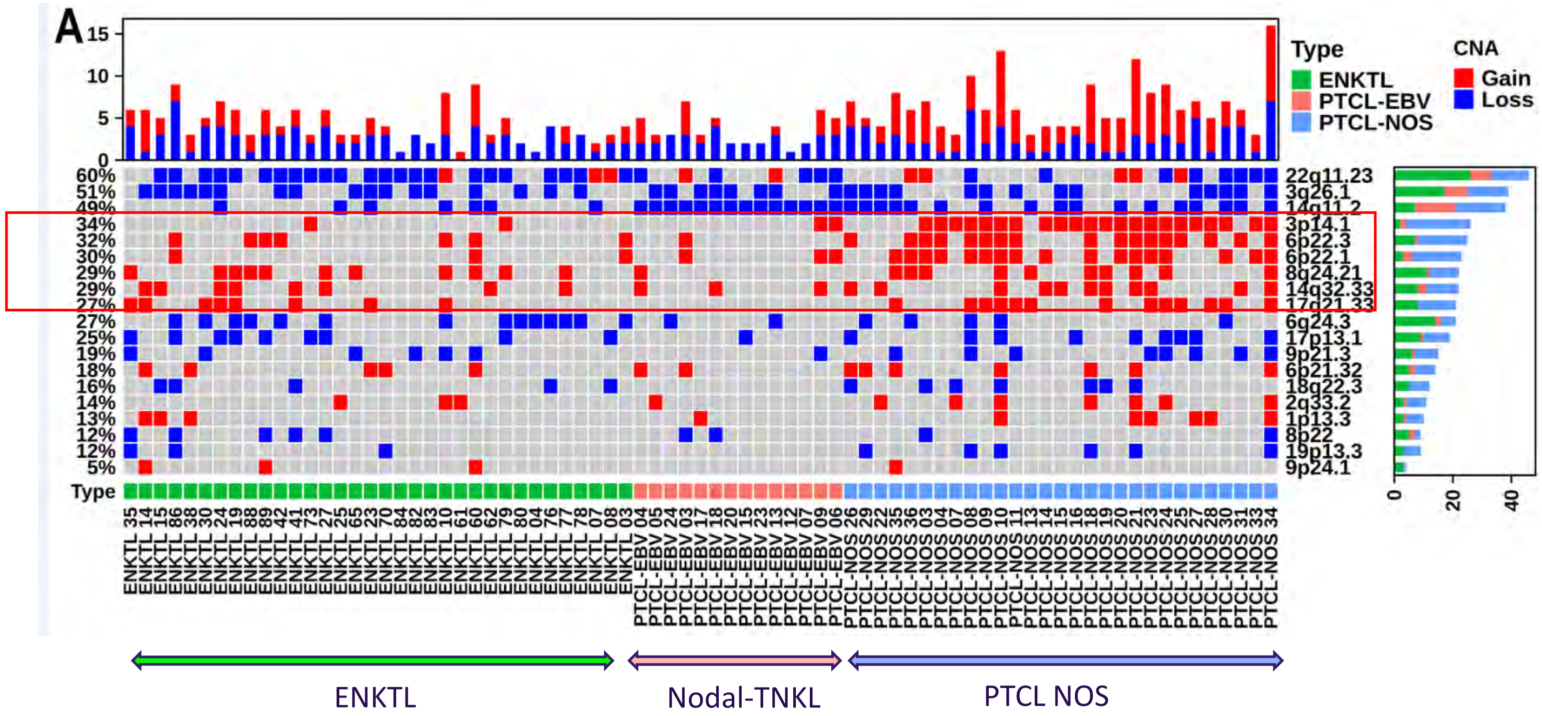
- T-cell markers: CD3+, CD2+
- Cytotoxic: TIA1+, granzyme B+, perforin+
- CD8+ / CD4- / CD56 –
- T-cell lineage (80%)
    - TCRβ (43-64%), TCRγ (0–13%), TCR silent (25%)
- NK-cell lineage (<20%)
- EBER positive (majority of tumour cells)





Ng SB. Haematologica 2018;103(2):278-287

Copy number alterations

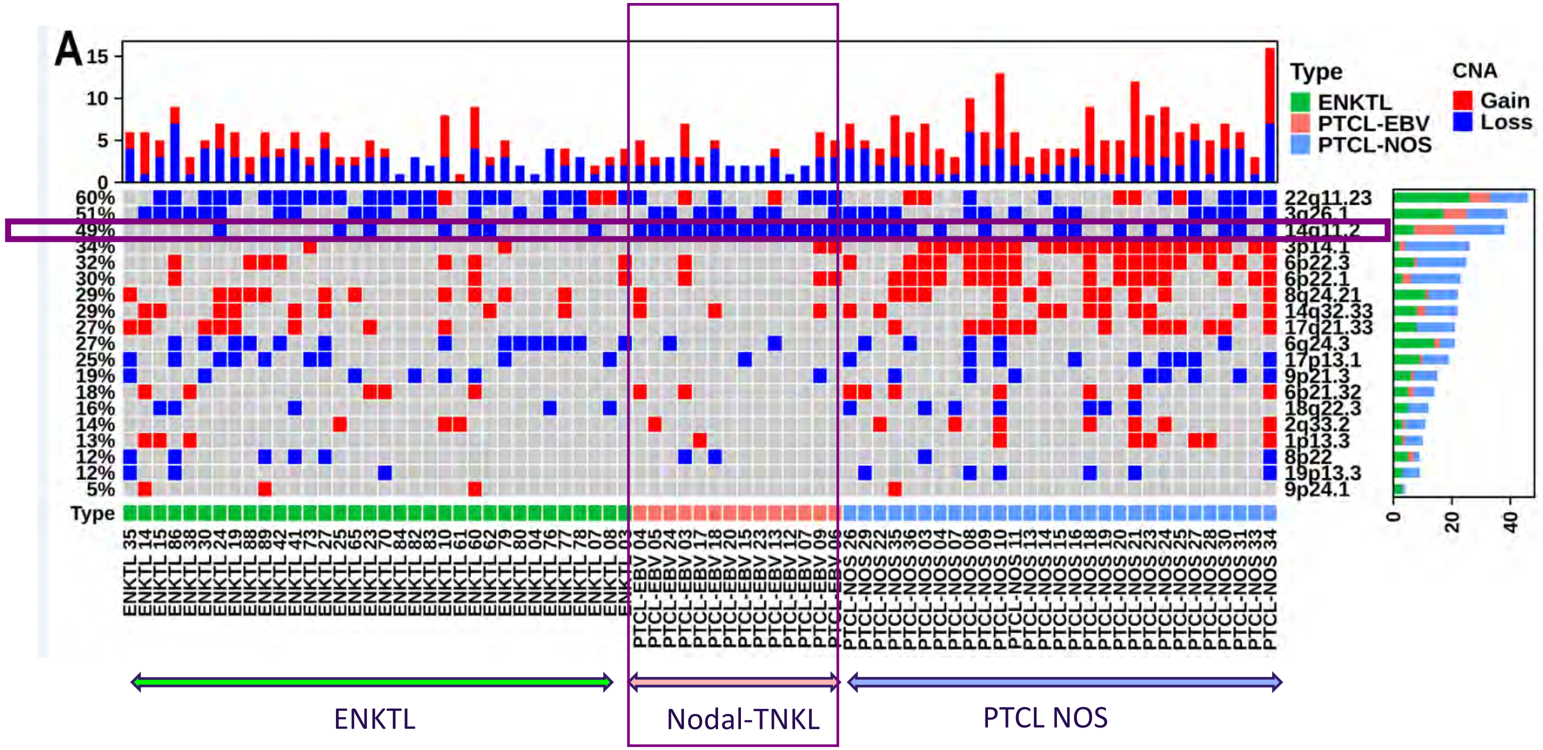


Recurrent gains more common in PTCL NOS than nodal TNKL and ENKTL:

- 3p14.1, 6p22.3, 6p22.1 and 17q21.33

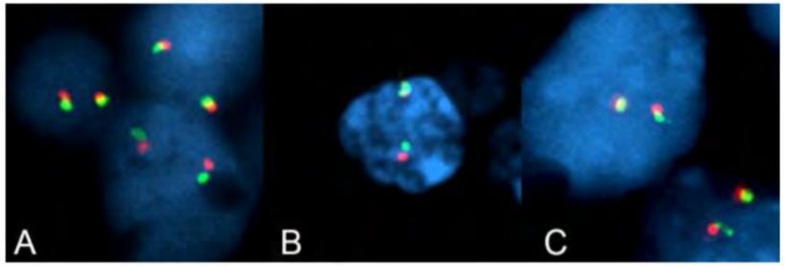
Wai CMM. Haematologica. 2022 Aug 1;107(8):1864-1879

Copy number alterations

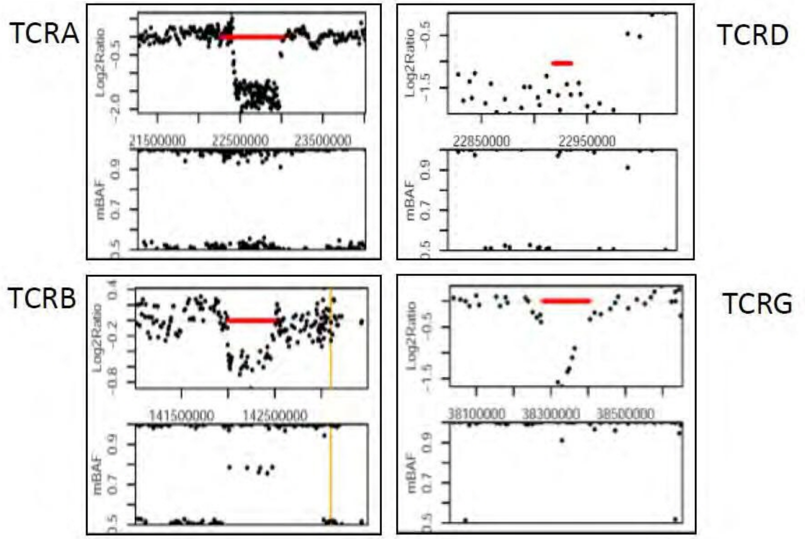


14q11.2 loss (TRA and TRD genes)

- common in nodal-TNKL (100%)
- Less common in PTCL NOS (59%) and ENKTL (21%)



FISH TCRA locus using breakapart probe



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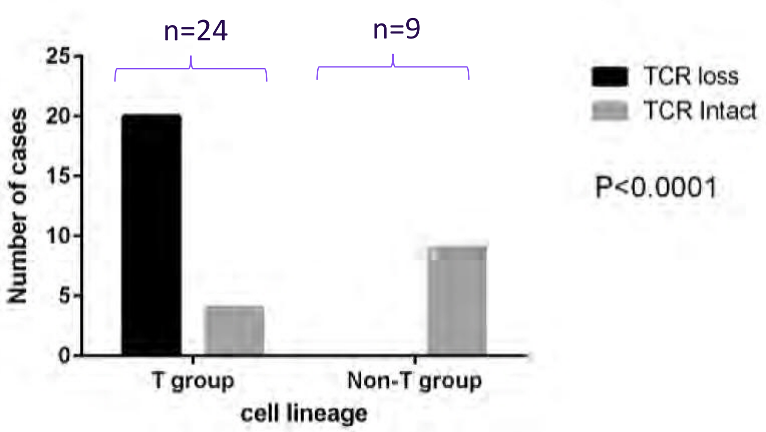
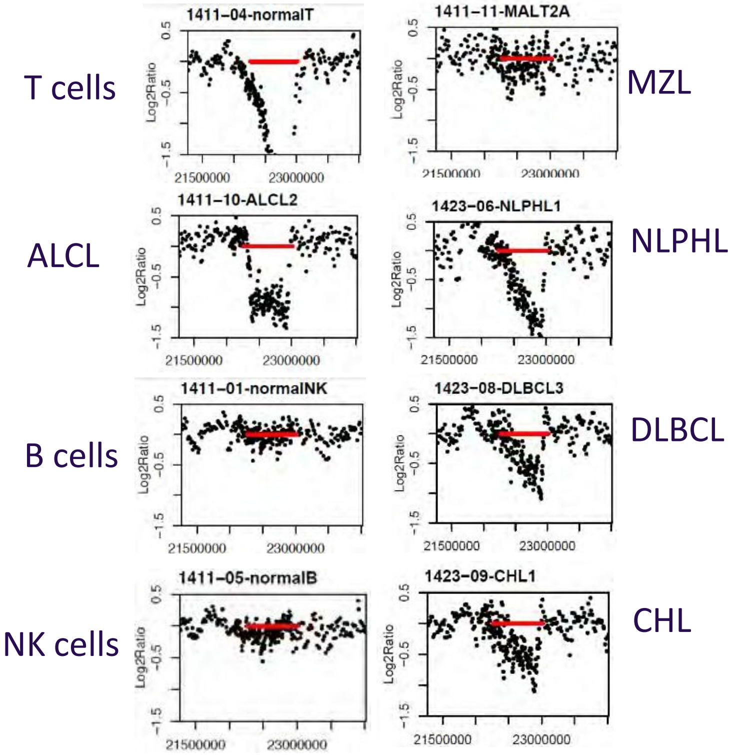
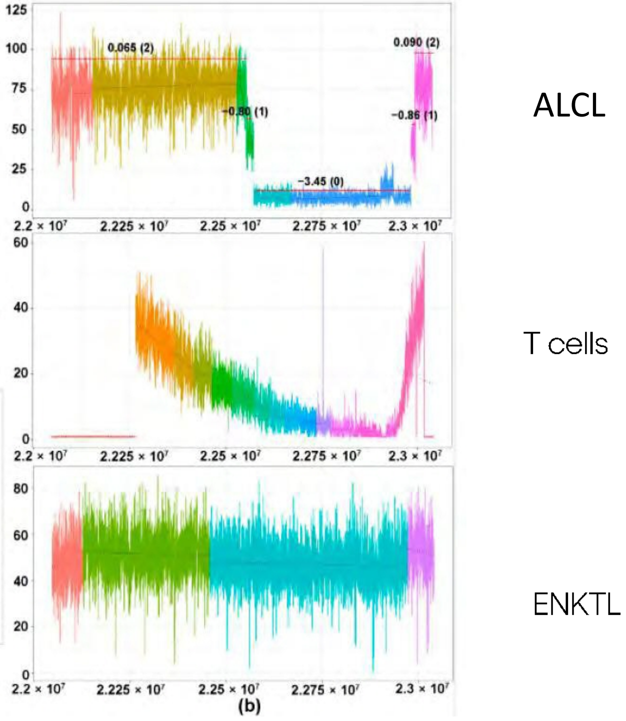
Is loss of 14q11.2 in nodal TNKL is reflection of T-cell lineage ?

Diagnosis	Diagnosis	TCRA	TCRB	TCRG	TCRD
AITL	T cell lymphoma				
ALCL	T cell lymphoma	-1			-1
PTCL	T cell lymphoma	-1			-1
PTCL	T cell lymphoma	-1/-2			-2
AITL	T cell lymphoma	-1			-1
ALCL	T cell lymphoma	-2	-2		-2
Tonsil	Benign				
Tonsil	Benign				
Lymph Node	Benign				
NK cells (1)	Benign				
NK cells (2)	Benign				
T cells (1)	Benign	-1/-2	-1		-2
T cells (2)	Benign	-1/-2	-1	-1	-2
B cells (1)	Benign				
B cells (2)	Benign				





Patterns of losses in TRA, TCD (OncoScan)



Patterns of losses in TRA gene (WGS)

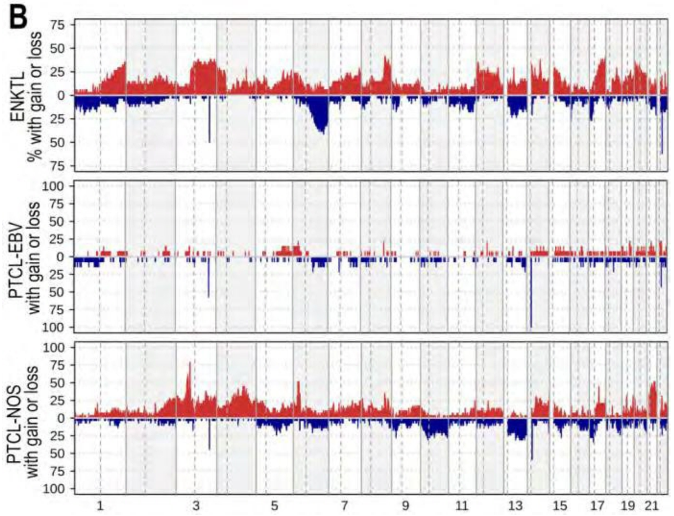
? Marker of T-cell lineage

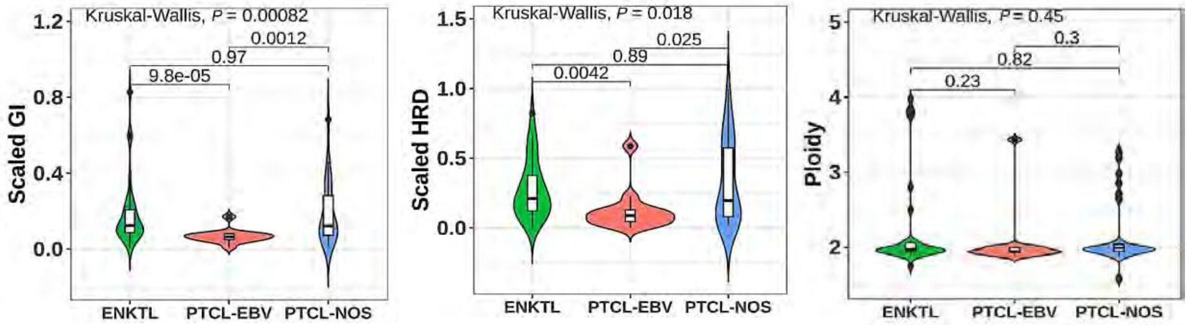
? additional critical driver event at TRA

? preferential TCR usage

Ng SB. Haematologica 2018; Oon ML. Cancers 2021, 13: 340

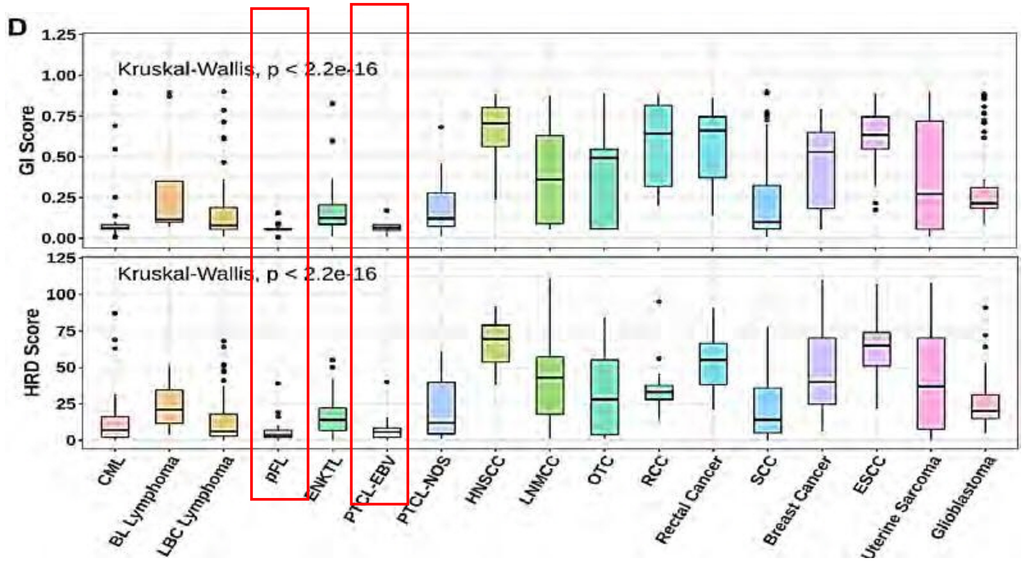
Low Genomic Instability despite aggressive behaviour





GI score: Ratio of the total length of regions with a CN other than 2 to a constant of 3.3×10^9

HRD score: LOH, telomere allelic imbalance, large-scale state transitions



Publicly available Oncoscan datasets:.

CML: Chronic Myeloid Leukemia;
BL: Burkitt-like;
LBC: Large B-Cell;
HNSCC: Head and neck SCC;
LNMCC: Lymph Node Metastases in Colon Cancer;
OTC: Oral tongue carcinoma;
pFL: pediatric-type follicular lymphoma;
RCC: Renal cell carcinoma;
SCC: synchronous colorectal cancer.
ESCC: Esophageal SCC;

Nodal-TNKL shows relatively low genomic instability

- Less genomic alterations
- Low Genomic Instability (GI) and homologous recombination deficiency (HRD) scores compared to ENKTL, PTCL NOS and solid cancers

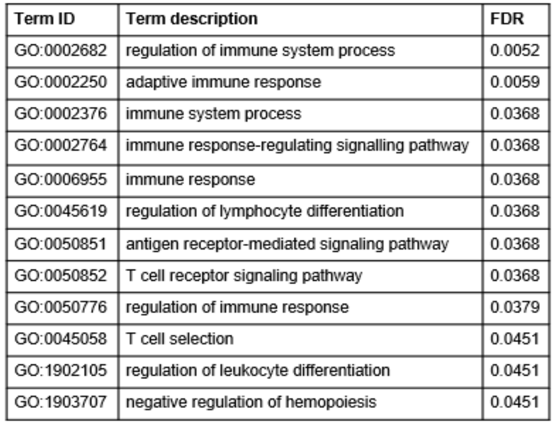
What is driving this tumour ?

PTCL-EBV = primary nodal TNKL

Wai CMM. Haematologica. 2022

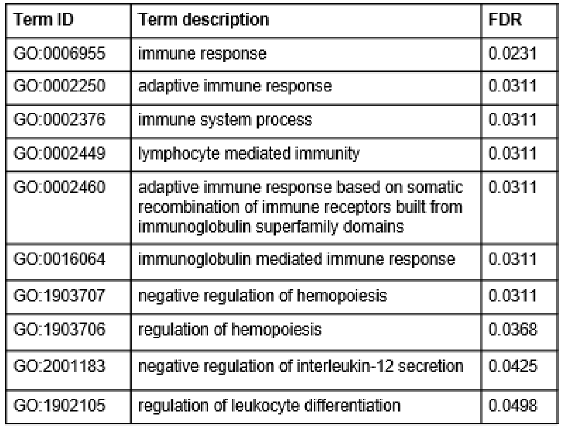
NFκB and immune pathways activation in nodal-TNKL

DEG between nodal TNKL and ENKTL

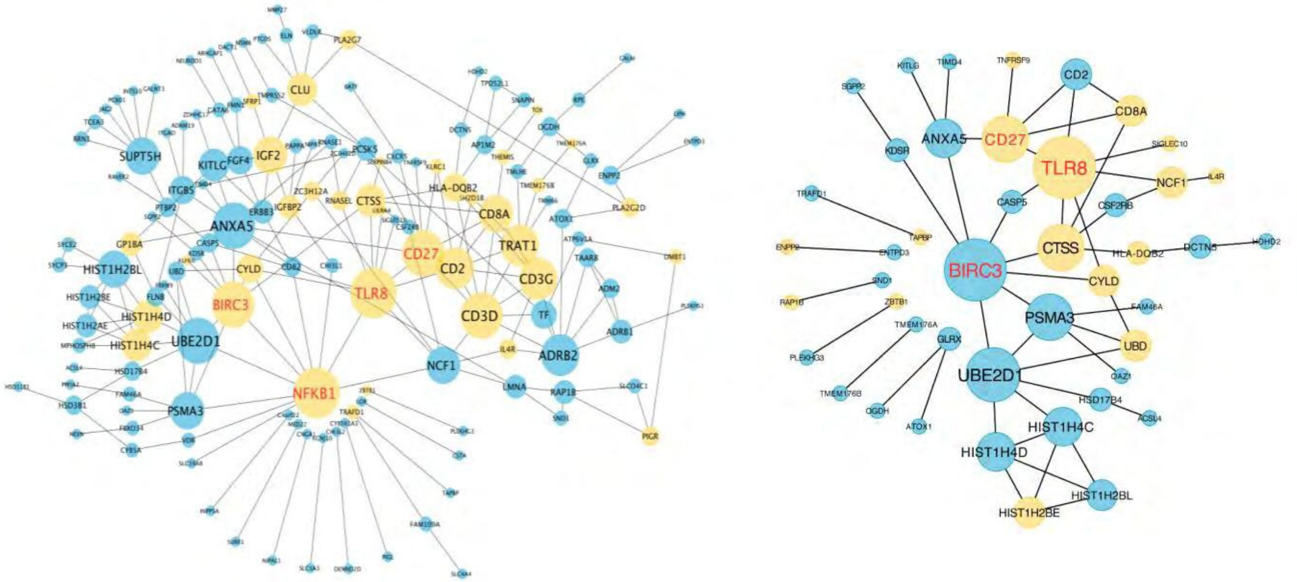


Term ID	Term description	FDR
GO:0002682	regulation of immune system process	0.0052
GO:0002250	adaptive immune response	0.0059
GO:0002376	immune system process	0.0368
GO:0002764	immune response-regulating signalling pathway	0.0368
GO:0006955	immune response	0.0368
GO:0045619	regulation of lymphocyte differentiation	0.0368
GO:0050851	antigen receptor-mediated signaling pathway	0.0368
GO:0050852	T cell receptor signaling pathway	0.0368
GO:0050776	regulation of immune response	0.0379
GO:0045058	T cell selection	0.0451
GO:1902105	regulation of leukocyte differentiation	0.0451
GO:1903707	negative regulation of hemopoiesis	0.0451

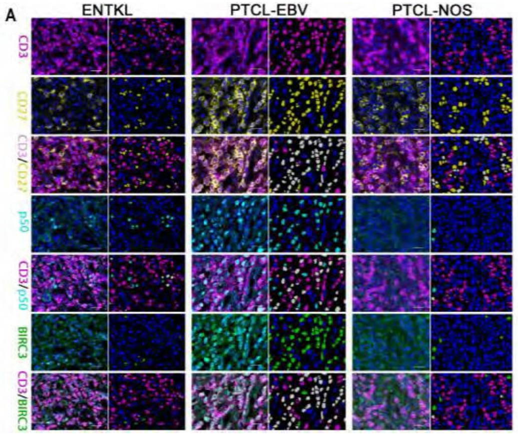
DEG between nodal TNKL and PTCL-NOS



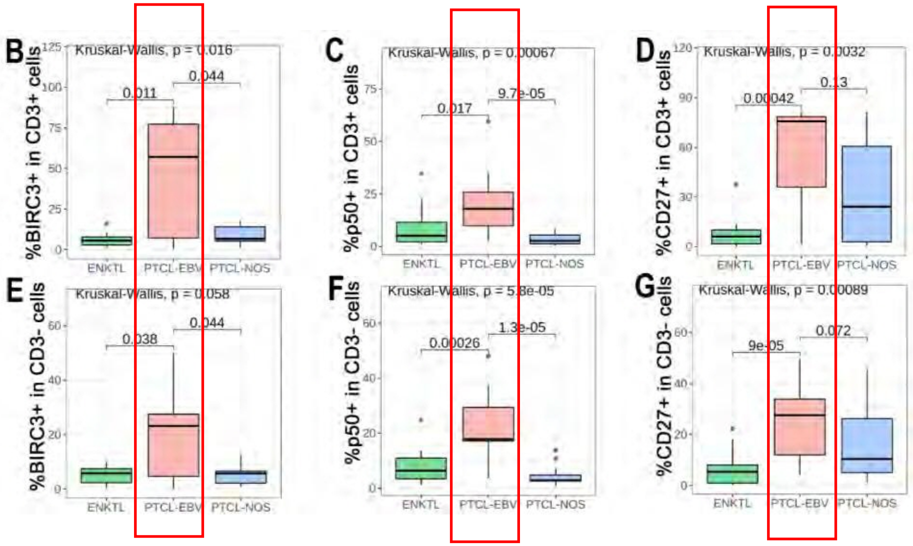
Term ID	Term description	FDR
GO:0006955	immune response	0.0231
GO:0002250	adaptive immune response	0.0311
GO:0002376	immune system process	0.0311
GO:0002449	lymphocyte mediated immunity	0.0311
GO:0002460	adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	0.0311
GO:0016064	immunoglobulin mediated immune response	0.0311
GO:1903707	negative regulation of hemopoiesis	0.0311
GO:1903706	regulation of hemopoiesis	0.0368
GO:2001183	negative regulation of interleukin-12 secretion	0.0425
GO:1902105	regulation of leukocyte differentiation	0.0498



NFκB-associated genes (*BIRC3, NFκB1, TLR8, CD27*)- central nodes in both DEG networks



Upregulated expression of BIRC3 and p50 (*NFκB1*) in tumor and non-tumor cells of PTCL-EBV compared to ENKTL and PTCL-NOS.



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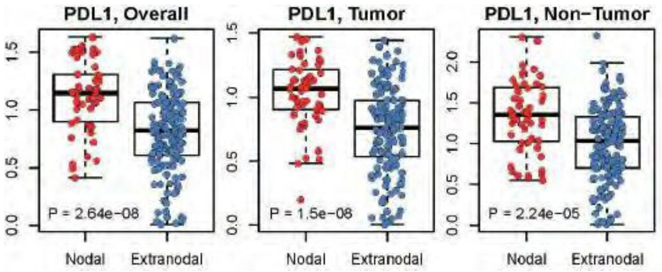
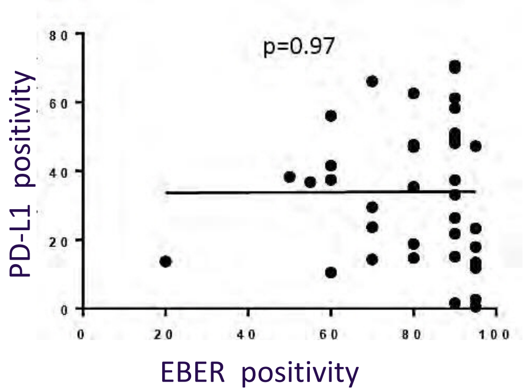
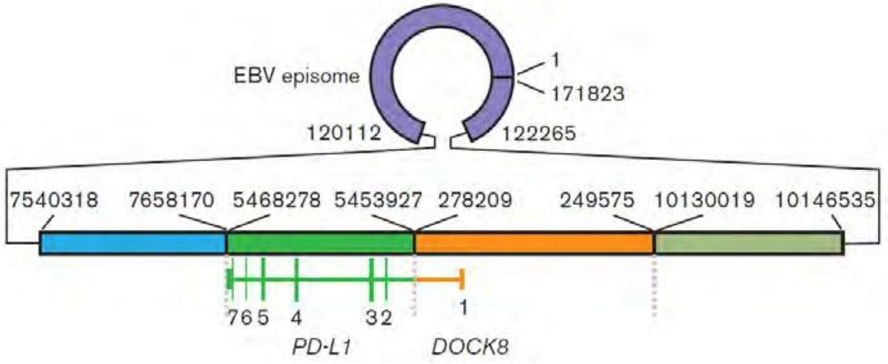
PDL1 is upregulated in primary nodal-TNKL

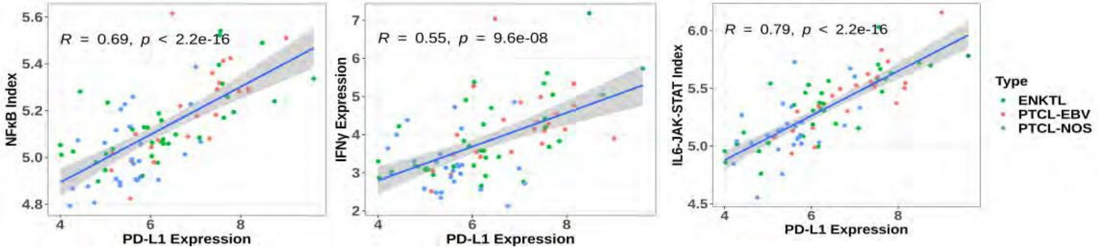


PD-L1 is expression is higher in tumour and non-tumor cells in nodal TNKL compared to ENKTL





- complex SV: 4 pieces of the part of human chr 9 tandemly incorporated into the EBV genome
- truncation of the 3' UTR of PD-L1



High PD-L1 gene expression and correlates with IFNγ, JAK-STAT and NFκB

What is driving upregulation of PDL1 in nodal-TNKL ?

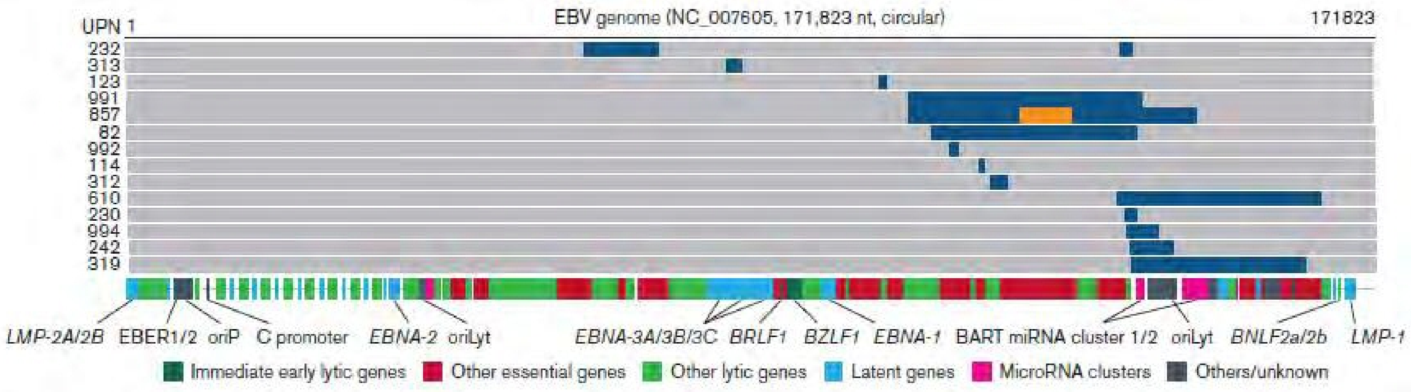
- No 9p24.1 gain
- No correlation with EBER expression
- Activation of IFNγ, IL6_JAK_STAT3 and NFκB
- 3' UTR of PD-L1 truncated

Targeting the PD-1/PD-L1 axis

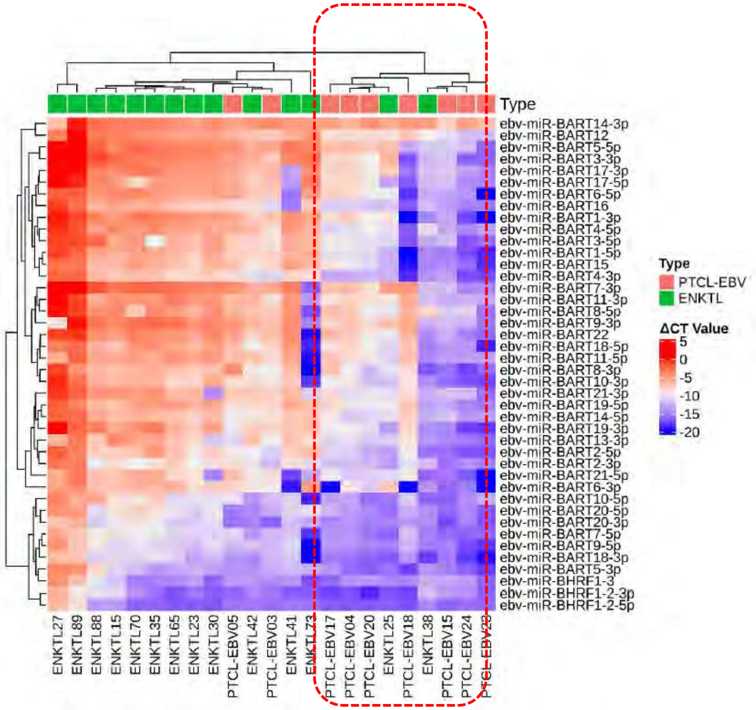
Wai CMM. Haematologica. 2022 Aug 1;107(8):1864-1879, Kato S. Blood Adv (2024) 8 (9): 2138–2147

Role of EBV

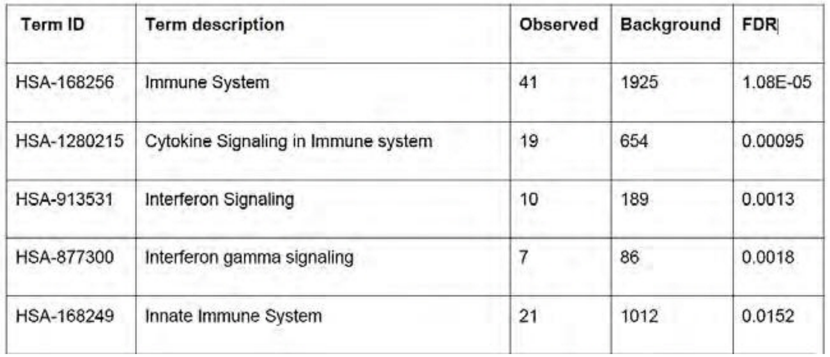
- Type II EBV latency pattern
- Type A strain (prevalent in Japan)
- No disease specific EBV strain
- Structural variations (del) common in EBV genomes (63%)
- Downregulation of EBV miRNAs compared to ENKTL
  - Target genes enriched in immune-related pathways



Gray bar- EBV genome from patients, Blue bar – deletions, Orange bar – inverted region



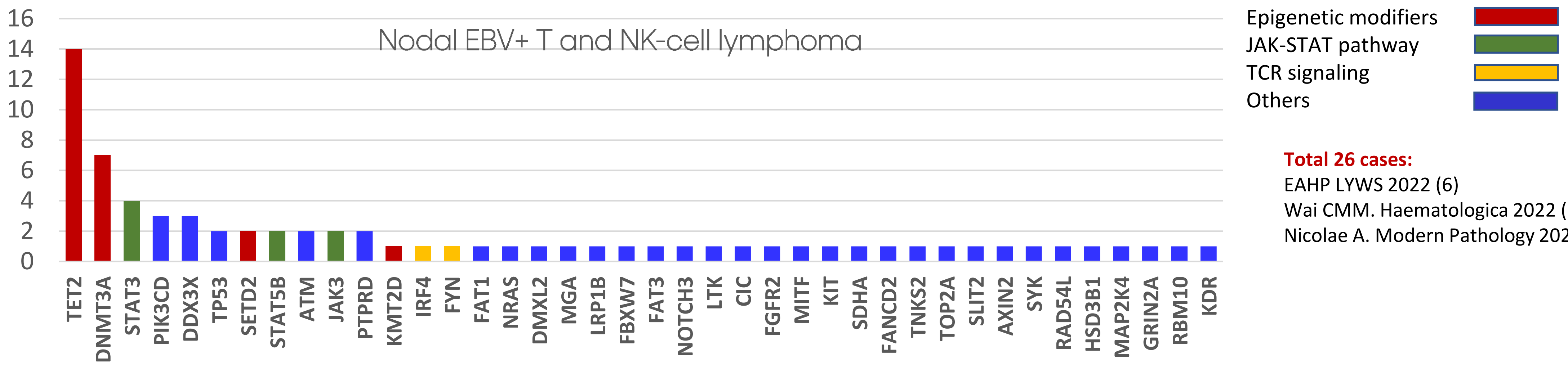
Reactome pathways enriched for target genes of differentially expressed EBV miRNA



Term ID	Term description	Observed	Background	FDR
HSA-168256	Immune System	41	1925	1.08E-05
HSA-1280215	Cytokine Signaling in Immune system	19	654	0.00095
HSA-913531	Interferon Signaling	10	189	0.0013
HSA-877300	Interferon gamma signaling	7	86	0.0018
HSA-168249	Innate Immune System	21	1012	0.0152

Wai CMM. Haematologica. 2022
Kato S. Blood Adv (2024) 8 (9): 2138–2147

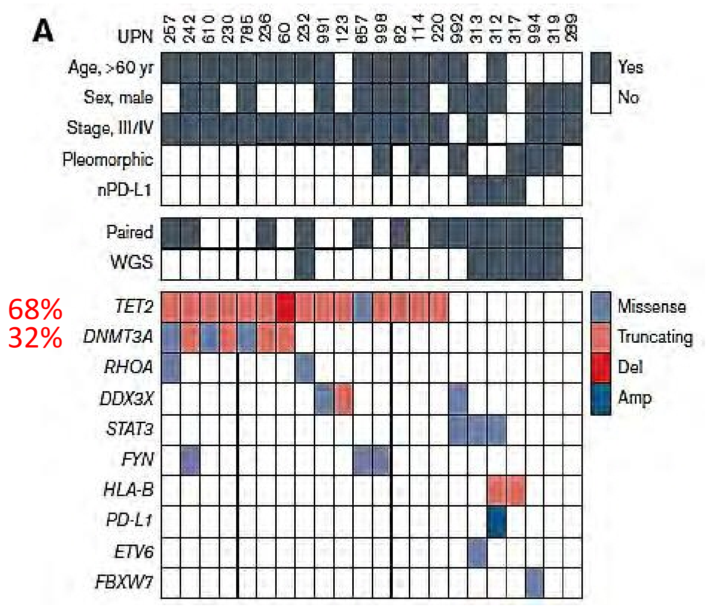
Mutational landscape



Total 26 cases:
EAHP LYWS 2022 (6)
Wai CMM. Haematologica 2022 (14)
Nicolae A. Modern Pathology 2022 (6)

Recurrent mutations

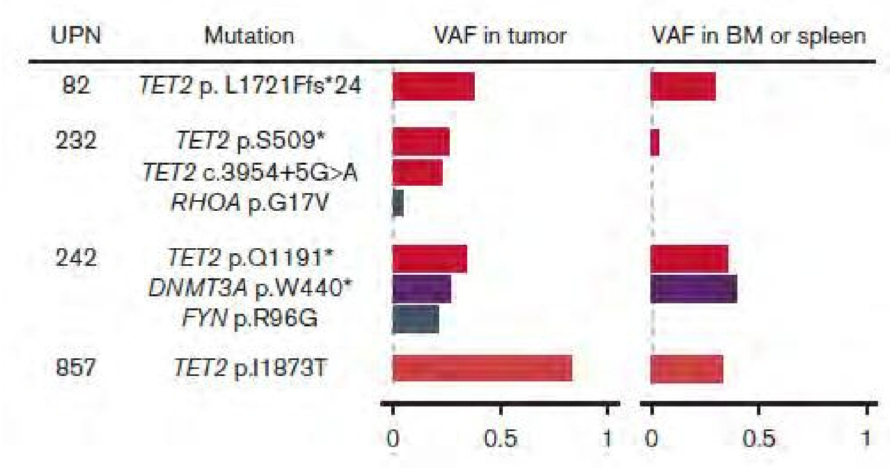
- Epigenetic modifiers: TET2, DNMT3A, KMT2D, SETD2
- JAK/STAT pathway: STAT5B, JAK3
- Others: DDX3X, TCR signaling



Climent F. Virchows Arch. 2023 Sep;483(3):333-348
Kato S. Blood Adv (2024) 8 (9): 2138–2147

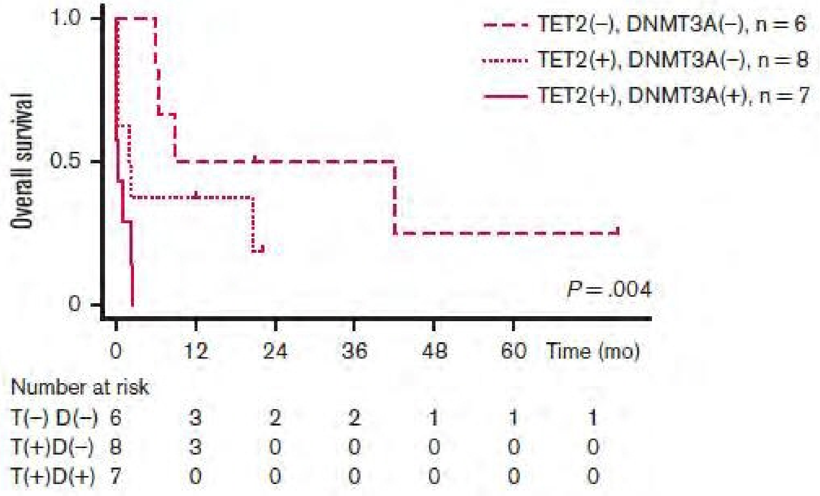
Association with clonal hematopoiesis

- High frequency of mutations of TET2 (58-68%), DMNT3A (27%-32%)



- 4 patients with TET2/DNMT3A alterations
- 3 of 4 cases had at least 1 identical TET2 and/or DNMT3A mutation in the BM or spleen tissue with VAF of >0.05

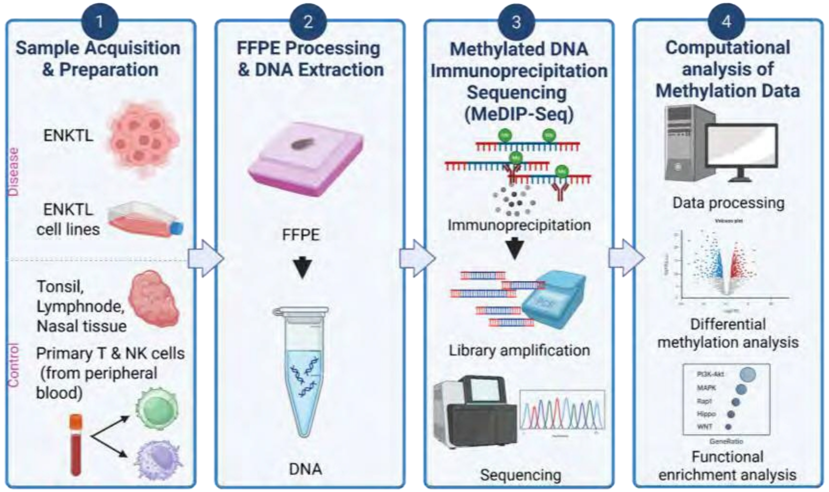
- TET2 mutations, especially with concurrent DNMT3A mutations, associated with shorter OS



Epigenetic Regulation of nodal TNKL through DNA Methylation

The Phyu

Ding Lingwen



ENKTL
n = 44

ENKTL Cell Lines *n* = 5

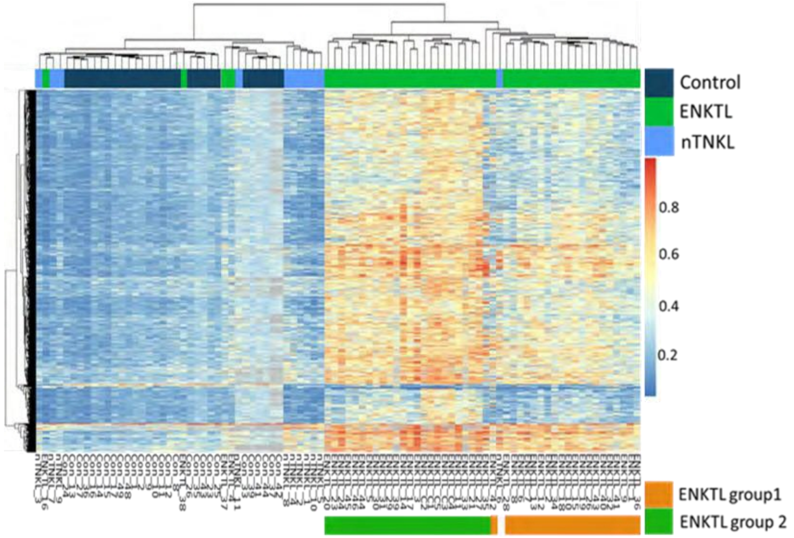
Nodal TNKL
n = 11

control tissues
n = 28

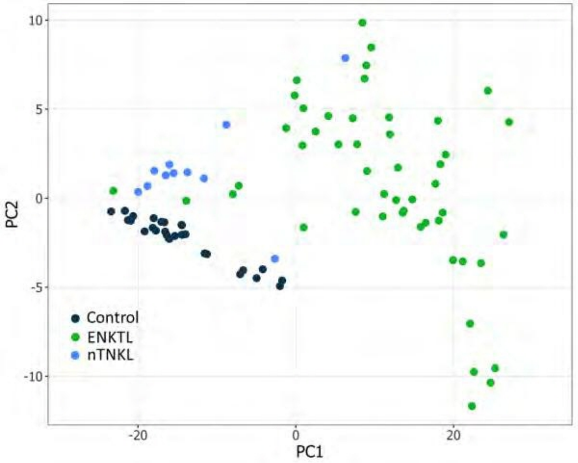
DNA Extraction

↓

Methylated DNA Immunoprecipitation Sequencing (MeDIP-Seq)



10207 DMRs of ENKTL, nTNKL and control



Distinct epigenetic profiles among between ENKTL, nTNKL, and control samples

ENKTL– hypermethylation
nTNKL– hypomethylation

nTNKL – EBV+nodal T and NK-cell lymphoma, DMR – Differentially Methylated Regions, DMG – Differentially Methylated Genes

Unpublished data. Do not post

ENKTL and nodal TNKL show different epigenetic signatures

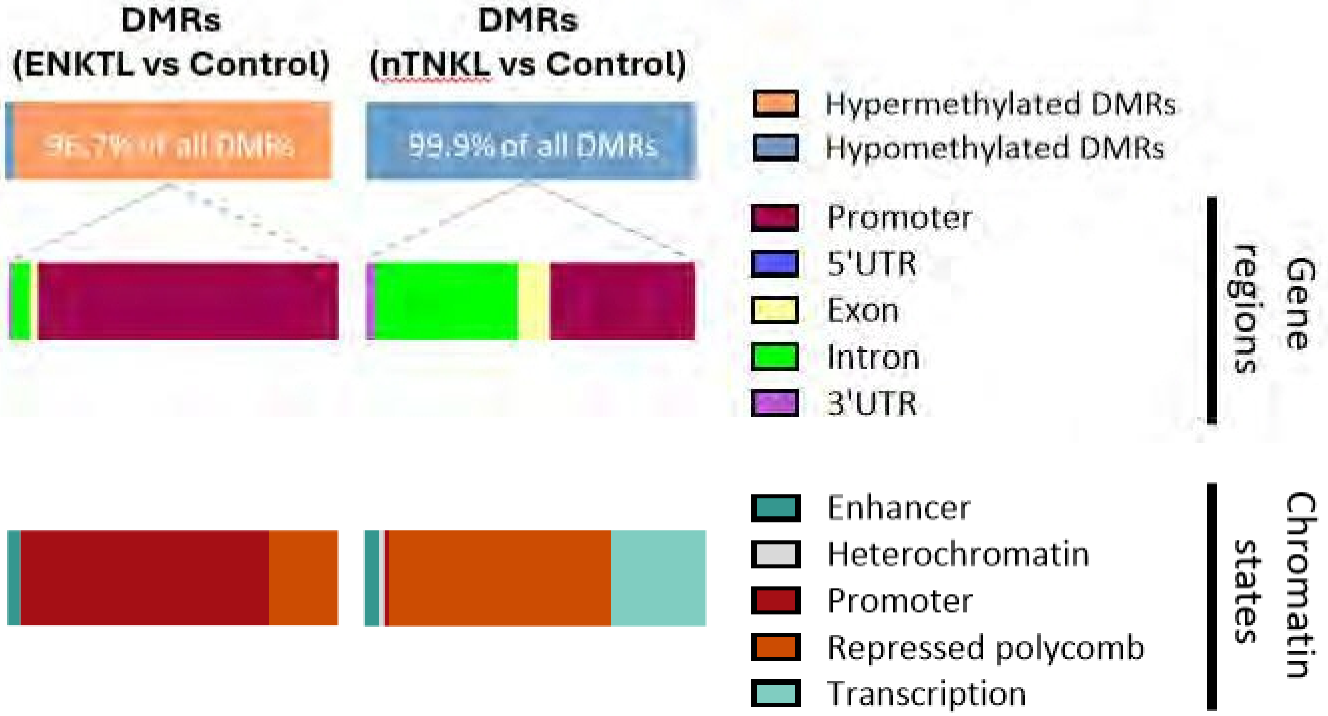
ENKTL hypermethylation
- predominantly promoter-centric

nTNKL hypomethylation
- evenly spread across promoter and intronic regions

nTNKL shows non-random hypomethylation in **Polycomb-repressed** and **transcription-**associated chromatin states

⬇

Abnormal differentiation program
Transcriptional dysregulation

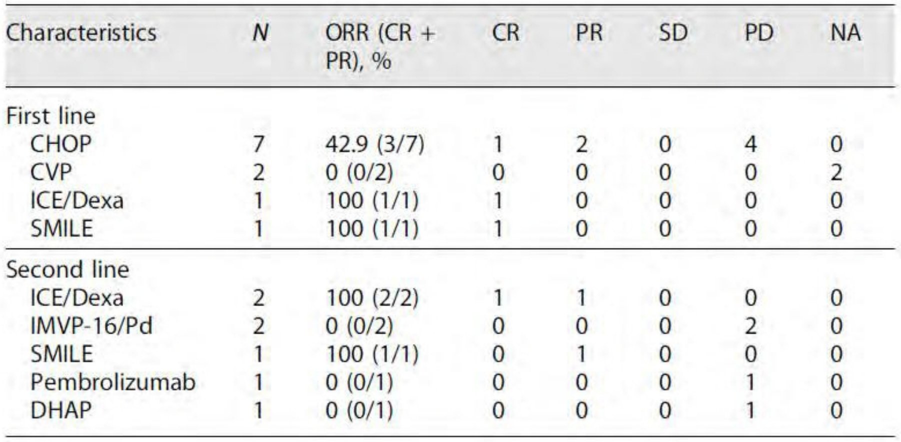


DMR – Differentially Methylated Regions

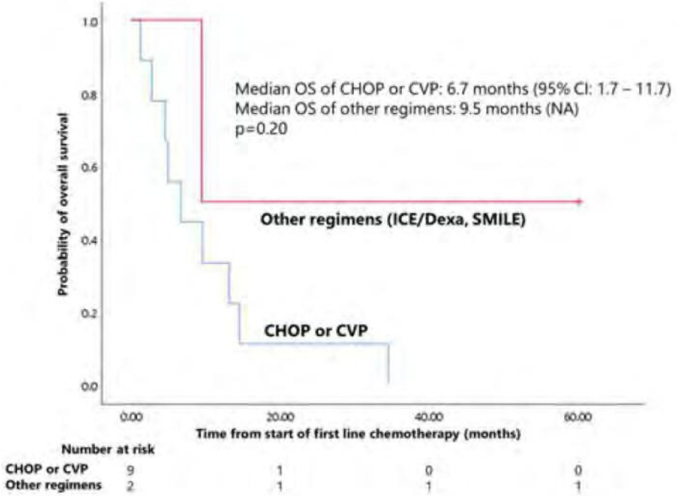
Unpublished data. Do not post

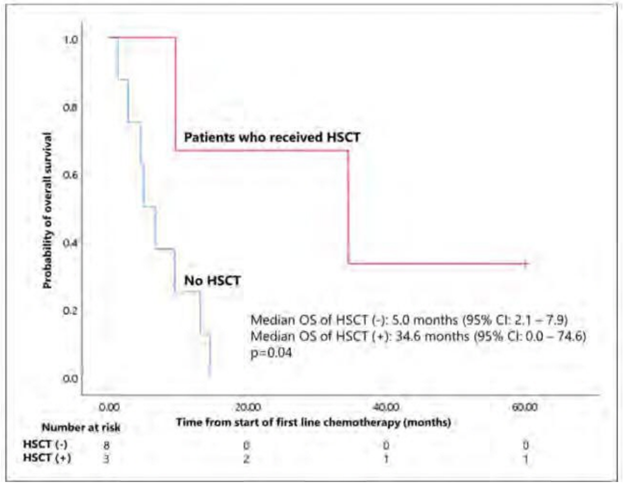
Treatment

- Limited data
- Choi DH et al (n=14)
    - Median age 52 yrs (range 32-88 yrs)
    - Advanced stage (93%, 13/14)
    - Treatment outcome in 11 patients
        - OS 9.5 months
        - Median PFS after 1st line chemotherapy 2.8 months



Characteristics	*N*	ORR (CR + PR), %	CR	PR	SD	PD	NA
First line							
CHOP	7	42.9 (3/7)	1	2	0	4	0
CVP	2	0 (0/2)	0	0	0	0	2
ICE/Dexa	1	100 (1/1)	1	0	0	0	0
SMILE	1	100 (1/1)	1	0	0	0	0
Second line							
ICE/Dexa	2	100 (2/2)	1	1	0	0	0
IMVP-16/Pd	2	0 (0/2)	0	0	0	2	0
SMILE	1	100 (1/1)	0	1	0	0	0
Pembrolizumab	1	0 (0/1)	0	0	0	1	0
DHAP	1	0 (0/1)	0	0	0	1	0



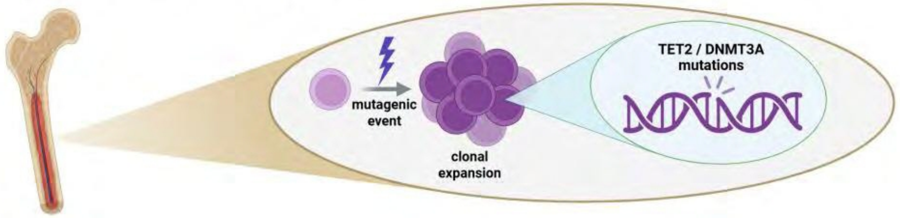


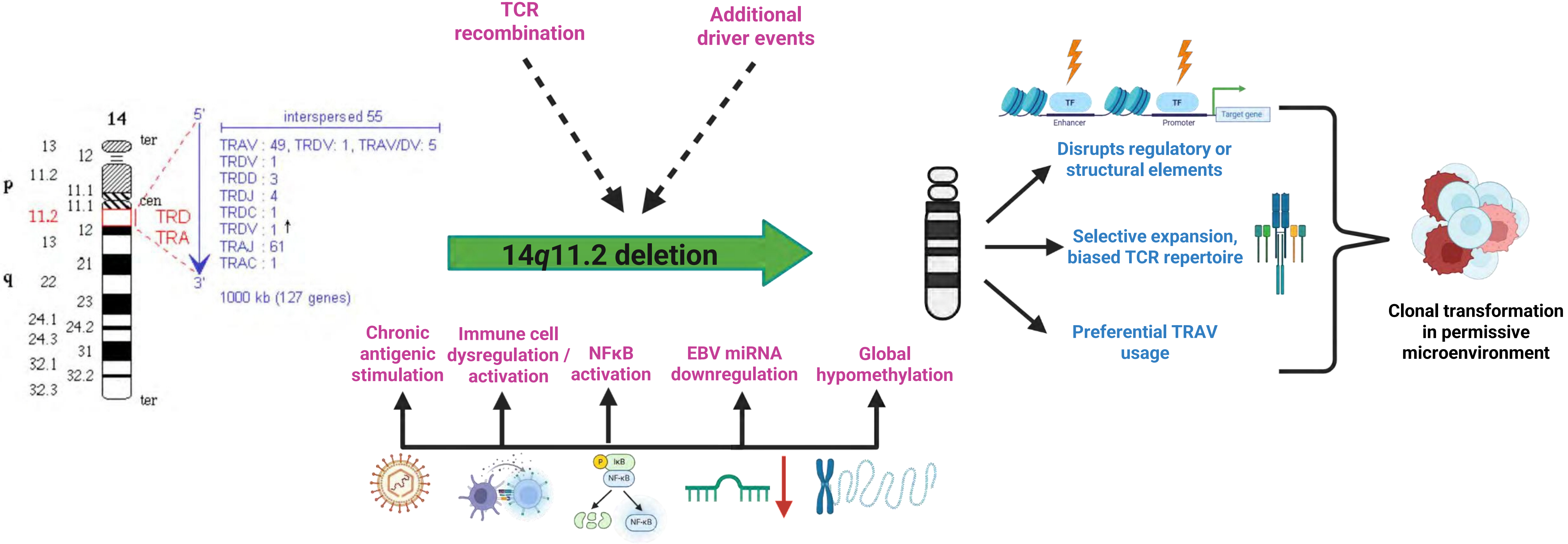
- CHOP and CVP regimens is not effective
- L-asparaginase-based chemotherapy (SMILE) may not work effectively (n=1)
- ICE / Dexa regimen shows potential benefits
    - median PFS (ICE/dexa): 7.4 months
    - Median PFS (CHOP or CVP): 2.2 months
- Undergoing HSCT after achieving a response helpful

Choi DH. Acta Haematol 2023

Proposed model of nodal TNKL pathogenesis

Clonal Hematopoiesis





TCR recombination

Additional driver events

14q11.2 deletion

Chronic antigenic stimulation

Immune cell dysregulation / activation

NFκB activation

EBV miRNA downregulation

Global hypomethylation

Disrupts regulatory or structural elements

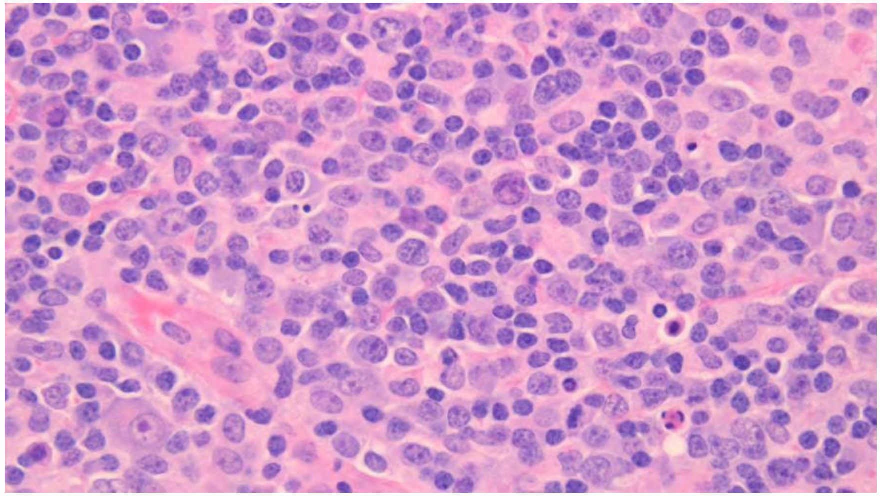
Selective expansion, biased TCR repertoire

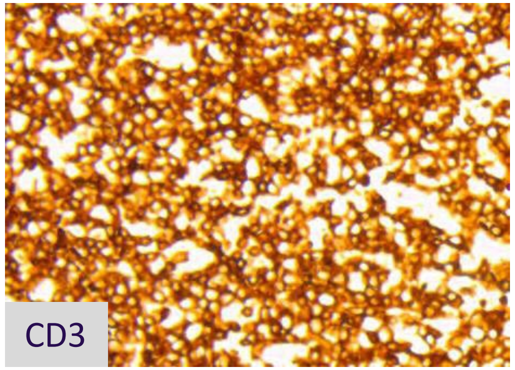
Preferential TRAV usage

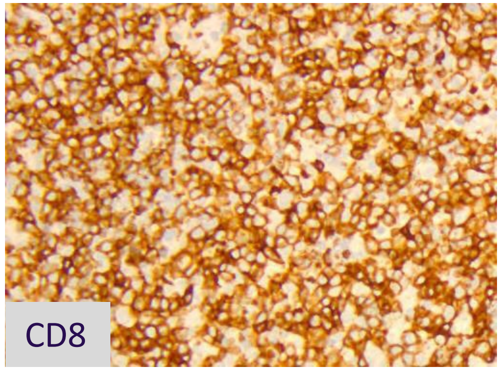
Clonal transformation in permissive microenvironment

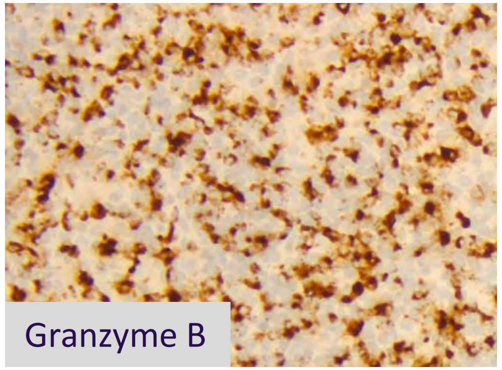
Diagnostic overlap between nodal TNKL and ENKTL

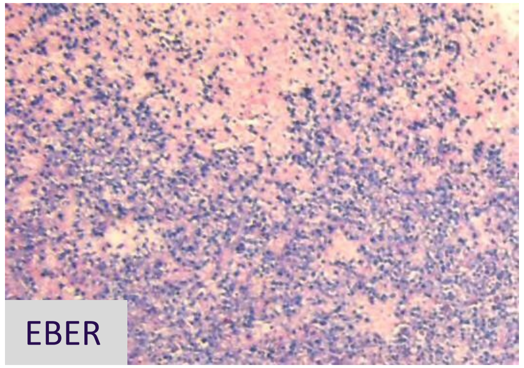
- 67/F, white, right neck masses
- PET scan: lymph nodes above and below the diaphragm, bilateral lacrimal glands, right tonsil, spleen. No nasal involvement.
- IHC: CD3+, CD8+, CD56-, cytotoxic, EBER+,
- T lineage: Monoclonal T cell rearrangement
- DNMT3A and TET2 mutations





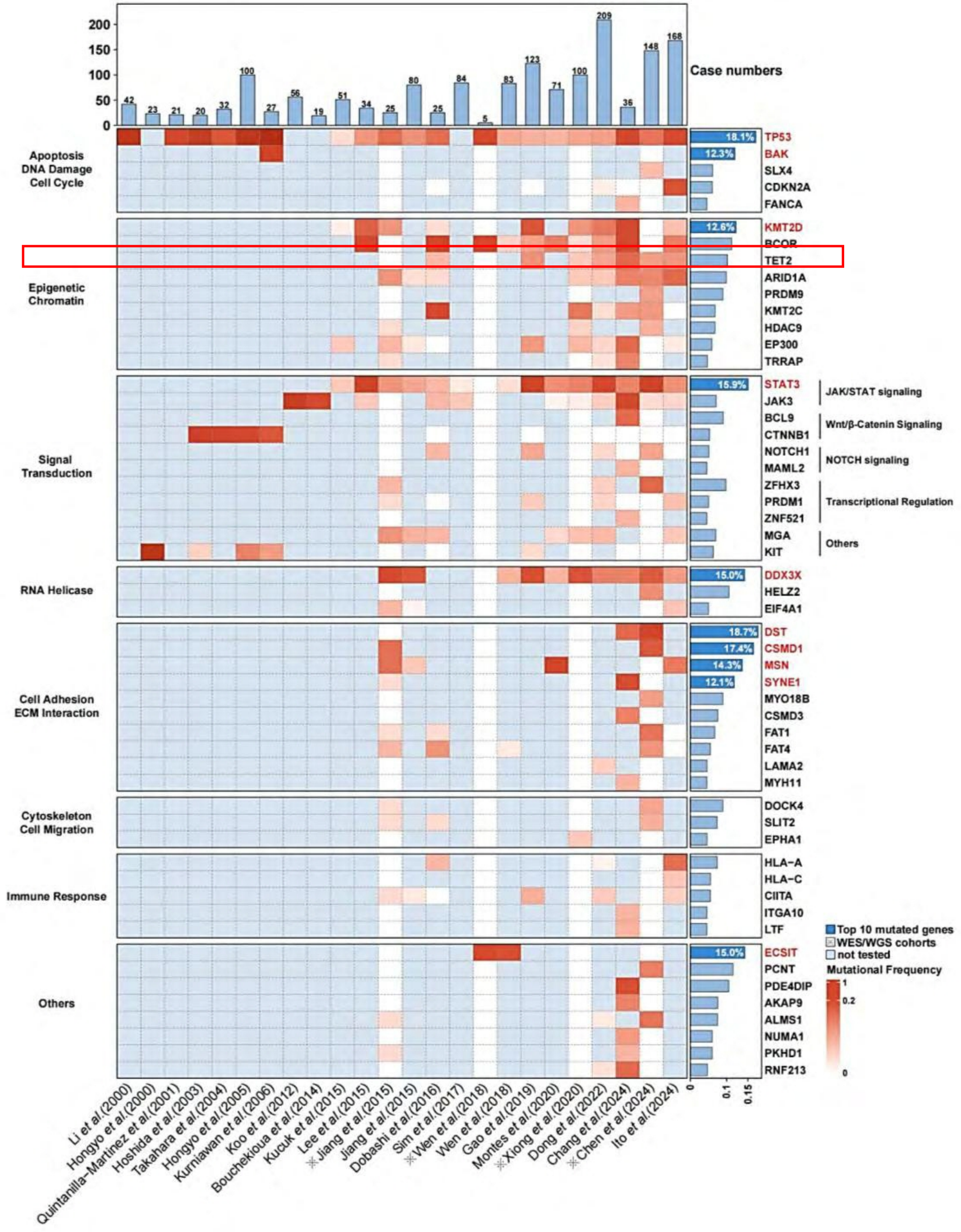






Do the presence of TET2 and/or DNMT3A mutations support diagnosis of nodal TNKL?

EAHP22-LYWS-1207, Kenneth Ofori, Columbia University Irving Medical Center



Mutational landscape of ENKTL

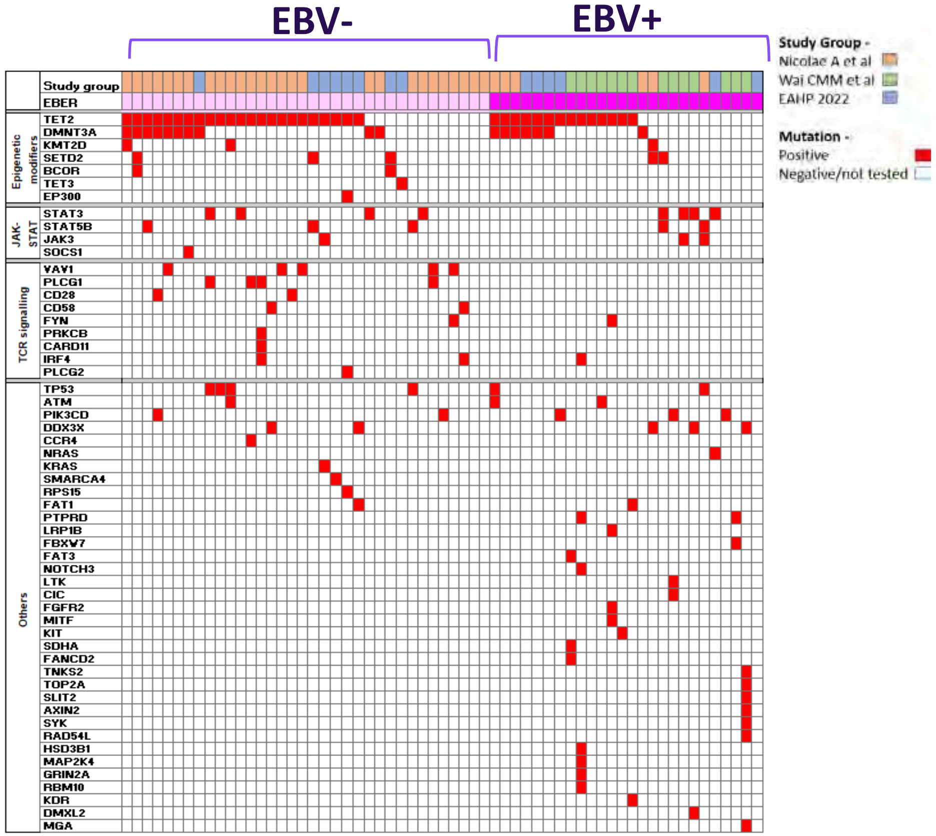
- Data from 22 published studies on ENKTL (2000-2024)
- Top 50 most frequently mutated genes (Top 10 genes in red) grouped according to functions

- Mutations of epigenetic modifier genes common
- TET2 mutations reported in 10.3% of ENKTL
  - Do these ENKTL involve LN?
  - Are they undiagnosed nodal TNKL?

Chan WL. The Lancet Regional Health - Western Pacific (Accepted for publication)

Is nodal TNKL distinct from EBV- cytotoxic PTCL NOS?

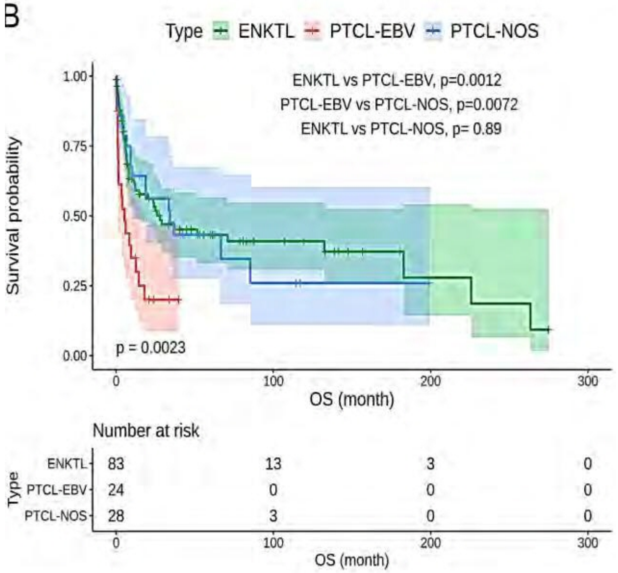
Similar mutational profile



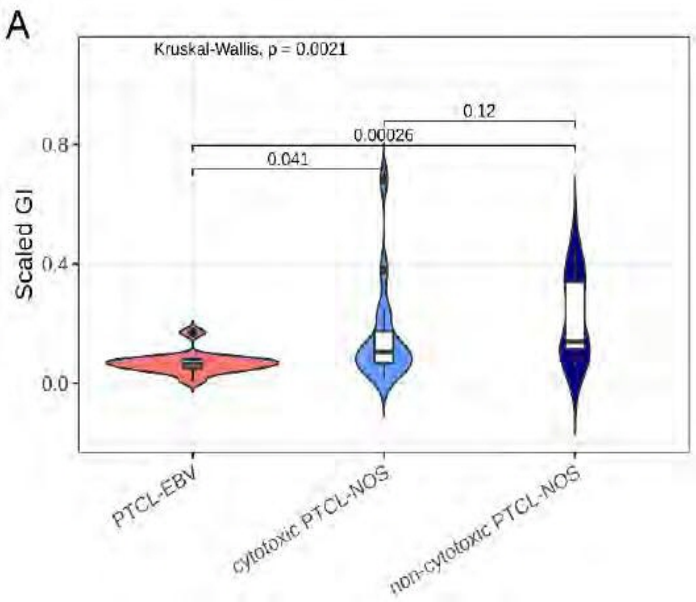
Study Group -
Nicolae A et al 🟧
Wai CMM et al 🟩
EAHP 2022 🟦

Mutation -
Positive 🟥
Negative ☐

Differences



Poorer OS compared to EBV- cytotoxic PTCL NOS



Lower genomic instability in EBV+ nodal TNKL

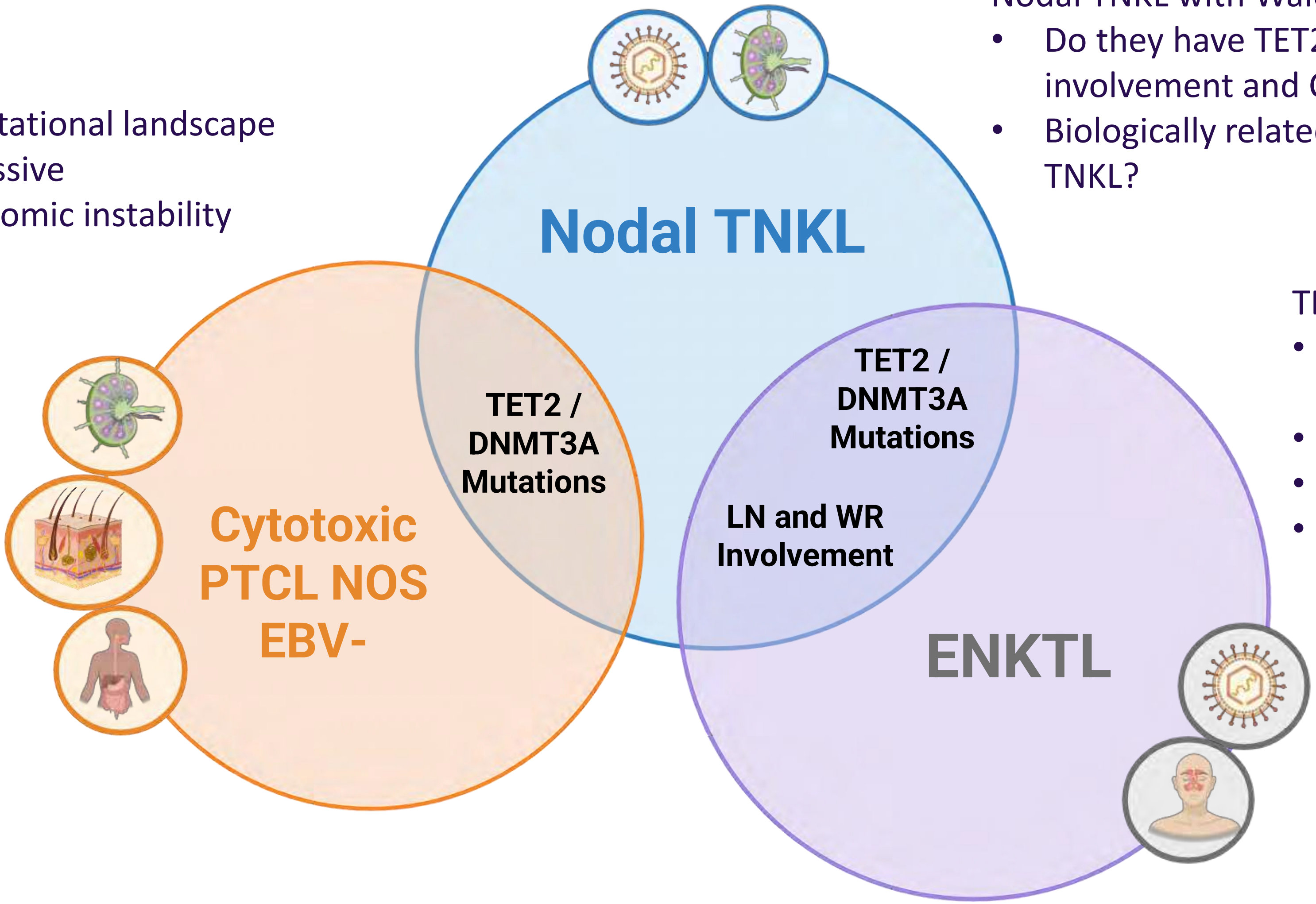
Climent F. Virchows Arch. 2023. Wai CMM. Haematologica 2022

Distinct entities or biologic continuum ?

Similar mutational landscape
Less aggressive
Higher genomic instability

Nodal TNKL with Waldeyer ring involvement
- Do they have TET2 and/or DNMT3A involvement and CHIP
- Biologically related to ENKTL or nodal TNKL?

TET2-mutated ENKTL
- Biologically distinct nodal variant of ENKTL?
- Extranasal ENKTL?
- Undiagnosed nodal TNKL?
- Do they have clonal hematopoiesis?



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