



EAHP 2026



23RD MEETING OF THE EUROPEAN ASSOCIATION FOR HAEMATOPATHOLOGY

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ABSTRACT BOOK



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Bone Marrow Abstracts – Oral

EA4HP26-ABS-560	Critical misclassifications compromise the reliability of automated digital bone marrow cytomorphology
EA4HP26-ABS-576	Automated CD34+ Blast Quantification in Bone Marrow Trephine Biopsies: A Deep Learning Approach for High-Sensitivity Pre-Screening.
EA4HP26-ABS-590	Using AI-augmented spatial transcriptomic analysis to characterise the megakaryocyte niche in myeloproliferative neoplasms
EA4HP26-ABS-653	AI-Assisted Continuous Indexing of Fibrosis Improves Reproducibility and Halves Reading Time in Diagnostic Bone Marrow Trephine Grading

Critical misclassifications compromise the reliability of automated digital bone marrow cytomorphology

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Background

The correct classification of individual bone marrow (BM) cells in closely related or similar morphological categories can be challenging. The clinical viability of automated digital cytomorphology (ADM) has not been fully explored as for the occurrence of critical BM cell confusions.

Principles/Methodology

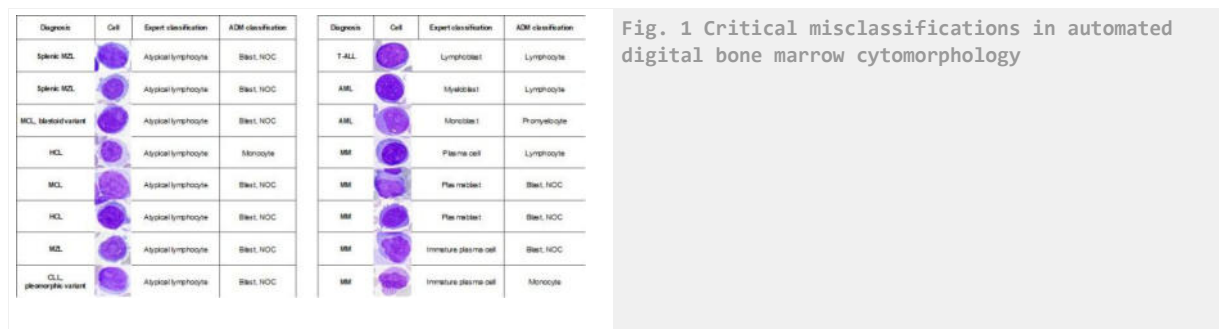
In 328 diagnostic BM smears, BM cell recognition using ADM (Morphogo system) was compared with expert optical microscopy in six diagnostic groups: MDN (15%), multiple myeloma (MM, 14%), mature B/T-cell neoplasms (13%), acute leukaemia and CMML (9%), MPN (8%) and reactive haemopoiesis (41%). To maintain clinical consistency, the percentage of correctly classified cells in individual patients was assessed. A detailed analysis was conducted for critical misclassification cases with clinical consistency of <80%. The patient similarity network (PSN) revealed differences between conventional and ADM myelograms.

Results

The relevant clinical consistency was 97.1%, with 5.5% of critical misclassifications, caused by a failure to recognise atypical neoplastic lymphocyte/blast, myeloblast/lymphocyte, lymphoblast/lymphocyte, immature plasma cell/blast and monoblast+promonocyte/promyelocyte. (Fig. 1) Atypical cytomorphology of misclassified cells was observed in all cases. Patients with critical misclassifications were only seen in the diagnostic categories AL+CMML, MM and B/T-lymphoma, not in the MPN, MDN and reactive groups. Using PSN, the majority of patients with low values of clinical consistency and all critical misclassifications occurred in three separate subgroups of AL+CMML (4/17), MM (5/46) and B/T-lymphoma (9/46) groups. (Fig. 2)

Conclusion

This study identified the most frequently misclassified cell types by ADM with potential critical impacts on BM diagnosis. These included atypical lymphocytes resembling blasts, small lymphoblasts and myeloblasts resembling lymphocytes, granular monoblasts resembling promyelocytes, dysplastic monocytic elements, plasmablasts, and immature plasma cells (candidate cells for future AI training). Although unfavourable outcomes are fully mitigated by expert supervision, we consider them to be a restriction of ADM's absolute reliability.



Misclassified neoplastic lympho/myelo/monoblasts and plasma cells are shown in cases of marginal zone lymphoma (MZL), mantle cell lymphoma (MCL), hairy cell leukemia (HCL), chronic lymphocytic leukemia (CLL), acute myeloid leukaemia (AML), acute T-lymphoblastic leukaemia (T-ALL) and multiple myeloma (MM). The confusion of atypical lymphocyte/blast was the most frequent misclassification. The main cytomorphological features of misclassified neoplastic lymphocytes were: medium size, finer chromatin, nucleoli, abundant basophilic or pale cytoplasm with projections, blastoid appearance.

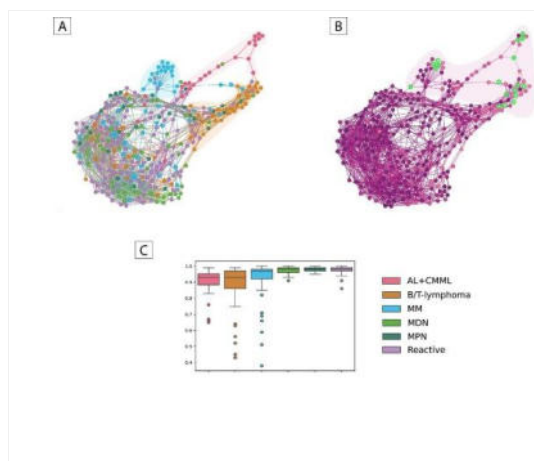


Fig. 2 Numerical differences of myelograms and clinical consistency across six diagnostic groups (A) Patient similarity network (PSN). The PSN was constructed based on similarities of patients as for the cell count differences. Patients closer together share more similar myelogram discrepancies in AL+CMML (red), MM (blue), B/T-lymphoma (orange), MDN (light green), MPN (dark green) and reactive (violet) groups. (B) Relevant clinical consistency values in the PSN. Patients with critical misclassifications highlighted in green occur exclusively in groups AL+CMML, MM and B/T-lymphoma. (C) Display of relevant clinical consistency values in the diagnostic groups (box plots).

EA4HP26-ABS-576

Automated CD34+ Blast Quantification in Bone Marrow Trephine Biopsies: A Deep Learning Approach for High-Sensitivity Pre-Screening.

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Background

Blast quantification in bone marrow biopsies (BMTBs) is fundamental for the classification of Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukemia (AML). CD34 immunohistochemistry is the accepted standard for identifying hematopoietic progenitor cells. However, manual assessment is demanding and prone to significant inter-observer variability. A major diagnostic pitfall is the co-expression of CD34 by vascular endothelial cells, which can lead to the overestimation of blast counts. This study evaluates a dedicated AI tool designed to automate CD34+ cell quantification while effectively distinguishing them from vascular structures.

Principles/Methodology

We developed an automated pipeline for whole-slide image (WSI) analysis presented on fig. 1.

The workflow includes:

1. **Vessel Exclusion:** A deep learning module trained to identify and mask vascular structures to prevent false-positive blast counts.
2. **Cell Detection:** An object detection model (YOLO-based) trained on over 21,000 manually annotated and 18,000 self supervised CD34+ and CD34- cells to quantify the blast percentage.
3. **Diagnostic classification:** An aggregation algorithm that translates cell-level data into slide-level diagnostic categories (<5%, ≥5 and <10%, ≥10 and <20%, ≥20%). The system was validated on an independent cohort of 231 WSIs to assess its performance as a clinical pre-screening tool.

Results

The AI tool demonstrated strong diagnostic reliability with an Area Under the Curve (AUC) of 0.75 at the WSI level. In quantifying CD34 expression within regions of interest, the difference between automated and manual expert measurements was within 1–2%. For the cell counting task, the model showed substantial agreement with pathologists (Cohen's kappa $\kappa = 0.76$). To test its utility as a pre-screening "safety-first" tool, the threshold was set to 100% sensitivity. At this level, the model successfully excluded 36.8% of negative cases from the manual review workflow without any false negatives. The cell detection component achieved high precision (0.92) and recall (0.93).

Conclusion

Deep learning-based analysis provides a reliable framework for objective CD34+ blast quantification in BMTBs. By effectively filtering vascular interference and providing high-sensitivity pre-screening, this tool has the potential to standardize MDS/AML diagnostics and significantly improve pathologist workflow. Further multi-center validation and refinement of the vascular detection modules are required before integration into the primary diagnostic routine.

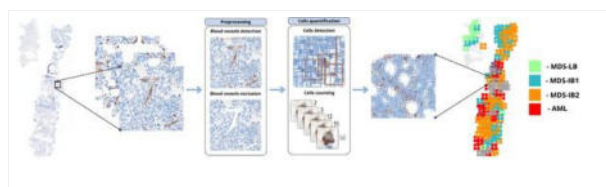


Fig. 1. Overview of the proposed pipeline for automated CD34-positive blast quantification.

EA4HP26-ABS-590

Using AI-augmented spatial transcriptomic analysis to characterise the megakaryocyte niche in myeloproliferative neoplasms

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Background

Myeloproliferative neoplasms (MPNs) are myeloid blood cancers characterised by well-defined driver mutations in *JAK2*, *CALR* or *MPL*. MPNs are typically characterised by abnormal megakaryopoiesis, with aberrant megakaryocyte numbers, abnormal cellular cytomorphology, and perturbed topological distribution. Megakaryocytes are also implicated in disease development, while emerging novel therapeutics now offer targeted approaches towards mutant

CALR (mCALR) expressed on neoplastic populations, most prominently by megakaryocytes. Despite this, the megakaryocytic niche is not yet fully characterised in the context of health or in MPN.

Principles/Methodology

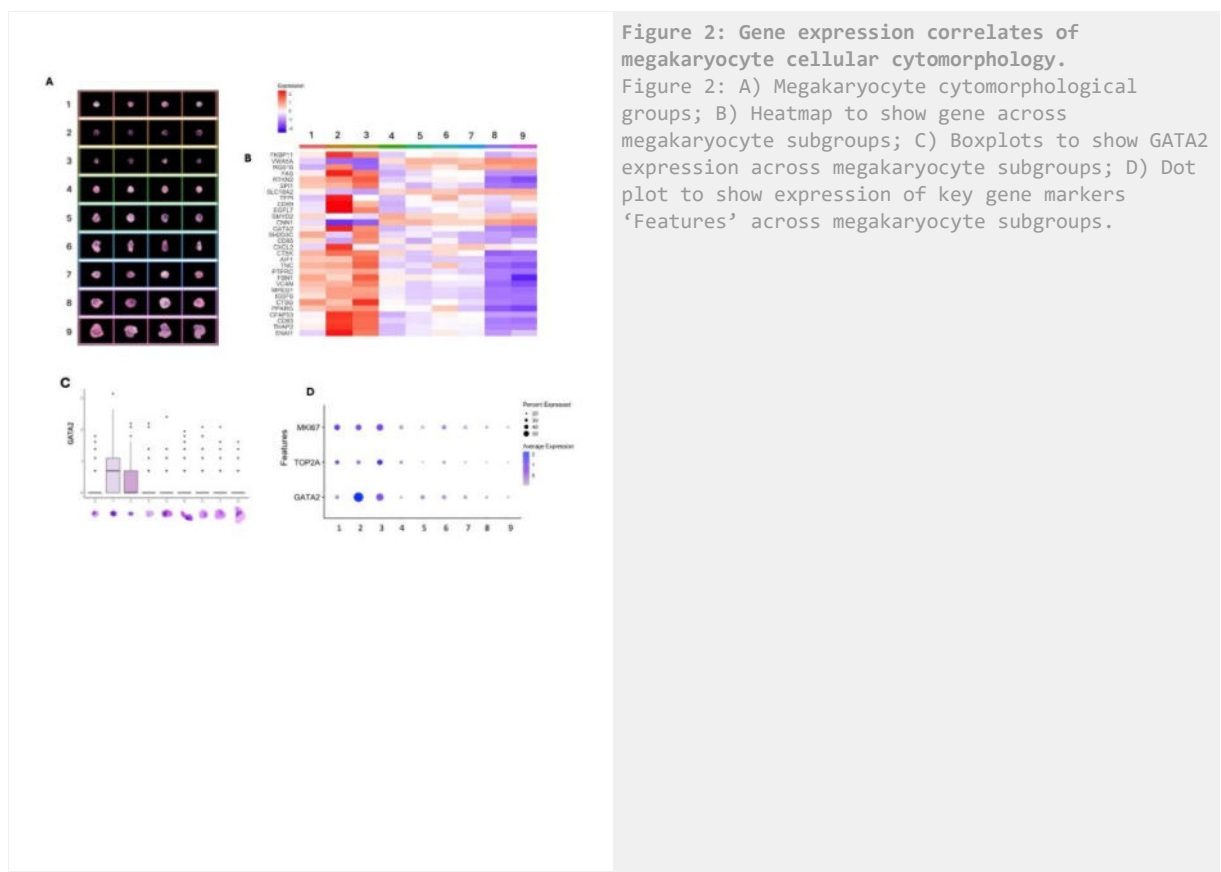
We perform spatial transcriptomic (ST) analysis using the 10x Xenium platform, analysing BM trephines from 27 patients with MPN and four healthy donors. We apply our AI-augmented bone marrow ST analysis workflow, performing histology-based annotation for QC, using (AI)-driven haematoxylin and eosin (H&E)-based feature detection to enhance megakaryocyte segmentation and to identify trabecular bone and adipocytes. Following cell type annotation using unsupervised ST-based clustering, we then use a toolkit of spatial biological analyses (cellular co-enrichment, global proximity) to map the topological distribution of megakaryocytes in the normal BM, across conditions, and in those cases harbouring a *CALR* mutation.

Results

We describe the topological distribution and cellular niche of megakaryocytes in those with an MPN, characterising differential pattern of cellular enrichment of stromal and immune cells (Figure 1). For the first time, we identify gene expression profiles associated with cellular cytomorphology across maturation states, with small megakaryocyte expressing an immature, cycling signature (Figure 2). We use trajectory inference (TI) approaches to quantitate and map spatial patterns of megakaryocyte maturation. We identify mCALR-expressing cells using a mutation-specific antibody, characterising the megakaryocyte niche in regions enriched for mCALR expression, and identifying differential microenvironmental and topological profiles in mCALR and non-mCALR MPN samples.

Conclusion

Our work extends our understanding of the megakaryopoiesis, the bone marrow niche, and has implications for future clinical translational therapeutic approaches.



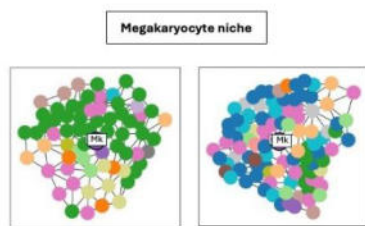


Figure 1: Schematic to demonstrate the megakaryocyte niche

Figure 1: Plots to demonstrate distribution of cells adjacent to megakaryocytes ('Mk'). Cells are represented by coloured dots and different cells are coloured by type.

EA4HP26-ABS-653

AI-Assisted Continuous Indexing of Fibrosis Improves Reproducibility and Halves Reading Time in Diagnostic Bone Marrow Trephine Grading

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Background

Assessment of fibrosis is central to evaluation of diagnostic bone marrow trephine (BMT) biopsies, yet conventional manual grading is subjective and qualitative. We previously developed an AI-based Continuous Indexing of Fibrosis (CIF) tool and demonstrated its capacity to detect fibrosis changes beyond manual sensitivity. Here we present the first large-scale clinical evaluation of CIF using consecutive, unselected diagnostic BMT samples.

Principles/Methodology

Nearly 1000 consecutive diagnostic BMTs from a tertiary referral centre underwent reticulin whole-slide image (WSI) quality assessment; 796 eligible slides were retained after stratified sampling. Fourteen experienced haematopathologists from 5 international centres performed fibrosis grading using three protocols across three rounds with washout periods: Manual read (reticulin WSI alone), Sequential-assisted read (manual grade then CIF heatmap review with opportunity to revise), and Concurrent-assisted read (CIF heatmap provided from the outset). Consensus MF grade was derived by majority vote from Round 1 Manual reads. Agreement was evaluated within a pre-specified non-inferiority framework with margin adopted from the FDA AIM-NASH biomarker qualification review.

Results

CIF scores correlated strongly with consensus MF grade (CIF P70 Spearman $\rho = 0.770$; AUC ≥ 0.85 at all adjacent grade boundaries; Fig. 1). Sequential-assisted reads improved inter-observer agreement by 12.6 percentage points (95% CI 10.5–14.8; Fig. 2) and agreement with the consensus reference by 3.0 percentage points (95% CI 0.4–5.7) versus Manual reads. Both Sequential and Concurrent assisted protocols improved intra-observer agreement by approximately 11–12 percentage points and reduced discordance at the clinically significant MF-1/MF-2 boundary. Concurrent-assisted reads halved median reading time (17 vs 30 seconds per slide; $p < 0.001$).

Conclusion

AI-assisted quantitative bone marrow fibrosis assessment using CIF is clinically deployable, reproducible, and efficient across the spectrum of routine diagnostic BMT samples. CIF assistance significantly improves intra- and inter-observer agreement among expert haematopathologists and reduces clinically relevant grading discordance. These findings support the role of quantitative fibrosis assessment in standardising diagnostic haematopathology and enhancing the sensitivity of fibrosis-based clinical trial endpoints.

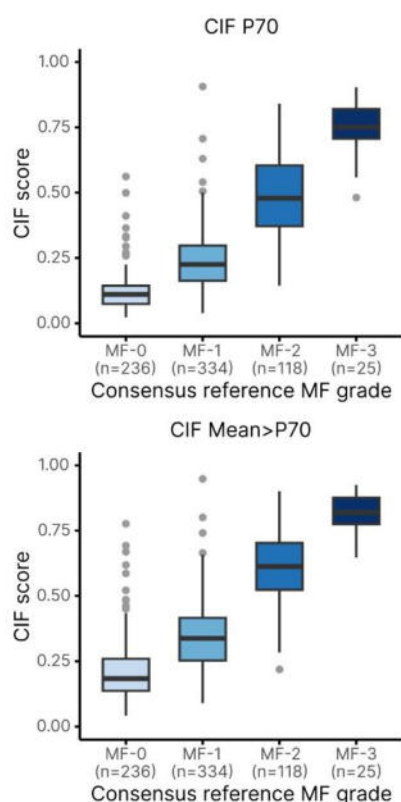


Figure 1. Slide-level CIF scores by consensus reference MF grade. CIF P70 (70th percentile of tile-level fibrosis scores) and CIF Mean>P70 (mean score among tiles above the P70 threshold) for 713 slides with consensus reference. Boxes show median and IQR; whiskers extend to 1.5× IQR. CIF P70 Spearman $\rho = 0.770$ (95% CI 0.733–0.803).

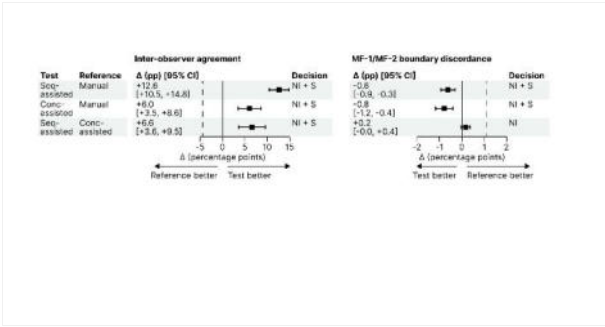


Figure 2. Inter-observer agreement by reading protocol. Left: difference in pairwise agreement rate (percentage points) between protocols. Right: difference in discordance across the MF-1/MF-2 boundary. Points and error bars show estimates and 95% CIs from generalised linear mixed models. Dashed lines indicate the pre-specified non-inferiority margin. NI = non-inferior; S = superior.

Bone Marrow Abstracts – Poster

EA4HP26-ABS-471	Leukemia maturation stages are associated with disease-permissive and immunosuppressive states in newly diagnosed AML
EA4HP26-ABS-474	Artificial intelligence-based segmentation of megakaryocytes in bone marrow biopsies
EA4HP26-ABS-501	AI-Assisted Diagnostic Classification of BCR::ABL-Negative Myeloproliferative Neoplasms Using a Pathology Foundation Model with Attention-Based Multiple Instance Learning
EA4HP26-ABS-509	Digital image analysis-guided quantification of bone marrow-infiltrating plasma cells in multiple myeloma
EA4HP26-ABS-518	A clinical-pathological characterization of erythroid sarcoma.
EA4HP26-ABS-541	Artificial intelligence-driven analysis of CD34-stained bone marrow trephines reveals limitations of conventional blast assessment and identifies prognostic histological motifs in a multicentric cohort of myeloid neoplasms.
EA4HP26-ABS-546	MegaQuant : Segment-Anything Model (SAM)-Assisted Segmentation Accelerates Annotations without Compromising Precision – A Case Study for Bone Marrow Megakaryocytes
EA4HP26-ABS-548	Automated Spatial Analysis of Immunohistochemistry Images of the Bone Marrow as an Economically Scalable Alternative to Spatial Omic Technologies
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EA4HP26-ABS-564	A multi-omic comparison of histiocytic sarcoma and its mesenchymal mimickers.
EA4HP26-ABS-565	The hematological Proteus: intralesional morphophenotypical heterogeneity and diagnostic mimickers in extramedullary chronic myelomonocytic leukemia.
EA4HP26-ABS-571	Philadelphia chromosome-positive acute leukemia with <i>BCR::ABL1</i> -positive cells significantly exceeding blasts may represent de novo blast phase of chronic myeloid leukemia with a silenced chronic phase
EA4HP26-ABS-584	Integrin expression profiling identifies a subgroup of acute myeloid leukemia with distinct transcriptional programs
EA4HP26-ABS-594	Characterising the topology of the human bone marrow in MPN using AI-augmented spatial transcriptomics
EA4HP26-ABS-598	Remodelling of the Bone Marrow Niche during Treatment of acute T-Lymphoblastic Leukaemia: A longitudinal Multi-Modal Analysis

EA4HP26-ABS-606	Development and Clinical Validation of an LLM-Based C-MICM Diagnostic Agent for Myeloid Neoplasms
EA4HP26-ABS-639	AI-Driven Detection and Quantification of CD34+ Progenitor Cells in Bone Marrow Biopsies using AIFORIA
EA4HP26-ABS-643	An AI-based system of fibrosis prediction from spatial transcriptomic data reveals bone marrow microenvironmental features associated with fibrosis in myeloproliferative neoplasia
EA4HP26-ABS-661	Genetic Landscape and Clinical Outcomes of <i>DDX41</i> -Mutated Myeloid Neoplasms: A Retrospective Single Institutional Analysis

Leukemia maturation stages are associated with disease-permissive and immunosuppressive states in newly diagnosed AML

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Background

AML-induced immunosuppression has emerged as cofactor in therapy resistance and relapse. To gain insights into potentially reversible mechanisms, we applied longitudinal single-cell and spatial analyses to bone marrow (BM) from newly diagnosed AML patients.

Principles/Methodology

43,000 single-cell RNA-seq profiles were generated from 12 paired samples of 6 AML patients at diagnosis (T1) and post-induction therapy (T2) using 5' scRNA/CITE-seq (140 TotalSeq-C antibodies). Spatial correlation was performed using Xenium transcriptomics integrated with histopathology on matched trephine biopsies, leveraging reference-guided label transfer, graph-based spatial modeling, and AI-based integration to map cell types, states, and interactions.

Results

8 different subtypes of AML cells were identified: 4 clusters with immature properties and 4 clusters with signs of maturation. Immature AML clusters were enriched in signatures for stemness, quiescence, homing and chemoresistance (e.g. β Catenin, Trail, TGF β) as well as proinflammatory pathways (e.g. NF κ B, TNF α , JAK/STAT). In contrast, more mature AML cells showed upregulation of PI3K, VEGF and metabolic pathway shift (OXPHOS, fatty acid homeostasis), possibly reflecting adaption of survival signals. Each AML cell cluster expressed distinct immune checkpoints, with most prominent expression of VISTA in monocyte-like AML cells. Spatially, VISTA+ monocyte-like AML cells surrounded other AML cells. The immune microenvironment at T1 was characterized by activation of inflammatory pathways and enrichment of MDSC-signatures in myeloid progenitors. Additionally, substantial reduction of classical dendritic cells type 1 (cDC1; $p=0.066$) and increased T cell senescence were found. Spatial and cell interaction analysis indicated disrupted interaction between cDC1 and CD8+ T cells. At T2, niche remodeling and reversal of immunosuppressive microenvironment was observed.

Conclusion

Our study shows that AML heterogeneity is linked to severe dysregulation of the BM immune microenvironment that appears reversible with standard chemotherapy. While highly immature AML cells sustain the leukemic stem cell pool, more mature AML cells may constitute the disease bulk. Particularly, monocyte-like AML cells undergo metabolic rewiring and shape a protective, VISTA-mediated immunosuppressive niche, potentially resulting in limited efficacy of immune checkpoint inhibitor therapy in AML. Defining AML-specific immune signatures may additionally inform future (immuno)therapeutic strategies.

EA4HP26-ABS-474

Artificial intelligence-based segmentation of megakaryocytes in bone marrow biopsies

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Background

Polycythemia vera(PV), essential thrombocythemia(ET), and primary myelofibrosis(PMF) are the three classical subtypes of BCR::ABL1-negative myeloproliferative neoplasms(MPNs). Although these entities share overlapping clinicopathological and molecular features, they differ in complications and disease progression, making their distinction challenging in routine practice. Artificial intelligence(AI) technologies have the potential to enhance conventional morphological assessment through quantitative image analysis. This study aimed to develop machine learning models to assist pathologists in the diagnosis of MPNs and the differentiation of their subtypes by identifying and analyzing megakaryocyte features.

Principles/Methodology

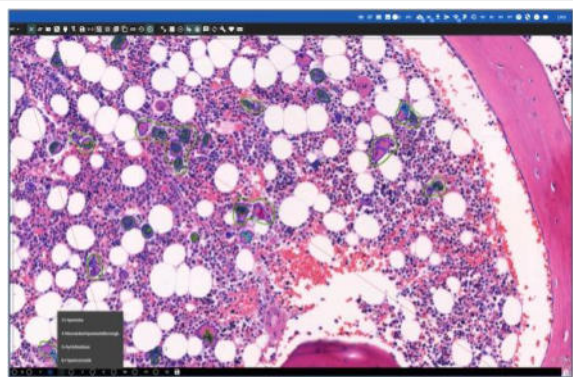
Hematoxylin and eosin (H&E)–stained bone marrow biopsy sections from 27 PV, 37 ET, and 17 prefibrotic PMF cases diagnosed between January 2012 and September 2024, along with 26 non-neoplastic bone marrow biopsies from patients without hematolymphoid disease or prior chemotherapy, were retrospectively reviewed and digitized using a 3DHISTECH scanner. In digital images, megakaryocyte nuclei (normal, bare, separated, bulbous, hyperchromatic, hyperlobated, and monolobated/hypolobated/micromegakaryocytes), cytoplasmic contours, and clusters were manually annotated using PACS. Based on these labeled data, AI algorithms were developed for megakaryocyte segmentation, classification, and MPN subtype differentiation using cytomorphologic and topographic features.

Results

The segmentation model detected megakaryocytes with an F1-score of 87.6%. The classification model distinguished dysplastic from normal megakaryocytes with an accuracy of 96.74% and predicted nuclear morphologic classes with an accuracy of 92.53%. Diagnostic models differentiated MPNs from non-neoplastic bone marrow with an average accuracy of 94%, reaching up to 100% in the best-performing model. Models developed for subtype differentiation achieved an accuracy of 81.48% using morphological features alone, which increased to 92.59% after incorporating clinical and laboratory data.

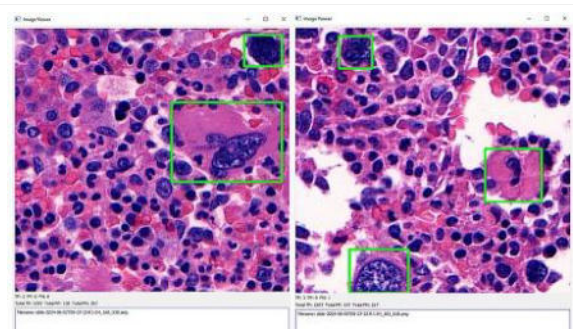
Conclusion

AI-based models demonstrated strong performance in megakaryocyte segmentation, classification, and the diagnosis and subtype differentiation of MPNs. These approaches may support pathologists by automating workflows, accelerating diagnostic processes, and providing objective results. The findings suggest potential clinical applicability and adaptability to other hematologic malignancies requiring quantitative histologic evaluation.



Model training process

The image shows the labeling interface of the Simplex PACS software during model training. Megakaryocytes were categorized based on their morphology and assigned to different label groups.



Segmentation model: cell detection validation process

In the left image, all megakaryocytes within the patch area were detected. In the right image, 3 out of 4 megakaryocytes were detected, while one bare megakaryocyte was not detected (FN: false negative; FP: false positive; TP: true positive).

EA4HP26-ABS-501

AI-Assisted Diagnostic Classification of BCR::ABL-Negative Myeloproliferative Neoplasms Using a Pathology Foundation Model with Attention-Based Multiple Instance Learning

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Background

BCR::ABL-negative myeloproliferative neoplasms (MPN) primarily include essential thrombocythemia (ET), polycythemia vera (PV), prefibrotic primary myelofibrosis (Pre-PMF), fibrotic primary myelofibrosis (Fibrotic PMF), and MPN, not otherwise specified (MPN, NOS). Accurate histopathological classification remains challenging owing to overlapping morphological features and substantial inter-observer variability. Artificial intelligence (AI) holds great potential for standardizing MPN diagnosis by capturing subtle morphological patterns.

Principles/Methodology

A total of 2,049 bone marrow trephine biopsies whole slide images (WSIs) were used for binary classification (MPN: n=1421; non-MPN: n=628), and a subset of 1,320 MPN WSIs were further used for five-class subtyping (ET: n=362, PV: n=284, Pre-PMF: n=251, Fibrotic PMF: n=410, MPN, NOS: n=13). All slides were annotated by senior hematopathologists and divided into training/test sets (80/20 split). The test set for five-class subtyping comprised 264 WSIs: ET (n=72), PV (n=57), Pre-PMF (n=50), Fibrotic PMF (n=82), and MPN, NOS (n=3). Feature extraction was performed using PanoPath (developed by Guangzhou Huayin Medical Laboratory Center Ltd.), a multimodal

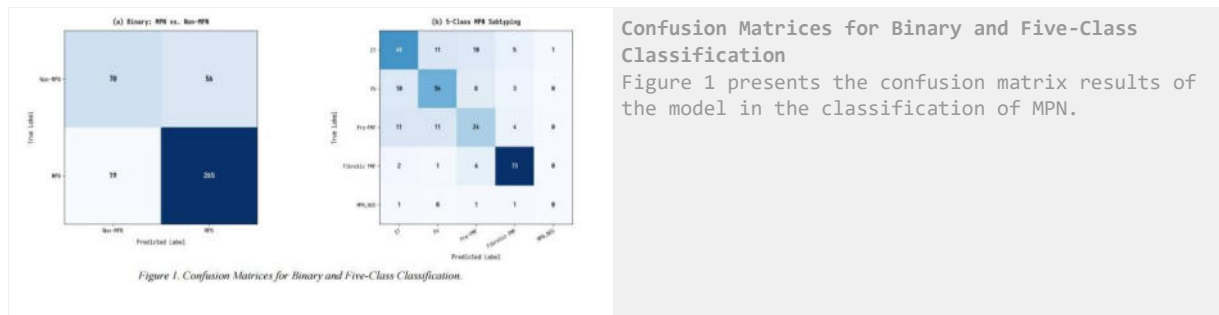
pathology foundation model pre-trained on >3M WSIs. An attention-based multiple instance learning (MIL) framework was applied for slide-level feature aggregation.

Results

Binary classification achieved 81.7% accuracy (weighted F1=0.81). For MPN, sensitivity was 93% and precision 83%; for non-MPN, precision was 79% and recall 56% (Figure 1, Table 1). In five-class subtyping, overall accuracy reached 67.4% (weighted F1=0.67; macro F1=0.52) (Figure 1, Table 1). Fibrotic PMF achieved the highest F1 (0.87), followed by ET (0.64) and PV (0.62). Pre-PMF showed moderate performance (F1: 0.48), reflecting morphological overlap with ET and PV. Because MPN, NOS had no predicted positives (F1 = 0), we also report macro F1 excluding this class, which increases from 0.52 to 0.65. The notable gap between weighted F1 (0.67) and macro F1 (0.52) reflects class imbalance.

Conclusion

The PanoPath-based MIL framework demonstrates robust screening performance and encouraging subtype classification results, particularly achieving high diagnostic accuracy for Fibrotic PMF (F1=0.87). Remaining challenges in differentiating morphologically similar subtypes (e.g., Pre-PMF vs. PV) support future work with larger datasets and multimodal fusion of histologic, clinical, and molecular data.



(a) Binary Classification (MPN vs. Non-MPN)
This matrix compares the classification performance between true labels and predicted labels for the MPN and Non-MPN classes. The results show that MPN samples achieve high classification accuracy.
(b) Five-Class MPN Subtyping
This matrix displays the classification results for five MPN subtypes (ET, PV, Pre-PMF, Fibrotic PMF, and MPN, NOS). Among these, Fibrotic PMF shows the best recognition performance.

Table 1. Performance Metrics for Binary and Five-Class Classification			
Subtype	Precision	Recall	F1-Score
Binary classification Performance Metrics			
Non-MPN	0.79	0.56	0.65
MPN	0.83	0.93	0.88
Five-Class MPN Subtyping Performance Metrics			
ET	0.65	0.62	0.64
PV	0.61	0.63	0.62
Pre-PMF	0.49	0.48	0.48
Fibrotic PMF	0.85	0.89	0.87
MPN, NOS	0.00	0.00	0.00

Performance Metrics for Binary and Five-Class Classification

Table 1 summarizes the precision, recall, and F1-score for each class across different classification tasks. Binary Classification: The MPN class achieves a precision of 0.83, a recall of 0.93, and an F1-score of 0.88. Five-Class MPN Subtyping: ET, PV, and Fibrotic PMF demonstrate moderate to good performance, with F1-scores of 0.64, 0.62, and 0.87, respectively. Pre-PMF shows weaker performance with an F1-score of 0.48. Due to the very limited amount of training data for MPN, NOS, the model fails to recognize this subtype.

Digital image analysis-guided quantification of bone marrow-infiltrating plasma cells in multiple myeloma

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Background

The quantification of clonal plasma cells in the bone marrow either by cytology or immunohistochemistry on bone marrow (BM) trephine biopsies is crucial for separation of multiple myeloma (MM) from its precursor lesion MGUS and therefore critical for therapy decisions. Furthermore, different spatial arrangements in the trephine biopsy, including interstitial clusters, nodules or diffuse sheets are relevant for diagnosis. Quantification of plasma cells in cytology or immunohistochemical stains depends on both the method of enumeration and representativity of the material. In this study, we aimed to compare digital image analysis (DIA) for plasma cell quantification with conventional visual assessment.

Principles/Methodology

BM trephine biopsies (n = 125) of patients diagnosed with MM were stained against CD138 or MUM1 and whole slide images (WSI) were created. A stepwise analysis was performed, including the visual estimation of infiltrating plasma cells both in the entire WSI and randomly selected areas of the BM section. Additionally, a DIA-guided quantification in the WSI and in the randomly selected areas was performed using QuPath. The results were also correlated with the corresponding plasma cell counts in bone marrow aspirate smears.

Results

Both stains were suitable for plasma cell enumeration by DIA. The visually assessed percentage of marrow-infiltrating plasma cells (whole biopsy) ranged from 1% to 97% compared to 0,06% to 91,89% assessed by DIA. Visual estimates were frequently higher than the percentages revealed by DIA, with discrepancies of up to 40% in single cases. Segmentation of the biopsy in random areas allowed to assess the influence of heterogenous distribution on plasma cell counts with both methods and an estimation of the necessary biopsy size for reliable plasma cell counts depending on the amount and distribution of the infiltrate.

Conclusion

Despite its high clinical relevance, quantification of BM-infiltrating plasma cells in MM and MGUS is not well standardized. With the spread of digital pathology, DIA for precise plasma cell quantification both in cytology and histology is a very promising tool, but requires further refinement in order to integrate heterogeneous distribution patterns into the diagnostic algorithm.

A clinical-pathological characterization of erythroid sarcoma.

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Background

Erythroid sarcoma is a rare subtype of myeloid sarcoma presenting with extramedullary mass-forming lesions whose pathological cells express erythroid markers. Both pediatric and adult patients are described. However, it is unclear whether such age groups differ, and how often ES is associated with other hematological malignancies (AHN). Due to its rarity and poor prognosis, ES poses major diagnostic and therapeutic challenges.

Principles/Methodology

We investigated the complete clinical, histopathological, and genetic profile of six ES cases, and systematically reviewed the international literature. We also performed statistical correlations to identify relevant features.

Results

Our study comprised 76 ES patients (70 cases from the literature), including 22 children and 54 adults. Female and male were the prevalent gender in adults and pediatric case respectively ($p=0.0438$). More frequently affected tissues/organs include CNS/orbit, and soft tissues in pediatric cases and lymph nodes and bone in adults ($p=0.0021$). Moreover, while all pediatric patients arose de novo, around 75% of adult cases were AHN ($p>0.0001$).

Histopathologically, ES showed a diffuse proliferation of large atypical epithelioid cells, with mild pleomorphism. Cell nuclei were large, with prominent nucleoli, and the cytoplasm was eosinophilic and occasionally vacuolated. The neoplastic cells expressed in all cases at least two erythroid markers, including CD71, E-cadherin, CD117, and Glycophorin A. CD34, MPO, and "histiocytic" markers, were rarely expressed. The phenotype slightly differed in pediatric and adults. Moreover, only adult patients expressed p53 in both primary and AHN cases.

At molecular analysis, the most frequent alterations involved TP53, JAK2 genes in adults ($p<0.0498$), and NF1A in children ($p<0.0001$). Nevertheless, in both cohorts a complex karyotype was often present ($>52\%$ of cases).

Survival was abysmal, with a median survival of 3,9 months. This was not different when comparing pediatric and adult cohorts. However, a significant advantage was observed in adult cases with primary vs AHN ES ($p=0.005$).

Conclusion

Overall, our data demonstrate that ES is a clinically and genetically heterogeneous disorder, with common histological findings. Highlighting the peculiarities of different subsets of patients, and especially primary vs AHN and pediatric vs adult cases, may help find possible tailored treatment strategies, improving the management of such a rare and aggressive neoplasm.

Artificial intelligence-driven analysis of CD34-stained bone marrow trephines reveals limitations of conventional blast assessment and identifies prognostic histological motifs in a multicentric cohort of myeloid neoplasms.

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Background

CD34+ blast quantitation in bone marrow trephines (BMTs) is relevant across myeloid neoplasms, particularly myelodysplastic neoplasms (MDS). Conventional assessment relies on subjective estimation and may affect disease classification. We developed an AI-driven framework integrating supervised blast quantification with unsupervised motif discovery and spatial mapping of CD34-stained BMTs, identifying histological patterns associated with disease category, MDS subtype, mutational profile, and prognosis. We validated this framework in an international multicentric cohort.

Principles/Methodology

The training set included 128 CD34-stained whole-slide images (WSIs) (38 MPN, 30 MDS, 10 AML, 50 controls) from Oxford University Hospital. Tissue-rich regions were annotated and tiled into 512×512-pixel patches (20× magnification). Two pathologists independently estimated blast percentages. A YOLOv7 model was trained for blast detection and quantification (absolute counts and blast area). For unsupervised analysis, we applied a UNI foundation model (>100,000 pretrained WSIs) followed by k-means clustering (k=50) to define histological motifs. Associations between motif enrichment, disease subtype, mutational status, and IPSS-R were assessed. External validation included 116 MDS and AML cases from two international centers.

Results

In training and validation cohorts, AI blast quantification showed concordance with bone marrow aspirate and flow cytometry, while pathological evaluation overestimated blast burden relative to AI models. This discrepancy decreased when blast area rather than absolute blast count was considered, supporting blast area as a relevant morphometric parameter in BMT evaluation. Unsupervised analysis identified recurrent motifs capturing biologically coherent spatial patterns across myeloid neoplasms. Motif enrichment profiles correlated with disease category, MDS subtype, mutational status, and IPSS-R stratification. Patient-level motif signatures predicted blast burden ($r \approx 0.6$). High-risk motifs were enriched in MDS cases with adverse prognostic features and accelerated leukemic evolution.

Conclusion

By integrating supervised blast quantification with unsupervised motif discovery, we propose a framework capturing bone marrow spatial heterogeneity and correlating with disease phenotype, prognosis, and leukemic evolution independently of conventional blast assessment. These findings support AI-derived representations of bone marrow biology as clinically relevant tools with prognostic value.

MegaQuant : Segment-Anything Model (SAM)-Assisted Segmentation Accelerates Annotations without Compromising Precision – A Case Study for Bone Marrow Megakaryocytes

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Background

Quantitative digital pathology relies on accurate cell segmentation and high-quality annotated datasets. Building on our MarrowQuant 2.0 workflow for quantitative bone marrow assessment, we evaluated whether AI-based image analysis could accelerate annotation tasks. Megakaryocytes in hematoxylin-eosin (H&E)-stained bone marrow trephine biopsies are diagnostically important but difficult to delineate due to their large size and morphological variability. Standard tools such as Cellpose fail to reliably identify megakaryocytes without extensive retraining. We therefore assessed whether the Segment Anything Model (SAM) could improve annotation efficiency while maintaining segmentation accuracy.

Principles/Methodology

Thirty-seven anonymized H&E-stained bone marrow biopsies were selected, and 21 image tiles (~700 megakaryocytes) were analyzed. A multi-annotator study included one pathologist (ground truth), four expert annotators, and three non-experts. Participants performed three annotation modes: manual, SAM-only, and SAM with manual correction (SAM-corrected). Annotation time, detected cells, and segmentation quality were evaluated using object-wise Intersection over Union (IoU) and true positive detection rates.

Results

SAM significantly reduced annotation time across all expertise levels while preserving high segmentation quality. For experts, mean annotation time decreased from 20.0 s/object manually to 9.7 s with SAM and 12.6 s with SAM-corrected annotation, representing over 50% time savings. Non-experts showed the greatest improvement (28.9 s vs 12.0 s), while the pathologist also benefited (10.1 s vs 8.6 s). Segmentation accuracy remained high, with an IoU of 0.990 compared with manual reference annotations. Total annotated area and object counts were consistent across methods, indicating no systematic bias. SAM performed best in images with well-defined cell boundaries. Optimized SAM segmentation also improved Cellpose performance, enabling intra-megakaryocyte nuclei detection, morphological feature extraction, and spatial analysis within the bone marrow microenvironment.

Conclusion

AI-assisted annotation using SAM substantially accelerates megakaryocyte segmentation in bone marrow histopathology without compromising accuracy. By extending the MarrowQuant 2.0 framework, this open-source workflow supports faster and more standardized generation of annotated datasets, facilitating broader adoption of AI-assisted quantitative pathology.

Automated Spatial Analysis of Immunohistochemistry Images of the Bone Marrow as an Economically Scalable Alternative to Spatial Omic Technologies

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Background

Recent developments in spatial (multi-)omic technologies offer novel biological insights from whole tissue sections. However, the cost and complexity of these technologies is currently prohibitive, precluding their use in larger cohorts or in diagnostic pathways. Automated spatial analysis of immunohistochemistry (IHC) sections offers an economically scalable method for deriving selected spatial information from routine diagnostic material. This work demonstrates such approach on bone marrow trephine samples of myeloproliferative neoplasms (MPN), comparing spatial insights from IHC and spatial transcriptomics (ST).

Principles/Methodology

Routine diagnostic whole slide images of selected IHC markers (CD3, CD20, CD79A, RET40F) were obtained for 23 bone marrow samples, spanning normal (n=4), non-fibrotic (ET/PV/prePMF, n=11) and fibrotic MPNs (n=8). The same samples underwent ST using 10x Xenium platform with 377 pan-cancer gene panel. The ST data was used to assign types to individual cells; the analysis has focused on subset of these to explore the spatial relationship between erythroids and lymphocytes. Each individual IHC image underwent automated detection of positive cells, using QuPath software for lymphocyte markers, and a bespoke computer vision algorithm to detect nucleated erythroid precursors on RET40F. To derive multi-marker spatial insights from IHC images, image registration was performed, using deeperhistreg, to bring the detections into a shared coordinate frame of RET40F for spatial analysis.

Results

The positive cell detection counts between IHC and their corresponding cell types on ST showed good level of agreement (Pearson $r=0.69-0.87$, all $p<0.05$), highlighting robustness of the IHC detections. The spatial relationships were explored using a range of spatial statistical methods, including Wasserstein distance, average nearest neighbour, Ripley's K and radial proportions, applied to both the ST and IHC cell locations, across cell type/marker pairs. There was agreement in spatial findings between ST and IHC. There was a degree of lymphocyte-erythroid adjacency seen in normal and non-fibrotic MPN samples, which was lost in myelofibrosis. This effect was most pronounced between CD3 and RET40F.

Conclusion

This work demonstrates a proof-of-concept for using routine IHC images to provide antigen-based validation of inferences derived from ST-based approaches, with the additional benefit of scalability into large cohorts of archival diagnostic material.

Quantification of key diagnostic bone marrow cells using automated digital cytomorphology

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Background

Correct quantification of bone marrow (BM) cells is crucial for diagnosis. The quantification of key diagnostic cells (blasts, monocytes, lymphocytes, and plasma cells) is possible through cytology, flow cytometry, histology, and immunohistochemistry. Automated digital morphology (ADM) using artificial intelligence is an innovative approach in this field.

Principles/Methodology

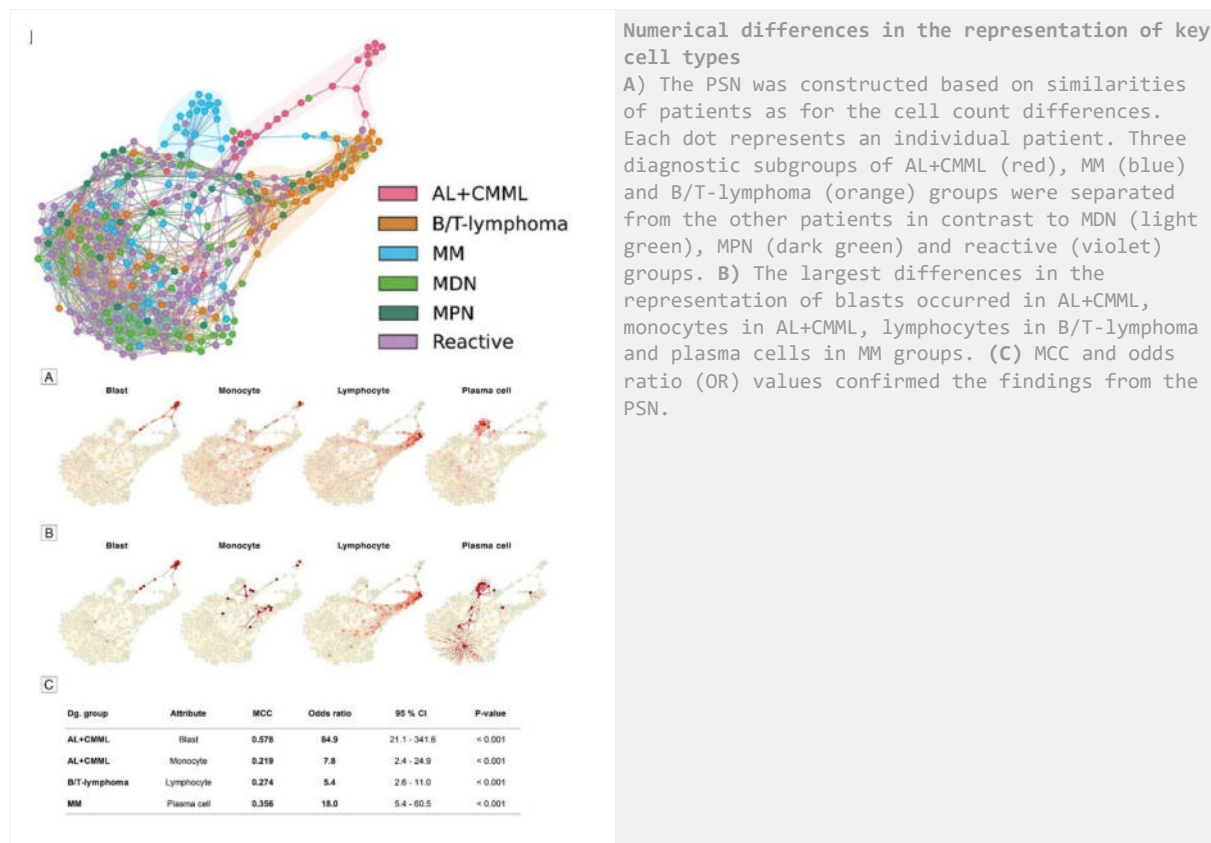
In 328 diagnostic BM smears, the quantification of BM cells using ADM (Morpho system) was compared with expert optical microscopy in six diagnostic groups: myelodysplastic neoplasms (MDN, 15%), multiple myeloma (MM, 14%), mature B/T-cell neoplasms (13%), acute leukaemia (AL) and chronic myelomonocytic leukaemia (CMML, 9%), myeloproliferative neoplasms (MPN, 8%), and reactive haemopoiesis (41%). Based on numerical myelograms, the differences were quantified for all cell types. 10 out of 25 cell attributes were selected for constructing the patient similarity network. Numerical differences in the representation of key diagnostically relevant cell types (see above) were displayed in the network. The Matthews correlation coefficient and odds ratio values were calculated.

Results

Based on the similarity, three separate subgroups of three diagnostic groups were identified: AL+CMML, B/T-lymphoma, and MM (Fig. 1). The largest differences in blast counts occurred in AL+CMML, monocyte counts in AL+CMML, lymphocyte counts in B/T-lymphoma, and plasma cell counts in MM groups (Fig. 1A, 1B). The Matthews correlation coefficient and odds ratio calculated for the key attributes in the diagnostic groups (AL+CMML, B/T-lymphoma, and MM) confirmed the findings from the network (Fig. 1C). The major reason for these differences is the automatic selection of the adaptive area for 100× immersion lens analysis by ADM, which is more representative and reliable than region selection in conventional light microscopy. ADM reduces the variability associated with user bias in region and cell identification by analysing a significantly larger number of slide areas and cells.

Conclusion

The largest numerical differences in the representation of blasts in AL+CMML, monocytes in AL+CMML, lymphocytes in B/T-lymphoma, and plasma cells in MM groups raise a major question regarding the accuracy of quantifying key diagnostic cells as well as a provocative question about the absolute correctness of expert opinion. The method used in this study has enormous potential to drive diagnostic transformation by reducing subjectivity and variability in assessments.



EA4HP26-ABS-561

Blind Diagnostician Language: A multimodal system for structured analysis of medical image data with application in haematological cytomorphology

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Background

Within modern diagnostics with large amounts of image data, the technical feasibility of automation, digitisation and the use of AI to capture diagnostically relevant features refine expert judgement. We present a multimodal system, termed *Blind Diagnostician Language* (BDL), which transforms visual data into structured, comprehensible textual descriptions, processed using language models.

Principles/Methodology

BDL integrates two AI models that perform specialised tasks using dedicated agents. A vision-language model (VLM) performed a semantic analysis of image data and converted it into a textual representation. A large language model (LLM) was used for subsequent logical deduction, summarisation and output generation. The VLM generated a structured image description according to a predefined template, which was then processed by the LLM into a concise narrative description. For querying, a retrieval-augmented generation (RAG) approach was employed. The entire workflow was controlled by orchestrated VLM/LLM-based agents that determined the sequence of steps, selected

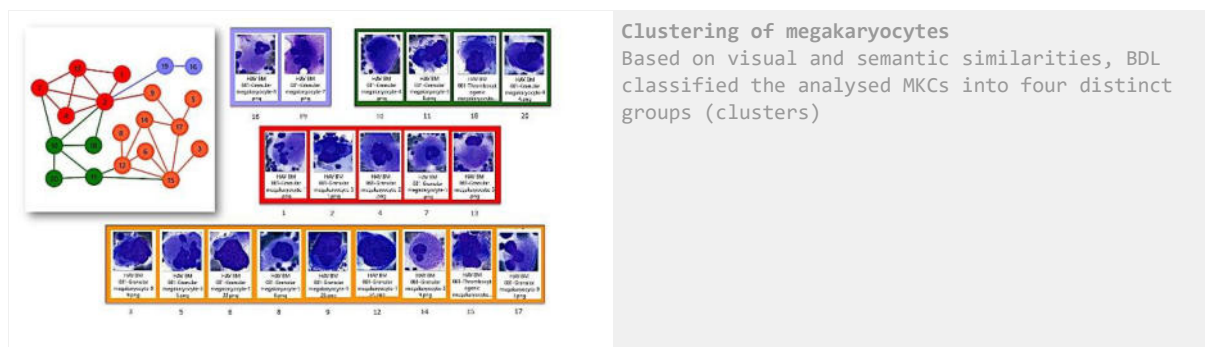
appropriate tools and interpreted results according to predefined goals. BDL was initially applied to the analysis of digital images of megakaryocytes (MKCs). In a bone marrow smear with reactive haemopoiesis, a digital record of all MKCs was obtained using Morphogo equipment. Twenty representative, high-quality MKC images were selected for analysis, and a precise semantic image-labelling template was defined. The objective of BDL was to classify MKCs into groups based on combined visual and semantic similarities.

Results

Based on visual and semantic similarities, BDL classified the analysed MKCs into four distinct groups (clusters) (Fig. 1). This approach demonstrates potential applications in haematological cytomorphology, including refinement of differential counts and morphological characterisation of cell populations, improved classification of indeterminate cells, evaluation of dysplasia, identification of cases with similar morphology and classification of myeloproliferative neoplasms.

Conclusion

BDL represents a multimodal system that integrates visual and linguistic models into a unified diagnostic framework. The conversion of image data into structured textual representations enables advanced search for similar morphological findings and contributes to the objectification and improvement of diagnostic processes in haematology, significantly enhancing decision-making in haematological morphology.



EA4HP26-ABS-564

A multi-omic comparison of histiocytic sarcoma and its mesenchymal mimickers.

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Background

The differential diagnosis between histiocytic sarcoma (HS) and undifferentiated pleomorphic sarcoma (UPS) is difficult, but bear relevant clinical consequences. Both are cytologically high-grade neoplasms involving soft tissue, skin, and gastrointestinal tract (among others) of adult patients. Of importance, their clinical-pathological overlap may be underrecognized, partly due to the subspecialization of surgical pathology practices. Still, UPS is managed according to international guidelines, while no standardized treatment approach currently exists for HS. Comparative data on their natural history, treatment, and prognosis remain limited.

Principles/Methodology

We comprehensively compared the clinical-pathological and genetic features of 17 patient diagnosed with HS, and 4 with UPS, through immunophenotyping, high-throughput sequencing (HTS), gene expression profiling (GEP), and methylome analysis. Malignant melanoma with loss of markers was carefully excluded.

Results

Our study demonstrates that among high-grade hematolymphoid neoplasms with and non-blastoid morphology, HS and UPS show a substantial morphophenotypic and methylome overlap. Nevertheless, we also identified distinct features within each group. We further highlight the specificity (even though not universal) of "histiocytic markers" (CD4, CD163, and PU.1) for HS. Genetically, HS displayed mutations in the MAPK-pathway, CDKN2A, TP53 and "myeloid" genes, with lower mutation burden, and a genetic signature at GEP of mononuclear phagocytes. At methylome analysis, HS cases displayed heterogeneous profiles, consistent with lymphoid, melanocytic, myeloid or histiocytic lineage. UPS otherwise showed a higher tumor mutational burden with heterogeneous molecular landscape specially enriched in gene amplification and alteration of RTK signaling pathway. GEP evidenced activation of Hippo and PI3K-Akt signaling pathway.

Conclusion

Our study demonstrate that the diagnostic difficulty in differentiate HS from its mesenchymal mimickers is partially reflected by the results of the single analyses we employed. Nevertheless, integration of multiomic clinical and histopathological data allows to confirm the identity of HS as "malignant histiocytosis", characterized by atypical proliferations of cells with histiocytic phenotype, recurrent alterations in MAPK pathway, cell cycle regulation, apoptosis, and DNA repair, and retention of a gene expression signature of mononuclear phagocytes.

EA4HP26-ABS-565

The hematological Proteus: intralesional morphophenotypical heterogeneity and diagnostic mimickers in extramedullary chronic myelomonocytic leukemia.

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Background

Chronic myelomonocytic leukemia (CMML) is a MDS/MPN presenting with persistent clonal monocytosis, and hematopoietic dysplasia. Around 30% of patients present with extramedullary involvement (eCMML), most frequently targeting the spleen, skin, lymph nodes and soft tissues. Such occurrence negatively affects patients prognosis and has been recently linked to underlying MAPK pathways genes mutation. Of importance, eCMML may represent the first sign of the disease, and may therefore represent a relevant challenge for histopathologists.

Principles/Methodology

We aim to investigate the morphological and phenotypical heterogeneity of a series of eCMML, putting emphasis on those cases meeting the criteria for other WHO-defined entities. For each patient, a complete characterization was performed on both the eCMML and paired bone marrow samples.

Results

We collected 10 cases of eCMML patients including 8 males, with involvement of skin, lymph node GI tract, and/or spleen. Most patients had MD-CMML. Three patients evolve toward AML. Four died of disease. In 5 cases the eCMML was the first manifestation. Most frequent molecular drivers were TET2 and SRSF2 and MAPK pathway genes. A common clonality was demonstrated in the eCMML and BM samples. Morphologically eCMML was extremely heterogeneous, ranging from blastoid infiltrated to very mature ones resembling Rosai-Dorfman disease.

Moreover in some eCMML infiltrates it was possible to identify morphologically and phenotypically different populations including blasts (CD34+/MPO+), monocytes (CD14+/CD163+/MPO-), myelomonocytic cells (CD14+/CD163+/MPO+) indeterminate dendritic cells (IDC, CD1a+/CD14+/CD163-/CD207-) and/or mature pDCs (CD4+/CD123+/CD303+/CD56-). A diagnosis of IDC histiocytosis and Rosai-Dorfman disease was possible in 4 and 1 cases respectively. Half of the eCMML infiltrates expressed CD56 in at least a fraction of the pathological cells.

Conclusion

eCMML may be the first manifestation of CMML and negatively impact patients prognosis. It is often driven by MAPK pathway genes mutation in association with other. Despite difficult, its prompt recognition by the pathologist allow to modulate patients' treatment and ameliorate their prognosis. eCMML needs to be differentiated from primary histiocytoses and reactive conditions involving targeted organs. In this setting, several morphological (e.g. cell distribution and morphology) and phenotypical features (e.g. CD56 expression), are helpful to rule out mimickers.

EA4HP26-ABS-571

Philadelphia chromosome-positive acute leukemia with *BCR::ABL1*-positive cells significantly exceeding blasts may represent de novo blast phase of chronic myeloid leukemia with a silenced chronic phase

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Background

Blast phase chronic myeloid leukemia (BP-CML) without chronic phase (CP) has been reported. Accurate diagnosis is critical, as therapy for BP-CML differs from *BCR/ABL1*-positive (Ph+) acute leukemia.

Principles/Methodology

We retrospectively analyzed 30 cases of acute leukemia with features suggestive of de novo BP-CML (lymphoid in 18, myeloid in 12), compared with 20 cases of Ph+ B-lymphoblastic leukemia (B-ALL).

Results

The median patient age was 46.5 years (range, 11–85). Circulating blasts ranged from 1–88% (median, 12%), compared with a median of 70% (range, 3–94.4%) in controls ($P<0.001$). Marrow blasts ranged from 5–90% (median, 40%) in BP-CML versus a median of 88% (range, 22.5–98%) in controls ($P<0.001$). Among 29 cases with karyotyping, 17 (58.6%) demonstrated abnormalities in addition to *BCR/ABL1*. Major-route abnormalities were identified in 11 cases (37.9%), compared with 1 case (5.3%) in controls ($P=0.037$). Cells harboring *BCR/ABL1* ranged from 51–100% (median, 94%) and exceeded blast percentages in all but one case. Ratio of *BCR/ABL1*-positive cells to blasts ranged from 0.84–19.4 (median, 2.43), significantly higher than controls (range, 0.25–1.24; median, 0.967) ($P=0.006$ for BP vs control and $P=0.005$ for lymphoid BP vs control). Twenty-six cases (89.7%) expressed p210 fusion transcripts, while three predominantly expressed p190 products. Among 20 cases evaluated by next-generation sequencing, 17 harbored pathogenic mutations. *TP53* mutations were present in 45.5% of myeloid BP cases but in none of the lymphoid BP cases. Following therapy, 7 cases (24.1%) demonstrated reversion to CP, characterized by persistently high *BCR/ABL1*-positive cells with low blast counts ($P=0.049$ vs controls; $P=0.041$, lymphoid BP vs control). Median follow-up was 15.5 months (range, 2–162), during which 16 patients died. Median survival was 21 months (95% CI, 13–112), with significantly shorter survival in myeloid BP (13 months) compared

with lymphoid BP (69 months; $P=0.03$). Of note, survival in lymphoid BP was significantly worse than controls ($P=0.047$) (Figure 1).

Conclusion

BCR/ABL1-positive cells discordantly more than blasts in *Ph+* acute leukemia suggest multilineage involvement and may be used as a major criteria in AI based algorithms for diagnosis of BP-CML (*BCR/ABL1*:blast ratio ≥ 1.07 ; accuracy, 91.3%; sensitivity, 96.6%; specificity, 82.4%). *de novo* myeloid BP-CML is associated with significantly worse outcomes than lymphoid BP-CML, while lymphoid BP-CML shows inferior survival compared with *Ph+* ALL.



Figure 1

EA4HP26-ABS-584

Integrin expression profiling identifies a subgroup of acute myeloid leukemia with distinct transcriptional programs

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Background

Acute myeloid leukemia (AML) is an aggressive and heterogeneous malignancy with persistently poor outcomes. Integrins are transmembrane adhesion receptors involved in cell adhesion, migration, survival and interactions with the microenvironment and are increasingly recognized as important regulators of AML biology. We investigated integrin expression patterns in AML blasts and related these to blast differentiation state, clinical features and transcriptional profiles.

Principles/Methodology

Based on initial flow cytometric screening using a broad integrin antibody panel, seven integrins ($\alpha 6$, $\alpha 7$, αM , $\alpha 9\beta 1$, $\beta 3$, $\beta 5$ and $\beta 7$) showing heterogeneous expression on peripheral blood leukemic blasts were selected for further analysis in a cohort of 46 non-APL AML patients. Integrin expression was analyzed across CD34/CD38-defined blast subpopulations. RNA sequencing was performed in the same cohort, followed by differential gene expression and Gene Ontology (GO) enrichment analyses.

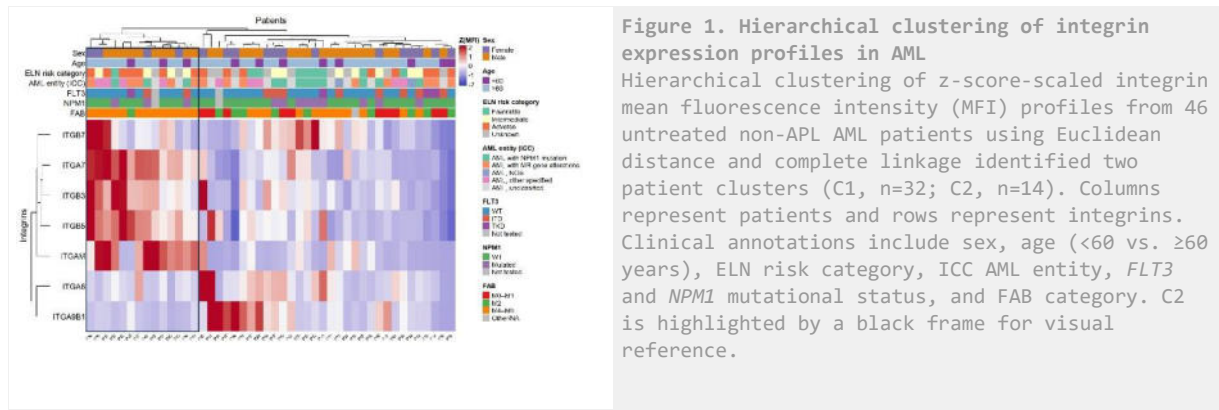
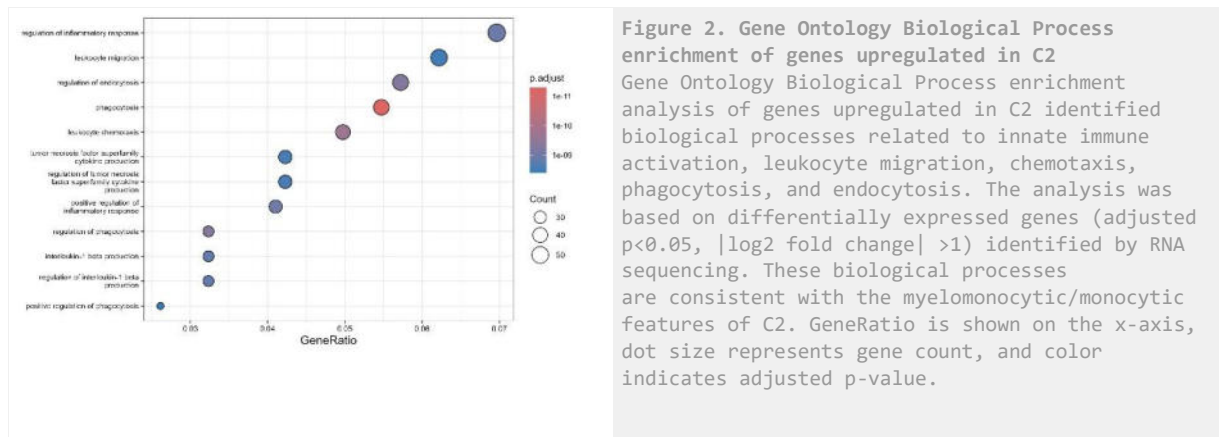
Results

Unsupervised hierarchical clustering of integrin mean fluorescence intensity (MFI) profiles identified two patient clusters: C1 ($n=32$) and C2 ($n=14$) (**Figure 1**). In univariate analyses, C2 showed higher expression of integrins $\alpha 7$, αM , $\beta 3$, and $\beta 5$ compared with C1 (adjusted $p < 0.01$) and was strongly associated with FAB category M4/M5 and the CD34⁺/CD38⁺ phenotype. Differences in integrin MFI between C2 and C1 and enrichment of the CD34⁺/CD38⁺

phenotype remained significant after adjustment for FAB M4/M5 status. Integrin differences also persisted after adjustment for the percentage of CD38-positive blasts. C1 showed more variable integrin expression and enrichment of CD34-positive blast populations. Transcriptomic analysis identified 2,036 differentially expressed genes between C2 and C1 (adjusted $p < 0.05$, $|\log_2 \text{ fold change}| > 1$). Upregulated genes in C2 included *MDM2*, *ZBTB7A*, and *IFNGR2*. GO enrichment analysis showed overrepresentation of pathways related to innate immune responses, leukocyte migration and chemotaxis, and phagocytosis in C2 (**Figure 2**). These transcriptional programs were preserved in analyses restricted to FAB M4/M5 AML.

Conclusion

Integrin profiling identified an AML cluster characterized by myelomonocytic/monocytic differentiation, low CD34 expression and enrichment of transcriptional programs related to innate immune activity, phagocytosis and chemotaxis, including upregulation of *MDM2*. Integrin profiling may refine biological stratification of AML and identify potential therapeutic targets.



Characterising the topology of the human bone marrow in MPN using AI-augmented spatial transcriptomics

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Background

The human bone marrow (BM) is the main site of haematopoiesis in adult life. Our understanding of the pathogenesis of myeloid malignancies including myeloproliferative neoplasms (MPNs) is limited by our lack of microenvironmental spatial contextualisation. We recognise the potential offered by emerging spatial transcriptomic (ST) approaches, which together with AI-based feature detection, can provide comprehensive topological descriptions of the human BM in health and in MPN.

Principles/Methodology

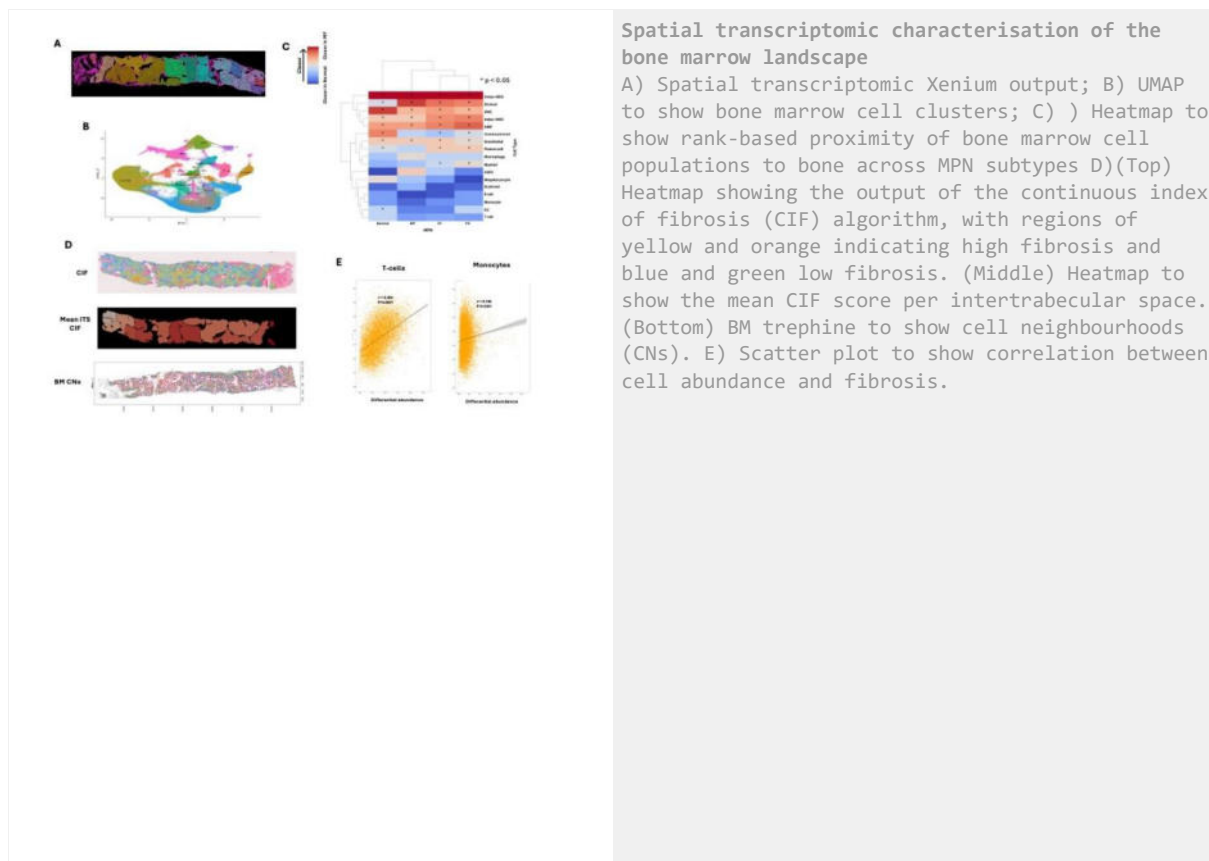
We present a comprehensive augmented spatial transcriptomics framework, integrating AI-based histological feature detection, ST-based spatial profiling and machine learning (ML)–based microenvironmental analysis. Applied to 30 human BM samples (>5 million cells), we use this framework to provide the most comprehensive topological characterisation of the human BM to date and provide novel insights into microenvironmental features of perturbation in MPN.

Results

We identify BM cell neighbourhoods defined by distinct patterns of cellular enrichment, some of which represent novel spatial trajectories of haematopoiesis and patterns of haematopoietic support. We then use our AI-augmented ST framework to capture microenvironmental perturbation in MPN. We show that myelofibrosis (MF), the most aggressive variant of MPN characterised by widespread BM fibrotic deposition, is defined by expansion of a small number of large cell neighbourhoods suggestive of clonal expansion. We then integrate ST data with AI-based fibrosis detection, finding that regions of BM fibrosis are characterised by stromal depletion and immune enrichment - signatures with potential diagnostic and therapeutic relevance. We also identify BM spatial signatures (or 'spatiotypes') that provide new microenvironmental definitions MPN subtypes. Finally, we present a novel machine-learning (ML) based approach to generate a quantitative metric of microenvironmental perturbation in MPN and revealing a previously unrecognised degree of intra- and inter-sample heterogeneity.

Conclusion

Our approach yields the most comprehensive topological characterisation of the human BM to date and provides a new paradigm for describing ecological remodelling in BM-based blood cancers. Our findings extend our understanding of bone marrow topology in health and in MPN.



EA4HP26-ABS-598

Remodelling of the Bone Marrow Niche during Treatment of acute T-Lymphoblastic Leukaemia: A longitudinal Multi-Modal Analysis

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Background

The bone marrow (BM) niche plays a pivotal role in the pathogenesis, therapy response, and relapse of acute leukaemia. Its dynamic remodelling over the treatment course, including allogeneic haematopoietic stem cell transplantation (allo-HSCT), remains poorly characterised. To comprehensively characterise the remodelling at the spatial and single-cell level, we present a longitudinal, multi-modal study of BM niche remodelling in patients with acute leukemia.

Principles/Methodology

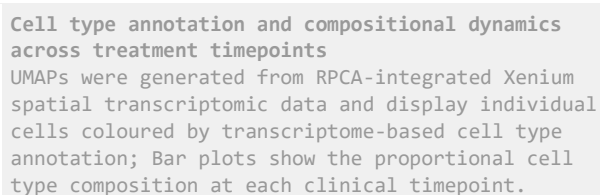
The cohort comprises four patients (P 1-4) with acute T-lymphoblastic leukemia. BM biopsies were obtained at diagnosis, prior to allo-HSCT, and at serial timepoints post-allo-HSCT. Leukaemia subtyping was performed by integration of flow cytometry, immunohistochemistry, and spatial transcriptomics (Xenium 5100 gene panel). Single-cell RNA sequencing, probe-based mutational analysis and multiplex protein imaging are currently being performed (pending).

P 1 (male, 39 years, pre-T-ALL/cortical T-ALL) remained MRD-positive pre-allo-HSCT and morphologically relapsed at 2.3 months post-allo-HSCT. P 2 (male, 23 years, cortical T-ALL) was likewise MRD-positive pre-allo-HSCT and relapsed as T-lymphoblastic lymphoma with concurrent complete remission (CR) in the BM. P 3 (male, 56 years, near-ETP-ALL) is currently receiving chemotherapy (GRAAL14), reducing blasts from 95% to 0.6%. P 4 (female, 9 years, ETP-ALL) achieved CR pre allo-HSCT, and has remained in CR for over two years. Xenium profiling of P 1 and P 2 yielded overall 679,156 cells after quality filtering. The majority of cells were annotated transcriptomically. Spatial data successfully replicated the clinical course of both patients, capturing blast reduction from diagnosis through treatment and relapse in P 1. Xenium alone was unable to detect blasts at very low disease burden (1.3 and 2.5 months post-transplant in P 2), where flow cytometry identified 0.1% residual blasts or assessment was not possible. T-cells from very low burden biopsies as well as unresolved cells will be reannotated once all pending data are available.

This study establishes the first spatial analysis of T-ALL and BM niche remodelling in this disease, integrating spatial, transcriptomic, mutational and proteomic modalities. Preliminary data demonstrate feasibility of high-resolution multi-modal tissue profiling. Final integration is expected to yield novel insights into niche–leukaemia interactions, engraftment biology, and mechanisms of therapy resistance.

Disease course, Xenium cell yield and blast content in two longitudinally profiled T-ALL patients

Two (of 4 in total) T-ALL patients are tracked across multiple timepoints – from diagnosis, through pre-allo HSCT, remission, and ultimately relapse – with Patient 1 relapsing in bone marrow and Patient 2 relapsing as cutaneous T-lymphoblastic lymphoma despite achieving CR in the bone marrow.



Development and Clinical Validation of an LLM-Based C-MICM Diagnostic Agent for Myeloid Neoplasms

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Background

Comprehensive interpretation of next-generation sequencing (NGS) reports for myeloid hematologic neoplasms relies heavily on the integrating multimodal data (clinical information, bone marrow morphology, immunology, cytogenetics, and molecular biology; C-MICM). The traditional manual diagnostic approach faces limitations such as dispersed data sources, lagging knowledge updates, and low reporting efficiency.

Principles/Methodology

We developed a large language model (LLM)-based C-MICM diagnostic agent to automate multimodal data integration and diagnosis (Fig. 1). Based on DeepSeek-R1 and RAGFlow, the agent automatically integrates C-MICM and NGS data using retrieval-augmented generation to produce WHO/ICC-compliant diagnostic opinions. We retrospectively enrolled 357 newly diagnosed patients, using independent consensus diagnoses from three hematopathologists as the gold standard. A two-level evaluation was established: Level 1 assessed binary screening performance (neoplasm vs. non-neoplasm) using a confusion matrix and Cohen's kappa. Level 2 evaluated neoplasm classification performance stratified by the completeness of expert diagnoses: Subset 1 (category only; category accuracy 1) and Subset 2 (category and type; category accuracy 2 and type accuracy). Workflow duration was compared between manual and AI-assisted workflows.

Results

Among the 357 cases, there were 263 myeloid neoplasms, 51 non-myeloid neoplasms, and 43 cases with undefined diagnosis due to insufficient evidence (excluded). For Level 1 myeloid neoplasm screening performance (N=314): sensitivity was 99.2% (95% CI, 97.3%–99.8%), specificity was 98.0% (95% CI, 89.7%–99.7%), and Cohen's kappa was 0.965 (Table 1). For Level 2 myeloid neoplasm classification, Subset 1 (n=55) achieved an overall category accuracy 1 of 90.9% (95% CI, 80.4%–96.1%). In Subset 2 (n=206), the overall category accuracy 2 was 96.6% (95% CI, 93.2%–98.3%), while the overall type accuracy was 81.6% (95% CI, 75.7%–86.3%). The highest type accuracies were observed in AML (88.9%) and MPN (91.9%). The average diagnostic workflow duration per case dropped from 35 min (manual) to 17 min (AI-assisted), improving efficiency by 51.4%.

Conclusion

The C-MICM agent demonstrated excellent screening performance and high category classification accuracy, while myeloid neoplasm subtype classification requires further refinement. The “AI preliminary diagnosis + expert review” model significantly improves diagnostic efficiency and supports precision diagnostics in hematology.

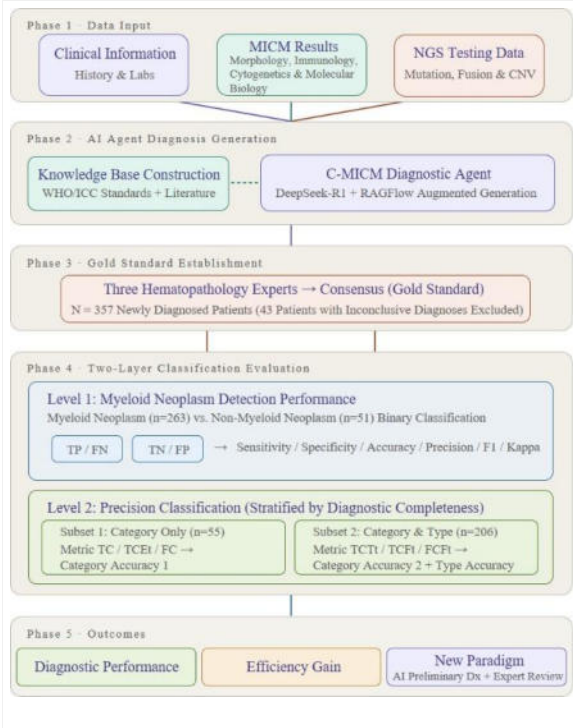


Fig. 1. Workflow for the Development and Clinical Validation of the C-MICM Diagnostic Agent

Table 1. Diagnostic Performance of the C-MICM Diagnostic Agent for Myeloid Neoplasm Detection and Precision Classification

A. Level 1: Myeloid Neoplasm Detection Performance

Diagnostic Category	N	Sensitivity % (95% CI)	Specificity % (95% CI)	Accuracy % (95% CI)	Precision % (95% CI)	F1-Score (95% CI)	Cohen's κ
Myeloid Neoplasms & Non-Myeloid Neoplasms	314 (263+51)	99.2 (97.3–99.8)	98.0 (89.7–99.7)	99.0 (97.2–99.7)	99.6 (97.9–99.9)	0.991 (0.987–1.000)	0.985

B. Level 2: Precision Classification Subset 1 (Category Only)

Diagnostic Category	TC	TCEI	FC	Total	Category Accuracy 1, % (95% CI)
AML	9	2	0	11	100 (74.1–100)
MDS	13	4	1	18	94.4 (74.3–99.0)
MPN	13	3	0	16	100 (80.6–100)
MDS/MPN	4	2	2	8	75.0 (40.9–92.9)
Others*	0	0	2	2	0 (0–65.8)
Total	39	11	5	55	90.9 (80.1–96.1)

C. Level 2: Precision Classification Subset 2 (Category and Type)

Diagnostic Category	TCTI	TCFI	FCFI	Total	Category Accuracy 2, % (95% CI)	Type Accuracy, % (95% CI)
AML	80	9	1	90	98.9 (94.0–99.8)	88.9 (80.7–93.9)
MDS	45	15	0	60	100 (94.0–100)	75.0 (62.8–84.2)
MPN	34	3	0	37	100 (90.6–100)	91.9 (78.7–97.2)
MDS/MPN	7	0	5	12	58.3 (32.0–80.7)	58.3 (32.0–80.7)
Others*	2	4	1	7	85.7 (48.7–97.4)	28.6 (8.2–64.1)
Total	168	31	7	206	96.6 (93.2–98.3)	81.6 (75.7–86.3)

95% CIs were calculated using the Wilson score method, and the F1-score was calculated using the bootstrap method.
TC: true category; TCEI: true category and evidence-exceeding type; FC: false category; TCTI: true category and true type; TCFI: true category and false type; FCFI: false category and false type.
Level 2 Subset 1 (Category only): Category Accuracy 1 = (TC + TCEI) / (TC + TCEI + FC).
Level 2 Subset 2 (Category and type): Category Accuracy 2 = (TCTI + TCFI) / (TCTI + TCFI + FCFI); Type Accuracy = TCFI / (TCTI + TCFI + FCFI).
| Confusion matrix: 261 true positives, 2 false negatives, 1 false positive, and 50 true negatives.
* Other myeloid neoplasms include: clonal hematopoiesis (CH, n=2), familial myeloid neoplasm (f-MN, n=6), mixed-phenotype acute leukemia (MPAL, n=1).

Table 1. Diagnostic Performance of the C-MICM Diagnostic Agent for Myeloid Neoplasms

EA4HP26-ABS-639

AI-Driven Detection and Quantification of CD34+ Progenitor Cells in Bone Marrow Biopsies using AIFORIA

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Background

The accurate assessment of blast cells using CD34 immunohistochemistry (IHC) in bone marrow biopsies (BMB) is essential for the diagnosis and classification of myelodysplastic syndrome (MDS) and acute myeloid leukemias (AML). CD34 IHC is also crucial for the detection of blast increase and accelerated phase in myeloproliferative neoplasms (MPN) and MDS/MPN. Guidelines for the morphologic readout of CD34+ blasts in the BM have been published, but the manual quantification remains subjective and is prone to interobserver variability. Using AIFORIA, we developed an AI strategy for the detection and quantification of CD34+ precursor cells in routine diagnostic samples.

Principles/Methodology

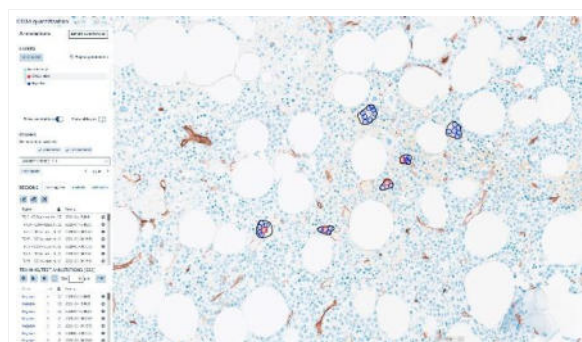
CD34-stained BMB from patients with MDS (6 MDS low blasts; 4 MDS with excess blasts), 13 AML, 5 CML, 8 classical MPN, and 3 MDS/MPN were used for the training of the AI model (AIFORIA Create Platform) based on 1085 single cell annotations (322 CD34-positive/763 CD34-negative cells; example shown in Fig.1). The automated quantification was correlated to the visual estimation of CD34+ blasts per whole slide image (WSI).

Results

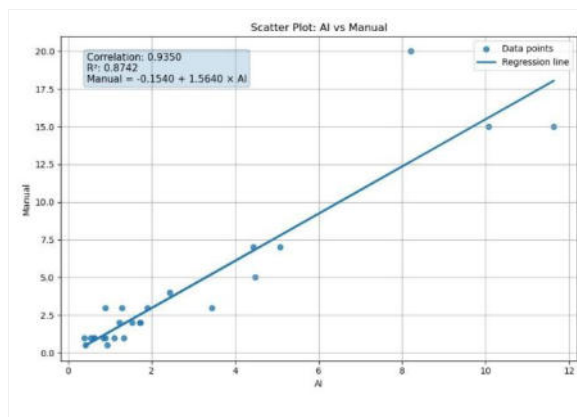
The analysis demonstrates a strong positive relationship between the AI-assisted and the manual cell counts (Pearson correlation coefficient 0.935; R^2 value 0.8742). The regression analysis indicates that the manual assessment systemically overestimates the number of CD34+ cells, particularly at higher cell counts (Fig. 2). The descriptive statistics show that the mean AI count was 2.71, whereas the mean manual count was higher at 4.08, again supporting the interpretation that manual counting tends to yield larger values. The medians were lower than the means for both variables (AI median = 1.34; Manual median = 2.00), and both distributions exhibited substantial positive skewness (AI skewness = 1.89; Manual skewness = 2.11), reflecting that most samples contained relatively low numbers of positive cells. The variability was greater in the manual counts (variance = 26.39) than in the AI counts (variance = 9.43), suggesting that the human assessments were more dispersed.

Conclusion

AI-assisted quantification of CD34+ progenitor cells in whole slide BM images enhances diagnostic precision and reproducibility in digital hematopathology. Manual assessment is prone to interobserver variability and overestimates CD34+ blast percentages with potential impact on final classification. The findings of this study will be validated in a larger cohort in a multicenter setting.



Training annotations in a bone marrow biopsy
Example of single cell annotations of CD34-positive/negative cells in smaller training regions in a bone marrow biopsy.



Correlation between the AI model and manual CD34+ count

EA4HP26-ABS-643

An AI-based system of fibrosis prediction from spatial transcriptomic data reveals bone marrow microenvironmental features associated with fibrosis in myeloproliferative neoplasia

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¹ University of Oxford, Nuffield Division of Clinical Laboratory Sciences, Oxford, UK; ² Oxford University Hospitals, Department of Cellular Pathology, Oxford, UK; ³ University of Oxford, Institute of Biomedical Engineering, Oxford, UK; ⁴ Unit of Pathology, ASST Santi Paolo e Carlo, Milan, Italy; ⁵ University of Oxford, Oxford Centre for Histopathological Research, Oxford, UK

Background

Myeloproliferative neoplasms are a group of myeloid malignancies associated with well-defined driver mutations and overproduction of blood cells. Some patients present with or develop bone marrow (BM) fibrosis, which is associated with a poor prognosis. BM microenvironmental constituents including immune populations, stromal cells and megakaryocytes are implicated in the development of fibrosis. However, our understanding of disease development is limited the lack of spatial contextualisation provided by conventional single cell RNA-seq and other molecular 'omic' approaches, and inability to integrate these approaches with conventional qualitative fibrosis detection methods.

Principles/Methodology

We have develop a model that predicts BM fibrosis using spatial transcriptomic (ST) data profiled by Xenium (377-gene panel). Using a large MPN ST cohort (n=30), each biopsy was divided into small tissue regions (512×512 pixels), and transcripts within each region were projected onto fine spatial per-gene density maps. A convolutional neural network was trained using a pairwise ranking objective to order regions according to fibrosis severity, using our previously established H&E morphology-based Continuous Indexing of Fibrosis (CIF) score as the reference standard. Model performance was evaluated using pairwise ranking accuracy and within-sample Spearman correlation. Model outputs were calibrated to the CIF scale, enabling generation of spatially resolved fibrosis maps across entire trephine biopsies (Figure 1). Genes associated with fibrosis severity and model prediction were identified.

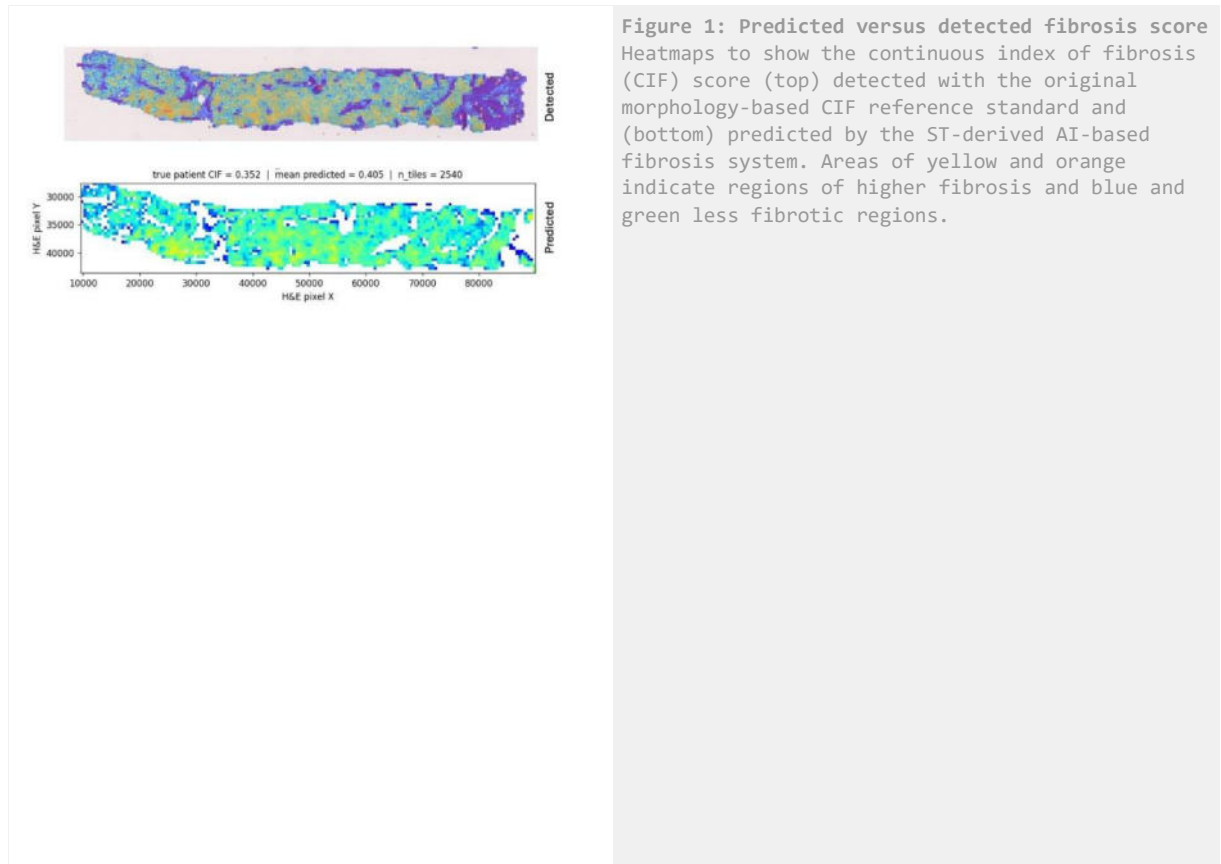
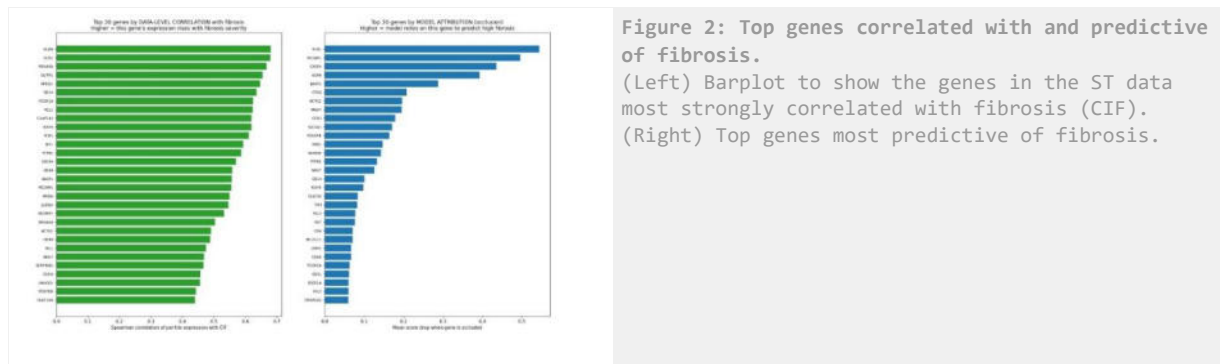
Results

The model achieved a validation pairwise ranking accuracy of 0.870 and a within-sample Spearman correlation of 0.55, demonstrating strong concordance with the morphology-based CIF reference standard. We found that

monocytes and macrophage-associated genes were most strongly correlated with fibrosis (CCR2, CD14, CD68) (Figure 2). Genes associated with supporting stromal and vascular populations including PDGFRB and PECAM1 were also prominently represented within this group. Transcripts associated with the stromal compartment had a greater model attribution capacity (CXCR4, VCAN, FBN1), as well as proliferation index marker MKI67, suggesting cell cycling activity is a discriminate feature for BM fibrosis.

Conclusion

We present a novel fibrosis prediction algorithm and demonstrate its potential for biological discovery, extending our understanding of microenvironmental features associated with fibrosis in MPN.



Genetic Landscape and Clinical Outcomes of *DDX41*-Mutated Myeloid Neoplasms: A Retrospective Single Institutional Analysis

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Background

DDX41 gene mutations are reported to be associated with myeloid neoplasms, notably acute myeloid leukemia (AML) and myelodysplastic neoplasms (MDS). Per WHO Classification it is recognized as one of "myeloid neoplasms associated with germline predisposition" as germline *DDX41* (pathologic/likely pathologic) mutations predispose to late-onset of diseases. However, large cohorts of *DDX41*-mutated myeloid neoplasms are limited. The objective of this study is to analyze the genetic characteristics and clinical outcomes of *DDX41* mutated MDS and AML patients at our quaternary referring cancer center.

Principles/Methodology

Patients harboring *DDX41* mutations between 2018-2026 from clinical charts and NGS data pool were retrospectively re-analyzed to confirm diagnoses, and correlate with their clinical outcomes.

Results

We identified 98 patients, AML (n=50) and MDS (n=48), with *DDX41* gene mutations (male-to-female ratio, 2.8, a median age of 68 years, range 31- 85 years). We observed 76 patients with presumed germline (77.6%) and 53 (54.1%) with presumed somatic mutations; 37 had both (37.8%). The most frequent germline mutation was p.M1I (20/74, 27%) followed by p.D140fs*2 (10/74, 13.5%), while the predominant somatic mutation was p.R525H (39/53, 73.6%), which frequently (29/39, 74.4%) co-occurred with germline variants. Co-mutations in other somatic variants were identified in 60% of patients (n=59), including *ASXL1* (n=12), *SRSF2* (n=12), *TP53* (n=11), *DNMT3A* (n=7), and *TET2* (n=7) and others (n=10) with 15 of 59 (25.4%) harboring ≥ 3 concurrent mutations. In our cohort a substantial proportion of patients (46/66, 69.6%) showed a Complete Response (CR) or Partial Response (PR) to initial therapies involving azacitidine with venetoclax or liposomal daunorubicin/cytarabine (Vyxeos). Overall survival (OS) analyses showed that MDS and AML patients had median OS of 74.0 months and 45.9 months; respectively (p=0.187). Patients received hematopoietic stem cell transplantation (n=44, 44.9%) showed significantly better OS (median 239.0 months vs 36.7 months; p<0.001). Patients with concurrent somatic mutations, particularly *TP53* and *DNMT3A*, had significantly worse OS (p=0.008, 0.001, 0.006, respectively).

Conclusion

In our study cohort the patients with concurrent somatic mutations had inferior OS, and the presence of *TP53* and *DNMT3A* co-mutations negatively impact OS. Transplantation would still be a better strategy to treat *DDX41* mutated myeloid neoplasms despite good responses to initial chemotherapy.

Lymphoma Symposium I | Microenvironment and B-cell lymphoma – Oral

EA4HP26-ABS-515	Interferon-gamma signaling influenced by SOCS1 and STAT6 mutations is a key feature in the microenvironment of predominantly diffuse follicular lymphoma
EA4HP26-ABS-534	CCL19 ⁺ fibroblasts define a nodal proliferative niche in chronic lymphocytic leukemia
EA4HP26-ABS-601	H&E-Derived STPath Maps Capture Immune Neighborhoods and Multiomic Context in Follicular Lymphomas and Diffuse Large B-cell Lymphomas
EA4HP26-ABS-617	Spatial immune ecosystems define CDKN2A-associated immune escape in mantle cell lymphoma
EA4HP26-ABS-625	Prognostic Significance and Spatial Characterization of Cytotoxic CD4 ⁺ T Lymphocytes in Diffuse Large B-cell Lymphoma

Interferon-gamma signaling influenced by SOCS1 and STAT6 mutations is a key feature in the microenvironment of predominantly diffuse follicular lymphoma

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Background

Follicular lymphoma with a predominantly diffuse growth pattern (dFL) lacks *BCL2* rearrangement in the majority of cases. However, the molecular landscape underlying dFL variants remains to be clarified.

Principles/Methodology

118 dFL cases (selected on the basis of more than 50 % diffuse areas) were characterized using IHC, FISH, WES, RNAseq, and clinical follow-up.

Results

The localization was inguinal in 105/118 cases (89 %). In 103/118 (87 %) cases, more than 75 % diffuse areas (supported by the absence of CD21+ FDCs) were observed. The cell population was composed of centrocytes in the vast majority of cases. Sclerosis and vascular hyperplasia were frequently observed. CD23 staining was positive to a various extent in all cases. Numerous CD3+/CD4+/PD1+ T-cells were present around vessels, whereas CD8+ and FOXP3+ T-cells were less abundant and more uniformly distributed.

FISH disclosed unrearranged *BCL2* (*BCL2UR*) in 103/117 (88%) and unrearranged *BCL6* in 93/104 (89%) cases. Among the 94 *BCL2UR* cases with evaluable WES, the most frequent pathogenic mutations targeted *CREBBP* (58%), *STAT6* (36%), *KMT2D* (33%), *TNFSFR14* (29%), *SOCS1* and *FOXO1* (20% each) (Fig 1). The copy number variation profile was characterized by frequent 16p13.3 losses (21/94) or copy neutral LOH (42/94), or both. Altogether, 63/94 (67%) samples displayed either 16p13.3 alteration or *SOCS1* mutation.

Among the 77 *BCL2UR* cases with evaluable RNAseq, unsupervised analysis showed segregation of samples into two main clusters (Fig 2). A common feature in both clusters was overexpression of genes involved in IFN-gamma (IFNG) signaling like *IFNG*, *CXCL10*, *CXCL11*, *IDO1*, especially in samples with *SOCS1* mutations. *STAT6* mutations were also associated with upregulation of IFNG signaling genes like *CXCL9* and *CXCL11* and down-regulation of *TNFSF14*. *EZH2* mutations were more frequent in Cluster #1 and were associated with additional upregulation of *CD70*, known to be involved in dendritic cells immune response. Partial nodularity was more frequent in cluster #2. Among the 53 patients with available clinical data (still ongoing collection), 48 (91%) patients presented with initial Ann-Arbor stage 1 (n= 37) or 2 (n=11).

Conclusion

BCL2UR dFLs share common molecular features including rarity of *BCL2R* and *BCL6R*, high frequency of 16p alterations as well as *SOCS1* and *STAT6* mutations, together with activation of the IFNG-signaling pathway in the microenvironment. These features are highly correlated with inguinal presentation and localized disease.



Fig1: Mutational landscape of BCL2UR, predominantly diffuse FL.

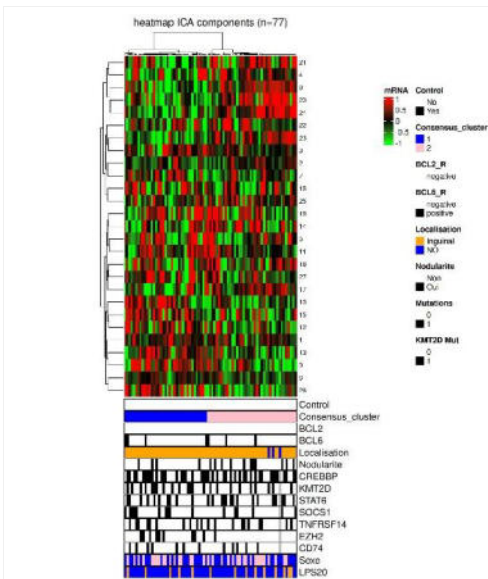


Fig2: Unsupervised RNAseq analysis of BCL2UR dFL. independent component analysis (ICA) of *BCL2UR* dFL (n=77) discloses 2 clusters of samples ("consensus cluster" bottom row) influenced by the expression of 27 gene components (numbered on the right side of the heatmap)

CCL19⁺ fibroblasts define a nodal proliferative niche in chronic lymphocytic leukemia

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Background

Lymph nodes (LNs) are a major site of proliferation in chronic lymphocytic leukemia (CLL), a tumor of mature B cells that is the most common leukemia in older adults. CLL cells proliferate in clusters called proliferation centers (PCs), within which CLL cells enlarge in size and accumulate MYC protein. The focal nature of PCs suggests that local stromal cues orchestrate their formation. The LN microenvironment is believed to provide stroma-derived survival signals that protect CLL cells from therapeutic pressure, thereby fostering persistence, suggesting that therapies that target components of the proliferative CLL LN microenvironment could complement tumor-directed therapies.

Principles/Methodology

To deeply characterize CLL cells and their proliferative LN microenvironment we performed single-cell RNA sequencing (scRNA-seq) in independently sorted CD45⁺ immune and CD45⁻ stromal cells to obtain high stromal cell representation. We then validated and tested major facets of the scRNA-seq results using spatial transcriptomics, multiplex immunofluorescence microscopy, and *ex vivo* 3D spheroid growth assays with human CLL cells.

Results

Our results indicate PCs, identified by *MYC/CD20* expression, contain a distinct fibroblast population expressing *CCL19*, termed CLL proliferation-associated (CPA) fibroblasts, and that CLL cells in the vicinity of PCs express the *CCL19* receptor *CCR7*, providing a recruitment/retention mechanism for CLL cells in PCs. Using informatic, spatial transcriptomic, and *in situ* analyses to identify ligand-receptor pairs involving PC CLL cells and nearby immune and stromal cells, we observe that PCs are enriched for macrophages expressing BAFF, the integrin $\alpha X\beta 2$ heterodimer, and Galectin9, factors implicated in cell growth, adhesion, and immunosuppression, respectively. The most prevalent predicted interactions in PCs involve CD74 and ligands such as MIF, and we find that CD74 blockade consistently inhibits primary human CLL cell growth in 3D cultures.

Conclusion

Altogether, these findings suggest that CPA fibroblasts recruit CLL cells to PCs, where they upregulate *MYC*, initiating cell cycle progression. Within PCs, CLL cells receive adhesion and growth support from CPA fibroblasts and macrophages, while galectin 9 expression contributes to local immune suppression. PCs therefore represent a progrowth, proadhesion, and immunoprotective niche. Our work highlights key features of this niche and provides a roadmap for identifying vulnerabilities and new therapeutic strategies.

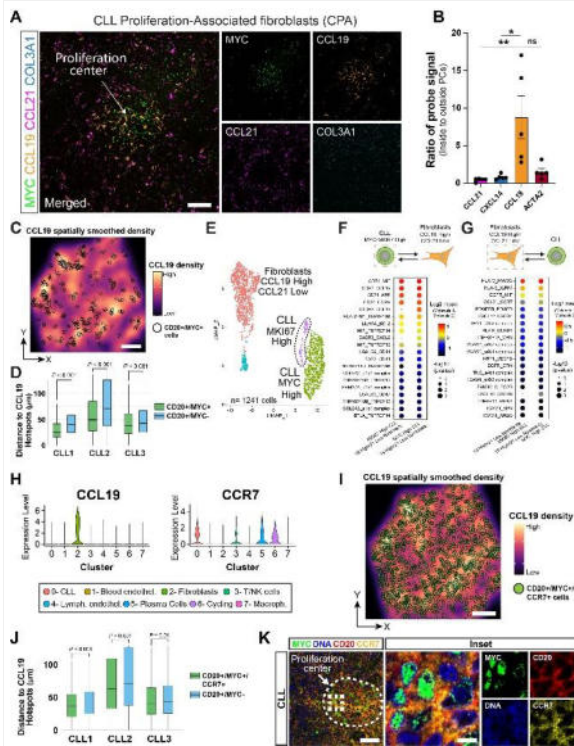


Figure 1. Identification and spatial organization of CLL proliferation-associated fibroblasts (CPAs)
Identification and spatial organization of CLL proliferation-associated fibroblasts (CPAs). A-B) Confocal IF and quantification showing enrichment of CCL19 within CLL PCs. C-G) Spatial transcriptomics, UMAP analysis, and CellPhoneDB prediction identifying CCL19-high/CCL21-low fibroblasts interacting with MYC⁺ and MKI67⁺ CLL cells. H,J) Expression levels, Spatial CCL19 density maps overlaid with proliferating CLL cells, including CCR7⁺ subsets. K) Confocal IF showing CCR7 expression on CD20⁺MYC⁺ CLL cells within PCs.

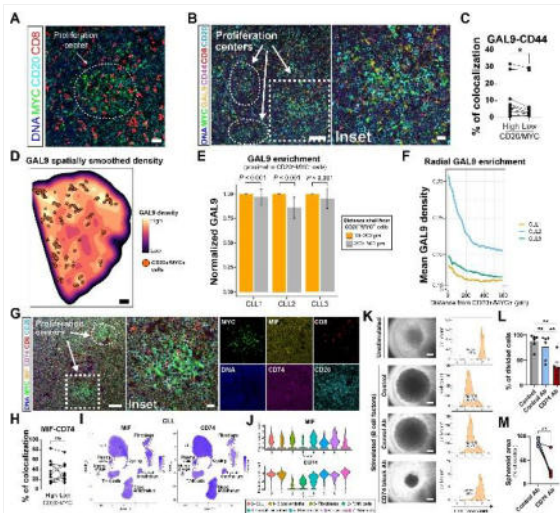


Figure 2. Galectin-9-mediated immune suppression and MIF-CD74 signaling in CLL proliferation centers
A-B,G) Multiplex and confocal IF showing GAL9, MIF, CD74, MYC, CD20, CD44, CD8, and DNA within CLL PCs. C,H) Quantification of GAL9-CD44 and MIF-CD74 colocalization near CD20⁺MYC⁺ cells. D-F) Spatial LGALS9 enrichment around proliferating MS4A1⁺MYC⁺ CLL cells. I-J) Single-cell expression of MIF and CD74. K-M) Spheroid cultures showing reduced CLL proliferation and spheroid size following CD74 blockade.

H&E-Derived STPath Maps Capture Immune Neighborhoods and Multiomic Context in Follicular Lymphomas and Diffuse Large B-cell Lymphomas

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Background

The lymphoma immune microenvironment reflects interactions among tumor genetics, morphology, and host immune response, but spatial assessment across modalities remains challenging. We evaluated whether H&E-derived STPath localized expression inference captures immune microenvironment patterns compared with sequencing and spatial profiling in follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL).

Principles/Methodology

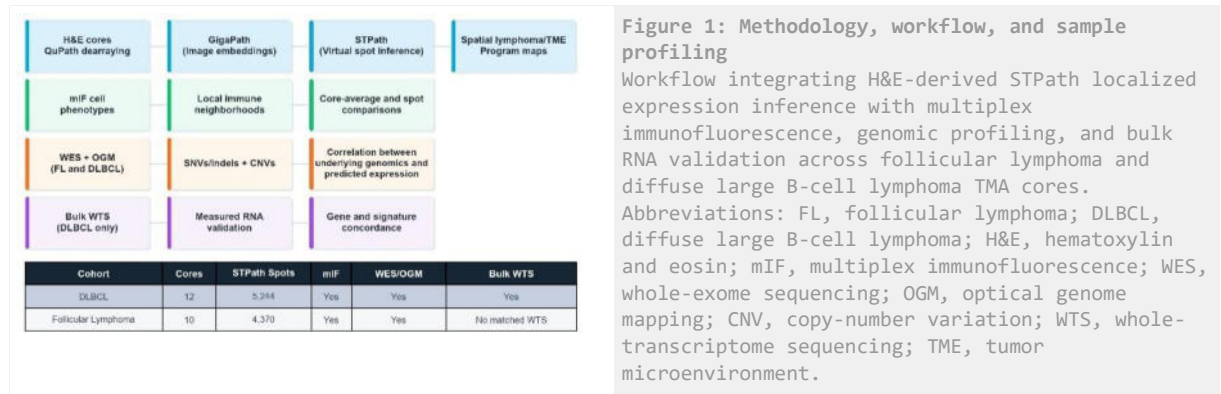
FL and DLBCL cases were profiled by H&E, multiplex immunofluorescence (mIF), whole-exome sequencing, and optical genome mapping; DLBCL cases also had bulk whole-transcriptome sequencing and were analyzed using LymphGen 2.0 web application based on integrated mutational, copy-number and structural alteration data. H&E cores were dearrayed, tiled, embedded with GigaPath, and analyzed with STPath to infer localized expression programs. STPath outputs were compared with local mIF neighborhoods, bulk RNA, and molecular features.

Results

In DLBCL, STPath gene-level predictions showed moderate concordance with matched bulk transcriptome, with strongest program-level agreement for cytotoxicity, ST2-associated LymphGen context, myeloid/DC, and B-cell/T-cell programs. STPath/mIF agreement was strongest when evaluated as local immune neighborhoods rather than whole-core fractions, with highest local concordance for T-cell, cytotoxic, and myeloid/DC programs. FL showed more modest local concordance, with relatively stronger checkpoint and T-cell neighborhood signals. Across cohorts, B-cell proportional abundance showed weak or inverse relationships with STPath B-cell/follicular scores, while DLBCL absolute local B-cell density correlated more positively with the STPath B-cell/tumor program. Genotype-spatial cross-checks provided complementary context: MYC rearrangement/high bulk MYC RNA was not recapitulated as high STPath MYC, whereas TP53 mutation/17p loss aligned with the highest STPath TP53 signal.

Conclusion

H&E-derived STPath maps captured regional immune-neighborhood states that partially aligned with mIF, bulk RNA, and genomic context in B-cell lymphomas. FL showed weaker B-cell program concordance than DLBCL, potentially reflecting tumor-to-microenvironment ratio. STPath signals varied by program (TP53 stronger than MYC), supporting validation in larger cohorts.



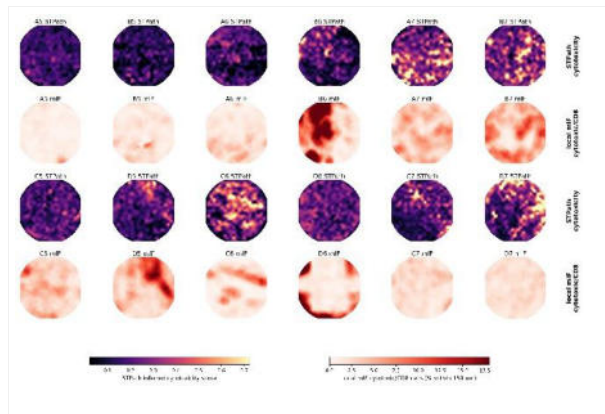


Figure 2: Paired STPath and mIF cytotoxic immune-neighborhood maps in DLBCL cores
Paired DLBCL core comparison. A5/B5, A6/B6, A7/B7, C5/D5, C6/D6, and C7/D7 are paired TMA cores from six DLBCL samples. Purple/yellow maps show STPath-inferred cytotoxicity; red maps show projected local mIF cytotoxic/CD8 cell density within 150 μ m. Warmer colors indicate higher signal.

EA4HP26-ABS-617

Spatial immune ecosystems define *CDKN2A*-associated immune escape in mantle cell lymphoma

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Background

Mantle cell lymphoma (MCL) is an aggressive B-cell lymphoma defined by the t(11;14)(q13;q32) translocation leading to cyclin D1 overexpression. Deletion of *CDKN2A* represents a strong adverse prognostic and predictive biomarker and is associated with inferior response to chemo-immunotherapy. Across multiple cancer entities, *CDKN2A* loss has been linked to an immunologically “cold” tumor microenvironment; however, the mechanisms by which *CDKN2A* deletion shapes the spatial immune landscape in MCL remain poorly understood.

Principles/Methodology

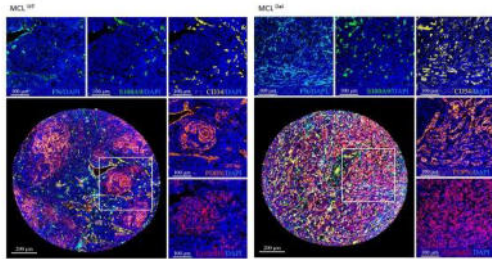
We applied a multimodal, spatially resolved approach to systematically compare *CDKN2A*-deleted MCL (MCL^{del}) and *CDKN2A*-wildtype MCL (MCL^{WT}). The cohort comprised 82 patients, including 12 MCL^{del} and 70 MCL^{WT} cases; 21 cases displayed blastoid morphology. Spatial transcriptomics was performed using Xenium 5K assays and complemented by protein analysis with 8 multiplex immunofluorescence panels. Spatial organization and tumor–microenvironment interactions were quantified using distance-based proximity metrics, neighborhood vector representations, and graph-based spatial statistics.

Results

MCL^{del} cases exhibited a distinct spatial transcriptional program characterized by upregulation of canonical cell-cycle regulators and proliferation-associated pathways. Spatial immune profiling revealed a profoundly altered tumor microenvironment consistent with an immunologically cold phenotype. Specifically, MCL^{del} samples showed enrichment of immunosuppressive cell populations (exhausted CD8 T-cells) and increased expression of multiple immune checkpoint and immune evasion markers (such as PDL1). Graph-based spatial analyses demonstrated disrupted immune neighborhood architecture in MCL^{del}, with decreased immune–tumor connectivity and increased segregation of effector immune cells from tumor regions. This correlated with stromal remodelling and storiform proliferation of podoplanin. These features indicating a convergence of high-risk genetic and microenvironmental phenotypes.

Conclusion

This comprehensive spatial multi-omics analysis reveals that *CDKN2A* deletion in MCL is associated with profound remodeling of the tumor immune microenvironment, resulting in spatial immune exclusion and enhanced immunosuppression. Our findings provide mechanistic insight into this high-risk subgroup and identify potential microenvironment-driven vulnerabilities that may be exploited for novel immunomodulatory treatment strategies.



Stromal remodeling in MCL with *CDKN2A* deletion
mIF imaging with Podoplanin, Fibronectin, S100A9,
CD34, and CyclinD1 show prominent desmoplastic
stromal remodeling in MCL^{DEL} versus MCL^{WT}

EA4HP26-ABS-625

Prognostic Significance and Spatial Characterization of Cytotoxic CD4+ T Lymphocytes in Diffuse Large B-cell Lymphoma

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Background

Cytotoxic CD4+ T lymphocytes (CD4+ CTLs) are a subset of CD4+ T cells that can directly eliminate cancer cells through cytotoxic molecules in an HLA class II (HLA II)-restricted manner. Although CD4+ CTLs constitute only a minor fraction of CD4+ T cells and their biological role remains incompletely understood, recent studies have identified them in several solid tumors. However, their presence and significance remain poorly characterized in lymphoma, a malignancy that frequently expresses HLA II. In this study, we investigated the distribution, spatial relationship, and clinical significance of CD4+ CTLs in association with HLA II-expressing tumor cells in patients with diffuse large B-cell lymphoma (DLBCL).

Principles/Methodology

We first analyzed publicly available single-cell RNA sequencing data (GSE242756) to identify the presence and candidate markers of CD4+ CTLs in lymphoma tissues. We then performed multiplexed immunohistochemistry (mIHC) using the Akoya Opal system on DLBCL tissue microarrays to visualize the spatial distribution of CD4+ CTLs and evaluate their associations with clinicopathologic features.

Results

Analysis of CD4+ T cells isolated from DLBCL, follicular lymphoma (FL), and tonsil tissues revealed the presence of CD4+ CTLs in all three tissue types. CD4+ CTLs were most abundant in DLBCL, followed by FL and tonsil. GZMB, NKG7, PRF1, GNLY, GZMK, and IFNG were identified as candidate markers of CD4+ CTLs. Based on these findings, GZMB and PRF1 were selected as mIHC markers for CD4+ CTLs. In the mIHC analysis, DLBCL cases with high

HLA II expression showed a higher abundance of CD4+ CTLs, with a significant positive correlation. However, spatial proximity analysis demonstrated that individual CD4+ CTLs were located closer to HLA II-negative B cells than to HLA II-positive B cells, suggesting possible HLA II loss in tumor cells adjacent to CD4+ CTLs. In addition, CD4+ CTL abundance was reduced in the MYC/BCL2 double-expressor group. Survival analysis showed that CD4+ CTL abundance was associated with prognosis in the HLA II-high group, whereas CD8+ CTL abundance was associated with prognosis in the HLA II-low group.

Conclusion

This study demonstrates the presence of CD4+ CTLs in DLBCL and suggests that they are associated with HLA II expression and may have distinct prognostic significance depending on the HLA II status of DLBCL. These findings suggest that CD4+ CTLs may play an important role in the tumor microenvironment and influence survival outcomes in patients with DLBCL.

Lymphoma Symposium I | Microenvironment and B-cell lymphoma – Poster

EA4HP26-ABS-460	A SINGLE CASE OF SYNCHRONOUS CHRONIC MYELOMONOCYTIC LEUKEMIA AND DIFFUSE B CELL LYMPHOMA- CASE REPORT LABORATORY VIEW
EA4HP26-ABS-466	Multidimensional Assessment of MYC Status in Diffuse Large B-Cell Lymphoma Using Histopathological Specimens
EA4HP26-ABS-483	Exposure to TNF- α and IFN- γ changes the expression of circadian cell activators in primary mediastinal B-cell lymphoma cells
EA4HP26-ABS-510	Nodal marginal zone lymphomas with an expansion of PD1-positive T cells are enriched for KLF2 mutations and show an IFN γ -associated inflammatory microenvironment
EA4HP26-ABS-511	Immunosenescence and T-cell remodeling in diffuse large B-cell lymphoma: highly multiplexed spatial profiling in elderly and frail patients from the Italian Lymphoma Foundation (FIL)
EA4HP26-ABS-512	Microenvironment characterization in EBV-positive and EBV-negative diffuse large B-cell lymphomas in post-solid-organ transplantation and in immunocompetent settings
EA4HP26-ABS-519	Microenvironmental Remodeling During Follicular Lymphoma Progression and Transformation Revealed by Spatial and Bulk Transcriptomics
EA4HP26-ABS-521	Small/Large Follicle-Size Pattern as a Key CD21-Based Classifier in Follicular Lymphoma: Clinicopathological and Prognostic Implications
EA4HP26-ABS-525	Differential Immune Cell Patterns and Fibrosis in Patient-Matched Bone Marrow and Extramedullary Sites in Multiple Myeloma
EA4HP26-ABS-527	Spatial Transcriptome Profiling in follicular lymphoma and distinct subgroups
EA4HP26-ABS-528	Breast Implant-Associated Diffuse Large B-Cell Lymphoma (BIA-DLBCL), EBV+: Report of a case and meta-analysis of rare previously published cases. Is it a distinct entity ?
EA4HP26-ABS-529	T-cell/histiocyte-rich large B-cell lymphoma revisited: A single institution retrospective review of 28 cases diagnosed from 2002-2025
EA4HP26-ABS-538	PD-1 expression in chronic lymphocytic leukemia (CLL) significantly correlates with its aggressive variant irrespective of p53 gene status.
EA4HP26-ABS-545	Prognostic significance of EZH2 and PD-L1 in the tumor microenvironment of DLBCL
EA4HP26-ABS-552	Spatial Transcriptomics Reveals Microenvironmental Reprogramming and Non-Linear Immune Ecosystems Across Fan Patterns in Nodular Lymphocyte-Predominant Hodgkin Lymphoma
EA4HP26-ABS-555	HHV-8 AND ONCOGENIC MICROENVIRONMENT: A CLINICOPATHOLOGIC SPECTRUM FROM CASTLEMAN DISEASE TO PLASMABLASTIC LYMPHOMA

EA4HP26-ABS-577	Ki67 Proliferation Index by AI-Assisted Digital Assessment in Nodal Marginal Zone Lymphoma: Morphological Correlates and T-Cell Microenvironmental Parameters
EA4HP26-ABS-580	CD10 expression in nodular lymphocyte-predominant B-cell lymphoma (NLPBL)/nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) : a diagnostic pitfall
EA4HP26-ABS-581	Primary Breast Diffuse Large B-cell Lymphoma: A report from the real-life series at the Institute of Oncology Ljubljana, Slovenia
EA4HP26-ABS-586	Abundant T-cells of low diversity distinguish B-cell precursor lymphoblastic lymphoma from leukemia
EA4HP26-ABS-593	Clinical utility of automated mRNA dual in situ hybridization (DISH) for B-cell clonality in primary cutaneous follicle centre lymphoma and its mimickers: A study of 51 cases
EA4HP26-ABS-595	Assessment of Immunoglobulin Light Chain Restriction Patterns via Kappa and Lambda Dual In Situ Hybridization in Diffuse Large B-cell Lymphoma
EA4HP26-ABS-599	Immunohistochemical Profiling of PD-L1 and CD47 in Treatment-Naïve Diffuse Large B-Cell Lymphoma, NOS
EA4HP26-ABS-609	CD30-Positive Diffuse Large B-Cell Lymphoma of the Breast in a Patient with Lymphocytic Mastitis: Evidence Suggestive of High-Grade Transformation from Extranodal Marginal Zone Lymphoma
EA4HP26-ABS-611	CPT1A inhibition sensitizes diffuse large B-cell lymphoma to venetoclax treatment
EA4HP26-ABS-631	Immune Microenvironment Programs Shape Biological Heterogeneity in Diffuse Large B-Cell Lymphoma
EA4HP26-ABS-635	Dissecting lymphoma complexity by spatially resolved hyperplex profiling
EA4HP26-ABS-638	Burkitt Lymphoma (BL): the darkest Lymphoma Microenvironment?
EA4HP26-ABS-642	Immune disparities in the TME of EBV+ and EBV- BL: role of ROS generation pathways and platelet activation
EA4HP26-ABS-650	Follicular lymphoma with amorphous deposits within neoplastic follicles
EA4HP26-ABS-652	Integrated Profiling of the Microenvironment for the Investigation of Subtype-Specific Phagocytic Checkpoint Biology in DLBCL
EA4HP26-ABS-657	SEQUENTIAL CUTANEOUS TRANSFORMATION OF CYCLIN D1– POSITIVE WALDENSTRÖM MACROGLOBULINEMIA INTO DIFFUSE LARGE B-CELL LYMPHOMA: A CASE REPORT

A SINGLE CASE OF SYNCHRONOUS CHRONIC MYELOMONOCYTIC LEUKEMIA AND DIFFUSE B CELL LYMPHOMA- CASE REPORT LABORATORY VIEW

PhD/MD Nahla Ibrahim

*KING ABDULAAH MEDICAL CITY, laboratory and blood bank, *, Saudi Arabia*

Background

Hematopoietic stem cells differentiate into myeloid and lymphoid lineages. Leukemia and lymphomas can arise from one of these progenitor stem cells. Concomitant myeloid leukemia and non-Hodgkin's lymphoma (NHL), rarely occur in the single patient.

Principles/Methodology

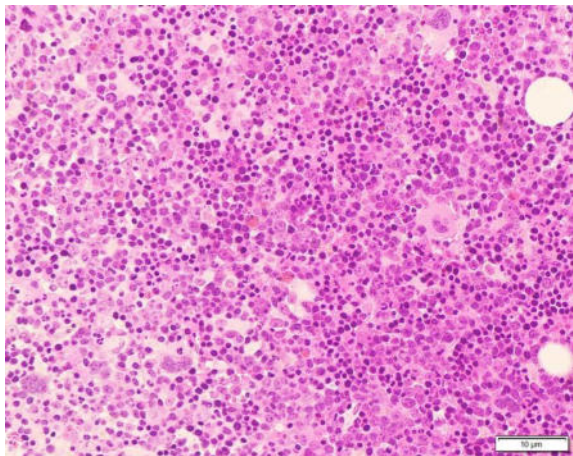
we report 69-year-old man with a synchronous diagnosis of both bone marrow chronic myelomonocytic leukemia (CMML) and nodal Epstein - Barr virus (EBV) positive diffuse large B cell lymphoma. He is long-term diabetic patient with no family history of blood disorders. On examination prominent left sided cervical and axillary lymphadenopathy with hepatosplenomegaly proofed by computed tomography (CT) imaging.

Results

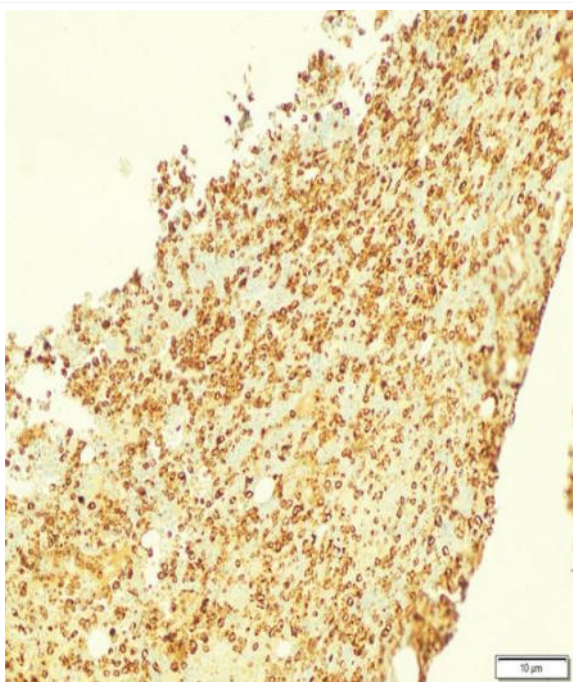
peripheral blood:Hgb 9.9 g/dl; RBC $3.5 \times 10^{12}/L$; WBC $35.8 \times 10^9/L$; PLT $18 \times 10^9/L$. Leukocytosis, with prominent monocytes. The Blast count $< 3\%$. Bone marrow hypercellular having trilineage dysplastic features, Monocytes prominent with atypical morphology. Blast cells $< 5\%$. Bone Marrow Trephine: Focal paratrabecular infiltration by atypical large lymphoid cells with mixed inflammatory infiltrate and mild fibrotic changes, comprises 15-20% of the cellularity. Other laboratory investigations positive serum EBV IgM and IgG. The cervical lymph node showed lymph node architecture is effaced by a lymphohistiocytic infiltrate consisting of scattered large B-cells with irregular nuclear contour and hyperchromatic nuclei. By immunostaining, the large cells are positive for CD20, EBV-LMP1, PAX5, CD79a, CD45, and BCL2 and negative for CD10, cyclin D1 and CD15. The ki-67 proliferative index is $\sim 40\%$. His Next Generation Sequencing (NGS) revealed that he carries variants in the genes ASXL1 44%, NRAS 41%, and TET2 45%. Other molecular testing showed negative PML-RARA, BCR/ABL p210, FLT3, NPM1, CEBPA mutation. Chromosomal analysis: 46 X, loss of the Y-chromosome normal FISH analysis for MDS and AML panels. Flowcytometry analysis on bone marrow specimen revealed no clear phenotypic evidence of increase in CD34 positive cells, and around 26- 30% monocytic population.

Conclusion

This case report described rare synchronous bone marrow chronic myelomonocytic leukemia (CMML) and EBV positive large B-cell lymphoma. The synchronous occurrence in a single patient is rare and may be attributed to a genetic mutation common to both. Furthermore In the setting of a diagnosed myeloid neoplasm, the presence of Epstein - Barr virus positive B-cell lymphoma raises the possibility of immunosuppression associated lymphoproliferative disease.



Bone Marrow biopsy: Hypercellular bone marrow, with prominent granulocytic and monocytic population,



Bone marrow biopsy show positive staining of monocytic and myeloid population for lysozyme

EA4HP26-ABS-466

Multidimensional Assessment of MYC Status in Diffuse Large B-Cell Lymphoma Using Histopathological Specimens

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Background

MYC plays a central role in the pathobiology of diffuse large B-cell lymphoma (DLBCL), and MYC rearrangement and protein overexpression are associated with aggressive clinical behavior. However, immunohistochemical (IHC) evaluation of MYC expression remains problematic due to interobserver variability, differences in antibody clones, and uncertainty regarding optimal cut-off values. More objective and biologically relevant approaches to MYC assessment are required.

Principles/Methodology

We analyzed 326 cases of DLBCL diagnosed between 2002 and 2016 using formalin-fixed paraffin-embedded tissue. MYC status was evaluated in a multidimensional manner, including genetic alterations (rearrangement, mutation, and amplification), MYC mRNA expression assessed by RNAscope, and protein expression assessed by conventional MYC IHC (clone Y69) and phosphorylated MYC at serine 62 (pMYC[S62]), a marker of MYC activation.

Results

MYC rearrangement showed a significant association with MYC protein expression assessed by clone Y69 ($p < 0.05$), whereas no clear correlation was observed between MYC expression and gene mutation or amplification. MYC mRNA expression demonstrated a moderate correlation with MYC protein expression ($R^2 = 0.33$); however, accurate evaluation by RNAscope was challenging in older archival specimens. Notably, MYC protein expression $\geq 60\%$ was associated with increased MYC activation, as reflected by pMYC (S62) expression. pMYC (S62) proved to be a useful surrogate marker for MYC activation, although cases harboring mutations near amino acid 62 showed reduced reliability of pMYC-based assessment.

Conclusion

Our results indicate that MYC activation in DLBCL cannot be adequately captured by genetic alterations or conventional MYC IHC alone. A multidimensional approach incorporating MYC expression level and phosphorylation status provides a more biologically meaningful assessment of MYC activity. pMYC (S62) represents a promising marker of MYC activation, although its limitations in cases with specific MYC mutations should be recognized.

EA4HP26-ABS-483

Exposure to TNF- α and IFN- γ changes the expression of circadian cell activators in primary mediastinal B-cell lymphoma cells

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Background

All living organisms adapt to 24-hour cycle through a sophisticated network - circadian clock regulation. Circadian clock works as a main regulator of each cell's gene expression and enables each cell's specific physiology and function. In humans, it is regulated by two transcription-translation feedback loops: the first feedback loop consists of activators CLOCK and its partners BMAL1 or BMAL2, while the second loop, regulated by the first, regulates the activators' expression, as well as the expression of other downstream genes. Additionally, BMAL1 is known to act as a negative regulator of NF- κ B signalling, it is involved in cellular responses to cytokines TNF- α and IFN- γ and regulates PD-L1 expression. In this way, it connects the circadian rhythm control and elements of the immune response, such as interactions of lymphoma cells with their microenvironment.

Principles/Methodology

In this study, we aimed to analyse the expression of circadian clock transcription activators in primary mediastinal B-cell lymphoma (PMBCL) in response to cytokines TNF- α and IFN- γ . TNF- α and IFN- γ are cytokines that are crucial for communication between tumour cells and cells from their surroundings, e. g. T-cells. For PMBCL model we used cell line MedB1, and for control cell line HeLa (non-lymphocytic cell line). Both cell lines were treated with different concentrations of TNF- α and IFN- γ over 24 and 48 hours, which was followed by the *CLOCK* and *BMAL1* gene expression quantification using qPCR.

Results

Our results show that TNF- α and IFN- γ do not influence *CLOCK* and *BMAL1* expression in non-lymphocytic cell line, while they influence circadian rhythm activators in PMBCL cells. The treatment with TNF- α during 24 hours decreases *CLOCK* expression in lymphoma cells, but only in the presence of higher concentration of the cytokine. *CLOCK* expression also decreases after the 48 hours treatment with the lower concentration of TNF- α , which is not the case with the higher cytokine concentration. On the other hand, IFN- γ increases *BMAL1* expression regardless of the cytokine concentration or period of exposure.

Conclusion

The combined results show a disruption in circadian clock activators expression in PMBCL cells as a result of exposure to TNF- α and IFN- γ , cytokines that are the main communication molecules between lymphoma cells and their microenvironment.

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Nodal marginal zone lymphomas with an expansion of PD1-positive T cells are enriched for *KLF2* mutations and show an IFN γ -associated inflammatory microenvironment

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Background

Nodal marginal zone lymphoma (NMZL) remains a challenging diagnosis due to the absence of disease-defining morphologic and/or genetic features. Moreover, a subset of NMZL is accompanied by an expansion of PD1+ T cells with T follicular helper (TFH) features, raising the differential diagnosis of TFH lymphoma. The biological basis for this T-cell expansion remains unknown. This study aimed to explore the genetic alterations and tumor microenvironment (TME) associated with this phenomenon.

Principles/Methodology

FFPE tissue was retrieved from cases diagnosed as NMZL rich in PD1+ T cells (PD1+ NMZL). PD1 staining patterns were categorized according to Egan *et al.* (Am J Surg Pathol 2020). Cases underwent NGS of 78 lymphoma-relevant genes and NanoString-based gene expression profiling (GEP) using the nCounter® PanCancer Immune Profiling Panel.

Results

The study included 39 NMZL cases: 23 with and 16 without expansion of PD1+ T cells. Among PD1+ NMZL, the pattern was follicular peripheral in 8, follicular central in 4, and diffuse in 11 cases. Mutational analysis revealed a significant association between PD1 enrichment and *KLF2* mutations, detected in 15/23 versus 2/16 cases ($p < 0.01$; OR, ~13). GEP, including CIBERSORT deconvolution, revealed significant differences between PD1+ and PD1- NMZL. While PD1- NMZL were enriched for memory B cells, reflecting higher tumor cell abundance, PD1+ NMZL

showed increased inflammatory cell populations and signatures. Notably, although an increased TFH component was present in PD1+ NMZL (*ICOS*, *IL21*), the dominant features included IFN γ -associated genes (*IFNG*, *STAT1*, *CXCL9/10/11*), NK/cytotoxic activity (*NCR1*, *GZMA/B*), and macrophage-related immune responses (*CHIT1*, *MSR1*), consistent with an IFN γ -associated inflammatory TME. Importantly, GEP-based comparison of *KLF2*-mutated and wild-type cases identified no significantly enriched genes or cell types.

Conclusion

This study provides the first integrated mutational and GEP analysis of PD1+ NMZL, demonstrating enrichment for *KLF2* mutations and an IFN γ -associated TME with TFH enrichment rather than a predominantly TFH-driven profile. A similar TME-rich signature associated with *KLF2* mutations has been described in a subset of splenic MZL. Intriguingly, despite their association with this TME, the lack of *KLF2*-specific GEP differences in our cohort questions the role of *KLF2* mutations as its primary driver. Further studies are warranted to elucidate how NMZL interacts with its TME and assess potential clinical implications.

EA4HP26-ABS-511

Immunosenescence and T-cell remodeling in diffuse large B-cell lymphoma: highly multiplexed spatial profiling in elderly and frail patients from the Italian Lymphoma Foundation (FIL)

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Background

Diffuse large B-cell lymphoma (DLBCL) mainly affects older adults, who often present with comorbidities and frailty that limit the use of full-dose chemoimmunotherapy. T-cell redirecting therapies are promising alternatives, but their efficacy may be impaired by age-related immune remodeling. Understanding how immune aging shapes the tumor microenvironment (TME) is thus critical to refine risk stratification and guide targeted therapies in elderly and frail patients.

Principles/Methodology

We applied highly multiplexed spatial multi-omics profiling to dissect the TME of 76 nodal and extranodal DLBCL cases from two FIL cohorts: the "Elderly Project" (≥ 65 years, stratified by geriatric assessment) and the "RI_CHOP" trial (< 65 years). FFPE samples from 44 elderly and 32 adult patients were analyzed using single-cell spatial transcriptomics (CosMx SMI, 6000-plex) and proteomics (CODEX, 50-plex). Major cell types and T-cell subsets were identified by unsupervised clustering followed by curated annotation based on lineage-marker expression.

Results

We focused on 44 nodal DLBCL cases, identifying 8 major cell populations: tumor B-cells, T-cells, macrophages, dendritic cells, fibroblasts, plasma cells, red blood cells, and stromal cells. T-cell analysis resolved 9 subtypes: CD4⁺ naive, CD4⁺ central memory (T_{cm}), CD4⁺ effector memory (T_{em}), CD4⁺ regulatory T cells (T_{reg}), CD8⁺ naive, CD8⁺ T_{cm}, CD8⁺ T_{em}, exhausted T cells (T_{ex}), and Tγδ cells. Compared with adult cases, elderly DLBCL showed enrichment of T_{ex} and CD8⁺ T_{em} cells, together with depletion of CD8⁺ T_{cm} and CD8⁺ naive T-cells, consistent with a shift toward effector and dysfunctional states. Stratification by fitness in elderly patients revealed a frailty-associated immunosuppressive and chronically stimulated T-cell landscape: fit patients exhibited higher proportions of CD4⁺ T_{reg} and CD8⁺ T_{em}, whereas unfit/frail patients were enriched in CD4⁺ T_{cm} and T_{ex} cells. Spatial analyses also identified distinct age- and fitness-related cellular organization patterns.

Conclusion

Aging and frailty in nodal DLBCL are associated with profound remodeling of the T-cell compartment, supporting the development of immunosenescence-oriented biomarkers to guide the use of T-cell redirecting therapies in this setting. Transcriptomic data will be integrated with CODEX spatial proteomics on the same cells, while analyses will be extended to extranodal cases and other immune cell populations towards a comprehensive understanding of tumor-immune interactions *in situ*.

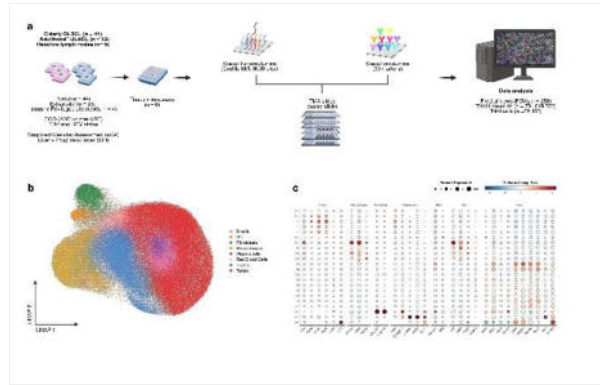


Figure 1
a. Schematic workflow of the experimental design and bioinformatic analysis (created with Biorender.com). Tissue cores from 78 DLBCL samples and 5 reactive lymph nodes were assembled into six TMAs and profiled on the same slide using CosMx SMI for single-cell spatial transcriptomics and CODEX for spatial proteomics. b. Uniform manifold approximation and projection (UMAP) of the major cell types identified in the CosMx SMI dataset. c. Dot plot showing the expression of canonical marker genes associated with each cell type shown in panel b.

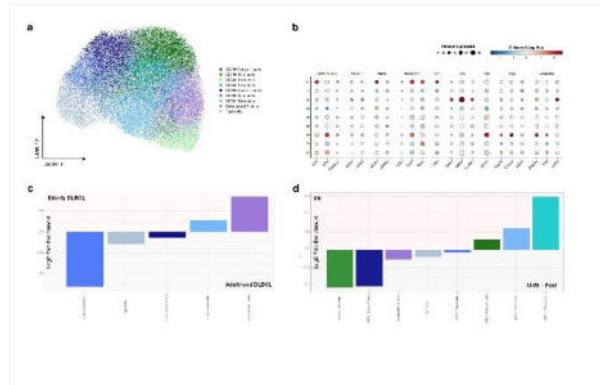


Figure 2
a. UMAP visualization of the nine T-cell subsets identified through in-depth analysis of the T-cell cluster. b. Dot plot illustrating the expression of canonical marker genes across the T-cell subsets defined in panel a. Log₂-fold enrichment of T-cell subtypes comparing elderly vs adulthood DLBCL cases (c), and fit vs unfit/frail elderly DLBCL cases (d).

Microenvironment characterization in EBV-positive and EBV-negative diffuse large B-cell lymphomas in post-solid-organ transplantation and in immunocompetent settings

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Background

Diffuse large B-cell lymphomas (DLBCLs) represent the most frequent group of post-transplant lymphoproliferative disorders (PTLD) occurring after solid organ or allogeneic hematopoietic cell transplantation. The pathogenesis of PT-DLBCLs is complex with a group associated with an Epstein-Barr virus (EBV) and another without viral association. Molecular data indicate that EBV+ PT-DLBCL bear less frequent genetic abnormalities compared to EBV- PT-DLBCL, possibly caused by the oncogenic activity of EBV contributing substantially to lymphoma development. EBV-positive DLBCLs can also occur in individuals without underlying immune deficiency or dysregulation other than immune senescence. Currently, data on the microenvironment of EBV+ and EBV- PT-DLBCL and EBV+ DLBCL in immunocompetent patients (EBV+ IC-DLBCL) are limited, a direct comparison of these entities is missing.

Principles/Methodology

For the present study, PT-DLBCL samples were retrieved from both the German PTLD-Registry and German centers, while samples from EBV+ and EBV- IC-DLBCL were collected from the latter. By using the nCounter® PanCancer Immune Profiling Panel we characterized the immune response related gene expression profile of 60 samples (PT-DLBCL EBV- n=15; PT-DLBCL EBV+ n=15; IC-DLBCL EBV- n=16; IC-DLBCL EBV+ n=14). *In silico* immune cell phenotyping was performed by CIBERSORTx. Additionally, immunostains were performed on tissue microarrays (TMAs) of a partially overlapping cohort.

Results

Our data corroborates that the T-cell population and signalling are lessened in PT-DLBCLs compared to EBV+ IC-DLBCLs. In line with an immunotolerogenic microenvironment, gene expression of the immune checkpoint protein PD1 is reduced in PT-DLBCLs compared to IC-DLBCLs. Our dataset confirms a previously published “inflamed” phenotype characterized by a cytotoxicity gene signature present in EBV+ PT-DLBCLs, and the *in silico* and TMA analysis indicates more plasma cells and less memory B-cells. Our results also demonstrate no major effect on microenvironment composition by different EBV latency patterns and reveal a significantly higher gene expression profile related to the extracellular matrix and adhesion in PT-DLBCL EBV- cases in comparison to IC-DLBCL EBV-.

Conclusion

In conclusion our detailed investigation of the immune cells in the tumour microenvironment of PT-DLBCLs compared to IC-DLBCLs reveals distinct differences in these histologically similar entities. Further analysis of our data is ongoing.

Microenvironmental Remodeling During Follicular Lymphoma Progression and Transformation Revealed by Spatial and Bulk Transcriptomics

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Background

Follicular lymphoma (FL) follows an indolent course, but relapse and histological transformation remain unpredictable. As FL evolution is shaped by tumor–microenvironment (TME) interactions, we aimed to characterize transcriptional and spatial remodeling of the TME during progression.

Principles/Methodology

We analyzed lymph node biopsies from 5 reactive lymphoid tissues (RLT) and 65 samples from 55 FL patients, including indolent, pre-relapse, pre-transformation, progression and transformed **samples**. Bulk RNA-seq data were analyzed by differential expression and ssGSEA. A local snRNA-seq reference from RLT was used for CIBERSORTx deconvolution. Spatial transcriptomics (10x Genomics Visium) was performed in 14 samples, 2 RLT, 1 indolent FL, 1 transformed and 2 progression cases with paired samples. Spots were deconvolved using SpaceXR/RCTD with the same RLT reference.

Results

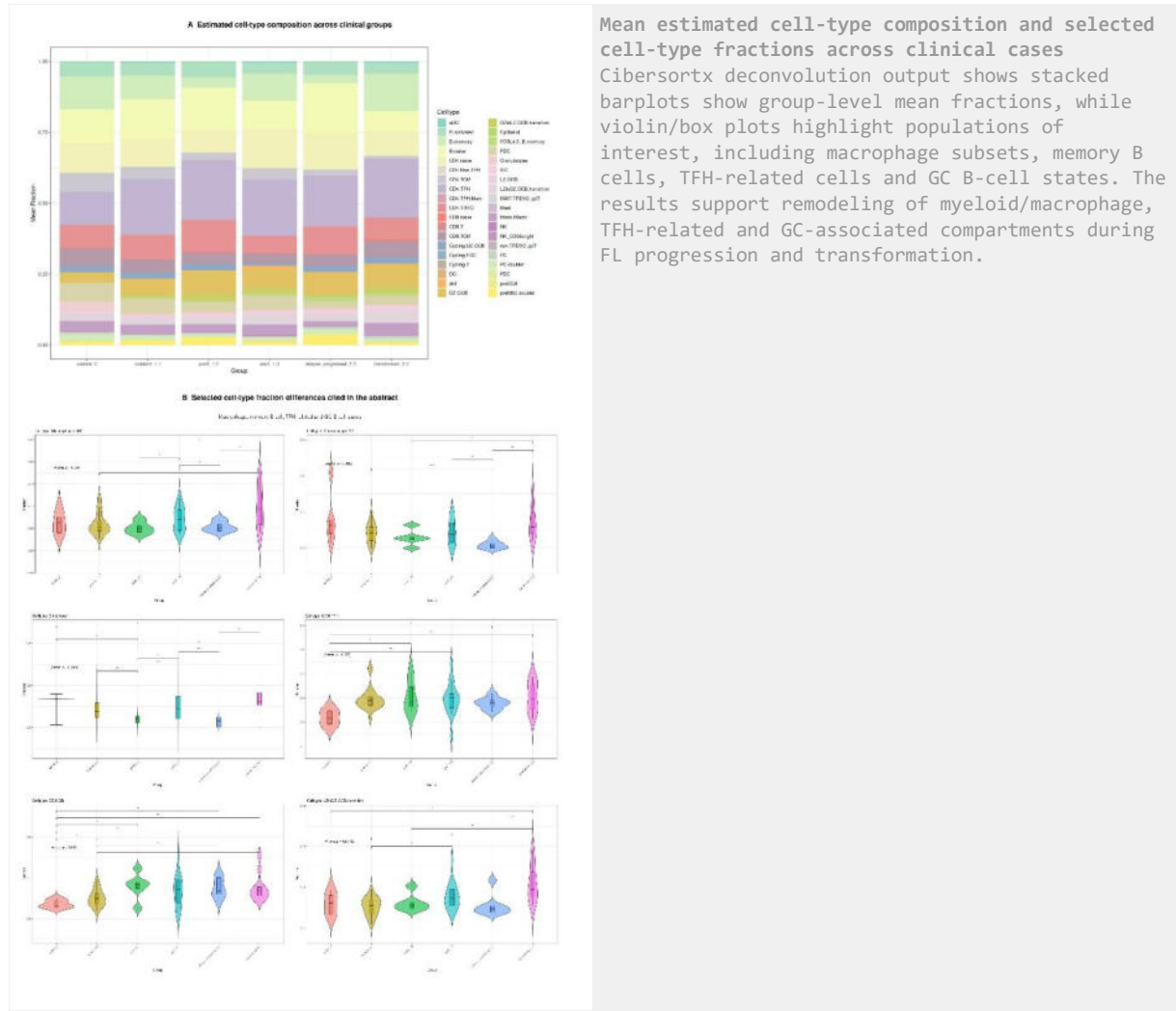
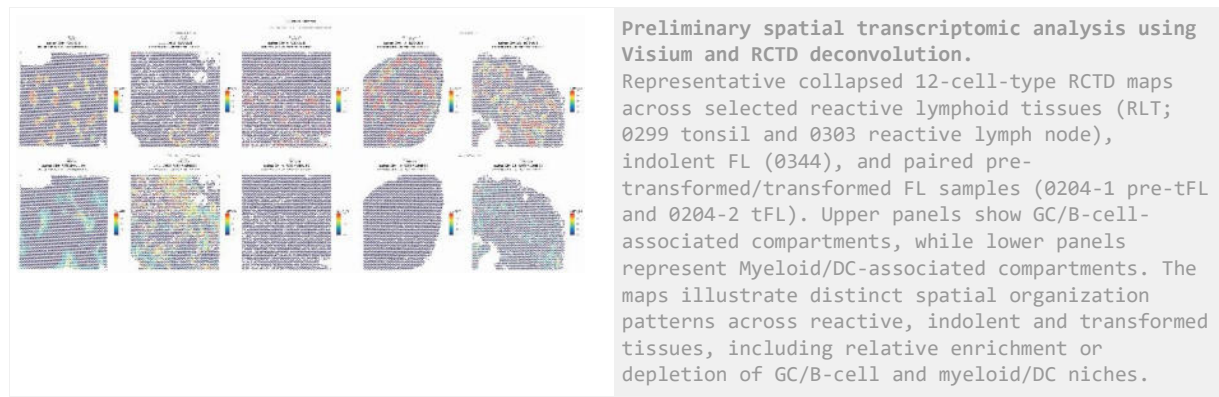
Bulk RNA-seq revealed transcriptional changes across FL evolution, with progressed and transformed cases showing altered germinal center (GC) B-cell programs, MYC/metabolic activity, stress-response signatures, and immune/TME remodeling. Immune deconvolution analyses supported alterations in GC B-cell states, TFH-related signals and myeloid/macrophage programs (Fig. 1).

Spatial transcriptomics added tissue-level resolution. The indolent FL also showed B-cell predominance, indicating that B-cell enrichment alone is insufficient to define progression without spatial and longitudinal context (Fig. 2). In longitudinal cases, the pre-relapsed sample displayed heterogeneous GC/cycling, Myeloid/FDC, and T/immune niches. In contrast, progression samples shifted toward more continuous B_non_GC-enriched regions with reduced Myeloid_DC, FDC, Cycling_GC_B and T/TFH components. Spatial metrics showed reduced mixing among immune niches, consistent with loss of GC-associated microenvironmental organization. In the paired transformation case,

the transformed sample showed relatively increased Myeloid/FDC remodeling rather than convergence toward the progression-associated B_non_GC pattern (Fig. 2).

Conclusion

These data support coordinated transcriptional and spatial remodeling during FL evolution. Bulk transcriptomics revealed disruption of GC- and immune-related programs, while spatial profiling showed reduced microenvironmental complexity and expansion of homogeneous B_non_GC-enriched regions. Progression involves compositional and spatial TME changes, whereas transformation may follow distinct Myeloid/FDC-associated remodeling trajectories



Small/Large Follicle-Size Pattern as a Key CD21-Based Classifier in Follicular Lymphoma: Clinicopathological and Prognostic Implications

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Background

Although clinical indices such as the Follicular Lymphoma International Prognostic Index (FLIPI) are used for prognostic prediction in follicular lymphoma (FL), no histological variable reliably associated with prognosis has been established. We evaluated two CD21-based follicular dendritic cell (FDC) meshwork classifiers—the Small/Large (S/L) follicle-size pattern and the Fragmented/Intact (F/I) meshwork integrity pattern—focusing on their clinicopathological correlates and prognostic relevance.

Principles/Methodology

We retrospectively analyzed 156 FL patients classified by S/L pattern using CD21 immunohistochemistry: Small (≤ 1 mm, n=100) and Large (> 1 mm, n=48). The F/I pattern (Fragmented, n=74; Intact, n=73), reflecting FDC meshwork integrity, served as a secondary classifier. Continuous variables were compared using the Mann–Whitney U test; binary categorical variables with Fisher's exact test; and multi-category variables with chi-square. Overall survival (OS) and relapse-free survival (RFS) were assessed by Kaplan–Meier/log-rank test.

Results

The S/L pattern demonstrated greater disease burden across multiple clinicopathological variables: Ann Arbor stage III–IV (81.1% vs 48.9%, $p < 0.001$), elevated serum $\beta 2$ -microglobulin (37.0% vs 6.7%, $p = 0.002$), high-risk FLIPI1 and FLIPI2 groups ($p = 0.002$ and $p = 0.009$, respectively), involvement of > 4 nodal sites ($p = 0.005$), bone marrow involvement (40.9% vs 21.1%, $p = 0.043$), splenomegaly (26.8% vs 9.3%, $p = 0.025$), and radiotherapy utilization ($p = 0.036$). The F/I pattern showed limited differences (involvement of > 4 nodal sites and CD10 positivity only). No OS or RFS differences were observed (all $p > 0.05$).

Conclusion

The predominance of CD20-positive tumor cells outside follicles in the Small pattern likely reflects impaired follicular retention due to disrupted FDC–B cell interactions, facilitating systemic dissemination and correlating with advanced-stage disease. The S/L follicle-size pattern, defined by a 1 mm CD21-based FDC meshwork threshold, is a clinically informative classifier consistently associated with advanced disease stage and higher disease burden in FL. The absence of significant OS and RFS differences likely reflects the low event rate (OS events: 5.6%, n=7/125) and limited follow-up duration. Validation in larger, prospective cohorts is warranted. CD21-based FDC meshwork classification represents an accessible immunohistochemical tool applicable in routine diagnostic practice.

Differential Immune Cell Patterns and Fibrosis in Patient-Matched Bone Marrow and Extramedullary Sites in Multiple Myeloma

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Background

Extramedullary plasma cell myeloma (EPM) is an advanced-stage disease, inextricably linked to poor therapeutic response and unfavorable prognosis. The microenvironment and its interaction with neoplastic cells seem to play the major role in the multiple myeloma (MM) clonal evolution and the emergence of EPM, turning the spotlight on the investigation of possible differences in the synthesis and spatial distribution of “tumor ecosystem” between MM and EPM.

Principles/Methodology

Formalin-fixed, paraffin-embedded tissue samples of matched cases from bone marrow (BM) and extramedullary disease (EMD) of patients with plasma cell neoplasm were retrieved from the archives of the Pathology Departments of Aristotle University of Thessaloniki and University of Ioannina. Quantitative and quality characteristics of both neoplastic cells and the tumor microenvironment were evaluated using hematoxylin-eosin stained sections and histochemical and immunohistochemical stains. Comparative assessment between the BM and EMD niches was accomplished. Statistical analysis was conducted using SPSS, v.31.0.1, and significance was defined as $p < 0.05$.

Results

The study population comprised 23 patients (9 men/14 women; median age 68 years), with synchronous MM and EMD. Paired analyses indicated a general tendency toward higher eosinophil, neutrophil, mast cell, and CD8+ densities in BM compared with EMD. A strong inverse correlation was observed between BM infiltration and neutrophil and eosinophil densities ($p = -0.711$, $p < 0.001$ and $p = -0.770$, $p < 0.001$). Lambda light chain monoclonality was significantly associated with increased BM stromal fibrosis (MF grading) ($p = 0.004$), including all MF grade 3 cases. Fibrosis was also associated with mast cell density in both BM and EMD niches. A moderate negative correlation between age at diagnosis and CD8+ density in extramedullary sites was revealed ($p = -0.50$, $p = 0.015$). No further significant associations were identified.

Conclusion

The microenvironmental composition in the bone marrow (BM) consistently exceeded that observed in extramedullary disease (EMD), indicating a stable distinction between the two compartments. Older age at diagnosis is associated with lower CD8+ T-cell density in the EMD microenvironment, indicating a weaker anti-tumor immune response in these cases. The reduced and heterogeneous representation of mast cells, neutrophils, eosinophils, and CD8+ T cells in EMD suggests an immune-depleted milieu, potentially associated with its more aggressive clinical behavior.

Spatial Transcriptome Profiling in follicular lymphoma and distinct subgroups

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Background

Follicular lymphoma (FL) is highly heterogeneous both in biological and clinical features. These features are known to depend on the tumor microenvironment (TME), which varies among different defined FL subgroups. Therefore, a deeper understanding of the interaction with the TME in FL in general, but also with focus on distinct FL subgroups is warranted.

Principles/Methodology

For this purpose, whole transcriptome gene expression profiles of selected regions of interest including all cell types as well as regions of interest limited to B-cells, T-cells, and follicular dendritic cells (FDCs) were analyzed in the germinal center (GC) and in the extra-tumoral mantle/marginal/interfollicular zones (M/M/IFZ) of 66 formalin-fixed and paraffin embedded FL samples with information on t(14;18) using Nanostring GeoMx Digital Spatial Profiling. Data analysis was accomplished using the commonly used GeoMxTools R-pipeline and the more recently published R package standR (Spatial transcriptomics analyses and decoding in R), which revealed more meaningful data by reducing batch effects, compared to the GeoMxTools R-pipeline in a recent comparison study with non-tumor GeoMx datasets.

Results

In line with previous findings, this study identified significant batch-associated variability in our FL GeoMx data using the common GeomxTools R-pipeline. Applying the standR workflow substantially reduced technical variation. The improved sensitivity of StandR also made it possible to identify biologically more meaningful differences in gene expression profiles between t(14;18)-positive and t(14;18)-negative FL, with previously identified differentially expressed molecular markers (e.g., NF-κB-associated genes and CD10) serving as a reference and as confirmation of the validity of this approach. Preliminary analyses revealed substantial differences among different compartments, cell types and FL with and without t(14;18), which are currently analyzed in more detail.

Conclusion

In conclusion, this spatial whole transcriptome approach provides insights into FL heterogeneity and pathogenesis that cannot be obtained using bulk whole transcriptome approaches. Moreover, the results from this approach will serve as a guide for further, more detailed high- and low-plex analysis at the single-cell and protein level to further improve the understanding of the oncogenic signaling between the tumor and its microenvironment in different molecular and clinical FL subgroups.

Breast Implant-Associated Diffuse Large B-Cell Lymphoma (BIA-DLBCL), EBV+: Report of a case and meta-analysis of rare previously published cases. Is it a distinct entity ?

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Background

Breast implant-associated diffuse large cell lymphoma (BIA-DLBCL), EBV+, is a rare type of high grade, large B-cell lymphoma with most of the previously published cases being classified as "DLBCL associated with chronic inflammation" (CI-DLBCL) and less frequently as "fibrin-associated DLBCL" (FA-DLBCL) according to the 5th edition of the World Health Organization (WHO) classification of haematolymphoid tumors. In certain published cases, BIA-DLBCL was initially misclassified as BIA-anaplastic large cell lymphoma (BIA-ALCL) due to incomplete immunohistochemistry (IHC). Here, we present a typical case of BIA-DLBCL, EBV+, and a meta-analysis of the rare published cases.

Principles/Methodology

Pre-operative management and diagnostic work up were based on the clinical practice for breast seroma. Gross examination and sectioning followed the published guidelines for BIA-ALCL. An extensive IHC analysis including B- and T-cell panels as well in situ hybridisation (ISH) for EBER1/2 transcripts of EBV were performed. NGS analysis using the lymphoma gene panel (>200 genes) as applied at Karolinska University Hospital is ongoing. A systematic review and meta-analysis of the rare published cases is performed.

Results

A 48-year-old woman received subpectoral cosmetic breast implants in 2013. In the past year, she developed swelling, deformity, and pain of the left breast. Mammography and ultrasound showed fluid around the left implant without implant rupture. FNA cytology and FACS analysis performed twice were not diagnostic for lymphoma. "En bloc" removal of the implant and capsule was performed. The initial pathology assessment favored BIA-ALCL, however complete IHC showed that the CD30+ lymphoma cells were negative for CD2, CD3, CD4, CD5, CD8, CD10, CD56, GATA3, GRB, PD1, ALK, BCL6, CD68, p53 and positive for CD19, CD20, CD79a, PAX5, MYC (30%), EBER1/2 with very high proliferation (> 95%). Based on IHC and ISH findings, the diagnosis of BIA-DLBCL, EBV+ was made. Review and meta-analysis of 26 well-documented, published cases of BIA-DLBCL, EBV+, revealed common histopathologic and IHC patterns that may justify for a distinct entity in the next edition of the WHO classification .

Conclusion

BIA-DLBCL, EBV+, is a distinct large B-cell lymphoma type that mimics BIA-ALCL both clinically and histologically. Based on relevant meta-analysis of case reports/case series, we propose that this rare entity should be separated from CI-DLBCL and FA-DLBCL in the upcoming revision of the WHO classification.

T-cell/histiocyte-rich large B-cell lymphoma revisited: A single institution retrospective review of 28 cases diagnosed from 2002-2025

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Background

T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL) is defined as an aggressive B-cell lymphoma in which <10% of the cells present are large, neoplastic B cells in a background of small T cells and histiocytes without follicular dendritic cell (FDC) meshworks. THRLBCL may arise de novo or as progression of nodular lymphocyte predominant B-cell lymphoma (NLPBCL). THRLBCL shares many features with variant patterns of NLPBCL, and with growing recognition of variant NLPBCL patterns in recent years, it has become unclear to what extent NLPBCL and THRLBCL represent two distinct entities. The aim of this study was to examine the frequency of de novo THRLBCL in the context of current classification systems.

Principles/Methodology

Cleveland Clinic archives were reviewed for cases receiving a definitive diagnosis of THRLBCL or in which a diagnosis of THRLBCL was favored. Relapsed THRLBCL was excluded. 28 cases (10 needle and 18 excisional samples) diagnosed between 2002 and 2025 were identified. Cases were reviewed by two hematopathologists and reclassified according to ICC and WHO5 criteria.

Results

The 28 cases included 21 males and 7 females, with a median age of 50.5 years. After review, 14 (50%) cases were reclassified as NLPBCL (12 excisions; 2 needle biopsies), 5 (18%) were reclassified as diffuse large B-cell lymphoma, not otherwise specified (DLBCL, NOS; all were excisional specimens), and 3 cases (11%; all needle biopsies) were consistent with a B-cell lymphoma but could not be further classified. Of the cases reclassified as NLPBCL, 8 had grade 2 histology (Fan patterns D, E, and/or F), 2 had grade 1 histology (all were pattern C), and 4 could not be assigned a Fan pattern. 6 cases (21%) showed pathologic findings compatible with THRLBCL, including 2 needle biopsies and 4 excisions. Because NLPBCL cannot be excluded in needle biopsies, only 4 cases (14%) remained that met current criteria for THRLBCL.

Conclusion

De novo THRLBCL is rare, and a majority of cases that were previously classified as THRLBCL at our institution would be classified as a variant pattern of NLPBCL or DLBCL, NOS using current classification schemes. Many cases that were previously diagnosed as THRLBCL were diagnosed on needle biopsy specimens; the current classifications strongly discourages making this diagnosis in limited/needle specimens. Studies are ongoing to compare our cases that meet current criteria for THRLBCL to a series of NLPBCL pattern D/E specimens.

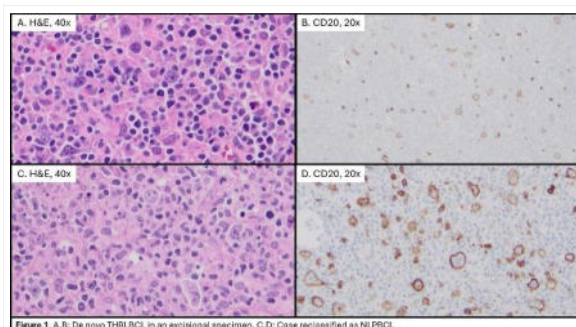
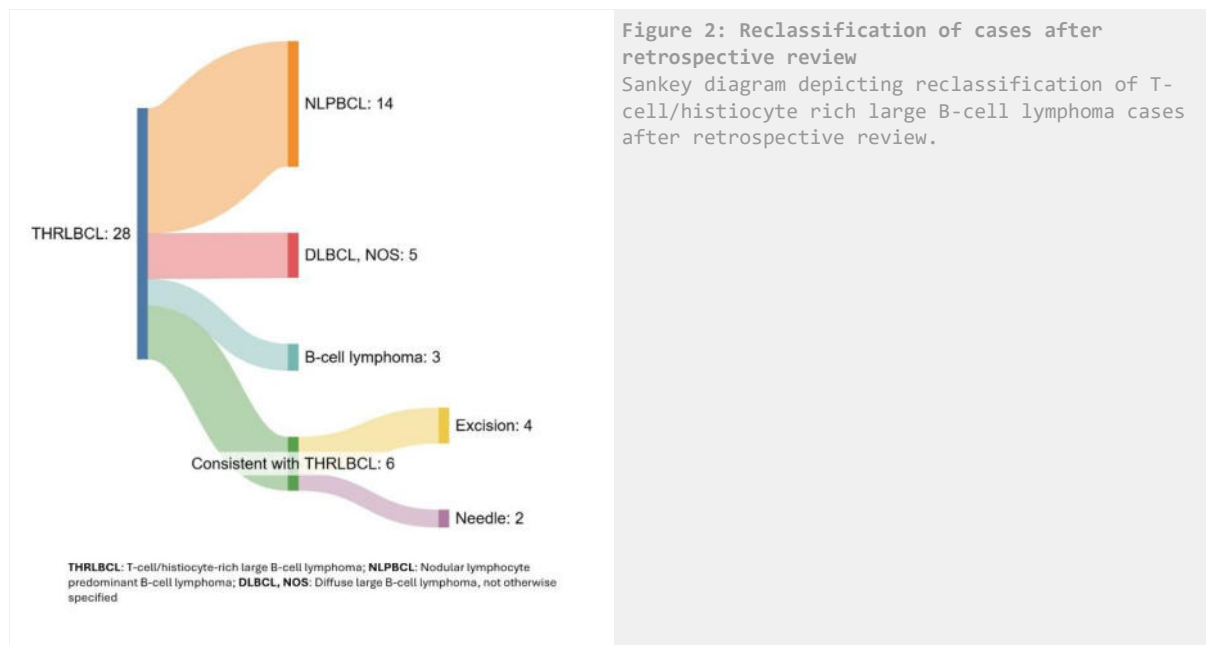


Figure 1
Representative photographs of histologic findings and CD20 immunohistochemistry in an excisional specimen considered to be compatible with T-cell/histiocyte-rich large B-cell lymphoma after rereview (THRLBCL; A, B), and a case that was originally diagnosed as THRLBCL and reclassified as a variant pattern of nodular lymphocyte predominant B-cell lymphoma after retrospective review (C, D).



EA4HP26-ABS-538

PD-1 expression in chronic lymphocytic leukemia (CLL) significantly correlates with its aggressive variant irrespective of p53 gene status.

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Background

Aggressive chronic lymphocytic leukemia (CLL) as defined in World Health Organisation classification of lymphoid tumours is characterised by confluent and merging proliferation centers and higher cell proliferation (around 40%) as compared to typical CLL. However no specific immunohistochemical markers have been identified to correlate with the aggressive variant of CLL to date. In a previous report, PD-1 expression was consistently positive in the lymphoma cells of large cell (Richter's) transformation (DLBCL-RT) but not in CLL or other DLBCL types (He et al. Am J Surg Pathol, 2018). However, PD-1 expression in the lymphoma cells of aggressive CLL remains still unknown.

Principles/Methodology

The study group included 18 aggressive CLL and 26 typical CLL with diagnostic biopsies. Of these, 6 patients with CLL progressed to DLBCL-RT and 3 to classical Hodgkin lymphoma (cHL). PD-1 expression was assessed by immunohistochemistry (IHC) using an autostainer (Ventana) and a protocol previously optimised for the diagnostic use of PD-1. Any intensity of PD-1 staining in the CLL cells was considered positive. In typical CLL, positivity was restricted to the proliferation centers as previously described and served as an internal positive control in each case.

Results

PD-1 expression in the lymphoma cells was found in 18/18 (100%) aggressive CLL, 6/26 (23%) typical CLL and 9/9 (100%) transformed CLL (DLBCL-RT or cHL) ($p < 0.0001$, Fisher's exact test). Of the 6 CLL cases progressed to DLBCL-RT, three (3) were originally diagnosed as aggressive CLL and thus, they were positive for PD-1 and 3 were diagnosed as typical CLL (1/3 positive for PD-1). Mutated p53 gene was found in 3/26 (15%) typical CLL

and 2/18 (11%) aggressive ALL and did not correlate with PD-1 expression in the lymphoma cells. None of the p53-mutated cases progressed to DLBCL-RT, however, 3 p53-mutated cases (2 typical and 1 aggressive CLL), progressed to cHL. The latter were remarkably positive for PD-1 in the CLL but not in the neoplastic Hodgkin and Reed-Sternberg cells.

Conclusion

In conclusion, PD-1 is consistently expressed in aggressive CLL (as in DLBCL-RT) but not in the majority of typical CLL irrespective of p53 gene status. Therefore, PD-1 may represent a novel marker for CLL progression to aggressive CLL as well as to DLBCL-RT or cHL transformation. However, serial biopsies of CLL and long follow up are required to establish PD-1 as a predictive marker for large cell transformation.

EA4HP26-ABS-545

Prognostic significance of EZH2 and PD-L1 in the tumor microenvironment of DLBCL

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Background

Diffuse large B-cell lymphoma (DLBCL) is an aggressive, molecularly heterogeneous malignancy. Recent studies emphasize the tumor microenvironment (TME) in treatment response, where EZH2 acts as a key epigenetic regulator and PD-L1 serves as a major immune evasion mechanism. Preclinical models show that EZH2 inhibitors can reprogram lymphoma cells toward an immunogenic phenotype and enhance checkpoint blockade. This study systematically characterizes the clinical relationship between EZH2 overexpression, PD-L1-mediated immune escape, cell of origin (COO), and prognosis.

Principles/Methodology

We retrospectively analyzed 95 DLBCL patients diagnosed at the Department of Medical Pathology, Balcalı Hospital, Çukurova University (2011–2022). Diagnoses were confirmed by two experienced hematopathologists based on WHO criteria. EZH2 expression was quantified using the H-score formula $1 \times (\% \text{ weak}) + 2 \times (\% \text{ moderate}) + 3 \times (\% \text{ strong})$, with a cutoff of ≥ 100 defining high expression. PD-L1 expression was categorized by Tumor Proportion Score (TPS) as negative ($<1\%$), low ($1\text{--}29\%$), or high ($\geq 30\%$). COO was determined via the Hans algorithm (GCB vs. non-GCB). Categorical variables were compared using Pearson's chi-square or Fisher's exact tests.

Results

High EZH2 expression (H-score ≥ 100) was detected in 31.6% (30/95) of patients and was significantly associated with the GCB subtype ($p = 0.016$; 63.3% of EZH2-high cases were GCB). A striking positive correlation was observed between high EZH2 and elevated PD-L1 levels ($p = 0.004$); high PD-L1 (TPS $\geq 30\%$) was present in 80.0% of EZH2-high patients, while only 3.3% were PD-L1-negative. Conversely, high EZH2 expression showed no significant correlation with traditional clinical markers or tumor burden, including age/NCCN-IPI ($p = 0.388$), sex ($p = 0.652$), Ann Arbor stage ($p = 0.565$), B symptoms ($p = 0.867$), extranodal involvement ($p = 0.978$), bone marrow involvement ($p = 0.320$), bulky disease ($p = 0.581$), Ki67 index ($p = 0.208$), or double-expressor status ($p = 0.959$).

Conclusion

EZH2/PD-L1 co-expression defines a distinct, biologically homogeneous GCB-DLBCL subgroup that evades the immune system independently of conventional prognostic risk factors. This coordinated "epigenetic-immune regulatory axis" highlights the need for molecular stratification in routine pathology. Evaluating EZH2 and PD-L1 via IHC represents a rational biomarker approach to select ideal candidates for combined epigenetic reprogramming and immune checkpoint blockade therapies in precision oncology.

Spatial Transcriptomics Reveals Microenvironmental Reprogramming and Non-Linear Immune Ecosystems Across Fan Patterns in Nodular Lymphocyte-Predominant Hodgkin Lymphoma

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Background

Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a germinal center (GC)-derived B-cell lymphoma with variable architectural patterns and clinical behavior. Increasing evidence suggests that disease heterogeneity may reflect differences in the immune microenvironment rather than LP cell-intrinsic programs alone. However, how immune ecosystems vary across Fan patterns and whether these represent linear progression or distinct biologic states remains unclear.

Principles/Methodology

Spatial transcriptomic profiling was performed using the GeoMx Digital Spatial Profiler on formalin-fixed NLPHL tissues from 10 patients. Total 94 regions of interest (ROIs) included LP cell-rich areas highlighted by MEF2B with rosetting T cells and non-LP-cell regions across A/B, C, and D patterns were selected. Gene expression was analyzed using z-score-based pathway signatures for GC programs, T-cell subtypes, interferon signaling, antigen presentation, and myeloid/macrophage-associated pathways. Differential expressions were assessed with False Discovery Rate (FDR) correction.

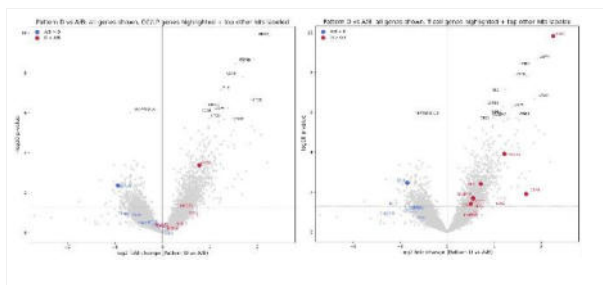
Results

LP-cell-rich ROIs from A/B-patterns demonstrated enrichment of GC/mantle B cell and T follicular helper (Tfh) programs with minimal inflammatory features. In contrast, cases of pattern D exhibited a distinct inflammatory ecosystem characterized by cytotoxic T-cell activation, interferon signaling, antigen processing, and myeloid/macrophage-associated gene expression. Pattern D cases showed a statistically significant overexpression of MYC ($p=0.005$), JAK/STAT ($p=0.03$), and NFkB ($p=0.001$) pathway genes compared to pattern A/B.

Importantly, T-cell subtype composition and immune pathway activity were largely preserved in non-LP ROIs, supporting a tissue-wide ecosystem model rather than phenomena restricted to LP-cell-rich areas. However, MYC pathway loses its significance in non-LP-cell-rich regions ($p=0.057$), while JAK-STAT/interferon and NF- κ B pathway activation extends beyond the immediate LP-cell niche. A single case with predominance of patterns A/B and focal pattern D displayed a hybrid transcriptional profile.

Conclusion

NLPHL comprises distinct, non-linear immune ecosystems spanning GC/Tfh-dominant, hybrid APC/interferon-enriched, and cytotoxic/myeloid inflammatory states. These findings support a model in which immune microenvironmental reprogramming, rather than linear morphologic progression, underlies disease heterogeneity. Hybrid cases may represent transitional states linking the Fan A/B with THRLBCL-like variant.



Differential gene expression between pattern D and pattern A/B LP-rich ROIs. Volcano plots demonstrating differential gene expression between pattern D and pattern A/B LP-rich ROIs. The left panel highlights GC/B cell-associated genes, while the right panel highlights T-cell-associated genes. Genes enriched in pattern D are shown in red, whereas genes enriched in pattern A/B are shown in blue.

HHV-8 AND ONCOGENIC MICROENVIRONMENT: A CLINICOPATHOLOGIC SPECTRUM FROM CASTLEMAN DISEASE TO PLASMABLASTIC LYMPHOMA

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Background

Human herpesvirus-8 (HHV-8) is an oncogenic virus linked to Kaposi sarcoma, multicentric Castleman disease, and HHV-8–related lymphomas. Through cytokine dysregulation, angiogenesis, plasma cell proliferation, and immune evasion, HHV-8 creates a distinctive inflammatory tumor microenvironment that may promote progression from reactive proliferation to lymphoma. This study aimed to characterize the clinicopathologic spectrum of overlapping HHV-8–associated disorders, with emphasis on lymphoma development and microenvironmental features.

Principles/Methodology

We retrospectively evaluated six HHV-8–positive patients (47–74 years; five males, one female) with overlapping HHV-8–associated disorders, including combinations of Kaposi sarcoma, multicentric Castleman disease, multiple myeloma, and plasmablastic lymphoma. Clinical and pathological data, including HIV status and involved sites, were reviewed from medical records and biopsy specimens. Histomorphologic and immunohistochemical analyses, including HHV-8 LANA-1 staining, were performed on lymph node, bone marrow, and tissue samples. Special emphasis was placed on tumor microenvironment features such as plasmacytosis, vascular proliferation, stromal inflammation, and architectural effacement, within the context of HHV-8–related inflammatory and oncogenic mechanisms.

Results

All cases demonstrated HHV-8 positivity by LANA-1 immunohistochemistry within lesional cells and shared prominent inflammatory and vascular microenvironmental features despite differing clinicopathologic presentations. Kaposi sarcoma was identified in five patients and frequently coexisted with plasma cell–rich Castleman disease lesions. Castleman disease cases showed marked plasmacytosis, expanded interfollicular zones, increased vascular proliferation, and inflammatory stromal changes. Two patients with concomitant multiple myeloma exhibited diffuse plasma cell infiltrates, suggesting a possible association between chronic HHV-8–mediated immune stimulation and plasma cell neoplasia. One HIV-positive patient developed plasmablastic lymphoma in association with Kaposi sarcoma, representing the most aggressive end of the HHV-8–associated disease spectrum.

Conclusion

Recognition of this rare association is crucial for accurate diagnosis and appropriate clinical management. We propose that such cases represent a biologic continuum of HHV-8–associated neoplasms, emphasizing the importance of routine HHV-8 evaluation in plasmablastic and plasma cell-rich malignancies.

Ki67 Proliferation Index by AI-Assisted Digital Assessment in Nodal Marginal Zone Lymphoma: Morphological Correlates and T-Cell Microenvironmental Parameters

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Background

Nodal marginal zone lymphoma(NMZL) is a rare indolent B-cell lymphoma lacking reliable markers for risk stratification. Ki67 proliferation index is routinely assessed by immunohistochemistry but remains poorly characterised in NMZL, with known inter-observer variability limiting reproducibility. To evaluate Ki67 distribution in a multi-institutional NMZL cohort, compare conventional pathologist assessment with AI-assisted digital quantification(AADQ), and investigate correlations with morphological parameters, T-cell tumour microenvironment(TME) features, and clinical outcomes including Richter transformation(RT).

Principles/Methodology

Sixty-three newly diagnosed NMZL cases(2016–25; 61.9% female, median age 59) from three tertiary centres; 71.4% excisional, 28.6% core biopsies. Ki67 categorised as low(0–20%), intermediate(21–40%), or high(>40%) by pathologist report and re-evaluated using Virapis AADQ platform(10 hotspot fields/excision; 5/core biopsy), germinal centres excluded. Morphological parameters: growth pattern(nodular, diffuse, interstitial, combined) and LCF(large cell focus, cohesive clusters of ≥ 10 large cells). TME parameters: intratumoral CD3/CD4/CD8 proportion, PD1 scatter, CD10 scatter. BM involvement, plasma cell proliferation, recurrence, and RT recorded(clinical data: 36/63).

Results

Ki67 low in 60.3%, intermediate 17.5%, high 22.2%. Pathologist-AADQ concordance moderate($\kappa=0.444$, $p<0.001$), greatest discordance in intermediate group. Diffuse growth associated with high Ki67($p=0.041$); nodular and combined patterns predominated in low-Ki67. LCF present in 49.2%; 92.9% of high-Ki67 harboured LCF versus 26.3% of low-Ki67($p<0.001$). T-cell proportion correlated with AADQ Ki67($p<0.001$) but not RT status. CD10 scatter ($p=0.019$) and PD1 scatter($p=0.016$) associated with higher AADQ Ki67; PD1 scatter also correlated with pathologist-reported Ki67($p=0.002$). High Ki67 associated with RT($p=0.004$); AADQ values trended similarly($p=0.08$). No significant associations with BM involvement, recurrence, or plasma cell proliferation.

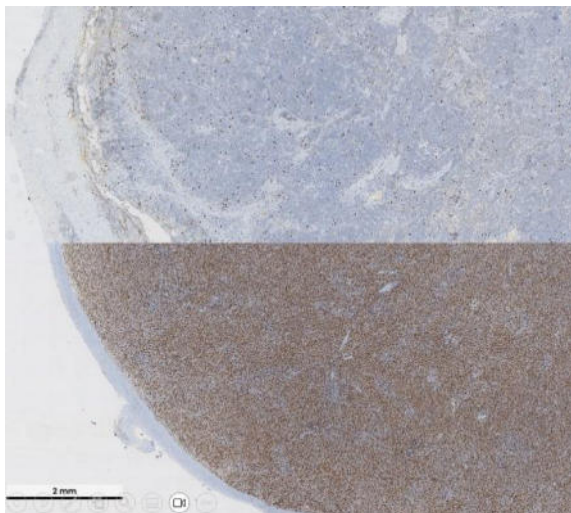
Conclusion

Ki67 in NMZL correlates with morphological features -diffuse growth and LCF - and TME parameters including intratumoral T-cell proportion, PD1 scatter, and CD10 scatter. Association with RT highlights potential as a marker of aggressive evolution. AADQ achieves moderate concordance with expert assessment, with greatest value in the high-Ki67 group. Integrated Ki67 reporting alongside morphological and TME parameters is recommended in routine NMZL evaluation



CD10 scatter in NMZL

In a subset of NMZL cases, CD10-positive cells exhibit aberrant expression or scatter beyond the boundaries of recognisable germinal centres



Ki67 low and high

In NMZL, Ki67 proliferative activity demonstrates considerable inter-case variability, ranging from markedly low levels in some cases to substantially elevated values in others, the latter occurring independently of demonstrable large-cell foci or histological transformation to Richter syndrome

EA4HP26-ABS-580

CD10 expression in nodular lymphocyte-predominant B-cell lymphoma (NLPBL)/nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) : a diagnostic pitfall

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Background

Nodular lymphocyte-predominant B-cell/Hodgkin lymphoma (NLPB/HL) is a distinct entity characterized by large often multinucleated tumor (LP) cells, retaining an intact B-cell program, arranged within a reactive microenvironment containing a variable number of B and T lymphocytes and often surrounded by T follicular helper (TFH) cell rosettes. On immunohistochemistry (IHC), LP cells express germinal center markers such as BCL6 and LMO2, in the absence

of CD10. They co-express OCT2 and BOB1, are commonly EMA+ and show weak or absent CD30 expression, a key feature in the differential diagnosis with classic Hodgkin lymphoma.

Here we present a unique case series of NLPB/HL with typical morphology but in which LP cells show unusual CD10 expression, representing a diagnostic pitfall.

Principles/Methodology

This retrospective single-center study included 406 patients diagnosed in our institution with NLPB/HL between 2015 and 2026, as primary diagnosis or second opinion. IHC and molecular analyses were performed in referring laboratories and completed in our institution.

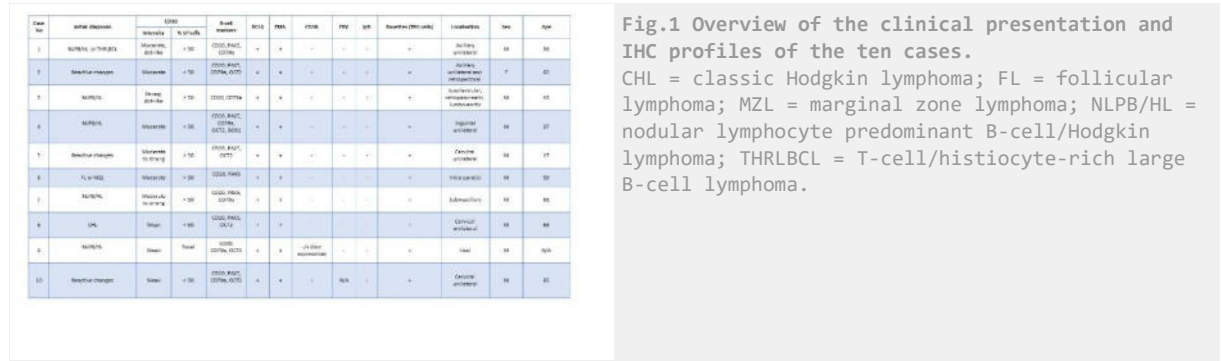
Results

Among the 406 NLPB/HL cases, 10 showed CD10 positivity in LP cells by IHC, with weak to strong staining intensity relative to internal positive controls. Nine cases were diagnosed on excisional biopsy and one on core-needle biopsy. There were 9 male and 1 female patient, with a median age of 56 years (range: 37–68 years). The predominant clinical presentation was unilateral supradiaphragmatic lymphadenopathy, single or multiple. Collection of clinical data on patient outcome is ongoing.

All cases showed a strong and harmonious expression of B-cell markers in LP cells, including CD20+, PAX5+, and when performed CD79a+ and OCT2+. BCL6 and EMA were positive in all cases. Only one case showed weak focal CD30 positivity. LP cells were IgD-negative in all cases. All cases displayed typical rosettes of TFH cells surrounding LP cells, characterized by expression of PD1 and/or CD57. Two cases with Fan pattern D raised differential diagnostic concern with follicular lymphoma, which was excluded by molecular studies.

Conclusion

CD10 expression in LP cells represents an unusual phenotypic variant of otherwise typical NLPB/HL. Although rare, this finding is an important diagnostic pitfall, as 6/10 cases were initially referred with another diagnosis. We show that CD10 expression may occur in typical NLPB/HL cases and that this diagnosis can be retained when clinical presentation, morphology, and overall immunophenotype are consistent with NLPB/HL.



Primary Breast Diffuse Large B-cell Lymphoma: A report from the real-life series at the Institute of Oncology Ljubljana, Slovenia

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¹ Institute of Oncology Ljubljana, Department of Pathology, Ljubljana, Slovenia; ² Institute of Oncology Ljubljana, Department of cytopathology, Ljubljana, Slovenia; ³ Faculty of Medicine, University of Maribor, Maribor, Slovenia; ⁴ University hospital Basel, Institute of Medical Genetics and Pathology, Basel, Switzerland; ⁵ Institute of Oncology Ljubljana, Department for Hemato-oncology, Ljubljana, Slovenia; ⁶ Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Background

Primary breast diffuse large B-cell lymphoma (PB-DLBCL) is a rare extranodal DLBCL, usually aggressive and reported to be mainly non-GC type by Hans algorithm (HA). We aimed to characterize clinicopathological features of 15 PB-DLBCL cases diagnosed over 22 years.

Principles/Methodology

FFPE tissues were analysed by immunohistochemistry (CD20, CD10, CD5, CD30, c-MYC, BCL2, BCL6, MUM1, pSTAT3, pSTAT5, MIB-1), EBV-ISH, and FISH probes (*MYC*, *BCL2*, *BCL6*, *IRF4*, locus 9p24.1). Multiplex immunofluorescence for tumour microenvironment analysis is ongoing. PFS and OS were determined; survival comparison used log-rank test and Cox regression for parameter impact.

Results

Fifteen female patients (32–93 years, median 63) were included; all but one were stage IAE. IPI scores were 0 in 7 and 1 in 8 patients. Diagnosis was via surgical resection (1), excisional (8), or percutaneous biopsy (6). Tumour size ranged 2–12 cm. Histology showed DLBCL in 13 cases and blastoid morphology in 1. According to HA, 9 were GC-type; however, 7 of 9 CD10+ cases expressed MUM1 and BCL6 (triple positive, TP) and 1 case was CD10 and MUM1 double positive. Only 1 case was pSTAT3 positive; all were pSTAT5 negative. *MYC* translocation was present in 3 cases, *BCL6* in 2 (1 with both *MYC* and *BCL6*); no *BCL2*, *IRF4*, or locus 9p24.1 translocations or amplifications of any of the studied genes were identified.

Thirteen patients received anthracycline-based chemotherapy, 10 with rituximab; 1 received radiotherapy and 1 declined treatment. Eleven received intrathecal chemotherapy; 9 of 13 treated patients had consolidation radiotherapy. Complete response (CR) was achieved in 13; 1 had partial response. Progression occurred in 5 treated patients; no CNS involvement was observed. At analysis, 6 patients were alive. Median follow-up was 83 months (range 19–235). Five-year PFS for treated patients was 76.2% (95% CI 64–88.4%), 5-year OS 76.0% (95% CI 63.8–88.2%), and lymphoma-specific survival 84.4% (95% CI 74.2–94.6%). Causes of death: lymphoma (4), infection (2), ischemic heart disease (1), colon cancer (2).

Conclusion

IPI, tumour size >5 cm, HA classification, Ki-67, or *MYC/BCL6* translocations did not significantly influence OS. A relatively high proportion of TP cases was observed compared to literature. Statistical significance was limited by small cohort size and incomplete results. Early-stage disease and optimal therapy are associated with favourable outcomes.

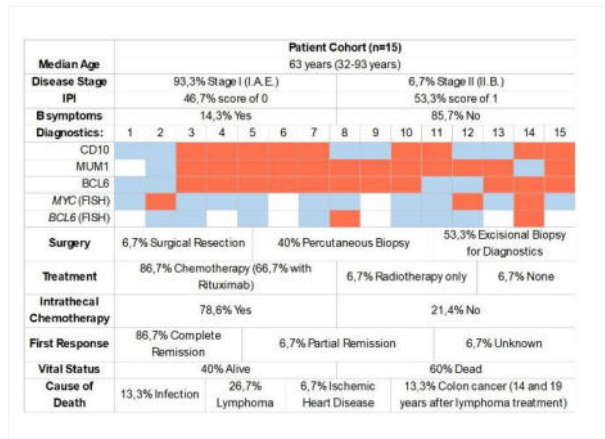


Figure 1. Clinicopathological characteristics of PB DLBCL patients.

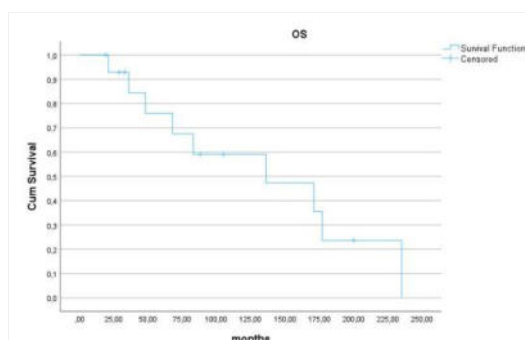


Figure 2. Overall survival for the PB DLBCL cohort

EA4HP26-ABS-586

Abundant T-cells of low diversity distinguish B-cell precursor lymphoblastic lymphoma from leukemia

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Background

B-cell precursor lymphoblastic leukemia (B-ALL) and B-cell precursor lymphoblastic lymphoma (B-LBL) show limited genomic and transcriptomic differences, yet distinct clinical presentations. B-LBL typically presents as tissue-resident tumors whereas B-ALL predominantly involves blood and bone marrow. We hypothesized that differences in the tumor microenvironment (TME) may be associated with these disease patterns.

Principles/Methodology

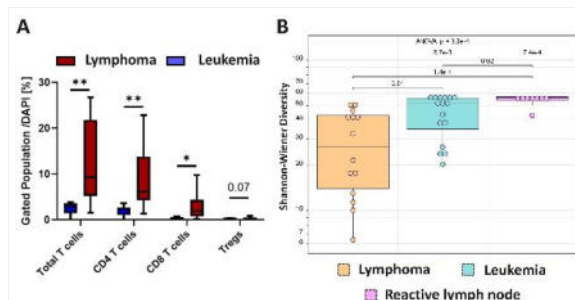
Single-cell spatial immune phenotypisation was performed on FFPE from pediatric B-LBL and B-ALL. The analysis was restricted to extramedullary manifestations to avoid localization/tissue confounders. We established a 50-marker Imaging Cyclic Staining workflow on the MACSima platform and applied it to tissue microarrays. Image analysis included *StarDist*-based single-cell segmentation and *Nimbus*-based marker prediction, followed by *QuPath* quantification and spatial neighborhood analysis. Bulk TCR sequencing was performed to assess TCR-repertoire-diversity.

Results

Single-cell immune profiling revealed a T-cell-enriched microenvironment in B-LBL compared with extramedullary B-ALL. B-LBL showed higher infiltration by CD4-positive and CD8-positive T cells compared to B-ALL (Fig. A). Moreover, B-LBL presented with higher content of CD163-positive macrophages. Interestingly, despite the abundance of T-cell in B-LBL, TCR sequencing demonstrated a significantly lower TCR-diversity of B-LBL compared to B-ALL (Fig. B).

Conclusion

Despite limited molecular-genetic differences, B-LBL and B-ALL differ in their TME when analyzed in identical tissues. We hypothesize, that abundant T-cells of limited diversity represent expansion of selected T-cell in the TME of B-LBL and that lymphoma-immune interaction may explain differences in clinical presentation between B-LBL and B-ALL. Ongoing high-plex spatial proteomic validation will define functional T-cell phenotypes, immune checkpoint expression and cellular interaction networks associated with disease manifestation and localization.



Comparison of T cell count and TCR-Diversity between B-LBL and extramedullary B-ALL.

Figure A: Single-cell quantification of T-cell populations in lymph node biopsies from B-LBL (n = 13) and extramedullary B-ALL (n = 10). Groups were compared using an unpaired Student's t-test. * < 0.05; ** < 0.01.

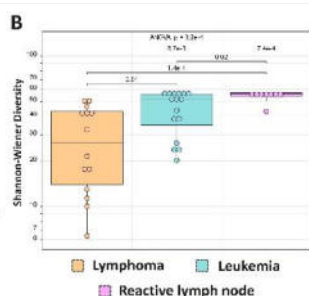


Figure B: Shannon-Wiener diversity of the TCR β repertoire in lymph node biopsies from B-LBL (n = 14), extramedullary B-ALL (n = 18), and reactive lymph nodes (n = 8). Groups were compared using one-way ANOVA with Bonferroni-corrected post hoc tests.

EA4HP26-ABS-593

Clinical utility of automated mRNA dual in situ hybridization (DISH) for B-cell clonality in primary cutaneous follicle centre lymphoma and its mimickers: A study of 51 cases

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Background

Primary cutaneous follicle centre lymphoma (PCFCL) is challenging due to morphological variability and overlapping features with reactive follicular hyperplasia (RFH). Differential diagnosis with primary cutaneous marginal zone lymphoproliferative disorder/lymphoma (PCMZLD/L) and various T-cell lesions, especially primary cutaneous CD4-positive small/medium T-cell lymphoproliferative disorder (PCSM-LPD), is also difficult. Detecting light chain restriction (LCR) via immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) tissues often yields low sensitivity and high background noise. Ultrasensitive branched-chain RNA in situ hybridization (DISH) has emerged as an alternative.

Principles/Methodology

We evaluated the diagnostic utility and sensitivity of automated Kappa/Lambda (K/L) mRNA DISH compared to conventional IHC. A retrospective analysis was conducted on 51 cases (2014–2025): PCFCL (n=23), systemic follicular lymphoma (sFL, n=5), PCFCL vs sFL (n=3), RFH (n=8), PCMZLD/L (n=7), secondary MZL (sMZL, n=2), and PCSM-LPD (n=3). Previous diagnoses relied on morphology and IHC in the clinical setting, with molecular tests (FISH, IGVH) when required. DISH was performed using the automated VENTANA® Kappa and Lambda Dual ISH mRNA Probe Cocktail. LCR was assessed based on monotypic mRNA signals or overwhelming signal ratios. (Note: VENTANA is a trademark of Roche.)

Results

DISH was technically successful in 49 of 51 cases (96.1%); two small, aged samples were non-evaluable due to tissue degradation and signal excess. DISH was conclusive in 83.7% of cases compared to 28.6% by IHC; major improvements were detected in PCFCL, sFL and RFH, where IHC evaluation is typically less sensitive, whereas LCR or polytypia is easier to assess in the other entities. Implementing K/L DSH resulted in a diagnostic change or refinement in 55.1% of cases when compared to IHC: 63.7% PCFCL, 57.1% RFH, 80% sFL, 66.7% PCFCL vs. sFL, 28.6% PCMZLD/L, 0% sMZL, and 33.3% PCSM-LPD. DSH achieved conclusive results in 83.7% of cases: 77.3% PCFCL, 71.4% RFH, 80% sFL and 100% in all other categories. It enabled the reclassification of one PCFCL case to RFH (polytypic pattern in a polyclonal IGVH context). High IGLL5 signal was identified in 36.7% (18/49) of biopsies.

Conclusion

Automated mRNA DSH provides superior sensitivity for assessing B-cell clonality in skin biopsies compared to IHC. Despite limitations related to sample age, size, and IGLL5 confounding, K/L DSH enabled diagnostic refinement in over 50% of cases.

Table. Comparison of the clinical utility of K/L detection by IHC and DSH in 51 skin lesions			
Initial diagnosis (N)	K/L IHC	K/L DSH	Diagnostic change
PCFCL (22)	3/22	17/22	14/22
sFL (5)	0/5	4/5	4/5
PCFCL vs. sFL (3)	1/3	3/3	2/3
RFH (7)	1/7	5/7	4/7
PCMZLD/L (7)	5/7	7/7	2/7
sMZL (2)	2/2	2/2	0/2
PCSM-LPD (3)	2/3	3/3	1/3
Total number	14/49	41/49	27/49

Abbreviations: PCFCL: primary cutaneous follicle centre lymphoma; sFL: systemic follicular lymphoma; RFH: reactive follicular hyperplasia; PCMZLD/L: primary cutaneous marginal zone lymphoproliferative disorder/lymphoma; sMZL: secondary marginal zone lymphoma; PCSM-LPD: primary cutaneous CD4-positive small or medium T-cell lymphoproliferative disorder; IHC: immunohistochemistry; DSH: mRNA dual in situ hybridization.

Table
Comparison of the clinical utility of K/L detection by IHC and DSH in 51 skin lesions

Assessment of Immunoglobulin Light Chain Restriction Patterns via Kappa and Lambda Dual In Situ Hybridization in Diffuse Large B-cell Lymphoma

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Background

B-cell lymphomas often exhibit clonal restriction of immunoglobulin light chains, a key diagnostic feature. Dual in situ hybridization (ISH) for kappa and lambda light chain mRNA can effectively demonstrate this clonality. We applied dual ISH in diffuse large B-cell lymphoma (DLBCL) cases to evaluate light chain restriction patterns and assess their clinical significance.

Principles/Methodology

We identified 91 DLBCL cases diagnosed between 2007 and 2016, collecting clinicopathological data including age, sex, progression-free survival (PFS), and overall survival (OS). Tissue microarrays were constructed from formalin-fixed, paraffin-embedded tumor samples. Cases were classified as germinal center B-cell (GCB) or non-GCB subtypes using immunohistochemistry for CD10, BCL6, and MUM1. Dual ISH for kappa and lambda was performed, and cases were categorized as kappa-restricted, lambda-restricted, dual-expressed, or exhibiting low expression with undetermined restriction. Off-target *IGLL5* signals were also recorded, and associations between ISH results and clinicopathological features were analyzed.

Results

The median patient age was 61 years (range, 18-83), with a male predominance (56.0%, n=51). According to Hans' algorithm, 22 cases (24.2%) were classified as the GCB subtype. Dual ISH revealed kappa restriction in 49 cases (53.8%), lambda restriction in 29 cases (31.9%), dual expression in 4 cases (4.4%), and low expression with undetermined restriction in 9 cases (9.9%), with widely varying signal intensities. These restriction patterns did not differ significantly between the GCB and non-GCB groups and showed no correlation with age, PFS, or OS. Notably, off-target *IGLL5* signals were observed in approximately one-third of the cases (38.5%, n=35), which had no significant impact on survival outcomes.

Conclusion

Dual kappa/lambda ISH in DLBCL demonstrates diverse light chain expression patterns. The frequent occurrence of off-target *IGLL5* signals may complicate clonality interpretation, necessitating cautious evaluation. In this cohort, ISH patterns were not associated with clinical outcomes. Further studies are warranted to clarify the broader clinical relevance of light chain restriction patterns identified via dual ISH in DLBCL.

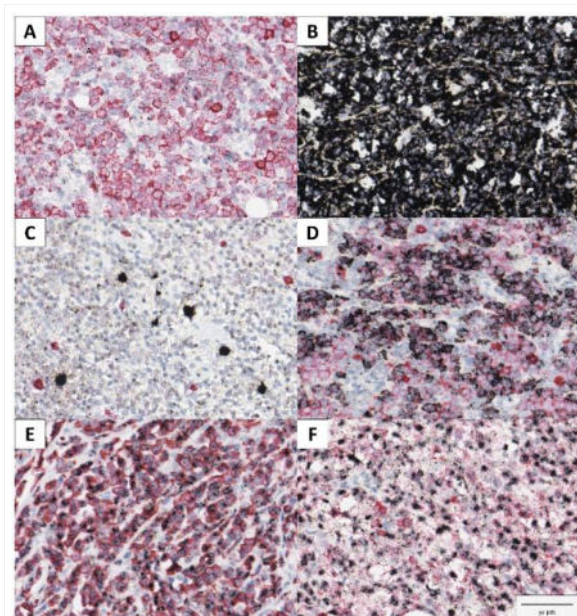


Figure 1. Representative kappa/lambda dual ISH patterns in diffuse large B-cell lymphoma. (A) Kappa restricted pattern, (B) Lambda restricted pattern, (C) low expression with undetermined restriction pattern, (D) Dual expression pattern, (E) Kappa restricted pattern with low IGLL5 off-target signals, and (F) Kappa restricted pattern with high IGLL5 off-target signals.

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Immunohistochemical Profiling of PD-L1 and CD47 in Treatment-Naïve Diffuse Large B-Cell Lymphoma, NOS

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Background

DLBCL NOS is the most common aggressive B-cell non-Hodgkin lymphoma. The tumor microenvironment (TME) plays a critical role in DLBCL pathogenesis, with immune evasion driven by checkpoint molecules PD-L1 and CD47. Within the TME, PD-L1 suppresses adaptive immunity by dampening cytotoxic T-cell activity, while CD47 disables innate immunity by preventing macrophage phagocytosis via SIRPα signalling. Co-expression of these checkpoints may synergistically remodel the TME toward immune tolerance and promote adverse outcomes.

Principles/Methodology

A retrospective cohort of 128 histopathologically confirmed DLBCL patients (January 2015–December 2021) was analyzed. Immunohistochemistry (IHC) was performed on tissue microarray sections using PD-L1 clone 22C3 (Dako), PD-L1 clone SP263 (Ventana), CD47 (D307P, Cell Signaling), and Ki-67 (MIB-1). Immunopositivity was defined as ≥1% tumor cell staining. Statistical analyses included Spearman rank correlation, chi-square/Fisher's exact tests, Mann-Whitney U test, and Kaplan-Meier survival analysis.

Results

PD-L1 positivity differed markedly between clones: 22C3 13.3% (15/113) versus SP263 56.4% (62/110). CD47 positivity was 15.3% (17/111). PD-L1(22C3) and CD47 showed strong co-expression ($p=0.448$, $p<0.001$; OR=11.05, $p=0.0002$), indicating coordinated suppression across both innate and adaptive TME compartments. The two PD-L1 clones were not interchangeable at binary threshold ($p=0.075$). Ki-67 (median 80%) was independent of all

checkpoint markers ($p>0.45$). CD47 positivity was associated with higher IPI risk group ($p=0.011$). No biomarker significantly predicted survival (composite event rate 21.1%).

Conclusion

The strong CD47/PD-L1(22C3) co-expression reflects a coordinated immunosuppressive TME programme operating through complementary innate (macrophage) and adaptive (T-cell) axes, providing biological rationale for dual checkpoint blockade in DLBCL. PD-L1 clone selection critically affects positivity rates and must be specified. Larger prospective studies with comprehensive TME profiling are needed to clarify prognostic and therapeutic implications.

EA4HP26-ABS-609

CD30-Positive Diffuse Large B-Cell Lymphoma of the Breast in a Patient with Lymphocytic Mastitis: Evidence Suggestive of High-Grade Transformation from Extranodal Marginal Zone Lymphoma

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Background

The 2022 WHO HAEM5 classification introduced a category for high-grade transformation of indolent B-cell lymphomas, including extranodal marginal zone lymphoma (EMZL/MALT). Transformation is believed to result from cumulative (epi)genetic alterations but the molecular mechanisms remains unknown. Primary breast lymphomas are rare, and transformation of EMZL in the setting of lymphocytic mastitis has only exceptionally been described.

Principles/Methodology

We report a rare case of extranodal CD30-positive diffuse large B-cell lymphoma (DLBCL) of the breast arising in a female patient with bilateral chronic lymphocytic mastitis. Histopathological and immunohistochemical analyses were performed on formalin-fixed paraffin-embedded (FFPE) tissue sections from surgically excised bilateral breast masses using standard histological techniques and PT LINK immunoperoxidase staining. B-cell monoclonality was assessed by multiplex fluorescent PCR amplification and capillary electrophoresis. Comprehensive genomic profiling was performed using the Illumina TruSight Oncology 500 panel. Detected variants were analyzed using Illumina Connected Insights software, with evaluation of tumor mutational burden (TMB) and microsatellite instability (MSI) status.

Results

Histological examination demonstrated severe bilateral lymphocytic mastitis characterized by confluent periductal CD3-positive mature lymphocytic infiltrates with prominent “onion-skin” fibrosis. Scattered large atypical lymphoid cells were identified in the background and showed positivity for CD20, CD30, and BCL2, while being negative for BCL1, BCL6, MUM1, E-cadherin, ER, PR, and p53. The Ki-67 proliferation index reached 80%. Molecular testing confirmed B-cell monoclonality. Genomic profiling identified two pathogenic alterations with oncogenic or likely oncogenic significance: REL amplification/duplication at 2p16.1 and a frameshift mutation in the FAS gene (p.Val220GlyfsTer6). Additional variants of uncertain significance highlighted the molecular complexity of the disease. The tumor demonstrated microsatellite stability and a low TMB of 3.2 mut/Mb.

Conclusion

Herein we report a potential relationship between chronic lymphocytic mastitis and high-grade transformation to CD30-positive DLBCL, possibly arising from occult EMZL. Comprehensive genomic profiling may provide important

insights into the molecular mechanisms underlying transformation and could contribute to improved classification and therapeutic stratification in rare extranodal lymphomas.

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CPT1A inhibition sensitizes diffuse large B-cell lymphoma to venetoclax treatment

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Background

First-line immunochemotherapy fails to achieve a durable response in up to one third of patients with diffuse large B-cell lymphoma (DLBCL). Therapy resistance is mediated, in part, by ineffective elimination of B-cells protected by metabolic tumor-stroma crosstalk. Carnitine palmitoyltransferase 1A (CPT1A), a component of the prognostic DLBCL-LAMI signature (Staiger et al. 2020), plays a major role in transporting fatty acids – partly provided by stromal cells – into mitochondria to support B-cell metabolism. We investigated the CPT1A inhibitors ST1326 and perhexiline for their ability to eliminate lymphoma cells in the presence of stromal cells and the potential of sensitizing them to the therapeutic BCL2 inhibitor venetoclax.

Principles/Methodology

CPT1A protein expression was evaluated in primary tumor specimens by IHC using TMAs. CPT1A inhibitors and venetoclax were tested on different pre-clinical DLBCL models. Therapeutic efficacy was assessed using MTT cytotoxicity and CellTiter Glo 2.0 assays. Effects on lipid metabolism, molecular signaling and induction of cell death were investigated by oil red O staining, immunoblots and AnxV flow cytometry, respectively.

Results

CPT1A protein was expressed in 51% (95/186) of primary DLBCL (cut-off 20%). Both CPT1A inhibitors impaired the viability of lymphoma cells: perhexiline was cytotoxic in 4/4 cell lines (IC₅₀ = 6–7 μM), ST1326 in 9/13 cell lines (IC₅₀ = 2–10 μM). Viability of normal fibroblasts was not affected by either drug. Perhexiline lost its cytotoxic effect against lymphoma cells when co-cultured with stromal cells. In contrast, ST1326 remained effective in killing DLBCL cells also in stromal environment. Suppression of fatty acid transport into mitochondria by ST1326 resulted in cytoplasmic lipid accumulation and induction of apoptotic cell death. The combination of ST1326 with the BCL2 inhibitor venetoclax increased its cytotoxic efficacy. Three DLBCL cell lines that were BCL2-positive but resistant to venetoclax were sensitized by ST1326 to the BCL2 inhibitor, an effect that persisted in the presence of stromal cells. In patient-derived DLBCL models, CPT1A inhibition impaired the viability of primary DLBCL cells and enhanced the antitumor activity of venetoclax.

Conclusion

Targeting fatty acid metabolism using ST1326 effectively eliminates lymphoma cells in the presence of stromal cells and increases significantly the efficacy of the BCL2-specific inhibitor venetoclax, thus providing a promising new therapeutic approach for treating DLBCL.

Immune Microenvironment Programs Shape Biological Heterogeneity in Diffuse Large B-Cell Lymphoma

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Background

Diffuse large B-cell lymphoma (DLBCL) shows marked biological heterogeneity influenced by both tumor genetics and the lymphoma microenvironment (LME). We investigated the relationships among genetic subtypes, cell-of-origin (COO), immune composition and the spatial organization of the LME.

Principles/Methodology

FFPE biopsies from 5 reactive lymphoid tissues (RLT) and 44 DLBCL cases, including 9 HGBL-DH-*BCL2* were analyzed by targeted DNA sequencing, bulk RNA-seq deconvolution with CIBERSORTx and spatial transcriptomics (Visium) in 4 representative cases. Genetic subtypes were assigned using the 2-S algorithm. Bulk RNA-seq data were used to determine COO, Dark Zone (DZ) status and LME composition using published algorithms.

Results

Bulk transcriptomic analyses identified distinct immune LME programs across molecular and genetic DLBCL subtypes. Compared with RLT, DLBCL samples shifted from GC-like toward mesenchymal and LME-depleted microenvironmental programs (Fig. 1).

Distinct immune profiles emerged across COO and genetic subtypes. GCB-DLBCL displayed a relatively immune-cold microenvironment enriched in naïve B cells, non-proliferative DZ-like cells and resting dendritic cells. In contrast, ABC-DLBCL showed a more immune-activated profile characterized by increased myeloid populations, plasmacytoid and follicular dendritic cells (DCs), plasma cell (PC) precursors and resting CD4⁺ memory T cells. DZ-pos cases were associated with LME-depleted program, enrichment of GC B-cells and increased M0/M2-like signatures, consistent with a more immunosuppressive LME.

At the genetic subtype level, MCD cases showed depletion of TFH-related signals and enrichment of plasmacytoid DCs, EZB showed reduced activated mast-cell signatures, and BN2 were enriched in myeloid and neutrophil-related programs. Relapsed/refractory cases showed increased M2 and decreased PC signatures.

Spatial transcriptomics revealed interpatient heterogeneity and distinct intratumoral immune architectures. Peripheral regions were enriched in activated and proliferating GC-like B cells, whereas central regions proliferative CD4 and myeloid populations, including activated DCs. DH cases exhibited heterogeneous spatial immune programs, ranging from inflammatory, myeloid-rich niches to more immunosuppressive environments (Fig. 2).

DLBCL exhibits a spectrum of LME states linked to molecular, genetic and spatial heterogeneity, highlighting the relevance of both immune composition and tissue architecture in DLBCL biology.

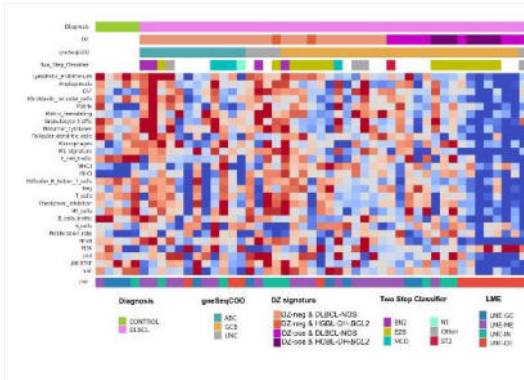


Figure1. Heatmap representation of lymphoma microenvironment programs (Kotlov et al. Cancer Discov. 2021) and immune-related signatures across reactive lymphoid tissues (RLT) and DLBCL samples. Cases are annotated according to diagnosis, Dark Zone (DZ) status, cell-of-origin (COO), genetic subtype (2-S classifier), and LME category (Germinal Center (GC)-like, Mesenchymal (ME), Immune-Depleted (DE), or Immune-Inflamed (IN)).

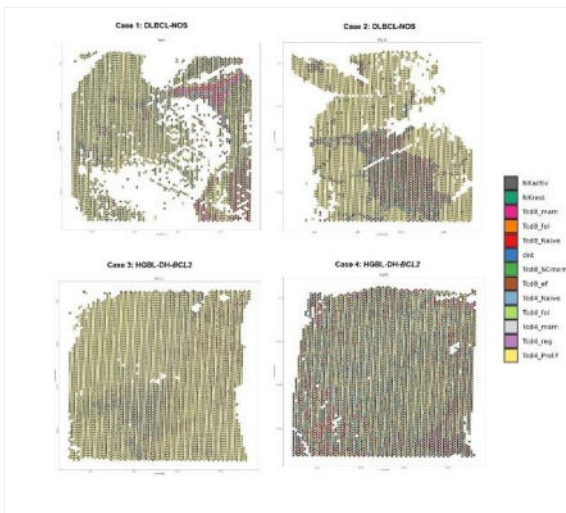


Figure 2. Spatial transcriptomic mapping of the T-cell compartment in representative DLBCL cases
Spatial deconvolution analyzed with the Visium platform (10X Genomics) identified heterogeneous distributions of T-cell and NK-cell populations across tumor regions, including memory, follicular, CD4⁺ and CD8⁺ T cells, effector and Stem Cell-like memory (SCmem) CD8⁺ T cells, regulatory and proliferative CD4⁺ T cells double-negative T cells (dnT), and NK activated and resting cells.

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Dissecting lymphoma complexity by spatially resolved hyperplex profiling

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Background

Lymphomas are hematologic malignancies displaying biological and clinical diversity. This heterogeneity manifests across disease presentation, genetic alterations, tumor microenvironment (TME) composition, and therapeutic response, making accurate diagnosis and risk stratification particularly challenging (PMID 34956859).

Lymphoma evaluation relies on histopathology, single-plex immunohistochemistry, and flow cytometric immunophenotyping, complemented by genetic and molecular profiling. These approaches exhaust specimens, yet fail to fully resolve the spatial and functional complexity of the TME, central to lymphoma pathogenesis, immune evasion, and treatment resistance (PMID 24510338).

Here, we leverage hyperplex immunofluorescence to achieve, on a single section, simultaneous characterization of the cellular composition, TME spatial architecture and heterogeneity across lymphoma subtypes.

Principles/Methodology

A formalin-fixed paraffin-embedded human lymphoma tissue microarray (TMA) was processed on a fully automated tissue staining and imaging system for multiplexed protein profiling (PMID 37813886). The resulting images were analyzed using dedicated software to extract phenotypic features and explore spatial relationships at the cellular level.

Results

The proteomics panel, comprising over 60 targets, including diagnostic, prognostic, and structural biomarkers, enabled concomitant in situ depiction of cellular phenotypes and tissue organization within the same tissue section. Multiplexed immunophenotyping confirmed lineage-specific expression patterns delineating substantial distinctions among the diverse lymphoma subtypes.

Our approach revealed meaningful heterogeneity in tumor and microenvironmental cellular composition, biomarker expression, immune-cell abundance and activation states.

Tissue spatial analysis unveiled cellular localization and interactions, neighborhood structures, and stromal architecture to functionally characterize the complex ecosystem across lymphoma subtypes.

Conclusion

This automated hyperplex workflow reduces sample consumption, offering a path toward scalable translational research and clinical applicability in lymphoma diagnostics. By preserving the tissue architecture while enabling multiplexed biomarker profiling, this method can overcome the limitations of traditional approaches and provide spatially resolved insights to support precise disease stratification and ultimately contribute to personalized treatments.

EA4HP26-ABS-638

Burkitt Lymphoma (BL): the darkest Lymphoma Microenvironment?

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Background

The tumor microenvironment (TME) plays a pivotal role in regulating the biology of germinal center (GC) B-cell lymphomas and immune escape strategies. Although lymphoma microenvironment (LME) has been extensively investigated in B-cell lymphomas, whether TME differences reflect the stage of GC differentiation from which each lymphoma may originate remains poorly understood.

Principles/Methodology

We compared the LME of 8 HGBCL-11q, 8 EBV-negative BL, 5 EBV-positive BL, 4 HGBCL-BCL2r/MYCr and 5 EBV-negative DLBCL-GCB. Gene expression profiling was performed on FFPE samples using the NanoString PanCancer

Immune Profiling Panel and validated by multiplex immunohistochemistry. A newly identified dark zone/light zone (DZ/LZ) spatial signature, including 201 LZ and 169 DZ genes, was applied to further characterize the cohort.

Results

Principal component analysis showed that HGBCL-11q, EBV-negative BL and HGBCL-BCL2r/MYCr grouped together, whereas EBV-positive BL and DLBCL-GCB showed a more distinct profile, based on ANOVA analysis of 365 differentially expressed genes ($p < 0.05$). Supervised clustering analysis of the DZ/LZ spatial signature revealed enrichment of DZ genes in EBV-negative BL, HGBCL-11q and HGBCL-BCL2r/MYCr. Conversely, LZ genes were enriched in DLBCL-GCB and, to a lesser extent, in EBV-positive BL.

Conclusion

Based on the DZ/LZ spatial signature, our results suggest that EBV-positive and EBV-negative BL may arise from different steps of the GC reaction. Further analyses are ongoing to determine whether the observed gene expression differences are mainly related to the TME or to lymphoma cells.

EA4HP26-ABS-642

Immune disparities in the TME of EBV+ and EBV- BL: role of ROS generation pathways and platelet activation

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Background

We previously investigated the immune landscape of Burkitt lymphoma (BL) cases with typical “starry sky” pattern or rare granulomatous reaction, showing distinct immune microenvironments. Here, we extended our gene expression profiling dataset to explore whether EBV status identifies biological differences among BL cases with typical “starry sky” morphology.

Principles/Methodology

Differential gene expression analysis was performed using DESeq2 on 8 EBV-positive and 8 EBV-negative BL cases, selecting genes with $|\log_2 \text{fold change}| > 0.58$ and adjusted $p\text{-value} < 0.05$. Immune deconvolution was performed using CIBERSORTx. Findings were validated by immunohistochemistry in 30 primary BL cases, including 15 EBV-positive and 15 EBV-negative cases, evaluating BZLF1, PSGL-1/SELPLG and P-selectin/SELP. Results were supported by an independent dataset.

Results

EBV-positive and EBV-negative BL showed distinct transcriptional and microenvironmental profiles. EBV-positive cases showed enrichment of NF- κ B, JAK–STAT, B-cell receptor and epigenetic regulation-related genes, together with up-regulation of oxidative stress-related genes, including PTGS2, ARG1 and MAP3K5, consistent with ROS generation, MAPK signaling and viral lytic cycle reactivation. Immune deconvolution suggested a more cytotoxic/innate profile in EBV-positive BL, while EBV-negative cases showed M2 macrophage enrichment. Platelet-related genes were differentially expressed in both groups, but with distinct patterns. EBV-positive cases showed up-regulation of ITGA2B, ITGB3, TREM1, CEACAM8 and SELPLG, suggesting adhesion, aggregation, platelet–endothelial interaction and leukocyte recruitment. Conversely, EBV-negative cases showed higher expression of TGFB1, APOE and NOS2A, suggesting immunoregulation, nitric oxide signaling and stromal remodeling. Focal BZLF1 expression was detected in 7/15 EBV-positive cases. PSGL-1 expression in perivascular lymphoid infiltrates, together with P-selectin staining on endothelial cells and adherent platelets, was observed in 9/15 EBV-positive and 0/15 EBV-negative cases.

Conclusion

EBV status identifies distinct biological programs in BL with typical “starry sky” morphology. Platelet-associated pathways appear to be involved in EBV-positive and EBV-negative BL, but through different molecular profiles. These data suggest that platelet–tumor and platelet–endothelial interactions may represent relevant mechanisms and potential therapeutic opportunities.

EA4HP26-ABS-650

Follicular lymphoma with amorphous deposits within neoplastic follicles

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Background

Follicular lymphoma (FL) with amorphous deposits is a rare variant of FL characterized by the presence of dense, eosinophilic substance within the neoplastic follicles. Recognizing this morphologic aspects is imperative to avoid misdiagnosis like reactive follicular hyperplasia or hyaline-vascular follicles of Castleman disease.

Principles/Methodology

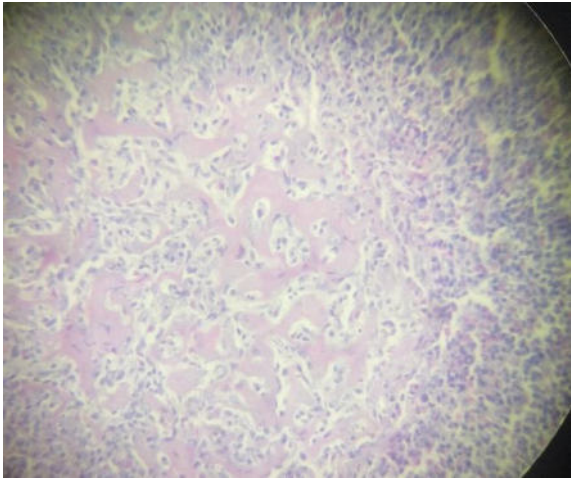
We report the case of a 48-year-old man who presented with abdominal pain. Radiological examination revealed large mesenteric nodes. A laparoscopic excisional biopsy was performed. The initial diagnosis was hyaline-vascular form of Castleman disease. A paraffin-embedded block was sent to us for review. We used hematoxylin-eosin staining and immunohistochemical methods: CD20, CD5, bcl2, CD10, CD23 and CD34.

Results

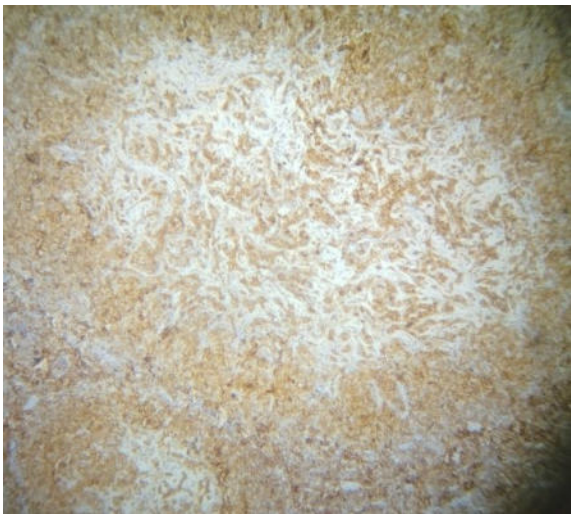
On HE stained slides, the node had a predominantly follicular pattern with effacement of the normal architecture by enlarged follicles with attenuated mantle zones and spread beyond the nodal capsul with sclerosis. The follicles were composed mainly of small centrocytes and a few centroblastes without tingible-body macrophages. Many follicles contained amorphous eosinophilic extracellular material. This material was PAS-positive and negative with Congo red. The neoplastic follicles were positive for CD20 and over-expressed bcl-2. CD5 was negative and the expression of CD10 was low between neoplastic follicles and beyond capsul. The abundance of amorphous substance may resemble the hyaline-vascular follicles of Castleman disease. Bcl2 expression may be difficult to interpret owing to the surrounding of tumoral cells by amorphous deposits

Conclusion

The acknowledgement of these morphological aspects helps to avoid misdiagnosis like reactive follicular hyperplasia, hyaline-vascular form of Castleman disease especially since the traitement and prognosis are different in each disease.



amorphous deposits in neoplastic follicle



BCL2+ in germinal center

Integrated Profiling of the Microenvironment for the Investigation of Subtype-Specific Phagocytic Checkpoint Biology in DLBCL

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Background

Phagocytic immune checkpoints (PICs) are emerging immunotherapeutic targets in diffuse large B-cell lymphoma (DLBCL). However, the spatial contexture of pro- and anti-phagocytic signals, and their relationship with adaptive T-cell neighbourhoods, has not yet been systematically investigated in DLBCL stratified by cell-of-origin subtype (germinal-centre B-cell-like/GCB versus activated B-cell-like/ABC) and underlying genetics. This effort is relevant to informing precision immunotherapy strategies aimed at overcoming resistance to current immune-based therapies in DLBCL.

Principles/Methodology

20 DLBCL cases (10 GCB, 10 ABC) were analysed by multiplex immunofluorescence using panels with 4–6 antibodies assessing pro-phagocytic (e.g. calreticulin, SLAMF7) and anti-phagocytic (e.g. CD24, PD-L1) molecules, and T-cell (e.g. CD4, CD8, PD-1) phenotypes (Fig. 1A). Targeted lymphoid NGS and integrated tissue metrics were used to compare tumour-cell phenotypes, PIC expression, and phagocytic/T-cell immune contexture across DLBCL genomic subtypes.

Results

GCB-DLBCL showed a more phagocytosis-permissive tumour microenvironment, with enrichment of phagocytic effector populations and CD20+ tumour cells expressing higher levels of “eat-me” molecules. In contrast, ABC-DLBCL showed increased expression of anti-phagocytic molecules together with a less favourable tumour-to-phagocyte balance, suggesting a more pronounced phagocytic immune-evasive programme associated with this DLBCL subtype. T-cell profiling further supported an immune-hot GCB microenvironment, with enrichment of activated cytotoxic and checkpoint-associated T-cell phenotypes showing increased inhibitory marker expression (Fig. 1B-D). NGS provided complementary biological insights, revealing distinct mutational patterns between DLBCL subtypes and their association with PIC signalling, as well as with the myeloid and T-cell niches.

Conclusion

This study identifies subtype-specific phagocytic checkpoint biology in DLBCL. GCB-DLBCL appears to be characterised by an immune-hot microenvironment, marked by activated pro-phagocytic signals, whereas ABC-DLBCL is characterised by anti-phagocytic checkpoint programmes together with a T-cell-depleted/immunologically suppressed microenvironment. These findings may provide a rationale for precision immunotherapy approaches with phagocytic checkpoint blockade in DLBCL.

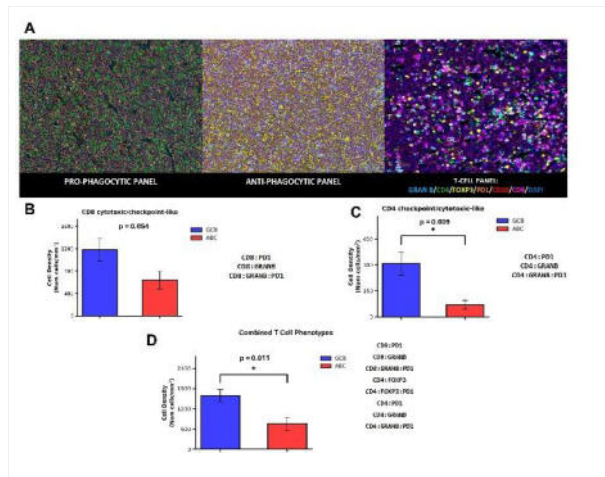


Figure 1. Multiplex immunofluorescent images and T-cell immune profiling
(A) Representative multiplex immunofluorescent images of pro-/anti-phagocytic signals in DLBCL; (B-D) Histograms showing T-cell immune profiling in DLBCL cases characterised for phagocytic signalling, stratified according to cell-of-origin subtypes (GCB versus ABC-like) (Bars show mean $\pm$ SEM; n=10 per group).

EA4HP26-ABS-657

SEQUENTIAL CUTANEOUS TRANSFORMATION OF CYCLIN D1-POSITIVE WALDENSTRÖM MACROGLOBULINEMIA INTO DIFFUSE LARGE B-CELL LYMPHOMA: A CASE REPORT

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Background

Waldenström macroglobulinemia (WM), classified as lymphoplasmacytic lymphoma (LPL), is an indolent mature B-cell neoplasm characterized by bone marrow (BM) infiltration and monoclonal IgM gammopathy. Histologic transformation into diffuse large B-cell lymphoma (DLBCL) is rare, particularly with secondary skin involvement, and is associated with poor prognosis. We report a rare case of Cyclin D1-positive WM with sequential progression from BM disease to cutaneous involvement and subsequent transformation into DLBCL through an intermediate plasmablastic phase.

Principles/Methodology

A 78-year-old male underwent sequential BM and skin biopsies during disease progression between 2024 and 2025. Histopathologic evaluation and immunohistochemical analyses included B-cell markers (CD20, PAX5, BOB1), plasma cell markers (CD38, CD138, MUM1), Cyclin D1, SOX11, BCL2, BCL6, c-MYC, IgM, and Ki67. FISH analyses assessed CCND1 and MYC abnormalities.

Results

Initial BM biopsy demonstrated diffuse infiltration involving 70–80% of marrow cellularity by small lymphoid cells expressing PAX5, BOB1, CD20, IgM, and Cyclin D1, while SOX11 and CD138 were negative. FISH showed no CCND1 rearrangement, supporting Cyclin D1-positive LPL/WM. During disease progression, cutaneous lesions developed. The first skin biopsy showed dermal infiltrates composed predominantly of plasmacytic/plasmablastic cells with partial PAX5 and CD19 expression, loss of CD20, positivity for IgM and MYD88, kappa restriction, and high proliferative activity (Ki67 >80%). FISH demonstrated MYC rearrangement and trisomy of CCND1, suggesting an aggressive plasmablastic phenotype. Subsequent biopsies showed diffuse infiltrates of medium-sized to large

atypical lymphoid cells with centroblastic/immunoblastic morphology expressing PAX5, BOB1, CD20, BCL2, BCL6, and c-MYC, consistent with DLBCL. Additional lesions showed admixed large B-cell and plasmacytic/plasmablastic populations with persistent MYC rearrangement and high proliferative activity, supporting progressive clonal evolution. The disease progressed rapidly, and the patient ultimately died due to cardiac decompensation.

Conclusion

This case demonstrates rare sequential transformation of Cyclin D1-positive WM into DLBCL through an intermediate plasmablastic phase associated with MYC abnormalities. Repeated tissue sampling and integrated morphologic, immunophenotypic, and molecular analyses were essential for identifying disease progression and transformation.

Lymphoma Symposium II | Microenvironment and T-cell lymphoma – Oral

EA4HP26-ABS-544	New advances in the genomic landscape of primary cutaneous CD30+ T-cell lymphoproliferative disorders
EA4HP26-ABS-562	Clonal Architecture and Evolution of T-Follicular Helper Lymphoma Revealed Through Paired Multisite and Longitudinal Sampling.
EA4HP26-ABS-588	Tumor microenvironment analyses of primary intestinal peripheral T-cell lymphomas reveal that enteropathy-associated T-cell lymphoma (EATL) and intestinal peripheral T-cell lymphoma, not otherwise specified (ITCL-NOS), display an immunosuppressive tumor microenvironment
EA4HP26-ABS-597	Deciphering Tumor Microenvironmental Ecosystems in Nodal T-follicular Helper Cell Lymphoma and Peripheral T-cell Lymphoma, NOS by Single-Nucleus Transcriptomics
EA4HP26-ABS-622	β -catenin-independent LEF1 activation in <i>DUSP22/IRF4</i> -rearranged ALK-negative anaplastic large cell lymphoma
EA4HP26-ABS-648	Exploring Heterogeneity in Systemic Anaplastic Large Cell Lymphoma Through Spatial Immune Profiling

New advances in the genomic landscape of primary cutaneous CD30+ T-cell lymphoproliferative disorders

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Background

Primary cutaneous CD30+ T-cell lymphoproliferative disorders (pcLPTCD30), encompassing lymphomatoid papulosis (LyP) and primary cutaneous anaplastic large cell lymphoma (pcALCL), represent the second most frequent group of cutaneous T-cell lymphoid neoplasms/disorders. Despite their usually indolent course, a subset of patients develops aggressive disease with nodal spread or systemic relapse. There are no known genomic biomarkers of aggressive disease, and previous reports have been based on small series.

Principles/Methodology

We report an integrated analysis of a cohort of 43 pcLPTCD30 patients, comprising 22 pcALCL and 21 LyP. Tumor skin biopsies of 17 patients (7 LyP, 9 pcALCL, 1 borderline case) were analyzed using whole-exome sequencing (WES) at a depth of 200× with matched constitutional DNA. To confirm the mutational profiles and altered pathways, we designed a 67-gene targeted panel, which was subsequently applied to pcLPTCD30 skin biopsies in a second cohort of 26 patients. The molecular profile was completed by immunohistochemical staining for pSTAT3, p53, LEF1, and β -catenin, as well as FISH for *DUSP22-IRF4* translocation.

Results

In the WES cohort (n=17), mutational signature analysis revealed a UV-type signature (predominant C>T transitions) in most (80%) of tumors. This UV signature was absent in a series of breast implant-associated ALCL (PMID: 31774495), establishing its specificity for pcLPTCD30. Pathway analysis identified recurrent mutations in genes of the JAK-STAT (53% of cases) and WNT/ β -catenin (47% of cases) pathways. In the validation cohort (n=26), JAK-STAT and WNT/ β -catenin alterations were confirmed in 28% and 33% of cases, respectively. *TP53* mutations were identified in 4 (9.3%) patients across both cohorts and correlated consistently with p53 overexpression by IHC and with aggressive clinical behavior (nodal involvement and/or cutaneous relapse). *DUSP22* rearrangements were detected in 4 cases (9.3%) and were associated with a favorable outcome, with none of the 4 patients requiring chemotherapy or radiotherapy. No correlation was observed between IHC expression of pSTAT3 or β -catenin and underlying pathway mutations.

Conclusion

We highlight, in our study, the novel presence of a UV mutational signature as a hallmark of pcLPTCD30, consolidate the role of JAK-STAT pathway alterations, and underscore the importance of WNT/ β -catenin pathway alterations in their pathogenesis. *TP53* mutations emerge as a candidate molecular biomarker of aggressiveness.

Clonal Architecture and Evolution of T-Follicular Helper Lymphoma Revealed Through Paired Multisite and Longitudinal Sampling.

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Background

T-follicular helper cell lymphoma (TFHL) frequently harbors mutations in *TET2/DNMT3A* commonly associated with clonal hematopoiesis (CH). We aimed to spatially map TFHL clonal architecture and its longitudinal evolution.

Principles/Methodology

22 samples from 82 patients (pts) (47 M, 35 F, median age 66) with TFHL (70 angioimmunoblastic, 5 follicular, 7 NOS) with staging bone marrow (BM) (26 pts), sequential (58 pts) or simultaneous tumor sites (12 pts) were analyzed by high-throughput sequencing (tNGS, 9 to 142 genes) and T-receptor clonality assessment.

Results

The 82 diagnostic biopsies harbored mutations in *TET2* (77/82 pts), *RHOA* (66/82), *DNMT3A* (34/82), *IDH2* (25/82), *CD28* (12/82), and *VAV1* (11/82).

Of the 26 patients with staging BM, 92% (24/26) harboured *TET2* and/or *DNMT3A* mutations (Fig. 1). Eighteen cases showed BM infiltration by pathology: in 11, both *TET2/DNMT3A* and *RHOA/VAV1* mutations in BM were shared with the tumor, whereas in the remaining 7 cases, BM samples contained only CH-associated *TET2/DNMT3A* mutations without tumor-specific *RHOA/VAV1* mutations. In negative (5) or suspicious (3) BM, *TET2/DNMT3A* mutations were frequently detected without tumor-specific signaling mutations. Finally, *TET2* mutations more often contributed to non-CH events than *DNMT3A*, consistent with ongoing acquisition of *TET2* mutations during TFHL progression.

In pts with simultaneous tumor samples, signaling profiles were identical (7/12 pts) or showed only minor subclonal differences (4/12 pts). One case harboured additional lesions associated with concurrent B-cell proliferations.

144 sequential samples (2 to 262 months post-diagnosis) from 58 pts showed four differential patterns: 36% showed stable mutational profiles; 47% had limited gains or losses of mutations (including *IDH2*, *CD28* and *PLCG1*), consistent with subclonal evolution; 9% developed another hematologic neoplasm, mostly B-cell proliferations; and 10% displayed complex evolution, including two patients who developed a second TFHL clonally unrelated with identical CH-associated *TET2/DNMT3A* mutations (Fig. 2).

Conclusion

In summary, while *TET2* and/or *DNMT3A* mutations were found in 94% of our primary TFHL tumors, evidence of CH was missing in 30% of staging-BM. The observed range of courses suggests that TFHL progression follows, in vast majority, stable or branching evolutionary trajectories, reflecting subclonal evolution. Occasionally, divergent clonal tumors emerge from a similar CH background.

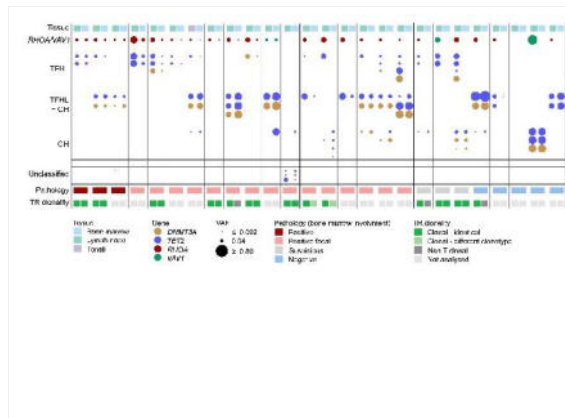


Figure 1 – TET2/DNMT3A and signaling mutations in paired tumor-bone marrow samples (n=26). *RHOA/VAV1* and *TET2/DNMT3A* mutations in samples from 26 pts. BM involvement was classified as positive, focal positive, suspicious, or negative. Assessed TR clonality showed similar clonotypes, different clonotypes or clonal vs non-clonal paired biopsies. *TET2/DNMT3A* mutations were stratified into 4 categories: (1) TFHL-associated, when *TET2/DNMT3A* mutations were detected in both tumor and BM at similar VAFs as *RHOA/VAV1* mutations; (2) TFHL-associated and CH, when present at VAFs $\geq$ than *RHOA/VAV1* mutations; (3) CH only, when VAFs are $<$ than *RHOA/VAV1* mutations; and (4) unclassified.

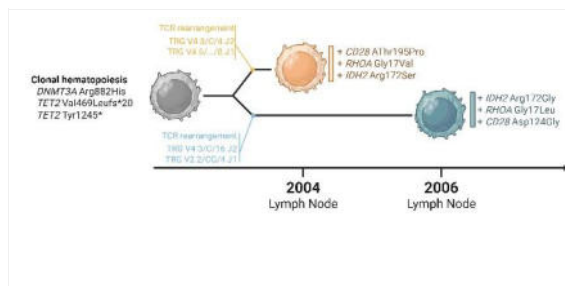


Figure 2 – Divergent clonal evolution from clonal hematopoiesis. Early clonal hematopoiesis, followed by at least two independent TR rearrangements. This divergence gives rise to distinct clonotypes that subsequently evolve into separate tumors: one harboring canonical *RHOA*, *IDH2*, and *CD28* mutations, and a second, clonotypically distinct tumor carrying alternative mutations in *RHOA*, *IDH2*, and *CD28*.

EA4HP26-ABS-588

Tumor microenvironment analyses of primary intestinal peripheral T-cell lymphomas reveal that enteropathy-associated T-cell lymphoma (EATL) and intestinal peripheral T-cell lymphoma, not otherwise specified (ITCL-NOS), display an immunosuppressive tumor microenvironment

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Background

Primary intestinal T-cell lymphomas (ITCLs) are aggressive malignancies comprising enteropathy-associated T-cell lymphoma (EATL), monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL), and ITCL-NOS, which designates tumors that do not fulfill the diagnostic criteria for EATL or MEITL. Our previous works demonstrated distinct genetic, epigenetic, and transcriptional signatures in EATL and MEITL, as well as an immunosuppressive tumor microenvironment (TME) in EATL. Here, we performed a comparative analysis of the TME across the three entities.

Principles/Methodology

A multi-omic dataset of 30 EATL and 52 MEITL was previously published (PMID: 41057685). Twenty-one ITCL-NOS were characterized by IHC including TME-associated markers (CD68, CD163, PD-L1) and HLA-I staining. RNA sequencing was performed in 7 ITCL-NOS cases. Gene expression signatures were analyzed by gene set

Deciphering Tumor Microenvironmental Ecosystems in Nodal T-follicular Helper Cell Lymphoma and Peripheral T-cell Lymphoma, NOS by Single-Nucleus Transcriptomics

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Background

Peripheral T-cell lymphomas (PTCL) are aggressive malignancies associated with poor responses to conventional therapies and frequent relapse. While prior studies have identified altered immune populations within the tumor microenvironment (TME), the multicellular ecosystems and signaling networks that support PTCL progression remain incompletely understood.

Principles/Methodology

We performed integrated multiomic analysis of archived frozen biopsies from 18 nodal T-follicular helper cell lymphoma (TFHL), 9 PTCL, NOS, 2 EBV+ nodal T-cell lymphomas, and 6 reactive lymph node/tonsil controls, using single-nucleus and bulk RNA sequencing and targeted mutational profiling. Unsupervised clustering, cell type annotation, differential expression, and pathway enrichment analyses identified malignant T-cell populations and transcriptionally distinct TME cell states. Matrix factorization and ligand-receptor interaction analyses were performed to define coordinated multicellular ecosystems and signaling programs.

Results

We identified 45 distinct non-malignant cell states in PTCL and controls, revealing extensive remodeling of the PTCL microenvironment. Compared to reactive lymphoid tissue, both TFHL and PTCL, NOS showed reduced total B-cells and antigen-presenting macrophages, enrichment of lymphoma-associated macrophages and exhausted CD8+ T-cells, and stromal remodeling with expansion of lymphoma-specific stromal populations. TFHL additionally demonstrated increased regulatory T-cells and blood endothelial cells, along with marked B-cell remodeling characterized by increased immunoblasts and plasma cells.

Integrated analysis identified recurrent multicellular ecosystems associated with distinct cytokine signaling programs, including two mutually exclusive TME states: an inflammatory macrophage/FDC-associated ecosystem and an immunosuppressive M2 macrophage/cancer-associated fibroblast (CAF) ecosystem enriched for TGFβ signaling. Ligand-receptor analysis further highlighted enhanced TGFβ-TGFBR interactions as a potential driver of PTCL TME remodeling. Functional validation using a murine PTCL model demonstrated that TGFβ blockade significantly reduced tumor burden and cellular proliferation.

Conclusion

Collectively, these findings suggest that PTCL is associated with coordinated tumor-microenvironment interactions shaped by distinct cytokine signaling programs. TGFβ-TGFBR signaling may represent a potential mediator of lymphoma-TME communication and a possible therapeutic target in aggressive T-cell lymphomas.

β-catenin-independent LEF1 activation in *DUSP22/IRF4*-rearranged ALK-negative anaplastic large cell lymphoma

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Background

DUSP22/IRF4-rearranged ALK-negative anaplastic large cell lymphoma (ALCL) is a biologically distinct subgroup characterized by frequent LEF1 expression. The mechanisms underlying LEF1 upregulation remain unclear and may be related either to retained T-cell receptor signaling or to alternative Wnt pathway activation. Recurrent LINC-PINT/miR-29 alterations and DUSP22 downregulation further support a potential role for Wnt-related signaling in this subgroup. We therefore investigated the molecular mechanisms associated with LEF1 expression in DUSP22-rearranged ALCL.

Principles/Methodology

Gene expression profiling was performed in 35 ALK-negative ALCL samples (23 *DUSP22/IRF4*-rearranged) using the TruSight RNA PanCancer Panel (1385 genes) sequenced on an Illumina MiSeq platform. LEF1 immunohistochemistry was available in 32 cases. Normalized expression data were analyzed using GSEA, STRING and Morpheus to identify enriched pathways and explore transcriptional interactions.

Results

Two complementary analyses (*DUSP22*-rearranged vs. non-rearranged, and LEF1-positive vs. LEF1-negative by immunohistochemistry) identified a shared transcriptional signature characterized by overexpression of *WNT10A*, *YAP1*, *EZH2*, *FGF9*, *PLCG1* and *PIK3R1*, with GSEA enrichment of PLC Signaling, Polycomb PRC2 and E2F Target pathways in both phenotypes. *WNT10A* emerged as the most significantly overexpressed gene in LEF1-positive cases and showed the strongest correlation with LEF1, followed by *EZH2*, *FGF9* and *YAP1*. STRING identified a significant protein-protein interaction network including a direct YAP1-LEF1 interaction, while Morpheus demonstrated coordinated clustering, supporting a convergent oncogenic transcriptional program. These findings support complementary β-catenin-independent mechanisms: WNT10A may promote non-canonical Wnt/Ca²⁺/PLC signaling, reinforced by FGF9-associated crosstalk; PIK3R1-associated signaling may contribute to EZH2 activation as a transcriptional cofactor at TCF/LEF regulatory sites; and YAP1 may enhance LEF1 transcriptional activity through direct coactivator interactions.

Conclusion

Our findings suggest a coordinated β-catenin-independent Wnt transcriptional program associated with LEF1 expression in *DUSP22/IRF4*-rearranged ALK-negative ALCL, highlighting *WNT10A*, *YAP1* and *EZH2* as candidate regulatory drivers. Further functional validation is warranted.

Exploring Heterogeneity in Systemic Anaplastic Large Cell Lymphoma Through Spatial Immune Profiling

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Background

Systemic anaplastic large cell lymphoma (ALCL) is a rare, aggressive and biologically heterogeneous T-cell lymphoma. ALK status and recurrent genetic subgroups are clinically informative but do not fully explain outcome heterogeneity. We investigated whether multiplex immunofluorescence (mIF) can identify tumour-immune microenvironment features that refine biological understanding of ALK-positive and ALK-negative systemic ALCL.

Principles/Methodology

Archival FFPE systemic ALCL biopsies were analysed using mIF panels for T-cell, macrophage, NK-cell and myeloid/neutrophil-associated compartments. The T-cell, macrophage and NK-cell panels were tested on a small cohort of cases for optimising antibody markers such as CD8, CD4, CD68, CD56, and establishing the image analysis pipeline. The pipeline was then applied for an exploratory analysis using the neutrophil panel focused on 10 cases (5 ALK-positive, 5 ALK-negative), staining for markers such as CD16. Slide images were acquired by multispectral imaging (Fig 1.) and analysed in QuPath using deep-learning-assisted segmentation and fixed positivity thresholds. Cell phenotypes and densities were compared between ALK groups and, where available, across paired tissue sites.

Results

The mIF workflow detected spatially resolved immune heterogeneity across multiple immune compartments. Exploratory myeloid/neutrophil-associated profiling identified recurrent but variable inflammatory signals, supporting a heterogeneous tumour-immune ecology rather than a simple ALK-defined immune phenotype. Several myeloid-associated features showed differential trends between ALK-positive and ALK-negative cases, suggesting that activated or transitional inflammatory states may contribute to ALCL microenvironmental diversity. In paired tissue-site analysis between the skin and lymph-node, immune readouts varied substantially despite shared disease context, highlighting the importance of tissue site, spatial architecture and tumour-aware analysis in interpreting the ALCL microenvironment.

Conclusion

This exploratory study establishes an mIF/QuPath workflow for in-situ immune microenvironment profiling in systemic ALCL and highlights substantial immune heterogeneity not fully explained by ALK status alone. The findings suggest that myeloid/neutrophil-associated and site-specific inflammatory patterns may contribute to ALCL microenvironmental diversity, warranting validation in larger cohorts with tumour-aware spatial analysis and orthogonal molecular characterisation.

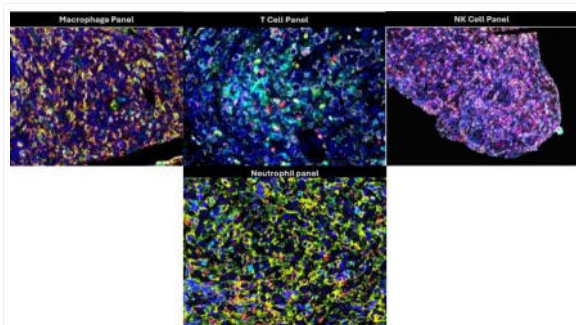


Figure 1: Representative Images of Multiplex Immunofluorescence Panels
Figure 1: Representative Images of Multiplex Immunofluorescence Panels

Lymphoma Symposium II | Microenvironment and T-cell lymphoma – Poster

EA4HP26-ABS-468	Peripheral T-cell lymphoma NOS with dual TCR- β and TCR- δ expression presenting as an incidental anterior mediastinal mass
EA4HP26-ABS-477	COMPLEX KARYOTYPE GAMMA/ DELTA T -CELL PROLYMPHOCYTIC LEUKEMIA LABORATORY VIEW :CASE REPORT
EA4HP26-ABS-490	Loss of SAMHD1 expression, a novel tumour suppressor, is significantly associated with large-cell transformation of mycosis fungoides.
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Peripheral T-cell lymphoma NOS with dual TCR- β and TCR- δ expression presenting as an incidental anterior mediastinal mass

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Background

PTCL-NOS is a heterogeneous group of mature T-cell lymphomas that cannot be assigned to a specific entity according to the latest WHO classification of haematolymphoid tumours. PTCL-NOS mainly affects adults, and can occur in nodal and extranodal sites. The three most common patterns of T-cell receptor (TCR) expression in PTCLs are TCR $\alpha\beta$, TCR $\gamma\delta$ and TCR-silent, although rare instances of dual TCR expression have been documented. PTCL-NOS with dual expression of TCR $\alpha\beta$ and TCR $\gamma\delta$ has only been reported once in a post-heart transplant patient. We present a case of PTCL-NOS with dual TCR β/δ expression in a 58-year-old female with an incidental anterior mediastinal mass.

Principles/Methodology

FFPE tissue of the anterior mediastinal mass was stained with haematoxylin and eosin and relevant immunohistochemical markers for microscopic examination. T-cell clonality for TCRB and TCRG gene rearrangement was performed by PCR.

Results

The patient presented with non-specific symptoms and was discovered to have an incidental mediastinal mass on chest X-ray. PET/CT showed an FDG-avid, anterior mediastinal soft tissue mass measuring 9cm in largest dimension without lymphadenopathy and a VATS biopsy was performed. Microscopically, there were multiple fragments of fibroadipose tissue diffusely infiltrated by a neoplastic lymphoid population comprising medium sized cells with irregular nuclei and scant pale cytoplasm. Focal remnant thymic epithelial elements were present, highlighted by MNF116 immunohistochemical stain. The neoplastic lymphoid cells were diffusely positive for the T-cell markers, CD2 and CD3, with aberrantly weak expression of CD7 and loss of CD5. The neoplastic T-cells were diffusely positive for both T-cell hemireceptors-betaF1 and -delta and the cytotoxic marker, TiA-1. There was weak positivity for CD8 and the Ki-67 proliferative index was 70%. CD4, CD1a, CD10, CD34, TdT and granzyme-B were negative while in situ hybridisation for EBV-encoded small RNA was negative. T-cell clonality yielded monoclonal TCRB and TCRG gene rearrangements supporting the diagnosis of PTCL-NOS with dual TCR β/δ expression. No bone marrow involvement was present. The patient underwent multiple cycles of various chemotherapeutic regimens and radiotherapy showing only a partial radiological response.

Conclusion

This is a unique case of PTCL-NOS with dual TCR β/δ protein expression occurring primarily in the thymus. The mechanism and prognostic significance of dual TCR expression remains to be determined.

COMPLEX KARYOTYPE GAMMA/ DELTA T -CELL PROLYMPHOCYTIC LEUKEMIA LABORATORY VIEW :CASE REPORT

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Background

T-cell prolymphocytic leukemia (T-PLL) is a rare T-cell disorder. Lymphadenopathy, splenomegaly, skin lesions, a high white blood cell count, and aggressive clinical course characterize the disease. We experienced a 67 year –old man diagnosed as TCR gamma/delta T –cell lymphoproliferative neoplasm .

Principles/Methodology

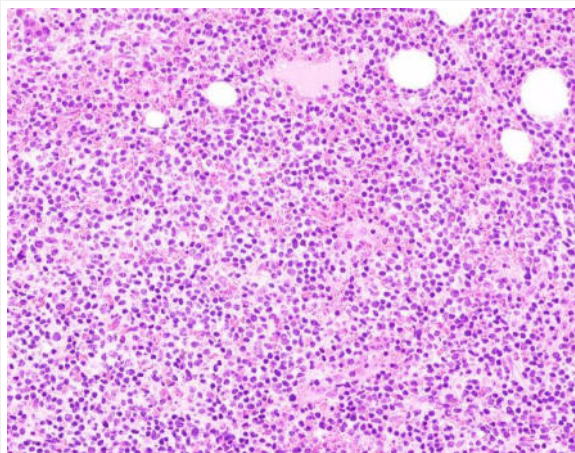
Fever, shortness of breath, and renal impairment, skin eruption at lower abdomen together with lymphadenopathy and splenomegaly were encountered.

Results

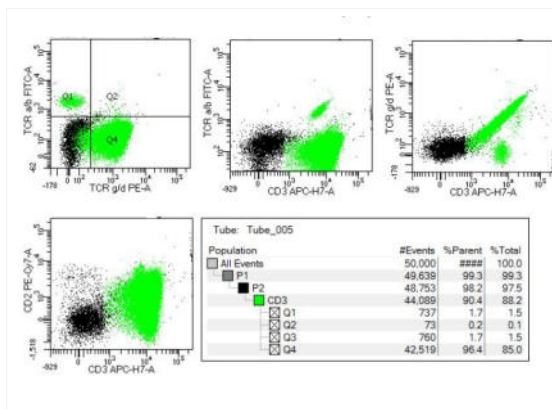
His peripheral blood showed leucocytosis, mainly lymphocytes and thrombocytopenia, platelets $32 \times 10^9/L$ and leukocytes $129 \times 10^9/L$, and hemoglobin values were 9.3 g/dL. Both peripheral blood and bone marrow aspirate were occupied with atypical lymphocytes more than 90%, these lymphocytes are medium to large size with atypical nuclei, high N/C ratio showing basophilic cytoplasm and marked nuclear indenting and clefting (cerebriform cells), with some cytoplasmic blebs some of the lymphocytes show prominent nucleoli and appear more immature (blast-like). The immunophenotype was performed on bone marrow and pleural fluid specimens revealed: positive CD3, CD5, CD7, CD2, CD57, gamma/delta T cell receptor, CD28, CD58, CD27, CD99, CD4, negative markers: CD8, CD30, TDT, CD1a, HLADR, Myeloid, B markers. Other tissue involved with the same infiltration includes cutaneous and lymph node involvement. Bone marrow biopsy: hypercellular for age around 95%, showing sheets of atypical lymphoid cells. No evidence of fibrosis. Immunohistochemical stains show positive: CD2, CD3, CD4, CD5, CD57, CD7, and TCL1. Negative CD20, CD30, TDT, EBER1. Cytogenetic, FISH, and TCR studies conducted and revealed: abnormal FISH results: trisomy 9 and deletion of (9p21) (CDKN2A), T cell receptor (TCR) Gamma gene rearrangement: positive. Cytogenetics and karyotyping: 49,XY,+X,+4,add(5)(p1?3),add(9)(p21),der(9)add(p21)del(9)(q13q22),+der(9)add(p21)del(9)(q13q22),-20,+21[cp3]/46,xy[13]. Serological detection of Human T-cell lymphotropic virus- I and II was negative. This case was typical T-PLL except for the lack of the typical chromosome aberration, where complex chromosomal abnormality detected.

Conclusion

T-PLL is a distinctive post-thymic T-cell malignancy a diagnosis possible in almost all cases based on characteristic clinical, light microscopic, phenotypic, and cytogenetic features. While a chromosome 14 inversion is highly characteristic, additional cytogenetic differences, such as complex karyotype in this case, may account for some case-to-case variation in clinical course.



bone marrow biopsy H&E
Bone marrow biopsy (H&E) with hypercellular marrow for age around 95%, showing diffuse sheets of atypical lymphoid cells.



Bone marrow dot blot Flow cytometry
flowcytometry dot blot shoe abnormal T population with gamma/delta expression

EA4HP26-ABS-490

Loss of SAMHD1 expression, a novel tumour suppressor, is significantly associated with large-cell transformation of mycosis fungoides.

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Background

Mycosis fungoides (MF) is the most common primary cutaneous T-cell lymphoma, clinically characterized by the evolution of patches, plaques and tumours and pathologically by clonal atypical T-cell infiltrates, usually with epidermotropism. Although early-stage MF typically follows an indolent course, large-cell transformation (tMF) is associated with disease progression and adverse outcome. CD30 may be expressed in transformed cells, but its expression is variable and may be absent, underscoring the need for biomarkers that may predict transformation. SAMHD1 is a dNTP hydrolase with multiple roles, including a potential tumour-suppressor role. A limited number of previous studies have shown loss of SAMHD1 expression in primary cutaneous CD30+ T-cell lymphoproliferative disorders (pcCD30+ LPD), however, SAMHD1 protein status has not been investigated in MF or tMF to date.

Principles/Methodology

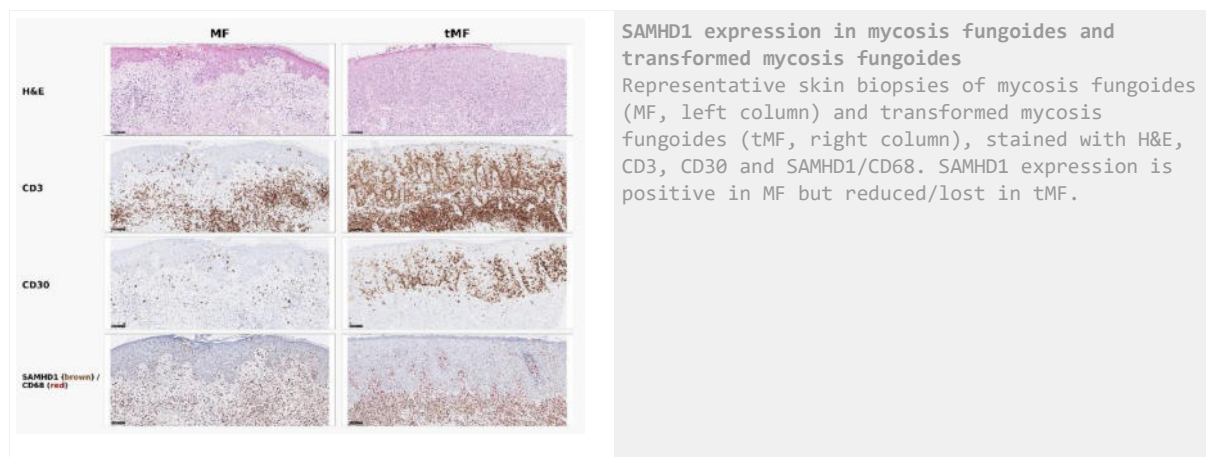
The study group included 12 MF samples, 15 tMF samples and 18 pcCD30+ LPD. Formalin-fixed, paraffin-embedded skin samples were retrospectively reviewed. tMF diagnosis was based on established morphologic criteria according to the 2022 WHO classification. SAMHD1/CD68 double immunohistochemistry was performed using a validated SAMHD1 antibody and Ventana autostainer as reported previously (Rassidakis et al. Blood Cancer J, 2018). CD68-positive histiocytes served as an internal positive control. In lymphoma cells, SAMHD1 expression was scored as positive (>10% positive cells) or reduced/lost (negative).

Results

SAMHD1 was positive in 10/12 (83.3%) MF and 1/15 (6.7%) tMF samples. The difference was highly statistically significant ($p < 0.0001$, Fisher's exact test). In the control group of pcCD30+ LPD SAMHD1 was positive in 2/18 (11.1%) samples and overall, the SAMHD1 expression was significantly less frequent in tMF and pcCD30+ LPD as compared to MF ($p < 0.00001$, Fisher's exact test). Comparison of patients with paired MF and tMF samples ($n=3$) showed that SAMHD1 expression was lost in tMF but not MF. In another patient SAMHD1 expression was lost both in MF and tMF, however the MF sample showed increased number of large cells (10-25%). Taken together, these preliminary results show that loss/reduction of SAMHD1 expression strongly correlates with large-cell transformation.

Conclusion

Loss of SAMHD1 expression strongly correlates with large-cell transformation of MF and therefore it may represent a promising novel prognostic marker in patients with MF.



EA4HP26-ABS-507

Acquisition of a FOXP3-positive regulatory-like state by malignant TFH cells in relapsed TFH lymphoma

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Background

Nodal T follicular helper cell lymphomas (nTFHLs) are mature T-cell neoplasms with substantial morphologic and biological heterogeneity. Whether malignant TFH cells can acquire a regulatory-like phenotype during disease evolution is unknown. We describe a case of nTFHL angioimmunoblastic-type in which the neoplastic TFH population evolved to express FOXP3 at relapse, and we assess the frequency of this phenotype in additional AITL cases.

Principles/Methodology

A 60-year-old woman with advanced AITL underwent serial lymph node biopsies at diagnosis and relapse. The patient was HTLV-1/2 seronegative. Tissue was evaluated by histology, immunohistochemistry, sequential immunofluorescence, PCR-based clonality studies, targeted genomic profiling, and single-cell RNA sequencing. To determine whether the phenotype was recurrent, seqIF analysis was performed in 8 additional nodal AITL cases.

Results

Initial biopsies showed classic AITL with an interfollicular atypical TFH infiltrate expressing CD3, CD4, BCL6, ICOS, and PD-1, with subset CD10 expression, expanded follicular dendritic cell (FDC) meshworks, scattered EBV-positive immunoblasts, and clonal TCR rearrangements. After multiple therapies and relapse, the malignant T-cells acquired a FOXP3-positive regulatory-like phenotype (Fig 1). The tumor cells remained ICOS and CTLA-4 positive, lost PD-1, showed CXCL13 in only a minor subset, and were CD7-dim/negative with reduced sCD3, larger cytology, and high proliferative activity. scRNA seq confirmed a dominant malignant TFH population containing a FOXP3-positive subset enriched for regulatory-associated genes including CTLA4, TNFRSF18, IKZF2, and PRDM1. VIRTUS2 RNA-seq pipeline used to detect and quantify viral sequences detected EBV but no HTLV-1/2 sequences further confirming the absence of HTLV-1/2. The relapse specimen harbored multiple *TET2* mutations (low VAF), together with *PLCG1* and *TP53* alterations. This phenotypic shift coincided with contraction of FDC networks, expansion of EBV-positive B cells, and increased M2-polarized macrophages. In the validation cohort, 2 of 8 additional AITL cases showed similar FOXP3-positive, CD7-negative malignant subsets.

Conclusion

Malignant TFH cells can evolve into a FOXP3-positive regulatory-like state, supporting biologic plasticity within nTFHL recapitulating physiologic programs observed during germinal center contraction. The same phenotype was identified in a subset of additional AITL cases, suggesting it is a recurrent state rather than an isolated event.

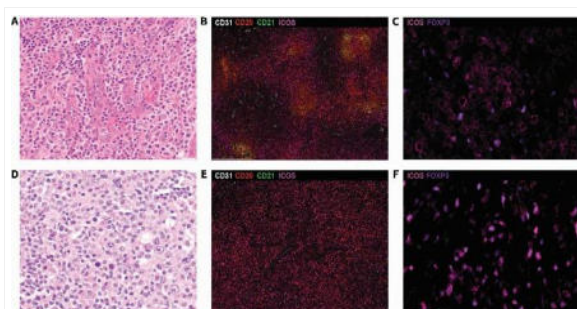


Figure 1. Phenotypic evolution of the malignant TFH population in AITL from diagnosis to relapse (A-C) Baseline nodal biopsy shows AITL with interfollicular atypical T cells, expanded perivascular FDC meshworks, and a rich microenvironment of B cells, ICOS⁺ T cells, and CD31⁺ vessels; only rare neoplastic TFH cells express FOXP3. (D-F) At relapse, AITL persists with marked microenvironmental remodeling, including loss of FDC meshworks and B-cell expansion, while ICOS⁺ T cells and rich vasculature remain. Notably, a major subset of neoplastic TFH cells acquires FOXP3 expression. Markers: CD31, CD20, CD21, ICOS and FOXP3.

Overlap: mycosis fungoides / Sezary syndrome and inflammatory dermatosis, a case series

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Background

To some extent, mycosis fungoides (MF), Sezary syndrome (SS) and inflammatory skin diseases (ISD) share their biology, as being mostly driven by skin homing T-cells, which reflects on overlapping clinic-pathologic features.

Principles/Methodology

Series of patients with ambiguous/overlap features between ISD and MF/SS, routinely diagnosed at a single Institution. *TCRG* clonality assessed according to BIOMED-2 guidelines. Nosologic ambiguity was challenged at clinic-pathologic correlation. Definition of the ISD profile was based on the dominant ISD-like picture, comprising pattern of acanthosis, spongiosis and inflammatory background, exceeding the common histologic traits of MF/SS.

Results

Major presenting features of 11 patients (M/F, 7/4; median age 64y): erythematous/keratotic plaques (6/11), mostly as multiple lesions in the trunk and limbs, and erythroderma (5/11), over a variable clinical course (0.5-31 yrs). Histology showed an eczematous/spongiotic profile in 7 cases, psoriasis or localized scleroderma (LS) in 2 cases each. Mayor clues to lymphoma diagnosis, mostly MF (10/11), with a single psoriasis/SS, included cytologic atypia (10/11), phenotypic aberration (9/11), epidermotropism (10/11), eccrinotropism (2/11) and density of the dermal infiltrate. *TCRG* proved monoclonal in all cases, with evidence of clonal identity at different sites and/or different timepoints in 10/11 cases. Three cases (2 eczema/MF, 1 LS/MF overlap) experienced MF progression, 1 eczema/MF case after cyclosporine administration, but followed by very good response to brentuximab; 1 eczema/MF patient died of septic complications.

Conclusion

Whether the ISD-like manifestations depend on the immunologic function of the neoplastic cells, predispose to neoplasm or act as independent bystanders, possibly favoring the homing of the MF/SS clones, is a matter of debate. The diagnostic conundrum requires close monitoring of the patient, multidisciplinary discussion and careful assessment of histologic pattern and morphology and phenotype of the lymphocytes, in the puzzling setting of inflammation. *TCRG* clonality does not prove neoplasm, but clonal persistence at spaced sites and/or timepoints may supports the diagnosis.

Therapies for MF/SS mostly aim at targeting the malignant cells, but do not address the ISD-like symptoms, which may benefit from a composite approach. On the contrary, with the notable exception of TNF-inhibitors, biologic therapies for ISD are acknowledged as carrying safe profiles.

	Initial suspicion	Final diagnosis	Lesion type and distribution	Major clues to MF/SS diagnosis	Follow up (yrs), status
1	PSO	SS/PSO	Erythroderma, PPK, alopecia	Dense dermal infiltrate, SS-type cells; sequential TCRG assessment	0.5, lost
2	MF	MF/SD	Patches	Epidermotropism, antigen loss	32, AWD
3	LS	MF/LS	Erythematous patches Ulcerated plaques at transformation	Dermal lymphocyte density, pleomorphism at transformation; sequential TCRG assessment	11, tMF, AWD
4	PSO	MF/E	Erythroderma	Lymphocyte atypia, antigen loss; sequential TCRG assessment	0.5, AWD
5	LS	MF/LS	Erythematous plaques	Epidermotropism, CD7-/CD8+ phenotype	0.5, AWD
6	PSO	MF/SD	Erythroderma, keratotic plaques Nodules at transformation	Dermal lymphocyte density and atypia; sequential TCRG assessment	29, tMF, AWD
7	PSO	MF/E	Erythroderma, keratotic plaques	Lymphocyte atypia, epidermotropism and eccrine involvement; sequential TCRG assessment	31, tMF, DUC
8	AD/E	MF/SD	Erythroderma, keratotic plaques	Lymphocyte atypia, epidermotropism and eccrine involvement, antigen loss; sequential TCRG assessment	4, AWD
9	PSO	MF/PSO	Erythematous plaques	Epidermotropism, antigen loss; sequential TCRG assessment	8, AWD
10	AD/E	MF/SD	Erythematous plaques	Lymphocyte atypia, epidermotropism and adnexal involvement; sequential TCRG assessment	5, AWD
11	MF	MF/E	Erythematous plaques	Lymphocyte atypia, epidermotropism	25, AWD

Legend: AD, atopic dermatitis; AWD, alive with disease; DUC, died of unrelated cause; E, eczema; LS, localized scleroderma; MF, mycosis fungoides; PPK, palmo-plantar keratosis; PSO, psoriasis; SD, spongiotic dermatitis; SS, Sezary syndrome; tMF, transformed mycosis fungoides

Table
Clinic-pathologic features of the present series

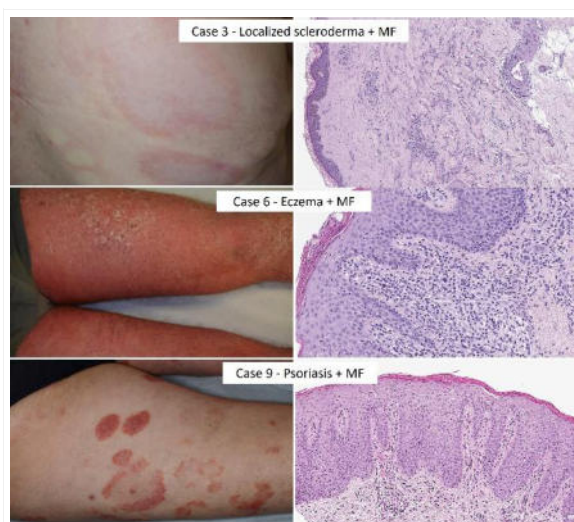


Figure
Representative clinic-morphologic pattern of current series.

EA4HP26-ABS-579

Lennert lymphoma: Clinicopathological study of 11 cases from the Institute of Oncology Ljubljana

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Background

Lennert lymphoma (LL) is a morphologic variant of peripheral T-cell lymphoma (PTCL) defined by diffuse growth, abundant epithelioid histiocytes, and sparse neoplastic cells. Its clinicopathologic and molecular features remain incompletely characterized. We studied LL cases from the Institute of Oncology Ljubljana (1994–2024).

Principles/Methodology

Eleven cases underwent extensive IHC analysis, including T-cell, TFH, TH1/TH2, cytotoxic, and EBV markers. All cases were analyzed by BIOMED-2 PCR, 7 by flow cytometry, and 8 by high-throughput sequencing (HTS; Ion AmpliSeq™ 172-gene panel); 3 remain under analysis.

Results

Six females and 5 males (age 29–85, median 59) were included, all immunocompetent except one with methotrexate-treated sarcoidosis. Clinical data were available for 8 patients. Six patients were stage 4, one stage 1, and one stage 2. All diagnoses were based on excisional lymph node biopsy. Morphology showed medium-sized cells in 6 cases, small-to-medium in 2, and medium-to-large in 3; clear cells were largely absent. Focal high endothelial venules (HEV) and follicular dendritic cell (FDC) remnants were seen in 5 cases each. Lymphoma cells were focally CD30+ in 2 cases, while 8 showed CD30+ IB/RS-like cells; EBV-ISH was positive in B-IB in 5 cases.

Immunophenotype: 9 CD4+, 1 CD8+, 1 CD4+/CD8+. Among TFH markers, PD-1 and ICOS were the most frequently expressed; CXCL13 and BCL6 were partially positive in 4 and 6 cases, CD10 generally negative. All cases were TCRD-negative. TH1/TH2 markers aligned all cases with PTCL-TBX21, with CXCR3 more consistently expressed than TBX21. Cytotoxic granules were detected in two CD4+ and both CD8+ cases. HTS failed in 1 case; 2 showed no mutations. Four cases harbored epigenetic regulator mutations.

Based on IHC, 7 cases were nodal TFH lymphoma, NOS (nTFH NOS), 4 were PTCL, NOS (including 2 cytotoxic). Two CD4+ cases with partial TFH-marker expression were reclassified as nTFH NOS based on HTS. In several TFH cases, dense reactive CD8+/TIA-1+/granzyme B+ infiltrates obscured neoplastic cells. HEV, FDC remnants, CD30+/EBV+ cells, PD-1, and ICOS were more frequent in nTFH NOS. Five of eight patients received COP/modified COP, 2 CHOP-like therapy; 1 missing. Median 5-year OS (n=8) was 60 months (95%CI 57.5–62.4%).

Conclusion

LL morphology may occur across PTCL subtypes; most cases appear nTFHL. Molecular testing may aid classification when tumor cells are sparse or TFH-marker expression limited. Median 5-year OS in this cohort exceeded literature reports.

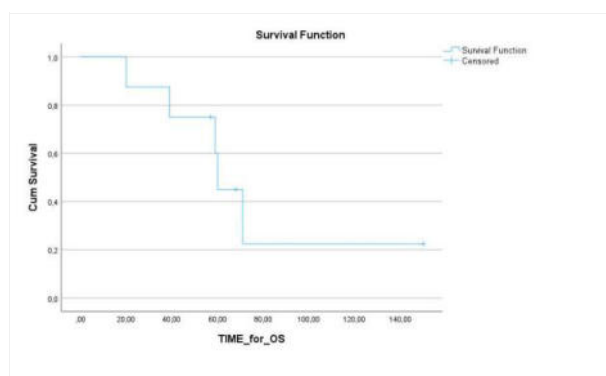
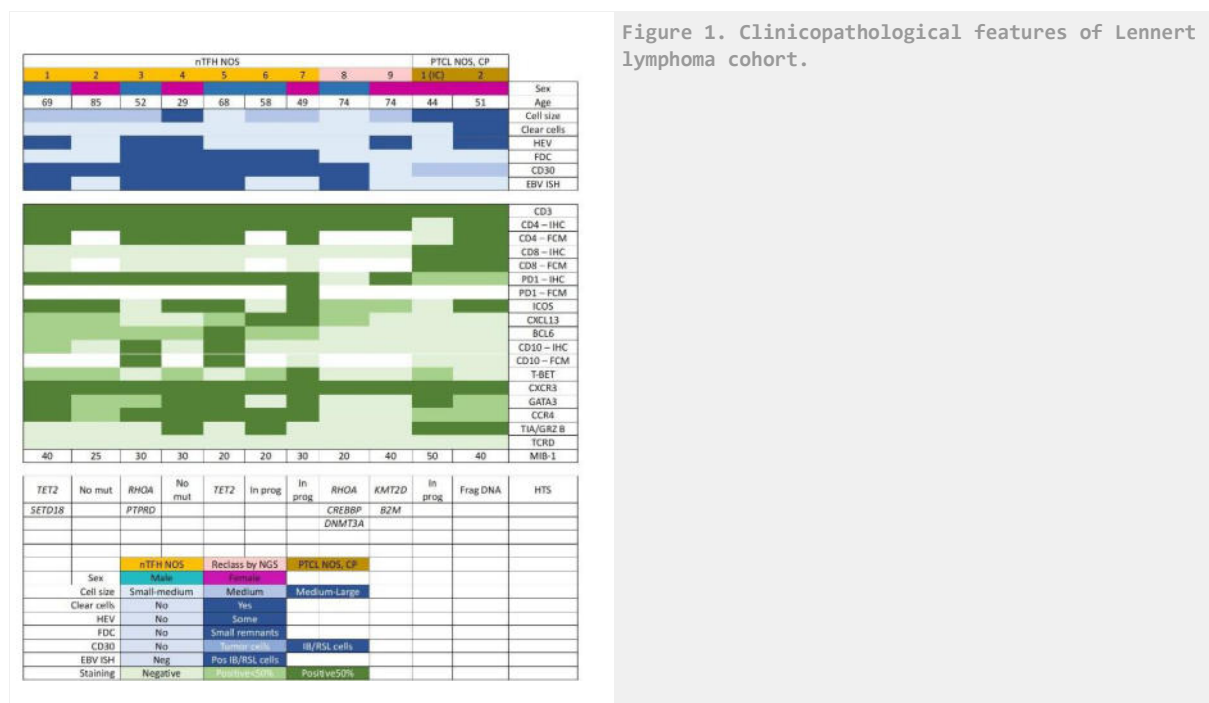


Figure 2. Overall survival of Lennert lymphoma patients



EA4HP26-ABS-583

CCL18⁺CD163⁺ Macrophage-Characterized Eosinophilic Microenvironments: Implications for Inferior PTCL Prognosis

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Background

Peripheral T-cell lymphoma (PTCL) is a rare, aggressive non-Hodgkin lymphoma with high clinicopathological heterogeneity. Some patients frequently experience severe pruritus and allergy-like manifestations, and massive tissue eosinophil accumulation is a prominent tumor microenvironment feature on histopathology. Although eosinophilia is widely linked to such allergic reactions, the deeper clinical implications of this dense infiltration beyond these symptoms, and potential molecular factors affecting this microenvironmental recruitment in PTCL, remain unclear. This study aimed to identify molecular factors associated with eosinophil accumulation in PTCL and investigate their clinical significance and prognostic value.

Principles/Methodology

We utilized three GEO datasets (GSE6338, GSE19069, and GSE58445) to perform transcriptomic profiling and survival analysis. Furthermore, immunohistochemistry (IHC), and multiplex IHC (mIHC) were implemented in real-world clinical PTCL samples.

Results

Transcriptomic intersecting of the GSE6338 and GSE19069 datasets yielded 244 shared up-regulated genes, which were functionally enriched in the “eosinophil chemotaxis” pathway involving CCL2, CCL8, and CCL18 in the GSE58445 dataset. Survival analysis of GSE58445 revealed that high CCL18 expression significantly correlated with shorter overall survival ($P = 0.0014$), but CCL2 or CCL8 were not. In clinical samples, H-score analysis showed that CCL18 protein levels positively correlated with microscopic eosinophil counts ($\rho = 0.499$, $P < 0.001$). Spatial

evaluation via mlHC further revealed that CCL18 protein co-localized with CD163⁺ M2 macrophages, and the tissue proportion of these CCL18⁺CD163⁺ cells was associated with eosinophil recruitment ($p = 0.903$, $P = 0.002$). Notably, in the GSE58445 dataset, patients characterized by concurrent high expression of both CD163 and CCL18 exhibited a significantly worse prognosis ($P = 0.0089$).

Conclusion

Our findings demonstrate that a dense eosinophilic microenvironment in PTCL closely reflects the presence of underlying CCL18-secreting CD163⁺ macrophages, and this combination of histological features effectively identifies a distinct high-risk patient subgroup. In clinical practice, abundant eosinophils on a routine histological slide may thus serve as a simple visual clue, suggesting that the addition of CCL18 staining could help refine risk stratification and identify patients who might ultimately benefit from therapies targeting this specific microenvironmental axis.

EA4HP26-ABS-607

Indolent T-cell lymphoma of the gastrointestinal tract in the CELAC network: frequent CD4-positive T-cell phenotype

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Background

Indolent T-cell lymphoma of the gastrointestinal tract (iTCL-GI) is a rare mature T-cell neoplasm often underdiagnosed because of the nonspecific symptoms and overlap with inflammatory enteropathies and intestinal T-cell lymphomas.

Principles/Methodology

We retrospectively identified patients diagnosed within the French CELAC network mainly in a context of differential diagnosis or refractory celiac disease type 2, diagnostic between 1991 and 2026. Eleven additional patients are under analysis and will be added to the initial 24 cases for the EAHP meeting.

Results

In the initial 24 patients, median age was 51 years (range 22-83). Main symptoms were chronic diarrhea (95.8%), weight loss (83.3%), and malabsorption (70.8%) with a mean diagnostic delay of 55.2 months (range 4-312).

The duodenum was involved in 20/24 patients, and 54.2% had multifocal gastrointestinal disease involving stomach (11/24), ileum (3/24) and colon (6/24). Extra-digestive involvement occurred in 46% of patients, including blood (n=6), lung (n=3), liver (n=4), lymph node and Waldeyer's ring (n=4), skin (n=1) and Kidney (n=1).

Endoscopic showed villous atrophy in 17/24 cases. Histology frequently mimicked celiac disease, with villous atrophy in 20/21 evaluable cases, epitheliotropism in 21/24, crypt hyperplasia in 12/24, lymphocytic cryptitis in 19/24. Tumor cells were mostly small sized with mild irregular nuclei. The phenotype was mainly CD4-positive (17/24), CD8-positive (5/24), or CD4/CD8 double-positive (2/24); CD7 loss was observed in 9/21 cases. JAK-STAT assessment showed pSTAT3-negative pSTAT5-positive profile in 7/9 cases. Clonal TRG rearrangement was consistently detected

Targeted NGS, available for 14 cases showed no pathogenic variant in 8 cases. Main mutations identified were *STAT3*, *JAK3*, *KRAS*, *DNMT3A*, *PRKCB*, *SOCS1*, *TET2*, *CD58* and *SETD2*.

Gluten-free diet, anti-TNF therapy and conventional chemotherapy were largely ineffective. Corticosteroids often induced only clinical improvement, whereas JAK-inhibition achieved symptom control in five treated patients. One patient developed transformation 15 years after diagnosis.

Conclusion

This cohort refines the diagnostic spectrum of iTCL-GI, emphasizing its frequent CD4 phenotype during the diagnostic work-up for refractory celiac disease type 2, potential extra-digestive dissemination, and therapeutic relevance of molecular targeted strategy, particularly JAK-STAT inhibition.

EA4HP26-ABS-608

Molecular genetic diagnosis of pediatric T-cell acute lymphoblastic leukemia in Ukraine: results of a comprehensive diagnostic approach.

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Background

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic malignancy characterized by pronounced molecular heterogeneity and a complex genetic profile. The presence of specific molecular alterations and the aberrant activation of signaling pathways directly influence the clinical course of the disease, making their investigation a key focus for patient molecular stratification and the development of personalized targeted therapies. **The aim** of the study was to characterize the cytogenetic and molecular profile of a pediatric cohort of patients with de novo T-ALL in Ukraine using a comprehensive diagnostic algorithm.

Principles/Methodology

The study analyzed the results of 21 pediatric patients (n=21) under 18 years of age with newly diagnosed T-ALL. The cohort included 17 boys and 4 girls. Bone marrow aspirate samples obtained during the primary diagnosis stage served as the research material. The diagnostic protocol included cytogenetic analysis (GTG-banding), polymerase chain reaction (PCR) for the identification of chimeric oncogenes, and targeted next-generation sequencing (NGS) using a specialized lymphoid panel.

Results

It was established that the most common pathogenic mechanism in the cohort was the activation of the NOTCH signaling pathway: mutations in *NOTCH1* were identified in 11 cases (n=11; 52.4%), *NOTCH2* in 3 (n=3; 14.3%), and *FBXW7* in 7 (n=7; 33.3%). The *STIL::TAL1* chimeric oncogene was verified in 2 cases (n=2; 9.5%), with both patients exhibiting concurrent mutations in NOTCH pathway genes. In 9 cases (n=9; 43%), additional alterations were identified in *CDKN2A*, *BRAF*, *CREBBP*, *KMT2D*, *ETV6*, *NRAS*, *SMARCA4*, *PTEN* and *TP53*. Cytogenetic abnormalities via routine karyotyping were detected in only 8 out of 21 patients (n=8; 38%) in combination with mutations in the NOTCH signaling pathway.

Conclusion

The study results confirm pronounced molecular heterogeneity in the pediatric T-ALL subgroup and indicate a high frequency of co-occurrence between structural chromosomal aberrations, translocations (specifically *STIL::TAL1*), and gene mutations in the NOTCH signaling pathway. Identifying these concurrent genetic events through an integrated approach underscores the critical need for comprehensive molecular genetic testing for accurate patient stratification and the personalization of therapeutic strategies

Clinicopathological and Molecular Spectrum of Type E Lymphomatoid Papulosis: A Series of 8 Cases with Mucosal and Deep Subcutaneous Involvement

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Background

Type E lymphomatoid papulosis (LyP) is a rare primary cutaneous CD30-positive T-cell lymphoproliferative disorder characterised by angiocentric and angiodestructive infiltrates with variable necrosis. Recognition remains challenging owing to histological overlap with angiocentric T-cell lymphoma.

Principles/Methodology

Retrospective review of all type E LyP cases diagnosed at our institutions between 2014 and 2025. Clinical, pathological, immunohistochemical (IHC) and molecular data were analysed.

Results

Eight patients (4 females and 4 males with median age of 45 years) were identified (Table 1).

Lesions predominantly presented as nodules on the extremities (6/8); two patients presented oral mucosa involvement and one had a breast lesion treated with radiotherapy. Management consisted of watchful waiting (n=5) or methotrexate for relapsing disease (n=3). After a median follow-up of 85 months, all remained alive, although one developed mycosis fungoides at 3 years and Hodgkin lymphoma at 8 years post-LyP diagnosis.

Histologically, all cases showed angiocentric medium-to-large atypical lymphoid cells involving the dermis (4/8), dermis and subcutis (3/8), subcutis alone (1/8) or mucosa (2/8); ischaemic necrosis was seen in 2/8.

In 3/8 cases, neoplastic lymphocytes rimmed hypodermal adipocytes. Neoplastic cells uniformly expressed CD3 and CD30, with variable CD4 (5/8) and CD8

(4/8) expression. TCR clonality was demonstrated in all evaluable cases. ALK,

EBER and *DUSP22* rearrangement were negative (Table 2). In all cases, the atypical infiltrate was accompanied by a dense reactive background of small lymphocytes and eosinophils.

Conclusion

Our series confirms that type

E LyP can present with atypical mucosal and deep subcutaneous involvement (rimming), mimicking aggressive lymphomas.

It may precede or coexist with other lymphomas, such as mycosis fungoides and Hodgkin lymphoma. Probably, chronic antigenic stimulation and prolonged immune dysregulation may create a permissive soil that can undergo malignant transformation. Critically, the constant presence of a prominent microenvironment rich in eosinophils and small lymphocytes suggests a host immune response that may modulate the characteristically indolent course of this angiocentric disorder.

Case	Age at Onset (y)	Sex	Type of Lesion	Localization	Onset/Duration (months)	Treatment	Response	Relapse	Follow-up Time (months)	Current Status	Additional Comments
1	12	F	Neuritic	occipital	10	No	Yes	Yes	12	Alive (N)	Increased from 10 to 12 months of follow-up
2	15	M	Neuritic	frontal	10	No	Yes	Yes	18	Alive (N)	Increased from 10 to 18 months of follow-up
3	27	M	Diffuse	frontal and parietal	10	No	Yes	Yes	10	Alive (N)	Increased from 10 to 12 months of follow-up
4	31	F	Neuritic	temporo-parietal	10	No	Yes	Yes	18	Alive (N)	Increased from 10 to 18 months of follow-up
5	43	F	Neuritic	frontal	10	No	Yes	Yes	12	Alive (N)	Increased from 10 to 12 months of follow-up
6	45	M	Neuritic	frontal	10	No	Yes	Yes	12	Alive (N)	Increased from 10 to 12 months of follow-up
7	39	F	Diffuse	frontal	10	No	Yes	Yes	12	Alive (N)	Increased from 10 to 12 months of follow-up
8	9	M	Neuritic	occipital	10	No	Yes	Yes	10	Alive (N)	Increased from 10 to 12 months of follow-up

Table 1: demographic and clinical data of patients
Table 1 contains the summary of demographic and clinical data of patients included into the case series.

	CSE	CDA	CME	CSO	TOL	ALX	BBB	Charity	Corporate management	Governing
1	+	0 ¹	+	+	+	+	+	FCI of professional	Yes	Yes
2	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
3	+	+	+	+	+	+	+	Corporate being professional	Yes	Yes
4	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
5	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
6	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
7	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
8	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
9	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
10	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
11	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
12	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
13	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
14	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
15	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
16	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
17	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
18	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
19	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
20	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
21	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
22	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
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26	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
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43	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
44	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
45	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
46	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
47	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
48	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
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50	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
51	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
52	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
53	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
54	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
55	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
56	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
57	+	+	+	+	+	+	+	FCI of professional	Yes	Yes
58	+	+	+	+	+	+	+	FCI of professional	Yes	Yes

Table 2: immunohistochemical and molecular data
Table 2 contains the summary of
immunohistochemical and molecular data of patients
included into the case series

EBV-Related Biomarkers Define a Clinically Aggressive Subset of Angioimmunoblastic T-Cell Lymphoma

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Peripheral T-cell lymphomas are aggressive neoplasms with limited prognostic biomarkers. Angioimmunoblastic T-cell lymphoma is characterized by neoplastic follicular helper T cells within a complex immune microenvironment frequently enriched in Epstein–Barr virus (EBV)-positive B cells. We specifically investigated the prognostic significance of EBV-related markers in AITL.

A retrospective cohort of 67 AITL patients was analyzed for survival outcomes and clinicopathological features. An independent cohort of 93 additional AITL cases underwent immunohistochemical, mutational and gene-expression analyses to assess EBV-related biomarkers, including EBER and LMP1/LMP2 expression, and their association with clinical outcome.

Results

In the first cohort, moderate/high EBER positivity was associated with poorer overall survival (OS) and progression-free survival (PFS) ($p=0.03$ and 0.06 , respectively), and significantly predicted disease progression within 24 months (POD24). This association remained significant in multivariate analysis ($p=0.022$; $RR=12.57$).

In the second cohort, mRNA expression analysis revealed a significant association between *LMP1/LMP2* (EBV markers), *TP53* expression, cytotoxic markers (*NKG7*, *GZMA*, *PRF1*), and *CD30*, *Ki-67*, and *PD-1*. *LMP1/LMP2* expression was significantly associated with EBER positivity (adjusted BH $p=0.0417$).

Univariate logistic regression identified EBV markers, *TP53* mRNA expression, and *CD30* expression as predictors of a lower probability of achieving complete response (CR) to first-line treatment ($p\leq 0.05$), although these variables did not retain significance in multivariate analysis.

Using Cox proportional hazards models, EBV status (*LMP1/LMP2* expression), *TP53*, *CD30* (mRNA and protein), and TET2 mutations were associated with poorer OS and PFS in univariate analysis ($p\leq 0.05$). In multivariate models including International Prognostic Index (IPI), TET2 mutations together with *TP53* and *CD30* expression remained independent prognostic factors for OS and PFS ($p\leq 0.05$).

Conclusion

These findings demonstrate that EBV-related biomarkers identify a biologically and clinically aggressive subset of AITL characterized by immune activation, cytotoxic signatures, and poor clinical outcome. EBV assessment may provide clinically relevant prognostic information and improve risk stratification in AITL.

EA4HP26-ABS-626

Clonal Hematopoiesis as a potential link between solid tumors and development of Nodal TFH T-cell lymphoma, angioimmunoblastic-type

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Background

Nodal-TFH-cell-lymphoma, angioimmunoblastic-type (AITL) is a mature T-cell neoplasm thought to arise through a multistep process involving early Clonal Hematopoiesis of Indeterminate Potential (CHIP)-mutations in *TET2* and/or *DNMT3A*, followed by secondary mutations in *RHOA* and/or *IDH2*.

Close relationships between AITL and other B, T and myeloid neoplasms have been reported, and recent studies also suggest an increase in certain solid tumors.

Principles/Methodology

We retrospectively recruited 67 patients diagnosed with AITL and reviewed their clinical records to identify secondary solid tumors.

Targeted next-generation sequencing (SureSelect) was performed on 54 AITL samples and 6 solid tumors.

Results

The cohort included 30 males and 37 females (average age at AITL diagnosis of 67 years) (Table 1). Fourteen patients (20.9%) developed an additional solid tumor, and five presented more than one (35.7%). In 10/14 patients (71.4%), solid tumors took place before the AITL diagnosis (Table 2).

44/54 of AITL (81%) harbored *TET2*-variants, and 25 of these 44 (56.8%) presented multiple *TET2*-variants. *DNMT3A*-variants were detected in 21/54 patients (38%), with three cases (3/21, 14%) showing multiple mutations. 47/54 cases (87%) carried CHIP-variants (*TET2* and/or *DNMT3A*) with 18/47 (38.3%) presenting mutations in both genes simultaneously.

Among patients with solid tumors, 12/13 (92%) carried CHIP-mutations. Nine of 12 (75%) harbored more than one CHIP-mutation and 8/12 (66%) showed co-occurrence of *TET2* and *DNMT3A*. CHIP-variants shared with AITL samples were also detected in 2/6 sequenced solid tumors.

The presence of multiple CHIP-variants was associated with solid malignancy (8/54 cases, 14%, p-value=0.02). Similarly, the presence of *DNMT3A*-variants was associated with solid tumors (9/54 patients, 16%, p-value=0.01). The latter association was further supported by an independent cohort.

Conclusion

Our findings reinforce the association between AITL and solid tumors, and suggest for first time a possible role for CHIP in mediating this relationship.

Patients with solid tumors are known to have higher rates of CHIP variants, increasing the risk of hematologic neoplasms. Since solid tumors preceded AITL diagnosis in most of our patients, these AITL could potentially be considered therapy-related neoplasms. Further studies are needed to fully understand how cytotoxic exposure promotes the selection and expansion of CHIP-associated clones leading to AITL development.

Variant of Study		Whole cohort (n=67)	%	AITL with solid tumor (n=14, 20.9%)		AITL without solid tumor (n=53, 79.1%)		χ ² p-value	
				n of cases	%	n of cases	%		
Age (mean, range)		67.13 (26-93)		NA				0.082	
Sex	Male	30	44.8	6	42.85	24	42.18	0.871	
	Female	37	55.2	8	57.14	29	54.71		
Time of solid tumor		Before AITL	NA	10	71.43	NA	NA	NA	
		After AITL	NA	4	28.57	NA	NA		
Presence of multiple solid tumours	Yes	NA	5	35.7	NA	NA	NA		
	No	NA	9	64.3	NA	NA			
Presence of CHIP mutations (<i>TET2</i> and/or <i>DNMT3A</i>)		Yes	47	87.04	12	92.31	35	85.36	1
		No	7	12.96	1	8.33	6	14.63	
Presence of multiple CHIP mutations (<i>TET2</i> and <i>DNMT3A</i>)		Yes	18	33.33	8	61.53	10	24.39	0.02
		No	26	66.66	5	38.46	31	75.61	
Presence of <i>TET2</i> mutations	Yes	44	81.3	11	84.61	33	80.49	1	
	No	10	18.5	2	15.38	8	19.51		
Presence of <i>DNMT3A</i> mutations		Yes	21	38.89	9	69.23	12	28.27	0.01
		No	33	61.11	4	30.77	29	70.73	

Table 1. Resume table of the main descriptives and analyzed results from the study.

Table 1

Resume table of the main descriptives and analyzed results from the study

Results

Comparisons between entities identified genes and pathway alterations consistent with prior reports, confirming cohort representativity. We also confirmed subgrouping within PTCL-NOS consistent with TBX21 and GATA3 gene signatures. To avoid transplant influence on survival, we used a 3-month censoring approach for progression-free survival (PFS), to develop a 35-gene prognostic metagene signature, with the top two tertiles showing a significantly lower risk than the bottom tertile (HR 15.1 95%CI 4.4-51.8 p<0.001). This signature performed similarly for full PFS and overall survival (OS) and remained an independent prognostic factor within histotypes or when combined with the International Prognostic Index (IPI) in multivariable analysis. Its performance was further validated in two independent cohorts from Europe and the US, with consistent and significant association with PFS and OS. The metagene risk score was enriched for B-cell and immune response related genes.

Conclusion

Analyzing a trial-based PTCL cohort molecularly consistent with previous public datasets, we developed a robust prognostic 35-gene metagene signature independent from the standard risk index IPI. This improves risk stratification and expands previous reports highlighting the role played by specific immune components (particularly infiltration by B cells) in patient outcomes.

EA4HP26-ABS-633

Systemic PD1-positive small T-cell lymphoma/lymphoproliferative disorder with intestinal tropism: clinicopathological and molecular characterization

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Background

Clonal indolent T-cell lymphoproliferative disorder of the gastrointestinal tract (ITLPD-GIT) is a rare entity with distinct CD4/CD8 immunophenotypes, recurrent genetic alterations, low proliferation and an indolent course. Atypical transmural or focal nodal involvement is recognized as part of the spectrum. In contrast, PD1-positive ITLPD-GIT cases tend to have systemic dissemination but no apparent aggressive behavior. We aimed to characterize these cases.

Principles/Methodology

We collected cases of indolent PD1-positive T-LPDs with gastrointestinal and/or systemic involvement from multiple institutions. Clinicopathological data, immunohistochemistry, clonality and targeted mutation/fusion analyses were assessed.

Results

Seventeen cases were identified. Clinical data were available in 10 patients: 6 males and 4 females, with a mean age of 51 years (range 19-92). The gastrointestinal tract was involved in 15/17 cases (88%), most often the

colorectum (6/17), followed by stomach and small intestine/ileum (5/17 each). Extraintestinal sites included lymph nodes (4/17), bone marrow (3/17), liver and skin (2/17 each), and single cases involving the epiglottis, esophagus, retroperitoneum, tonsil and orbit. Autoimmune or hematologic conditions accounted for 50% of cases. All cases showed moderate to strong PD1 expression. Most were CD4+/CD8- (10/16, 63%), followed by CD4-/CD8+ (3/16, 19%), double-negative (2/16, 13%) and double-positive (1/16, 6%). Ki-67 was generally low (<10%) in gastrointestinal lesions, but was increased (>10%) in 4 cases, mainly among CD4+/CD8- cases (3/4). All cases showed clonal rearrangements, with identical clones across distinct sites in multifocal cases. Targeted molecular analysis revealed recurrent JAK/STAT pathway alterations, including *STAT3* mutation in 3 cases, a *JAK1* variant of uncertain significance and a *TFG::JAK2* rearrangement. Additional alterations involved *ATM* in 2 cases, as well as in *KMT2D* and *DDX3X*. Two cases transformed to aggressive T-cell lymphoma after 3 and 5 years, one with CD4+ and one with CD4/CD8+ phenotype.

Conclusion

This study shows that PD1-positive ITLPD-GIT, particularly cases with a CD4+ phenotype, may display Ki-67 >10%, systemic dissemination and, rarely, transformation to aggressive T-cell lymphoma. Together with the frequent autoimmune/hematologic background, these findings suggest a distinctive clinicopathological spectrum that should be distinguished from conventional ITLPD-GIT, while avoiding overdiagnosis as PTCL, NOS.

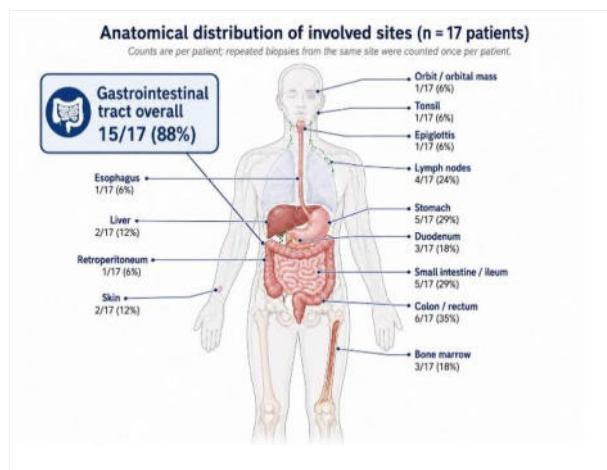


Figure 1. Anatomical distribution of involved sites

EA4HP26-ABS-649

T-cell large granular lymphocytic leukemia (T-LGLL)-like marrow reaction pattern in patients developing cytopenias during immune checkpoint inhibitor therapy

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Background

Immune checkpoint inhibitors (ICIs) have transformed cancer therapy by enhancing tumor-specific T-cell responses, but may disrupt peripheral tolerance and cause immune-related adverse events, including uncommon but potentially severe hematologic toxicities. Because ICI-related cytopenias have a broad differential diagnosis, bone marrow

evaluation is essential to establish the diagnosis, clarify the mechanism and guide treatment. We identified a reproducible marrow reaction pattern mimicking T-cell large granular lymphocytic leukemia (T-LGLL), which may explain pathogenesis and guide management.

Principles/Methodology

Bone marrow biopsies from three patients with clinically significant cytopenias during ICI therapy were reviewed using routine histochemistry and immunohistochemistry. T-cell clonality was assessed by PCR-based TCRB/TCRG assays and by next-generation sequencing (NGS) (Oncomine TCR Pan-Clonality Assay). Hotspot regions of *STAT3* and *STAT5B* were assessed by NGS.

Results

All three biopsies showed markedly hypercellular marrow for age (approximately 90–100%) with nodular and interstitial lymphoid infiltrates comprising ~30–40% of nucleated cells. The infiltrate was predominantly T-cells. CD4+ T-cells and CD20+ B cells formed nodular aggregates, while interstitial CD8+ T-cells co-expressed TIA-1 and CD57 and were negative for perforin and ICOS; PD-1 highlighted nodular CD4+ T-cells but not the interstitial CD8+ population. The interstitial T-cell population was TCRβF1+ and TCRδ-. Hematopoietic changes were consistent with clinical cytopenias, including markedly reduced granulopoiesis, severely decreased erythropoiesis consistent with pure red cell aplasia, and disorganized erythropoiesis with megaloblastoid features. No increase in blasts or metastatic involvement was identified. TCR clonality testing by PCR and NGS demonstrated polyclonal patterns in all cases. Targeted mutation analysis showed no *STAT3* or *STAT5B* mutations. Overall, the findings supported a reactive T-cell expansion closely mimicking T-LGLL.

Conclusion

We describe a reproducible T-LGLL-like marrow reaction pattern, characterized by prominent interstitial cytotoxic phenotype CD8+ T-cell infiltrates with accompanying nodular CD4+ T-cell aggregates and polyclonal TCR profiles. Recognition of this pattern is key to avoid misdiagnosis as chronic T-cell neoplasia and supports a T-cell-mediated mechanism that may justify consideration of T-cell-directed immunosuppression in selected steroid-refractory cases.

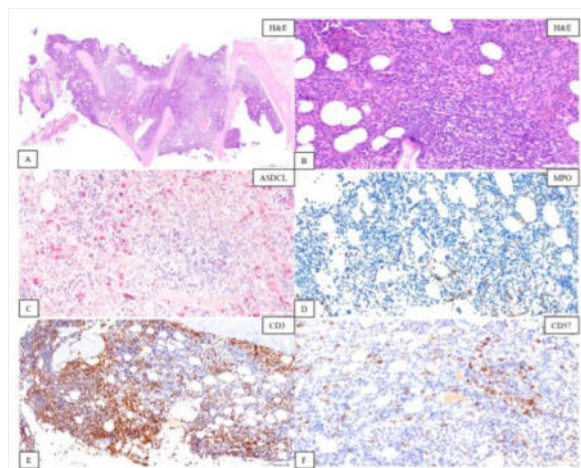


Figure. 1: Case 1.

Case 1. Hypercellular bone marrow biopsy with nodular and interstitial lymphoid infiltrates on H&E (A, B). The lymphoid infiltrate consisted of small lymphoid cells with no significant atypia (B). A marked reduction in granulopoiesis was demonstrated by ASDCL stain and MPO immunostain (C, D). The lymphoid infiltrate predominantly consisted of CD3+ T-cells (E). Expression of CD57 in the interstitial T-cells (F).

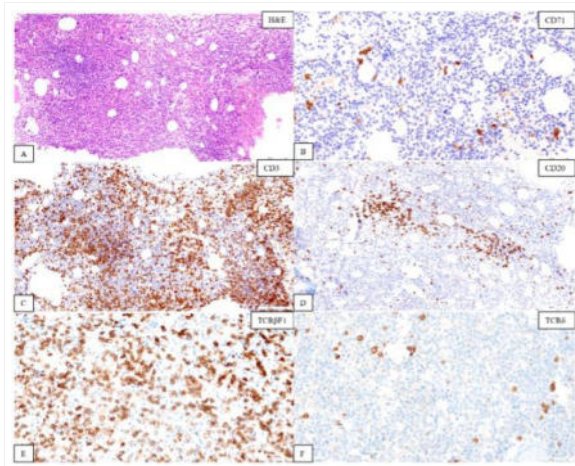


Figure 2. Case 2.

Hypercellular bone marrow biopsy with nodular and interstitial lymphoid infiltrates accounting for 30% of nucleated cells on H&E (A). A marked reduction in erythropoiesis was demonstrated by CD71 (B). Predominant CD3+ T-cells were present in the interstitial and nodular infiltrate (C). CD20+ B-cells in the nodular aggregates (D). The majority of the interstitial T-cells expressed TCRβF1 with scattered TCRδ+ T-cells (E, F).

EA4HP26-ABS-658

Adult T-cell leukemia/lymphoma in French Guiana: preliminary clinicopathologic and microenvironmental profiling with molecular-transcriptomic integration

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Background

Adult T-cell leukemia/lymphoma (ATLL) is an aggressive HTLV-1-associated T-cell malignancy. French Guiana is highly endemic for HTLV-1, and ATLL has been systematically documented. Although genomic studies have mainly focused on Japanese cohorts, recent data indicate distinct Western ATLL molecular patterns. Tissue-based data from French Guiana remain limited.

Principles/Methodology

We included 24 newly diagnosed ATLL cases from French Guiana referred for expert hematopathology review through the LYMPHOPATH network between 2019 and 2026. FFPE specimens underwent centralized re review including morphology, immunohistochemistry, EBER in situ hybridization and selected microenvironment-associated features. Targeted NGS was performed at diagnosis in a subset. Whole-exome capture RNA sequencing (WERS) was completed in 5 cases, with sequencing planned for the remaining cohort.

Results

Median age at diagnosis was 54.7 years (range, 30.3-71.4), with 13 females and 11 males. Available classification included acute and lymphoma-type ATLL; some subtypes remain pending due to ongoing clinical data collection. FFPE specimens involved nodal and extranodal sites, including bone marrow, skin, gastrointestinal tract, nasal fossa and breast. Diffuse architecture predominated (20/28), and median Ki67 was 90% (range, 30-100). Tumor cells were CD3+, CD4+ and CD25+, with frequent CD7 loss or abnormal expression. EBER was negative all specimens. CD30 and PD1 were variable, and background CD20 positive B cells were present in a subset. Among 13 NGS-profiled cases, pathogenic/likely pathogenic variants most frequently involved CCR4 and TP53 (4/13 each), while recurrent copy-number alterations involved CD58 (6/13), CCR4, CDKN2A/B and RHOA (4/13 each). Preliminary WERS profiles showed T-cell transcripts, including CD3 and CD4, and ATLL-relevant transcripts such as IL2RA/CD25 and

CCR4, with case-to-case differences in proliferation, checkpoint, interferon, cytotoxic, B-cell and macrophage-related transcripts.

Conclusion

This preliminary cohort is an expert-reviewed FFPE tissue resource integrating morphology, phenotype, microenvironment features and molecular profiling. Data show tissue heterogeneity, high proliferation, variable microenvironment features and recurrent ATLL related alterations. Completion of WERS across the cohort should strengthen correlations between morphology, immune microenvironment, molecular alterations and expression profiles, and support comparison with published Japanese and Western ATLL cohorts.

Lymphoma Symposium III | Microenvironment and Hodgkin lymphoma – Oral

EA4HP26-ABS-493	Detection of traces of EBNA2 in the Microenvironment of Pediatric Classical Hodgkin Lymphoma Reveals Its Immunomodulatory Role
EA4HP26-ABS-517	Diagnostic ctDNA assay enables HLGEn-based molecular classification of classic Hodgkin lymphoma with preserved tumor microenvironment features.
EA4HP26-ABS-554	Immune topography and dynamics of cytotoxicity in Hodgkin lymphoma.
EA4HP26-ABS-604	Dissecting the Single-Cell Spatial Transcriptomic and Proteomic Landscape of Classic Hodgkin Lymphoma in People Living with HIV
EA4HP26-ABS-628	Multiplex immunohistochemistry profiling reveals immune microenvironment heterogeneity in untreated Hodgkin lymphoma in older patients from the clinical trial PVAB (LYSA)

Detection of traces of EBNA2 in the Microenvironment of Pediatric Classical Hodgkin Lymphoma Reveals Its Immunomodulatory Role

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Background

The detection of Epstein–Barr virus (EBV) traces using ultrasensitive techniques in B-cell lymphomas previously classified as EBV-negative by conventional methods suggests a broader role for the virus in lymphomagenesis. Its contribution in classical Hodgkin lymphoma (cHL) remains unexplored. This study aimed to characterize viral transcripts expression by sensitive methods.

Principles/Methodology

A total of 48 pediatric cHL cases were analyzed. EBV status was evaluated with the conventional approach: EBER in situ hybridization (ISH). LMP1 and EBNA2 transcripts were assessed using highly sensitive dual ISH combined with CD30 immunohistochemistry (IHC) to distinguish tumor cells from cells of the tumor microenvironment (TME). IHC evaluated proteins expression for LMP1, CD4, CD8, CD56, CD68, CD163, PD-1, PD-L1 (TME and tumor cells), IL-10, FOXP3, Granzyme B (GrB), TGF-β, and T-bet. Event-free survival was analyzed using the Kaplan-Meier method.

Results

Among the 32 EBERS+ cases, 32 expressed LMP1 transcripts in both tumor cells (LMP1t+) and the TME (LMP1m+), none expressed EBNA2 transcripts in tumor cells (EBNA2t-), and 23 expressed EBNA2 transcripts in the microenvironment (EBNA2m+). In the 16 EBERs-, 10 cases displayed LMP1t+ cells, 11 cases LMP1m+ and 6 EBNA2m+. Remarkably, EBNA2 transcripts were exclusively observed in the TME in all cases. 5 cases did not express any of the analyzed transcripts (Table 1).

PD-L1 expression in the TME was significantly higher in cases expressing EBNA2 transcripts compared to those without EBNA2 expression (p=0.0373, MW test). Similarly, GrB expression was also significantly increased in EBNA2m+ cases (p=0.0012, MW test) (Graphs A-B). The count of IL10+ cells in the groups defined by EBNA2 transcript expression showed a trend (p=0.0768, MW test). Only cases expressing traces of LMP1 in both tumor cells and the TME exhibited a predominant M1 profile (CD68+cells/CD163+ cells> 1.5) (p=0.0339, p=0.0096, Fisher’s exact test). No significant differences in event-free survival were observed according to viral transcript expression (p>0.05).

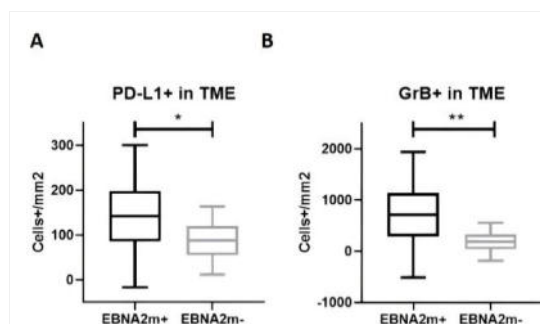
Conclusion

EBNA2 expression in the TME, even in EBV- cases, might be associated with immune checkpoint regulation and cytotoxic responses, highlighting a previously underrecognized role of EBV and its viral traces in shaping the cHL microenvironment. Additionally, LMP1 expression in tumor cells and the TME could contribute to the M1-polarized profile previously described in pediatric EBV- associated cHL.

EBERS by ISH (cases)		Double ISH combined with CD30 by IHC (cases)				Non detection
		LMP1 transcripts in tumor cells (LMP1t+)	LMP1 transcripts in TME (LMP1m+)	EBNA2 transcripts in tumor cells (EBNA2t+)	EBNA2 transcripts in TME (EBNA2m+)	
Positive	32	32 Mean: 29.43 cells/cx/mm2	32 Mean: 24.88 cells/cx/mm2	0	23 Mean: 14.13 cells/cx/mm2	0
Negative	16	10 Mean: 18.28 cells/cx/mm2	11 Mean: 20.22 cells/cx/mm2	0	6 Mean: 15.00 cells/cx/mm2	5

Table 1: Summary of EBERS ISH vs. LMP1/EBNA2 transcripts expression

Positive and negative cases for conventional HIS EBERs, compared with cases expressing the LMP1 and EBNA2 transcripts in tumor cells and in the TME, by dual HIS using the ViewRNA ISH Tissue 2-Plex Assay combined with CD30 by IHC. Abbreviations: LMP: latent membrane protein; EBNA: Epstein-Barr nuclear antigen; EBERs: non-codingRNA associated with the Epstein-Barr virus; ISH: in situ hybridization; IHC: immunohistochemistry.



Graphs: Marker expression.
(A) PD-L1 expression in the tumor microenvironment according to EBNA2 transcript expression status.
(B) Granzyme B (GrB) expression in the tumor microenvironment according to EBNA2 transcript expression status.

EA4HP26-ABS-517

Diagnostic ctDNA assay enables HLGen-based molecular classification of classic Hodgkin lymphoma with preserved tumor microenvironment features.

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Background

Biological stratification of classic Hodgkin lymphoma (cHL) has been challenging due to the scarcity of Hodgkin/Reed-Sternberg (HRS) cells and tissue-related technical limitations.

Aoki et al. recently developed HLGen tool from HRS-cell enriched tissue data, defining four molecular clusters with distinct tumor microenvironment (TME) features and potential therapeutic relevance. HLGen was subsequently applied to ctDNA, but biological and TME validation of derived clusters is lacking.

Our aim was to reclassify patients from a large cHL cohort according to HLGen using diagnostic ctDNA data and to test whether the derived clusters preserved distinctive clinical and TME features.

Principles/Methodology

HLGen was applied to 217 patients from Piroso et al. 2025 cohort with suitable ctDNA molecular data. Patient characteristics and cluster distribution are shown in Fig. 1A-B. Associations with large mediastinal mass (LMM), refractory/relapse (RR) status and B2M mutations were evaluated in the full cohort. Tissue microarrays were constructed for 53 reclassified cases with available tissue, and several TME markers were scored by two

hematopathologists (FJ, AT) (Fig. 1C). Cluster-associated features were assessed using variable-appropriate statistical tests with FDR correction.

Results

76% of cases were assigned to HLGen clusters using our ctDNA assay, while 24% remained unclassifiable. In the full cohort, B2M mutations were enriched in STB cluster, consistent with Aoki findings. RR status did not significantly differ across clusters. LMM was lower in CN913 rather than enriched in CN2P, as previously reported. In the tissue subset, HLGen-derived clusters showed coherent histologic and TME features. Nodular sclerosis histology was enriched in CST, EBER-positive cases in CN913, PD1+ T cells were enriched in CN2P and low in CST, concordant with Aoki findings. Additional patterns included enrichment of TFH cells in CN2P and TIA1+ T cells in CN913. Several features, including Tregs, macrophage polarization and PD-L1 expression, showed concordant trends without reaching statistical significance, likely due to limited sample size.

Conclusion

Our ctDNA assay enables HLGen classification in most cHL patients and recapitulates biological features within clusters. These findings support HLGen classification using our ctDNA assay as a practical upfront molecular classification tool in cHL. Defining biologically driven clusters may provide a comprehensive framework for future tailored therapeutic strategies.

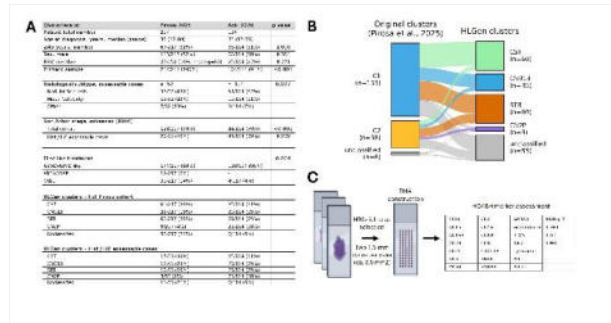


Figure 1
A. Patient characteristics of our cohort compared with Aoki et al. 2026 cohort. B. Alluvial plot showing reclassification according to HLGen. C. Tissue microarray (TMA) construction and immunohistochemistry panel.

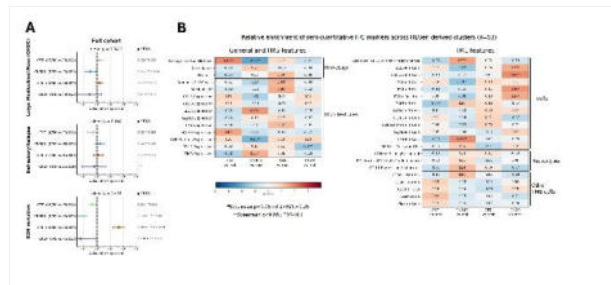


Figure 2
A. Forest plots for clinical and molecular variables in the full cohort. B. Relative enrichment matrix for HRS and TME immunophenotypic features.

Immune topography and dynamics of cytotoxicity in Hodgkin lymphoma.

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Background

Classical Hodgkin lymphoma (cHL) is characterized by a complex tumor microenvironment in which Hodgkin and Reed–Sternberg (HRS) cells coexist with abundant immune infiltrates. Although CD8+ T cells and natural killer (NK) cells are expected to mediate antitumor responses, their contribution in cHL remains paradoxical because of their low abundance, frequent loss of MHC class I expression by tumor cells, and immune dysfunction. We aimed to define the functional states, spatial organization, and clinical relevance of cytotoxic immune populations in cHL.

Principles/Methodology

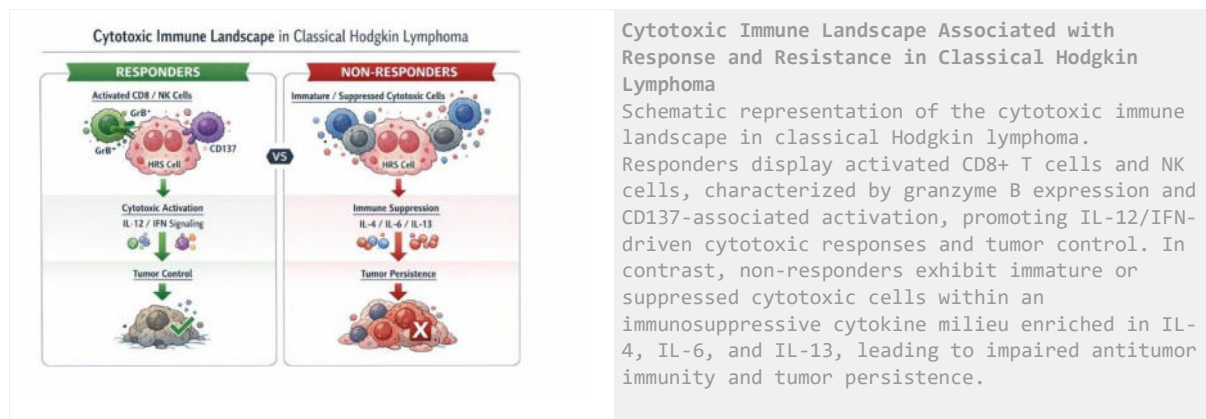
We analyzed 140 cHL cases using multiplex immunofluorescence and digital image analysis on tissue microarrays. Two panels characterized cytotoxic CD8+ T-cell and NK-cell phenotypes using markers of activation, cytotoxicity, inhibition, exhaustion, and immune evasion, including CD30, CD8, granzyme B, LAG3, CD137, PD-L1, and NKG2A. Spatial relationships between cytotoxic cells and HRS cells were assessed and correlated with EBV status, MHC-I/B2M expression, and treatment response. Spatial transcriptomics using GeoMx was performed in an independent validation cohort of 24 cases.

Results

We identified heterogeneous cytotoxic immune landscapes across cHL patients, reflecting patient-specific immune fingerprints. Non-responding patients showed enrichment of immature, inactive, and immunosuppressive CD8+ T-cell and NK-cell populations, whereas activated cytotoxic subsets were associated with favorable outcome. Tumors with MHC-I or B2M loss displayed increased CD8+ T-cell infiltration without parallel NK-cell expansion. EBV-positive cases were enriched in immunosuppressive cytotoxic phenotypes. Spatial analysis revealed distinct immune niches, with activated cytotoxic cells preferentially localized near tumor regions in responding patients. Transcriptomic validation supported these observations and identified cytokine programs associated with cytotoxic activation or local immunosuppression.

Conclusion

The cytotoxic immune landscape of cHL is shaped by functional maturation and spatial organization of CD8+ T cells and NK cells rather than by cell abundance alone. This spatial-phenotypic model identifies immune topographies associated with treatment response, EBV infection, and MHC-I deficiency, providing a framework for improved risk stratification and therapeutic strategies aimed at restoring cytotoxic immune function in cHL.



EA4HP26-ABS-604

Dissecting the Single-Cell Spatial Transcriptomic and Proteomic Landscape of Classic Hodgkin Lymphoma in People Living with HIV

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Background

In the combination antiretroviral therapy (ART) era, the relative risk of classic Hodgkin lymphoma (CHL) among people living with HIV (PLWH) has risen from 8-fold to 13-fold. The CHL tumor microenvironment (TME) in PLWH is unique, showing depleted CD4⁺, CD56⁺, CD57⁺, CD123⁺, and B cells, alongside increased CD8⁺/FOXP3⁺ cells and CD163⁺ macrophages. Using single-cell spatial transcriptomics and multiplex fluorescence immunohistochemistry (mFHC), we mapped the proteogenomic landscapes of CHL in PLWH versus immunocompetent controls to evaluate HIV cellular persistence, transcriptomic variance in Hodgkin and Reed-Sternberg (HRS) cells, and altered TME spatial architecture.

Principles/Methodology

Tissue microarrays from 25 HIV⁺ CHL cases and 21 HIV⁻ CHL controls were analyzed via single-cell spatial transcriptomics (10x Genomics Xenium; 380-gene immuno-oncology panel plus a 100-probe custom add-on for EBV, HIV, and CHL genes). Complementary 6-antibody mFHC panels (including CD30, CD20, CD3, CD4, CD8, GZMB, PD-L1, PD-1, TIM-3, LAG-3, FOXP3, and CD68) are ongoing. HIV⁺ CHL specimens were provided by the AIDS and Cancer Specimen Resource, funded by the NCI.

Results

Low-level HIV transcripts were detected in sparse, non-canonical cell types (CD8⁺ T, plasma, endothelial, myeloid/dendritic, and stromal cells). The HIV⁺ CHL TME showed significant macrophage enrichment and B-cell scarcity compared to HIV⁻ CHL. EBV⁺ HRS cells in HIV⁺ cases significantly upregulated IFNG, CXCL9, CXCL10, GZMB, MX1, C1QB, IL2RA, and NKG7, while TOX2 was enriched in HIV⁻ cases. Within HIV⁻ cohorts, EBV⁻ HRS cells expressed higher CXCL13 than EBV⁺ cells. HIV status also dictated distinct T-cell profiles: in HIV⁺ cases, CD4⁺ T cells were enriched for VSIG4, C1QA, C1QB, and CCL8 (vs. CCL22, CCR4, CCR7 in HIV⁻), and CD8⁺ T cells preferentially expressed CXCL9, CXCL10, VSIG4, MSR1, and C1QB (vs. IL7R, CCR7 in HIV⁻). Detailed spatial transcriptomic and proteomic analyses are ongoing.

Conclusion

Spatial transcriptomics confirms a highly distinct, macrophage-enriched TME in HIV+ CHL and suggests low-level HIV presence in non-canonical cell compartments. Integrating these data with ongoing mIHC profiles will elucidate how HIV and EBV cooperatively reshape the spatial architecture and immune evasion mechanisms of CHL.

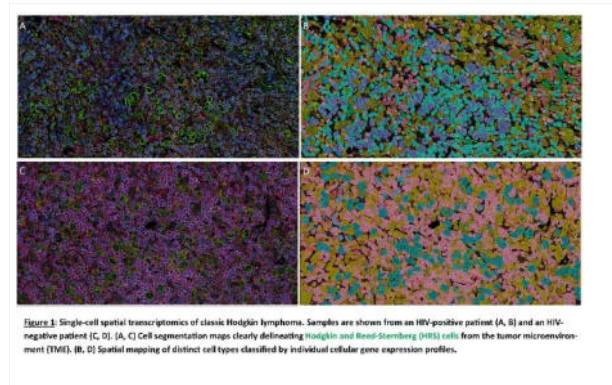


Figure 1
Single-cell spatial transcriptomics of classic Hodgkin lymphoma. Samples are shown from an HIV-positive patient (A, B) and an HIV-negative patient (C, D). (A, C) Cell segmentation maps clearly delineating Hodgkin and Reed-Sternberg (HRS) cells from the tumor microenvironment (TME). (B, D) Spatial mapping of distinct cell types classified by individual cellular gene expression profiles.

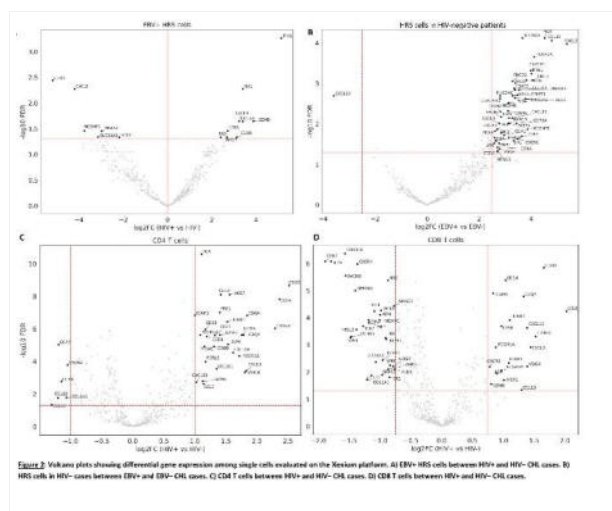


Figure 2
Volcano plots showing differential gene expression among single cells evaluated on the Xenium platform. A) EBV+ HRS cells between HIV+ and HIV- CHL cases. B) HRS cells in HIV- cases between EBV+ and EBV- CHL cases. C) CD4 T cells between HIV+ and HIV- CHL cases. D) CD8 T cells between HIV+ and HIV- CHL cases.

Multiplex immunohistochemistry profiling reveals immune microenvironment heterogeneity in untreated Hodgkin lymphoma in older patients from the clinical trial PVAB (LYSA)

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Background

Classical Hodgkin lymphoma (cHL) is characterized by rare Hodgkin and Reed–Sternberg (HRS) cells within a rich tumor microenvironment (TME). Older cHL patients (>60 years) have worse outcomes than younger patients suggesting a different pathogenesis with for instance a frequent association with Epstein-Barr Virus (EBV). The composition of TME has been reported to be associated with patient outcomes but few studies have focused on older patients.

Principles/Methodology

We retrospectively analyzed 67 cHL patients aged >60 years of the 89 included in the LYSA PVAB phase II trial (NCT02414568). Centralized pathological review was performed for all cases. Pretreatment FFPE biopsies were analyzed using six-panel multiplex immunohistochemistry (mIHC) on the Akoya platform, enabling assessment of immune cell subsets (CD3, CD4, CD8, CD68, CD163, CD20, XCR1, Nkp46, CD80, CXCR5, CXCL13, FoxP3, GrzB), immune checkpoint expression (PD1, LAG3, TIM3, TIGIT, IDO1, VISTA, CTLA4, GITR) and HRS cells (CD30, MUM1, CMH-I, CMH-II, PD-L1). AI-assisted image analysis (Indica Labs) was applied. Clinical and histological (including EBV status and cHL subtypes) characteristics, response to therapy and outcome were collected.

Results

Median overall survival (OS) was 39 months and median progression-free survival (PFS) was 28.5 months. Most patients were <70 years (64%). Nodular sclerosis was the predominant subtype (n=46, 69%), followed by mixed cellularity (n=11, 16%). Tumor associated with EBV was found in 32 cases (48%). Unsupervised clustering of mIHC data revealed marked TME heterogeneity. Preliminary analyses suggest distinct immune profiles, one more immune-enriched TME characterized by increased infiltration of CD3 T-cells (CD4, FOXP3, CD8, GrzB), expression of immune checkpoint molecules (PD-L1, CTLA4, LAG3, TIM3, VISTA, IDO1, PD1), macrophage-associated populations (CD163, CD68, CXCL13), and CD20 B-cell. Another profile was tumor-cell-oriented, characterized by a higher density of CD30+MUM1+ tumor cells with reduced immune cell density.

Conclusion

These data demonstrated substantial TME heterogeneity regarding immune-reactive cells, the expression of immune proteins, and HRS cell density among older cHL patients. Further analyses integrating mIHC data with clinical characteristics, metabolic response to the PVAB regimen, and outcomes are ongoing to identify patient subgroups and refine prognostic and therapeutic stratification.

Lymphoma Symposium III | Microenvironment and Hodgkin lymphoma – Poster

EA4HP26-ABS-530	STING Expression by the T-Cells of the Tumor Microenvironment (TME) Surrounding the Neoplastic Hodgkin and Reed-Sternberg Cells May Reflect the Systemic T-cell Responses in Classical Hodgkin Lymphoma with Potential Therapeutic Implications.
EA4HP26-ABS-531	Capabilities and Limitations of Open-Source Vision–Language Models in Hodgkin Lymphoma Diagnosis and Education in Hematopathology
EA4HP26-ABS-537	Immunohistological comparison of lymphocytes in the tumour microenvironment between EBV-positive and EBV-negative classical Hodgkin lymphoma.
EA4HP26-ABS-570	PAX5-negative Classic Hodgkin-like Lymphoma: A Diagnostic Challenge
EA4HP26-ABS-587	The crucial role of CD4 T cells in the development of Hodgkin lymphoma: a novel disease-defining framework
EA4HP26-ABS-636	The tumor microenvironment of EBV+ Diffuse Large B-cell Lymphoma and EBV+ classical Hodgkin Lymphoma

STING Expression by the T-Cells of the Tumor Microenvironment (TME) Surrounding the Neoplastic Hodgkin and Reed-Sternberg Cells May Reflect the Systemic T-cell Responses in Classical Hodgkin Lymphoma with Potential Therapeutic Implications.

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Background

Classical Hodgkin lymphoma (CHL) is characterized by the presence the neoplastic Hodgkin and Reed Sternberg cells (HRS) (usually <1%) and inflammatory, T cell-rich, tumor microenvironment (TME) that seems to contribute to cHL pathogenesis. In particular, T-cells of the TME are often localized in close proximity to HRS cells forming "rosettes". STING, an adaptor protein involved in anti-tumor immune responses through activation of the cGAS-STING pathway, is known to be expressed by normal and neoplastic T-cells. Here, we investigated STING expression in the TME of cHL, specifically focusing on the reactive T-cells surrounding the HRS ("rosettes") and correlated the expression patterns with T-cell populations in peripheral blood (PB) as well as circulating immune response mediators prior to treatment.

Principles/Methodology

The study group represents a previously published prospective study of cHL (Mulder et al, Hemasphere 2022) and included 54 previously untreated patients. STING expression was assessed by immunohistochemistry using a previously validated antibody. STING expression was evaluated in the T-cells of the TME focusing on the "rosettes". Any intensity of STING immunostaining was considered positive. The results of STING expression in the T-cells of the TME were correlated to the T-, B- and NK-cell subpopulations in PB samples analyzed by flow cytometry (FC), immune response mediators assessed by plasma proteomics (Olink platform) as well as clinical outcome including event-free survival (EFS).

Results

STING expression in the T-cells of the TME was observed in 36/54 (67%) cHL tumors. From the STING-negative group, 14 cHL tumors showed STING expression in HRS cells as compared to 3 cases in the STING+ group ($p < 0.0001$, Chi-square test). STING-negativity in the T-cells of the TME significantly associated with lower number of CD4+ naive, CD4+PD-1+ naive and CD4+ CM (central memory) T-cell populations in PB ($p < 0.05$, Mann-Whitney test). No significant associations between STING expression in T-cells of the TME were observed with cytokine profile (Olink), and various clinical and laboratory parameters of this HL cohort. However, a statistical trend towards inferior EFS was noted for patients with STING- T-cells of the TME as compared to STING+ group (p , not significant).

Conclusion

Expression of STING in the T-cells of the TME is inversely associated with its expression in the neoplastic HRS cells and may reflect the systemic T-cell mediated immune responses in cHL.

Capabilities and Limitations of Open-Source Vision–Language Models in Hodgkin Lymphoma Diagnosis and Education in Hematopathology

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¹ Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Pathology, Berlin, Germany; ² Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Clinical Study Center, Berlin, Germany; ³ Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Biometry and Clinical Epidemiology, Berlin, Germany; ⁴ German Cancer Research Center (DKFZ), German Cancer Consortium (DKTK) Partner Site Berlin, Heidelberg, Germany; ⁵ Vivantes Netzwerk für Gesundheit GmbH, Department of Pathology, Berlin, Germany; ⁶ Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Nuclear Medicine, Berlin, Germany; ⁷ Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin Institute of Health, Clinician Scientist Program, Berlin, Germany

Background

Hematopathology faces rising diagnostic demands amid a shortage of specialists. Open-source vision–language models (OS-VLMs) may support image analysis and education. We assess their usability using Hodgkin lymphoma (HL), a challenging entity with heterogeneous morphology.

Principles/Methodology

272 H&E and immunohistochemical (IHC) images from 15 HL and 1 control were embedded in 9 prompts/case and evaluated using a local version of MedGemma (MG4B vs. 27B, Google LLC). Subtypes included nodular sclerosis (NSHL), mixed cellularity (MCHL), lymphocyte-rich (LRHL), lymphocyte-depleted (LDHL), and nodular lymphocyte predominant HL (NLPHL). 4 temperatures (0, 0.2, 0.5, 0.7) were tested. Prompts covered interpretation, diagnosis, and reporting. Responses were scored for correctness (+1 correct, 0 partly correct, –1 incorrect) and commitment (+1 yes, 0 no) using pathology report ground truth. Blinded evaluation was performed by an experienced hematopathologist (HP). Also, 7 educational text-only prompts on HL diagnostics were posed to MG27B and ChatGPT-5, benchmarked against a human supervisor (reference standard; +1/0/–1). Models were compared by a rank-based ANOVA-type test.

Results

Prompting yielded 17 decisions/case, 272/temperature and 1088/model. Mean correctness was –0.21 (MG4B) vs. 0.02 (MG27B) vs. 0.79 (HP) ($p < 0.001$). Best model performance was at temperature 0.2 in MG27B. Mean correctness was highest for H&E-stain (1.00 vs. 0.95 vs. 1.00) and malignancy recognition (0.27 vs. 0.40 vs. 1.00) and lowest for tissue recognition (–0.97 vs. –0.93 vs. 1.00). IHC interpretation improved in MG27B vs. MG4B (–0.05 vs. –0.22). Report structure was better in MG27B. Mean case-level correctness varied by subtype in MG27B vs. HP with highest performance in MCHL (0.16 vs. 0.79) and lowest in NLPHL (–0.13 vs. 0.79). Mean commitment increased in MG27B across questions and cases (0.89), approaching human levels (0.94). For education, MG27B was comparable to ChatGPT, but both struggled with IHC selection and naming correct differential diagnosis, e.g. AITL.

Conclusion

MG27B improved over MG4B, reflecting rapid progress in OS-VLMs. Yet image recognition remains outperformed by pathologists and further training with HL images is needed. Relative to correctness, commitment was disproportionately high, which may be misleading in hematopathology, where complex diagnoses require caution. VLMs may support report generation and education but require supervision and prior pathology experience of users.

Immunohistological comparison of lymphocytes in the tumour microenvironment between EBV-positive and EBV-negative classical Hodgkin lymphoma.

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Background

While classical Hodgkin lymphoma (cHL) is one of the most common lymphomas in Western countries, its incidence in East Asian regions, including Hong Kong, remains low. Studies stated that 40% cases of cHL are found to harbour Epstein-Barr virus (EBV) in Western countries. Yet, reports of up to 60% of cHL cases in Hong Kong are EBV-positive, often linked to early childhood viral exposure. EBV is well known to modulate the tumour microenvironment of various tumours, including cHL, which is rich in various immune cells, including T-lymphocytes. Investigating the infiltration of CD3-positive T-lymphocytes in EBV-positive cHL may shed light on the immune landscape associated with EBV-related oncogenesis and on immunotherapeutic strategies.

Principles/Methodology

All cHL cases issued at the Division of Anatomical Pathology of Queen Mary Hospital, Hong Kong, from Jan 2015 to April 2026 were collected. The haematoxylin and eosin (H&E)-stained slides, immunohistochemical slides and Epstein-Barr Virus (EBV)-Encoded Small RNAs (EBER) in situ hybridisation slides were reviewed by two pathologists (R.Y.O and K.K.Y). For each case, the reviewed diagnosis, the positivity of EBER and the percentages of CD3-positive and CD20-positive lymphocytes were recorded. Statistical analysis were performed using IBM SPSS Software.

Results

Among the 44 cases of cHL, 12 (27.27%) were positive for EBV. Patients with EBV-positive cHL were significantly older, with a median age of 70.5 vs. 33.5 years in EBV-negative cases ($p<0.001$) (Table 1.). There is a slight male predominance in EBV-positive cases (10/12, 83.33% vs. 16/32, 50%), but not reaching statistical significance. 6/25 (24%) cases of nodular sclerosis cHL, 5/10 (50%) cases of mixed-cellularity cHL and 1/4 (25%) cases of lymphocyte-rich cHL were positive for EBV.

In the tumour microenvironment, EBV-positive cHLs had a significantly higher percentage of CD3-positive T-lymphocytes than EBV-negative cHLs (median 75% vs. 65%, Mann-Whitney $U = 113.5$, $p = 0.037$) (Figure 1.). There was no significant difference in the percentages of CD20-positive B-lymphocytes between the two groups.

Conclusion

EBV-positive cHLs have a higher percentage of CD3-positive T-lymphocytes in the tumour microenvironment. Further studies regarding the composition of CD3-positive T-lymphocytes, particularly CD8+ cells, may aid the exploration of immune-mediated therapy against Hodgkin lymphomas.

		EBV-positive cHL	EBV-negative cHL	p-value
Age (years) median		70.5	33.5	<0.001
Sex	Male	10 (83.33%)	16 (50%)	0.083
	Female	2 (16.67%)	16 (50%)	
Histological subtype	Nodular sclerosis	6 (50%)	19 (58.38%)	0.329
	Mixed cellularity	5 (41.67%)	5 (15.63%)	
	Lymphocyte-rich	1 (8.33%)	3 (9.38%)	
	Lymphocyte-poor	0	0	
	Not available	0	5 (15.63%)	

Table 1. Demographics and histological subtypes of cHL patients

Patients with EBV-positive cHL were significantly older, with a median age of 70.5 vs. 33.5 years in EBV-negative cases.

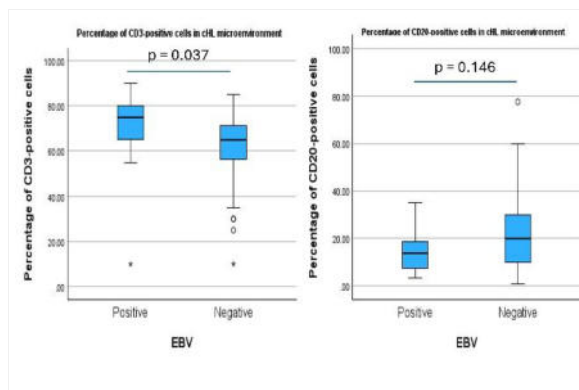


Figure 1. Percentages of CD3- and CD20-positive cells in the tumour microenvironment of cHLs. EBV-positive cHLs had a significantly higher percentage of CD3-positive T-lymphocytes than EBV-negative cHLs.

EA4HP26-ABS-570

PAX5-negative Classic Hodgkin-like Lymphoma: A Diagnostic Challenge

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Background

Classic Hodgkin Lymphoma (CHL) shows a profoundly disrupted B-cell program, with dim and variably expressed PAX5 as the only positive B-cell marker in most cases. Rare PAX5-negative cases are described that may also express cytotoxic and/or T-cell markers and/or EMA. Conversely, CD30 and CD15 coexpression is reported in ALK-negative anaplastic large cell lymphoma (ALK- ALCL), so that a poorly defined gray zone exists between PAX5neg CHL with aberrant T-associated phenotype and ALK- ALCL.

Principles/Methodology

A multicenter series of 52 PAX5 negative CHL-like lymphomas was comprehensively immunophenotyped (including T and cytotoxic markers, and EMA). T-cell clonality was assessed in 48 cases (with NGS and/or GeneScan Analysis), and *JAK2* and *DUSP22* gene rearrangements by FISH in 38 and 14 cases, respectively. Morphology and phenotype were defined as Typical (T) or Atypical (A) for CHL; atypical morphology included sheets of mononucleated or too pleomorphic or partly anaplastic cells and very few Reed-Sternberg cells; atypical phenotype was defined if any T and/or cytotoxic markers and/or EMA were expressed. Cases were subclassified in 4 groups: All typical (TT), all atypical (AA), typical morphology/atypical phenotype (TA), atypical morphology/typical phenotype (AT).

Results

Results are shown in Table 1. Most cases showed typical CHL morphology and phenotype with the exception of PAX5 negativity, and polyclonal TCR, although an atypical phenotype was not uncommon. With current information, TT cases and most TA cases are best classified as CHL with marker aberrancy. Cases with more T-cell marker expression (i.e. at least one antigen out of ≥2 main subgroups of T-markers, cytotoxic markers, EMA), despite typical CHL morphology were considered as “grey zone CHL vs CHL-like T-NHL”. Cases of the AA group +/- T-clonality were considered ALCL. 8/12 cases from the AA group and 3/19 cases of the T/A group showed T-cell clonality. AT cases are few and heterogenous.

Conclusion

The majority of PAX5neg CHL-like lymphomas are best classified as CHL even in the presence of other minor phenotypic aberrancies. Since PAX5 represses the T-cell program in lymphoid precursors, its absence may explain partial T-cell phenotype reprogramming. However, a subset of cases, some of which show T-cell clonality, cannot be classified without further studies including analysis at the single cell level and currently represent a grey zone between CHL and ALCL.

Table 1: Subclassification of cases upon morphology, phenotype, TCR clonality, JAK2/DUSP22 rearrangement

Morphology/ Phenotype (acronym)	Total n. cases	TCR ^a Polyclonal	TCR ^a Monoclonal	JAK2 Rearrange- ment (36 cases)	DUSP22 Rearrange- ment (14 cases)
Typical/Typical (TT)	12	11	1	0/9	Not tested
Atypical/Atypical* (AA)	12	4	8	0/10	0/5
Typical/Atypical* (TA)	19	16	3	25/15	0/5
Atypical**/Typical (AT)	5	3	2	0/4	0/4
	46	34	14	2/38	0/14

^aInclude cases with expression of any T-cell (CD3, CD5, CD2, CD7, CD4, CD8) and/or cytotoxic (Tia1, Granzyme B, perforin) marker, EMA

^{**}Include morphologic features that would question a straightforward diagnosis of CHL (i.e. predominant mononuclear cells, anaplastic looking cells, minimal HRS cells) in an overall HL-like histology

^bby NGS and/or Geno Scan Fragment analysis

§ one case had polyclonal TCR; one case 21 had monoclonal TCR

Table 1.
Subclassification of cases upon morphology,
phenotype, TCR clonality, JAK2/DUSP22
rearrangement

EA4HP26-ABS-587

The crucial role of CD4 T cells in the development of Hodgkin lymphoma: a novel disease-defining framework

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Background

Classic Hodgkin lymphoma (cHL) is an enigmatic malignancy in which a small number of Hodgkin Reed-Sternberg (HRS) cells persist within a large and complex tumor microenvironment (TME). While immune evasion has been widely studied, the supportive role of the TME is less well appreciated. CD4 T cells are highly interesting in this respect, because they are consistently abundant and form intimate physical interactions with the HRS cells that are often visible as characteristic rosette structures (figure 1). Here, we propose a novel pathogenetic framework that links these interactions to the origin, development and diagnostic features of cHL.

Principles/Methodology

We developed an integrative conceptual framework centered on the interaction between CD4 T cells and HRS cells, based on evidence from *in vitro* coculture systems, gene expression data, immunohistochemistry and established immunology principles.

Results

Our framework highlights the exceptionally high and characteristic production of the chemokine TARC (CCL17) by HRS cells, which selectively recruits Th2 and regulatory T cells and starts to increase already years before diagnosis. Recruited CD4 T cells establish immunological synapses with HRS cells through HLA class II and CD58 mediated interactions. The HRS cells orchestrate a chronic state of intermediate T-cell activation, marked by low-level expression of immune checkpoint molecules and the transcription factor TOX2. This phenotype resembles early commitment toward the T follicular helper (Tfh) lineage and connects the immune landscape at diagnosis to the germinal center niche from which HRS cells originate. Within this niche, germinal center B cells depend on strong survival signals from Tfh cells to escape apoptosis. Dysregulation of this tightly controlled system may enable the accumulation of genetic aberrations and facilitate the earliest stages of lymphoma development.

Conclusion

We propose that cHL is best understood not as a malignancy of HRS cells alone, but as a disease driven by a functional unit consisting of malignant B cells and cooperating CD4 T cells. Within this unit, germinal center B cells acquire the capacity to recruit and modulate CD4 T cells through TARC production and controlled chronic activation, thereby securing Tfh-like support signals that promote their survival, growth and malignant transformation.

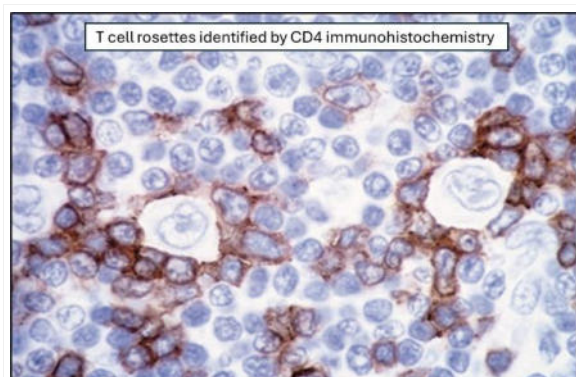


Figure 1

EA4HP26-ABS-636

The tumor microenvironment of EBV+ Diffuse Large B-cell Lymphoma and EBV+ classical Hodgkin Lymphoma

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Background

EBV+ Diffuse Large B-cell Lymphoma, not otherwise specified (EBV+ DLBCL, NOS) and EBV+ classical Hodgkin Lymphoma (cHL) form two ends of a spectrum ranging from textbook tumor dense cases towards cases with scarce tumor cells dispersed in an inflammatory rich environment (Nagase et al., 2026). Although both entities are classified as distinct diseases receiving different therapies, extensive overlap exists between immune environment-rich EBV+ DLBCL NOS and EBV+ cHL. Both entities have the EBV virus in common, regulating the pathogenesis and the tumor

microenvironment (Keane et al., 2019; Murray & Young, 2019; Tan et al., 2018; Yeo et al., 2024), leading us to think that therapies affecting the tumor microenvironment could be beneficial for EBV+ B-cell lymphoproliferative entities such as EBV+ DLBCL NOS and EBV+ cHL.

Principles/Methodology

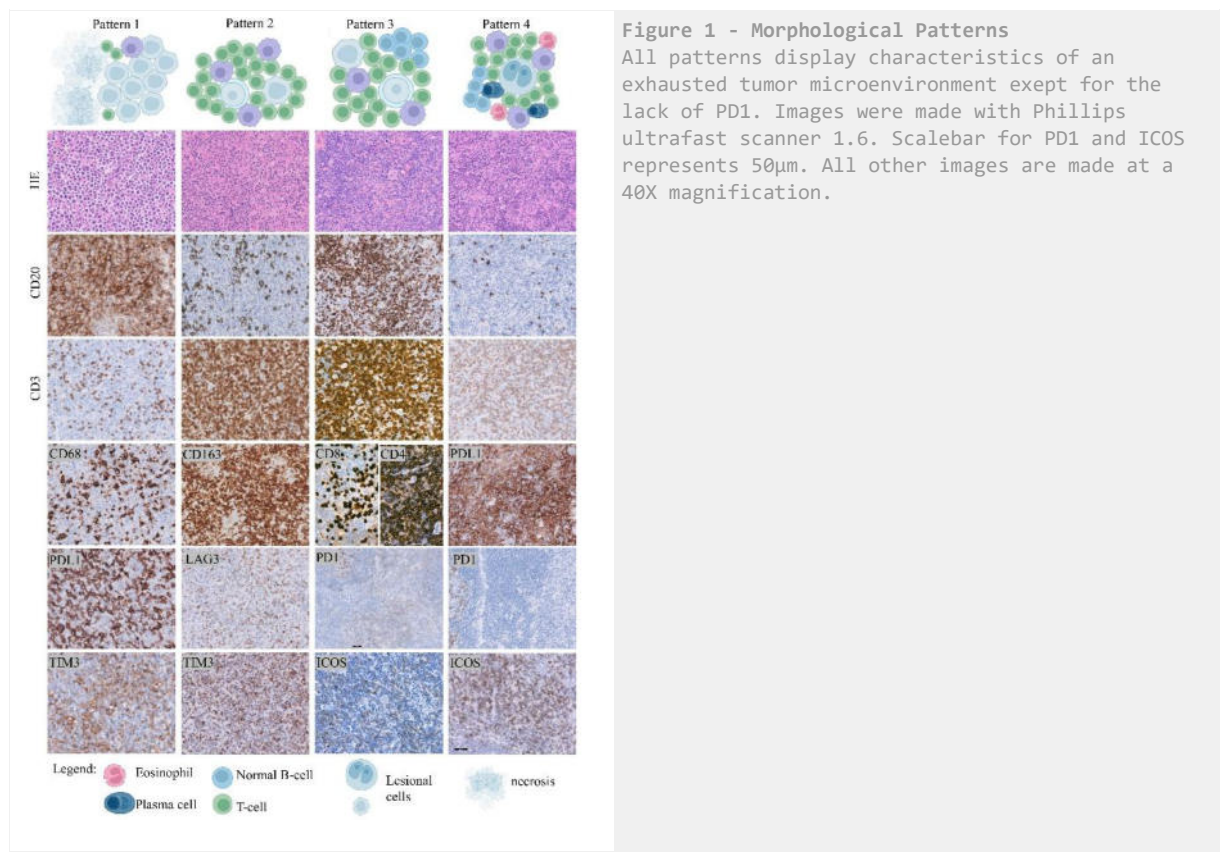
We investigated a cohort of 59 EBV+ DLBCL, NOS (ICD-O code 9680/3) and cHL, EBV+ (ICD-O code 9650/1). We evaluated tumor microenvironmental markers on immunohistochemistry.

Results

We investigated a cohort of 59 patients in which we recognized 4 immune environment patterns. The first pattern included “monomorphic” EBV+ DLBCL NOS patients (n=16) in which the large amount of the tumor was taken up by sheets of large neoplastic cells and in which necrosis was common (Figure 1). Pattern 2 and 3 include immune rich cases with B-cell marker preservation on the HRS(-like) cells. In this group of patients, we recognized a microenvironment, either T-cell histiocyte rich (pattern 2), or enriched with normal small B-cells (pattern 3) (Figure 1). Pattern 4 included EBV+ cHL cases displaying an inflammatory rich environment with prominent eosinophils and plasma cells. PD-L1 was upregulated in tumor population, but also in macrophages of the tumor microenvironment. EBV+ cHL had the highest PD-L1 H-score (255) (Bourbon *et al*, 2021) in tumor cells, significantly higher compared to Pattern 1 “monomorphic” (114) and pattern 3 “B-cell enriched” (140) (Figure 2). All patterns showed signs of exhaustion (TIM3, LAG3, PDL1, CD163, see Figure 1). However, PD-1 expression was lacking in almost all patients, whereas ICOS was regularly found expressed by environmental T-cells (Figure 1).

Conclusion

In conclusion, EBV+ cHL showed highest PD-L1 upregulation by lesional cells. EBV+ DLBCL NOS and EBV+ cHL show both similar signs of exhaustion in their tumor microenvironment, but lack PD1 expression, which needs further investigation. A bulk RNA sequencing experiment on this cohort is ongoing.



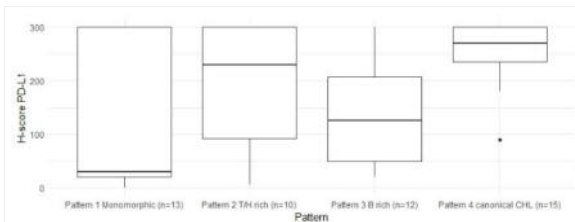


Figure 2 - H-score of neoplastic PD-L1 expression per pattern

Boxplot representing the H-scores of neoplastic PD-L1 expression per pattern. Significant differences (using t-test) were between canonical Hodgkin Lymphoma pattern 4 and "monomorphic" pattern 1 ($p=0.0027$) and between B-enriched "immune-rich" pattern 3 and canonical classical Hodgkin Lymphoma pattern 4 ($p=0.0028$). This graph was created using R version 4.5.1.

Lymphoma Symposium IV | Microenvironment and immune privilege sites – Oral

EA4HP26-ABS-566	Tumor microenvironment and gene expression profile of lymphomas involving immunoprivileged sites: primary and secondary CNS lymphomas
EA4HP26-ABS-637	Comprehensive histo-molecular characterization of a cohort of peripheral T-Cell lymphomas with primary central nervous system presentation.

Tumor microenvironment and gene expression profile of lymphomas involving immunoprivileged sites: primary and secondary CNS lymphomas

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Background

Primary central nervous system lymphomas (PCNSL) are rare, aggressive B-cell lymphomas with poor prognosis. The immunoprivileged CNS niche is thought to shape the tumor microenvironment (TME) and select for specific lymphoma mutational profiles enabling immune evasion. The molecular basis of CNS tropism, particularly whether the mechanisms driving PCNSL lymphomagenesis overlap with those in secondary CNS lymphoma (SCNSL) remains largely unknown. Integrated characterization of tumor and TME transcriptomics across these entities may uncover oncogenic mechanism, early biomarkers and therapeutic targets.

Principles/Methodology

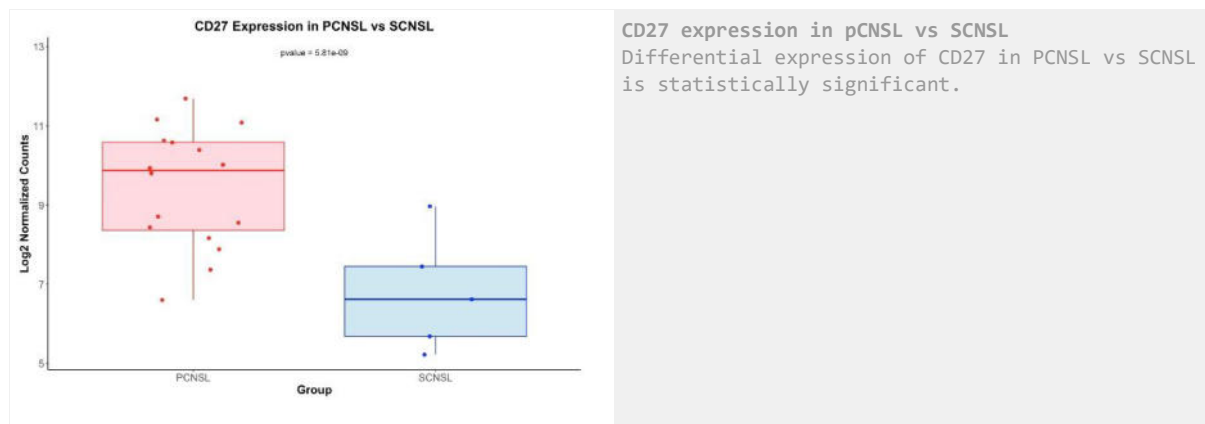
We retrospectively collected FFPE material from 57 patients, including 23 PCNSL, 8 SCNSL, 13 systemic DLBCL from patients with CNS involvement, 13 systemic DLBCL from patients without CNS involvement, plus 7 reactive brain tissue controls. Seven patients had paired samples from CNS and systemic lesions. In order to compare CNS samples from patients with PCNSL and SCNSL, whole gene expression profile (GEP) was investigated using an RNA-seq free methodology (HTG-EdgeSeq) and data was processed with HTGAnalyzer (Díez-Ahijado, 2025), enabling differential expression and TME analysis.

Results

Differential expression analysis identified two significantly upregulated genes in PCNSL, versus SCNSL: CD27, a stimulatory co-receptor involved in B/T-cell regulation (Fig1), and FDCSP (Follicular Dendritic Cell Secreted Protein), a marker of the follicular immune response. Gene set enrichment analysis revealed a transcriptomic profile consistent with an active “hot” TME in PCNSL, with significant upregulation of interferon alpha and gamma response pathways and significant downregulation of TGF- β signaling. Canonical pathway analysis confirmed enrichment of CTLA4, IL2RB, and NO2IL12 pathways in PCNSL. TME deconvolution, through different signatures (quanTIseq and xCell) consistently and significantly demonstrated higher levels of CD4⁺ Th2 lymphocytes in PCNSL and a trend towards a higher proportion of myeloid dendritic cells.

Conclusion

In summary, we found relevant and significant differences in both GEP and TME between PCNSL and SCNSL. Our preliminary results suggest that the distinct GEP may reflect adaptation of the tumor to the CNS immunoprivileged environment while TME differential composition supports an increased antigen presentation capacity in PCNSL.



EA4HP26-ABS-637

Comprehensive histo-molecular characterization of a cohort of peripheral T-Cell lymphomas with primary central nervous system presentation.

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Background

Primary central nervous system lymphomas (PCNSL) account for 4–6% of all extranodal lymphomas. Over 95% are of B-cell origin. Primary T- and NK-cell lymphomas (PTCLs) of the CNS are estimated to represent as few as 1% of CNS lymphomas when strict criteria are applied. We report the largest multicenter cohort to date of 34 cases with associated clinical annotations, expert histopathological review, and integrative interpretation of ancillary findings.

Principles/Methodology

We included cases of PTCL with primary CNS involvement recorded in the LYMPHOPATH registry between 2013 and 2023. Diagnoses were reviewed according to most recent classifications (WHO5/ICC). Immunohistochemical profile was completed during pathology review. FISH for *DUSP22/IRF4* rearrangement was performed to all ALK-anaplastic large cell lymphomas (ALCL). Targeted NGS was performed using a 67-gene panel.

Results

The 34 patients included 26 men and 8 women, with a median age of 60 years (range 25–79). Supratentorial lesions were predominant (25/34); 9 cases were infratentorial or in other locations. A history of immune dysregulation (autoimmune disease, coeliac disease, prior B-lymphoma, transplant) was documented in 11/34 patients (32%). The final diagnosis distribution comprised: PTCL not otherwise specified (PTCL-NOS) (n=21), ALK- ALCL (n=4, and 3 *DUSP22* rearranged), ALK+ ALCL (n=2), extranodal NK/T-cell lymphoma nasal-type (ENKTL, n=5), and adult T-cell leukaemia/lymphoma (ATLL, n=2). Cytotoxic phenotype was observed in all but one PTCL-NOS case. In 12/24 cases with interpretable NGS results, we identified mutations in the *JAK/STAT* pathway (*JAK1*, *JAK3*, *STAT3*, *STAT5B*) and in other pathways. Four patients classified as PTCL-NOS displayed morphological, immunophenotypic, and molecular profiles consistent with those reported in enteropathy-associated T-cell lymphoma (EATL, n=3) or monomorphic epitheliotropic T-cell lymphoma (MEITL, n=1). None of these 4 patients disclosed digestive tumors, but the 3 patients with an “EATL profile” had a past history of celiac disease.

Conclusion

We report the largest series of PTCL with primary CNS involvement. We found a unique distribution of T-cell lymphomas, characterized by the absence of primary CNS T Follicular Helper (TFH) lymphoma, an overrepresentation of ALCL *DUSP22* rearranged, and a predominance of PTCL NOS with cytotoxic phenotype. Also, we report 4 cases with morphological, immunohistochemical, and molecular features overlapping with those of primary intestinal T-cell lymphomas.

Lymphoma Symposium IV | Microenvironment and immune privilege sites – Poster

EA4HP26-ABS-472	Primary Central Nervous System Lymphoma in an Immune-Privileged Site: Predominant Activated B-Cell Phenotype With Frequent MYC and BCL2 Expression in a Hispanic-Caribbean Cohort
EA4HP26-ABS-479	Where Lymphomas Hide: Epigenetic Clues to Immune-Privileged Site DLBCL Origins
EA4HP26-ABS-624	Isolated EBV-Positive Diffuse Large B-Cell Lymphoma in Cerebrospinal Fluid: A Case Report

Primary Central Nervous System Lymphoma in an Immune-Privileged Site: Predominant Activated B-Cell Phenotype With Frequent MYC and BCL2 Expression in a Hispanic-Caribbean Cohort

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Background

Primary central nervous system lymphoma (PCNSL) is an aggressive extranodal B-cell lymphoma arising within the immune-privileged central nervous system microenvironment. While clinicopathologic features have been described in predominantly North American and European populations, contemporary data in Hispanic-Caribbean patients remain limited, particularly regarding integrated biologic characterization.

Principles/Methodology

A retrospective review of PCNSL cases diagnosed between 2023 and 2025 at a tertiary referral center in Puerto Rico was performed. Clinical features, lesion distribution, immune status, histologic subtype, immunophenotype, Epstein-Barr virus status by in situ hybridization, proliferation index, and available fluorescence in situ hybridization (FISH) studies were analyzed. Comprehensive molecular testing, including expanded cytogenetic panels and MYD88 mutation analysis, was limited by test availability, reflecting real-world diagnostic practice in the region.

Results

Fourteen cases of PCNSL were identified, including twelve diffuse large B-cell lymphomas and two high-grade B-cell lymphomas. Patients ranged from 44 to 80 years of age and were predominantly immunocompetent. Lesions most commonly involved deep brain structures, periventricular white matter, and corpus callosum and were usually solitary. Among diffuse large B-cell lymphomas, ten demonstrated an activated B-cell immunophenotype, while two showed germinal center phenotype. Epstein-Barr virus in situ hybridization was negative in all cases. BCL6 expression was present in thirteen cases. MYC protein expression was identified in eleven cases, frequently ranging from 40% to 70%, and BCL2 expression was observed in ten cases. Ki-67 proliferation indices ranged from 70% to 100%. FISH studies performed in a subset of cases did not reveal recurrent rearrangements.

Conclusion

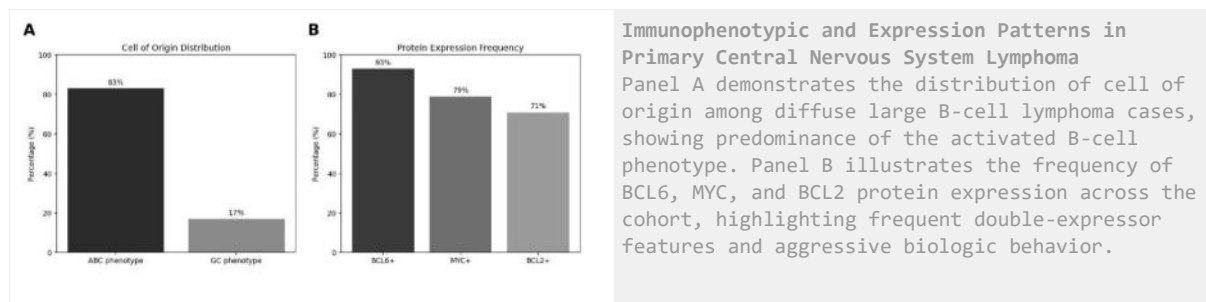
Within the immune-privileged central nervous system microenvironment, PCNSL in this Hispanic-Caribbean cohort demonstrated predominant activated B-cell phenotype, frequent MYC and BCL2 protein overexpression, and high proliferative activity, consistent with aggressive biologic behavior. The absence of recurrent cytogenetic rearrangements in tested cases suggests oncogenesis driven largely by dysregulated protein expression. These findings expand biologic characterization of PCNSL in an underrepresented population while highlighting real-world diagnostic limitations in resource-dependent settings.

Clinicopathologic Features of Primary CNS Lymphoma in a Hispanic-Caribbean Cohort (2023-2025)				
Parameter	Findings	n (%)	Notes	
Age (years)	Older adult predominance	—	44-80	
Immune status	Immunocompetent	13 (93)	1 autoimmune disease	
Lesion distribution	Deep brain/white matter/corpus callosum	12 (86)	Mediocranially solitary	
Histology: subtype	Diffuse large B-cell lymphoma	12 (86)	2 high-grade B-cell	
Cell of origin (CD20/CD22)	Activated B-cell phenotype	10/12 (83)	2 germinal center	
Epstein-Barr virus	Negative by in situ hybridization	14 (100)	—	
BCL6 expression	Positive	13 (93)	—	
MYC expression	Positive	11 (79)	40-70%	
BCL2 expression	Positive	10 (71)	—	
Ki-67 index	High	—	70-100%	

Abbreviations: DLBCL, diffuse large B-cell lymphoma; CNS, central nervous system.

Clinicopathologic Features of Primary Central Nervous System Lymphoma in a Hispanic-Caribbean Cohort

Summary of clinical, anatomical, and immunophenotypic features of primary central nervous system lymphoma in a Hispanic-Caribbean cohort, demonstrating predominant activated B-cell phenotype, frequent MYC and BCL2 expression, and high proliferation index.



EA4HP26-ABS-479

Where Lymphomas Hide: Epigenetic Clues to Immune-Privileged Site DLBCL Origins

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Background

Diffuse large B-cell lymphoma (DLBCL) arising in immune-privileged sites (IPS), including central nervous system (CNS), testis, ocular, breast, and ovary exhibit distinct clinical and biological behavior. We compared clinicopathologic features and DNA methylation profiles of IPS-DLBCL with systemic DLBCL to define site-specific patterns and underlying biology.

Principles/Methodology

75 cases were analyzed: CNS-DLBCL (n=55), T- DLBCL (n=3), other IPS-DLBCL (n=5), and systemic DLBCL (n=12). Clinical data, phenotype, FISH results, treatments, and outcomes were reviewed. DNA methylation profiling (EPIC array) was performed on 16 cases (ocular n=1, testicular n=3, breast n=2, CNS n=4, systemic n=6).

Results

Patients with testicular and other IPS-DLBCL were older (mean 73.3 and 73.8 years) than those with CNS-DLBCL (64.7 years) and systemic DLBCL (63.3 years) ($p=0.0002$). Serum LDH was highest in systemic DLBCL (677.1 U/L; $p<0.0001$). Double-expressor phenotype was common in CNS (45.5%) and systemic DLBCL (50.0%), but rare in testicular (0%) and other IPS-DLBCL (20%). *MYC* gain was enriched in CNS-DLBCL (38.2%; $p=0.0159$), whereas *BCL2* gain was more frequent in systemic DLBCL (25.0%). HD-MTX-based therapy was used exclusively for CNS-DLBCL, while R-CHOP and DA-R-EPOCH predominated in systemic and non-CNS IPS cases. Systemic DLBCL showed the poorest outcomes (PFS 8 months; OS 18 months) (Table 1).

Methylation profiling showed that CNS and systemic DLBCL share a globally hypomethylated, tightly clustered profile consistent with high proliferative activity and epigenetic instability. Breast DLBCL displayed mixed hyper- and hypomethylation, indicating heterogeneity; testicular cases demonstrated broad variability consistent with germ-cell epigenetic plasticity. Ocular DLBCL displayed relatively stable, moderately methylated patterns. Promoter CpGs were better preserved in CNS and ocular DLBCL, while systemic and T-DLBCL exhibited stronger promoter hypomethylation. CpG island patterns further separated tissue of origin groups, with CNS/ocular cases aligning near neuroectoderm signatures and breast DLBCL clustering with epithelial tissues.

Conclusion

IPS-DLBCLs differ substantially from systemic DLBCL in clinical, genetic, and epigenetic profiles. Distinct methylation signatures highlight site specific biology and support the utility of methylation-based markers for tissue of origin classification and precision-guided therapies.

Feature	Primary CNS DLBCL (n=55)	Primary Testicular DLBCL (n=3)	Other IPS-DLBCL (n=5)	Systemic DLBCL (n=12)	P-value
Age (mean, yrs)	64.7	73.3	73.8	65.3	<0.01
Gender					
Male (n, %)	27 (49.1%)	3 (100.0%)	1 (20.0%)	5 (41.7%)	0.08
Female (n, %)	28 (50.9%)	0 (0.0%)	4 (80.0%)	7 (58.3%)	0.08
Prior malignancy (n, %)	6 (10.9%)	0 (0.0%)	0 (0.0%)	3 (25.0%)	0.49
Hematologic (n, %)	1 (1.8%)	0 (0.0%)	0 (0.0%)	1 (8.3%)	0.65
Non-hematologic (n, %)	5 (9.1%)	0 (0.0%)	0 (0.0%)	2 (16.7%)	0.48
Serum LDH (high >320 u/L; mean)	255.37	246.3	432.4	677.1	<0.01
B2 microglobulin (0.8–2.4 mg/L; mean)	2.0	N/A	2.16	N/A	N/A
Location	Parietal: 6 (10.9%) Frontal: 21 (38.2%) Basal ganglia: 2 (3.6%) Cerebellum: 4 (7.2%) Periventricular: 3 (5.4%) Occipital: 3 (5.4%) Temporal: 3 (5.4%)	Left testis: 2 (66.7%) Right testis: 1 (33.3%)	Eye: 1 (20.0%) Left breast: 1 (20.0%) Right breast: 2 (40.0%) Left ovary: 1 (20.0%)	Lymph nodes: 9 (75.0%) Femur: 1 (8.3%) Spleen: 1 (8.3%) Parotid: 1 (8.3%)	N/A
CNS, multiple sites (Yes, %)	30 (54.5%)	N/A	N/A	N/A	N/A
Supratentorial (n, %)	49 (89.1%)	N/A	N/A	N/A	N/A
Infratentorial (n, %)	6 (10.9%)	N/A	N/A	N/A	N/A
Deep structures (n, %)	19 (34.5%)	N/A	N/A	N/A	N/A
BM involvement (n, %)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (16.7%)	0.02
Histo Algorithm					
GCB (n, %)	17 (30.9%)	1 (33.3%)	1 (20.0%)	3 (25.0%)	0.94
Non-GCB (n, %)	38 (69.1%)	2 (66.7%)	4 (80.0%)	9 (75.0%)	0.98
Double-expressor (n, %)	25 (45.5%)	0 (0.0%)	1 (20.0%)	6 (50.0%)	0.30
Ki-67 (mean)	84.0	83.3	88.3	80.0	<0.01
EBV-positive (n, %)	4 (7.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.67
FISH studies					
BC12-R (n, %)	2 (3.6%)	0 (0.0%)	0 (0.0%)	1 (8.3%)	0.41
MYC-R (n, %)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.16
BC12-R (n, %)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.16
Gain of BC12 (n, %)	3 (5.5%)	1 (33.3%)	0 (0.0%)	3 (25.0%)	0.07
Gain of BC14 (n, %)	1 (1.8%)	0 (0.0%)	0 (0.0%)	1 (8.3%)	0.30
Gain of MYC (n, %)	21 (38.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.01
Loss of MYC (n, %)	4 (7.3%)	0 (0.0%)	0 (0.0%)	1 (8.3%)	0.99
Initial response					
CR (n, %)	21 (38.2%)	1 (33.3%)	1 (20.0%)	8 (66.6%)	0.09
PD (n, %)	38 (69.1%)	2 (66.7%)	4 (80.0%)	4 (33.3%)	0.38
Progression/Relapse (n, %)	15 (27.0%)	0 (0.0%)	3 (60.0%)	3 (25.0%)	0.85
PFS (mean, months)	33	N/A	45	8	
SCT (n, %)	14 (25.5%)	0 (0.0%)	0 (0.0%)	3 (25.0%)	0.76
Final outcome					
CR (n, %)	28 (50.9%)	3 (100.0%)	2 (40.0%)	9 (75.0%)	0.15
PD (n, %)	27 (49.1%)	0 (0.0%)	3 (60.0%)	3 (25.0%)	0.16
Deceased (n, %)	21 (38.2%)	0 (0.0%)	2 (40.0%)	3 (25.0%)	0.51
Overall survival (mean, months)	28	48	43	18	

Figur 1: Comparison of clinicopathological findings among primary CNS, other primary immune-privileg

EA4HP26-ABS-624

Isolated EBV-Positive Diffuse Large B-Cell Lymphoma in Cerebrospinal Fluid: A Case Report

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Background

Epstein Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL) is a rare disease; it can involve nodal and extranodal sites. Herein, we report a case in which neoplastic lymphoid cells were identified exclusively in the cerebrospinal fluid (CSF).

Principles/Methodology

A 54-year-old woman presented with memory loss and cognitive impairment in 2022. These symptoms occurred every 1–2 months and worsened progressively. By June 2023, the patient developed headaches, mania, irritability and other abnormal psychiatric manifestations. Lymphoma was suspected at a local hospital, and she was subsequently referred to our hospital. Lumbar puncture was performed to collect cerebrospinal fluid (CSF) for routine examination, flow cytometry, cytology and next-generation sequencing (NGS).

Results

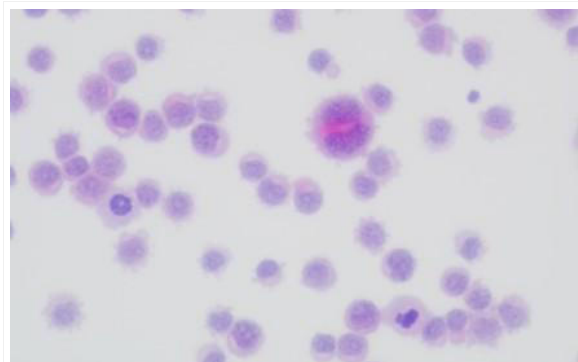
Cytological examination of the CSF revealed numerous large atypical cells (Fig 1), characterized by abundant cytoplasm, slightly irregular nuclei, occasional multinucleation (≥2 nuclei), and frequent mitotic figures.

Immunohistochemically, these neoplastic cells were positive for CD20, CD79a, PAX5 and LMP1 (Fig 2). CSF flow cytometry identified a total of 227 cells, of which 25.39% were abnormal B-cells expressing CD20dim and CD22, with negative expression of CD19, CD10, CD5, monoclonal kappa, monoclonal lambda and CD38. CSF EBV-DNA load was 2.3×10^3 copies/mL. Next-generation sequencing (NGS) of CSF detected mutations in TP53, FAT1, MAF, TNFRSF13B and ITGB3.

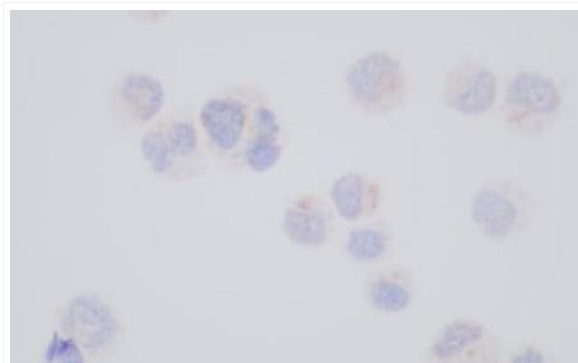
Computed tomography (CT) and magnetic resonance imaging (MRI) showed no abnormal lesions or pathological enhancement. The final diagnosis was **Epstein-Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL)**.

Conclusion

EBV-positive DLBCL in elderly patients frequently involves extranodal sites, and B symptoms are common. Our patient had no detectable solid lymphoma lesions; neoplastic cells were identified exclusively in the CSF, representing an extremely rare presentation and special microenvironment. Following chemotherapy, the patient's psychiatric symptoms resolved completely. However, EBV-DNA remained detectable in the CSF for more than one year, and TP53 gene mutation was persistently identified. Therefore, we report this unique case to highlight the clinical, pathological and molecular features, and to discuss the diagnostic and therapeutic challenges with pathologists and clinicians.



HE
the abnormal cells in CSF (100X)



LMP1

Lymphoma Symposium V | Recent advances – Oral

EA4HP26-ABS-464	Interpretation of T-cell receptor gene rearrangements using next-generation sequencing in nodal T follicular helper cell lymphoma, a comparison with the EuroClonality/BIOMED-2 assay
EA4HP26-ABS-494	Somatic Hypermutation as a Surrogate for <i>IG::BCL2</i> and <i>IG::MYC</i> and Its Role in MYC-Driven Oncogenesis in DLBCL
EA4HP26-ABS-498	Integrated spatial multiomics profiling identifies inflammatory tumor-macrophage niches defining prognostically distinct ENKTL subgroups
EA4HP26-ABS-526	Misinterpretation in IG/TR clonality diagnostics: findings from the EuroClonality EQA scheme
EA4HP26-ABS-553	Clonal CAR T-Cell Lymphoproliferative Disorder of the Gastrointestinal Tract: Histopathologic, Immunophenotypic, and Molecular Characterization Following BCMA-Directed Therapy
EA4HP26-ABS-557	Functional characterization of STAT6 mutations in classical Hodgkin Lymphoma reveals oncogenic addiction through neomorphic transcription factor activity
EA4HP26-ABS-602	<i>IRF4</i> rearrangements in adult diffuse large B-cell lymphoma in clinical trial cohorts of the German Lymphoma Alliance (GLA)
EA4HP26-ABS-627	Low-Level CHIP Populations in Reactive Lymphoid Tissue as Potential Precursors of B-Cell Lymphoma
EA4HP26-ABS-651	SOX11 Expression in CD5-Positive Large B-Cell Lymphomas
EA4HP26-ABS-663	Lymphoma specific foundation model predicts dark zone signature from digital H&E-stained tissue images

Interpretation of T-cell receptor gene rearrangements using next-generation sequencing in nodal T follicular helper cell lymphoma, a comparison with the EuroClonality/BIOMED-2 assay

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Background

Nodal T follicular helper (TFH) cell lymphoma (nTFHL) can be difficult to diagnose since it often shows features of immune dysregulation and can have a low number of neoplastic T-cells in involved lymph nodes. The analysis of T-cell receptor (TR) gene rearrangements using next-generation sequencing (NGS) and the conventional EuroClonality/BIOMED-2 was performed to compare their performance on this challenging diagnosis.

Principles/Methodology

DNA was extracted from 32 nTFHL samples (FFPE) which included nTFHL, angioimmunoblastic type (AITL) and nTFHL, NOS. NGS amplicon-based analysis of TRBV-TRBD-TRBJ, TRBD-TRBJ, TRGV-TRGJ rearrangements was performed using the EuroClonality two-step PCR protocols and the conventional EuroClonality assay. Sequences were analyzed for abundance, clonotype, and functionality using ARResT/Interrogate. 23 nTFHL cases were analyzed for mutations in lymphoma-associated genes using Trusight Oncology 500 or whole-exome sequencing.

Results

Both the NGS-based and the conventional clonality assays resulted in a high detection of clonality (97% and 94%, respectively), including both monoclonal and bi-clonal cases. The NGS-based approach detected two or more clonal TR targets in all clonal samples, whereas the conventional assay detected a single dominant rearrangement in three cases (table 1). Among the 29 clonal productive TRBV-TRBD-TRBJ rearrangements in nTFHL, the genes TRBV20-1 and TRBV19 were most frequently represented. Examination of reactive lymph nodes and tonsils showed that these TRBV genes were also most frequently represented in reactive T-cell populations. The analysis of follow-up samples of two bi-clonal cases showed identical clonotypes at diagnosis and follow-up, but also clonotypes specific to one of the time-points (figure 1). Among the cases analyzed for gene mutations, 17 harbored a *RHOA* mutation and 10 of these also carried an *IDH2* mutation. The polyclonal case showed a *DNMT3A* mutation.

Conclusion

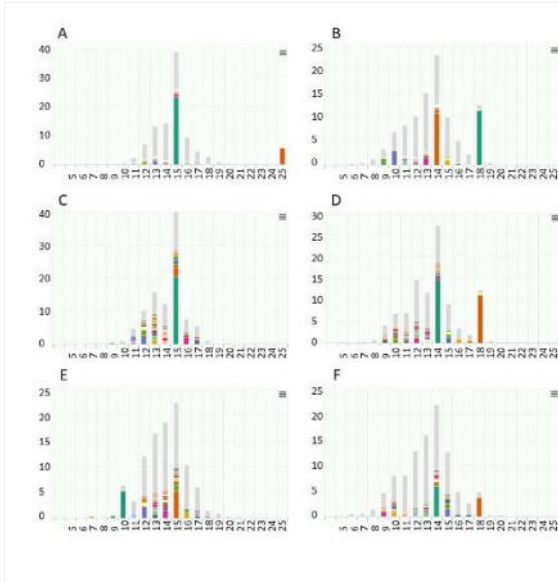
NGS enables reliable detection of even small clones, as is frequent in nTFHL. The number of clonal targets per case was higher than that obtained with the conventional assay. In nTFHL, clonal productive TRBV-TRBD-TRBJ rearrangements showed frequent use of TRBV20-1 and TRBV19, likely reflecting their overall high prevalence rather than selective subfamily usage.

Follow-up analysis of bi-clonal cases indicated dynamic changes, with additional small clones emerging and disappearing over time alongside one persistent clone.

AITL/ nTFHL	TRB clonal rearrangements				TRG clonal rearrangements				TRB + TRG clonal rearrangements			
	Total	1 R	2 R	≥3 R	Total	1 R	2 R	≥3 R	Total	1 R	2 R	≥3 R
NGS	30 (94%)	10 (31%)	11 (34%)	9 (28%)	30 (94%)	5 (16%)	21 (64%)	4 (12%)	31 (97%)	0 (0%)	4 (13%)	27 (84%)
GS	27 (84%)*	12 (41%)	11 (41%)	4 (15%)	27 (84%)	5 (16%)	22 (80%)	0 (0%)	30 (94%)**	3 (9%)***	7 (22%)	22 (69%)

Number of clonal TR gene recombinations detected by the NGS-based and the conventional EuroClonality

For both assays, the targets TRBV-TRBD-TRBJ, TRBD-TRBJ, and TRGV-TRGJ were evaluated. *In 7 cases with scoring of the TRBD-TRBJ target as polyclonal, the DNA quality was suboptimal and did not allow evaluation of TRBD1-TRBJ rearrangements. **The total number of cases with TRB and TRG clonal rearrangements represent the cases that were scored as clonal. ***Isolated rearrangements include the following: a clear clonal TRG rearrangement but polyclonal TRB gene rearrangements. In addition, there were 2 cases with a dominant peak of very weak signal intensity detected in a single target TRB-VJ.



Clonality results of diagnosis case angioimmunoblastic T-cell lymphoma 01 and follow-up (FU) samples

The plots of TRBV-TRBD-TRBJ (A) and TRGV-TRGJ (B) at diagnosis; these targets at FU-1 (C and D, respectively) and at FU2 (E and F, respectively). The top 50 clonotypes are indicated in separate colors, and less prevalent clonotypes are indicated in gray. The most abundant clonotype is always depicted as green, and the second most abundant clonotype in orange. The mutual abundances of the two clonal TRGV-TRGJ rearrangements (green and orange colored clonotypes) slightly change in time.

Somatic Hypermutation as a Surrogate for *IG::BCL2* and *IG::MYC* and Its Role in MYC-Driven Oncogenesis in DLBCL

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Background

Diffuse large B-cell lymphoma (DLBCL) shows variable responses to immunotherapy. Despite molecular subtyping allowing stratification of DLBCL into distinct biological and prognostic groups, FISH analysis for *MYC* and *BCL2* rearrangement remains the standard of care in clinical laboratories, present in ~10% and ~20% of cases, respectively. *MYC* translocations involve immunoglobulin (*IG*) (~55%) or non-*IG* partners (~45%), with different effects on *MYC* expression and clinical outcome. As *MYC* and *BCL2* become targets of somatic hypermutation when transactivated by translocation, we investigated whether somatic hypermutation could serve as a surrogate for these events. We also examined the genomic configuration of *MYC* translocation and evaluated the oncogenic activity of recurrent *MYC* hotspot mutations.

Principles/Methodology

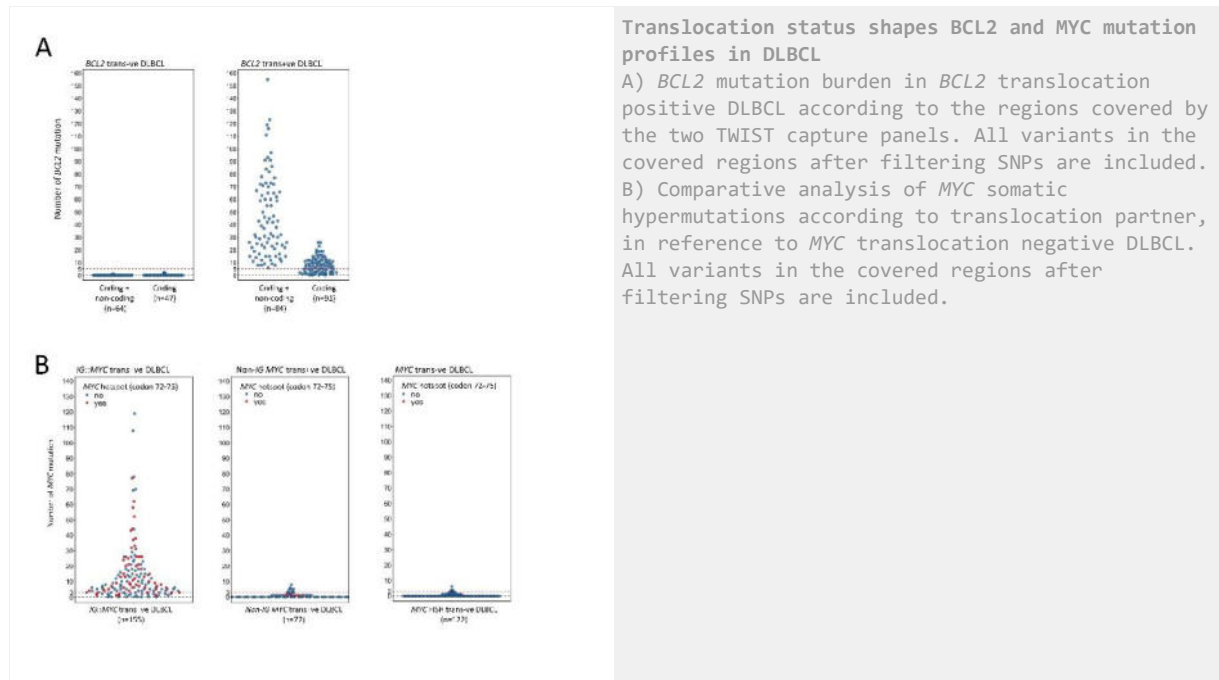
The study included 384 DLBCLs: 251 *MYC* translocation–positive (150 *BCL2* translocation–positive), 122 *MYC* translocation–negative (16 *BCL2* translocation–positive), and 11 with unknown *MYC* status (9 *BCL2* translocation–positive). Mutation profiling and *MYC* translocation genomic configurations were investigated by targeted next-generation sequencing (NGS). *MYC* hotspot mutations were assessed for transforming activity *in vitro*.

Results

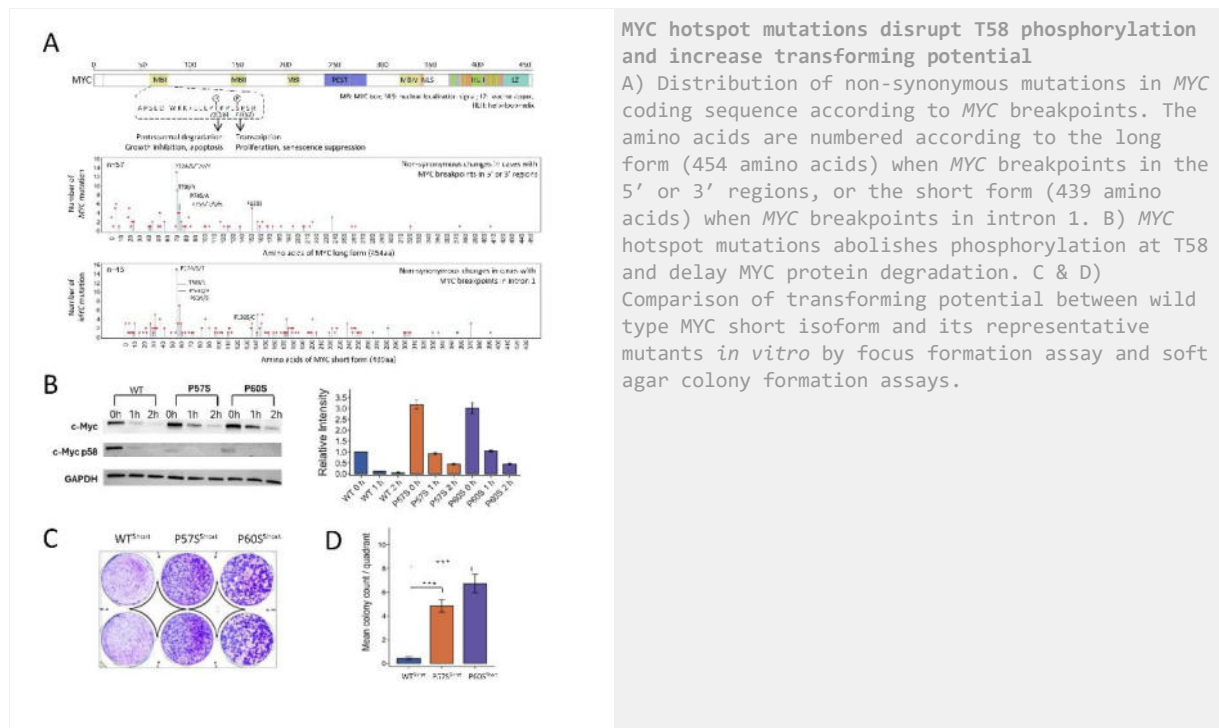
All *BCL2* translocation–positive cases showed high levels of *BCL2* somatic hypermutation (≥5 mutations), clearly separating from *BCL2* translocation–negative cases. Similarly, 81% (125/155) of *IG::MYC* cases had *MYC* somatic hypermutation (≥3 mutations) well above those seen in *MYC* translocation–negative cases. *MYC* somatic hypermutation occurred predominantly in *IG::MYC* cases (94%) but less frequently in non-*IG::MYC* cases (38%). *MYC* hotspot mutations clustered at the phosphorylation sites (T58 and S62) in the *MYC* Box 1 domain and were enriched in cases with *MYC* breakpoints in intron 1, particularly those with *BCL2* translocation and/or *TP53* mutation, resulting in exclusive expression of the short *MYC* isoform. Functionally, P57S and P60S mutations abolished T58 phosphorylation, impaired protein degradation, and markedly increased transforming activity compared with wild-type *MYC*.

Conclusion

Somatic hypermutation in *BCL2* and *MYC* is strongly associated with *BCL2* and *IG::MYC* translocations respectively and may serve as a surrogate marker for these events. *MYC* breakpoint configuration may shape somatic hypermutation and hotspot mutations that enhance *MYC* stability and oncogenic activity, highlighting a previously underappreciated mechanism of *MYC* activation in DLBCL.



tr-ve: translocation negative; tr+ve: translocation positive.



Integrated spatial multiomics profiling identifies inflammatory tumor-macrophage niches defining prognostically distinct ENKTL subgroups

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Background

Extranodal natural killer/T-cell lymphoma (ENKTL) is a rare, aggressive Epstein–Barr virus-associated malignancy with limited treatment efficacy and a heterogeneous tumor microenvironment (TME). Macrophages/myeloid cells are key TME regulators with substantial inflammatory and immunoregulatory plasticity, yet their heterogeneity in ENKTL remains poorly defined. How EBV-associated tumor transcriptional programs relate to spatial tumor–macrophage organization is also central to understanding ENKTL biology.

Principles/Methodology

We defined spatial architecture and cellular interactions of ENKTL TME using integrated single-cell spatial multiomics, combining Digital Spatial Profiling for whole-transcriptome and immuno-oncology protein analysis with CosMx 6k single-cell spatial transcriptomics.

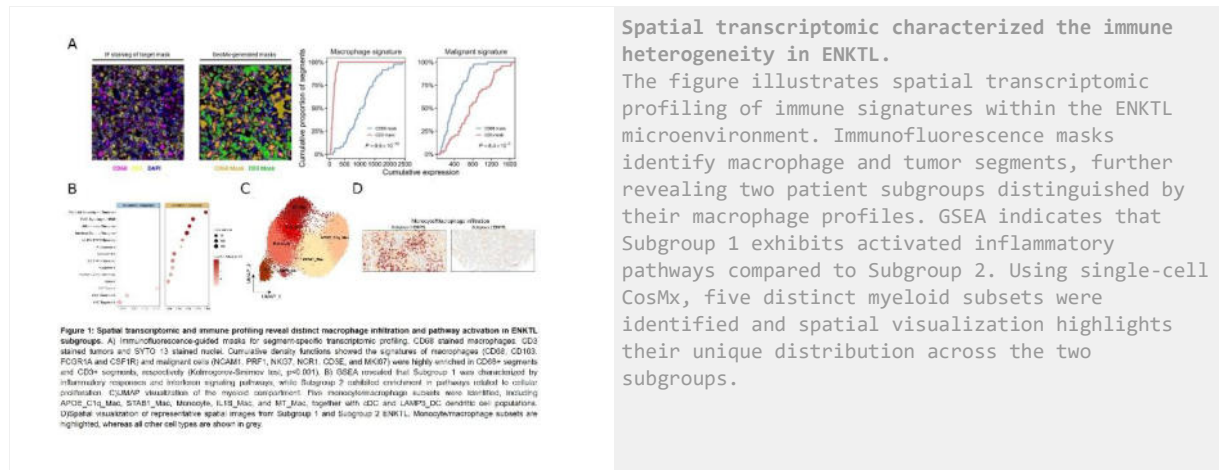
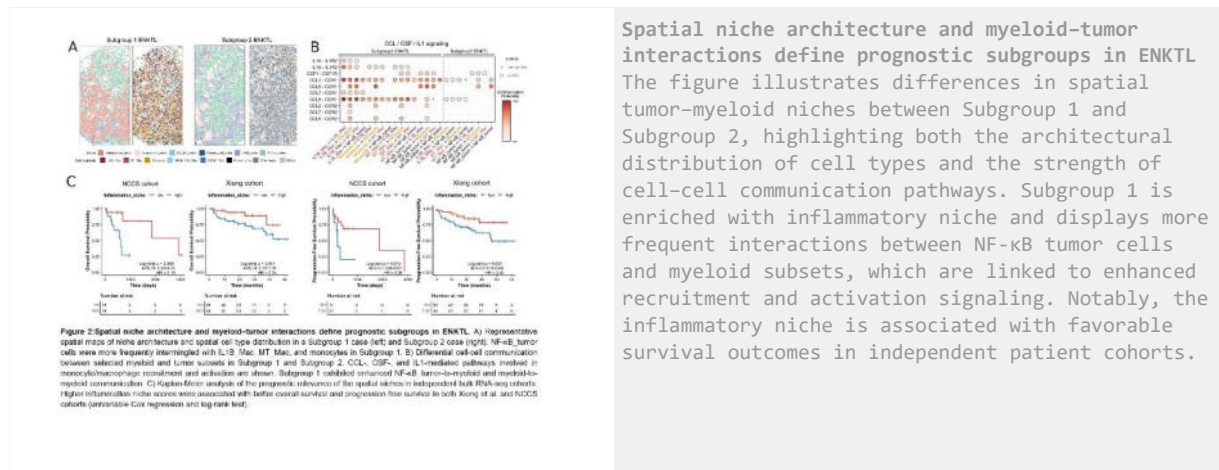
Results

Spatial profiling identified two patient subgroups distinguished by distinct macrophage programs. Subgroup 1 was characterized by inflammatory macrophages with prominent IFN- α/γ response programs and elevated expression of immunoregulatory molecules, including IDO1 and CD274. This subgroup also harbored an EBV-associated NF- κ B tumor subset with parallel immunostimulatory and immunoregulatory features, suggesting coordinated inflammatory activation across tumor and myeloid compartments. In contrast, Subgroup 2 displayed an immune-quiescent, macrophage-sparse phenotype dominated by STAB1+ macrophages associated with scavenger functions. Spatial neighborhood analysis uncovered an inflammatory niche in Subgroup 1, where EBV-associated NF- κ B tumor subset was closely juxtaposed with inflammatory myeloid populations. This spatial configuration was accompanied by increased tumor-myeloid and myeloid-myeloid communication, linking tumor-derived IFN- γ signaling to inflammatory macrophage recruitment and activation. Within the myeloid compartment, CCL- and IL1-mediated signaling pointed

to local propagation and reinforcement of the inflammatory macrophage state. Clinically, this inflammatory niche is associated with favorable survival across independent cohorts, supporting its prognostic relevance in ENKTL.

Conclusion

Our findings define spatially organized tumor-myeloid niches as clinically relevant functional units of the ENKTL TME. By integrating tumor-cell states, myeloid programs, and patterns of cellular colocalization, these niches provide prognostic information and may inform therapeutic strategies that move beyond tumor-centric approaches to consider the functional architecture of the immune microenvironment.



Misinterpretation in IG/TR clonality diagnostics: findings from the EuroClonality EQA scheme

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Background

Clonality analysis of immunoglobulin (IG) and T-cell receptor (TR) gene rearrangements is central to the diagnostic work-up of suspected lymphoproliferations. Accurate clonality assessment requires robust technical performance and expert interpretation. The EuroClonality External Quality Assessment (EQA) scheme evaluates laboratories' ability to identify IG/TR rearrangement patterns and supports the diagnosis of lymphoid malignancies.

Principles/Methodology

Wet (DNA-based) and paper (GeneScan plots) cases are distributed annually. Laboratories apply routine commercial or in-house multiplex PCR assays based on EuroClonality/BIOMED-2 protocols and interpret results according to EuroClonality guidelines. Next-generation sequencing (NGS)-based clonality results may also be submitted. Certification is based on final molecular interpretation, with an additional educational score reflecting detailed interpretation.

Results

From 2014 to 2025, 620 participants submitted results to the EuroClonality EQA IG/TR schemes. Overall performance improved with repeated participation. Misinterpretation of clonality patterns remains a key limitation, particularly in cases with multiple clonal peaks in TRB and IGK loci, frequently leading to incorrect distinction between biallelic rearrangements and biclonality. Oversized TRB signal, resulting from downstream J usage, may be erroneously interpreted as an additional clonal rearrangement. In IGK, an IGKV-IGKJ rearrangement may be followed by an intron RSS-Kde rearrangement on the same allele. A distal inverted IGKV gene cluster further increases complexity, allowing rearrangements to be retained rather than deleted, resulting in up to six rearrangements per clone. Distal IGK rearrangements are not recognized by GeneScan. However, the utility of NGS enables precise identification of IG/TR gene segments, including the recognition of distal IGK rearrangements, and resolves complex rearrangement patterns.

Conclusion

Despite improved EQA performance, misinterpretation of complex IGK and TRB rearrangement patterns remains a recurrent diagnostic challenge. These findings underscore the need for strict adherence to locus-specific interpretative principles. Integration of NGS into diagnostic workflows and EQA schemes will improve the standardization and diagnostic accuracy of clonality analysis in hematopathology.

Clonal CAR T-Cell Lymphoproliferative Disorder of the Gastrointestinal Tract: Histopathologic, Immunophenotypic, and Molecular Characterization Following BCMA-Directed Therapy

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Background

BCMA-directed CAR T-cell therapy has transformed management of relapsed/refractory multiple myeloma. A subset of recipients develops gastrointestinal (GI) symptoms attributable to immune effector cell-associated enterocolitis, yet tissue pathology and mechanisms of clonal T-cell expansion remain poorly defined.

Principles/Methodology

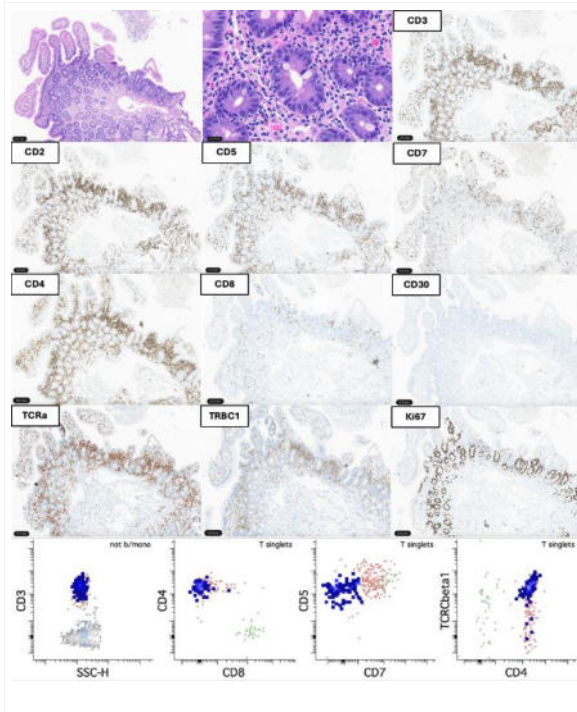
GI biopsies from patients with new-onset luminal symptoms following ciltacabtagene autoleucel (cilta-cel) at Memorial Sloan Kettering Cancer Center (2022–2025) underwent hematopathology evaluation. An IHC panel (CD3, CD4, CD8, TCR α , TCR δ , CD103, TRBC1, MUM1, CD138, BCMA) was applied. Clonality was assessed by TCR fragment analysis. CAR transgene was quantified by droplet digital PCR (ddPCR) in tissue and blood. In a subset, targeted NGS, lentiviral integration site analysis, and single-cell RNA sequencing were performed.

Results

Among 20 patients with post-infusion GI symptoms undergoing biopsy, 5 (25%) harbored CAR transgene-positive infiltrates. The duodenum was involved in all cases; extraduodenal sites included jejunum, ileum, antrum, and colon. Histology demonstrated monotonous small-to-medium lymphocytes expanding lamina propria without atypia or destructive growth. The infiltrate expressed CD3 and TCR α with CD4 or CD8 positivity, lacked CD103 and TCR δ , and showed TRBC1 restriction in all cases. Plasma cell markers were negative. TCR fragment analysis showed monoclonal rearrangements in 3/5 cases; 2 showed dominant sub-threshold peaks. Tissue CAR burden (1.7–25.1%) exceeded blood levels (0.03–1.32%), indicating tissue-tropic accumulation. In one fully characterized case (Perica et al., NEJM 2025), integration site analysis identified lentiviral insertion within *TP53*. NGS revealed *DNMT3A* p.Y284* and *SOCS1* p.S206F mutations with strong pSTAT3 positivity by IHC. In a second case, NGS identified a *KMT2A* (*MLL1*) T1679Nfs*15 frameshift mutation. Integration site analysis and NGS for remaining cases are ongoing.

Conclusion

We define the pathologic features of a clonal CAR T-cell lymphoproliferative disorder preferentially involving duodenal lamina propria. The combination of ddPCR, TRBC1 restriction, and TCR clonality distinguishes this entity from reactive infiltrates. In one case, lentiviral integration within *TP53* and a *SOCS1* mutation with JAK–STAT activation suggest one potential mechanism of transformation. Genomic characterization of remaining cases is ongoing.



Duodenum with atypical CD4+ T-cell infiltrate
Increased small T cells are noted in the lamina propria, expressing CD3. CD2 and CD5 are preserved, while CD7 is partially lost. Neoplastic cells are positive for CD4 and negative for CD8 and CD30. TCRα is positive, and TRBC1 has monotypic positive expression. The Ki-67 proliferative index is low, approximately 5%. Flow cytometry shows neoplastic cells (dark blue) positive for CD3 and CD5 (dim) while negative for CD7. Background CD4 and CD8 T cells can be seen in red and green, respectively.

Case	Site of Involvement	TRBC1/CD3 Ratio (IHC)	CAR ddPCR Tissue	CAR ddPCR Blood	TCR Fragment Analysis	NGS / Integration site	Outcome
1*	Duodenum	7.3%	25.1%	NA	Clonal	SOC57 mut. TP53 integration	Death
2	Duodenum Jejunum	84.7%	6.6%	1.30%	Borderline clonal	KMT2A mut.	Alive
3	Duodenum	31.6%	14%	0.25%	Clonal	Pending	Death
4	Duodenum Antrum Ileum Colon	88.4%	22.4%	1.32%	Clonal	Pending	Alive
5	Duodenum	26.2%	1.7%	0.03%	Borderline clonal	Pending	Alive

*Previously reported (Perica et al., N Engl J Med 2025;392:577-83)

Summary Table of Cases

EA4HP26-ABS-557

Functional characterization of STAT6 mutations in classical Hodgkin Lymphoma reveals oncogenic addiction thorough neomorphic transcription factor activity

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Background

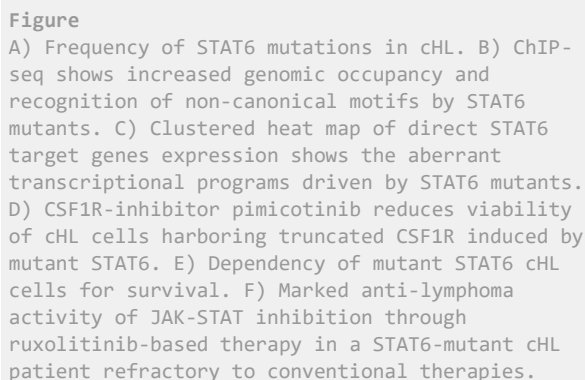
cHL is largely curable but a subset of patients still dies of this lymphoma, underscoring the need to better define its drivers to enable targeted therapies. We identified recurrent mutations in the DNA-binding domain of STAT6, a transcription factor downstream of IL4/IL13, in ~30% of cHL cases (Tiacci et al. Blood 2018), but their functional role is unclear.

We studied the most frequent STAT6 mutations (N417Y, D419G/H/N, and double mutants, Fig. A) in cHL cell lines expressing endogenous or exogenous wild-type (WT) or mutant STAT6 through an integrated multi-omics approach combining chromatin binding (ChIP-seq), chromatin accessibility and regulatory profiling (ATAC-seq and H3K27ac Cut&Run), 3-dimensional genome organization (Hi-C-seq), and transcriptomic analyses (RNA-seq).

Compared to STAT6 WT, all mutants showed stronger and expanded genomic occupancy, at least in part through neomorphic binding to non-canonical (“alternative”) STAT6 DNA motifs (Fig. B), leading to increased chromatin accessibility, increased active histone marks associated with regulatory elements including new super-enhancers (SEs), and increased long-range chromatin interactions at multiple loci. These alterations established an aberrant core transcriptional program (Fig. C) promoting proliferation, metabolic adaptation, immune evasion (including upregulation of PDL1/PDL2) and lineage plasticity, while repressing interferon signaling and tumor suppressors. Notably, mutant STAT6 activated a SE within the locus of the myeloid receptor tyrosine kinase CSF1R, leading to the expression of a novel truncated isoform lacking the ligand-binding domain but retaining the kinase domain, consistent with potential constitutive kinase activity. Indeed, viability of STAT6-mutant cHL cells was impaired by the clinical-grade CSF1R-specific inhibitor pimicotinib (Fig. D).

Finally, a young patient with STAT6-mutant cHL not responding to 9 prior therapies (including nivolumab) achieved a rapid and marked clinical response to treatment with the JAK/STAT inhibitor ruxolitinib combined with nivolumab.

We functionally dissected the role of STAT6 mutations in cHL pathogenesis and provided a mechanistic rationale for therapeutic strategies targeting the JAK/STAT axis and key downstream effectors such as CSF1R.



***IRF4* rearrangements in adult diffuse large B-cell lymphoma in clinical trial cohorts of the German Lymphoma Alliance (GLA)**

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Background

Advances in molecular profiling have led to the recognition of distinct entities of large B-cell lymphoma (LBCL), among them LBCL with *IRF4* rearrangement (LBCL-*IRF4*). This tumor typically arises with distinct clinico-pathological features, such as predominantly in children and young adults in the head and neck region, a follicular and/or diffuse growth pattern, strong *IRF4*/MUM1 expression, a germinal center B-cell phenotype, and a favorable prognosis following chemotherapy. However, reports to date have included only a limited number of adult DLBCL cases.

Principles/Methodology

We performed fluorescence in-situ hybridization and gene expression profiling using the DLBCL COO assay to analyse 63 samples with the characteristic co-expression of CD10⁺BCL6⁺MUM1⁺ out of 659 unselected patients enrolled in seven prospective clinical trials of the GLA (9.5%). In addition, 147 DLBCL samples enrolled in two prospective GLA clinical trials were investigated for *IRF4* translocation frequency as a control group without preselected CD10⁺BCL6⁺MUM1⁺ expression status.

Results

In 210 samples attempted, FISH was evaluable in 179 cases, including 56 DLBCL with CD10⁺BCL6⁺MUM1⁺ and 123 unselected DLBCL. *IRF4* translocation was observed in 21/179 samples altogether (12%), encompassing 6/56 (11%) of the CD10⁺BCL6⁺MUM1⁺ cohort and 15/123 (12%) unselected DLBCL. From the latter cohort, co-expression of CD10⁺BCL6⁺MUM1⁺ was observed in only 1/15 samples, while the remaining 14 samples did not show CD10⁺BCL6⁺MUM1⁺. COO subtyping was feasible in 4/7 *IRF4*-rearranged CD10⁺BCL6⁺MUM1⁺ samples, all of which were assigned to the GCB subtype. In the 14 DLBCL cases without CD10⁺BCL6⁺MUM1⁺, COO was available in nine samples, 6/9 with GCB- and 3/9 with ABC-subtype. In the seven *IRF4*-rearranged CD10⁺BCL6⁺MUM1⁺ samples, no other gene was affected by translocation, while double or triple hits with *BCL2*, *BCL6* and/or *MYC* occurred simultaneously with *IRF4* translocation only in the samples without CD10⁺BCL6⁺MUM1⁺ co-expression. The median expression of MUM1 was 40% (range from 0 to 100%), 17/21 *IRF4*-rearranged samples had high expression (>40%).

Conclusion

IRF4 rearrangement is a recurring event in adult DLBCL. In our series, all *IRF4*-rearranged samples with aberrant CD10⁺BCL6⁺MUM1⁺ expression were of GCB-subtype by GEP-COO and did not show additional translocations of either *BCL2*, *BCL6* and/or *MYC*. However, *IRF4* rearrangements in adult DLBCL do not exclusively occur in CD10⁺BCL6⁺MUM1⁺ co-expressing samples.

Low-Level CHIP Populations in Reactive Lymphoid Tissue as Potential Precursors of B-Cell Lymphoma

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Background

Clonal haematopoiesis of indeterminate potential (CHIP) is increasingly recognised as a condition predisposing to lymphoma, although the absolute risk remains low. This may partly reflect the reliance of population-based studies on peripheral blood (PB) samples for CHIP detection. However, as lymphoid cells primarily transit through the PB while homing to and maturing within secondary lymphoid organs, PB may represent a suboptimal proxy for lymphoma precursor populations.

Principles/Methodology

To elucidate the presence of CHIP in lymphoid organs, we examined 42 biopsies of unclear lymphoid hyperplasias without proven lymphoma diagnosis. Additionally, we identified a cohort of 24 patients with B-cell lymphoma for which both tumour biopsy and previous reactive lymph node (LN) or bone marrow samples were available (matched pairs). All specimens underwent immunohistochemistry (H3K27me3) and targeted high-throughput sequencing. All findings were contextualized with clinical history and follow up data.

Results

CHIP populations with lymphoma-associated mutations were identified in 15/42 (35.7%) samples that showed reactive findings by morphology, predominantly involving genes essential for DNA methylation (*TET2*) and histone-modifiers (*KMT2C*, *KMT2D*) (Fig.1A). Their presence was associated with overexpression of H3K27me3, independently of age and immunodeficiency. In 6/24 (25%) matched-pairs, identical mutations were present in both biopsies, detectable up to 20 years before lymphoma onset (median time to lymphoma 56 months). Affected genes included histone modifiers (*KMT2D*, *CREBBP*) as well as canonical tumour suppressors and signalling regulators (*TP53*, *SGK1*) (Fig.1B). One illustrative case demonstrated a *SGK1* mutation (variant allelic frequency [VAF] 0.07%) in an enlarged reactive abdominal LN excised during pancreatic surgery 8 years prior to the diagnosis of a tonsillar DLBCL harbouring the identical mutation (VAF 83.6%).

Conclusion

Clinically and morphologically ambiguous lymphadenopathies are enriched for clonal lymphopoiesis, with a prevalence of 35.7%, markedly exceeding the rate reported in PB (1–2%). Additionally, low-level CHIP populations (VAF<5%) were detected in previous reactive LN samples in 25% of patients with B-cell lymphoma, showing identical mutations. Both cohorts exhibited alterations in histone-modifier pathways. The detection of low-level CHIP in lymphoid tissues might help identify individuals at a genuinely increased risk of developing lymphoma years before overt malignancy emerges.

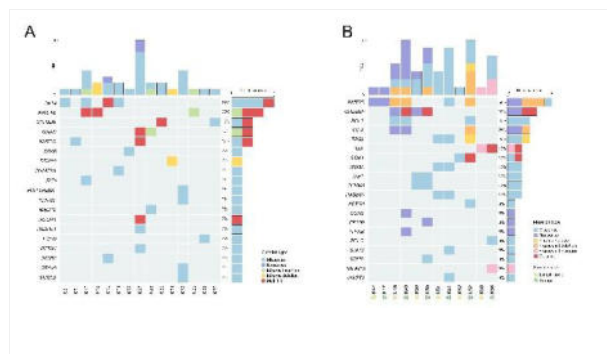


Figure 1. Mutational Landscape
(A) OncoPrint with variants identified in the unmatched cohort of unclear lymphoid hyperplasias, (B) OncoPrint with variants identified in the matched-pairs cohort (B-cell lymphoma versus previous reactive lymph node). The mutations in the reactive lymph node samples have been included even if under the usual cutoff of VAF 5% (down to 1 read). Mutation class, number of samples harbouring mutations in each gene, and tumour mutational burden (TMB) are shown.

EA4HP26-ABS-651

SOX11 Expression in CD5-Positive Large B-Cell Lymphomas

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Background

SOX11 is a neural transcription factor overexpressed in 90% of mantle cell lymphomas (MCL) and is a useful marker in the diagnosis of the cyclin D1-negative variant. It is also expressed in a subset of Burkitt lymphomas (BL) and lymphoblastic lymphomas (LBL). Cyclin D1-negative MCL harbor *CCND2* or *CCND3* rearrangements which may in some cases be cryptic and undetectable by routine cytogenetic or fluorescence *in situ* hybridization (FISH). Nevertheless, the rearrangements lead to *CCND2/D3* mRNA overexpression detectable by molecular methods. In this study, we report and characterize a cohort of CD5-positive large B-cell lymphomas expressing SOX11 that do not meet the criteria for MCL, LBL or BL.

Principles/Methodology

Fifteen mature B-cell lymphomas were selected based on blastoid or large cell morphology, cyclin D1 negativity and SOX11 expression, in the absence of features of BL or LBL. FISH was performed to evaluate *CCND1*, *CCND2*, *BCL2*, *BCL6*, *MYC*, and *IRF4* rearrangements. *SOX11* and *CCND1/D2/D3* mRNA expression were studied by qPCR

and/or NanoString. Genomic profiling was studied by whole-genome sequencing in two cases and targeted next-generation sequencing in 14/15.

Results

Ten tumors had large cell and five blastoid morphology. Three patients had a previous follicular lymphoma diagnosis. All cases were positive for B-cell markers, CD5 and SOX11, with high Ki67 index. By Hans Algorithm, eight cases were classified as GCB and seven as non-GCB. *CCND1*, *D2* and *D3* mRNA overexpression was negative in all cases studied. FISH assays were negative in all tumors studied with the exception of *BCL6*, rearranged in 3/12 tumors. Recurrent pathogenic mutations were detected in *TP53* and *RB1* in three tumors and in *CCND3*, *CARD11*, *BTG2*, *NOTCH2*, *KMT2D* and *PRDM1* in two each. The cohort included 11 males and 4 females with median age of 66 (43-93 years). Eight patients presented with stage IV, two with III and one with I. The disease involved exclusively nodal sites in three patients, extranodal in six and both nodal and extranodal in six. First-line treatment was immunochemotherapy in 11 patients, three with intensive regimens. One patient received immunotherapy combined with radiotherapy and another palliative treatment. Follow up available showed that 4/12 patients died of the disease (15-64 months), three had only partial response and four were alive without disease (7-66 months).

Conclusion

SOX11 is expressed in a subset of CD5-positive large B-cell lymphomas without features of MCL or BL.

EA4HP26-ABS-663

Lymphoma specific foundation model predicts dark zone signature from digital H&E-stained tissue images

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Background

Identification of high-grade B-cell lymphomas is critical for therapy selection. However, definitions and criteria have been imprecise due to varied morphologic and genetic features and definitions. Gene expression profiling has identified a molecular high-grade signature that resembles that of germinal center dark zone (DZ) B-cells that has been reproduced by independent groups and identified high risk cases with DLBCL and “Burkitt-like” morphology. However, gene expression profiling is not routinely used or available in clinical laboratories, the aggressive nature of these lymphomas often requires immediate treatment. We have developed LymphoVision, an internally-developed image foundation model, trained with 46 million H&E patches from 12,919 archival Mayo Clinic cases. In proof-of-concept studies, we have shown it can be used to perform diagnostic tasks, accurately infer cell of origin in diffuse large B-cell lymphoma (DLBCL), and predict event free survival at 24 months (EFS24) in DLBCL all from an H&E-stained section. We hypothesized that LymphoVision could be used to identify diffuse aggressive large B-cell lymphomas with a dark zone (DZ) signature.

Principles/Methodology

Patch-level embeddings were extracted from H&E whole slide images using LymphoVision and aggregated to slide-level representations through multiple instance learning (MIL). Cell of origin and DZ signature are determined via gene expression profiling for cases enrolled in the Molecular Epidemiology Resource or Lymphoma Epidemiology of Outcomes studies including 8 US academic centers. The model was evaluated with 82 cases (27 positive and 55

negative). For classifying DZ cases, we performed randomized 10-fold cross-validation using a 50/50 train/test split. For predicting Linear Predictor Score (LPS) directly, we used 4 folds.

Results

For DZ classification, Lymphovision achieves a mean test AUC of 0.92, F1 of 0.78, with a mean sensitivity of 0.76 and a specificity of 0.91. For LPS value prediction, the model achieved a mean Pearson correlation of 0.76.

Conclusion

We present proof of concept that LymphoVision can recognize features that predict DZ gene expression signature in diffuse large B-cell lymphoma. The number of cases is admittedly small, and large cohorts need to be studied to further fine tune and validate the model. However, LymhoVision represents a major advance in AI tools that has many potential applications to advance the practice of hematopathology.

Lymphoma Symposium V | Recent advances – Poster

EA4HP26-ABS-469	FREQUENT <i>NPM1</i> MUTATION, MONOBLASTIC/MONOCYTIC ORIGIN AND PROGNOSTIC SIGNIFICANCE OF ORGAN AND SYSTEM INVOLVEMENT IN MYELOID SARCOMA – A MULTICENTER STUDY
EA4HP26-ABS-484	End-to-End Digital and Computational Hematopathology in Routine Clinical Practice at Memorial Sloan Kettering Cancer Center
EA4HP26-ABS-487	KMT2A-rearranged B-lymphoblastic lymphomas are skewed towards a more mature developmental stage
EA4HP26-ABS-492	Optical Genome Mapping Is a Powerful Diagnostic Tool in Non-Hodgkin Lymphoma
EA4HP26-ABS-495	Histiocytic Neoplasms with a Concurrent Haematological Neoplasm, a Single Institution's Experience
EA4HP26-ABS-497	Mutational Profiles of African Burkitt Lymphoma: Association with EBV Status Across Age Groups.
EA4HP26-ABS-499	Ultrasensitive kappa and lambda dual in situ hybridization has high diagnostic utility in the differential diagnosis of Hodgkin lymphoma
EA4HP26-ABS-516	Deep sequencing of B-cell lymphoma specific genes in molecular and clinical follicular lymphoma subgroups
EA4HP26-ABS-520	Amplification and overexpression of cyclin D1 in Epstein–Barr virus-positive inflammatory follicular dendritic cell tumor: A targeted next-generation sequencing study
EA4HP26-ABS-522	SAMHD1 gene promoter methylation is the mechanism for silencing of SAMHD1 gene and protein expression in precursor T-acute lymphoblastic leukemia but not in mature T-cell lymphomas
EA4HP26-ABS-532	TCR Alpha: A Novel, Powerful Clinical Tool for Diagnosis of Reactive and Neoplastic T-Cell Proliferations
EA4HP26-ABS-542	Genomic configuration of non- <i>IG::MYC</i> translocation in DLBCL and its biological impact
EA4HP26-ABS-550	<i>MYC</i> Mutations in Large B-Cell Lymphomas and Their Correlations with Gene Rearrangements and Copy Number Gain
EA4HP26-ABS-556	Clonally related and concurrently occurring Interdigitating Dendritic Cell Sarcoma (IDCS) and T-Lymphoblastic Leukemia/Lymphoma (T-LLL) in a four years old girl - a case report and review of IDCSs associated with other hematolymphoid neoplasms (HLN)-
EA4HP26-ABS-563	A TCRbeta1/TCRbeta2 (TRBC1/TRBC2) monoclonal antibody pair for determining T-cell monotypia as a surrogate for clonality in formalin-fixed paraffin-embedded material
EA4HP26-ABS-568	Analysis of vitreous cell-free DNA may improve molecular analysis in vitreoretinal lymphoma
EA4HP26-ABS-569	Immunoglobulin light chain mRNA in situ hybridization refines classification across the NLPHL–cHL spectrum
EA4HP26-ABS-582	Reciprocal <i>MYC::BCL6</i> translocation is frequent in “triple-hit” (<i>MYC-BCL2-BCL6</i>) and “double-hit” (<i>MYC-BCL6</i>) large B-cell lymphomas

EA4HP26-ABS-585	Angioimmunoblastic T-cell Lymphoma at King's College Hospital: Association with Clonal Haematopoiesis
EA4HP26-ABS-596	Mutational and Copy Number Landscape of High-Grade B-Cell Lymphoma, Not Otherwise Specified (HGBL-NOS): Integrated Genomic Profiling of an Indian Cohort
EA4HP26-ABS-605	Clinical impact of NGS Profiling in B- and T-Cell Lymphomas: A Single-Center Experience
EA4HP26-ABS-610	microRNAs expression in different subtypes of Castleman Disease
EA4HP26-ABS-612	PARP-1 Immunoexpression and Clinical Outcome in Aggressive Large B-Cell Lymphomas
EA4HP26-ABS-614	Molecular Profiling of Lymphoid Malignancies Using TruSight Oncology 500: Initial Experience from a Single Center in North Macedonia
EA4HP26-ABS-620	High-Grade B-Cell Lymphoma with 11q Aberration in Africa: A differential diagnosis in cases with features of endemic Burkitt lymphoma
EA4HP26-ABS-623	Classifier-oriented targeted NGS for molecular subtyping of large B-cell lymphomas
EA4HP26-ABS-632	Pseudo-Richter Transformation Following BTKi Interruption in CLL: A Clinico-Pathological and Molecular Characterization
EA4HP26-ABS-640	Multicentre evaluation of the EuroClonality-NDC next-generation sequencing assay in the real-world diagnosis of lymphoproliferative disorders
EA4HP26-ABS-641	Skin and non-GI mucosal lymphoproliferative disorders expand the spectrum of T-cell lymphoproliferative disorders with <i>STAT3-JAK2</i> fusion
EA4HP26-ABS-644	Non-alpha/beta T-cell lymphoma: a single Centre experience on gamma/delta and TCR-silent subsets
EA4HP26-ABS-647	Comparison of a commercial NGS-Based B-Cell Clonality Assay with the Euroclonality NGS primer sets across B-Cell Lymphoma Subtypes with and without Somatic Hypermutation
EA4HP26-ABS-654	T-bet Expression Defines a Distinct, Clinically Aggressive Subset of Diffuse Large B-Cell Lymphoma Potentially Related to "Aging" of B cells and has an Altered Immune Microenvironment
EA4HP26-ABS-662	Multisystem ALK-positive histiocytosis-a case report

FREQUENT *NPM1* MUTATION, MONOBLASTIC/MONOCYTIC ORIGIN AND PROGNOSTIC SIGNIFICANCE OF ORGAN AND SYSTEM INVOLVEMENT IN MYELOID SARCOMA – A MULTICENTER STUDY

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Background

Myeloid sarcoma (MS) is a tumorous extramedullary proliferation of blast or blast equivalent cells (e.g. promonocytes or promyelocytes). The most involved sites are the skin, also referred to as *leukaemia cutis* (LC), lymph nodes and gastrointestinal tract, but virtually any anatomical site can be affected. These lesions, especially without the clinical context of a known bone marrow disease, pose a differential diagnostic challenge.

Principles/Methodology

In this retrospective multicenter clinico-pathological study on 154 patients with MS or LC, 169 samples were analyzed by morphology, immunohistochemistry and fluorescent in situ hybridization, and a subset by additional sequencing [*TP53* & *NPM1*]. In cases in which bone marrow samples of patients were available (n=133), the diagnosis, phenotype, cytogenetic and molecular findings of the intramedullary disease was compared to the extramedullary samples. Overall survival (OS) (n=122) was calculated from the time of diagnosis to death or last follow-up.

Results

The majority of cases were lysozyme positive (diffuse in 91% and focal in 5%), 51% showed diffuse and 6% focal expression of CD56, and IRF8 was strongly positive in 31% of the lesions. Lack of myeloperoxidase (MPO), CD117 and CD34 expression was observed in 27%, 39% and 58%, respectively. PU.1 was positive in almost all instances (95%), but BRAF V600E was consistently negative. CD123 was diffusely (13%) or focally (25%) positive, which, in addition to frequent CD4 (73%) and CD56 expression, pointed to a phenotypic overlap with blastic plasmacytoid dendritic cell neoplasms. Survival analysis revealed that MS occurring at sanctuary sites (CNS, orbit, ovary, and testis) was characterized by excellent survival. Similarly to histiocytoses, there was a prognostic difference between isolated and multisystemic involvement by MS. Patients who underwent allogeneic hematopoietic stem cell transplantation showed significantly improved survival.

Conclusion

In conclusion, this multicenter study suggests that most MS are of myelomonocytic/monoblastic origin, a high proportion of them are *NPM1* mutated, and may lack expression of MPO and CD34. *NPM1* mutation-specific antibodies should be integrated into the diagnostic panels for MS or LC, while IRF8 and PU.1 are not recommended as they cannot distinguish MS from histiocytic neoplasms. Besides the phenotypic overlap with histiocytoses, MS cases show similarities in clinical outcomes when categorized analogously into uni- and multisystemic diseases.

End-to-End Digital and Computational Hematopathology in Routine Clinical Practice at Memorial Sloan Kettering Cancer Center

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Background

Digital and computational pathology tools, including artificial intelligence (AI) and large language models (LLMs), have the potential to transform hematopathology practice. However, most reported applications remain limited to isolated use cases rather than system-wide implementation in routine clinical care. We implemented a system-wide digital and computational hematopathology workflow using a modular design to accommodate diverse operational and diagnostic needs.

Principles/Methodology

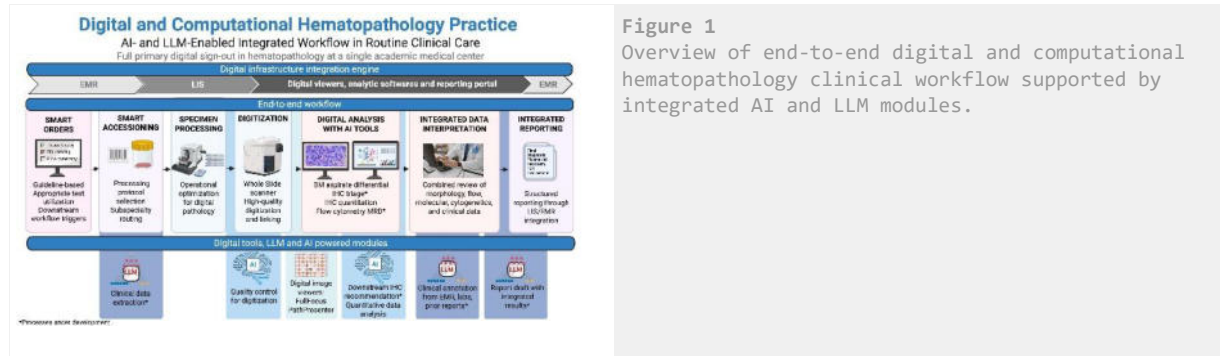
We established an integrated IT infrastructure linking the electronic medical record (EMR), laboratory information system (LIS), AI/LLM-enabled analytical platforms, and a reporting platform functioning as an integration engine. The workflow incorporated smart orders linked to clinical question and disease state; all major specimen types; order-driven accessioning and downstream testing protocols; digitally aligned laboratory processing; slide and data digitization with AI-based quality control; AI-assisted quantitative assessment for bone marrow aspirates and immunohistochemistry; LLM-based data extraction, annotation, and clinical decision support; and full primary digital sign-out. (Fig 1 and 2)

Results

End-to-end validation using 60 specimens demonstrated 100% concordance with conventional analog review. Clinical reporting accuracy was maintained relative to analog workflows, while reproducibility of analytical data improved by implementation of AI tools. The system improved test utilization by clinicians and pathologists, enabled immediate access to clinical, pathology, and quality data, and accelerated reporting reducing turnaround time. Additional benefits included improved office space utilization, remote access across locations and sign-out capabilities, longitudinal case review, rapid and easy case exchange including in intradisciplinary and multidisciplinary conferences and expanded educational access to archived cases. The digital workflow received positive feedback from attending hematopathologists and trainees including improved work-life balance.

Conclusion

System-wide implementation of a fully digital and computational hematopathology workflow is feasible in routine practice and improves access, collaboration, efficiency, and data integration while maintaining diagnostic accuracy. This model provides a practical and scalable framework for broader adoption of AI-enabled hematopathology workflows and creates new opportunities for data science-driven research.



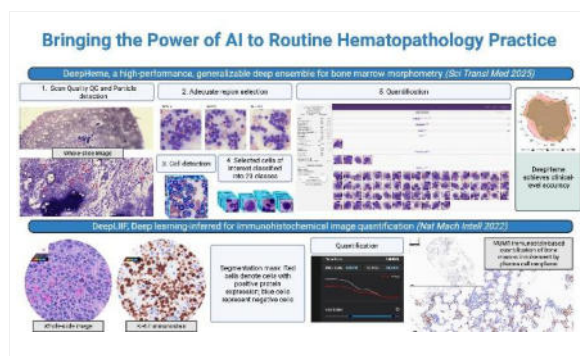


Figure 2

Examples of clinically validated AI-based modules seamlessly integrated into routine hematopathology workflow: 1) DeepHeme module provides automated real-time bone marrow differential counts by assigning each cell to a lineage and differentiation state. 2) DeepLIIF module provides real-time quantitative immunohistochemistry marker assessment for nuclear proteins commonly used in hematopathology including Ki67, MUM1, BCL6 and MYC.

EA4HP26-ABS-487

KMT2A-rearranged B-lymphoblastic lymphomas are skewed towards a more mature developmental stage

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Background

In B-lymphoblastic leukemia (B-ALL) rearrangements of the *KMT2A* gene (*KMT2Ar*) are associated with unfavourable outcome. In contrast, in B-lymphoblastic lymphoma (B-LBL), the non-leukemic counterpart of B-ALL, no differences in progression-free survival were observed in paediatric *KMT2Ar* patients. This discrepancy is remarkable, because clinical treatment of B-LBL and B-ALL is nearly identical. B-LBL differs from B-ALL by the presence of tissue manifestation and low bone marrow infiltration. Given the distinct clinical presentation, and the difference in outcome, we asked if B-LBL samples of the *KMT2Ar* subtype were characterized by specific molecular features.

Principles/Methodology

Differential gene expression analysis was performed by RNA-seq on 11 B-LBL *KMT2Ar* specimens and 25 B-LBL patients without *KMT2Ar*. Quantitative image analysis of TdT expression was performed 17 B-LBL *KMT2Ar* specimens and 18 B-LBL patients without *KMT2Ar*. Clonal rearrangements of the immunoglobulin (IG) loci were investigated in a total of 30 samples. Identification of the *KMT2A* fusion partner was performed either by FISH or by RNA-seq from in a total of 16 *KMT2Ar* samples.

Results

We found that B-LBL have a significant lower expression of TdT and transcriptionally resembles a more mature stage of B-cell differentiation. These data were supported by the analysis of IG rearrangements, showing an increased number of productive *IGH* and *IGL* rearrangements in *KMT2Ar* B-LBL. Furthermore, we found that in B-LBL translocation partners for *KMT2A* are the *MLLT1*, *MLLT3* and *MLLT10* genes, but never the *AFF1* gene. These data differ significantly from what has been observed in BM/blood-derived *KMT2Ar* pediatric B-ALL patients, where approximately 70% show an immature phenotype and approximately 50% carry a *KMT2A::AFF1* fusion.

Conclusion

The data here presented may suggest that KMT2Ar of more committed/mature cells are a prerequisite for high tissue dissemination accompanied by low BM infiltration. Furthermore, given that a high immature/multipotent cellular phenotype has been associated with an adverse outcome and slow decline in MRD under therapy, our findings may explain why the KMT2Ar subtype in B-LBL is not associated with unfavorable outcome. Therefore, our data suggest that tissue-based KMT2Ar cases may represent a biologically distinct, potentially less aggressive subtype, which could influence risk stratification and treatment monitoring.

EA4HP26-ABS-492

Optical Genome Mapping Is a Powerful Diagnostic Tool in Non-Hodgkin Lymphoma

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Background

Non-Hodgkin lymphoma (NHL) is a diverse and heterogeneous group of hematological malignancies. These lymphomas arise from the clonal proliferation of either B/T or natural killer lymphocytes, and their correct classification relies partly on identifying characteristic structural variants and copy number alterations. Current standard-of-care technologies for detecting these genomic features, chromosome banding analysis (CBA) and fluorescent in situ hybridization (FISH), are labor intensive and have specific limitations. CBA has low resolution and relies on viable cell culture, whereas the targeted approach of FISH does not provide the whole genome view required for comprehensive disease characterization. This highlights the need for higher-resolution nontargeted genomic methods. Previous studies have evaluated optical genome mapping (OGM) as a whole genome alternative for cytogenomic characterization in NHL diagnostics but were restricted in number and to cases with peripheral blood and/or bone marrow invasion.

Principles/Methodology

Here, we selected a comprehensive cohort of 110 NHL cases (79 B-NHL and 31 T-NHL/natural killer-NHL) derived from different types of tissue biopsies, all with established histopathological diagnoses and performed OGM. Seventy-eight samples were genomically well characterized at diagnosis by CBA and FISH. The remaining 32 cases were included because of previous CBA failure, although FISH data were available for 20 cases.

Results

OGM provided informative results in 94% of the cohort, with a high concordance rate of 97.6% compared with CBA/FISH in detecting clinically relevant aberrations. The 2 variants that were missed were both present at the detection threshold of OGM. In contrast, OGM successfully resolved 26 samples with previous CBA failure and detected 3 additional disease-defining events, resulting in diagnostic reclassification of 1 patient. Finally, OGM identified novel recurrent aberrations that warrant further investigation into their pathogenetic implications.

Conclusion

To conclude, OGM robustly detects clinically relevant structural variants and copy number alterations and presents a promising alternative to CBA and FISH in routine diagnostic evaluation of NHL.

Histiocytic Neoplasms with a Concurrent Haematological Neoplasm, a Single Institution's Experience

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Background

Histiocytic neoplasms are rare haematological disorders characterized by macrophage/histiocytic proliferation, some driven by clonal mutations and translocations, commonly in the MAPK pathway. Even more rarely, these can be associated with a second haematological malignancy, which some studies show share identical mutations, suggesting a common progenitor. We present a case series of histiocytic disorders including two with a second, concurrent haematological malignancy from a large, UK-based Haematological Malignancies Diagnosis Service (HMDS) centre.

Principles/Methodology

Cases were retrieved from the King's College Hospital electronic medical records. The search ranged from January 2022 to April 2026 using the search terms: 'Rosai Dorfman Disease' (RDD), 'histiocytosis' 'Langerhans cell histiocytosis' (LCH), 'Langerhans cell sarcoma' (LCS), 'Erdheim-Chester Disease' (ECD) and related terms. Searches were completed on SNOMED codes and the final diagnosis. Cases which met the inclusion criteria were reviewed.

Results

We identified 18 cases of histiocytic proliferations (Table 1): 11 cases of RDD, 5 cases of LCH, 1 case of LCS and 1 case of ECD. Two cases were identified with a second concurrent haematological malignancy. Patient 4 was a 68-year-old male who presented with anaemia, thrombocytopenia and a monocytosis. The bone marrow aspirate showed features of chronic myelomonocytic leukaemia. The biopsy also showed a proliferation of S100+/CD68+ histiocytes with emperipolesis, rendering a diagnosis CMML-0 with neoplasia-related RDD. Immunohistochemistry (IHC) for BRAF V600E was strongly positive in both the histiocytic and monocytic population (Image 1). The patient progressed to acute myeloid leukaemia and passed away 4 months later. Patient 11 was a 58-year-old male, who presented with acute kidney injury and serum free light chain ratio of <0.01 (Lambda light chain 20,070 mg/L). The bone marrow aspirate was in keeping with plasma cell myeloma and the biopsy demonstrated 20-25% clonal plasma cells, as well as S100+/CD68+ histiocytes with emperipolesis. IHC for BRAF V600E was negative in the histiocytic population. The patient is currently on second line therapy.

Conclusion

We identified 2 cases of neoplasia-related RDD. One case showed BRAF V600E was positive in both histiocytic and monocytic populations by IHC, suggesting a common mutation. Further studies are required to elucidate the clonal relationship and pathogenic impact of dual histiocytic and non-histiocytic haematological malignancies.

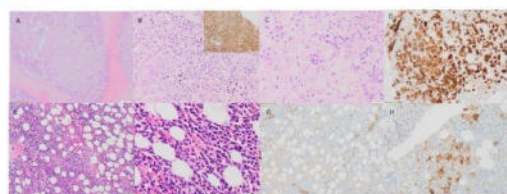


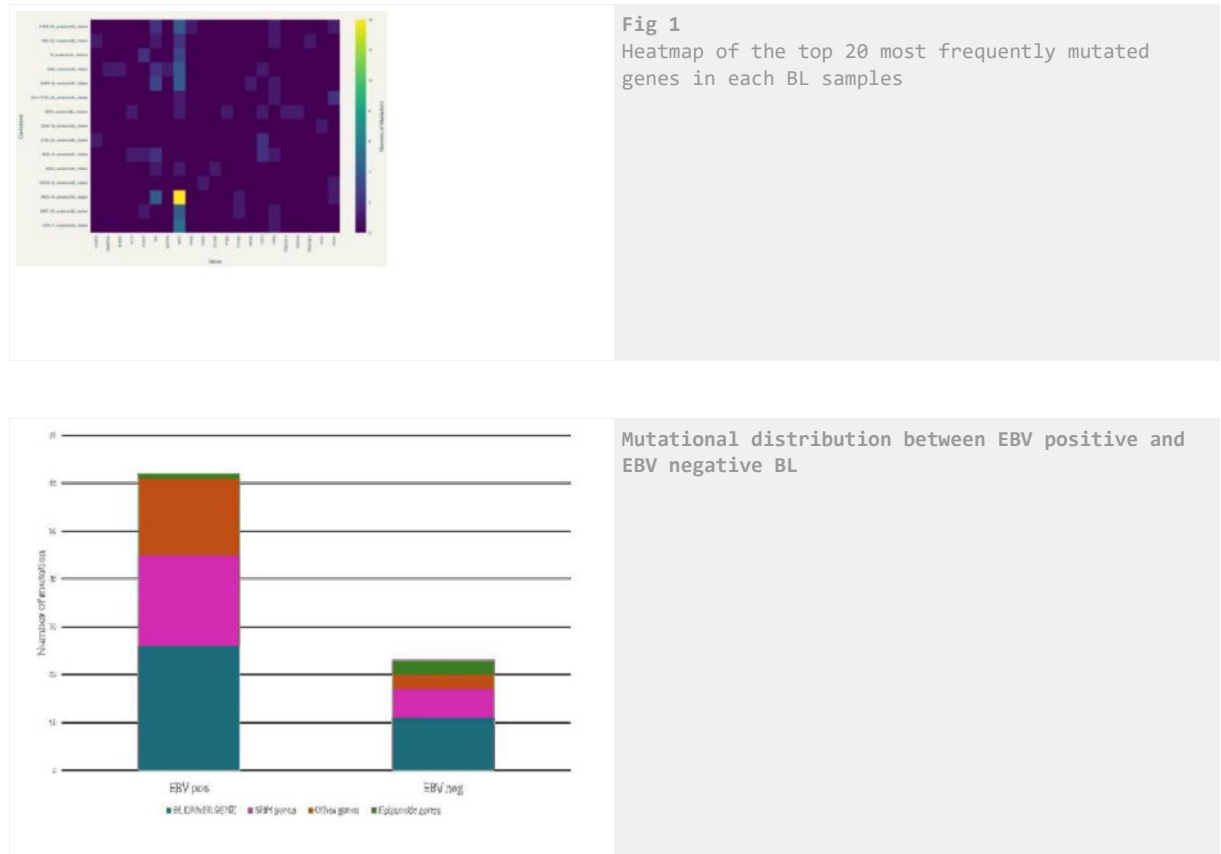
Image 1.

Patient 4, case of CMML and RDD. (A B and C) H&E shows hypercellular marrow with a histiocytic proliferation, emperipolesis and atypical monocytoid cells. (B-Inset) Lysozyme IHC shows positive staining of monocytoid cells. (D) BRAF V600E shows strong positive staining in both the histiocytic and the monocytic cells. Patient 11, case of plasma cell myeloma with RDD. (E and F) H&E shows hypercellular marrow with histiocytes, emperipolesis and an atypical plasma cell proliferation. (G) MUM-1 IHC highlights the plasma cell population. (H) S100 IHC positive staining in the histiocytic population.

MYC, *PAX5* while EBV negative BL had more driver gene mutations in *ID3*, *TCF* and more epigenetic mutations. These findings were independent of age.

Conclusion

Our results indicate that consistent with findings from European and American studies, the mutational status of BL in African patients is more strongly associated with the presence of EBV than the age of the patient. Our study provides new insights into the biology of BL in Africa. EBV negative BL in Africa may represent sporadic BL confirming that not all cases of African BL should be assumed to be endemic BL. This highlights the importance of considering EBV status in the molecular classification of BL. These results will be confirmed in a larger cohort.



EA4HP26-ABS-499

Ultrasensitive kappa and lambda dual in situ hybridization has high diagnostic utility in the differential diagnosis of Hodgkin lymphoma

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Background

Classic Hodgkin lymphoma (CHL) is derived from germinal center B cells that downregulate the B-cell program and lack immunoglobulin expression. Most CHLs can be differentiated from other B-cell neoplasms by assessing clinical presentation combined with morphologic and immunophenotypic features. However, in some situations it may be

difficult to distinguish CHL from other neoplasms such as nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) and primary mediastinal large B-cell lymphoma (PMBL).

Principles/Methodology

We investigated the utility of a highly sensitive kappa and lambda dual in situ hybridization (DISH) mRNA stain (Roche/Ventana Medical Systems, Tucson, AZ) in distinguishing CHL, NLPHL and PMBL. Whole tissue sections from a total of 99 cases (49 CHL, 22 NLPHL, and 28 PMBL) were examined.

Results

Three cases (1 NLPHL and 2 PMBL) were not interpretable due to crush artifact and/or insufficient neoplastic cells. The HRS cells of CHL were negative for both kappa and lambda in 47/49 (96%; 30/30 nodular sclerosis, 4/6 mixed cellularity (MCCHL), 5/5 lymphocyte-rich (LRCHL), 5/5 syncytial, 3/3 NOS), while neoplastic cells were positive in 20/21 (95%; 13 kappa, 6 lambda, 1 double kappa/lambda) NLPHLs and 26/26 (100%; 19 kappa, 6 lambda, 1 double kappa/lambda) PMBLs ($p < 0.0001$, two-tailed Fisher's exact test) (Figure 1). Two EBV(-) MCCHLs were kappa restricted, but otherwise had clinicopathologic features and treatment response consistent with CHL. The sensitivity and specificity to differentiate CHL from NLPHL was 91% and 98%, respectively, and to differentiate CHL from PMBL was 93% and 100%, respectively. Background non-neoplastic B cells showed skewed light chain staining (excess kappa) in 2/49 (4%) CHL and 4/22 (18%) NLPHL, a finding not detected by concurrent flow cytometry. In comparison, 3 CHL that arose as Richter transformation from chronic lymphocytic leukemia/small lymphocytic lymphoma contained monotypic background small B cells. Background plasma cells showed excess kappa staining in 1/49 (2%) CHL and 4/28 (14%) PMBL.

Conclusion

Determination of light chain expression by ultrasensitive kappa and lambda DISH staining is valuable in the evaluation of CHL and its mimics, with a high sensitivity and specificity. This assay appears useful in differentiating LRCHL from NLPHL and syncytial CHL from PMBL. Future studies are warranted to determine if this assay may be particularly helpful in needle core biopsy specimens that do not allow full assessment of associated architectural features.

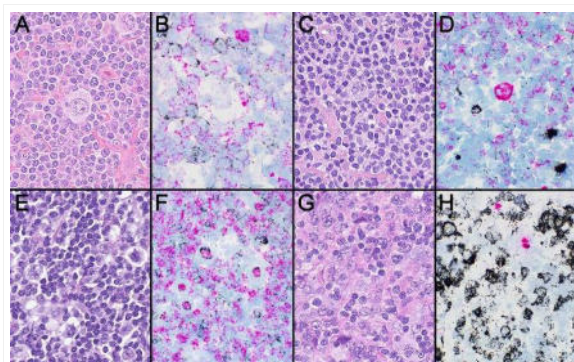


Figure 1. Immunoglobulin light chain staining with ultrasensitive kappa and lambda DISH

The HRS cells of a CHL (A, H&E x400) are negative for immunoglobulin light chain expression using ultrasensitive kappa and lambda DISH (B, x1000). The LP cells of a NLPHL (C, H&E x400) show monotypic kappa (magenta) staining (D, x500). One NLPHL case (E, H&E x400) demonstrates both lambda (silver) cytoplasmic and kappa (magenta) nuclear staining (F, x500). A PMBL case (G, H&E x400) shows monotypic lambda (silver) expression (H, x500).

EA4HP26-ABS-516

Deep sequencing of B-cell lymphoma specific genes in molecular and clinical follicular lymphoma subgroups

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Background

Follicular lymphoma (FL) is a malignant neoplasm of mature germinal center B cells. The majority of cases exhibit the t(14;18)(q32;q21) translocation, which is detected in approximately 85% of advanced FL cases and in approximately 50% of localized FL cases. Another distinctive molecular feature of FL biology is the acquisition of newly acquired N-glycosylation sites (NANGS) in the variable B-cell receptor domains, generated by somatic hypermutation (SHM). Previous studies revealed similarities but also significant differences among these molecular and clinical subgroups. However, mutation screenings were typically conducted with relatively low coverage and primarily on paraffin-embedded tissue, not optimal for the detection of subclonal mutations.

Principles/Methodology

Thus, we applied deep sequencing (mean coverage on target: >1.000x) of 40 genes typically altered in B-cell lymphoma to a well characterized FL cohort (n=67) with fresh frozen material available.

Results

This approach confirmed a significantly higher amount of *BCL2* and *MEF2B* mutations in t(14;18)-positive FL. *STAT6* mutations were more common in t(14;18)-negative FL, although the difference was not statistically significant. No significant differences were found between FL with and without NANGS regarding the occurrence of mutations in the genes examined here, nor was there an increased incidence of *CREBBP* and *STAT6* mutations in advanced or localized FL, as had been observed in an earlier, similar study comparing these groups. However, unlike previous approaches, the current strategy showed that certain pathogenic relevant gene mutations occurred significantly more frequently in localized FL at the clonal and subclonal level.

Conclusion

In summary, this deep-sequencing study has allowed us to identify a higher frequency of pathogenic and diagnostic relevant gene mutations that had not been detected in previous studies. Whether this difference can be validated in other cohorts and is reflected at the protein level remains to be investigated.

EA4HP26-ABS-520

Amplification and overexpression of cyclin D1 in Epstein–Barr virus-positive inflammatory follicular dendritic cell tumor: A targeted next-generation sequencing study

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Background

Follicular dendritic cell (FDC) sarcoma comprises a conventional type and an inflammatory pseudotumor-like variant (IFDCT). IFDCT is consistently associated with Epstein–Barr virus (EBV) infection and generally follows an indolent clinical course. Although EBV is believed to contribute to the pathogenesis of IFDCT, the underlying mechanisms remain incompletely understood. Recent comprehensive next-generation sequencing studies have identified recurrent somatic alterations involving the major histocompatibility complex and epigenetic regulatory pathways, as well as signaling pathways related to immune responses.

Principles/Methodology

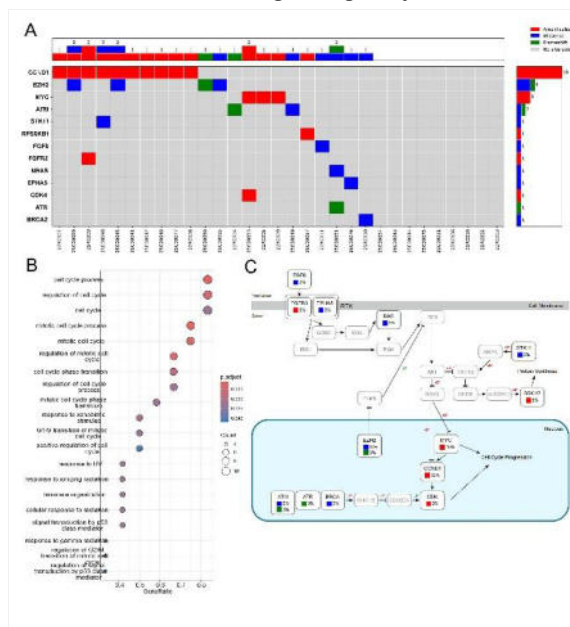
In this study, we performed targeted DNA sequencing of 523 cancer-associated genes (TruSight Oncology 500) in 31 cases of IFDCT. The cohort included 13 men and 18 women, with a mean age of 62.6 years (range, 36–84). Tumors arose in the spleen (n=20), liver (n=8), colon (n=2), and pancreas (n=1), with a mean tumor size of 5.8 cm (range, 1.4–11.7).

Results

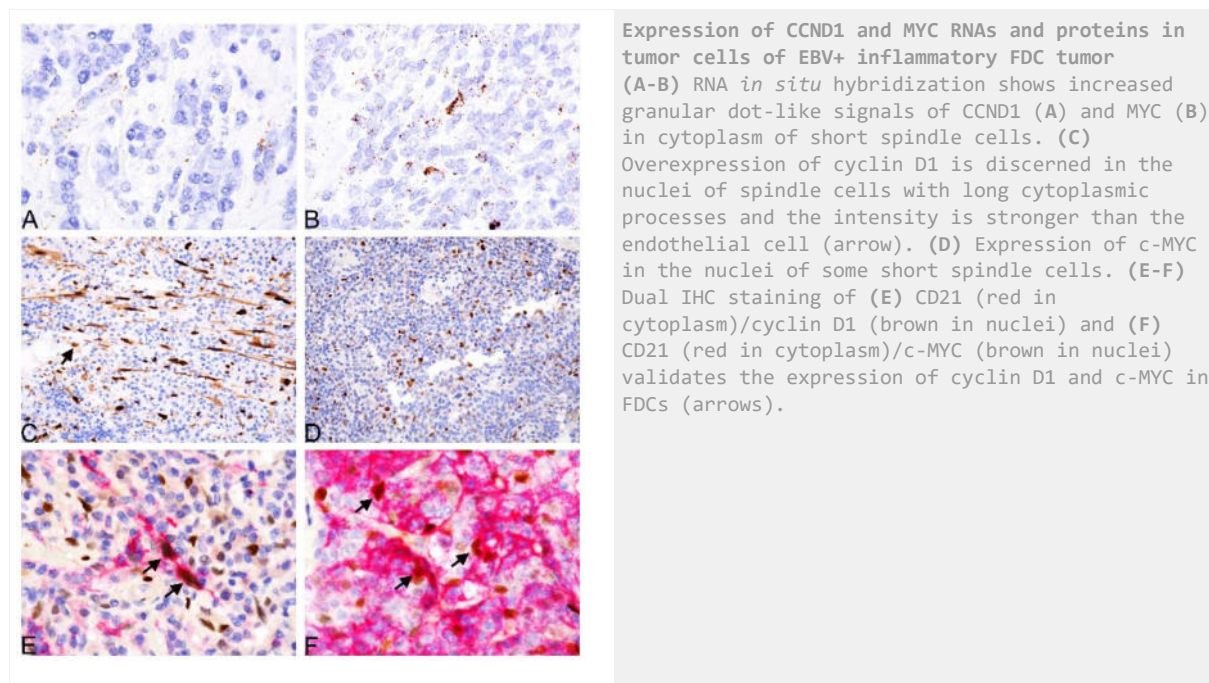
Genetic alterations were found in 71% of cases with *CCND1* amplifications (3-5 copies) being the most common (45.5%), followed by *EZH2* mutations (18.2%), *MYC* amplifications (3-4 copies, 13.6%) and *ATM* mutations (9.1%). Pathway analysis identified cell cycle progression as the main activated signaling in IFDCT. RNA *in situ* hybridization and double immunohistochemical staining confirmed the overexpression of cyclin D1 and MYC in FDCs. Clinically, overexpression of cyclin D1 was frequently found in both mutation-positive (88.9%) and mutation-negative (71.4%) cases and showed no association with tumor behavior. No significant clinicopathologic difference was present between mutation-positive and mutation-negative tumors. Notably, splenic origin was associated with female predominance (p=0.011) and larger tumor size (p=0.048). In addition, tumor cells exhibited a type II EBV latency pattern (LMP1+/EBNA2-) in 84.6% of cases, and LMP1 expression correlated with larger tumor size (p=0.021).

Conclusion

Taken together, these findings support a role for EBV-driven activation of the cyclin D1 pathway and suggest that EBV–LMP1–*CCND1* signaling may contribute to the pathogenesis of IFDCT.



Genetic mutation profile and bioinformatics analysis of EBV-positive inflammatory FDC tumor (A) Waterfall plot depicting the variant landscape identified by TS0500 sequencing. (B) Gene Ontology (GO) enrichment analysis for Biological Process (BP), performed using clusterProfiler reveals that enriched terms are predominantly associated with the mitotic cell cycle process. (C) Pathway map constructed from KEGG analysis and literature curation. The schematic illustrates the convergence of the FGF8/FGFR3/MYC axis, mTOR signaling, and DNA damage response genes (ATM, ATR, and BRCA) to drive mitotic cell cycle progression.



EA4HP26-ABS-522

SAMHD1 gene promoter methylation is the mechanism for silencing of SAMHD1 gene and protein expression in precursor T-acute lymphoblastic leukemia but not in mature T-cell lymphomas

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Background

The SAM and HD domain 1 (SAMHD1) protein is a dNTP hydrolase that depletes the intracellular dNTPs. SAMHD1 operates as a restriction factor for HIV infection, is involved in DNA damage and anti-tumor immune responses, and confers resistance to cytarabine in acute myeloid leukemia. SAMHD1 may also play a role in oncogenesis as a tumor suppressor. We have shown previously (EA4HP 2022) that the SAMHD1 is commonly but variably expressed in mature T-cell non Hodgkin lymphomas (NHL) but not in precursor T-acute lymphoblastic leukemia (T-ALL). Therefore, the present study aimed at investigating the mechanism responsible for silencing of SAMHD1 gene and protein expression in precursor T-ALL as opposed to mature T-NHL or preB-ALL.

Principles/Methodology

The *in vitro* system consisted of 23 cell lines, including 9 precursor T-ALL, 8 precursor B-ALL and 6 mature T-NHL cell lines. Bisulfite-converted genomic DNA was subjected to methylation-specific PCR amplification to assess the methylation patterns in the SAMHD1 promoter region. Jurkat cell line showing hypermethylation of SAMHD1 gene promoter served as a positive control and was treated, along with other cell lines, with methylation inhibitors Decitabine, 5-Azacytidine and GSK-3484862. Protein expression was analyzed by Western blot and gene expression by quantitative RT-qPCR (mRNA level). The *in silico* analysis was conducted on data from 51 childhood ALL cell

lines obtained from *Leo et al.* (Nature Communications 2022, PRIDE), including 29 B-ALL, as well as 22 T-ALL cell lines, for genome-wide evaluation of RNA and protein expression levels of targets of interest.

Results

SAMHD1 gene promoter methylation was found in 8/9 T-ALL, 0/8 preB-ALL and 0/6 mature T-NHL cell lines ($p < 0.0001$, Fisher's exact test). Similarly, methylation of SAMHD1 gene was observed in 9/13 patients with T-ALL but 0/22 patients with mature T-NHL ($p < 0.0001$, Chi-square test). Treatment of T-ALL cell lines with de-methylation agents restored SAMHD1 gene expression. In silico analysis showed increased SAMHD1 gene expression (>2 log2 fold) only in 2/22 T-ALL as compared to 22/29 preB-ALL cell lines ($p < 0.0001$, Chi-square test). All but one (TALL-104) T-ALL cell lines showed high gene expression of methyltransferases DNMT3A and TET1.

Conclusion

Methylation of SAMHD1 gene promoter seems to be the mechanism underlying silencing of the gene in T-ALL but not in mature T-NHL or preB-ALL cells. The use of de-methylation agents restores the gene expression of a promising tumor suppressor, SAMHD1.

EA4HP26-ABS-532

TCR Alpha: A Novel, Powerful Clinical Tool for Diagnosis of Reactive and Neoplastic T-Cell Proliferations

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Background

Detecting T-cell receptor expression is a critical component of diagnosis of T-cell lymphomas and distinguishing them from natural killer lineage malignancies. There are only limited tools for detection of TCR expression in TCL on FFPE specimens. This is especially challenging in TCL lacking surface CD3 expression which leads loss of TCR expression. To address this issue, we developed a novel immunohistochemistry assay that can detect TCR alpha (TCR α) chain expression.

Principles/Methodology

105 cases, including 20 cases with benign T-cells (reactive lymphoid tissues (n=10), B-cell lymphomas with reactive T-cell background (n=10)), mature TCLs of $\alpha\beta$ lineage (n=65; enriched for cases with loss of surface CD3 by flow cytometry [sCD3^{FC}]), and $\gamma\delta$ T-cell (n=10) / NK-cell (n=10) lymphoproliferative disorders were tested. All cases were stained with CD3 (LN10), TCR α (H-1), and TCR β (8A3). A subset of cases (n=13) were further evaluated by multiplex immunofluorescence, as well as thymic controls (n=2) to correlate expression patterns with T-cell maturation.

Results

(1) Validation: In benign/reactive cases (n=20), all showed concordant CD3^{IHC} and TCR α staining with expected membranous/cytoplasmic patterns, verifying antibody performance in non-neoplastic T-cells. All $\gamma\delta$ T/NK-cell LPDs (n=20) were negative for both TCR α and TCR β , confirming specificity.

(2) $\alpha\beta$ TCLs/LPDs: 65 mature $\alpha\beta$ TCLs (n=18 TFHL, n=7 ATLL, n=10 sALCL, n=3 BI-ALCL, n=7 T-PLL, n=2 PTCL-NOS, n=1 T-LGL, n=1 EATL, n=3 $\alpha\beta$ MEITL, n=7 MF/SS, n=6 other CTCL) were stratified by sCD3^{FC} as positive (n=12), dim (n=11) and negative (n=42). TCR α IHC was positive in 11/12 sCD3^{FC} positive and 10/11 sCD3^{FC} dim cases; TCR β was positive in 10/12 and 8/11, respectively. Among sCD3^{FC} negative cases, CD3^{IHC} was positive in 30 and negative in 12; TCR α was positive in 28/30 CD3^{IHC} positive and 9/12 in CD3^{IHC} negative cases, whereas TCR β was positive in

Conclusion

	Flow Cytometry	H&E	Tracking Marker	CD3	TCRa	TCR β
Case 1						
Case 2						
Case 3						
Case 4						
Case 5						
Case 6						

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Genomic configuration of non-*IG::MYC* translocation in DLBCL and its biological impact

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Background

MYC translocation occurs in ~12% of DLBCL, with ~55% involving *IG* and associating with poor prognosis. The remaining translocations involve non-*IG* genes, showing variable clinical and biological impacts, and their spectrum and effect on *MYC* transactivation remain unclear.

Principles/Methodology

We studied 153 DLBCLs including 148 with *MYC* translocation by FISH analysis (21 *IG::MYC*, 49 non-*IG::MYC*, 78 with unknown partners) and 5 with unknown *MYC* status. *MYC* translocations, copy number changes, and somatic mutations were investigated by next generation sequencing (NGS) using a custom TWIST panel.

Results

NGS detected *MYC* translocations in 18/21 cases with *IG::MYC*, 37/49 with non-*IG::MYC*, 68/83 with unknown *MYC* partners/status, and 4 with cryptic *MYC* translocation not identified by FISH.

Among *IG::MYC* cases, 73% were *IGH::MYC*, with *MYC* breakpoints clustered from ~2 kb upstream of the 5' UTR to intron 1, and *IGH* breakpoints located in switch regions encompassing super enhancers. The remaining *IG::MYC* cases involved *IGL* (21%) and *IGK* (6%), with *MYC* breakpoints in the 3' UTR or further downstream to *PVT1*, while light chain breakpoints lay in joining/constant regions with super enhancers. Immunohistochemistry revealed high *MYC* protein expression (≥70% tumour cells) in 85% (17/20) *IG::MYC* cases. One of the three low-expression cases harboured a somatic mutation likely disrupting the epitope recognised by *MYC* antibody.

Among non-*IG::MYC*, 30 translocation partners were identified, with the most frequent partner loci being *BCL6* (28%) and *PAX5/ZCCHC7* (15%). *MYC* breakpoints in non-*IG::MYC* spanned a broad region from ~200kb upstream of *MYC* to ~440kb downstream of *PVT1*. Five cases showed promoter substitution and 2/2 cases with *MYC* expression data exhibited high *MYC* protein levels. The remaining cases displayed no clear genomic configuration indicating super enhancer hijacking, nonetheless 40% (16/40) showed high *MYC* expression.

In *IG::MYC*, ad hoc deletions, copy number gains and somatic mutations occurred near translocation breakpoints, while in non-*IG::MYC*, these alterations spanned *MYC/PVT1* regions, without any clusters targeting *MYC* transcriptional control.

Conclusion

IG::MYC juxtaposes *MYC* with *IG* switch super enhancer, leading to *MYC* constitutive transactivation. Non-*IG::MYC* involves a wide spectrum of promiscuous partners with variable genomic configurations, a minority showing promoter substitution and a big majority lacking apparent genomic machinery to drive *MYC* transactivation.

MYC Mutations in Large B-Cell Lymphomas and Their Correlations with Gene Rearrangements and Copy Number Gain

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Background

MYC rearrangements are detected in 10–15% of large B-cell lymphomas (LBCL). They can occur alone or in association with rearrangements on *BCL2* with or without *BCL6* in the category of “high grade B-cell lymphomas, double/triple hit (HGBCL-DH/TH). *MYC* copy number gain (CNG) occurs in 15% of LBCL. Coding *MYC* point mutations are known to occur in approximately half of LBCL with *MYC* rearrangement, however their association with CNG is poorly understood and their prognostic impact little explored.

Principles/Methodology

MYC coding sequence has been investigated within a commercial targeted NGS lymphoma panel, in 78 DLBCL cases, retrieved from the archives of the Pathology Unit of ASST Spedali Civili di Brescia. These included 60/78 with diagnosis of DLBCL, NOS (77%) and 18/78 HGBCL-DH/TH or -NOS (23%). In addition to routine FISH analysis using *MYC*, *BCL2* and *BCL6* break apart probes, fusion probes for *MYC::IGH*, *MYC::IGK* and *MYC::IGL* were sequentially applied in *MYC* rearranged (-R+) cases. *MYC*-CNG was detected by NGS data analysis. Clinical data was available for all cases and statistical prognostic correlations were attempted.

Results

Among the investigated cases, 13/78 (17%) showed mutations in the *MYC* coding sequence (*MYC*-MUT). They occurred proportionally in HGBCL-DH/TH or -NOS (3/18, 17%) and DLBCL, NOS (10/60, 17%). The most frequent mutation detected was the p.(Thr73Ile), found in 3/13 cases (23%). Another two cases carried mutations in the adjacent Pro72 residue (Pro72Ser and Pro72His, respectively). All together, these are known pathogenic mutations affecting the *MYC* Box 1 (MB1) domain. All the 5 cases harboring MB1 mutations are also *MYC*-R+ with 4/5 displaying a *MYC::IGH* fusion. Notably, in 5/13 (38%) cases, more than 1 *MYC* mutation was detected (from 2 to 6) and all were *MYC*-R+ with 4/5 displaying a *MYC::IGH* fusion. Among the *MYC*-CNG cases, 1/9 was also *MYC*-MUT, although the unique identified variant was the p.(Asn26Ser), a known benign polymorphism. Notably, while no prognostic significance was detectable for *MYC* mutations, *MYC*-CNG in this cohort results significantly unfavorable and associated with higher risk of death for disease.

Conclusion

This single centre experience confirmed the strong association of *MYC*-MB1 pathogenic mutations and the occurrence of multiple *MYC* mutations with *MYC*-R+ cases, particularly in a *MYC::IGH* context. *MYC*-CNG, detected by NGS, was correlated with higher incidence of death for disease, but did not associate with *MYC* pathogenic mutations.

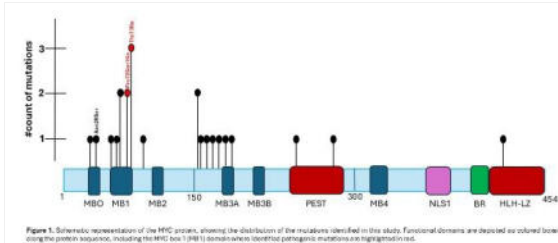


Figure 1
Figure 1. Schematic representation of the MYC protein, showing the distribution of the mutations identified in this study. Functional domains are depicted as colored boxes along the protein sequence, including the MYC box 1 (MB1) domain where identified pathogenic mutations are highlighted in red.

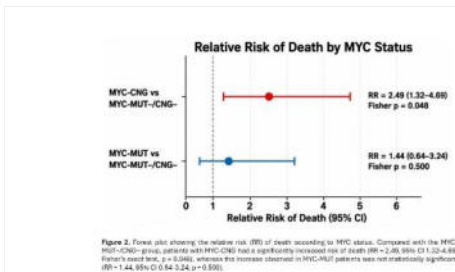


Figure 2
Figure 2. Forest plot showing the relative risk (RR) of death according to MYC status. Compared with the MYC-MUT-/CNG- group, patients with MYC-CNG had a significantly increased risk of death (RR = 2.49, 95% CI 1.32-4.69; Fisher's exact test, $p = 0.048$), whereas the increase observed in MYC-MUT patients was not statistically significant (RR = 1.44, 95% CI 0.64-3.24; $p = 0.500$).

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Clonally related and concurrently occurring Interdigitating Dendritic Cell Sarcoma (IDCS) and T-Lymphoblastic Leukemia/Lymphoma (T-LLL) in a four years old girl - a case report and review of IDCSs associated with other hematolymphoid neoplasms (HLN)-

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Background

IDCSs are rare and interesting HLNs. It has been reported that these tumors can be associated with second malignancies including other HLNs not surprisingly. Increasing molecular evidence suggests that some of these tumors share a common clonal origin reflecting lineage plasticity and transdifferentiation. We reported here a unique child case diagnosed as IDCS and T-LLL in the same lymph node (LN) and concurrently bone marrow (BM) biopsy which were proven to be clonally related.

Principles/Methodology

Beside routine examination of LN and BM biopsy, immunohistochemistry (IHC), flow cytometry (FC), and next generation sequence (NGS) were performed on LN biopsy and BM aspiration.

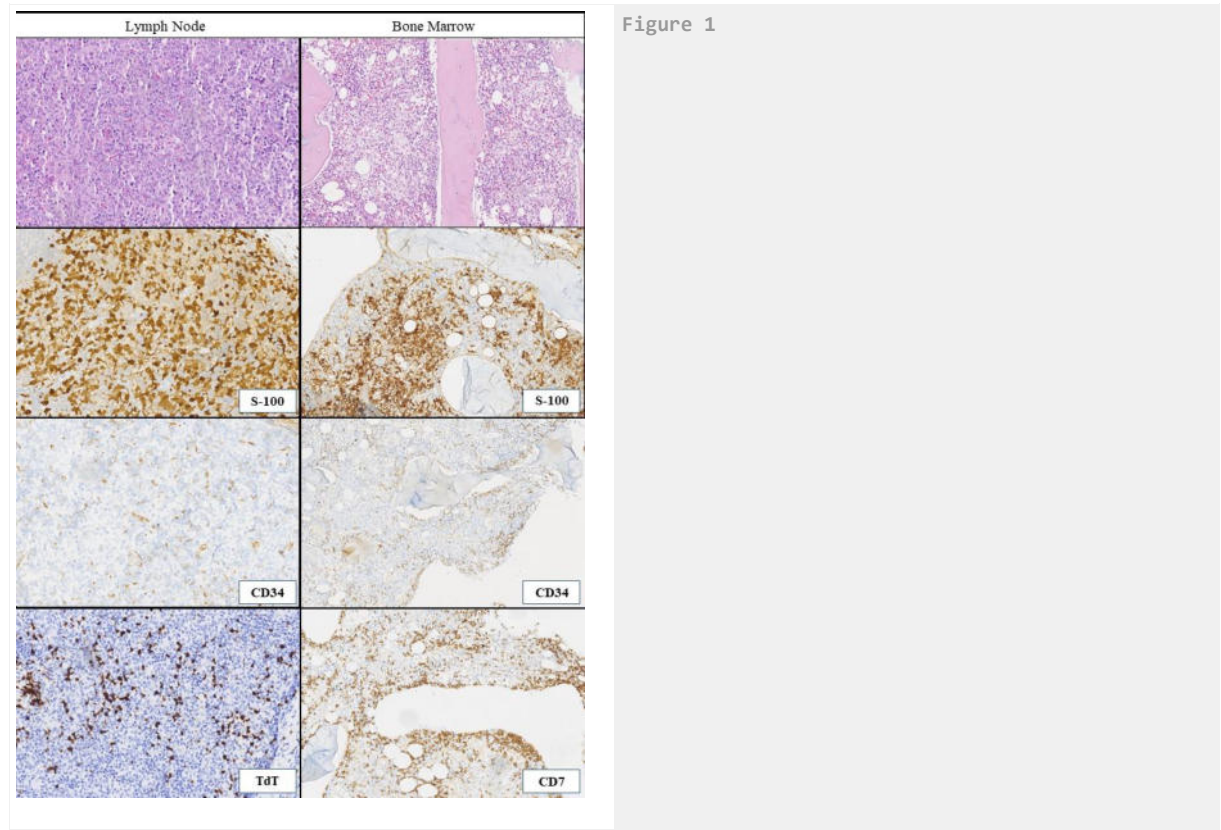
Results

The patient was presented with persistent multiple lymphadenopathies. Histopathological examination of excisional biopsy of servical LN revealed the tumor consisting of sheets of polygonal cells (Figure 1) which were strongly positive

for S-100, CD4, and CD43 by IHC. The antibodies being negative were CD45, all other T cell markers, all B cell markers, langerin, HMB-45, SOX-10, keratin AE1/AE3, EBER, CD68, and CD163. There was also a lymphoblastic component consisting of 10% of all cellular population which was positive for CD34 and TdT. Similarly, there were two neoplastic populations in BM biopsy. BM aspiration showed only T-LLL component because of the increased reticulin fibrosis in sarcoma areas probably. Therefore, FC analysis was reported only pre-T-Lymphoblastic leukemia with the 26% of blastic cells. Regarding of these FC findings, we may draw the conclusion of FC and NGS analyses of BM aspiration were reflecting the lymphoblastic component alone. NGS revealed identical pathogenic variants in WT1, ETV6, NRAS, and MPL across both specimens, supporting a shared clonal origin. Patient was treated by chemotherapy as T-LLL. After 30 months of diagnosis, patient is doing well.

Conclusion

According to the English literature (except one study with unknown number of similar cases), we have found 23 cases of IDCs with another HLN. Among these 23 cases, there is only one patient has T-LLL. There are few reports showing a clonal relationship between sarcoma and second HLN as in our case. These findings highlight the transdifferentiation of lymphoid neoplasm to/from IDCs, arising a result of MAPK pathway mutation. Therefore, the treatment of HLN may cure IDCs, too, similar to case presented here.



A TCRbeta1/TCRbeta2 (TRBC1/TRBC2) monoclonal antibody pair for determining T-cell monotypia as a surrogate for clonality in formalin-fixed paraffin-embedded material

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Background

T-cell lymphomas are often histologically indistinguishable from benign T-cell infiltrates. Clonality testing is frequently required for diagnosis. It lacks the spatial context and is slow and expensive, relying on complex, multiplexed PCR reactions, which are interpreted by scientists or pathologists with specialist molecular training. For B-cell and plasma cell neoplasms, the kappa/ lambda assay permits analysis of B-cell monotypia, as a surrogate for clonal status, avoiding the need for clonality testing in many cases. Prior to our work, no monoclonal antibody pair existed that could distinguish between the two differentially used T-cell receptor beta constant regions, TCRbeta1 and TCRbeta2 (TRBC1 and TRBC2), in formalin fixed paraffin embedded (FFPE) tissue.

Principles/Methodology

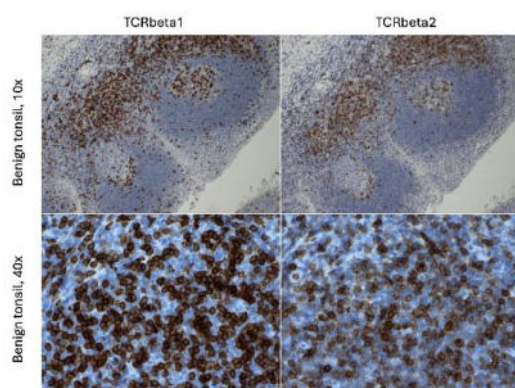
We produced specific rabbit monoclonal antibodies and demonstrated the feasibility of their use for immunohistochemical detection of TCRβ1 and TCRβ2 in formalin-fixed, paraffin-embedded (FFPE) tissue as a novel diagnostic strategy for T-cell lymphomas. We also made a chimeric mouse monoclonal anti-TCRβ2 antibody from the parent rabbit antibody.

Results

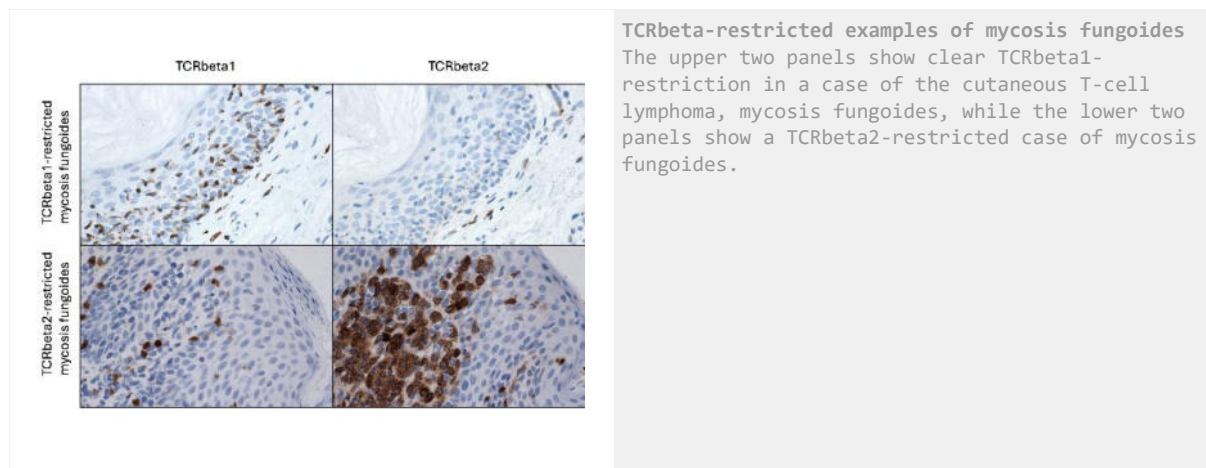
In our initial biopsy cohort, single immunostaining results permitted clear distinction between T-cell lymphoma (n=13) and benign T-cell populations (n=8). Both TCRβ1/2 double immunostaining and CD4/CD8 double immunostaining combinations with TCRβ1/2 appeared promising and might have utility in selected clinical diagnostic settings. We also showed that single immunostaining is amenable to automated cell counting using QuPath, permitting accurate calculation of the TCRβ2:TCRβ1 ratio. We are now expanding these cohorts.

Conclusion

This novel diagnostic assay for T-cell lymphoma can be used in an analogous way to the kappa/ lambda assay for B-cell and plasma cell neoplasms. It promises to be diagnostically useful in both haematopathology and dermatopathology.



TCRbeta1/2 staining in a benign tonsil section
Similar numbers of lymphocytes are positively stained with each antibody, indicating a TCRbeta1:2 ratio close to 1:1.



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Analysis of vitreous cell-free DNA may improve molecular analysis in vitreoretinal lymphoma

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Background

Vitreoretinal lymphoma (VRL) is a rare and aggressive subtype of diffuse large B-cell lymphoma (DLBCL) and is considered a variant of primary central nervous system lymphoma (PCNSL). Diagnosis remains challenging, as conventional cytology and immunophenotyping of vitreous aspirates has limited sensitivity. Molecular diagnostics^(1,2) have improved detection rates, and recent evidence suggests that vitreous cell-free tumor DNA (ctDNA) may represent a valuable diagnostic substrate. Therefore, we systematically compared mutation and copy number variation (CNV) patterns in vitreous cells and ctDNA of vitreous fluid, respectively.

Principles/Methodology

Using a customized amplicon-based next-generation sequencing (NGS) analysis targeting recurrent alterations in PCNSL, vitreous ctDNA was compared with paired genomic DNA (gDNA) derived from the cellular fraction of vitrectomy specimens from 46 VRL cases and 10 uveitis controls. Concordance of single nucleotide variants (SNVs) and CNVs, mutation profiles, and variant allele frequencies (VAFs) were evaluated.

Results

ctDNA analysis showed excellent concordance with paired gDNA results. Overall, 91% of SNVs and 95% of CNVs detected in VRL were identified in both sample types. The most frequently altered genes were *MYD88* and *PIM1* (84% each), followed by *KMT2D* (60%), *MPEG1* (55%), *IGLL5* (54%), *CD79B* (52%), and *ETV6* (45%). *CDKN2A/B* deletions represented the most common CNVs. Clinically relevant hotspot mutations in *MYD88* and *CD79B* were consistently detected in both materials. Additional private mutations were observed in 48% of paired samples, predominantly in ctDNA, mostly at low VAFs and involving genes associated with somatic hypermutation. Mean VAFs were higher in ctDNA than in gDNA. No pathogenic mutations or CNVs were detected in uveitis samples.

Conclusion

Vitreous ctDNA is a reliable and sensitive substrate to strengthen molecular diagnostics for VRL. The high concordance with cellular gDNA, robust detection of key driver alterations, and absence of alterations in reactive controls support its clinical usability. Higher cfDNA concentrations and VAFs in comparison to gDNA are likely due to high vulnerability and apoptotic rate of vitreous lymphoma cells. Therefore, vitreous ctDNA analysis may improve VRL detection in routine diagnostics.

1. Bonzheim I et al, Blood 2015;126(1):76-9
2. Bonzheim I et al, Blood Adv. 2022;6(5):1598-1607

EA4HP26-ABS-569

Immunoglobulin light chain mRNA in situ hybridization refines classification across the NLPHL–cHL spectrum

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Background

Nodular lymphocyte-predominant Hodgkin/B-cell lymphoma (NLPHL/BL) and classic Hodgkin lymphoma (cHL) are biologically distinct entities, exhibiting preserved versus lost B-cell differentiation and immunoglobulin (IG) transcription. However, cases with overlapping morphologic and immunophenotypic features between NLPHL and lymphocyte-rich cHL (cHL-LR), here designated as NLPHL-OL, remain biologically ambiguous.

Principles/Methodology

We applied a novel, highly sensitive dual κ/λ mRNA in situ hybridization assay to assess IG light-chain expression across this spectrum. We analyzed 53 lymph node biopsies, including NLPHL-OL cases (n=15), cHL-LR (n = 11), along with typical NLPHL (n = 13) and cHL of other subtypes (n = 14) as controls. Distribution, intensity, and patterns of κ/λ mRNA expression in neoplastic cells was systematically evaluated and correlated with histology, architecture, immunophenotype, and Epstein-Barr virus (EBV) status.

Results

Diffuse, strong cytoplasmic light chain mRNA expression, representing the canonical pattern, was detected in the tumor cells of all NLPHL (13/13) and the majority of NLPHL-OL cases (12/15, 80%). In contrast, faint perinuclear or granular cytoplasmic signals (non-canonical patterns) were detected in 6/11 (55%) cHL-LR and 4/14 (28%) of other cHL subtypes. Canonical κ/λ expression was thus restricted to NLPHL and NLPHL-OL, confirming their classification as NLPHL with aberrant features, whereas cHL was negative or displayed exclusively non-canonical patterns. Non-canonical staining was also seen in 3/15 (20%) NLPHL-OL, including two EBV-positive cases. Rare aberrant light chain profiles in NLPHL and NLPHL-OL cases included single-cell κ/λ coexpression, and distinct LP-cell populations with mutually exclusive κ and λ expression.

Conclusion

In summary, κ/λ mRNA ISH refines diagnostic classification across the NLPHL–cHL spectrum, is particularly useful in cases with overlapping features, and reveals canonical, non-canonical, and negative staining patterns that may reflect B-cell program integrity, placing these cases along a biological continuum.

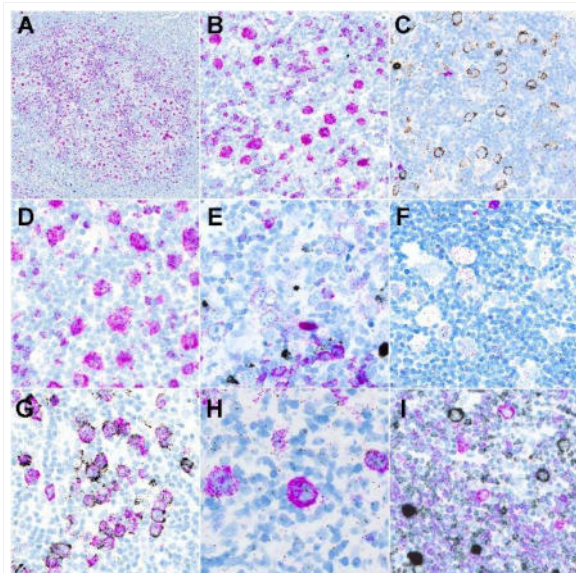


Figure 1. Spectrum of κ/λ mRNA ISH expression in LP and HRS cells
(A-C) Positivity in NLPHL and NLPHL-OL cases.

(D-F) Canonical and non-canonical κ/λ expression patterns.
(G-I) Atypical κ/λ expression profiles.

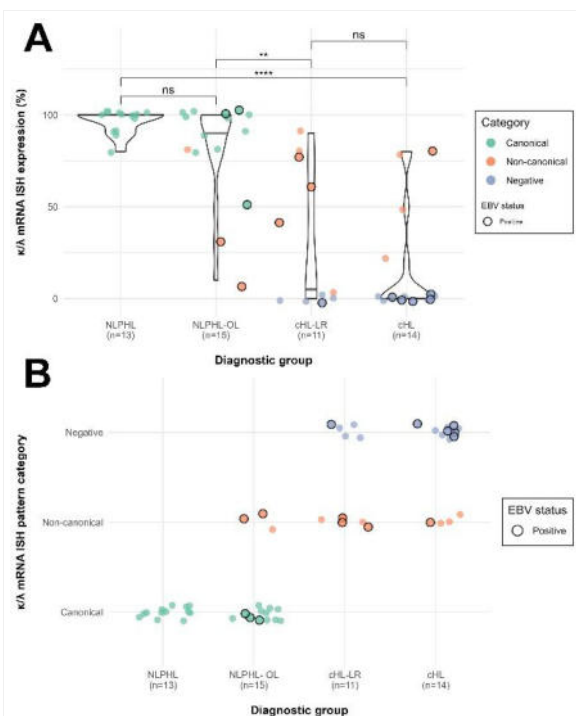


Figure 2. κ/λ mRNA ISH expression across diagnostic groups

(A) Violin plots showing the proportion of large tumor cells with κ/λ mRNA expression in NLPHL, NLPHL-OL, cHL-LR, and cHL. Each dot represents an individual case; horizontal lines indicate group medians. Dots are color-coded by pattern; black circles denote EBV-positive cases. Differences were assessed by Kruskal-Wallis test with Dunn's post hoc test using Benjamini-Hochberg correction. Significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****). (B) Dot plot illustrating the distribution of cases by κ/λ expression pattern across diagnostic groups

Reciprocal *MYC::BCL6* translocation is frequent in “triple-hit” (*MYC-BCL2-BCL6*) and “double-hit” (*MYC-BCL6*) large B-cell lymphomas

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Background

Determination of *MYC*, *BCL2* and *BCL6* translocation, is crucial for the classification of large B-cell lymphomas (LBCL). Lymphomas with *MYC-BCL2-BCL6* rearrangements (TH) comprise a group of lymphomas with dismal prognosis. However, the classification of lymphomas with “double-hit” (DH) *MYC-BCL6* without *BCL2* translocation (DH-*MYC-BCL6*) is controversial: it is considered a provisional entity in the ICC classification whereas it is classified as a subtype of LBCL in the 5th edition of the WHO classification. In the clinical practice, rearrangements are commonly identified using break-apart FISH tests. This approach is highly sensitive, but it does not provide information about the partners and the complexity of genomic alterations. For instance, reciprocal fusions between *MYC* and *BCL6* have been reported both in TH lymphomas and DH-*MYC-BCL6* (thus considered “pseudo-DH”). Of note, it has been demonstrated that some of those cases are in fact real DH lymphomas with a complex *MYC::IGH::BCL6* rearrangement reflecting the complexity of genetic alterations such cases. In this context, we aimed to evaluate the utility of *MYC-BCL6* fusion probes for detecting reciprocal rearrangements and to determine its frequency among TH and DH-*MYC-BCL6* lymphomas.

Principles/Methodology

LBCL with *MYC* and *BCL6* translocation (+/- *BCL2* rearrangement) were retrieved from the Pathology files of the H. Germans Trias i Pujol, Spain. All cases had been studied with FISH-BA probes at routine diagnosis. The reciprocal translocation was studied with *MYC* & *BCL6* FISH probes (Agilent) and DF *MYC-IGH* FISH assay was performed in cases with available tissue.

Results

A total of 34 LBCL with *MYC* and *BCL6* translocation were collected, and *MYC-BCL6* FISH assay could be studied in 26 cases. They comprised 15 TH and 11 DH *MYC-BCL6* cases. Overall, 10/26 cases (38%) had a *MYC::BCL6* rearrangement, been equally distributed in TH and DH-*MYC-BCL6* lymphomas (6/15, 40% and 4/11, 36% respectively). Of note, 3 of 6 cases (50%) with available results, a *MYC::IGH* was also demonstrated, suggesting a *MYC::IGH::BCL6* rearrangement.

Conclusion

The present study confirms the presence of reciprocal rearrangement between the *MYC* and *BCL6* genes in a high percentage of TH and DH-*MYC-BCL6* lymphomas. Notably, some of these cases correspond to cases with demonstrated *MYC::BCL6* rearrangement using DF probes, suggesting complex genetic recombinations such as the *MYC::IGH::BCL6* translocation. The biological impact of this genetic heterogeneity remains to be established.

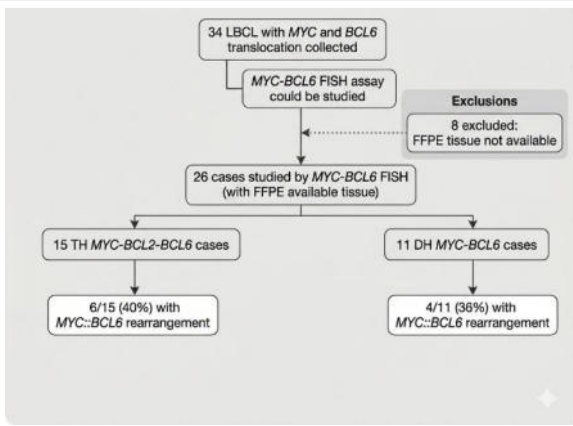


Figure 1.- Flow diagram of the cases

Table 1. FISH status profiles across the 26 studied cases.

Case	Subtype (DH vs. TH)	MYC-BA	MYC-IGH	FBCL2	FBCL6	MYC-BCL6
1	TH	T	NA	T	T	T
2	DH	T	NT	NT	T	T
3	TH	T	NT	T	T	NT
4	TH	T	NT	T	T	NT
5	ND	T	NA	NT	T	T
6	TH	T	NT	I	I	NT
7	TH	T	NT	T	T	T
8	DH	T	NA	NI	T	NT
9	DH	T	NT	NT	T	NT
10	DH	T	T	NI	T	NT
11	DH	T	NA	NT	T	NT
12	TH	T	T	T	T	NT
13	TH	T	NA	T	T	NT
14	TH	T	T	T	T	NT
15	DH	T	NT	NT	T	NT
16	TH	T	T	I	I	NT
17	TH	T	T	T	T	T
18	DH	T	NT	NI	I	NT
19	TH	T	T	T	T	NT
20	DH	T	T	NI	T	T
21	DH	T	T	NI	T	T
22	TH	T	NT	T	T	T
23	TH	T	T	T	T	NT
24	TH	T	NA	T	T	T
25	DH	T	NA	NT	T	NT
26	TH	T	NA	T	T	T

Notes: TH, double-hit lymphoma; DH, diffuse large B-cell lymphoma; ND, not determined; T, translocated/present status; NT, non-translocated/absent status; NA, data not available or not determined.

Table 1.- FISH status profiles across the 26 cases studied

EA4HP26-ABS-585

Angioimmunoblastic T-cell Lymphoma at King's College Hospital: Association with Clonal Haematopoiesis

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Background

Angioimmunoblastic T-cell lymphoma (AITL) is a peripheral T-cell lymphoma of CD4+ T follicular helper cell origin. Its mutational landscape involves the epigenetic regulators *TET2* and *DNMT3A*, *IDH2R172* and the small GTPase

RHOA. While *RHOA* and *IDH2* mutations are largely AITL-specific, *TET2* and *DNMT3A* alterations occur across haematological malignancies and are associated with clonal haematopoiesis (CH), implicating a shared mutated haematopoietic stem cell (HSC) origin. CH has been documented in only a few AITL series and its association with secondary myeloid neoplasms has been reported. Here we report our single-centre experience.

Principles/Methodology

Twelve AITL cases diagnosed between 2023 and 2026 at King's College Hospital, London were identified retrospectively. Clinical, morphological, and molecular data from lymph node (LN) biopsies and concurrent staging bone marrow biopsies (BMB) were collected. NGS was performed on available LN and matched BMB samples.

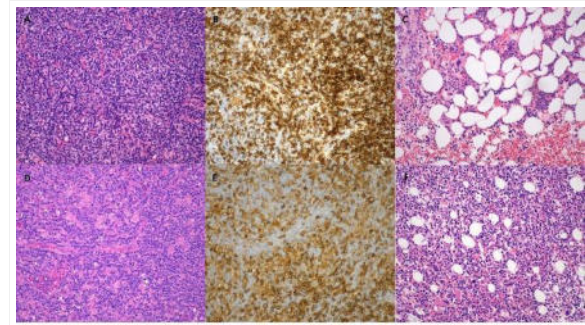
Results

Twelve cases were identified (age range 39–86 years; 8M:4F). Eight had LN material; NGS (LGP) was performed on seven, with one excluded due to insufficient DNA. Mutations were detected in 6/7: *TET2* (5/7), *RHOA* (4/7), *DNMT3A* (3/7), *IDH2* (2/7) and *PLCG1* (2/7). Nine staging BMBs were available: four involved by AITL, one suspicious, four uninvolved; three underwent LGP and two myeloid gene panel (MGP). Mutations in 4/5 BMBs tested: *TET2* (4/5), *DNMT3A* (2/5). Five cases had matched LN and BMB data: Cases 1 and 7 (uninvolved BMBs) showed identical *TET2* and *DNMT3A* mutations at similar VAFs, consistent with CH; Case 2 (uninvolved BMB, clonal plasma cells) showed identical *DNMT3A* and *TET2* mutations with an additional *RHOA* mutation exclusively in LN; Case 9 (involved BMB) showed identical *IDH2* and *RHOA* mutations; Case 11 (uninvolved BMB, LGP) showed *IDH2* and *TET2* mutations in LN only with no mutations in BMB.

Conclusion

In three cases with morphologically uninvolved BMBs, identical *TET2* and *DNMT3A* mutations were concordant between LN and BMB at similar VAFs, supporting a shared mutated HSC origin and establishing CH as integral to AITL biology. The additional *RHOA* mutation confined to the LN in Case 2 illustrates stepwise clonal evolution from CH to overt lymphoma. These findings support routine NGS of staging BMBs in AITL to characterise the clonal reservoir and identify patients at risk of secondary haematological neoplasms.

Molecular and Clinical Characteristics of AITL Cases									
Case	Age	Sex	Staging group	LN	BMB	LN	BMB	LN	BMB
1	78	Male	Uninvolved	LN	Uninvolved	LN	Uninvolved	LN	Uninvolved
2	78	Female	Uninvolved	LN	Uninvolved	LN	Uninvolved	LN	Uninvolved
3	78	Male	Uninvolved	LN	Uninvolved	LN	Uninvolved	LN	Uninvolved
4	78	Male	Uninvolved	LN	Uninvolved	LN	Uninvolved	LN	Uninvolved
5	78	Male	Uninvolved	LN	Uninvolved	LN	Uninvolved	LN	Uninvolved
6	78	Male	Uninvolved	LN	Uninvolved	LN	Uninvolved	LN	Uninvolved
7	78	Male	Uninvolved	LN	Uninvolved	LN	Uninvolved	LN	Uninvolved
8	78	Male	Uninvolved	LN	Uninvolved	LN	Uninvolved	LN	Uninvolved
9	78	Male	Uninvolved	LN	Uninvolved	LN	Uninvolved	LN	Uninvolved
10	78	Male	Uninvolved	LN	Uninvolved	LN	Uninvolved	LN	Uninvolved
11	78	Male	Uninvolved	LN	Uninvolved	LN	Uninvolved	LN	Uninvolved
12	78	Male	Uninvolved	LN	Uninvolved	LN	Uninvolved	LN	Uninvolved



Histological and immunohistochemical findings in two AITL cases

Image 1: Case 1: (A) Lymph node H&E showing polymorphous infiltrate with pale areas and arborising high endothelial venules. (B) CD4 IHC highlighting predominant CD4+ T-cell population. (C) Staging bone marrow H&E showing normocellular marrow with no morphological evidence of lymphoma infiltration. Case 2: (D) Lymph node H&E showing polymorphous infiltrate of small, medium and large lymphoid cells with prominent vascularity, consistent with AITL. (E) CD4 IHC showing diffuse CD4+ positivity. (F) Staging bone marrow H&E showing hypercellular marrow with clonal plasma cells with atypical features.

Mutational and Copy Number Landscape of High-Grade B-Cell Lymphoma, Not Otherwise Specified (HGBL-NOS): Integrated Genomic Profiling of an Indian Cohort

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Background

High-grade B-cell lymphoma, not otherwise specified (HGBL-NOS), is a newly defined aggressive mature B-cell neoplasm in WHO-HAEM5 (2022). It represents a diagnosis of exclusion, distinct from DLBCL-NOS, Burkitt lymphoma, lymphoblastic lymphoma, and “double-hit” HGBL. Its clinicopathological and genomic landscape, particularly in Indian patients, remains poorly characterised.

Principles/Methodology

In this ambispective study, large B-cell lymphoma biopsies (2022 onwards) were reviewed per WHO-HAEM5 criteria using immunohistochemistry (c-MYC, BCL2, Ki-67, SOX11, TdT) and fluorescence in situ hybridisation (MYC/BCL2 break-apart probes). Twenty-five confirmed HGBL-NOS cases underwent whole-exome sequencing (WES; Illumina NovaSeq 6000, GATK Mutect2 pipeline), annotated per AMP guidelines. Twenty-three cases were profiled on Affymetrix OncoScan CNV Plus for copy number variants (CNVs).

Results

WES identified 197 somatic variants across 95 cancer-associated genes. Tier 1 variants (strong clinical significance) = 15; Tier 2 = 8; Tier 3 = 173; Tier 4 = 1. Pathogenic/likely pathogenic variants = 45; VUS = 149. Loss-of-function alterations (stop-gain, frameshift, start-loss, stop-loss) accounted for 18.8% of variants. Recurrent driver genes included *STAT3* (4 cases), *SOCS1*, *FOXO1*, *TP53*, *EZH2*, *KMT2D*, *CREBBP*, *CD79B*, *STAT5B*, *TET2*, *BCL6*, *BCL2*, *JAK3*, *IRF8*, *FAT1*, *SETD2*, *BTG1/2*, *CCND3*, *IKBKB*, among others. Recurrent loss-of-function events were observed in *STAT5B*, *TET2*, *ASXL1*, *KRAS*, *IRF8*, *JAK3*, *CDKN2A*, *SOCS1*, *SETD2*, *FAT1*, *BCOR*. CNV profiling revealed recurrent whole-chromosome gains (X, 3, 4, 8, 9, 12, 21, 22) and losses (5, 16, 19, 20), with additional partial and copy-neutral LOH events. Approximately 10% of cases displayed a non-GCB immunophenotype, highlighting heterogeneity.

Conclusion

HGBL-NOS harbours a distinct mutational architecture spanning epigenetic regulators (*EZH2*, *KMT2D*, *CREBBP*, *SETD1B*, *TET2*), BCR/NF-κB (*CARD11*, *CD79B*, *SOCS1*), JAK–STAT (*STAT3*, *STAT5B*, *JAK3*), RAS/MAPK, and tumour-suppressor pathways, superimposed on chromosomal instability with recurrent whole-chromosome gains and losses. These findings confirm biological distinction from DLBCL-NOS and support integrated IHC, FISH, and molecular profiling for routine workup, with implications for targeted therapy and risk stratification.

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Clinical impact of NGS Profiling in B- and T-Cell Lymphomas: A Single-Center Experience

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Background

The role of NGS in routine hematopathology is increasingly relevant for the characterization of B- and T-cell lymphomas. The identification of recurrent genetic alterations may provide diagnostic, prognostic, and potentially therapeutic information, although its integration into daily clinical practice is still under evaluation.

Principles/Methodology

A total of 233 cases of suspected or confirmed B- or T-cell lymphomas from a single referral center underwent molecular profiling using a commercially available targeted 73-gene NGS panel. Of these, 121 cases were analyzed for research purposes, whereas 112 were profiled upon clinical request. Among the clinically requested cases, samples included 80 FFPE tissues, 31 blood-derived specimens, and 1 frozen sample. Specimens comprised lymph node (39), blood-derived (31; including 29 bone marrow and 2 peripheral blood specimens), gastrointestinal (10), splenic (6), otorhinolaryngological (6), skin (5), and mediastinal (5) biopsies. Overall, the cohort included large B-cell lymphomas (n=41), T-LGL (n=13), MCL (n=21), PMBCL (n=8), and AITL (n=7). Variant interpretation was performed manually by integrating data from the software provided, large online databases and scientific publications.

Results

Among DLBCL/HGBCL cases, the most frequently mutated genes were *TP53*, *KMT2D*, *PIM1*, *NFKBIE*, *SOCS1* and *STAT6*. In MCL, *TP53* mutations were detected in 27% of cases, supporting prognostic stratification. In 8 cases of mediastinal disease, NGS was performed to aid in the differential diagnosis between PMLBCL and other lymphomas. Activating mutations in oncogenes (*STAT6*, *XPO1*), loss-of-function mutations in tumor suppressor genes (*SOCS1*, *TNFAIP3*, *B2M*, *CIITA*, *CD58*) and loss-of-function mutations in *NFKBIE* allowed confirmation of the pathological diagnosis of PMBCL. Pathological suspicion of AITL was confirmed by the identification of mutations in *RHOA*, *IDH2*, *TET2* and *DNMT3A*. Among cases with clinical suspect of T-LGL, when *STAT3* mutations were detected, neutropenia and recurrent infections were observed; one case showed cutaneous progression and another developed a metachronous B-cell lymphoma. No *STAT5B* mutations were identified in this cohort.

Conclusion

Targeted NGS profiling is a feasible and informative tool in routine hematopathology. Molecular analysis may support diagnosis, improve prognostic stratification, and contribute to the biological characterization of B- and T-cell lymphomas, supporting its progressive integration into clinical practice.

microRNAs expression in different subtypes of Castleman Disease

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Background

Castleman Disease (CD) includes lymphoproliferative disorders sharing histopathological features and classified into unicentric (UCD) and multicentric CD (MCD). MCD comprises herpesvirus 8 (HHV8)-related CD (also associated with HIV); POEMS-associated CD and idiopathic CD (iMCD). Although CD pathogenesis remains unclear, multiple mechanisms are likely involved. MicroRNA (miRNA) are small regulatory molecules involved in immune regulation and oncogenesis, but data on miRNA expression in CD are limited. This study investigates the miRNome across UCD and MCD subtypes.

Principles/Methodology

miRNA expression was retrospectively analyzed in 24 patients diagnosed between 2000-2022 using QIAseq miRNA Library Kit (QIAGEN) and sequencing Platform. Expression profiles were correlated with clinical and histological features.

Results

Significant miRNA expression differences emerged across histotypes, IL-6 levels, B symptoms, autoimmune diseases and disease localization. let-7a-5p was upregulated in cases with elevated IL-6 and B symptoms and downregulated in plasmacell (PC) histotypes, suggesting associations with symptom onset, VEGF production and vascular proliferation, features typical of non-PC phenotypes. Among 4 patients with autoimmune diseases, miR-342-3p was downregulated, while higher levels were seen in patients without autoimmunity ($p=.03$), possibly reflecting better immune regulation. No correlation with Treg percentage or CD4/CD8 ratio was found. Comparing UCD vs MCD, miR-151a-5p was upregulated in MCD ($FDR=.08$) and is known to inhibit Rho GTPase regulators and E2F6; miR-92a-3p, downregulated in MCD ($FDR=.06$), targets PTEN and PHLPP2, negative regulators of mTOR. miR-34a-5p, involved in lymphocyte activation, showed higher expression in patients with elevated CD8 counts, supporting its role in immune response. We also observed differential expression of miR-199a-3p and miR-221-3p, both implicated in mTOR signaling. miR-199a-3p was more expressed in hyaline-vascular (HV) and mixed histotypes vs. PC, while miR-221-3p showed highest levels in HV. Comparing our results with literature on HHV8-associated miRNA, hsa-miR-21-5p was more expressed in HHV8+ cases, though without statistical significance due to small numbers.

Conclusion

This is the first study exploring the CD miRNome in relation to clinical and molecular features. Differential expression of some miRNA supports distinct pathogenic mechanisms and highlights potential biomarkers for diagnosis, monitoring, and future therapeutic strategies.

PARP-1 Immunoexpression and Clinical Outcome in Aggressive Large B-Cell Lymphomas

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Background

Aggressive large B-cell lymphomas (aLBCL), represent the most common subtype of lymphoma and are clinical and molecular heterogeneous. While 60–70% of patients achieve cure with first-line chemotherapy, 30–40% develop relapse/refractory disease.

A hallmark of these neoplasms is the frequent disruption of DNA damage response pathways. Poly (ADP-ribose) polymerases, particularly PARP-1 and PARP-2, play central roles in DNA repair and maintenance of genomic stability. In parallel, LMO2 has been implicated in the regulation of double-strand break repair through its interaction with 53BP1, impairing BRCA1 recruitment and thereby influencing DNA repair pathway choice. Notably, increased LMO2 expression has been associated with enhanced sensitivity to PARP-1/2 inhibition. Previous studies in mice have demonstrated that PARP isoforms may have distinct roles in lymphomagenesis, suggesting potential biological and therapeutic implications.

Principles/Methodology

A total of 127 aLBCL were analysed, including 100 DLBCL, 18 DLBCL transformed from FL (DLBCL-FLt), 1 HGBL-NOS, 6 HGBL DH/TH, and 2 cases of Burkitt lymphoma.

Formalin-fixed, paraffin-embedded biopsy specimens were analysed using B- and T-cell markers and FISH. PARP-1, LMO2, and MYC protein expression were evaluated by immunohistochemistry (cut-off for LMO2 and MYC at 30% and 40%, respectively).

PARP-1 expression was assessed according to the percentage of positive tumour cells and staining intensity, and an H-score was subsequently calculated. MYC rearrangement status was determined by FISH, and TP53 mutational status was also evaluated. mRNA expression levels of *PARP1*, *PARP2*, *MYC*, and *LMO2* were assessed in 24 cases.

Results

The cohort included 57 women (44.9%) and 70 men (55.1%), with an age range of 20–96 years (mean 64 years). 74 cases (58.3%) were nodal and 53 (41.7%) extranodal.

Correlation analyses demonstrated a significant positive association between PARP-1 H-score and LMO2 RNA expression as well as LMO2 IHC expression.

Clinical follow-up was available for 71 patients, comprising 56 DLBCL and 15 DLBCL-FLt cases. Within this cohort, treated with curative chemotherapy schemes, PARP-1 H-score stratified patients into a high expression group (n=48) and a low expression group (n=23). Low PARP-1 IHC expression associated with significant inferior outcomes (overall and progression-free survival; $p=0.016$ and $p=0.015$, respectively).

Conclusion

PARP-1 expression status may have biological and potential therapeutic implications.

Molecular Profiling of Lymphoid Malignancies Using TruSight Oncology 500: Initial Experience from a Single Center in North Macedonia

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Background

Comprehensive genomic profiling (CGP) using next-generation sequencing (NGS) has become increasingly important in the diagnosis, prognostic stratification, and therapeutic management of hematologic malignancies. The TruSight Oncology 500 (TSO-500) assay enables simultaneous detection of single nucleotide variants (SNVs), insertions/deletions, copy number variations (CNVs), tumor mutational burden (TMB), and microsatellite instability (MSI). The aim of this study was to evaluate the molecular landscape of lymphoma patients analyzed with the TSO-500 panel in a single molecular pathology center.

Principles/Methodology

A total of 19 patients with various lymphoid malignancies were analyzed using the Illumina TSO-500 panel at the Institute of Pathology, Faculty of Medicine, UKIM, Skopje. Formalin-fixed paraffin-embedded (FFPE) tumor samples were subjected to CGP. Detected variants were classified according to their clinical significance, and TMB/MSI status was assessed.

Results

Pathogenic or likely pathogenic genomic alterations were identified in the majority of cases. Frequently detected alterations involved genes associated with lymphomagenesis and targeted therapeutic relevance, including *EZH2*, *BRAF*, *NOTCH2*, *TNFAIP3*, *TNFRSF14*, *ERBB2*, *CDK4*, and *MDM2*. Multiple clinically relevant CNV alterations were also observed, such as *ERBB2*, *AKT3*, and *NTRK1* gains, and *TNFRSF14* loss. One patient with diffuse large B-cell lymphoma (DLBCL) demonstrated a *BRAF* p.(Lys601Asn) alteration accompanied by high TMB (10.3 mut/Mb), while another case showed combined alterations in *NOTCH2*, *TNFAIP3*, and *TNFRSF14*. A third patient with metastatic CNS DLBCL exhibited CGP with *ERBB2* amplification, *EZH2* oncogenic mutation, and additionally alterations in *NBN*, *CREBBP*, and *GNA13*. Most samples were microsatellite stable and with low TMB values, although isolated TMB-high cases were identified. Several variants of uncertain significance were also detected, emphasizing the complexity of interpretation in large-panel sequencing.

Conclusion

TSO-500 CGP provides valuable molecular information in lymphoid malignancies, enabling identification of clinically actionable alterations, prognostic biomarkers, and potential therapeutic targets. Our initial experience demonstrates the feasibility and clinical utility of broad NGS testing in routine molecular diagnostics of lymphoma patients in North Macedonia. Larger studies are needed to further evaluate the clinical impact of these findings on personalized treatment strategies in hematologic oncology.

High-Grade B-Cell Lymphoma with 11q Aberration in Africa: A differential diagnosis in cases with features of endemic Burkitt lymphoma

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Background

Burkitt lymphoma (BL) is a highly proliferative germinal center B-cell malignancy whose epidemiology in Africa is tightly linked to Epstein-Barr virus (EBV) and malaria exposure. However, a subset of aggressive B-cell lymphomas in endemic regions clinically and morphologically resembles BL but lacks IG::MYC rearrangements and instead harbors characteristic 11q copy-number imbalances (high-grade B-cell lymphoma with 11q aberration, HGBCL, 11q).

Principles/Methodology

We analyzed African BL biopsies and cell lines using cytogenetics (FISH for MYC and 11q probes), copy-number arrays, genome/exome sequencing, and immunohistochemistry for phenotype and EBV. We synthesized evidence from African biopsy cohorts and BL cell lines using: interphase FISH for MYC/BCL2/BCL6/IG loci; targeted probes for 11q gain/loss; high-density copy-number platforms (OncoScan/CytoScan); whole genome/exome sequencing and fusion validation; and immunohistochemistry to confirm BL phenotype and EBV status.

Results

In our study of the cases from Kenya over a 4-year period, 19 were confirmed as BL (11 EBV-positive, 8 EBV-negative), and 4 as high-grade B-cell lymphoma, not otherwise specified (HGBL, NOS). Ancestry mapping using SNP data from WGS and CytoScan confirmed that the BJAB cell line clusters genetically between Luhya individuals from Kenya and Bantu populations from Uganda, consistent with its reported Kenyan origin within the BL-endemic Great Lakes region of Africa. Notably, one EBV-negative, MYC non-rearranged case with BL morphology exhibited the canonical 11q aberration, representing the first documented primary HGBCL, 11q in Africa.

Conclusion

These findings are compelling evidence that HGBCL, 11q is an important differential diagnosis to lymphomas with BL-like morphology and high-grade morphology in Africa, particularly in cases which are EBV –negative and lack the MYC rearrangement. •We therefore believe that some of the cases previously diagnosed as BL or BL-like lymphoma may represent HGBL, 11q.

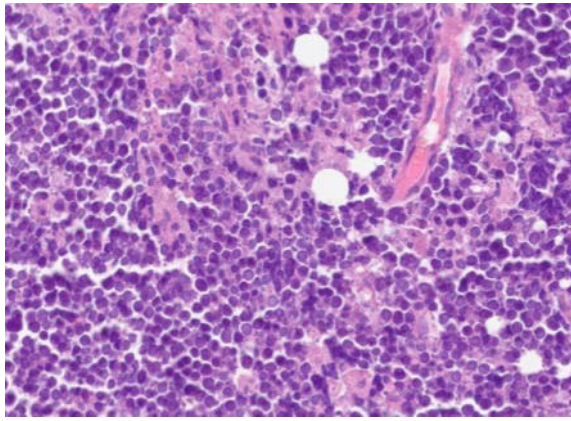


Figure 1
Diffuse proliferation of monomorphic medium sized cells with round nuclear contours, scanty cytoplasm and inconspicuous nucleoli

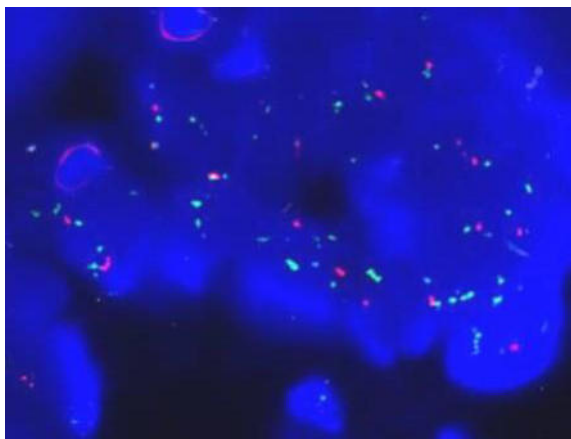


Figure 2
Typical 11q aberration with gain of 11q23 region (green), loss of 11q24 region (orange) and normal CEP 11 (aqua)

EA4HP26-ABS-623

Classifier-oriented targeted NGS for molecular subtyping of large B-cell lymphomas

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Background

Large B-cell lymphomas (LBCL), including DLBCL and HGBCL, are biologically heterogeneous and genomic classes are increasingly relevant for hematopathology reporting and subtype-adapted trials. Although WES-based classifiers remain a reference, LySeqST data show that robust DLBCL classification is achievable with targeted assays capturing lymphoma-defining mutations and CNVs. We tested whether commercial NGS panels can be refined into a transferable classifier-oriented assay.

Principles/Methodology

We compared three commercial targeted NGS panels covering 54, 60, and 73 lymphoma-associated genes with a manually curated 173-gene panel. In silico analyses used a dataset of 574 annotated LBCL cases. LymphGen 2.0 applied to the complete genomic feature set provided reference labels; each panel was then evaluated by restricting input to its genes. Concordance was assessed by accuracy, Cohen's kappa, confusion matrices and class-wise sensitivity/specificity. A multilabel elastic-net framework with nested cross-validation and feature stability analysis was applied to the 173-gene panel to derive a reduced gene panel.

Results

Commercial panels showed limited-to-moderate concordance with LymphGen 2.0 labels. Accuracy/kappa were 49.30%/0.401 for 54 genes, 53.48%/0.448 for 60 genes, and 56.10%/0.474 for 73 genes, with variable class recognition and incomplete capture of BN2, ST2, A53 and composite categories. The curated 173-gene panel improved assignment, reaching 95.64% accuracy and kappa 0.946. The reduced 100-gene panel retained high performance, with 85.01% accuracy and kappa 0.814. Specificity remained high for all categories, including A53, BN2, composite, EZB, MCD, N1 and ST2. Sensitivity was also preserved for A53, MCD, composite, N1, EZB and BN2, supporting classifier-informed feature selection.

Conclusion

A classifier-oriented 173-gene panel substantially outperforms commercial hematopathology NGS panels for LBCL molecular subtyping. These data support an integrated diagnostic strategy in which panel design is guided by histopathology, immunophenotype, cytogenetics and subtype-specific genomic biology. To enhance clinical feasibility, a robust multilabel elastic-net approach was used to derive a 100-gene panel while maintaining high classification accuracy, suggesting that biologically informed reduced panels may enable routine LBCL molecular classification.

EA4HP26-ABS-632

Pseudo-Richter Transformation Following BTKi Interruption in CLL: A Clinico-Pathological and Molecular Characterization

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Background

Pseudo-Richter transformation (pRT) is a recently described, transient phenomenon in chronic lymphocytic leukemia (CLL) occurring after interruption of Bruton tyrosine kinase inhibitors (BTKi), such as ibrutinib. It mimics true Richter transformation (RT) clinically and histologically, with rapid lymphadenopathy and aggressive morphology, but

resolves upon drug reintroduction. Distinguishing pRT from true RT is essential to avoid unnecessary intensive chemotherapy.

Principles/Methodology

We analyzed 7 patients from different centers with suspected transformation after BTKi discontinuation. Paired CLL and pRT samples were available in 5 cases, while only pRT samples were analyzed in 2. Investigations included histopathological review including morphology and Ki67 proliferation index, and capture-based next-generation sequencing interrogating the IG loci (IGH/IGK/IGL) as well as the most frequent driver alterations in CLL and other mature B-cell neoplasms to assess clonal relationship and genomic evolution. Two patients were studied by WGS.

Results

Ibrutinib was discontinued for oncologic reasons in 5 cases (breast n=1, lung n=3, skin n=1), tonsillectomy in 1 case, and infection in 1 case. Pseudo-RT cases showed heterogeneous morphology: DLBCL-like transformation with centroblastic features (n=4), prolymphocytoid progression (n=1), and expanded proliferation centers resembling accelerated CLL (n=2). Ki67 index was significantly elevated, ranging from 40% to 80%. IGHV status was unmutated in 5/7 cases; the remaining showed 97.92% and 97.25% homology, including one stereotyped subset 2 case with IGLV3-21^{R110}. No recurrent VH family usage was observed among the cases. NGS demonstrated clonal stability between CLL and pRT, with the same shared mutations (*NOTCH1*, *XPO1*, *EGR2*, *TP53*) and no additional mutated genes, copy number alterations (CNAs) or structural variants (SVs). Notably, one pRT case exhibited a t(14;19) [*IGHM::BCL3*] rearrangement. This alteration was not identified in its paired CLL because it was not covered with the panel used in this sample.

Conclusion

This study expands the morphological spectrum of pRT beyond the previously described large-cell morphology. Despite aggressive morphology, the genomic profile of pRT was similar to that of the underlying CLL with absence of de novo mutated genes, CNAs or SVs typical of true RT, supporting a transient “flare” phenomenon. Preservation of the baseline molecular profile and temporal association with BTKi interruption are key diagnostic features.

EA4HP26-ABS-640

Multicentre evaluation of the EuroClonality-NDC next-generation sequencing assay in the real-world diagnosis of lymphoproliferative disorders

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Background

Genetic testing plays a central role in the assessment of lymphoproliferative disorders. Comprehensive NGS assays such as EuroClonality-NDC (EC-NDC), combining assessment of structural and point aberrations with clonality assessment, have the potential to streamline diagnostic assessment but have yet to be evaluated in real-world diagnostic practice.

Principles/Methodology

A retrospective analysis of EC-NDC results and associated pathology integrated reports was performed from 09/2023 to 09/2025 in two haematological malignancy diagnostic services in the UK. Failure rates, concordance with parallel testing, diagnostic impact, and the generation of clinically relevant information were evaluated.

Results

A total of 583 cases were reviewed, comprising multiple tissue types (lymph node n=257 (44%); bone marrow n=110 (19%); extranodal sites n=216 (37%)), including reactive conditions (n=85 (14.5%)), B-cell non-Hodgkin lymphomas (NHL) (n=390 (66.5%)), T-NHL (n=88 (15%)), Hodgkin lymphomas (HL) (n=15 (3%)) and other (n=5 (1%)). The assay failed in 55 (9.4%) cases, with a failure rate of 6.4% in lymph node tissue, 9.9% in bone marrow, and 17.4% in skin. Parallel FISH testing was performed in 191 cases, with an overall concordance of 20%, in which FISH missed 15 aberrations, FISH failed in 17 cases, and EC-NDC missed 7 cases. B-cell clonality studies showed a sensitivity of 91.1% in B-NHL and 46.7% in HL. T-cell clonality studies showed a sensitivity of 82% of T-NHL. EC-NDC informed subtyping in 157 (30%) cases, including 72 diffuse large B-cell lymphomas, 20 lymphoplasmacytic lymphomas, 12 marginal zone lymphomas and 11 follicular lymphomas. EC-NDC aided diagnostic reclassification from reactive to malignant diagnoses in 17 cases (3%), malignant to reactive diagnoses in 28 cases (5%), B-cell to T-cell neoplasm in 5 cases (1%), T-cell to B-cell neoplasm in 4 cases (<1%), and highlighted HL-NHL discrepancies in 3 (<1%) cases. Results leading to unexpected findings or diagnoses were present in 26 (4%) cases. Clinically informative data were generated in 319 (55%) cases, contributing to management change in 64 (11%) cases, management optimisation in 204 (35%) cases, and changes from no treatment to treatment, or vice versa, in 35 (6%) cases.

Conclusion

EC-NDC facilitated enhanced evaluation of suspected lymphoproliferative disorders, generating diagnostic and clinically informative data in a substantial proportion of cases, leading to improved patient safety and outcomes.

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Skin and non-GI mucosal lymphoproliferative disorders expand the spectrum of T-cell lymphoproliferative disorders with *STAT3-JAK2* fusion

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Background

STAT3-JAK2 fusions have been described in chronic, persistent, clinically challenging T-cell lymphoproliferative disorders involving the small intestine, colon, and skin, most often with a CD4+ phenotype. The best-recognized presentation is indolent T-cell lymphoproliferative disorder (LPD) of the gastrointestinal (GI) tract. In our practice, however, we have more often identified this process in patients with cutaneous lymphoma/LPD. We thus aimed to better characterize *STAT3-JAK2* fusion-positive T-cell lymphoproliferative disorders at our tertiary care institution.

Principles/Methodology

An institutional database of clinical samples previously analyzed by DNA- and/or RNA-based next-generation sequencing was searched for *STAT3-JAK2* fusions. Ten unique cases were identified and reviewed for clinical, histopathologic, immunophenotypic, and molecular features.

Results

Ten patients were identified: 7 male and 3 female, with a mean age of 55.4 years (range, 40–71). Sites included skin, parotid gland, and breast implant capsule. Diagnoses included clonal CD4+ T-cell LPD (parotid, n=1), cutaneous T-cell lymphoma/mycosis fungoides (n=7), CD8+ primary cutaneous aggressive epidermotropic T-cell lymphoma

(pcAETCL, n=1), and implant-associated CD30+ anaplastic large cell lymphoma (ALCL, n=1). Follow-up from first diagnostic biopsy (mean, 45 months; median, 10 months; range, 2–224 months) showed one disease-related death (pcAETCL). The parotid case later developed persistent low-grade systemic, stomach, and liver inflammation with elevated liver function tests; the remaining skin lymphoma/LPD cases had persistent skin inflammation. No other patients had GI symptoms. Non-ALCL cases were CD3+ (9/9); 8/9 showed monotypic TRBC1 expression and 1/9 was TCR-delta+. ALCL was CD3-/CD30+. T-cell subsets were CD4+/CD8- (n=5), CD4-/CD8+ (n=2), CD4+/CD8+ (n=1), and CD4-/CD8- (n=2). All cases harbored in-frame *STAT3-JAK2* fusions, most commonly *STAT3* exon 23-*JAK2* exon 19; the parotid case showed *STAT3* exon 23-*JAK2* exon 17. DNA sequencing in 4 cases revealed only non-recurrent mutations, including of *EPHB1*, *TNFAIP3*, *ASXL1*, *TP53*, *MGAM*, *PAK7*, *SMARCB1*, and *NOTCH1*.

Conclusion

STAT3-JAK2 fusion-positive T-cell lymphoproliferative disorders involve mucocutaneous sites beyond the GI tract and show clinical and immunophenotypic heterogeneity. Recognition of this fusion may support classification as a distinct biologically defined group and has potential therapeutic relevance because JAK2-mediated signaling may be targetable.

EA4HP26-ABS-644

Non-alpha/beta T-cell lymphoma: a single Centre experience on gamma/delta and TCR-silent subsets

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Background

Within non- α/β proliferations, γ/δ + cutaneous T-cell lymphomas (CTCL) is formally recognized, but a T-cell receptor (TCR)-silent phenotype (non- α/β and non- γ/δ) can be encountered, as an elusive subtype.

Principles/Methodology

Retrospective, continue series of CTCL patients routinely diagnosed from 2019 to 2024 and followed at a single Institution, featuring either a BetaF1-/TCR δ + or BetaF1-/TCR δ -, EBV-negative phenotype, and demonstrating monoclonal *TCRG* rearrangement.

Results

36 patients: 25 γ/δ and 11 TCR-silent; a median follow up, 33 months (range 7-240)

γ/δ subset

Eight patients presented with "classical" γ/δ -CTCL, including deep dermal/hypodermal $\pm$ superficial/epidermal involvement, and tendency to aggressive behavior (3 died-of-disease; median follow-up 17 months). One patient featured a coexistent refractory celiac disease, with intraepithelial, gamma/delta+ duodenal lymphocytosis, and one patient shifted to a TCR-silent phenotype.

Ten patients featured the morphologic and clinical profile typical of mycosis fungoides (MF), all alive with disease (median follow-up 84 months), except for one patient progressing to tumor-stage TCR-silent CTCL after a 12-year follow-up. Six patients (median age: 9 years) featured typical lesions and behavior of lymphomatoid papulosis (LyP). One patient displayed a CD4+/TCR δ + proliferation, with a clinic-pathologic profile fully recapitulating Sezary syndrome.

TCR-silent subset

Two cases demonstrated pleomorphic TCL, with deep dermal-hypodermal involvement and rapid progression with systemic, non-nodal disease. One patient presented MF-type picture, over a 17-year period. Remaining cases fell within typical CD30+ lymphoproliferative disorders (LPD), including seven LyP (two of which, CD30-negative) and one primary cutaneous anaplastic large cell lymphoma (pcALCL).

Conclusion

We highlight that γ/δ -CTCLs encompass a broad clinicopathological spectrum, including indolent variants that challenge the conventional prognostic framework.

TCR-silent phenotype is proper to CD30+ LPD/lymphomas, which share a heavily disrupted canonical T-cell differentiation and signaling program (PMID:20511667), but it is also observed in aggressive, extranodal TCL (PMID:21753698). Our series delineates a continuum between γ/δ and TCR-silent subsets, including tendency to a progressive course in patients outside the boundaries of LyP/pcALCL. Also, it stresses the need to regard the diagnosis of CTCL as a matter of clinic-pathologic pattern, rather than adherence to strict criteria.

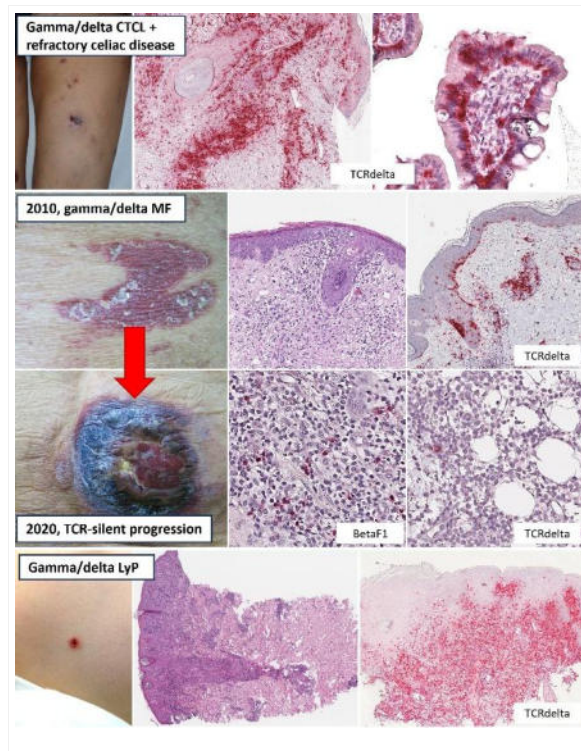


Figure
Representative clinic-pathologic picture of gamma/delta and TCR-silent cutaneous T-cell lymphoma.

EA4HP26-ABS-647

Comparison of a commercial NGS-Based B-Cell Clonality Assay with the Euroclonality NGS primer sets across B-Cell Lymphoma Subtypes with and without Somatic Hypermutation

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Background

Clonality assessment is an important tool in the diagnosis of B-cell lymphomas (B-NHL). However, detection sensitivity varies depending on lymphoma subtype and the degree of somatic hypermutation (SHM), which can impair

primer binding in immunoglobulin (IG) gene rearrangement assays. This study evaluated the performance of the Oncomine™ BCR Pan-Clonality Assay across different B-NHL entities and compared its results with established reference methods.

Principles/Methodology

The Oncomine™ BCR Pan-Clonality Assay (Thermo Fisher Scientific, Waltham, MA, USA), which covers IGH, IGK, IGL, and KDE/Cint rearrangements, was evaluated using formalin-fixed paraffin-embedded (FFPE) tissue samples from a B-cell lymphoma cohort including 30 cases with no/low SHM burden (MCL and CLL/SLL), 30 cases with an expected high SHM rate (DLBCL and FL), and bone marrow biopsies with minimal B-NHL infiltration. In cases with a polyclonal IGH result, an analysis with an additional primerset which targets the FR3 region more distally (IGH FR3(d)-J) was performed. Results were compared with EuroClonality NGS assays. Concordance between methods and clonality detection rates were assessed for the final overall interpretation, as well as the single primersets. The impact of SHM was evaluated.

Results

The assay demonstrated high sensitivity for detection of B-cell clonality, including cases with low tumor burden. Detection rates were highest in MCL/CLL (100%), where SHM burden is generally lower, with near-complete concordance with the EuroClonality standard. In contrast, lower sensitivity was observed in germinal center (GC)-derived lymphomas such as DLBCL/FL (83%), likely reflecting impaired amplification due to SHM. The inclusion of complementary IGK primer sets already in the Pan-BCR approach and IGH FR3(d)-J in a second step significantly improved clonality detection in these entities (IGH 30%, IGH FR3(d) 53%, IGK 77%). Cases with discrepant or pseudoclonal results were predominantly observed in SHM-associated lymphomas.

Conclusion

The Oncomine™ BCR Pan-Clonality Assay is a sensitive and robust method for clonality assessment in routine lymphoma diagnostics, including samples with minimal infiltration. Combined analysis of heavy- and light-chain IG targets improves clonality detection in B-NHL with extensive SHM while preserving limited material through a single NGS-based workflow. Addition of primers improves diagnostic yield and reduce false-negative or pseudoclonal results in GC-derived lymphomas.

EA4HP26-ABS-654

T-bet Expression Defines a Distinct, Clinically Aggressive Subset of Diffuse Large B-Cell Lymphoma Potentially Related to “Aging” of B cells and has an Altered Immune Microenvironment

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Background

T-bet (encoded by *TBX21*) regulates Type 1 immunity and drives age-associated B-cell (AgeBC) accumulation, fueling immunosenescence and inflammaging. Historical data suggested diffuse large B-cell (DLBCL) and follicular (FL) lymphomas are T-bet-negative. Given recent insights into AgeBCs, we used multiplex immunohistochemistry (m-IHC) to re-evaluate T-bet expression and characterize the tumor microenvironment (TME) in FL and DLBCL.

Principles/Methodology

Tissue microarrays from FL (n=63) and DLBCL (n=64) samples were evaluated using a six-color fluorescent m-IHC panel (CD20, CD3, T-bet, GATA3, granzyme B, CD56) and digital pathology software to quantify T-bet expression and TME immune subsets. Clinical characteristics and patient outcomes were retrieved from the Cancer Surveillance System and the Washington State Cancer Registry.

Results

T-bet⁺ B cells (>3%) were found in 14% of DLBCLs (9/64) vs. 0% of FLs (0/63; $p=0.003$). In T-bet⁺ DLBCLs, T-bet⁺ B cells ranged from 3.8% to 30.4% (4 GCB, 5 non-GCB; $p=ns$); 1/3 EBV⁺ DLBCLs was T-bet⁺. T-bet⁺ DLBCL patients were younger (45 [41] vs. 72 [19] yrs; $p=0.01$) and more frequently presented with stage 4 disease (5/9 vs. 9/52; $p=0.043$) and B symptoms (5/9 vs. 9/48; $p=0.019$). T-bet⁺ DLBCLs showed a highly infiltrated TME with higher proportions of CD3⁺ T cells (41% [36.4%] vs. 12.95% [21.2%]; $p=0.026$), T-bet⁺ T cells (10.25% [27.5%] vs. 0.74% [1.64%]; $p<0.001$), GATA3⁺ T cells (0.62% [1.37%] vs. 0.03% [0.13%]; $p=0.001$), T-bet⁺GATA3⁺ T cells (0.5% [0.57%] vs. 0.01% [0.03%]; $p<0.001$), and granzyme B⁺ cells (0.78% [1.73%] vs. 0.24% [0.9%]; $p=0.06$). Mean disease-specific survival was shorter for T-bet⁺ vs. T-bet⁻ DLBCL (47.3 vs. 72.9 months; the difference did not reach statistical significance). All values unless otherwise specified, are median values [interquartile range].

Conclusion

T-bet is expressed in a small but distinct subset of DLBCLs (14% in this study), defining a clinically aggressive phenotype (younger age, advanced stage, shorter survival) with a T-cell-rich TME. T-bet may serve as a valuable prognostic biomarker, suggesting AgeBC-related pathways and potentially “aging” of B cells contribute to aggressive DLBCL pathogenesis. While these preliminary findings from our pilot study require validation in larger cohorts, work is currently underway to expand the sample size and evaluate spatial correlations.

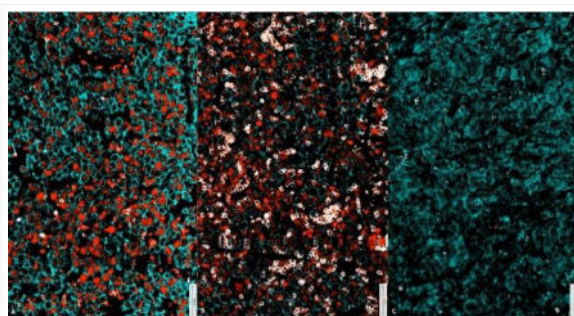
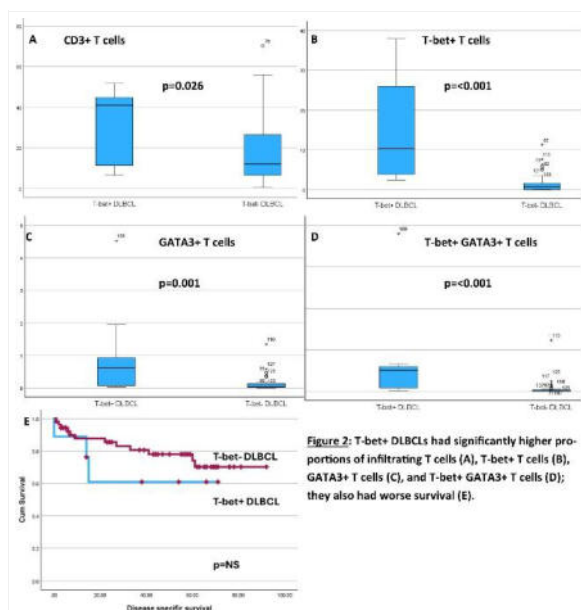


Figure 1
mFISH panel using CD20, CD3, T-bet, CD56, granzyme B and Gata3. CD20, and T-bet are depicted in the images. A: T-bet⁺ DLBCL with sparse T cells; B: T-bet⁺ DLBCL with abundant T-bet⁺ and T-bet⁻ T cells; C: T-bet⁻ DLBCL with sparse T cells.



Multisystem ALK-positive histiocytosis-a case report

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Background

The more recent International Consensus Classification (ICC) and World Health Organization (WHO) classifications include anaplastic lymphoma kinase (ALK)-positive histiocytosis as a distinct disease type, characterized by ALK gene rearrangement and ALK immunoreactivity in neoplastic histiocytes.

Principles/Methodology

Morphological, immunohistochemical (IHC) and molecular analyses were performed on a trephine bone marrow biopsy sample. The molecular analysis, in particular, included Fluorescence In Situ Hybridization (FISH) using locus-specific dual-color ALK break-apart probes.

Results

Case report: A 28-year-old female patient presented with a persistent low-grade fever and night sweats followed by migratory polyarthritides, paresthesias of the left lower third of the face and irregular vaginal bleeding. Blood count indicated mild normocytic anemia and leucopenia. Initial brain magnetic resonance imaging (MRI) showed three granulomatous foci affecting the left trigeminal nerve. Whole body positron emission tomography/computed tomography (PET/CT) scan detected multiple hypermetabolic areas in the brain, lymph nodes, lungs, liver, pancreas, peritoneum, uterine cervix and bones. The pathohistological examination of the trephine bone marrow biopsy identified the diffuse infiltration of all medullary spaces by storiform arrangement of histiocytes represented by spindle-shaped cells, clear cytoplasm, surrounded by dense collagenous stroma, with pronounced capillary proliferation. IHC stained positive for OCT2, HLA-DR, CD68, CD163, F13a, CD4, ALK, cyclin D1, with low Ki-67 proliferation fraction (10%). Molecular analysis revealed ALK rearrangement. The morphological, IHC, and molecular findings were consistent with the diagnosis of ALK-positive histiocytosis. Other histiocytic neoplasms, atypical ALK-rearranged histiocyte-rich tumors (such as inflammatory myofibroblastic tumor and epithelioid fibrous histiocytoma), endometrial stromal sarcoma and IgG4-related disease were excluded. The patient started therapy with ALK inhibitors, after which she achieved a partial response to treatment.

Conclusion

A rare case of multisystemic ALK-positive histiocytosis in an adult is presented. Molecular analysis for the detection of ALK gene rearrangement is necessary to confirm the diagnosis and start targeted therapy

Unravelling the molecular heterogeneity of BCL2-R–negative, CD23-positive - follicle center lymphomas

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Background

The pathogenesis of conventional BCL2-rearranged (BCL2-R) follicular lymphoma (cFL) is well characterized, but FL subtypes without BCL2-R (BCL2-nR) are less studied. BCL2-nR, CD23+, follicle center lymphoma (FCL) is a provisional entity with distinct clinical, morphological and genomic features. This study aims to further characterize the clinicopathological and molecular features of BCL2-nR, CD23+ FCL using a multimodal approach.

Methodology

We studied 67 patients with diagnosis of CD23+ FL diagnosed between 2021 and 2024 (32F:35M; mean age: 62.5 years). The intensity of immunohistochemical CD23 expression was graded as weak, intermediate, and strong. BCL2 and BCL6 rearrangements were evaluated by FISH. Next generation sequencing (NGS) was performed using two custom panels interrogating 59 (n=6) and 246 lymphoma-related genes (LymphKey, n=32), respectively, and paired tumor/normal whole-genome sequencing (n=29). Copy number alterations (CNA) were studied using Oncoscan CN arrays (n=47).

Results

All patients were assigned to 4 groups according to presence/absence of BCL2-rearrangements (+/-) and STAT6 mutations (wt/mut) (Fig.1A). BCL2-nR/STAT6mut FCL comprised 22/67 cases (33%), 13 (59%) of them with inguinal lymph node involvement (32% in the rest, not significant). Growth pattern was predominantly diffuse (8/22) or mixed (7/22) but 7 tumors had predominantly follicular growth pattern. CD23 staining was strong in 18 (82%) BCL2nR/STAT6mut tumors; 6 (75%) BCL2R/STAT6mut, 12 (41%) BCL2R/STAT6wt, and 3 (38%) BCL2nR/STAT6wt. STAT6 mutations in BCL2nR/STAT6mut tumors showed a VAF ranging from 2% to 37% (median 20%), and all but one mutation were located in the DNA binding domain (Fig.1B). BCL2nR/STAT6mut FCL compared to the rest carried significantly more mutations in TNFRSF14 (64%, 14/22, p .035), FOXO1 (59%, 13/22, p .0003) and POU2F2 (21%, 4/19, p .03). Mutations in CREBBP were distributed across all groups but mutations in the KAT domain were enriched in the BCL2-nR/STAT6mut (16/19, 84%, p .07, Fig. 1C). CN array showed significantly more frequent CNN-LOH in 16p13.3/CREBBP (8/16, 50%, p= .001), all with concomitant damaging mutations in CREBBP; LOH/Loss in 1p36/TNFRSF14 was frequent in BCL2-nR/STAT6mut (43.8%, 7/16) but did not distinguish this group from the others.

Conclusion

Overall, our findings indicate that BCL2-nR/STAT6mut FCL has a distinct genomic profile, may present with follicular pattern and in other locations than inguinal region, and CD23 alone may not identify all patients.