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Ο ΡΟΛΟΣ ΤΩΝ microRNAs ΣΤΑ ΝΟΝ-HODGKIN ΛΕΜΦΩΜΑΤΑ: ΣΥΣΧΕΤΙΣΗ ΜΕ ΤΗΝ ΠΡΟΓΝΩΣΗ ΚΑΙ ΤΗΝ ΑΠΟΚΡΙΣΗ ΣΤΗ ΘΕΡΑΠΕΙΑ

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UNIVERSITY OF IOANNNINA
SCHOOL OF HEALTH SCIENCES
DEPARTMENT OF MEDICINE

SECTOR OF INTERNAL MEDICINE
DEPARTMENT OF HEMATOLOGY

THE ROLE OF microRNAs IN NON-HODGKIN LYMPHOMAS: ASSOCIATION WITH PROGNOSIS AND RESPONSE TO TREATMENT

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«Η έγκριση της διδακτορικής διατριβής από το Τμήμα Ιατρικής του Πανεπιστημίου Ιωαννίνων δεν υποδηλώνει αποδοχή των γνωμών του συγγραφέα Ν. 5343/32, άρθρο 202, παράγραφος 2 (νομική κατοχύρωση του Ιατρικού Τμήματος)»

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«Ο ρόλος των microRNAs στα Non-Hodgikin λεμφώματα: συσχέτιση με την πρόγνωση και την απόκριση στη θεραπεία»

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Η Γραμματέας του Τμήματος

Αικατερίνη Λαγού

Αφιερώνω με ευγνωμοσύνη και αγάπη στην μητέρα μου, στον πατέρα μου (που θα ήταν πολύ χαρούμενος) και στα αδέρφια μου.

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Το Λέμφωμα από Μεγάλα Β-κύτταρα αποτελεί τη συχνότερη μορφή μη Hodgkin λεμφώματος και, παρά τη σημαντική πρόοδο που έχει σημειωθεί στη θεραπευτική του αντιμετώπιση, εξακολουθεί να χαρακτηρίζεται από έντονη βιολογική και κλινική ετερογένεια. Ένα σημαντικό ποσοστό ασθενών εμφανίζει υποτροπή ή ανθεκτικότητα στα καθιερωμένα θεραπευτικά σχήματα, γεγονός που υπογραμμίζει την ανάγκη για βαθύτερη κατανόηση των μηχανισμών της νόσου και για την αναζήτηση νέων προγνωστικών εργαλείων που θα μπορούσαν να συμβάλουν σε πιο εξατομικευμένες θεραπευτικές προσεγγίσεις.

Η παρούσα διατριβή εκπονήθηκε με αφετηρία το ενδιαφέρον για τον ρόλο των μοριακών βιοδεικτών στην αιματολογική κακοήθεια και ειδικότερα για τη δυναμική των κυκλοφορούντων microRNA ως μη επεμβατικών δεικτών διάγνωσης και πρόγνωσης. Τα microRNA, ως κρίσιμοι ρυθμιστές της γονιδιακής έκφρασης, έχουν αναδειχθεί τα τελευταία χρόνια ως πολλά υποσχόμενα εργαλεία στην ογκολογία, προσφέροντας νέες προοπτικές στη διαστρωμάτωση κινδύνου και την πρόβλεψη της έκβασης των ασθενών.

Στο πλαίσιο αυτό, η παρούσα μελέτη εστιάζει στη διερεύνηση της κλινικής και προγνωστικής σημασίας επιλεγμένων microRNA στον ορό ασθενών με νεοδιαγνωσμένο Λέμφωμα από Μεγάλα Β-κύτταρα, επιχειρώντας να συμβάλει στη διεύρυνση της υπάρχουσας γνώσης και να υποστηρίξει την ανάγκη ενσωμάτωσης νέων μοριακών παραμέτρων στα ήδη καθιερωμένα προγνωστικά μοντέλα. Η εργασία αυτή φιλοδοξεί να αποτελέσει ένα μικρό αλλά ουσιαστικό βήμα προς την κατεύθυνση της πιο στοχευμένης και εξατομικευμένης φροντίδας των ασθενών.

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1. 1.Epameinondas Koumpis, Alexandra Papoudou-Bai, Konstantina Papathanasiou, Evangelos Kolettas, Panagiotis Kanavaros, and Eleftheria Hatzimichael. Unraveling the Immune Microenvironment in Diffuse Large B-Cell Lymphoma: Prognostic and Potential Therapeutic Implications. *Curr Issues Mol Biol. 2024 Jul 5;46(7):7048–7064*
2. Epameinondas Koumpis, Vasileios Georgoulis, Konstantina Papathanasiou, Alexandra Papoudou-Bai, Panagiotis Kanavaros, Evangelos Kolettas and Eleftheria Hatzimichael. The Role of microRNA-155 as a Biomarker in Diffuse Large B-Cell Lymphoma. *Biomedicines 2024 Nov 21;12(12):2658.*
3. Προφορική ανακοίνωση στο 36ο Πανελλήνιο Αιματολογικό Συνέδριο, Αθήνα, 2025 (δημοσίευση στον τόμο περιλήψεων του συνεδρίου: περίληψη 28: Ε. Κουμπής, Ε. Παντελή, Β. Γεωργούλης, Δ. Λεονάρδος, Ε. Αποστολίδου, Θ. Δημητρίου, Κ. Παπαθανασίου, Η. Τάσση, Κ. Κελαϊδώνη, Λ. Κυριαζοπούλου, Δ. Γουγωπούλου, Λ. Μπενετάτος, Π. Καναβάρος, Ε. Κωλέττας, Ε. Καψάλη, Ε. Χατζημιχαήλ. Προγνωστική αξία των κυκλοφορούντων miR-155-3p και miR-142-3p στην ολική επιβίωση ασθενών με ΔΛΜΒΚ: προοπτική μονοκεντρική μελέτη. *Haema 2025; 16(suppl. 1)*
4. Eirini Panteli, Epameinondas Koumpis, Vasileios Georgoulis, Georgios Petros Barakos, Evangelos Kolettas, Panagiotis Kanavaros, Alexandra Papoudou Bai, Eleftheria Hatzimichael. The Role of microRNAs as Potential Biomarkers in Diffuse Large B-cell Lymphoma, *Non-Coding RNA 2026, 12(1), 2*

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Table of Contents

1. An Integrative Overview of Lymphomas, with a Focus on Diffuse Large B-Cell Lymphoma

Lymphomas constitute a heterogeneous group of malignant neoplasms arising from lymphoid cells at various stages of differentiation. They represent a major category of hematologic malignancies and are broadly divided into Hodgkin lymphoma (HL) and non-Hodgkin lymphomas (NHL), each encompassing multiple biologically and clinically distinct entities. Advances in immunophenotyping, cytogenetics, molecular diagnostics, and transcriptomic profiling have significantly refined our understanding of lymphoma pathobiology, underscoring the need for updated, internationally harmonized classification systems (1, 2). In 2022, two major classification frameworks were published: the 5th edition of the World Health Organization (WHO) Classification of Hematolymphoid Tumors and the International Consensus Classification (ICC) of mature lymphoid neoplasms (1, 2). Both systems aim to integrate morphologic, immunophenotypic, genetic, and clinical criteria, but they differ in structure, terminology, and emphasis (1, 2).

NHL represent the most common subgroup of lymphomas. According to the latest epidemiological data from the United States, the estimated number of new NHL cases in 2025 was 80,350, whereas that of HL was 8,720. Furthermore, the projected number of deaths were 19,390 new deaths among patients with NHL and 1,150 deaths among patients with HL (3). Large B-cell lymphoma (LBCL) accounts for approximately one-third of adult lymphoma cases (4). In Europe, the annual incidence of LBCL is approximately 28,000 cases (5), with males being affected more frequently than females. The incidence also shows a marked increase with advancing age (4). Several risk factors associated with the development of LBCL have been identified, including immunodeficiency, B-cell–activating autoimmune diseases, viral infections, and a family history of lymphoma (6). Diffuse large B-cell lymphoma (DLBCL) is a highly aggressive type of LBCL, marked by extensive molecular and pathological heterogeneity. This variation is reflected in its clinical behavior, resulting in diverse therapeutic responses and prognoses (7, 8). DLBCL represents the most frequent form of NHL, with a rising incidence over recent years.

Rituximab, a monoclonal antibody targeting the CD20 antigen on B-cell surfaces, plays a central role in the treatment of DLBCL. Its addition to the CHOP regimen—comprising

cyclophosphamide, doxorubicin, vincristine, and prednisone—markedly improved 5-year overall survival (OS) rates (58% compared with 45%) for CHOP alone. As a result, the R-CHOP combination became the established first-line therapy for DLBCL patients for many years (9). R-CHOP achieves cure in approximately 50–70% of patients (10). Nevertheless, around 30% of those who initially achieve complete remission (CR) will eventually experience a relapse, while another 20% will be found to have disease that is primarily refractory to treatment (11, 12). Polatuzumab vedotin is an antibody–drug conjugate directed against CD79b, a molecule broadly expressed on the surface of malignant B cells (13). Recently, a modified version of the R-CHOP regimen, known as pola-R-CHP—where vincristine is replaced by polatuzumab vedotin—was evaluated as first-line therapy in the POLARIX trial. The pola-R-CHP arm demonstrated superior progression-free survival (PFS) compared standard R-CHOP; however, no significant difference in OS was observed between the two treatment groups (13).

1.1. DLBCL classification

DLBCL may develop through the transformation of a pre-existing indolent, slow-growing low-grade NHL, such as follicular lymphoma (FL). Nonetheless, in most cases, DLBCL emerges spontaneously, without any prior history of lymphoma, a scenario referred to as “de novo” onset (14).

With respect to molecular characteristics, gene expression profiling has classified DLBCL into three distinct molecular subtypes: the germinal center B-cell–like (GCB) subtype, the activated B-cell–like (ABC) subtype, and a third category referred to as type 3 or unclassified cases (15). More recently, comprehensive molecular and cytogenetic analyses have enabled the development of more refined classification systems (16). One such framework defines the following genetic subtypes: (a) MCD, characterized by co-mutations in MYD88 (L265P) and CD79B; (b) BN2, defined by BCL6 fusions or NOTCH2 mutations; (c) N1, associated with NOTCH1 mutations and (d) EZB, involving EZH2 mutations or BCL2 translocations. Despite these advances, a substantial subset of patients still does not fit into any of these defined categories (17). An independent genomic analysis of 304 DLBCL cases proposed of an alternative molecular classification comprising five distinct clusters (C):

- **C1**, characterized by *NOTCH2* mutations and linked to favorable prognosis;

- **C2**, associated with aneuploidy and biallelic inactivation of *TP53*, correlating with poor clinical outcomes;
- **C3**, defined by *BCL2* mutations and translocations, along with mutations in genes involved in epigenetic regulation, also linked to adverse prognosis;
- **C4**, marked by alterations in signaling pathways such as Jak/STAT, and associated with better outcomes; and
- **C5**, which includes cases with 18q chromosomal gains and co-occurring *MYD88* and *CD79B* mutations, typically linked to poor prognosis.

In this study, the prognostic relevance of various genetic alterations was also examined. Notably, gain of 13q31.3—which includes the *miR-17-92* cluster—was identified as an independent predictor of worse PFS, highlighting the potential role of microRNA (miRNA) dysregulation in the pathogenesis and progression of DLBCL (18). The recently proposed classification frameworks for DLBCL, which delineate increasingly precise disease subtypes through integrated genetic, molecular, and immunophenotypic profiling, aim to capture the biological heterogeneity of the lymphoma more accurately and to facilitate a more individualized and effective therapeutic strategy. Despite their potential to enhance diagnostic precision and guide targeted treatment approaches, these classification systems depend heavily on advanced molecular technologies that are not yet widely accessible in routine clinical practice and, in several instances, still require further validation before they can be fully implemented on a broad clinical scale (1, 2).

Fluorescence in situ hybridization (FISH) is recommended for the detection of poor-prognosis DLBCL subtypes, particularly high-grade B-cell lymphomas (1, 2, 19). The WHO-HAEM4R (fourth edition) category of high-grade B-cell lymphoma, characterized by dual rearrangements of MYC and BCL2 and/or BCL6 has undergone conceptual revision and reclassification. Based on the recognition that these neoplasms display heterogeneous cytomorphology yet share uniform dark-zone biological properties and gene expression signatures, the WHO-HAEM5 designates this group as DLBCL/high-grade B-cell lymphoma with MYC and BCL2 rearrangements (DLBCL/HGBL-MYC/BCL2) (1). This definition encompasses tumors harboring concurrent MYC and BCL2 rearrangements, irrespective of whether their morphology consists of large, intermediate, or blastoid cells. Accordingly, the primary morphological interpretation can be retained after establishing the underlying genetic profile (2). This group represents a biologically cohesive entity with a consistently

germinal-center (GC)–derived gene expression pattern and a close pathogenetic relationship to FL and molecular GC-like DLBCL subsets. Furthermore, gene expression signatures associated with DLBCL/HGBL-MYC/BCL2 [such as double-hit(DHIT)sig] substantially overlap with those observed in Burkitt lymphoma (1). In contrast, lymphoid neoplasms exhibiting dual rearrangements of MYC and BCL6 constitute a more heterogeneous category with variable gene expression and mutational landscapes, distinctly different from DLBCL/HGBL-MYC/BCL2. Consequently, these cases are no longer included within the DLBCL/HGBL-MYC/BCL2 entity and are now classified as either a subtype of DLBCL, NOS or HGBL, NOS, depending on their cytomorphological features (1). Outside the context of a clinical trial, treatment is typically administered with dose-adjusted R-EPOCH (rituximab, etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone) or another intensive regimen, a practice supported by a meta-analysis of 11 studies demonstrating improved clinical outcome (20).

1.2. DLBCL risk stratification

To improve risk stratification in patients with DLBCL and enhance the accuracy of clinical outcome prediction, several prognostic scoring systems have been developed. The most widely used among them is the International Prognostic Index (IPI), although it was developed prior to the introduction of rituximab. This index considers factors such as patient age, serum lactate dehydrogenase (LDH) levels, Ann Arbor stage, ECOG performance status, and the number of disease sites. Based on these parameters, patients are classified into four risk categories—low, low-intermediate, high-intermediate, and high—with corresponding 3-year OS estimates ranging from 91% in the low-risk group to 59% in the high-risk group (21). Following the integration of rituximab into standard treatment protocols, the revised IPI (R-IPI) was introduced. While it retained the original risk factors, it simplified patient stratification into three prognostic groups: very good (no risk factors), good (one to two risk factors), and poor (three to four risk factors) (22). Additionally, the NCCN-IPI scoring system was developed specifically for newly diagnosed DLBCL patients intended to receive R-CHOP therapy (23). The NCCN-IPI was developed using data from the National Comprehensive Cancer Network database and was largely based on the same adverse prognostic factors as earlier scoring systems. However, it introduced more refined risk stratification, categorizing patients once again into four risk groups: low, low-intermediate, high-intermediate, and high.

Among the three scoring models, the NCCN-IPI appears to offer the greatest predictive accuracy for both PFS and OS. Nonetheless, none of these tools have been able to clearly identify a subgroup of patients with very poor prognosis, highlighting a continuing unmet need in the risk stratification of DLBCL (24, 25). A more recent prognostic tool for DLBCL patients, known as the International Metabolic Prognostic Index, has been introduced. This model incorporates patient age, disease stage, and baseline metabolic tumor volume (MTV), as assessed through positron emission tomography (PET) imaging (26).

There is a clear and ongoing need to enhance prognostic evaluation in patients with DLBCL. Progress in this direction is being driven by efforts to identify innovative biomarkers across various research disciplines. Among the most promising of these, circulating tumor DNA (ctDNA) has emerged as a biomarker of substantial interest, especially following initial studies that demonstrated its potential clinical relevance in non-hematologic solid tumors. ctDNA consists of fragmented DNA—ranging in size from approximately 70 base pairs to 21 kilobases—that is released into the bloodstream by malignant cells through processes such as apoptosis, necrosis, or active secretion (27). Multiple studies have highlighted the potential of ctDNA as a non-invasive biomarker for predicting prognosis, tracking treatment response, and enabling early detection of relapse in patients with DLBCL (28). A systematic review evaluating the utility of ctDNA as a “liquid biopsy” in DLBCL reported that immunoglobulin heavy chain (IGH) gene rearrangements and tumor-specific somatic mutations detectable in ctDNA currently represent the most robust and clinically informative biomarkers for monitoring therapeutic response (29). These molecular features offer a non-invasive means of tracking disease dynamics and may enhance early assessment of treatment efficacy. In contrast, the prognostic relevance of overall ctDNA concentration and the clinical implications of its methylation patterns remain insufficiently understood. The review emphasizes that these parameters require more comprehensive and methodologically consistent investigations before their potential prognostic value can be fully established (29). Notably, several clinical trials evaluating novel therapeutic agents, including polatuzumab vedotin and odronextamab, have incorporated ctDNA as a biomarker of molecular response. Emerging evidence indicates that patients who achieve undetectable ctDNA levels during therapy or at its completion tend to demonstrate improved survival outcomes. Consequently, ctDNA holds considerable promise as a tool to inform clinical decision-making, potentially enabling treatment escalation or de-escalation at defined points during the therapeutic course (30).

1.3. DLBCL therapy

In the frontline treatment of newly diagnosed DLBCL, the standard therapeutic approaches include either R-CHOP or, alternatively, Pola-R-CHP (19). For patients with primary refractory disease or those who experience an early relapse of DLBCL—defined as occurring within one year after completing initial therapy—chimeric antigen receptor (CAR) T-cell therapy is the preferred treatment approach (31, 32). In contrast, individuals with late relapses may be managed with either salvage chemotherapy followed by autologous stem-cell transplantation (ASCT) or CAR T-cell therapy (4). For patients who are not candidates for transplantation, alternative therapeutic options include polatuzumab vedotin combined with rituximab and bendamustine, tafasitamab in combination with lenalidomide, and, in cases of second relapse, bispecific antibodies such as glofitamab (alone or in combinations) or epcoritamab (4, 19).

1.4. Other LBCL

Additional clinically significant large B-cell lymphoma subtypes that warrant mention include primary mediastinal B-cell lymphoma and primary central nervous system (CNS) B-cell lymphoma. Primary DLBCL of the CNS, commonly referred to as primary CNS lymphoma (PCNSL), is an aggressive malignancy characterized by disease confined to the CNS (33). PCNSL was first recognized as a distinct entity in the 2017 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues. In the 2022 WHO Classification, it is placed within the category of 'Large B-cell lymphomas of immune-privileged sites,' whereas the ICC considers it a separate, well-defined diagnostic entity (1, 2, 33). It represents an aggressive malignancy that is characteristically restricted to the CNS at diagnosis and at recurrence, with systemic dissemination being an uncommon event. The disease predominantly affects adults over the age of 60 (1, 34). They are treated differently comparing to DLBCL, NOS. High-dose methotrexate (HD-MTX) in combination with an alkylating agent and rituximab, with or without high-dose cytarabine (HD-AraC), constitutes the standard induction therapy for primary CNS lymphoma. Current evidence supports the use of combination regimens that have been evaluated in randomized clinical trials. For patients who are medically fit and exhibit a responsive or stable disease following induction, ASCT is recommended as

consolidation therapy, with thiotepa-based conditioning regimens representing the preferred approach (33, 34).

Primary mediastinal B-cell lymphoma (PMBCL) is a rare and aggressive subtype of mature LBCL that is clinically and biologically distinct from DLBCL (35). It is believed to originate from thymic medullary B cells and exhibits clinicopathologic characteristics that differ from DLBCL, while sharing certain clinical and biological features with nodular sclerosing classic HL (35). PMBCL constitutes roughly 5–10% of LBCL cases and predominantly affects younger individuals, with a marked female predominance and a median age of approximately 37 years (36). It is characterized by a bulky anterior mediastinal mass, rapid progression, and a distinct gene expression profile (4). PMBCL is associated with a favorable prognosis, with five-year survival rates surpassing 80% (35). Long-term outcomes with regimens such as R-CHOP14 or more intensive approaches—including DA-EPOCH-R—in patients under 80 years of age are generally favorable, with comparable complete response rates and durable remission outcomes (4).

2. MicroRNAs — Definition and Biogenesis

Non-coding RNAs (ncRNAs) represent a heterogeneous group of RNA transcripts that, despite not encoding proteins, fulfill vital regulatory and structural roles in cellular processes. Based on their length, ncRNAs are broadly categorized into short ncRNAs (generally <200 nucleotides) and long non-coding RNAs (lncRNAs) (>200 nucleotides) (37, 38, 39). ncRNAs are divided into two functional categories: housekeeping and regulatory. Housekeeping ncRNAs—including ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), small nuclear RNAs (snRNAs), and small nucleolar RNAs (snoRNAs)—perform indispensable roles in core cellular processes (40, 41). In contrast, regulatory ncRNAs function predominantly in the modulation of gene expression. Within this regulatory framework, long non-coding RNAs (lncRNAs)—encompassing both linear lncRNAs and circular RNAs (circRNAs)—represent a predominant class of ncRNA species and are essential for sustaining normal cellular function and tissue homeostasis. Small non-coding RNAs, including microRNAs (miRNAs), small nucleolar RNAs (snoRNAs), and PIWI-interacting RNAs (piRNAs), have traditionally been viewed as post-transcriptional repressors of gene expression; however, accumulating evidence indicates that they also participate in the regulation of RNA metabolism at both transcriptional and

translational levels (41, 42). Moreover, fragments originating from housekeeping RNAs—such as tRNA-derived fragments (tRFs) and rRNA-derived fragments (rRFs)—are increasingly recognized as discrete functional entities rather than mere degradation products (43) as shown in **Figure 1**.

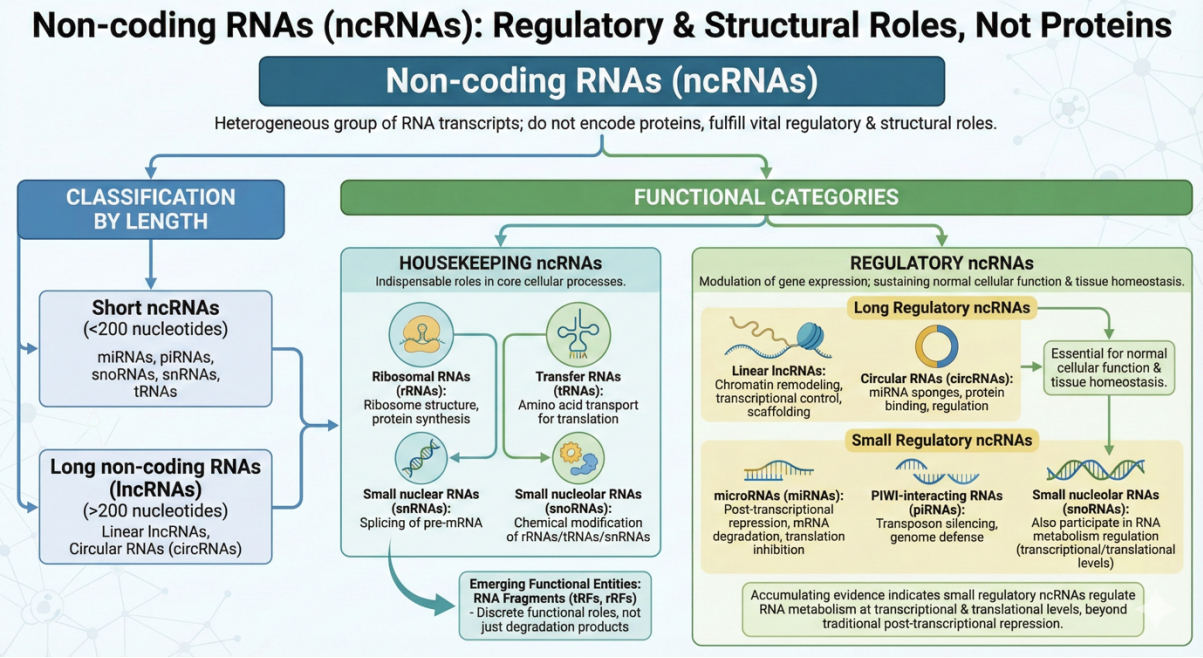

Figure 1. *Classification of Non-Coding RNAs. Created with Gemini*

Among the various classes of small ncRNAs, miRNAs constitute one of the most functionally significant groups. miRNAs belong to a large family of naturally occurring, endogenous, single-stranded ~22-nucleotide-long RNAs that interact with their target RNA in a sequence-dependent manner and therefore are significant regulators of posttranscriptional gene expression (44, 45). In 2024, Victor Ambros and Gary Ruvkun were awarded the Nobel Prize in Physiology or Medicine for their pioneering discovery and characterization of microRNAs, first identified in the roundworm *Caenorhabditis elegans* (46, 47, 48). Since their discovery, it is estimated that microRNAs regulate nearly two-thirds of all human genes (49, 50). These molecules participate in the regulation of numerous biological processes (51), including cell growth, differentiation, development, and apoptosis (52). Their dysregulation has been implicated in a wide range of human diseases, such as diabetes, cardiovascular disorders, and cancer (53, 54, 55, 56, 57). They are potentially useful biomarkers since they can be detected both in tumor tissue and in peripheral blood and they are stable during sample handling and storage (58).

miRNAs are preferentially transcribed by polymerase II into primary mi-RNAs (pri-miRNA) that are subsequently processed in the nucleus by the enzyme Drosha and its cofactor binding protein, DiGeorge syndrome critical region 8 (DGCR8) to become pre-miRNAs (**Figure 2**) (59, 60, 61). Pre-miRNAs are transported to the cytoplasm by Exportin-5, where they are subsequently recognized and processed by the RNase III enzyme Dicer, generating small double-stranded RNA molecules (62). The resulting miRNA duplex is then incorporated into an Argonaute protein, facilitating the formation of the ribonucleoprotein complex known as the RNA-induced silencing complex (RISC). RISC is composed of the transactivation-responsive RNA-binding protein (TRBP) and Argonaute 2 (Ago2) (63). The RISC complex facilitates the identification of the specific mRNA target by recognizing complementary sequences in the 3' untranslated region. It can result in mRNA degradation, destabilisation, or translational suppression without the need for precise pairing in humans. (52). Alternative or noncanonical pathways for miRNA biogenesis have been identified including Drosha-independent and Dicer independent pathways, where pri-miRNAs are processed to pre-miRNAs by the spliceosome machinery and debranching enzyme (52, 64). The biosynthesis of miRNAs is illustrated in **Figure 2.**

Figure 2. *The synthesis of miRNAs. miRNAs are transcribed by RNA polymerase II as primary miRNA transcripts (pri-miRNAs), which undergo nuclear processing by the RNase III enzyme Drosha in conjunction with its essential cofactor DGCR8, yielding precursor miRNAs (pre-miRNAs). These pre-miRNAs are subsequently exported to the cytoplasm via Exportin-5, where they are recognized and cleaved by the RNase III enzyme Dicer to generate short double-stranded RNA intermediates. The resulting miRNA duplex is then incorporated into an Argonaute protein, facilitating the assembly of the RNA-induced silencing complex (RISC). Once formed, RISC directs sequence-specific recognition of target mRNAs, ultimately leading to their degradation, destabilization, or translational repression. In addition to this canonical pathway, several non-canonical miRNA biogenesis routes, such as Drosha-independent and Dicer-independent mechanisms, have also been described. Ago2: Argonaute 2 , DGCR8: Di George syndrome critical region 8, RISC: RNA-induced silencing complex, TRBP: Transactivation response element RNA-binding protein. (E. Koumpis et al, Biomedicines. 2024 Nov 21;12(12):2658). Created with BioRender.*

Since imperfect cleavage of pri- or pre-miRNA can result in diverse 5’ or 3’ ends, a single miRNA gene can produce numerous miRNA isoforms (isomiRs). Distinct isomiRs derived from the same miRNA precursor can exhibit differential target specificity, thereby regulating diverse genes and biological pathways in a context-dependent manner. Moreover, certain isomiRs display cancer-type specificity, underscoring their potential utility as highly discriminative biomarkers for disease detection, classification, and prognosis (52, 65).

miRNAs can be extracted directly from lymph node tissue or obtained easily from peripheral blood (liquid biopsy), rendering them valuable biomarkers that can serve as potential diagnostic, prognostic or predictive biomarkers as shown in **Figure 3**.

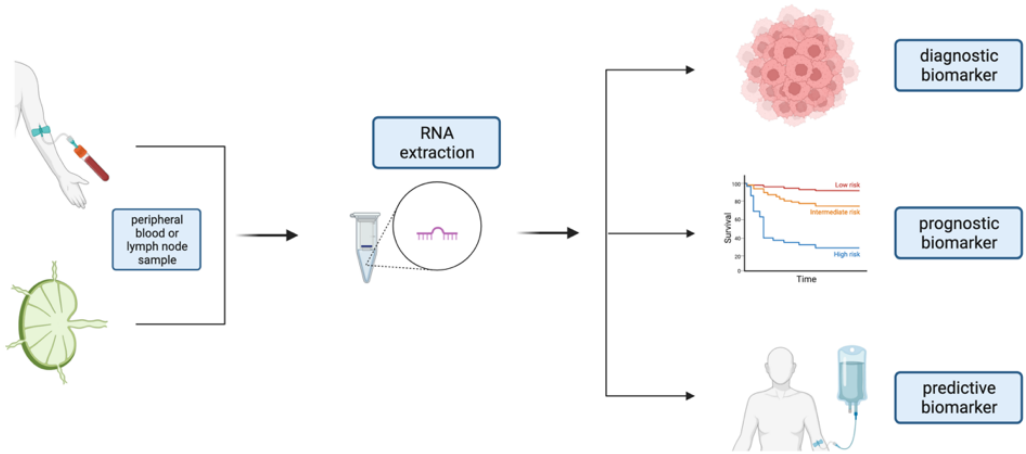

Figure 3. *miRNAs as biomarkers. miRNAs may be isolated from lymph node tissue or, with greater ease, from peripheral blood through liquid biopsy techniques. Their accessibility and stability make them highly valuable molecular indicators with potential utility as diagnostic, prognostic, or predictive biomarkers. (E. Koumpis et al, Biomedicines. 2024 Nov 21;12(12):2658). Created with BioRender*

A biomarker is an objectively measured and evaluated indicator of normal biological or pathogenic processes, or pharmacologic responses to a therapeutic intervention (66). A diagnostic biomarker detects or confirms the presence of a disease or condition of interest, or identifies an individual with a subtype of the disease (67). The predictive value of biomarkers assesses the impact of therapy on a patient with a particular condition, whereas their prognostic value predicts the outcome regardless of the treatment approach (66, 68).

In an effort to enhance prognostic stratification and the evaluation of therapeutic response in patients with DLBCL, numerous research groups have intensified their search for novel, clinically informative biomarkers. Traditional invasive diagnostic and monitoring methods are increasingly being supplanted by non-invasive strategies such as liquid biopsy, which offer greater safety, accessibility, and longitudinal monitoring capability (69). While circulating tumor cells can offer tumor-specific information, their analysis is less appealing in DLBCL because this lymphoma type typically lacks circulating tumor cells (70). The emerging clinical use of cell-free RNAs (particularly miRNAs and circRNAs) has shown substantial

promise in advancing precision medicine approaches for DLBCL. Owing to their stability in circulating biofluids, disease-specific expression patterns, and capacity to reflect underlying molecular alterations, these cell-free RNA species are increasingly recognized as valuable biomarkers for improving diagnosis, prognostic assessment, and treatment stratification (71).

3. miRNAs in hematopoiesis

Hematopoietic stem cells (HSCs) are multipotent progenitor cells endowed with the remarkable ability to self-renew and to give rise to the full spectrum of mature blood cell lineages. Through tightly regulated differentiation processes, these stem cells serve as the foundational source from which all cellular components of the hematopoietic and immune systems are ultimately derived (72). A wide array of genetic and epigenetic regulatory mechanisms contributes to the maintenance of homeostasis and the controlled differentiation of the normal hematopoietic system. Among these regulatory layers, miRNAs play an important role by modulating gene expression programs essential for proper hematopoietic development and function (73, 74). As previously noted, miRNAs are endogenous, single-stranded noncoding RNAs approximately 22 nucleotides in length that bind target RNAs in a sequence-dependent manner, leading to their degradation or translation inhibition. Through this mechanism, they serve as important regulators of post-transcriptional gene expression (44, 45). Each individual miRNA has the capacity to regulate multiple distinct mRNA transcripts, reflecting the broad and interconnected nature of miRNA-mediated gene control. Conversely, a single mRNA may be subject to regulation by several distinct miRNAs, creating a complex and multilayered post-transcriptional regulatory network. To date, more than 3,700 human miRNAs have been identified, underscoring the extensive scope of this regulatory system and its potential impact on numerous cellular processes (75). miRNAs function as key regulatory molecules that shape the development, maintenance, and functional balance of the normal hematopoietic system, and their dysregulation plays a critical role in the initiation and progression of hematologic malignancies (76, 77).

Early work in the field of hematopoiesis identified specific miRNAs that are preferentially expressed in blood-forming cells and whose levels change dynamically during the initial stages of blood cell development (77). Among these, miR-181, miR-223, and miR-

142 were highlighted as hematopoietic-specific miRNAs with regulated expression patterns during early lineage commitment, underscoring their potential roles in the control of hematopoietic differentiation and function (77). The miRNAs implicated in the self-renewal of HSCs in mouse models include miR-33 (78), miR-99 (79), and miR-125a (80). Furthermore, studies have identified at least 33 different miRNAs expressed in $CD34^+$ HSCs, highlighting the complexity of post-transcriptional regulation within this compartment. These miRNAs are involved in multiple fundamental cellular processes, including the control of proliferation, survival, and self-renewal. Importantly, many of them contribute to preserving the undifferentiated state of HSCs by suppressing pathways that drive maturation, thereby ensuring proper regulation of hematopoietic lineage commitment (81). In addition to their physiological roles, oncogenic miRNAs (oncomiRs) function by downregulating the expression of key tumor-suppressor genes, thereby promoting cellular transformation and malignant progression. Conversely, tumor-suppressor miRNAs act as inhibitory regulators of oncogenes, helping to restrain uncontrolled cell growth and maintain normal cellular homeostasis. The balance between these two classes of miRNAs is therefore essential for preventing the development of cancer (54, 82, 83). The first oncomiRs identified in cancer were miR-15a and miR-16-1 which were frequently deleted or downregulated in chronic lymphocytic leukemia (CLL) associated with deletion 13q14 (53).

Abnormal patterns of miRNA expression have been strongly implicated in the initiation and progression of several hematologic malignancies (84, 85). In particular, dysregulated miRNAs play critical roles in the development of myelodysplastic syndromes (MDS)/acute myeloid leukemia (AML), where they contribute to disrupted hematopoietic differentiation and increased genomic instability (85). Similarly, altered miRNA expression is a key driver in lymphomagenesis, influencing signaling pathways that govern lymphocyte survival, proliferation, and transformation (84). Beyond hematologic cancers, aberrant miRNA regulation has also been linked to the pathogenesis of a wide spectrum of solid tumors, highlighting their broad impact on oncogenic processes across diverse tissues (86, 87). For instance, miR-150 plays an important role in the regulation of erythropoiesis and megakaryocytopoiesis, and its dysregulation has been linked to MDS development (88, 89). The main target of miR-150 is MYB, also known as c-Myb, which is a regulatory transcription factor in the hematopoietic system and gastrointestinal tract that preserves the balance between cell division, differentiation, and survival (90). Dysregulation of MYB activity has

been associated with several hematologic disorders (91). Moreover, miR-145 plays an important role in regulating both megakaryocytic and erythroid differentiation, primarily through its ability to target Fli-1, a transcription factor that is essential for the proper development and functional maturation of these hematopoietic lineages. By modulating Fli-1 expression, miR-145 helps direct lineage commitment and maintain balanced blood cell production (92). The miR-17-92 cluster is a polycistronic miRNA cluster, consisting of miR-17, miR-18a, miR-19a, miR-19b, miR-20a, and miR-92a, which is often overexpressed in certain malignancies. This cluster targets the tumor suppressor PTEN and the pro-apoptotic protein Bim by inhibiting their expression (93). By targeting the pro-apoptotic protein Bim, the miR-17-92 cluster ensures survival of hematopoietic stem and progenitor cells, playing a crucial role in hematopoiesis (94). miR-143/145 differentially modulate HSC and progenitor activity via suppression of canonical transforming growth factor (TGF)-β signaling and loss of expression of these miRNAs can lead to MDS development (95). The interaction between HSCs, progenitor cells and bone marrow stromal cells is modulated by CXCL12, a chemokine that is regulated by several different miRNAs (96). miR-10a and miR-10b have been shown to be overexpressed in $CD34^{+}$ hematopoietic progenitor cells, where their increased levels promote the upregulation of the transcription factor TWIST-1. This alteration contributes to reduced sensitivity to apoptosis, thereby supporting the survival of these cells under conditions that would normally trigger programmed cell death (97). miRNAs play a critical role in lymphomagenesis by regulating key genes involved in lymphocyte development, survival, and proliferation. In normal hematopoiesis, miRNAs help maintain balanced differentiation and immune homeostasis; however, their dysregulation can disrupt these processes and promote malignant transformation (84, 98). OncomiRs may enhance lymphomagenesis by suppressing tumor-suppressor genes, while tumor-suppressor miRNAs normally restrain oncogenic pathways and prevent uncontrolled lymphoid expansion. Altered miRNA expression profiles have been observed across lymphoma subtypes, influencing pathways such as NF-κB, PI3K–AKT, and MYC signaling (84, 98). Collectively, these changes contribute to abnormal cell growth, impaired apoptosis, and the acquisition of malignant characteristics, underscoring the importance of miRNAs as both drivers and potential biomarkers of lymphoma development (86). **Figure 4** illustrates the role of miRNAs in normal hematopoiesis and in cancer development.

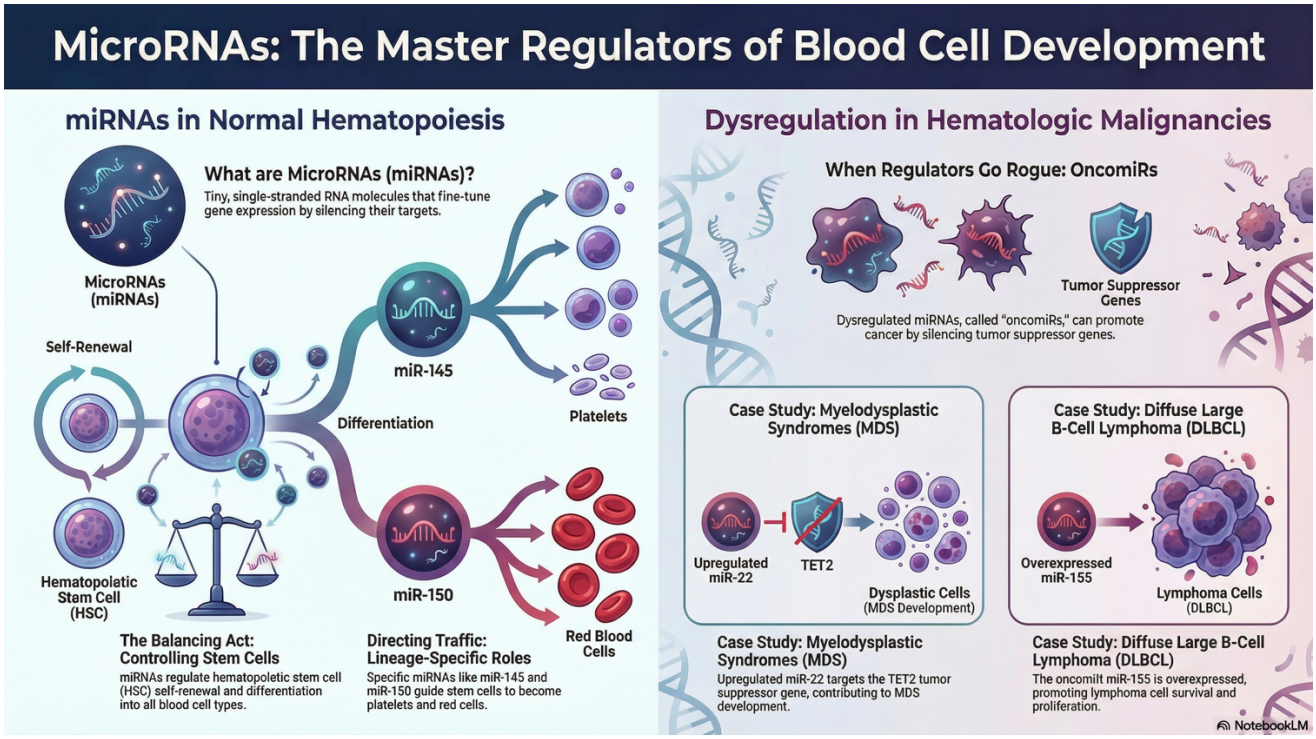

Figure 4. *miRNAs in Hematopoiesis and in malignancies. (Created with NotebookLM).*

4. miRNAs and their role in DLBCL pathogenesis and prognosis

MiRNAs function as critical post-transcriptional regulators in the pathogenesis of DLBCL, where they modulate multiple molecular pathways that govern disease development, progression, and response to treatment (**Figure 5**) (99). Several miRNAs, such as miR-155, miR-21, and the miR-17-92 cluster, act as oncogenes by suppressing tumor-suppressor genes, thereby enhancing cell growth, survival, and immune evasion. In contrast, miRNAs such as miR-34a, miR-144, and miR-181a serve as tumor suppressors by downregulating oncogenes such as SIRT1, BCL6, and CARD11 (99, 100). **Figure 5** presents an overview of several important miRNAs and their associations with the pathogenesis of DLBCL.

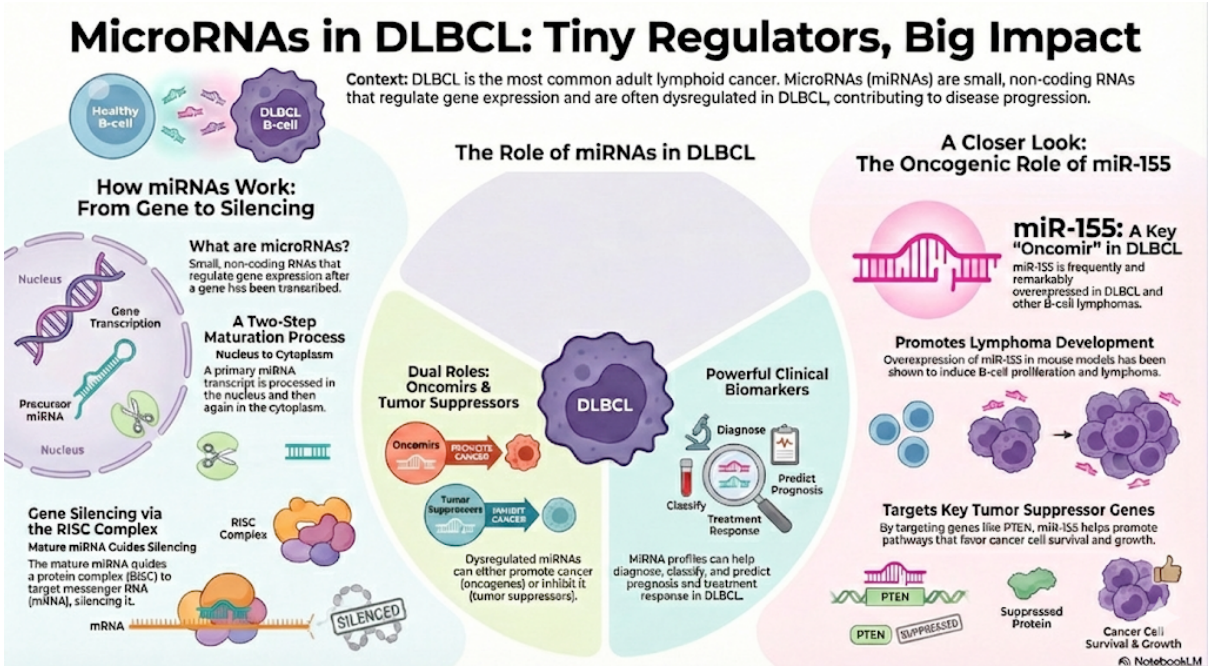

Figure 5. *miRNAs in DLBCL. miRNAs are, small non-coding RNAs that regulate gene expression and are often dysregulated in DLBCL, contributing to disease progression. (Created with NotebookLM and Gemini).*

4.1. miR-155

miR-155 is a highly conserved and functionally versatile miRNA that originates from the non-coding transcript B-cell Integration Cluster (BIC), which is encoded on chromosome 21 (101). Notably, the insertion of the avian leukosis virus (ALV) into the promoter region of the BIC gene activates its expression in B-cell lymphomas. miR-155, which is encoded by BIC, plays a role in regulating immune function, hematopoiesis, and inflammatory processes (102). miR-155 has been shown to influence the differentiation of T helper cells and the germinal center (GC) response, helping to generate an effective T cell-dependent antibody response, partly by controlling the production of various cytokines (103). miR-155 also plays a central role in regulating myeloid cell differentiation and modulating the production of inflammatory cytokines, primarily through its repression of SOCS1 and SHIP1—key negative regulators of the PI3K/AKT signaling cascade. Dysregulated expression of miR-155 has been implicated in the pathogenesis of a wide spectrum of hematologic malignancies and solid tumors, thereby supporting its classification as an oncogenic miRNA (oncomiR) (104).

Analyses of splenocytes derived from Eμ-miR-155 transgenic mice, which are engineered to overexpress miR-155 specifically in B cells, reveal a marked reduction in IKKβ

mRNA compared with wild-type animals. This finding suggests that miR-155 may regulate the expression of both IKKβ and IKKε, two key kinases involved in NF-κB pathway activation. By downregulating these components, miR-155 appears to contribute to a negative feedback circuit that attenuates NF-κB signaling. Such a mechanism would restrain excessive inflammatory and immune responses, thereby maintaining immune homeostasis (105, 106). MiR-155 directly targets transcripts encoding several proteins involved in LPS signaling, such as the Fas-associated death domain protein (FADD), IKKε, and the receptor (TNFR superfamily)-interacting serine-threonine kinase 1 (Ripk1) while simultaneously enhancing TNF-alpha translation, thereby promoting anti-apoptotic effects (107). Additionally, miR-155 targets histone deacetylase 4 (HDAC4) and impairs the transcriptional activity of B-cell lymphoma 6 (BCL6) (108). Reduced BCL6 levels subsequently lead to de-repression of some of the known BCL6 targets, including inhibitor of differentiation (Id2), IL-6, cMyc, Cyclin D1, and Mip1α/ccl3, all of which promote cell survival and proliferation (108). Furthermore, miR-155 directly targets the bone morphogenetic protein (BMP)-responsive transcriptional factor SMAD5, a modulator of transforming growth factor- beta (TGF-B) signaling (109). In DLBCL models, miR-155 expression inhibited activation of the retinoblastoma protein (RB), reducing the abundance of the inhibitory pRB-E2F1 complex and limiting G0/G1 cell cycle arrest (110).

MiR-155 also functions as a modulator of immune responses (111). Small lipid-enclosed extracellular vesicles known as exosomes have emerged as important mediators of intercellular communication, enabling the transfer of biologically active molecules through the extracellular environment. These vesicles carry a diverse array of molecular cargos, including proteins, lipids, and nucleic acids. Notably, microRNAs such as miR-155 can be selectively incorporated into exosomes and transported to neighboring or distant recipient cells, where they modulate gene expression and cellular behavior. This mode of miRNA trafficking represents an additional layer of post-transcriptional regulation that contributes to the complexity of intercellular signaling networks (112, 113). Colorectal carcinoma metastasis has been linked to exosomal miR-155-mediated interaction between dendritic cells and cancer cells, as well as between tumor-associated fibroblasts. (113, 114). These roles establish miR-155 as a crucial determinant of both tumor progression and tumor microenvironment formation. Furthermore, macrophages and dendritic cells upregulate miR-155 expression following exposure to danger signals. Elevated miR-155 also influences the function of myeloid-derived suppressor cells (MDSCs), a subset of immature myeloid cells

with potent immunosuppressive activity (111, 115). Notably, the presence of MDSCs in the tumor microenvironment is often associated with worse prognosis (116). A key aspect of miR-155's function in the immune system involves its FOXP3-dependent regulation within regulatory T cells (Tregs). Through this regulatory axis, miR-155 enhances the competitive fitness and survival of specific Treg subsets. Mechanistically, it achieves this by targeting and suppressing the expression of Suppressor of Cytokine Signaling-1 (SOCS1), a negative regulator of cytokine signaling. By limiting SOCS1 activity, miR-155 supports optimal cytokine responsiveness in Tregs, thereby promoting their stability and immunoregulatory capacity (117). Additionally, miR-155 plays a significant role in shaping humoral immunity by influencing the development of class-switched plasma cells. It exerts this regulatory function in part by targeting the transcription factor PU.1 and modulating the expression of activation-induced cytidine deaminase (AID), both of which are essential for effective immunoglobulin class-switch recombination and plasma cell differentiation (118, 119). **Figure 6** illustrates key targets of miR-155 implicated in the development of DLBCL.

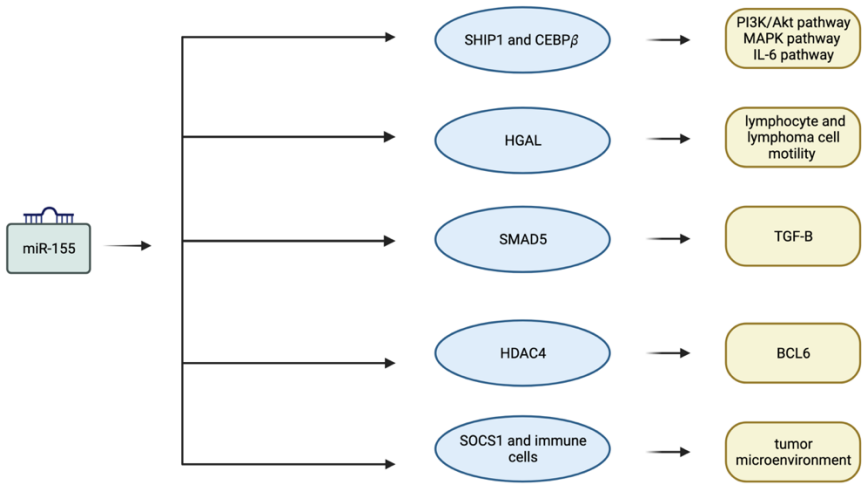

Figure 6. *miR-155 targets. miR-155 targets a wide range of genes, proteins, and cells, playing a crucial role in DLBCL pathogenesis. It regulates key pathways, transcription factors, cytokine expression, and shapes the tumor microenvironment in DLBCL (E. Koumpis et al, Biomedicines. 2024 Nov 21;12(12):2658). Created with BioRender.*

Cell-free RNAs, including miRNAs and circRNAs, have shown potential as biomarkers for precision medicine approaches in DLBCL (120). Among these, miR-155 stands out as a promising diagnostic, prognostic, and predictive biomarker (121). As a candidate biomarker, the expression of miR-155 has been studied in both tissue and peripheral blood.

Research investigating the role of miR-155 as a diagnostic biomarker in both tissue and blood samples has yielded mixed findings (122, 123, 124). Multiple studies have demonstrated elevated miR-155 levels in both tumor tissues and the circulating blood of patients with DLBCL, compared with various non-malignant controls, including reactive (non-cancerous) lymph nodes, normal peripheral B cells, and serum, plasma, or exosome-derived samples from healthy individuals (125, 126, 127). These findings suggest that increased miR-155 expression may be associated with lymphomagenesis or may reflect underlying disease biology. In contrast, several other investigations have reported no significant differences in miR-155 expression between DLBCL patients and matched control groups, indicating that the diagnostic utility of miR-155 remains uncertain and may depend on differences in study design, sample type, or analytical methods. (128, 129). Importantly, circulating miR-155 concentrations have been reported to correlate positively with expression levels within lymphoma tissue, suggesting that serum measurements may serve as a reliable and minimally invasive surrogate for tumor-derived miR-155 (130). Additionally, serum miR-155 levels appear to fluctuate throughout the disease course, with variations observed at diagnosis, during treatment, and upon disease progression [104]. Studies comparing patients responding to therapy with those who esperiencing progressive disease have demonstrated a general decrease in circulating miRNAs in former group (131).

Multiple studies have shown that miR-155 expression, whether measured in lymphoma tissue or serum, is consistently higher in patients with the non-GCB (ABC) subtype compared with those classified as GCB. Multiple studies have shown that miR-155 expression, whether measured either in lymphoma tissue or in serum, is consistently higher in patients with the non-GCB (ABC) subtype compared with those classified as GCB (124, 125, 126, 128, 130). This subtype-specific elevation is thought to reflect underlying biological mechanisms, including direct suppression of the *HGAL* gene by miR-155, as previously discussed (132). Beyond molecular subtype classification, miR-155 has also been investigated as a potential marker for distinguishing de novo DLBCL from transformed disease. One study reported that miR-155 expression was significantly higher in de novo DLBCL compared with transformed

aggressive B-cell lymphoma (133). However, a separate investigation focusing on the transformation of FL into DLBCL identified alterations in several other microRNAs, including miR-223, miR-217, miR-222, miR-221, and members of the let-7i/let-7b family but found no differential expression of miR-155 between de novo and transformed DLBCL (125). Consequently, miR-155 does not appear to be a reliable marker for distinguishing FL from DLBCL or for predicting FL transformation into DLBCL (125). Well designed, prospective, randomized studies are essential to further clarify the potential diagnostic utility of miR-155 in DLBCL patients and to establish standardized protocols for miRNA detection and analysis.

The role of miR-155 as a prognostic biomarker in DLBCL remains a subject of ongoing debate. In one study involving tissue samples from 90 patients with de novo DLBCL, reduced miR-155 expression was associated with a higher 5-year progression-free survival rate (134). Similarly, two other studies reported that elevated miR-155 levels were associated with worse overall survival (135). In one such investigation, real-time PCR analysis of tumor samples from 118 lymphoma patients enabled the classification of individuals into high- and low–miR-155 expression groups using the median expression level as a cutoff. This stratification demonstrated that patients with elevated miR-155 expression experienced significantly reduced overall survival compared with those exhibiting lower levels (136). In contrast, another study found that in patients with the GCB subtype of DLBCL treated with R-CHOP, high miR-155 levels were associated with more favorable outcomes, independently of IPI score (137). Beyond tissue-based studies, miR-155 has also been examined in peripheral blood to o assess its potential as a circulating prognostic marker. Although one study reported that serum miR-155 levels did not demonstrate prognostic significance in patients with DLBCL (125), subsequent investigations provided contrasting evidence. More recent studies have found that higher circulating miR-155 levels were independently linked to poorer clinical outcomes, and this association remained significant even when accounting for IPI risk categories (129, 138). Similar to its proposed diagnostic role, the prognostic relevance of miR-155 in DLBCL remains to be fully established and will require validation through well-designed prospective, randomized studies. Furthermore, resolving the inconsistencies observed across existing reports, will require the implementation of standardized methodologies for miRNA measurement and data analysis to ensure that findings can be reliably compared and reproduced.

The predictive value of a biomarker reflects its ability to inform therapeutic decision-making by indicating whether a patient is likely to respond to a specific treatment. miR-155 has emerged as a promising predictive biomarker detectable in both tissue and peripheral blood. In one study, miR-155 expression served as an independent predictor for choosing appropriate chemotherapy regimens in de novo DLBCL, indicating its potential usefulness as a tissue-based biomarker with predictive value (139). Patients with high miR-155 expression who received R-CHOP had improved OS and PFS compared with those treated with the CHOP alone (139). Notably, this difference was not observed in patients with low miR-155 expression (139). Conversely, another study reported that high miR-155 expression was associated with R-CHOP treatment failure (140).These apparently contradictory findings may reflect differences in patient populations, methodological approaches, or the complex context-dependent roles of miR-155 in DLBCL biology. More recently, elevated miR-155 expression has been shown to increase the sensitivity of DLBCL cell lines to vincristine through the direct suppression of the WEE1 gene (141).

In an analysis of circulating miRNAs in patients with DLBCL, serum miRNA levels closely mirrored those present in tumor tissues, suggesting that circulating miRNAs could function as dependable, non-invasive biomarkers. However, miR-155 levels in peripheral blood did not demonstrate any association with treatment sensitivity or resistance in that study (142). In contrast, more recent evidence indicates that exosomal miR-155 is significantly elevated in patients with refractory or relapsed DLBCL, compared with both treatment-responsive individuals and those undergoing R-CHOP therapy (143). Conversely, another investigation reported that miR-155 levels in serum-derived extracellular vesicles were markedly altered in some hematologic malignancies when compared with healthy controls, yet no significant differences were observed in patients with DLBCL (144).

Therapeutic inhibition of miR-155 using the anti-miR oligonucleotide Cobomarsen is currently being investigated in patients with chronic lymphocytic leukemia (CLL), following promising clinical activity observed in a limited cohort of patients with cutaneous T-cell lymphoma (CTCL) (86). In addition to CLL, Cobomarsen has emerged as a potential therapeutic candidate for DLBCL (145). Preclinical studies demonstrated that Cobomarsen significantly reduced cellular proliferation and promoted apoptosis in ABC-DLBCL cell lines (145). Moreover, intravenous administration of Cobomarsen in a xenograft model of ABC-DLBCL using immunoompromised NSG (NOD Scid Gamma) mice resulted in a marked reduction in

tumor volume, activation of apoptotic pathways, and de-repression of established direct miR-155 target genes (145). Notably, in the same study, a patient who received five cycles of Cobomarsen treatment exhibited substantial tumor reduction and subsequent disease stabilization, without evidence of treatment-related toxicity (145).

4.2. miR-21

miR-21 is one of the most prevalent and evolutionarily conserved miRNAs. It is present in nearly all cell types and plays essential regulatory functions in both normal physiology and various disease conditions (146). Furhermore, miR-21 is one of the most consistently overexpressed microRNAs across a wide range of malignant tumors (147, 148), and it has been implicated in various cancer-associated processes. miR-21 is closely associated with DLBCL as an oncomiR; however, only few studies have analyzed its mechanism.

Significant overexpression of miR-21 has been observed in DLBCL tissues and cell lines, where elevated miR-21 levels have been shown to promote cellular proliferation, migration, and invasion while simultaneously suppressing apoptosis in SU-DHL-8 cells (149). In addition, miR-21 is markedly elevated in patients with newly diagnosed DLBCL and holds potential as a circulating biomarker for assessing therapeutic response. Notably, patients who became PET/CT negative following four cycles of R-CHOP demonstrated a substantially greater reduction in circulating miR-21 levels compared with those who continued to exhibit PET/CT positivity (150).

Several studies have clarified the oncogenic role of miR-21 in DLBCL, showing that it promotes tumor development partly by suppressing FOXO1 and activating the PI3K/AKT pathway. PTEN, a key tumor suppressor and negative regulator of PI3K/AKT signaling, is also a direct target of miR-21(151). FOXO1 regulates tumor-suppressive genes such as p27, p21, FasL, and Bim, and its activity is inhibited when AKT-mediated phosphorylation drives FOXO1 from the nucleus to the cytoplasm for proteasomal degradation (152, 153). Expression analyses in DLBCL cell lines showed that miR-21, FOXO1, and PTEN levels are closely linked (154).

miR-21 has been implicated in tumor progression by downregulating tumor suppressor genes such as PDCD4, PTEN, and TPM1 (155). Elevated miR-21 levels have been observed in hepatocellular carcinoma tissues, in the cerebrospinal fluid of primary CNS

lymphoma patients, and in the serum of DLBCL patients (125, 156, 157). Moreover, high miR-21 levels have been associated with poorer overall survival, and multivariate analyses have identified miR-21 as an independent prognostic marker in DLBCL (154, 158). Similar findings have been reported in primary CNS lymphoma and cutaneous T-cell lymphoma (159, 160). These observations underscore the role of miR-21 as a key contributor to lymphoma development and progression.

4.3. miR-34

The miR-34 family, comprising miR-34a and the miR-34b/c cluster, is closely linked to p53 activity, as these microRNAs function downstream of the p53 tumor-suppressor pathway. Loss of miR-34 expression has been associated with reduced sensitivity to p53-dependent apoptotic responses induced by chemotherapeutic agents (161, 162). In DLBCL models, suppression of c-MYC has been shown to upregulate miR-34a, decrease FOXP1 expression, and trigger apoptosis DLBCL cells (163). Additionally, miR-34 family members modulate the PI3K/AKT signaling pathway, a central regulator of cell growth and survival, thereby inhibiting proliferation and promoting apoptosis through the repression of key pathway components (164).

miR-125a-5p and miR-34a-5p have been proposed as predictive biomarkers of Richter syndrome, the transformation of CLL into aggressive large-cell lymphoma. Notably, elevated miR-125a-5p or reduced miR-34a-5p can predict nearly 50% of Richter syndrome cases up to 0.5–5 years before clinical onset, offering clinicians valuable guidance for early therapeutic decision-making (165) . In a study of DLBCL cases investigators found that the co-occurrence of TP53 mutations and miR-34a methylation was associated with a significantly poorer prognosis, with a median survival of 9.4 months, whereas single alterations in TP53 or miR-34A/B/C promoter methylation did not impact survival (166). miR-34a expression is also significantly reduced in MALT lymphoma and DLBCL compared with normal tissues, and low miR-34a levels along with increased FOXP1, p53, and BCL2 coexpression—correlated with adverse prognosis (167). On the other hand, higher miR-34a expression has been linked to improved overall survival and increased sensitivity to doxorubicin in DLBCL (168).

The ability of miR-34 family members to modulate gene expression and regulate key cellular processes,including proliferation, apoptosis, and metastasis has positioned them as

compelling candidates for the development of targeted cancer therapeutics. Their broad influence on oncogenic signaling networks underscores their potential utility in the treatment of lymphomas and other malignancies (164).

4.4. miR-17-92 cluster

The miR-17-92 cluster is a polycistronic microRNA unit composed of miR-17, miR-18a, miR-19a, miR-19b, miR-20a, and miR-92a. This cluster is frequently overexpressed in a range of solid tumors and lymphoid malignancies. Functionally, miR-17-92 contributes to oncogenesis by suppressing key tumor suppressors, including PTEN and the pro-apoptotic protein Bim (93). Often referred to as “oncomiR-1”, this cluster plays a central role in regulating cell-cycle progression, proliferation, apoptosis, and other fundamental cellular processes (169). The transcription factor MYC activates the expression of miR-17-92 by directly binding to its genomic locus (170). In addition, miR-17–92 modulates the expression of critical cell-cycle regulators such as E2F1, and in some contexts, E2F2 and E2F3, thereby further influencing proliferative and survival signaling pathways (169).

Among the molecular subtypes of DLBCL identified through gene-expression profiling (GEP), GCB-DLBCL is characterized by elevated levels of miR-17-92 and frequent amplification of the 13q31.3 region, where the miR-17-92 locus resides (170, 171). Expression profiling of the miR-17–92 cluster has emerged as a valuable diagnostic tool, offering potential for distinguishing germinal center–derived DLBCL (GC-DLBCL) from high-grade follicular lymphoma (FL), as this cluster is typically overexpressed in DLBCL relative to FL (172). Increasing attention has also been directed toward the prognostic relevance of individual members of the miR-17–92 cluster. For example, elevated miR-18a expression has been associated with significantly reduced overall survival in patients with DLBCL undergoing R-CHOP therapy (173).

Similarly, overexpression of miR-18a, miR-19a, and miR-92a correlates with markedly poorer overall survival in individuals with B-cell non-Hodgkin lymphoma, including those with DLBCL, while increased levels of miR-19a and miR-92a are additionally linked to decreased event-free survival (EFS) (174). Collectively, these findings underscore the potential importance of the miR-17-92 cluster as both a diagnostic discriminator and a prognostic biomarker across B-cell lymphomas.

4.5. miR-142

miR-142 represents the only human miRNA gene currently known to undergo recurrent somatic mutation in a substantial subset of DLBCL, with alterations reported in approximately 20% of cases. Notably, these mutations frequently localize to the seed regions of both miR-142-3p and miR-142-5p—the short, evolutionarily conserved nucleotide sequences that are indispensable for precise target mRNA recognition. Because the seed sequence plays a central role in directing miRNA–mRNA interactions, mutations within this domain have the potential to profoundly alter the regulatory capacity of the mature miRNAs. Such alterations may disrupt normal post-transcriptional gene silencing, generate aberrant target specificities, and ultimately contribute to the dysregulation of signaling pathways implicated in lymphomagenesis (175). Both previously established and newly discovered targets of miR-142 include genes such as CFL2, CLIC4, STAU1, TWF1, AKT1S1, CCNB1 (Cyclin B1), LIMA1, and TFRC. These downstream effectors participate in key biological processes—including regulation of the cell cycle, control of cytoskeletal dynamics, and maintenance of iron homeostasis—all of which have significant potential to modulate tumor development and malignant phenotypes (176). In a comprehensive pan-cancer assessment, Urbanek-Trzeciak and colleagues analyzed publicly accessible datasets from The Cancer Genome Atlas (TCGA) and determined that miR-142 in DLBCL exhibits the highest mutation frequency among all hypermutated miRNAs across the surveyed cancer types, reaching nearly 22% (177). Loss of miR-142 expression in DLBCL cell lines results in widespread proteomic alterations, characterized by increased abundance of proteins that facilitate oncogenic processes and, conversely, reduced levels of proteins essential for mounting an effective immune response. Notably, components of the major histocompatibility complex class I (MHC-I) antigen presentation pathway are among the downregulated factors, suggesting that miR-142 deletion may simultaneously enhance tumor-promoting mechanisms and impair immune surveillance (176). Both miR-142-3p and miR-142-5p are highly expressed in DLBCL, but mutations can lead to loss of function and altered regulation of cellular pathways (175, 178, 179) **(Figure 7)**. Although miR-142 is one of the most B-cell-enriched and frequently mutated miRNAs in DLBCL, current clinical studies have not consistently demonstrated that its expression alone has independent prognostic value (180).

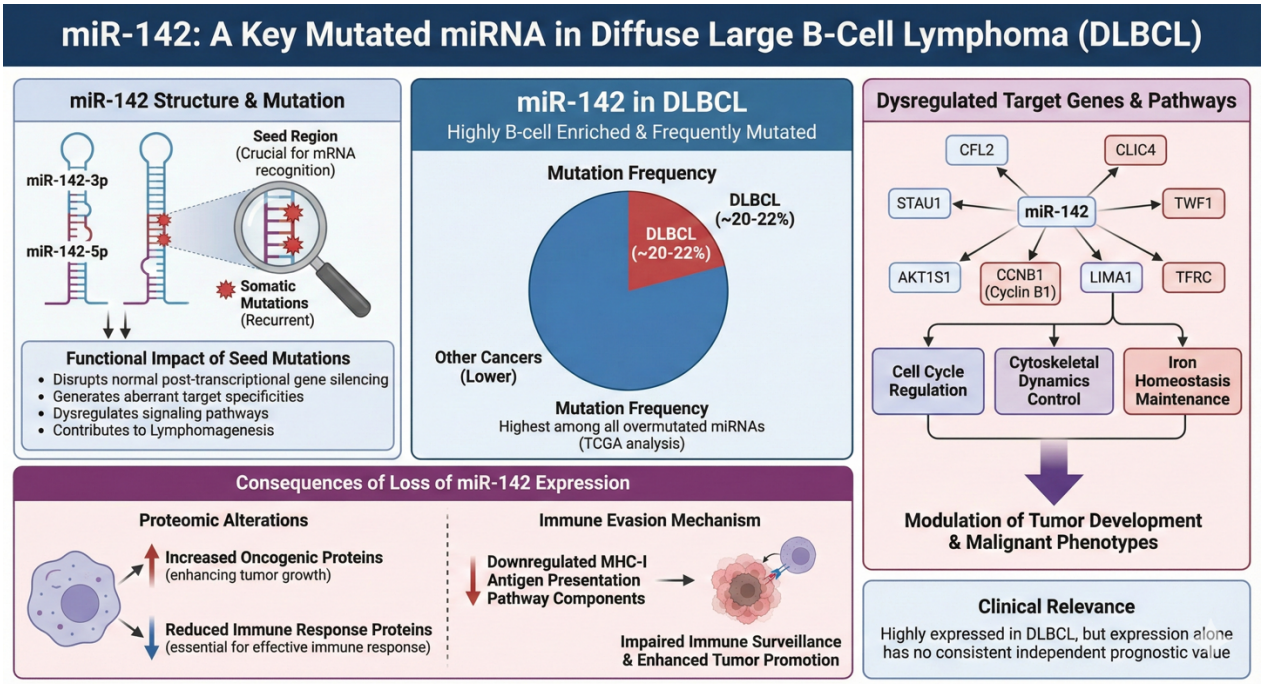

Figure 7. *miR-142, A key mutated miRNA in DLBCL. (Created with Gemini)*

4.6. miR-22

Another microRNA of relevance is miR-22, which has gained attention as a modulator of therapeutic responses in DLBCL. Its intracellular levels and extracellular release appear to change in accordance with R-CHOP administration, with increases observed after treatment that may indicate shifts in tumor cell behavior or reflect the biological impact and effectiveness of therapy (181). At a mechanistic level, miR-22 modulates several critical signaling networks, including components of the p53 pathway. It exerts its effects by targeting cell-cycle-associated genes such as CDK6 and CDKN1A (p21), thereby affecting cell-cycle arrest and the induction of apoptotic responses (181, 182). Furthermore, miR-22 influences the c-MYC/MYCBP axis as well as the PTEN–AKT signaling pathway, underscoring its broader involvement in regulating cell survival and proliferative capacity. Collectively, these observations point to miR-22 as a potential indicator of therapeutic responsiveness and as a plausible target for intervention, particularly in DLBCL molecular subtypes that exhibit elevated miR-22 expression (182). In DLBCL, high serum miR-22 expression was associated with adverse prognostic outcomes (182). These observations were substantiated by a more recent investigation in which miR-22 was identified as a serum-based predictor of adverse clinical outcome and therapeutic response in DLBCL. In particular, elevated circulating miR-

22 levels were associated with shorter PFS and with poor response to R-CHOP therapy (181) **(Figure 8).**

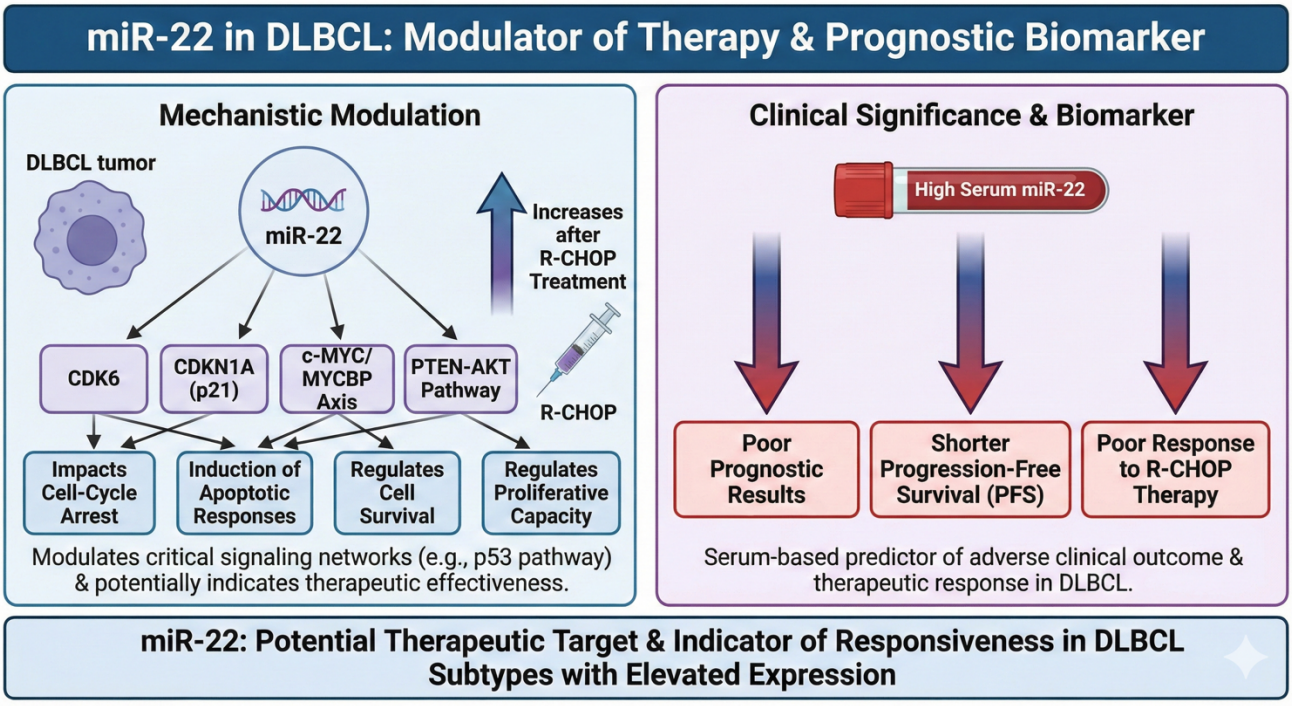

Figure 8.*miR-22 and its role in DLBCL. (Created with Gemini)*

4.7. Other important miRNAs in DLBCL

Additional miRNAs are increasingly recognized as important contributors to DLBCL pathogenesis and as markers of prognostic significance, owing to their influence on critical signaling pathways that regulate lymphoma cell survival, proliferation, and treatment response.

In DLBCL, miR-181a acts as a tumor suppressor, particularly in the ABC subtype. Its expression is lower in ABC-like tumors than in GCB cases, and restoring miR-181a inhibits growth, promotes apoptosis, and suppresses NF-κB signaling by targeting CARD11, IκBα, p105/p50, and C-Rel (183, 184). Together, these observations position miR-181a as a key modulator of NF-κB activity and underscore its potential as both a prognostic biomarker and a therapeutic target in DLBCL, particularly within the ABC-like subtype (185).

miR-144 also functions as a tumor suppressor in DLBCL, with its expression markedly reduced in both patient tissues and cell lines relative to normal controls. This diminished expression is linked to enhanced tumor cell proliferation and invasiveness. Mechanistically,

miR-144 directly targets and suppresses BCL6, a key transcriptional regulator that drives DLBCL progression (186), thereby limiting the proliferative and invasive capacity of DLBCL cells (185). However, its value as an independent diagnostic biomarker in DLBCL has not yet been firmly established and will require validation in larger, well-designed clinical studies. Collectively, the available evidence highlights miR-144 as an important upstream regulator of BCL6 and positions it as a promising candidate for therapeutic targeting in DLBCL, given its influence on tumor cell growth and invasiveness (180, 186).

5. miRNAs and their role in TME in DLBCL

Another key element in the pathobiology of DLBCL, with emerging prognostic and predictive relevance, is the tumor microenvironment (TME) (187). The TME represents a highly complex and dynamic network of cellular and acellular components that both surround and interact with the tumor, thereby influencing its initiation, maintenance, and progression (187, 188, 189). It encompasses an immune compartment—including T and B lymphocytes, natural killer (NK) cells, tumor-associated macrophages (TAMs), neutrophils, myeloid-derived suppressor cells (MDSCs), and dendritic cells (DCs)—as well as a non-immune compartment composed of cancer-associated fibroblasts (CAFs), the extracellular matrix (ECM), mesenchymal stromal cells, and a range of secreted factors such as cytokines and chemokines (189, 190) (**Figure 9**).

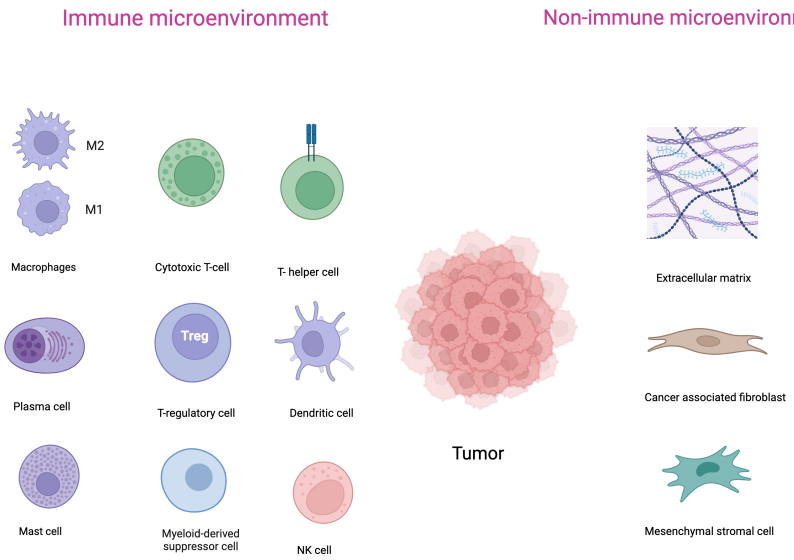

Figure 9. *Components of immune and non-immune microenvironment in DLBCL.*

miRNAs are central to DLBCL pathogenesis through their regulation of oncogenic pathways and their impact on the TME. Specifically, miR-155 plays a pivotal role not only in promoting tumor progression but also in actively shaping the TME, where it influences the behavior of both malignant cells and surrounding stromal and immune cell populations. Through these combined effects, miR-155 contributes to the establishment of a microenvironment that supports lymphoma growth, survival, and immune evasion (134). Moreover, in response to danger signals—such as pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) like IFN-β—macrophages and dendritic cells (DCs) exhibit increased expression of miR-155. This upregulation also influences the behavior of myeloid-derived suppressor cells (MDSCs), a group of immature myeloid cells known for their potent immunosuppressive functions (134, 191). Importantly, the accumulation of MDSCs within the tumor microenvironment is commonly associated with poor clinical outcomes (192). Furthermore, elevated miR-155 expression is associated with decreased peripheral $CD8^+$ T-cell levels and disruption of T-cell receptor signaling. In addition, miR-155 upregulates PD-L1 on lymphoma cells, thereby facilitating immune evasion by suppressing $CD8^+$ T-cell activity through the PD-1/PD-L1 pathway. Therapeutically, blockade of PD-L1 can counteract this effect, restoring $CD8^+$ T-cell function and inhibiting tumor growth, with particularly notable benefit in Epstein-Barr Virus (EBV)-associated DLBCL (129). miR155 also influences cytokine production and cellular migration within the TME. For example, miR-155 targets DEPTOR, thereby modulating pro-inflammatory cytokine expression and enhancing the migratory capacity of DLBCL cells (193).

miR-21 has emerged as a critical regulator of B-cell lymphoma biology, where it substantially contributes to disease progression. Experimental models demonstrate that enforced overexpression of miR-21 can drive the transformation of normal pre-B cells into a malignant, lymphoma-like state, underscoring its potent oncogenic capacity within early B-cell developmental stages (194, 195). From a clinical perspective, increased circulating miR-21 levels in the serum of patients with DLBCL closely mirror its expression within tumor tissues and are consistently associated with more advanced disease at diagnosis. Moreover, these elevated serum concentrations have been correlated with inferior overall survival, highlighting miR-21 as a clinically meaningful indicator of both tumor burden and prognosis (158, 196). An altered or dysfunctional tumor microenvironment plays a decisive role in shaping the chemosensitivity of cancer cells (197). Forced overexpression of miR-21 has been

shown to markedly increase chemoresistance in B-lymphoma cells, with this effect becoming even more pronounced when the cells are co-cultured with immune and endothelial components of the tumor microenvironment. These observations align with earlier findings indicating that miR-21 promotes the adhesion of myeloma cells to bone marrow stromal cells, creating a supportive niche that contributes to resistance against chemotherapy. (198). Collectively, these results underscore miR-21 as a critical mediator linking microenvironmental interactions to drug resistance in hematologic malignancies.

Several additional miRNAs help coordinate the complex cellular interactions within the DLBCL TME, shaping immune cell activity, driving tumor progression, and modulating therapeutic responses. Among these, miR-142-5p is notably upregulated in immunosuppressive TAMs in DLBCL. Functional inhibition of miR-142-5p has been shown to reprogram these macrophages toward a more pro-inflammatory, anti-tumor phenotype, thereby enhancing antibody-dependent cellular phagocytosis and improving the efficacy of immunotherapeutic strategies. Clinically, elevated levels of miR-142-5p, together with an increased abundance of $CD206^{+}$ TAMs, are associated with poorer responses to immunochemotherapy, underscoring their relevance as potential biomarkers of treatment outcome (199). Furthermore, a prognostic model based on four circulating miRNAs (miR-21, miR-130b, miR-155, and miR-28) has been associated with increased levels of MDSCs and Th17 cells within the DLBCL TME. The expansion of these immunosuppressive cell populations contributes to weakened anti-tumor immunity and is associated with poorer clinical outcomes. These miRNAs not only participate in regulating key oncogenic signaling pathways, including Ras signaling, but also play an important role in shaping the immune landscape of DLBCL by influencing the balance and function of various immune cell subsets within the TME (200). Overall, targeting dysregulated miRNAs within the TME, such as by suppressing miR-155 or miR-142-5p, represents a promising therapeutic approach for strengthening anti-tumor immunity and enhancing the effectiveness of immunotherapy in DLBCL (129, 199, 201).

6. Aim of the Study

As is evident from the introductory section, LBCLs—and DLBCL in particular—represent aggressive malignancies for which there is an ongoing effort to refine molecular classification systems, develop more effective therapeutic strategies, and improve prognostic assessment through the identification of reliable biomarkers. Among the various molecular candidates under investigation, miRNAs have garnered considerable attention due to their biological stability and their well-documented involvement in the pathogenesis of lymphoid neoplasms. In this context, we selected three specific miRNAs for evaluation, based on evidence from the existing literature. The study focuses on **miR-155-3p** and **miR-22-3p**, both of which have been associated with adverse clinical outcomes when overexpressed, as well as **miR-142-3p**, one of the most frequently mutated and dysregulated miRNAS in DLBCL. Collectively, these miRNAs represent biologically and clinically relevant candidates for further exploration in the setting of lymphoma.

The objective of this study was to evaluate the expression levels of miR-155-3p, miR-142-3p, and miR-22-3p in serum samples collected at diagnosis and to investigate whether these circulating miRNAs correlate with clinical and biological parameters as well as with overall survival (OS), thereby exploring their potential prognostic significance in patients with LBCLs.

7. Materials and Methods

The study was conducted in the Department of Hematology at the University Hospital of Ioannina, Greece. All participants provided written informed consent prior to enrollment. The research adhered to the principles of the Declaration of Helsinki, and the study protocol received approval from the Hospital's Scientific and Bioethics Committee. Patients with a newly diagnosed, treatment-naïve LBCL were eligible for inclusion.

Serum samples were collected from 45 patients with LBCL and 26 healthy controls. Although plasma is frequently considered the preferred matrix for circulating miRNA analysis because it avoids coagulation-related artifacts, emerging evidence suggests that serum-based miRNA measurements may offer greater temporal stability (202). In particular, it has been reported that no significant time-dependent changes have been observed in serum levels across a large panel of miRNAs, indicating that serum may provide more stable and reproducible miRNA measurements compared with plasma (202). This stability has been attributed to the relative resistance of serum miRNAs to pre-analytical variability once coagulation has occurred, provided that sample handling is standardized. Therefore, while plasma may reduce confounding related to cellular activation during clotting, serum remains a valid and reliable biological matrix for circulating miRNA analysis when pre-analytical variables are carefully controlled and consistently applied across study groups (202).

Total RNA was isolated using the miRNeasy Serum/Plasma Kit (QIAGEN, Cat. No. 217184). Complementary DNA (cDNA) synthesis was performed with the TaqMan Advanced miRNA cDNA Synthesis Kit (Applied Biosystems, Cat. No. A28007). Subsequently, quantitative real-time polymerase chain reaction (qRT-PCR) was carried out on the LightCycler 480 system (Roche). Detection of miR-155-3p (477926_mir), miR-142-3p (477910_mir), and miR-22-3p (477985_mir) was performed using the respective TaqMan™ Advanced miRNA assays (Applied Biosystems, Cat. No. A25576). An exogenous spike-in control, ath- miR-159 from the eukaryote *Arabidopsis thaliana* was used as a reference for normalization.

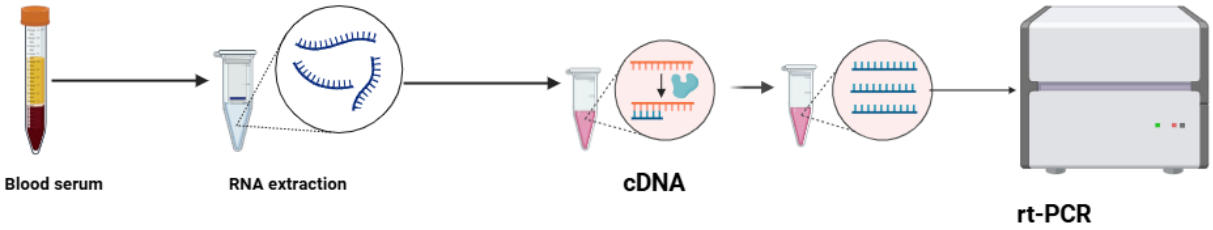

Figure 10. *All protocols that were conducted miRNeasy Serum/Plasma Kit (QIAGEN, Cat. No. 217184). Created with BioRender.*

Below, all protocols are described from the phase of isolation up to the extraction of Real-Time PCR results. RNA isolation using the miRNeasy Serum/Plasma (QIAGEN, Cat. No. 217184). Total RNA Isolation Kit.

Analysis of the RNA isolation process from serum:

1. Prepare serum or plasma or thaw frozen samples.

2. Add 5 volumes QIAzol Lysis Reagent. Mix by vortexing or pipetting up and down.

Serum/plasma (µl)	Protocol step 2: QIAzol Lysis Reagent(µl)	Protocol step 4:chloroform (µl)	Protocol step 6: approx. volume ofupper aqueous phase (µl)	Protocol step 7: 100% ethanol (µl)
≤50	250	50	150	225
100	500	100	300	450
200	1000	200	600	900

Table 1. *Volumes of each reagent that was used for the RNA extraction.*

In this circumstance, 200 µl of serum were used, 1000 µl of QIAzol Lysis Reagent, 200 µl of chloroform, 600 µl of approx. volume of upper aqueous phase and 900 µl of 100% ethanol respectively.

3. Place the tube containing the lysate on the bench top at room temperature (15–25°C) for 5 min. At this point 2 µl of the exogenous spike-in control miR-159 from the eukaryote *Arabidopsis thaliana* was added in each sample. The spike-in control miRNA from Arabidopsis thaliana is added to samples because it serves as an exogenous control that is not naturally present in the specimen. Its detection ensures that the RNA isolation and RT-qPCR processes have worked efficiently. It also allows normalization across different samples, helping to

correct for RNA losses or variability in the reactions. Furthermore, if the spike-in is not detected, it indicates potential technical issues at any stage, such as RNA extraction or cDNA synthesis. In summary, it acts as an internal quality control to ensure the reliability of the results.

4. Add chloroform of an equal volume to the starting sample to the tube containing the lysate and cap it securely. Vortex or shake vigorously for 15 s. Thorough mixing is important for subsequent phase separation.

5. Place the tube containing the lysate on the bench top at room temperature for 2–3 min.

6. Centrifuge for 15 min at 12,000 x g at 4°C. After centrifugation, heat the centrifuge up to room temperature if the same centrifuge will be used for the next centrifugation steps. After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase. See previous Table for the approximate volume of the aqueous phase.

7. Transfer the upper aqueous phase to a new collection tube. Add 1.5 volumes of 100% ethanol and mix thoroughly by

pipetting up and down several times. Do not centrifuge. Continue without delay with step 9. A precipitate may form after addition of ethanol, but this will not affect the procedure.

8. Pipet up to 700 µl of the sample, including any precipitate that may have formed, into an RNeasy MinElute spin column in a 2 ml collection tube. Close the lid gently and centrifuge at 8000 x g (10,000 rpm) for 15 s at room temperature. Discard the flow-through. Reuse the collection tube in step 9.

9. Repeat step 8 using the remainder of the sample. Discard the flow-through. Reuse the collection tube in step 10.

10. Add 700 µl Buffer RWT to the RNeasy MinElute spin column. Close the lid gently and centrifuge for 15 s at 8000 x g (10,000 rpm) to wash the column. Discard the flow-through.

11. Pipet 500 µl Buffer RPE onto the RNeasy MinElute spin column. Close the lid gently and centrifuge for 15 s at 8000 x g (10,000 rpm) to wash the column. Discard the flow-through.

12. Pipet 500 µl of 80% ethanol onto the RNeasy MinElute spin column. Close the lid gently and centrifuge for 2 min at 8000 x g (10,000 rpm) to wash the spin column membrane. Discard the collection tube with the flow-through.

13. Place the RNeasy MinElute spin column into a new 2 ml collection tube. Open the lid of the spin column, and centrifuge at full speed for 5 min to dry the membrane. Discard the

collection tube with the flow-through. To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise). It is important to dry the spin column membrane because residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.

14. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.

As little as 10 µl RNase-free water can be used for elution if a higher RNA concentration is required, but the yield will be reduced by approximately 20%. Do not elute with less than 10 µl RNase-free water, as the spin column membrane will not be sufficiently hydrated.

The dead volume of the RNeasy MinElute spin column is 2 µl: elution with 14 µl RNase- free water results in a 12 µl eluate.

15. Storage at -80 ºC.

cDNA Synthesis – Reverse Transcription PCR using the TaqMan Advanced miRNA cDNA Synthesis Kit

cDNA synthesis was performed using the *TaqMan Advanced miRNA cDNA Synthesis Kit* (Applied Biosystems), according to the manufacturer's instructions. The resulting cDNA was subsequently used as a template for real-time PCR. All kit reagents were stored at –20 °C, with the exception of the miR-Amp Master Mix, which was maintained at 2–4 °C.

Thermocycler Programming

Prior to the synthesis procedure, the PCR instrument was programmed with the following cycling conditions:

- **Polyadenylation:** 37 °C for 45 min; 65 °C for 10 min; hold at 4 °C
- **Adaptor Ligation:** 16 °C for 60 min; hold at 4 °C
- **Reverse Transcription:** 42 °C for 15 min; 85 °C for 5 min; hold at 4 °C
- **miRNA Amplification:** enzyme activation at 95 °C for 5 min; denaturation at 95 °C for 3 min; annealing/extension at 60 °C for 30 sec, repeated for 14 cycles; final step at 99 °C for 10 min; hold at 4 °C

Sample Preparation

RNA sample concentrations were adjusted to ≤5 ng/µL, with a final input volume of 2 µL per reaction. RNA concentration and purity were determined spectrophotometrically, followed by dilution as required.

cDNA Synthesis Workflow

The described sequential steps ensured efficient polyadenylation, adaptor ligation, reverse transcription, and pre-amplification of miRNAs, generating high-quality cDNA for subsequent quantitative PCR analysis.

Poly(A)-tailing Reaction

Initially, the addition of the Poly(A)-tail to the total miRNA was performed.

More specifically, the steps of the reaction were as follows:

1. Vortexing for mixing and spin down of the samples and reagents.
2. Preparation of the Poly(A) Reaction Mix according to the instructions in the table below.

Materials	**Volume per sample**
10x Poly(A) Buffer	0,5 µl
ATP	0,5 µl
Poly(A) Enzyme	0,3 µl
RNase free water	1,7 µl
Total Poly(A) Reaction Mix	3 µl

Table 2. *Volumes of each reagent of the reaction.*

3. Vortex and spin down the Poly(A) Reaction Mix.
4. Add 2 µL of each sample and 3 µL of the Poly(A) Reaction Mix into the reaction Eppendorf tube.

   *Total reaction volume per sample: 5 µL
5. Vortex and spin down. Place the Eppendorf tubes in the thermal cycler.

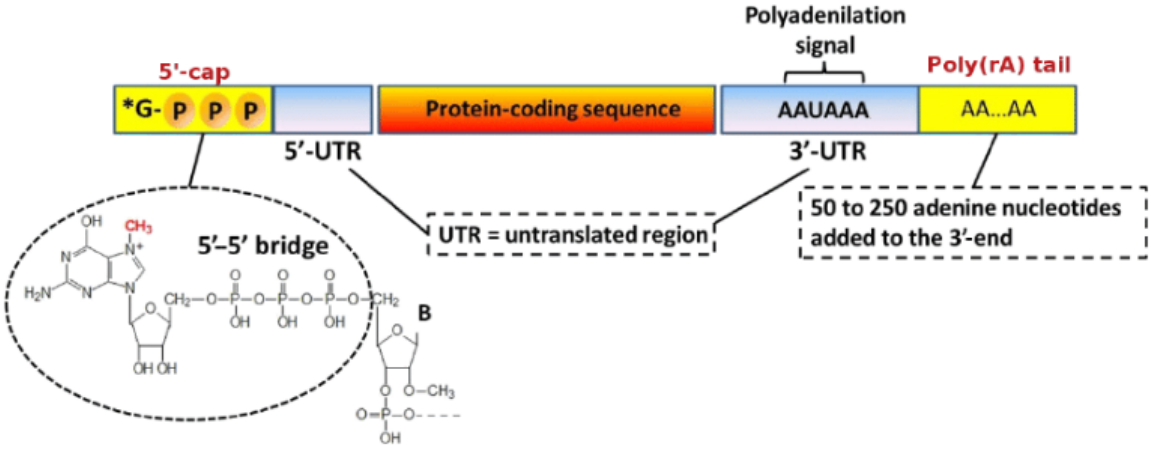

Figure 11. *Poly(A) tailing of miRNAs (Adapted from Roviello et al. 2015).*

Adaptor Ligation Reaction

After completion of the Poly(A)-tailing reaction, the adaptor ligation reaction was performed immediately. The adaptor served as the forward primer for the subsequent amplification of the cDNA.

More specifically, the steps of the reaction were as follows:

1. Preparation of the Ligation Reaction Mix according to the instructions of the table below.

Materials	**Volume per sample**
5x DNA Ligase Buffer	3 µl
50% PEG 8000	4,5 µl
25x Ligation Adaptor	0,6 µl
RNA Ligase	1,5 µl
RNase-free water	0,4 µl
Total Ligation Reaction Mix	10 µl

Table 3. *Volumes of each reagent of the reaction.*

2. Vortex and spin down the Ligation Reaction Mix.

3. Transfer 10 µl from the Ligation Reaction Mix into each Eppendorf tube from the previous Poly(A) reaction. The total volume of each sample is 15 µl.

4. Vortex and spin down. Place the Eppendorf tubes in the thermal cycler.

Reverse transcription reaction

After the adaptor ligation reaction, we proceed immediately with the reverse transcription reaction. A universal primer, which binds to the Poly-A tail, is required at this stage in order to enable the extension of all microRNAs.

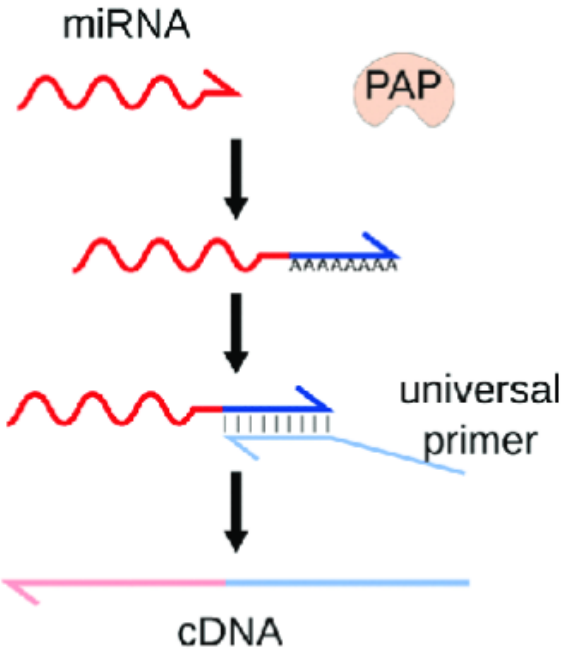

Figure 12. *Binding of the Universal RT primer to the Poly-A tail (Adapted from Jet et al. 2021).*

The cDNA obtained from the reaction can be used for the Real-time PCR reaction.

More specifically, the steps of the reaction were as follows:

Preparation of the Reverse Transcription Reaction Mix according to the instructions of the table below:

Materials	**Volume per sample**
5x RT Buffer	3 µl
dNTP Mix (5Mm)	4,5 µl
20x Universal RT Primer	0,6 µl
10x RT Enzyme Mix	1,5 µl
RNase free water	0,4 µl
Total RT Reaction Mix	10 µl

Table 4. *Volumes of each reagent of the reaction.*

2. Vortex and spin down the Reverse Transcription Reaction Mix.

3. Transfer 15 µl from the Reverse Transcription Reaction Mix into each Eppendorf tube from the previous reaction. The total volume of each sample was 30 µl.

4. Vortex and spin down. Place the Eppendorf tubes in the thermal cycler.

Amplification reaction

After the reverse transcription reaction, an amplification reaction was performed in order to obtain a greater amount of cDNA.

More specifically, the steps of the reaction were as follows:

Preparation of the miR-Amp Reaction Mix according to the instructions of the table below:

Materials	**Volume per sample**
2x miR Amp Master Mix	25 µl
20x miR Amp Primer Mix	2,5 µl
RNase-free water	17,5 µl
Total miR Amp Reaction Mix	45 µl

Table 5. *Volumes of each reagent of the reaction.*

2. Vortex and spin down the miR-Amp Reaction Mix.

3. Transfer 45 µl from the miR-Amp Reaction Mix into a new Eppendorf tube and add 5 µl from the Reverse Transcription reaction product. The total volume of each sample was 50 µl.

4. Vortex and spin down. Place the Eppendorf tubes in the thermal cycler.

Store the product at −20 °C (for more than two months).

Real-Time Polymerase Chain Reaction (Real-Time PCR)

The product of the reverse transcription reaction, which had been amplified, was subsequently used to assess the expression of the microRNA of interest using the Real-Time PCR method. The detection and quantification of miR-155-3p (477926_mir), miR-142-3p (477910_mir), and miR-22-3p (477985_mir) and ath-mir-159a were performed using TaqMan Advanced miRNA Assays, which contain a set of primers and probes necessary for analyzing microRNA expression levels. During PCR, the primers and probes bind to complementary sequences along the cDNA product clones. During polymerization, the DNA polymerase

cleaves only the probes that are bound to the target sequences. After the DNA polymerase passes, the dye is separated from the rest of the probe, becoming active and starting to fluoresce. As polymerization continues, the fluorescence increases. Polymerization of the clone continues, but since the 3' end of the probe is blocked, its extension does not occur during the PCR reaction.

Experimental procedure:

1. Add 5 µl of sample and 35 µl of DNase/RNase-free water.
2. Vortex and spin down in the PCR Master Mix.
3. Preparation of the Real-Time Reaction Mix according to the instructions of the table below:

Materials	**Volume per sample**
TaqMan Fast Advanced Master Mix (2x)	10 µl
TaqMan Advanced miRNA Assay (20x)	1 µl
RNase-free water	4 µl
Total PCR Reaction Mix	15 µl

Table 6. *Volumes of each reagent of the reaction.*

4. Vortex and spin down the RT-PCR Mix.
5. Transfer 15 µl of RT-PCR Mix and 5 µl of sample, for a total volume of 20 µl.
6. Mix and check to ensure there are no significant bubbles.
7. Place the plate in the machine.

The PCR cycles and temperatures are shown below:

Stage	**Temperature**	**Time**	**Cycles**
Enzyme Activation	95 °C	20 sec	1
Denaturation	95 °C	3 sec	40
Primer Annealing/Extension	60 °C	30 sec	

Table 7. *Conditions of each reaction of the PCR.*

At this point, it is useful to analyze some elements concerning the method of real-time polymerase chain reaction, in order to better understand the technique through which our measurements were obtained.

The difference between Real-time PCR and conventional quantitative PCR lies in the fact that, in the reaction, a fluorescent substance is added, which serves as a detector of the produced copies. The fluorescence intensity, which is proportional to the amount of the generated product, is recorded and allows the monitoring of amplification in real time. The number of cycles required to produce a detectable fluorescence signal is inversely proportional to the initial amount of DNA. During Real-time PCR, the fluorescence signal is continuously recorded throughout the performance of one or more PCR reactions over a series of cycles. It is a method of quantification with high accuracy, sensitivity, and reproducibility. The method is widely applied in studies of gene expression, in combination with reverse transcription. Of course, like any scientific method, Real-Time PCR also has certain limitations. Specifically, it requires beforehand the isolation of high-quality RNA and its precise conversion into cDNA. Moreover, errors that may arise due to human factors (such as unsuccessful primer design, incorrect handling of instruments and software, mistakes in dilutions, etc.) represent an important limitation of the method (203, 204).

The phases of Real-Time PCR are the same as those of conventional PCR. That is, we have the baseline phase, during which the increase in fluorescence intensity is not yet detectable, corresponding to approximately the first 18 cycles of the reaction. Subsequently, the amount of product generated becomes sufficient so that the fluorescence signal surpasses the baseline and becomes detectable. The cycle at which this occurs is called the quantification cycle or threshold cycle (Ct). The Ct value is measured during the second phase, the exponential phase, and can be used for the accurate determination of the initial amount of DNA present in the sample. The greater the initial amount of DNA in the sample, the earlier the signal becomes detectable and the lower the Ct value. After the exponential phase, the reaction enters the linear phase, during which the signal continues to increase, but no longer exponentially, as the products are generated at a slower rate. Finally, in the plateau phase, product amplification decreases sharply until the last cycle of the reaction (205).

As for the probe used, this was the TaqMan probe, as previously mentioned. It consists of a reporter dye molecule at the 5’ end and a quencher at the 3’ end, which selectively bind, through complementarity, to an internal region of the amplified sequence. When Taq

polymerase reaches the probe, it hydrolyzes it through its 5’ nuclease activity, thereby separating the reporter dye from the quencher. As a result, the dye begins to emit fluorescence. Probes of this category have the advantage of increasing the specificity of the results, since fluorescence is emitted only when the desired sequences are amplified, thus largely avoiding fluorescence from potential by-products (206, 207). This study was designed and reported in accordance with the REMARK (REporting recommendations for tumor MARKer prognostic studies) guidelines to ensure transparency and reproducibility of prognostic biomarker research (208).

7.1. Statistical analysis

For the statistical analysis, the Mann–Whitney U test was used to compare the serum expression levels of miR-142-3p, miR-155-3p, and miR-22-3p in patients with LBCLs with those of healthy controls. Percentile values of each miRNA’s expression were used to categorize samples as having high or low expression. Expression levels above the 75th percentile of healthy controls were defined as overexpression, whereas levels below the 25th percentile were defined as low expression.

Clinical and biological parameters, along with pathological findings from diagnostic biopsies of LBCL, were collected and correlated with the serum expression of miR-142-3p, miR-155-3p, and miR-22-3p. For statistical comparisons, the χ^2 test and the Mann–Whitney U test were applied to categorical and continuous variables, respectively. PFS was defined as the time from treatment initiation to documented disease progression or death from any cause, whichever occurred first. OS was defined as the time from treatment initiation to death from any cause or the date of last follow-up.

The Kaplan–Meier method was used to estimate OS, and survival curves were compared using the log-rank test in univariate analyses. Variables with p-values < 0.05, along with miR-142-3p, miR-155-3p, and miR-22-3p expression levels, were further assessed in multivariable analysis using a Cox proportional hazards model. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 29.0 for Windows (IBM Corp., Chicago, IL).

In addition, receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic accuracy of miR-155-3p, miR-142-3p, and miR-22-3p for discriminating LBCL patients (n=45) from healthy controls (n=26). The area under the curve (AUC) with 95% confidence interval (CI) was calculated using 2,000 bootstrap resamples. Optimal cutoff values were determined using Youden's J index (J = Sensitivity + Specificity - 1). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated at each optimal cutoff. For prognostic analysis, 2-year landmark ROC analysis was performed to assess the predictive value of each miRNA for PFS and OS. Patients were classified as experiencing an event if progression (PFS) or death (OS) occurred within 24 months; those event-free at 24 months were classified as non-events. Patients censored before 24 months were excluded, resulting in 30 evaluable patients for each outcome. Differences in miRNA expression between groups were compared using the Mann-Whitney U test. ROC curve analyses were performed using Python with scikit-learn and SciPy. A two-sided p-value <0.05 was considered statistically significant.

Given the heterogeneity of lymphoma subtypes in the initial cohort, which could introduce biological variability and potentially confound prognostic analyses, a separate analysis was performed after excluding patients with primary CNS lymphoma and PMBCL.

8. Results

8.1. Demographic Characteristics and miRNA Expression Compared with Healthy Controls

A total of 45 patients were enrolled in the present study, comprising 26 men (57.8%) and 19 women (42.2%). The median age was 67 years (mean: 65 years), reflecting the typical age distribution observed in LBCL. Of the enrolled participants, 41 were classified as having DLBCL not otherwise specified (NOS), two were diagnosed with primary CNS lymphoma, and two with primary mediastinal lymphoma. The median follow-up duration was 26 months. All patients received standard immunochemotherapy as initial treatment in accordance with established clinical guidelines. At the time of data collection, 13 patients (28.9%) had died, with 32 patients (71.1%) remaining alive. For comparative purposes, a control group of 26 healthy individuals was included, from whom serum samples were obtained.

Analysis of circulating miRNAs revealed that miR-155-3p and miR-142-3p expression did not differ significantly between patients and healthy controls, indicating no detectable dysregulation at diagnosis (**Table 8** and **Table 9**). In contrast, miR-22-3p was significantly overexpressed in patients with LBCL compared with controls ($p < 0.001$), suggesting a potential role for this miRNA in disease pathogenesis or as a diagnostic biomarker **(Table 10).**

miR-155-3p	Mean	N	Median
control	9.264841180332263	26	.087371735701375
patients	14.215544237874072	45	.273573425315185

Table 8. *The expression of miR-155-3p did not differ significantly between patients and controls (p = 0.41).*

miR-142-3p	Mean	N	Median
control	8.001322448233170	26	.033865270676152
patients	5.062798216970871	45	.000782294802334

Table 9. *The expression of miR-142-3p did not differ significantly between patients and controls (p = 0.245).*

miR-22-3p	Mean	N	Median
control	8.567220646316015	26	.009568275336775
patients	5.402456861351724	45	.817902058557782

Table 10. *The expression of miR-22-3p was significantly higher in patients compared with controls ($p < 0.001$).*

Receiver operating characteristic (ROC) analysis was performed to evaluate the diagnostic accuracy of miR-155-3p, miR-142-3p, and miR-22-3p for discriminating LBCL patients (n=45) from healthy controls (n=26) **(Figure 13)**.

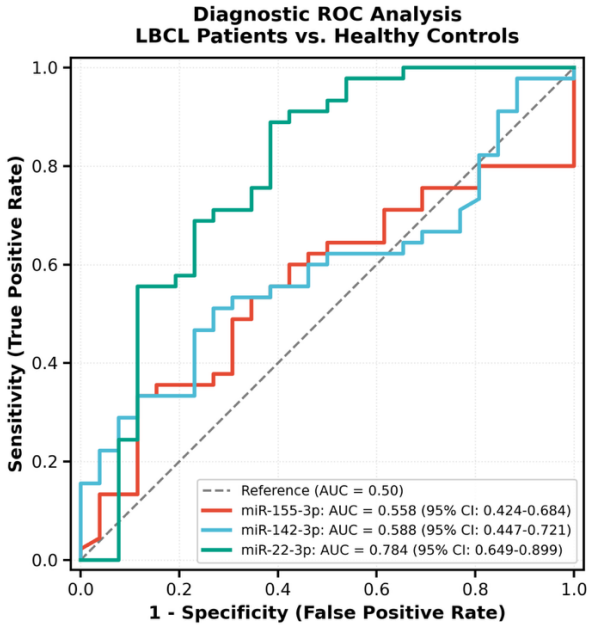

Figure 13. *Diagnostic ROC Analysis of miRNAs for LBCL Detection. Receiver operating characteristic curves for miR-155-3p, miR-142-3p, and miR-22-3p in discriminating LBCL patients (n=45) from healthy controls (n=26). miR-22-3p demonstrated the highest diagnostic accuracy (AUC=0.784, 95% CI: 0.649-0.899). The diagonal dashed line represents random classification (AUC=0.50).*

Among the three miRNAs, miR-22-3p demonstrated the best diagnostic performance with an area under the curve (AUC) of 0.784 (95% CI: 0.649-0.899), indicating acceptable discrimination. Higher miR-22-3p expression levels were observed in LBCL patients compared to controls (median 0.800 vs. 0.010, p=0.0001).Using Youden's J index to determine the optimal cutoff point (expression level ≥0.045), miR-22-3p achieved a sensitivity of 88.9% and specificity of 61.5%, with positive and negative predictive values of 80.0% and 76.2%,

respectively **(Figure 13)**. miR-142-3p showed modest diagnostic utility (AUC=0.588, 95% CI: 0.447-0.721), with notably lower expression in LBCL patients compared to controls (median 0.0007 vs. 0.034). At the optimal cutoff (≤0.0007), sensitivity was 51.1% and specificity was 73.1%. miR-155-3p demonstrated poor diagnostic discrimination (AUC=0.558, 95% CI: 0.424-0.684), with higher expression in patients but no statistically significant difference (p=0.424).

8.2. Clinical and biological characteristics of patients with newly diagnosed LBCL and their association with progression/death

In this cohort of newly diagnosed patients with LBCL, several clinical and biological variables were found to be significantly associated with both disease progression and mortality. Hypoalbuminemia (albumin <3.5 g/dL) emerged as a strong adverse prognostic indicator, occurring more frequently among patients who experienced progression or death (57.1% vs. 12.9%, p = 0.004) and among those who died compared with survivors (53.8% vs. 15.6%, p = 0.022). A high International Prognostic Index (IPI 3–5) demonstrated a consistent association with unfavorable outcomes, being present in all patients who progressed or died (vs. 61.3% of progression-free patients, p = 0.009) and likewise in all deceased patients (vs. 62.5% of progression-free patients, p = 0.010). The presence of B symptoms was also significantly correlated with both endpoints, occurring more frequently among patients who progressed or died (78.6% vs. 35.5%, p = 0.011) and among those who died (76.9% vs. 37.5%, p = 0.023). Moreover, neutropenic fever during treatment was markedly more common in patients with adverse outcomes, occurring in 42.9% of those who progressed or died versus 9.7% of event-free patients (p = 0.017), and in 46.2% of deceased patients versus 9.4% of survivors (p = 0.011), suggesting that treatment-related complications or heightened disease burden may contribute to poorer prognosis.

A borderline association was observed between decreased miR-142-3p expression and adverse outcomes, showing a trend toward significance for both progression or death (p = 0.051) and for mortality alone (p = 0.098), suggesting a potential biological role that warrants further investigation. In contrast, no significant associations were identified for age, sex, double-expressor phenotype, elevated LDH or β2-microglobulin, bulky or extranodal disease, hyponatremia, hypercalcemia, miR-155-3p overexpression, or increased miR-22-3p

expression in relation to either progression or mortality. The clinical and biological characteristics underlying these findings are presented in **Tables 11 and 12**.

Clinical and biological characteristics of patients with newly diagnosed LBCL and their association with progression or death			
Characteristic	**Progression free**	**Progression or death**	**p-value (χ^2)*or Fisher exact test**
Age at diagnosis >60	21/31 (67.7%)	10/14 (71.4%)	1.0
Male	16/31(51.6%)	10/14 (71.4%)	0.33
Double expression	7/30 (23.3%)	6/14 (42.9%)	0.33
Albumin<3.5 g/dL	4/31 (12.9%)	8/14 (57.1%)	**0.004**
Increased LDH	25/31 (80.6%)	14/14 (100%)	0.156
β2-microglobulin increased	14/31 (45.2%)	6/11 (54.5%)	0.854
IPI score 3-5	19/31 (61.3%)	14/14 (100%)	**0.009**
B symptoms	11/31 (35.5%)	11/14 (78.6%)	**0.011**
Bulky disease	14/31(45.2%)	8/13 (61.5%)	0.509
Neutropenic fever	3/31 (9.7%)	6/14 (42.9%)	**0.017**
Extranodal disease	19/31 (61.3%)	12/14 (85.7%)	0.165
Hyponatremia	4/31 (12.9%)	5/14 (35.7%)	0.111
Hypercalcemia	5/31 (16.1%)	1/14 (7.1%)	0.648
miR-155 overexpressed	9/31 (29%)	7/14 (50%)	0.306
Decreased miR-142-3p	11/31 (35.5%)	10/14 (71.4%)	0.051
Increased miR-22-3p	21/31 (67.7%)	8/14 (57.1%)	0.519

Table 11. *Clinical and biological characteristics of patients with newly diagnosed LBCL and their association with progression or death.*

Clinical and biological characteristics of patients with newly diagnosed LBCL and their association with death			
Characteristic	**Progression free**	**Death**	**p-value (χ^2)*or Fisher exact test**
Age at diagnosis >60	22/32 (68.8%)	9/13 (69.2%)	1.0
Male	17/32(53.1%)	9/13 (69.2%)	0.507
Double expression	8/31 (25.8%)	5/13 (38.5%)	0.478
Albumin<3.5 g/dL	5/32 (15.6%)	7/13 (53.8%)	**0.022**
Increased LDH	26/32 (81.3%)	13/13 (100%)	0.16
β2-microglobulin increased	14/32 (43.8%)	6/10 (60%)	0.477
IPI score 3-5	20/32 (62.5%)	13/13 (100%)	**0.01**
B symptoms	12/32 (37.5%)	10/13 (76.9%)	**0.023**
Bulky disease	14/32 (43.8%)	8/12 (66.7%)	0.31
Neutropenic fever	3/32 (9.4%)	6/13 (46.2%)	**0.011**
Extranodal disease	20/32 (62.5%)	11/13 (84.6%)	0.178
Hyponatremia	4/32 (12.5%)	5/13 (38.5%)	0.12
Hypercalcemia	4/32 (16.1%)	2/13 (15.4%)	1
Increased miR-155-3p	9/32 (28.1%)	7/13 (53.8%)	0.169
Decreased miR-142	12/32 (37.5%)	9/13 (69.2%)	0.098
Increased miR-22	21/32 (65.6%)	8/13 (61.5%)	1

Table 12. *Clinical and biological characteristics of patients with newly diagnosed LBCL and their association with death.*

8.3. Associations Between of specific miRNAs Expression and Key Clinical and Laboratory Variables

8.3.1. miR-155-3p overexpression

Clinical and biological characteristics of newly diagnosed LBCL patients were examined in relation to high miR-155-3p expression (**Table 13**). Overall, no statistically significant

differences were observed between patients with high versus low miR-155-3p expression across the majority of evaluated parameters. The proportion of patients aged >60 years did not differ substantially between groups (75.0% vs. 62.5%, p = 0.738), nor did sex, high BCL-2 or MYC expression, or double-expressor status (all p = 1.0). Transformation from low-grade lymphoma appeared less frequent among patients with high miR-155 expression (6.3% vs. 31.0%), although this difference did not reach statistical significance (p = 0.071).

Similarly, laboratory variables—including hypoalbuminemia (37.5% vs. 20.7%, p = 0.296), elevated LDH (93.8% vs. 82.8%, p = 0.399), and increased β2-microglobulin (60.0% vs. 40.7%, p = 0.382)—were comparable between groups. No significant differences were identified with respect to high IPI score (3–5), presence of B symptoms, bulky disease, neutropenic fever, extranodal involvement, or hypercalcemia (all p > 0.05). The prevalence of the non-GCB phenotype was nearly identical between groups (45.8% vs. 43.8%, p = 1.0). High miR-155-3p expression was also not associated with decreased miR-142-3p or increased miR-22-3p expression.

Hyponatremia was the only parameter to demonstrate a borderline association, occurring more frequently among patients with high miR-155-3p expression compared with those with lower espression (37.5% vs. 10.3%, p = 0.050), suggesting a potential relationship that warrants further evaluation in larger cohorts.

Clinical and biological features of newly diagnosed LBCL patients and their association with high miR-155-3p expression			
Characteristic	**miR-155 no high expressor**	**miR-155 high expressor**	**p-value (χ^2)*or Fisher exact test**
Age at diagnosis >60	19/29 (62.5%)	12/16 (75%)	0.738
Male	15/29 (51.7%)	11/16 (68.8%)	0.429
BCL-2 high expressor	24/28 (85.7%)	13/15 (86.7%)	1
MYC high expressor	9/28 (32.1%)	5/14 (35.7%)	1
Double expressor	9/29 (31%)	4/15 (30.8%)	1

Transformation from low grade lymphoma	9/29(31%)	1/16 (6.3%)	0.071
Albumin<3.5 g/dL	6/29 (20.7%)	6/16 (37.5%)	0.296
Non-GCB	11/24 (45.8%)	7/16 (43.8%)	1
Increased LDH	24/29 (82.8%)	15/16 (93.8%)	0.399
β2-microglobulin increased	11/27 (40.7%)	9/15 (60%)	0.382
IPI score 3-5	19/29 (65.5%)	14/16 (87.5%)	0.164
B symptoms	12/29 (41.4%)	10/16 (62.5%)	0.296
Bulky disease	14/29 (48.3%)	8/15 (53.3%)	1
Neutropenic fever	5/29 (17.2%)	4/16 (25%)	0.7
Extranodal disease	19/29 (65.5%)	12/16 (75%)	0.738
Hyponatremia	3/29 (10.3%)	6/16 (37.5%)	**0.05**
Hypercalcemia	4/29 (13.8%)	2/16 (12.5%)	1
Decreased miR-142	13/29 (44.8%)	8/16 (50%)	0.983
Increased miR-22	19/29 (65.5%)	10/16 (62.5%)	1

Table 13. *Clinical and biological features of newly diagnosed LBCL patients and their association with high miR-155 expression.*

8.3.2. miR-142-3p low expression

Clinical and biological features of newly diagnosed LBCL patients were analyzed to determine their association with low miR-142-3p expression (**Table 14**). Most variables did not differ significantly between patients with low versus non-low miR-142-3p expression. The distribution of patients aged >60 years and sex were similar across groups (both p = 1.0). Likewise, no significant differences were observed for high BCL-2 expression, history of transformation from a low-grade lymphoma, hypoalbuminemia, elevated LDH, increased β2-microglobulin, high IPI score (3–5), presence of B symptoms, bulky disease, extranodal involvement, hypercalcemia, or increased expression of miR-155-3p or miR-22-3p (all p > 0.05).

Two variables demonstrated statistically significant associations with low miR-142-3p expression. **High MYC expression was more frequent among patients with low miR-142-3p levels compared with those without low expression (52.6% vs. 17.4%, p = 0.023).**

Additionally, the non-GCB phenotype was significantly less common in the low miR-142-3p group (25.0% vs. 65.0%, p = 0.026), suggesting a potential molecular distinction between these subgroups.

Neutropenic fever and hyponatremia each approached significance, occurring more frequently among patients with low miR-142-3p expression (33.3% vs. 8.3%, p = 0.061; and 33.3% vs. 8.3%, p = 0.061, respectively), indicating possible clinical associations that merit further investigation in larger cohorts.

Clinical and biological features of newly diagnosed LBCL patients and their association with low miR-142-3p expression			
Characteristic	**miR-142 no low expressor**	**miR-142 low expressor**	**p-value (χ^2)*or Fisher exact test**
Age at diagnosis >60	17/24 (70.8%)	14/21 (66.7%)	1
Male	14/24 (58.3%)	12/21 (57.1%)	1
BCL-2 high expressor	21/23 (91.3%)	16/20 (80%)	0.393
MYC high expressor	4/23 (17.4%)	10/19 (52.6%)	**0.023**
Transformation from low grade lymphoma	5/23 (20.8%)	5/21 (23.8%)	1
Albumin<3.5 g/dL	6/24 (25%)	6/21 (28.6%)	1
Non-GCB	13/20 (65%)	5/20 (25%)	**0.026**
Increased LDH	22/24 (91.7%)	17/21 (81%)	0.396
β2-microglobulin increased	14/23 (60.9%)	6/19 (31.6%)	0.114
IPI score 3-5	16/24 (66.7%)	17/21 (87.5%)	0.329
B symptoms	10/24 (41.7%)	12/19 (57.1%)	0.461
Bulky disease	13/24 (54.2%)	9/20 (45%)	0.762
Neutropenic fever	2/24 (8.3%)	7/21 (33.3%)	0.061
Extranodal disease	17/24 (70.8%)	14/21 (66.7%)	1
Hyponatremia	2/24 (8.3%)	7/21 (33.3%)	0.061
Hypercalcemia	4/24 (16.7%)	2/21 (9.5%)	0.67

Increased miR-155-3p	8/24 (33.3%)	8/21 (38.1%)	0.983
Increased miR-22-3p	16/24 (66.7%)	13/21 (61.9%)	0.983

Table 14. *Clinical and biological features of newly diagnosed LBCL patients and their association with low miR-142 expression.*

8.3.3. miR-22 high expression

The clinical and biological characteristics of newly diagnosed LBCL patients were evaluated to determine their association with high miR-22-3p expression (Table 15). Overall, most variables showed no statistically significant differences between patients with high versus low miR-22-3p expression. Age at diagnosis 60 years appeared more frequent among patients with high miR-22-3p expression (79.3% vs. 50.0%), although this difference did not reach statistical significance ($p = 0.090$). Similarly, sex distribution, high BCL-2 and MYC expression, history of transformation from low-grade lymphoma, hypoalbuminemia, elevated LDH, increased β2-microglobulin, high IPI score (3–5), presence of B symptoms, bulky disease, neutropenic fever, extranodal involvement, hyponatremia, decreased miR-142-3p expression, and increased miR-155-3p expression were comparable between groups (all $p > 0.05$).

A statistically significant association was observed for the non-GCB phenotype, which was more common among patients with high miR-22-3p expression compared with those with low expression (57.7% vs. 21.4%, p = 0.046), suggesting a potential biological relationship between miR-22-3p **overexpression and non-GCB molecular features**. Hypercalcemia showed a trend toward higher prevalence in the high miR-22 group (20.7% vs. 0%, $p = 0.075$), although this difference did not achieve statistical significance.

Taken together, these findings indicate that high miR-22 expression is not broadly associated with most conventional clinical or laboratory parameters in LBCL but may correlate with specific biological characteristics, particularly the non-GCB phenotype.

Clinical and biological features of newly diagnosed LBCL patients and their association with high miR-22-3p expression			
Characteristic	**miR-22 no high expressor**	**miR-22 high expressor**	**p-value (χ^2)*or Fisher exact test**
Age at diagnosis >60	8/16 (50%)	23/29 (79.3%)	0.09
Male	11/16 (68.8%)	15/29 (51.7%)	0.429
BCL-2 high expressor	12/14 (85.7%)	25/29 (86.2%)	1
MYC high expressor	6/15 (40%)	8/19 (29.6%)	0.733
Transformation from low grade lymphoma	3/16(18.8%)	7/29 (24.1%)	1
Albumin<3.5 g/dL	6/16 (37.5%)	6/29 (20.7%)	0.385
Non-GCB	3/14 (21.4%)	15/26 (57.7%)	**0.046**
Increased LDH	14/16 (87.5%)	25/29 (86.2%)	1
β2-microglobulin increased	6/16 (37.5%)	14/26 (53.8%)	0.476
IPI score 3-5	11/16 (68.8%)	22/29 (75.9%)	0.869
B symptoms	7/16 (43.8%)	15/29 (51.7%)	0.841
Bulky disease	8/16 (50%)	14/28 (50%)	1
Neutropenic fever	3/16 (18.8%)	6/29 (20.7%)	1
Extranodal disease	9/16 (56.3%)	22/29 (75.9%)	0.306
Hyponatremia	3/16 (18.8%)	6/29 (20.7%)	1
Hypercalcemia	0/16 (0%)	6/29 (20.7%)	0.075
Decreased miR-142-3p	8/16 (50%)	13/19 (44.8%)	0.983
Increased miR-155-3p	6/16 (37.5%)	10/29 (34.5%)	1

Table 15. *Clinical and biological features of newly diagnosed LBCL patients and their association with high miR-22 expression.*

8.4. Progression-Free Survival According to miR-155, miR-142, and miR-22 Expression

PFS was evaluated in relation to the expression levels of miR-155-3p, miR-142-3p, and miR-22-3p in patients with newly diagnosed LBCL. Distinct differences were observed across the three miRNA expression groups.

For miR-155-3p, patients with low expression demonstrated a longer mean PFS of 42.57 months (SE 4.35; 95% CI: 34.05–51.09) compared with 21.48 months (SE 4.40; 95% CI: 12.86–30.10) in those with high miR-155-3p expression. Although this difference reflected a numerically shorter PFS in the high-expression group, it did not reach statistical significance ($p = 0.076$). Median PFS could not be estimated due to censoring (**Figure 14).**

In contrast, miR-142-3p expression was significantly associated with PFS outcomes. Patients with high miR-142-3p expression had a mean PFS of 47.29 months (SE 3.98; 95% CI: 39.49–55.09), whereas those with low expression exhibited a markedly shorter mean PFS of 23.01 months (SE 5.92; 95% CI: 11.40–34.62) ($p = 0.009$). Median PFS estimable only for the low miR-142-3p group (30.0 months; SE 12.21; 95% CI: 6.07–53.93), as the largest PFS time in the normal-expression group was censored **(Figure 15).**

Analysis of miR-22-3p expression did not demonstrate significant differences in PFS. The non-high expression group had a mean PFS of 35.53 months (SE 6.55; 95% CI: 22.68–48.37), compared with 37.69 months (SE 4.25; 95% CI: 29.36–46.03) in the high-expression group ($p = 0.508$). Median PFS could not be estimated for either subgroup due to censoring **(Figure 16).**

Overall, these findings indicate that low miR-142-3p **expression is significantly associated with inferior PFS, high miR-155**-3p **expression shows a non-significant trend toward worse PFS, and miR-22**-3p **expression does not appear to influence PFS in newly diagnosed LBCL patients.**

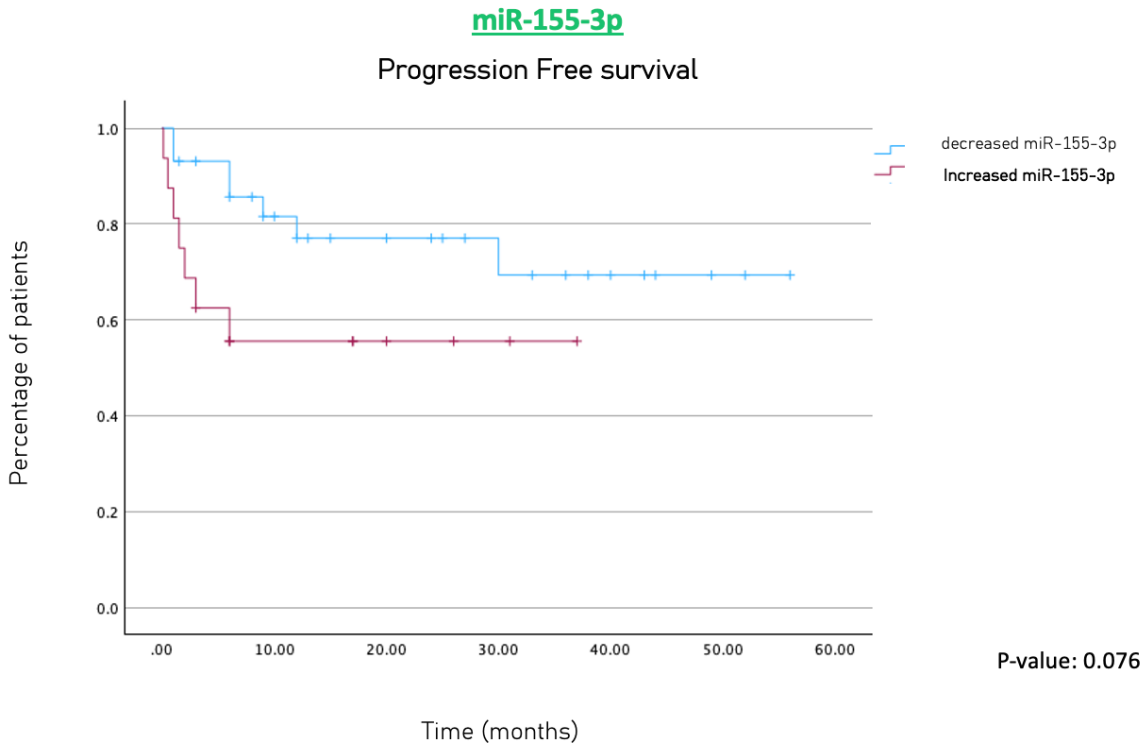

Figure 14. *miR-155-3p and PFS in LBCL.*

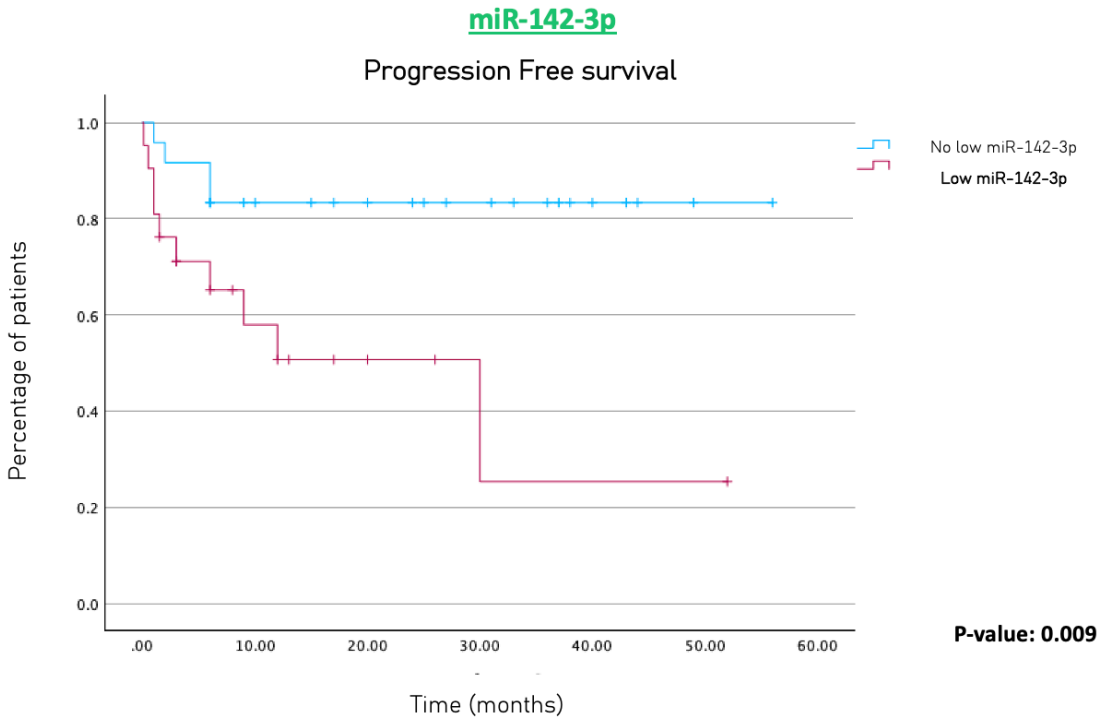

Figure 15. *miR-142-3p and PFS in LBCL.*

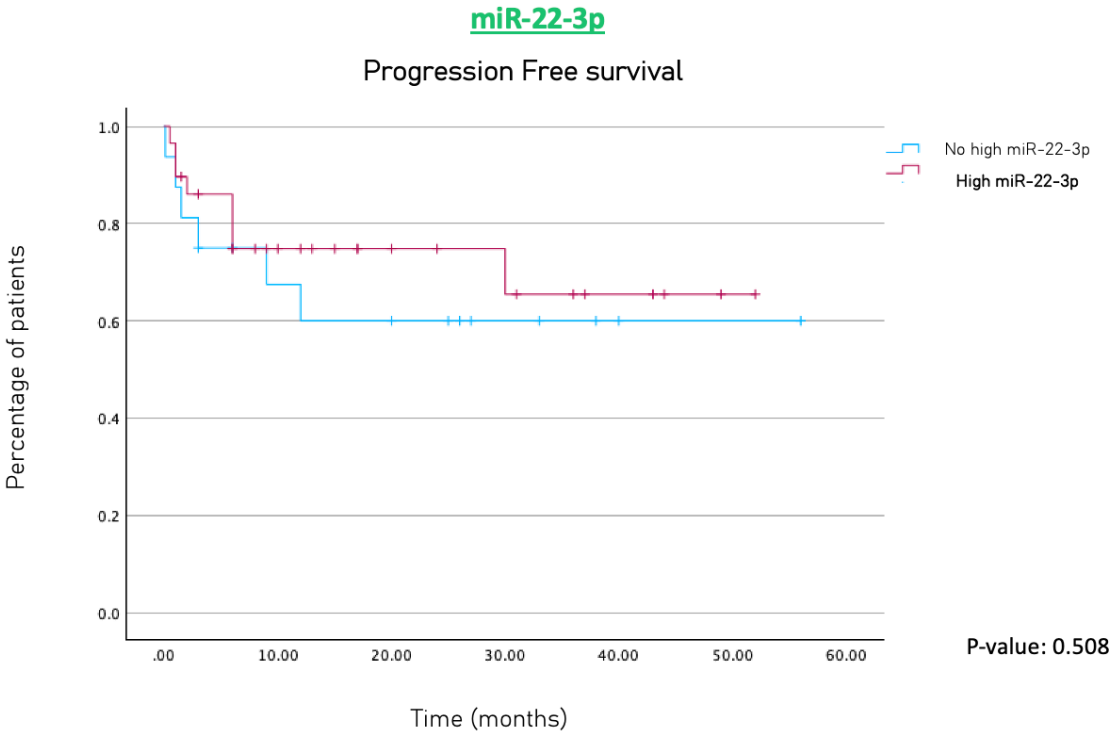

Figure 16. *miR-22-3p and PFS in LBCL.*

Univariate analysis was performed to evaluate the relationship between clinical and biological characteristics and 2-year PFS in patients with newly diagnosed LBCL (Table 16). Several factors were identified as significantly associated with differences in PFS. Hypoalbuminemia (albumin <3.5 g/dL) was strongly linked to inferior outcomes, with a 2-year PFS of 33% compared with 83% among patients with normal albumin levels ($p < 0.001$). A high International A high Prognostic Index (IPI 3–5) was likewise associated with reduced PFS, with arate of 56.8% versus 100% in patients with low-risk IPI scores (0–2) ($p = 0.009$). The presence of B symptoms was also associated with decreased PFS (52.9% vs 84.8%, $p = 0.005$). Additionally, patients who developed neutropenic fever exhibited a markedly lower 2-year PFS compared with those who did not (33% vs 78.9%, $p = 0.002$). Decreased miR-142-3p expression was similarly associated with inferior PFS, with a 2-year rate of 50.7% compared with 83.3% in patients without reduced expression ($p = 0.009$).

In contrast, several characteristics—including age at diagnosis >60 years, sex, elevated LDH, increased β2-microglobulin, bulky disease, extranodal involvement, bone marrow infiltration, hypercalcemia, and increased miR-22-3p expression—did not demonstrate statistically significant associations with PFS (all $p > 0.05$). Hyponatremia (44.4% vs 76%, p =

0.056) and increased miR-155-3p expression (55.6% vs 77%, p = 0.076) exhibited trends toward reduced PFS, although these did not reach statistical significance.

Taken together, univariate analysis identified hypoalbuminemia, high IPI, the presence of B symptoms, neutropenic fever, and decreased miR-142-3p expression as factors associated with significantly lower 2-year PFS in this patient cohort.

Univariate analysis PFS		
Characteristic	**2y-PFS (%)**	**p-value**
Age at diagnosis >60	69.9%	0.657
Male	59.5%	0.177
Albumin<3.5 g/dL	**33%**	**<0.001**
increased LDH	64.6%	0.129
β2-microglobulin increased	69.3	0.46
IPI score (3-5)	**56.8%**	**0.009**
B-symptoms	**52.9%**	**0.005**
Bulky disease	75%	0.66
Neutropenic fever	**33%**	**0.002**
Extranodal disease	62.3%	0.116
Bone marrow infiltration	60%	0.172
Hyponatremia	44.4%	0.056
Hypercalcemia	83.3%	0.498
Increased miR-155-3p	55.6%	0.076
Decreased miR-142-3p	**50.7%**	**0.009**
Increased miR-22-3p	74.8%	0.508

***Table 16**. Univariate analysis PFS*

Cox proportional hazards regression was performed to evaluate the independent association of selected clinical and molecular variables with PFS in patients with newly diagnosed LBCL (Table 17). Neutropenic fever was not included in the multivariable model because it represents a post-treatment event rather than a baseline prognostic characteristic. As an outcome that occurs after therapy initiation, it may act as an intermediate clinical event influenced by both disease biology and treatment toxicity, thereby introducing time-dependent bias and limiting its suitability for inclusion as an independent predictor in the Cox model. Low miR-142-3p expression was independently associated with an increased risk of disease progression, with a hazard ratio (HR) of 3.97 (95% CI: 1.18–13.3, p = 0.026). Hypoalbuminemia also retained statistical significance in the multivariable model, demonstrating an HR of 4.53 (95% CI: 1.46–14.02, p = 0.009), indicating a markedly elevated risk of progression among patients with albumin levels <3.5 g/dL.

The presence of B symptoms showed a trend toward an increased risk of progression (HR 2.95; 95% CI: 0.81–10.83), although this did not reach statistical significance (p = 0.102). High IPI score (3–5) did not demonstrate a significant association with PFS in this model (p = 0.965) and the estimated hazard ratio and confidence interval reflected substantial instability due to complete separation in the data, limiting interpretability.

Collectively, the Cox regression analysis identifies low miR-142 expression and hypoalbuminemia as independent adverse prognostic factors for PFS in this cohort.

Cox regression analysis (Progression Free Survival)			
Characteristic	**Hazard ratio**	**95% CI**	**p-value**
Low miR-142-3p	3.97	1.18-13.3	**0.026**
IPI score 3-5	458194.55	0-1.384 × 10^{260}	0.965
Hypoalbuminemia	4.53	1.46-14.02	**0.009**
B-symptoms	2.95	0.81-10.83	0.102

Table 17. *Cox regression analysis (Progression Free Survival).*

As indicated by the results, when high IPI score was included in the multivariable model, it produced an extremely large hazard ratio with an associated standard error approaching infinity, indicating severe model instability and quasi-complete separation. Consequently, the IPI parameter was excluded from the model to ensure statistical validity

and interpretability. In the adjusted analysis, low miR-142-3p expression showed a trend toward an increased risk of progression, with an estimated hazard ratio (HR) of 3.27 (95% CI: 0.98–10.88; p = 0.054). Hypoalbuminemia emerged as a statistically significant predictor, associated with a 3.67-fold increased risk (95% CI: 1.21–11.16; p = 0.022). B symptoms were also associated with an elevated risk (HR 3.26; 95% CI: 0.86–12.40), although this did not reach statistical significance (p = 0.083).

8.5. Overall Survival According to miR-155, miR-142, and miR-22 Expression

OS was evaluated in relation to the expression levels of miR-155-3p, miR-142-3p, and miR-22-3p in patients with newly diagnosed LBCL. Pronounced differences were observed for miR-155-3p and miR-142-3p, while miR-22-3p showed no association with OS.

For miR-155-3p, patients with low expression demonstrated significantly longer OS, with a mean survival time of 45.02 months (SE 3.87; 95% CI: 37.44–52.60), compared with 21.03 months (SE 4.45; 95% CI: 12.31–29.75) in those with high miR-155-3p expression (p = 0.015). Median OS could not be estimated due to censoring **(Figure 17).**

A significant difference in OS was also observed according to miR-142-3p expression. Patients with normal/high miR-142-3p expression exhibited a mean OS of 47.53 months (SE 3.80; 95% CI: 40.08–54.98), whereas patients with low miR-142-3p expression had a mean OS of 27.46 months (SE 5.85; 95% CI: 15.99–38.92). Median OS of 18.0 months (SE 7.57; 95% CI: 3.17–32.84) was estimable for the low miR-142-3p group, while censoring prevented estimation for the normal/high-expression group **(Figure 18).**

In contrast, miR-22-3p expression was not associated with differences in OS. The mean OS was 39.84 months (SE 5.93; 95% CI: 28.21–51.47) among patients with low miR-22-3p expression and 37.57 months (SE 4.23; 95% CI: 29.28–45.86) in those with high miR-22-3p expression (p = 0.916). Median OS could not be estimated for either group because the longest observed survival times were censored **(Figure 19).**

Taken together, these findings indicate that high miR-155-3p expression and low miR-142-3p expression are associated with significantly inferior OS, whereas miR-22-3p expression does not appear to influence OS in newly diagnosed LBCL patients.

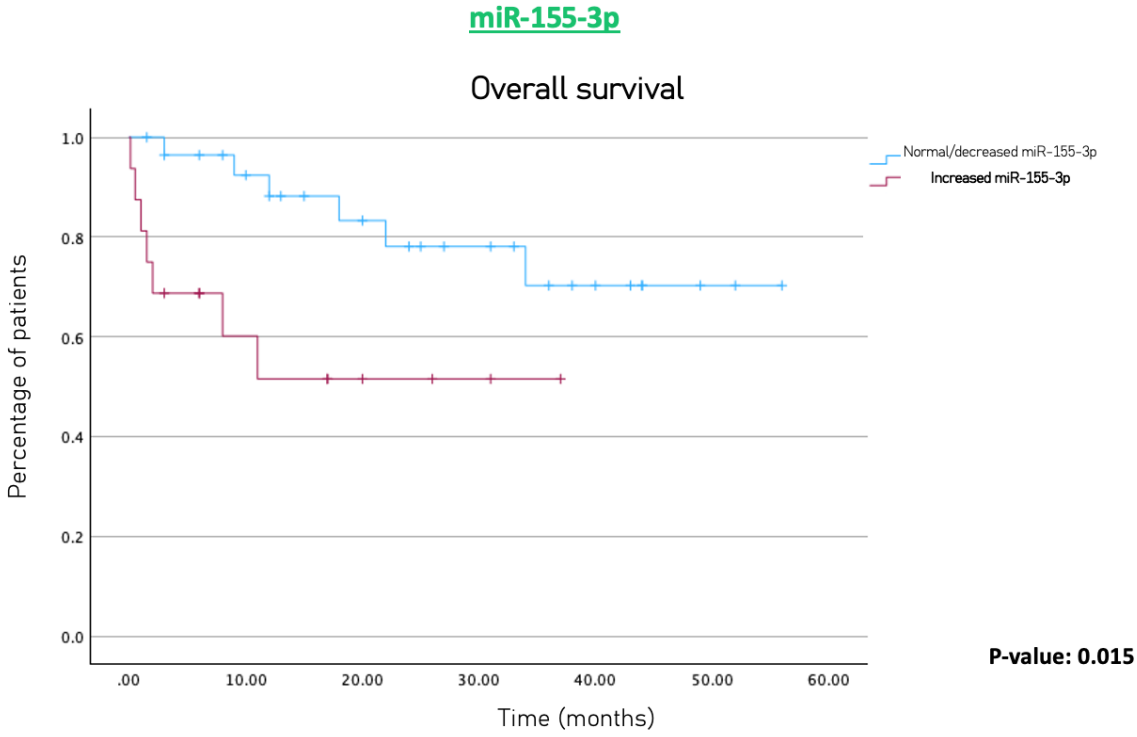

Figure 17. *miR-155-3p and OS in LBCL.*

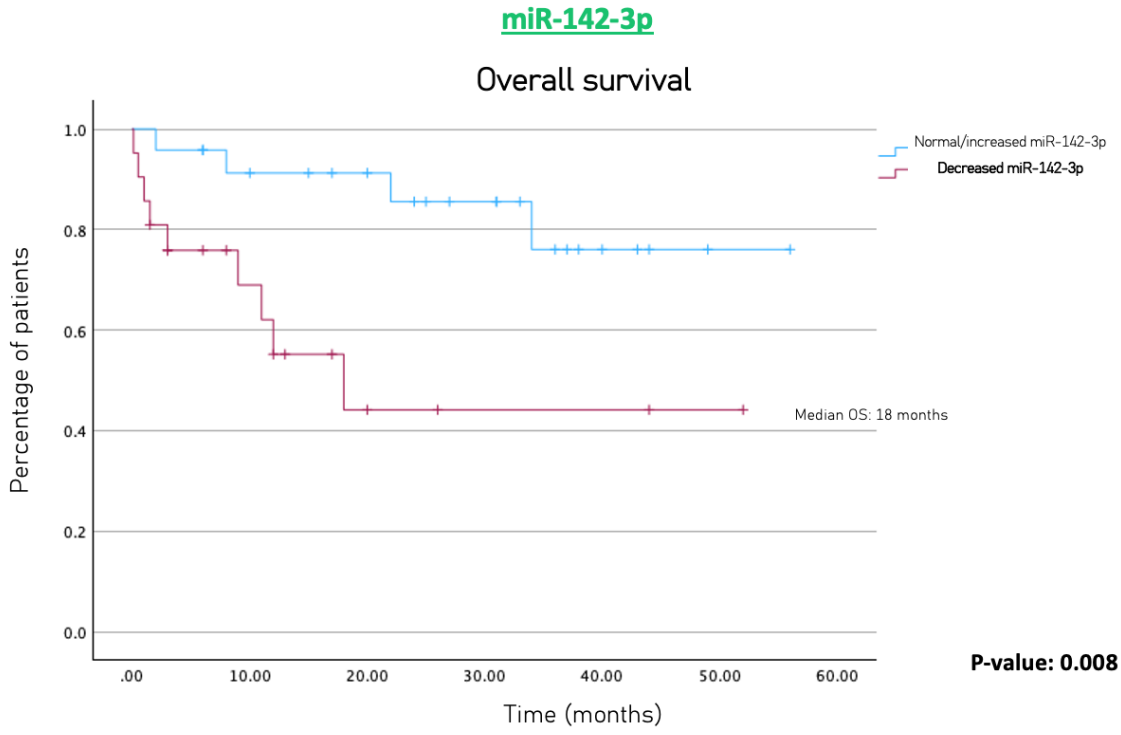

Figure 18. *miR-142-3p and OS in LBCL.*

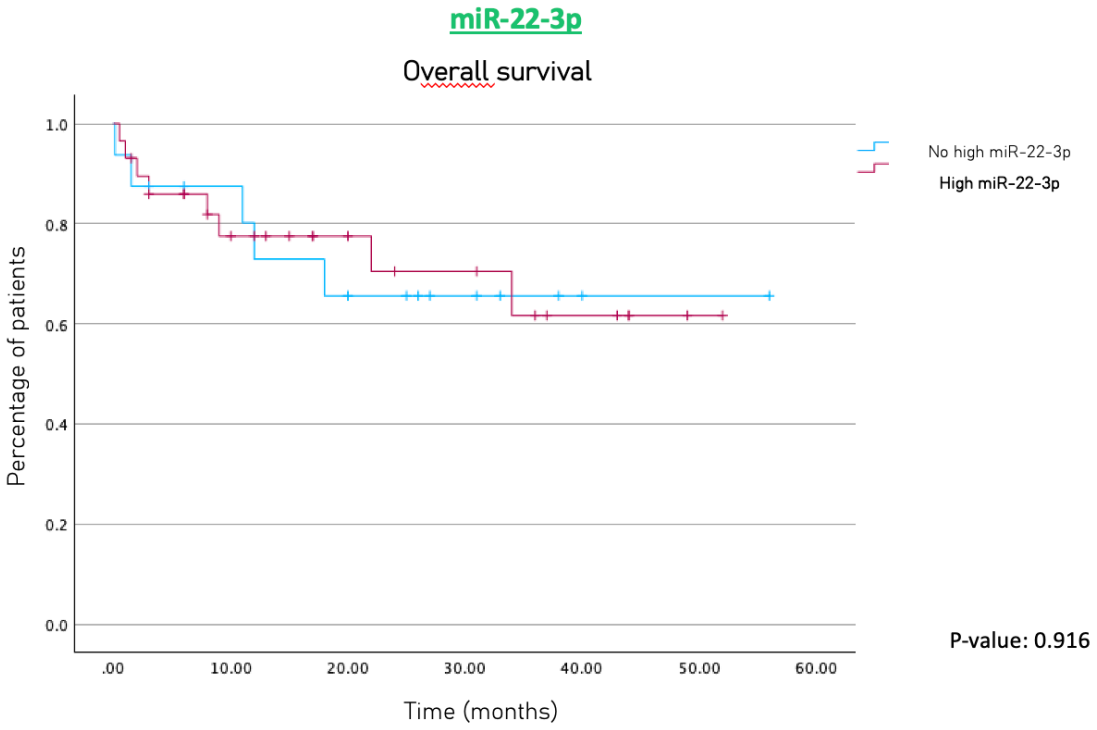

Figure 19. *miR-22-3p and OS in LBCL.*

8.6. Univariate Analysis of Overall Survival

Univariate analysis was conducted to examine the associations between clinical, laboratory, and molecular characteristics and 2-year OS in patients with newly diagnosed LBCL (Table 18). Several variables were significantly associated with reduced OS. Hypoalbuminemia (albumin <3.5 g/dL) demonstrated a strong association with inferior survival, with a 2-year OS of 35.2% compared with 82% in patients with normal albumin levels (p = 0.007). Similarly, a high International Prognostic Index (IPI 3–5) was associated with poorer outcomes, with a 2-year OS of 55.5% versus 100% among those with low-risk IPI scores of 0–2 (p = 0.012). B symptoms were also linked to decreased survival, with a 2-year OS of 55.9% compared with 80% in patients without B symptoms (p = 0.014). Patients who developed neutropenic fever during treatment exhibited a markedly reduced 2-year OS of 33% compared with 80.7% in those without this complication (p = 0.002).

Hyponatremia was also significantly associated with inferior survival, with a 2-year OS of 32.4% in affected patients compared with 76.7% in those with normal sodium levels (p =

0.007). Increased miR-155-3p expression was similarly associated with inferior OS (51.6% vs. 78.1%, p = 0.015), as was decreased miR-142-3p expression (44.2% vs. 85.6%, p = 0.008).

In contrast, other evaluated characteristics—including age at diagnosis >60 years, sex, elevated LDH, increased β2-microglobulin, bulky disease, extranodal involvement, bone marrow infiltration, hypercalcemia, and increased miR-22-3p expression—did not show statistically significant associations with 2-year OS (all p > 0.05).

Univariate analysis OS		
Characteristic	**2y-OS (%)**	**p-value**
Age at diagnosis >60	67.6%	0.758
Male	59.9%	0.265
Albumin<3.5 g/dL	**35.2%**	**0.007**
increased LDH	64.6%	0.158
β2-microglobulin increased	73.3%	0.306
IPI score (3-5)	**55.5%**	**0.012**
B-symptoms	**55.9%**	**0.014**
Bulky disease	65%	0.201
Neutropenic fever	**33%**	**0.002**
Extranodal disease	61.9%	0.165
Bone marrow infiltration	70%	0.488
Hyponatremia	32.4%	0.007
Hypercalcemia	83.3%	0.856
Increased miR-155-3p	**51.6%**	**0.015**
Decreased miR-142-3p	**44.2%**	**0.008**
Increased miR-22-3p	70.5%	0.916

Table 18. *Univariate analysis (Overall Survival).*

Cox regression analysis (Overall Survival)			
Characteristic	**Hazard ratio**	**95% CI**	**p-value**
Hypoalbuminemia	4.66	0.98-22.01	0.053
IPI score 3-5	533217	0-2.367 × 10^{230}	0.96
B-symptoms	1.83	0.44-7.53	0.41
Hyponatremia	0.39	0.052-2.97	0.36
Increased miR-155-3p	**6.24**	**1.38-28.17**	**0.017**
Decreased miR-142-3p	**7.52**	**1.62-34.8**	**0.01**

Table 19. *Cox regression analysis (Overall Survival).*

Cox proportional hazards regression was performed to assess the independent association of clinical and molecular characteristics with OS in patients with newly diagnosed LBCL (Table 19). Increased miR-155-3p expression remained significantly associated with an elevated risk of mortality, with a hazard ratio (HR) of 6.24 (95% CI: 1.38–28.17, p = 0.017). Decreased miR-142-3p expression also retained statistical significance in the multivariable model, demonstrating an HR of 7.52 (95% CI: 1.62–34.80, p = 0.010). Hypoalbuminemia showed a trend toward association with inferior OS (HR 4.66; 95% CI: 0.98–22.01), although this did not reach statistical significance (p = 0.053).

Other variables included in the model—specifically high IPI score (3–5), presence of B symptoms, and hyponatremia—did not demonstrate statistically significant associations with OS (all p > 0.05). Notably, the hazard ratio estimate for high IPI (3–5) was unstable due to complete or near-complete separation within the data, limiting interpretability.

When the International Prognostic Index (IPI) was included in the Cox regression model, it resulted in extreme coefficient estimates and substantial model instability; therefore, a subsequent Cox regression analysis excluding IPI was performed. In this adjusted model, low miR-142-3p expression was significantly associated with an increased risk of mortality, with a hazard ratio (HR) of 4.80 (95% CI: 1.12–20.54; p = 0.035). High miR-155-3p expression showed a trend toward increased risk (HR 3.61; 95% CI: 0.88–14.92; p = 0.076). Hypoalbuminemia was also associated with an elevated risk (HR 4.09; 95% CI: 0.83–20.23), although this did not reach statistical significance (p = 0.084). Hyponatremia was not significantly associated with mortality (HR 0.39; 95% CI: 0.05–3.04; p = 0.370). B symptoms

were associated with a higher risk (HR 2.86; 95% CI: 0.67–12.23), but this association was not statistically significant (p = 0.156).

8.7. Prognostic ROC analysis for 2-year outcomes

For prognostic assessment, 2-year landmark analyses were performed. Patients censored before 24 months without experiencing the event of interest were excluded, resulting in 30 patients eligible for both PFS and OS analyses **(Figure 20).**

For predicting 2-year mortality, miR-155-3p showed acceptable discriminatory performance (AUC=0.720, 95% CI: 0.531-0.900), with higher expression associated with increased mortality risk. At the optimal cutoff (≥0.41), sensitivity was 66.7% and specificity was 77.8%. miR-142-3p demonstrated comparable prognostic value (AUC=0.697, 95% CI: 0.480-0.901), with lower expression predicting worse outcomes. Using a cutoff of ≤0.000462, miR-142-3p achieved a sensitivity of 75.0% and a specificity of 83.3% for predicting 2-year mortality. For 2-year PFS prediction, miR-155-3p (AUC=0.667, 95% CI: 0.455-0.862) and miR-142-3p (AUC=0.605, 95% CI: 0.380-0.817) showed moderate discriminatory ability. In contrast, miR-22-3p demonstrated no prognostic value for either PFS (AUC=0.520) or OS (AUC=0.525).

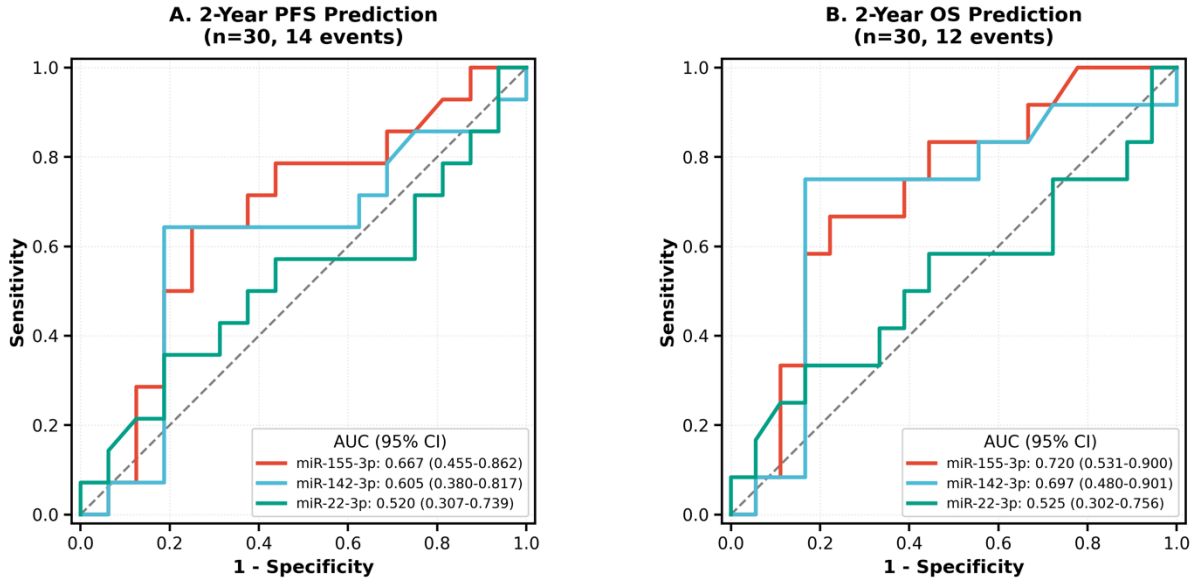

Figure 20. *Prognostic ROC Analysis for 2-Year Outcomes. Receiver operating characteristic curves for prediction of (A) 2-year progression-free survival (n=30, 14 events) and (B) 2-year overall survival (n=30, 12 events). Fifteen patients were excluded from each analysis due to censoring before 24 months. For OS prediction, miR-155-3p (AUC=0.720) and miR-142-3p*

(AUC=0.697) showed acceptable discriminatory ability, while miR-22-3p demonstrated no prognostic utility (AUC=0.525).

8.8. Associations between miRNA alterations and DLBCL prognosis (PFS and OS)

In the present study, a total of 45 patients were enrolled. Among these, two patients were diagnosed with primary mediastinal large B-cell lymphoma (PMBCL) and two with primary central nervous system (CNS) lymphoma. To minimize biological heterogeneity and avoid potential confounding effects, subsequent statistical analyses were restricted to patients with DLBCL-NOS. Primary CNS lymphoma and PMBCL were excluded due to their distinct biological features, clinical behavior, and therapeutic approaches. Notably, despite this restriction, the overall results were comparable to those observed in the full cohort, supporting the robustness and consistency of the identified prognostic associations.

PFS was evaluated according to the expression levels of miR-155-3p, miR-142-3p, and miR-22-3p in patients with DLBCL-NOS. Distinct patterns were observed among the three miRNA expression groups.

For miR-155-3p, patients with low expression demonstrated a mean PFS of 39.18 months (SE 4.45; 95% CI: 30.46–47.90), whereas those with high miR-155-3p expression had a shorter mean PFS of 21.48 months (SE 4.40; 95% CI: 12.86–30.10). This difference reflected a trend toward inferior PFS in the high-expression group; however, it did not reach statistical significance (p = 0.099). Median PFS could not be estimated due to censoring of the largest survival times (**Figure 21).**

In contrast, miR-142-3p expression demonstrated a statistically significant association with PFS. Patients with normal/high miR-142 expression exhibited a mean PFS of 37.85 months (SE 3.28; 95% CI: 31.42–44.28), compared with 23.01 months (SE 5.92; 95% CI: 11.40–34.62) in those with low miR-142-3p expression (p = 0.012). Median PFS estimable was available only for the low-expression group, calculated at 30.0 months (SE 12.21; 95% CI: 6.07–53.93) (**Figure 22).**

Analysis of miR-22-3p expression did not reveal significant differences in PFS. The mean PFS was 23.70 months (SE 4.97; 95% CI: 13.97–33.44) in the low-expression group and 38.24 months (SE 4.42; 95% CI: 29.57–46.91) in the high-expression group, but this difference

was not statistically significant (p = 0.264). Median PFS could not be estimated due to censoring.

Overall, these findings indicate that low miR-142-3p expression is significantly associated with inferior PFS, high miR-155-3p expresssion shows a non-significant trend toward reduced PFS, and miR-22-3p expression does not appear to influence PFS in patients with newly diagnosed DLBCL-NOS.

Following the miRNA expression analyses, univariate analysis was conducted to assess clinical and laboratory factors associated with PFS in DLBCL-NOS (**Table 20**). At 2 years, advanced age at diagnosis (>60 years) and male sex were not significantly associated with PFS (p = 0.944 and p = 0.441, respectively). Similarly, increased LDH levels, elevated β2-microglobulin, bulky disease, extranodal involvement, bone marrow infiltration, hyponatremia and hypercalcemia did not demonstrate statistically significant associations with PFS.

In contrast, several variables were significantly associated with inferior 2-year PFS. Patients with hypoalbuminemia (albumin<3.5 g/dL) exhibited a markedly reduced PFS compared with those with normal albumin levels (36.4% vs 81.0%, p = 0.005). A high International Prognostic Index (IPI) score (3–5) was also associated with poorer outcomes, with a 2-year PFS of 57.2% compared with 100% in patients with low-risk scores (0-2)(p = 0.019). B symptoms were associated with decreased PFS (52.9% vs 86.9%, p = 0.008), as was neutropenic fever during treatment (37.5% vs 76.7%, p = 0.011) (**Table 20**).

Multivariate Cox proportional hazards regression analysis was performed to identify independent predictors of PFS (Table 21). **Hypoalbuminemia** was independently associated with an increased risk of disease progression, with a hazard ratio (HR) of 3.99 (95% CI, 1.23–12.97; p = 0.021). Similarly, **decreased expression of miR-142-3p** emerged as an independent adverse prognostic factor for PFS (HR 4.86; 95% CI, 1.28–18.49; p = 0.021).

B symptoms showed a trend toward an increased risk of progression; however, this association did not reach statistical significance (HR 3.89; 95% CI, 0.85–17.90; p = 0.081). Although a high IPI score (3–5) yielded an extremely elevated hazard ratio, this estimate was not statistically significant (p = 0.967) and was accompanied by an implausibly wide confidence interval, suggesting model instability due to sparse events or complete separation.

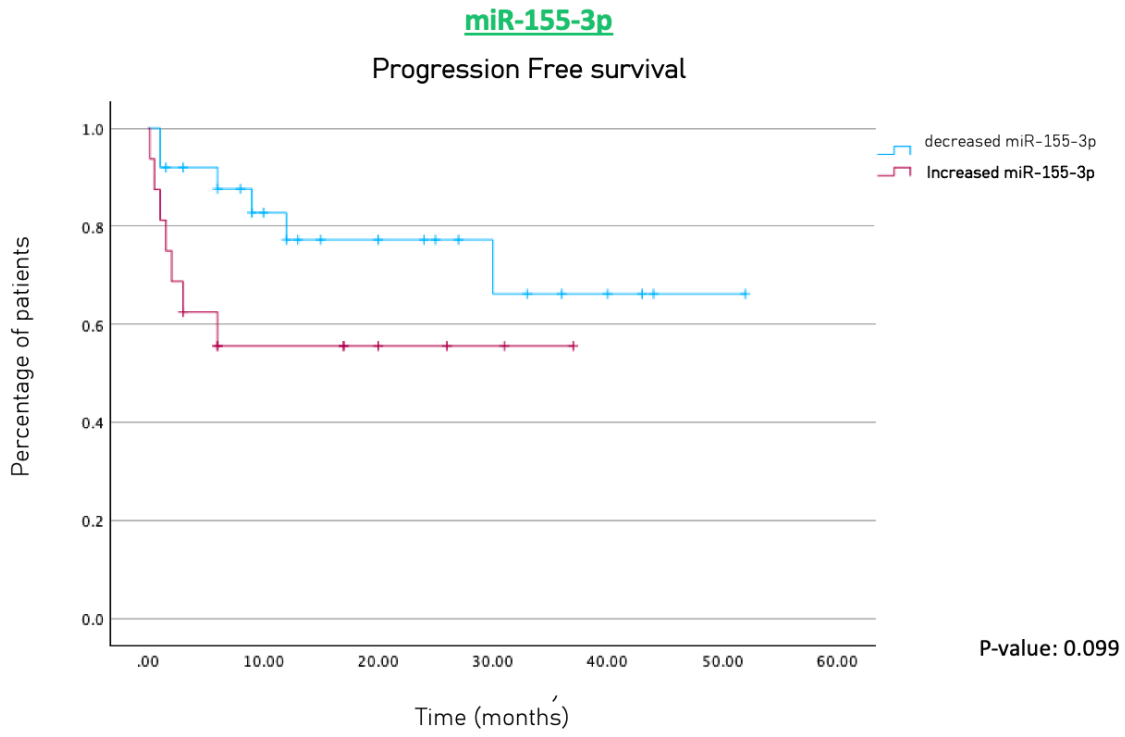

Figure 21. *miR-155-3p and PFS in DLBCL.*

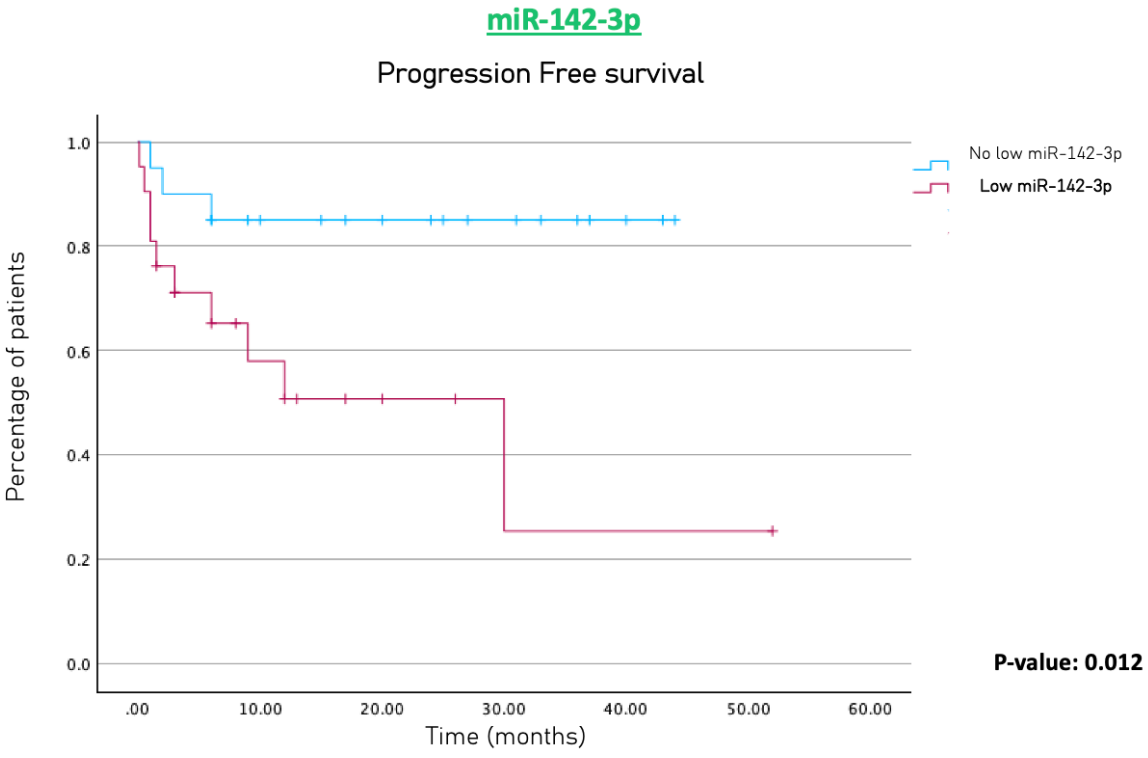

Figure 22.*miR-142-3p and PFS in DLBCL.*

Univariate analysis PFS		
Characteristic	**2y-PFS (%)**	**p-value**
Age at diagnosis >60	71.5%	0.944
Male	62.3%	0.441
Albumin<3.5 g/dL	**36.4% (vs 81%)**	**0.005**
increased LDH	63.3%	0.121
β2-microglobulin increased	69.3%	0.69
IPI score (3-5)	**57.2% (vs 100%)**	**0.019**
B-symptoms	**52.9% (vs 86.9%)**	**0.008**
Bulky disease	75%	0.66
Neutropenic fever	**37.5% (vs 76.7%)**	**0.011**
Extranodal disease	64.5%	0.321
Bone marrow infiltration	60%	0.189
Hyponatremia	44.4%	0.068
Hypercalcemia	83.3%	0.47
Increased miR-155-3p	55.6%	0.099
Decreased miR-142-3p	**50.7%**	**0.012**
Increased miR-22-3p	76.9%	0.264

Table 20. *Univariate analysis in PFS in patients with DLBCL..*

Cox regression analysis (PFS)			
Characteristic	**Hazard ratio**	**95% CI**	**p-value**
Hypoalbuminemia	**3.99**	1.23-12.97	**0.021**
IPI score 3-5	467043.84	0-1.544 × 10^{272}	0.967
B-symptoms	3.89	0.85-17.9	0.081
Decreased miR-142-3p	**4.86**	**1.28-18.49**	**0.021**

Table 21. *Cox regression analysis in PFS in patients with DLBCL.*

OS was evaluated in relation to the expression levels of miR-155-3p, miR-142-3p, and miR-22-3p in patients with DLBCL. Significant differences were observed for miR-155-3p and miR-14-3p 2, whereas miR-22-3p expression did not demonstrate an association with OS.

For miR-155-3p, patients with non-high expression exhibited a mean OS of 41.73 months (SE 3.96; 95% CI: 33.98–49.49), compared with 21.03 months (SE 4.45; 95% CI: 12.31–29.75) in those with high miR-155-3p expression. This difference was statistically significant ($p = 0.026$). Median OS could not be estimated for either subgroup because the longest observed survival times were censored **(Figure 23).**

Similarly, miR-142-3p expression was significantly associated with OS. Patients with high miR-142-3p expression had a mean OS of 38.40 months (SE 2.97; 95% CI: 32.58–44.21), whereas those with low expression demonstrated a mean OS of 27.46 months (SE 5.85; 95% CI: 15.99–38.92) ($p = 0.018$). Median OS was estimable only for the low-expression group and was calculated at 18.0 months (SE 7.57; 95% CI: 3.17–32.84) **(Figure 24).**

In contrast, miR-22-3p expression did not show a significant association with OS. Patients with lower miR-22-3p expression demonstrated a mean OS of 27.63 months (SE 4.41; 95% CI: 18.98–36.28), compared with 38.44 months (SE 4.33; 95% CI: 29.96–46.92) in those with high expression, but this difference did not reach statistical significance ($p = 0.569$). Median OS could not be estimated for either subgroup due to censoring.

Collectively, these findings indicate that high miR-155-3p expression and low miR-142-3p expression are associated with significantly shorter OS, whereas miR-22-3p expression does not appear to influence OS in patients with newly diagnosed DLBCL-NOS.

Following the miRNA expression analyses, univariate analysis was performed to evaluate clinical and laboratory factors associated with OS in DLBCL-NOS (**Table 22**). At 2 years, age at diagnosis >60 years and male sex were not significantly associated with OS ($p = 0.888$ and $p = 0.580$, respectively). Increased LDH levels, elevated β2-microglobulin, extranodal disease, bone marrow infiltration, double-expressor status and hypercalcemia also showed no significant association with OS**.** Bulky disease showed a trend toward worse OS but did not reach statistical significance ($p = 0.091$).

Several variables were significantly associated with inferior 2-year OS. Patients with **hypoalbuminemia** (albumin<3.5 g/dL) had reduced OS compared with those with normal albumin levels (41.6% vs 79.7%, $p = 0.026$). A **high IPI score (3–5)** was associated with poorer survival (57.9% vs 100%, $p = 0.027$). The presence of **B symptoms** was also correlated with

reduced OS (55.9% vs 84.0%, p = 0.021). **Neutropenic fever** during treatment was strongly associated with inferior OS (20.8% vs 78.5%, p = 0.002), as was **hyponatremia** (32.4% vs 78.4%, p = 0.012).

With respect to molecular characteristics, as previously described, elevated miR-155-3p expression was associated with poorer OS (51.6% vs 79.8%, p = 0.026), while reduced miR-142-3p expression was likewise significantly associated with inferior survival outcomes (44.2% vs 89.4%, p = 0.018).

All variables demonstrating statistical significance in univariate analyses were subsequently entered into a multivarable Cox proportional hazards regression model to evaluate their association with OS (**Table 23**). In this analysis, increased miR-155-3p expression was significantly associated with inferior OS (HR 6.86; 95% CI, 1.36–34.53; p = 0.019), while decreased expression of miR-142-3p also emerged as an independent adverse prognostic factor (HR 7.65; 95% CI, 1.58–37.12; p = 0.012). Hypoalbuminemia, high IPI score (3–5), B symptoms, and hyponatremia were not significantly associated with OS in the multivariable model.

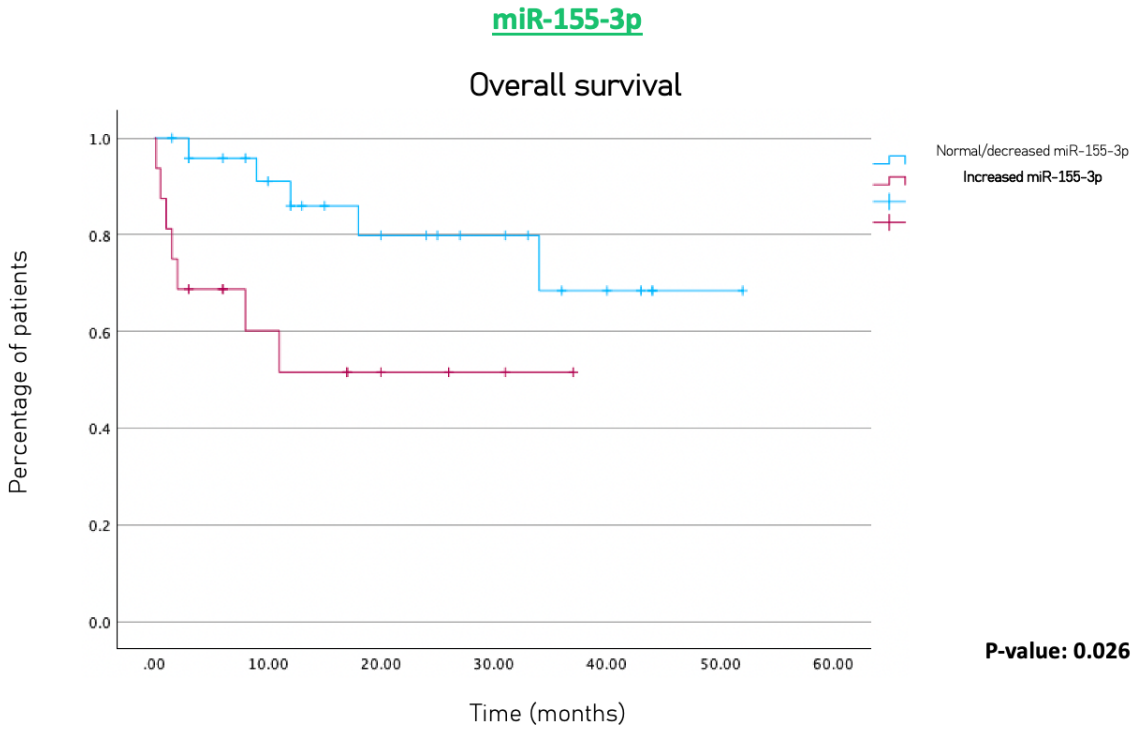

Figure 23. *miR-155-3p and OS in DLBCL.*

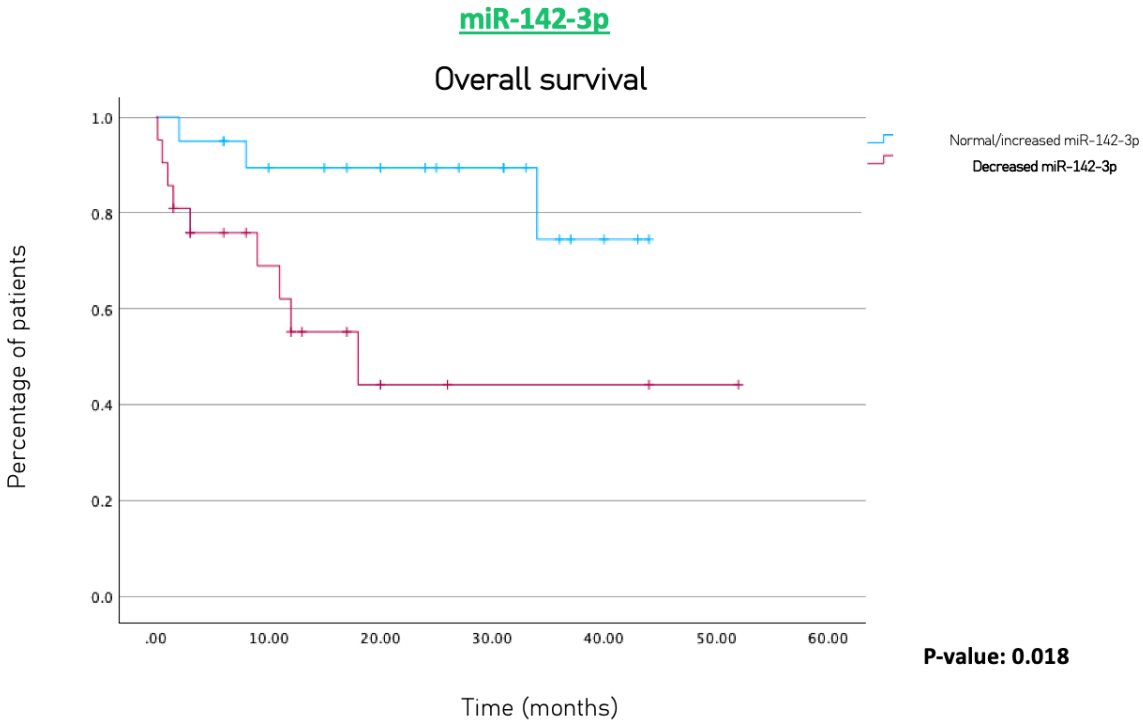

Figure 24. *miR-142-3p and OS in DLBCL.*

Univariate analysis OS		
Characteristic	**2y-OS (%)**	**p-value**
Age at diagnosis >60	72.3%	0.888
Male	65%	0.58
Albumin<3.5 g/dL	**41.6% (vs 79.7%)**	**0.026**
increased LDH	64%	0.148
β2-microglobulin increased	73.3%	0.513
IPI score (3-5)	**57.9% (vs 100%)**	**0.027**
B-symptoms	**55.9% (vs 84%)**	**0.021**
Bulky disease	61% (vs 81.8%)	0.091
Double expressor	52.1%(vs 75.4%)	0.352
Neutropenic fever	**20.8% (vs 78.5%)**	**0.002**
Extranodal disease	66.3%	0.45
Bone marrow infiltration	70%	0.567
Hyponatremia	**32.4%(vs78.4%)**	**0.012**
Hypercalcemia	83.3%	0.962
Increased miR-155-3p	51.6% (vs79.8%)	0.026
Decreased miR-142-3p	**44.2% (vs 89.4%)**	**0.018**
Increased miR-22-3p	75.7%	0.569

Table 22. *Univariate analysis (PFS) in patients with DLBCL.*

Cox regression analysis (Overall Survival)			
Characteristic	**Hazard ratio**	**95% CI**	**p-value**
Hypoalbuminemia	3.09	0.58-16.31	0.185
IPI score 3-5	512585	0-4.546 × 10^{243}	0.962
B-symptoms	2.83	0.55-14.71	0.216
hyponatremia	0.44	0.055-3.47	0.433
Increased miR-155-3p	**6.86**	**1.36-34.53**	**0.019**
Decreased miR-142-3p	**7.65**	**1.58-37.12**	**0.012**

Table 23. *Cox regression analysis (OS) in patients with DLBCL.*

A comprehensive overview of the principal findings of the present study is provided in **Figure 25**, which summarizes the key clinical and molecular factors identified as being associated with patient outcomes.

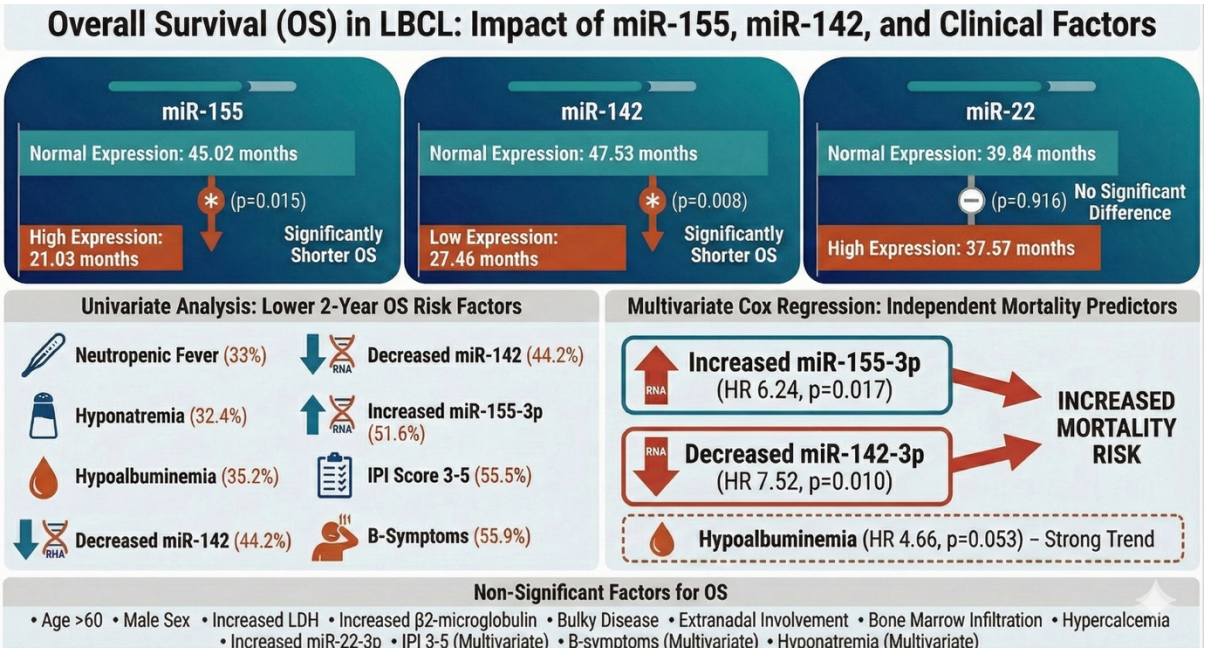

Figure 25. *A summary of key findings of our study. This figure summarizes the principal findings of our study concerning the prognostic role of miRNAs in LBCL.*

8.9. Association of Treatment Response with Survival and miRNA Levels

In this analysis of 45 patients with LBCL, complete response (CR) was achieved in 32 patients (71.1%), while 13 patients (28.9%) experienced treatment failure. CR achievement was strongly associated with superior outcomes: progression rate was only 6.2% in the CR group versus 100% in the non-CR group, and mortality was 3.1% versus 92.3%, respectively ($p<0.001$ for both). Similarly, median PFS was 22.0 months in the CR group versus only 2.0 months in the non-CR group, and OS was 22.0 versus 8.0 months ($p=0.004$).

Despite this strong association between CR and clinical outcome, serum levels of miR-155-3p, miR-142-3p, and miR-22-3p showed no statistically significant association with CR achievement ($p=0.153$, $p=0.802$, and $p=0.679$, respectively). ROC analysis demonstrated poor-to-modest discriminatory ability (AUC 0.525–0.638), and neither univariate nor multivariate logistic regression identified any of the three miRNAs as significant predictors. These findings should be interpreted with caution given the small sample size, the single-

center design, baseline-only miRNA measurement without serial sampling, and the absence of an independent validation cohort, factors that limit statistical power and generalizability of the conclusions.

9. Discussion

9.1 Overview

LBCL constitutes a biologically and clinically heterogeneous group of aggressive B-cell malignancies, encompassing entities with distinct molecular features, patterns of disease evolution, and clinical outcomes (19). Although the introduction of immunochemotherapy has significantly improved survival, considerable variability in treatment response and long-term prognosis remains (13). The International Prognostic Index (IPI) has long served as a cornerstone for risk stratification in LBCL, incorporating readily available clinical parameters to estimate outcomes. However, even within IPI-defined risk categories, marked heterogeneity in survival and disease behavior persists, underscoring the limitations of purely clinical prognostic models (4). MiRNAs, as post-transcriptional regulators of gene expression, have emerged as key modulators of lymphomagenesis and tumor progression (55, 86).

In the present study, we investigated the diagnostic and prognostic relevance of circulating miR-155-3p, miR-142-3p, and miR-22-3p in a cohort of patients with newly diagnosed LBCL. By integrating miRNA expression data with clinicopathological parameters, molecular features, and survival outcomes, we aimed to clarify the distinct biological roles of these miRNAs in LBCL.

Although no significant differences in circulating miR-155-3p and miR-142-3p levels were observed between patients and healthy controls in our study, both miRNAs demonstrated prognostic relevance within the patient cohort. This apparent discrepancy may be explained by tumor-specific heterogeneity among individuals with LBCL. The expression of miR-155 and miR-142 is known to vary according to molecular subtype and tumor microenvironmental features, such as activated B-cell versus germinal-centre phenotypes and the presence of MYC and BCL2 dysregulation. Consequently, while average circulating miRNA levels across patients and controls may overlap, variability within the patient cohort likely reflects biologically meaningful differences related to tumor aggressiveness, immune dysregulation, and disease behavior (1, 2, 209).

Additionally, differences in systemic versus local miRNA release dynamics may contribute to this finding. These miRNAs may not be uniformly released into the circulation

across all lymphoma cases, and their serum levels may become biologically informative only in the context of increased tumor burden, heightened cellular stress, or active exosomal secretion. Therefore, their prognostic value appears to derive from their association with disease activity and progression within affected patients rather than from baseline elevations compared with healthy individuals (209, 210).

9.2Diagnostic and prognostic role of miRNA Profiles in LBCL

9.2.1 miR-22

One of the most salient observations of the present study is the distinct behavior of individual miRNAs when evaluated in diagnostic versus prognostic contexts. Although the circulating expression levels of miR-155-3p and miR-142-3p did not differ significantly between patients with LBCL and healthy controls, miR-22-3p was markedly overexpressed in the patient cohort. This differential expression pattern suggests that miR-22-3p may be implicated in early disease-related biological processes rather than in determinants of disease aggressiveness. The observed elevation of miR-22-3p may reflect alterations in tumor-associated signaling pathways or systemic immune and inflammatory responses that accompany lymphoma development, highlighting its potential relevance as a disease-associated biomarker rather than a prognostic indicator.

In the present study, miR-22-3p was significantly elevated in LBCL compared with healthy controls and was also more prevalent in the non-GCB subgroup. A plausible explanation is that non-GCB (ABC-like) LBCL is characterized by distinct transcriptional and microenvironmental programs, including chronic activation of NF-κB–related survival signaling and stromal/immune interactions, which can shape circulating miRNA profiles (211, 212).

MiR-22 was initially identified through cloning studies in HeLa cells and is an evolutionarily conserved microRNA encoded by a gene located on chromosome 17p13 (213). MiR-22 has been shown to regulate multiple epigenetic modifiers, such as TET2 , thereby influencing chromatin remodelling and transcriptional regulation (213, 214). Given that TET2 is frequently mutated or functionally impaired in B-cell lymphomas, repression of TET2 by miR-22 may exacerbate epigenetic instability and promote aberrant transcriptional programs

associated with malignant transformation (215, 216). Dysregulation of these pathways is well documented in B-cell lymphomas (215, 216).

In addition to its effects on DNA hydroxymethylation, miR-22 has been shown to regulate chromatin remodelling by modulating histone acetylation and methylation. Specifically, miR-22 targets histone deacetylase 4 (HDAC4) and lysine demethylase 3A (KDM3A), both of which are involved in transcriptional control of genes governing cell cycle progression, apoptosis, and genomic stability (213). Furthermore, miR-22 has also been implicated in the regulation of inflammatory and survival signalling pathways relevant to lymphoma biology. By targeting TNFAIP8, a molecule involved in NF-κB–mediated survival signaling, miR-22 may exert tumor-suppressive effects (213).

Taken together, these findings are consistent with the possibility that circulating miR-22 reflects subtype-linked biology rather than a uniform LBCL-wide oncogenic signal. Its preferential expression in non-GCB LBCL suggests that miR-22-3p may reflect underlying molecular programs specific to this subtype rather than directly influencing clinical outcome.

In the present study, miR-22-3p expression was not associated with clinical outcome, as no significant relationship was observed with either PFS or OS. This finding contrasts with reports in the literature in which elevated serum miR-22 levels have been linked to adverse prognosis in patients with DLBCL (217, 218). Previous studies have suggested that increased circulating miR-22 expression may be associated with inferior treatment response and reduced survival, indicating a potential prognostic role for this microRNA in specific clinical or biological contexts (217, 218). The discrepancy between the present results and those of prior studies may reflect differences in patient cohorts, sample size, methodological approaches, or underlying disease biology, underscoring the context-dependent nature of miR-22 expression and its clinical significance.

The dissociation between the diagnostic elevation and the lack of prognostic significance of miR-22-3p in our cohort warrants further mechanistic consideration. Several hypotheses may explain this observation. First, miR-22 may be elevated as a consequence of early lymphomagenesis-associated cellular stress, metabolic reprogramming, or inflammatory signaling rather than serving as a direct driver of disease aggressiveness. In this model, miR-22 upregulation represents a reactive phenomenon accompanying malignant transformation but does not independently influence the trajectory of established disease. Second, the dual nature of miR-22 as both a tumor suppressor and an oncogene in different

cellular contexts—a phenomenon termed "miRNA duality"—may contribute to its inconsistent prognostic behavior (213). While miR-22 can suppress tumor growth by targeting oncogenic pathways such as c-MYC/MYCBP and PTEN-AKT, it may simultaneously promote survival through epigenetic reprogramming via TET2 inhibition (213, 214). The net effect of these opposing functions may be context-dependent, varying according to the molecular subtype, mutational landscape, and microenvironmental composition of individual tumors. Third, the significant association between high miR-22 expression and the non-GCB phenotype observed in our study suggests that miR-22 elevation may reflect subtype-specific biology rather than serve as a universal marker of aggressiveness. The non-GCB/ABC subtype is characterized by constitutive NF-κB activation and distinct gene expression programs that may independently determine prognosis, potentially masking any independent contribution of miR-22 to clinical outcomes (211, 212). Fourth, circulating miRNA levels represent a composite signal derived from both tumor cells and non-malignant cellular compartments, including immune cells, stromal elements, and platelets. The relative contribution of each source to circulating miR-22 levels remains poorly defined, and heterogeneity in these contributions across patients may dilute any tumor-specific prognostic signal. Finally, technical factors including the timing of sample collection relative to disease evolution, variability in tumor burden at diagnosis, and the inherent biological variability of circulating miRNA measurements may contribute to the observed lack of prognostic association. Future studies employing serial sampling, paired tissue-serum analyses, and larger patient cohorts stratified by molecular subtype will be necessary to definitively establish whether miR-22 possesses prognostic utility in specific clinical or biological contexts.

9.2.2 miR-155

miR-155 is a highly conserved microRNA encoded by the BIC locus on chromosome 21, with established roles in immune regulation, hematopoiesis, and inflammatory signaling, and is aberrantly activated in B-cell lymphomas (101, 102). In addition, miR-155 regulates T-helper cell differentiation and germinal center responses, thereby modulating T-cell–dependent antibody production through cytokine regulation (103).

In DLBCL, particularly in the ABC/non-GCB subtype, constitutive activation of the NF-κB pathway is a central pathogenic feature driving tumor cell survival, proliferation, and

resistance to apoptosis (211, 219). Mechanistically, miR-155 contributes to sustained NF-κB activation by repressing multiple negative regulators of upstream signaling pathways. In DLBCL models, miR-155 directly targets SHIP1 and SOCS1, both of which function as inhibitory checkpoints of PI3K/AKT and JAK/STAT signaling that converge on NF-κB transcriptional activity (104). Furthermore, in Eμ-miR-155 transgenic mice, B-cell–specific miR-155 overexpression reduces IKKβ expression, indicating that miR-155 modulates NF-κB signaling through negative feedback regulation (105, 106). Moreover, miR-155 promotes cell survival and proliferation by targeting key regulators of apoptotic, inflammatory, epigenetic, and cell-cycle pathways, including components of LPS/NF-κB signaling, HDAC4, BCL6, SMAD5, and RB-related cell-cycle control (86).

In the present study, miR-155 expression was not found to be associated with the ABC subtype of DLBCL. This observation contrasts with the prevailing evidence in the literature, which largely supports a diagnostic role for miR-155 in distinguishing between the ABC and GCB molecular subtypes. Numerous studies have reported consistently higher miR-155 expression in patients with the non-GCB/ABC subtype compared with those classified as GCB, irrespective of whether miR-155 levels were assessed in tumor tissue or in circulating serum samples (124, 125, 126, 128, 130).

Previous studies have suggested that miR-155 expression differs between de novo DLBCL and transformed aggressive B-cell lymphomas, with higher expression levels reported in de novo cases (133). However, this observation was not replicated in the present study. Our findings are in line with other published data examining the molecular changes associated with histologic transformation. In particular, a study investigating the transformation of follicular lymphoma (FL) to DLBCL identified differential expression of several miRNAs, including miR-223, miR-217, miR-222, miR-221, and members of the let-7i/let-7b family, whereas miR-155 was not among the miRNAs found to be significantly altered during transformation (125). Collectively, these observations suggest that miR-155 may not serve as a reliable biomarker for distinguishing de novo DLBCL from transformed disease or for predicting the risk of FL transformation to DLBCL (125).

In the present study, elevated circulating miR-155-3p expression was associated with poorer PFS, although this association did not reach statistical significance, and with significantly reduced OS. Notably, miR-155-3p remained an independent prognostic factor for OS in multivariable Cox regression analysis. These findings are consistent with previous

reports demonstrating that elevated miR-155 levels correlate with poor prognosis in DLBCL (129, 138). Several studies have investigated the prognostic significance of miR-155 expression in DLBCL, with largely consistent evidence supporting its association with adverse clinical outcomes. In a study analyzing tumor tissue from 90 patients with de novo DLBCL, reduced miR-155 expression was associated with a significantly higher 5-year progression-free survival rate, suggesting a protective effect of lower miR-155 levels (134). Two additional studies demonstrated that high miR-155 expression, stratified by median tumor levels, was associated with significantly inferior OS (135, 136). While one study did not demonstrate prognostic significance for serum miR-155 levels in patients with DLBCL , subsequent investigations have yielded contrasting results (125). More recent studies have shown that elevated circulating miR-155 expression is independently associated with adverse clinical outcomes, with this relationship remaining significant after adjustment for IPI risk categories (129, 138). High miR-155 expression was associated with improved progression-free and overall survival in patients treated with R-CHOP compared with CHOP alone, an effect not observed in cases with low miR-155 expression (139). In contrast, another study reported an association between elevated miR-155 expression and R-CHOP treatment failure (140).

The absence of differential expression between patients and controls suggests that miR-155-3p functions primarily as a progression-associated biomarker, reflecting tumor aggressiveness rather than disease initiation.

9.2.3 miR-142

miR-142 is the only human miRNA known to harbor recurrent somatic mutations in DLBCL, occurring in approximately 20% of cases and frequently affecting the seed regions of miR-142-3p and miR-142-5p. These mutations can disrupt miRNA–mRNA interactions, alter target specificity, and contribute to dysregulated signaling pathways involved in lymphomagenesis (175). miR-142 is predominantly expressed in hematopoietic cells and plays a critical role in B-cell development, cytoskeletal dynamics, and immune synapse formation (220, 221). Both miR-142-3p and miR-142-5p are typically expressed at high levels in DLBCL; however, recurrent mutations affecting these microRNAs have been shown to impair their normal function. Such alterations can lead to loss of regulatory activity and aberrant modulation of downstream cellular pathways, ultimately contributing to disrupted signaling networks

involved in lymphomagenesis and disease progression (175, 178, 179). Importantly, this study identified a significant association between low miR-142 expression and high MYC expression. The observed association between low miR-142 expression and increased MYC expression suggests a potential biological interaction between miR-142–mediated regulatory pathways and MYC-driven oncogenic programs in DLBCL. MYC is a central transcriptional regulator that promotes cell cycle progression, metabolic reprogramming, and genomic instability, and its overexpression is a well-established adverse prognostic factor in aggressive B-cell lymphomas (17, 211). Interestingly, low miR-142 expression was less frequently observed in non-GCB cases, suggesting that miR-142–defined biological subsets may transcend conventional cell-of-origin classification. This finding highlights the potential of miRNA profiling to capture biologically meaningful heterogeneity beyond immunophenotypic categories.

Among the miRNAs analyzed, miR-142-3p emerged as a particularly robust prognostic marker. In the present study, low miR-142 expression was consistently associated with inferior PFS and OS and retained independent prognostic significance in multivariable analyses. Although miR-142 is biologically relevant and recurrently mutated in a subset of DLBCLs, the existing literature has not consistently established its role as an independent prognostic or predictive biomarker in lymphoma (175, 222, 223). While some reports have suggested potential associations between miR-142 expression and treatment response or survival, these observations remain inconclusive and require validation in larger, well-characterized cohorts (222, 224). To our knowledge, this study represents one of the first to demonstrate a clear prognostic impact of miR-142 expression on clinical outcomes in LBCL, thereby providing novel evidence for its potential clinical relevance.

The biological mechanisms underlying the association between low miR-142 expression and adverse clinical outcomes in LBCL are likely multifactorial and interconnected. First, miR-142 functions as a critical regulator of immune surveillance within the tumor microenvironment. Loss of miR-142 expression has been shown to result in widespread proteomic alterations, including downregulation of major histocompatibility complex class I (MHC-I) components essential for antigen presentation, thereby impairing cytotoxic T-cell recognition and facilitating immune evasion (176). Second, miR-142 deficiency may promote oncogenic signaling through de-repression of its validated targets, including genes involved in cell cycle progression (CCNB1/Cyclin B1), cytoskeletal remodeling (CFL2, TWF1, LIMA1), and mTOR pathway activation (AKT1S1), collectively enhancing tumor cell proliferation and

survival (176, 220). Third, the observed correlation between low miR-142 expression and high MYC expression in our cohort suggests a potential functional interplay, whereby loss of miR-142-mediated post-transcriptional control may synergize with MYC-driven transcriptional programs to accelerate disease progression. MYC is known to globally repress miRNA biogenesis while selectively upregulating oncogenic miRNAs, potentially establishing a feed-forward loop that sustains aggressive tumor phenotypes (170). Fourth, miR-142 plays an essential role in normal B-cell development and germinal center dynamics; its loss may disrupt the tightly regulated balance between proliferation and apoptosis that characterizes normal lymphoid maturation, predisposing cells to malignant transformation and treatment resistance (220, 221). Finally, given that miR-142 is among the most frequently mutated miRNA genes in DLBCL, with mutations often affecting the seed region critical for target recognition, even modest reductions in circulating miR-142 levels may reflect underlying genetic alterations that fundamentally alter the miRNA's regulatory capacity (175, 177). Together, these mechanisms position miR-142 as a central node integrating immune evasion, proliferative signaling, and genomic instability pathways that collectively drive aggressive disease behavior.

9.3Discussion of other findings

Several established clinical and laboratory parameters were associated with inferior outcomes in patients with LBCL, underscoring the continued prognostic relevance of systemic disease burden, host factors, and treatment-related complications.

B symptoms -Fever (>38^{o}C), drenching sweats, weight loss (10% body weight over 6 months)- are among the key features used to assess patient status and guide prognosis (225, 226). B symptoms were significantly associated with worse prognosis, consistent with prior studies demonstrating that constitutional symptoms reflect high tumor burden, increased inflammatory cytokine production, and biologically aggressive disease behavior (225). B-symptoms have long been incorporated into lymphoma staging systems and are recognized markers of adverse outcome in aggressive lymphomas, including DLBCL (226).

A **high International Prognostic Index (IPI) score (3–5)** was strongly associated with inferior prognosis, consistent with its established role as the most widely validated prognostic

tool in DLBCL. The IPI integrates age, disease stage, performance status, extranodal involvement, and serum LDH, capturing both tumor burden and host-related risk factors (227). Despite advances in molecular classification, the IPI remains a robust predictor of survival and continues to provide clinically meaningful risk stratification in the immunotherapy era (228).

Hypoalbuminemia was identified as a strong adverse prognostic factor in this study. Pretreatment serum albumin levels have important prognostic significance in cancer (229). Low serum albumin levels reflect systemic inflammation, poor nutritional status, and advanced disease, and have consistently been associated with inferior survival in DLBCL (230, 231).

Hyponatremia was similarly associated with poor prognosis. It is the most common electrolyte disorder in clinical practice and is associated with increased morbidity and mortality (232). Hyponatremia may arise from inappropriate antidiuretic hormone secretion, renal dysfunction, or systemic inflammation, and is often indicative of advanced disease or severe illness (232, 233). Prior studies in lymphoma populations have shown that hyponatremia is associated with increased treatment-related toxicity and inferior survival outcomes (232, 233).

The occurrence of **neutropenic fever** during treatment was also associated with worse prognosis. Although neutropenic fever represents a treatment-related complication rather than a baseline disease characteristic, its association with adverse outcomes likely reflects increased treatment toxicity, therapy interruptions, dose reductions, and compromised treatment intensity (234, 235). Moreover, neutropenic complications may disproportionately affect patients with aggressive disease biology or limited physiological reserve, thereby indirectly contributing to poorer survival (234, 235).

Taken together, these findings emphasize the multifactorial nature of prognosis in LBCL, in which disease biology, host condition, and treatment-related factors interact to influence clinical outcomes. Importantly, the persistent prognostic significance of these clinical variables highlights the necessity of interpreting emerging molecular biomarkers, such as circulating miRNAs, within the broader framework of established clinical risk factors. In addition, the concordance of our findings with established prognostic indicators supports the robustness and internal validity of the study, indicating that the cohort and collected data

reliably reflect known patterns of disease behavior and outcome in LBCL. The following figure **(Figure 26)** summarizes the prognostic significance of additional factors in LBCL.

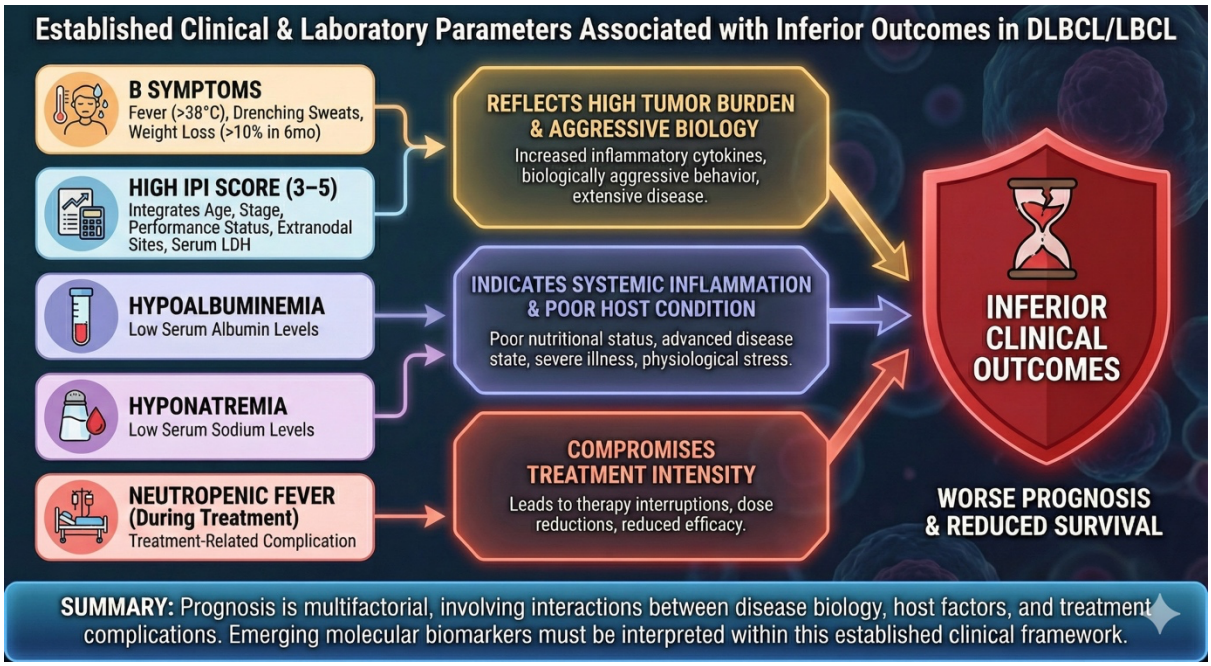

Figure 26. *Established parameters associated with inferior outcomes in LBCL.*

9.4Limitations and Methodological Considerations

The limitations of this study include its single-center design and relatively small sample size, which may limit generalizability and statistical power. Additioanlly, miRNA expression was assessed at diagnosis only, precluding evaluation of dynamic changes during therapy. Another important limitation of this study is the heterogeneity of the lymphoma subtypes included in the initial cohort, which may introduce biological variability and potentially confound the interpretation of prognostic analyses. However, when the analysis was restricted to patients with DLBCL-NOS in order to enhance biological homogeneity (41 of 45 patients), the results remained largely unchanged and were comparable to those obtained in the overall cohort. A key limitation of the Cox proportional hazards models in this study is the relatively small number of patients and outcome events. Survival analyses based on limited events are prone to reduced statistical power and may yield unstable hazard ratio estimates with wide confidence intervals. This constraint can affect the reliability of multivariable modeling, increase the risk of overfitting, and limit the ability to adjust adequately for multiple prognostic factors. Consequently, some associations that did not reach statistical significance in the Cox models may reflect insufficient power rather than a true absence of effect. The findings derived from these analyses should therefore be interpreted with caution and viewed as hypothesis-generating, underscoring the need for validation in larger, independent cohorts.

9.5Future Directions

Future studies should aim to validate these findings in larger, independent, and multicenter cohorts to ensure their robustness and generalizability across diverse patient populations. Particular emphasis should be placed on the adoption of standardized and harmonized protocols for circulating miRNA measurement, including sample processing, normalization strategies, and analytical platforms, to minimize technical variability and facilitate meaningful comparisons across studies worldwide. Ultimately, integrating miRNA profiling with genomic and transcriptomic data may improve personalized risk stratification in LBCL.

9.6Challenges for clinical implementation

Despite the promising results of this and other studies examining circulating miRNAs as biomarkers in LBCL, several substantial challenges must be addressed before clinical implementation can be realized. First, pre-analytical variability represents a major obstacle to reproducibility. Factors including sample collection methodology (serum versus plasma, tube type, processing time), storage conditions, freeze-thaw cycles, and hemolysis can significantly influence measured miRNA concentrations, necessitating rigorous standardization of protocols across institutions (202, 209). Second, analytical standardization remains incomplete; different quantification platforms (qRT-PCR, digital PCR, next-generation sequencing, microarrays), normalization strategies (endogenous controls, spike-in controls, global mean normalization), and data analysis pipelines can yield discordant results, complicating cross-study comparisons and the establishment of universal reference ranges (209). Third, the definition of clinically actionable cutoff values requires validation in large, independent, and ethnically diverse cohorts. The use of percentile-based thresholds derived from healthy controls, as employed in the present study, provides a reasonable starting point but may not optimally discriminate prognostic subgroups; receiver operating characteristic (ROC) curve analysis and outcome-based threshold optimization should be pursued in future investigations. Fourth, the integration of miRNA biomarkers into existing prognostic frameworks—such as the IPI, NCCN-IPI, or emerging molecular classifiers—will require demonstration of added value beyond established parameters, ideally through net reclassification improvement or decision curve analyses. Fifth, cost-effectiveness and turnaround time must be considered; while qRT-PCR–based miRNA quantification is technically feasible in most clinical laboratories, the added expense and time relative to routine staging and laboratory investigations must be justified by meaningful improvements in patient outcomes or resource utilization. Sixth, the biological complexity of miRNA regulation, including the influence of isomiRs, competing endogenous RNAs, and context-dependent target selection, may limit the generalizability of single-miRNA biomarkers argueing for for the development of multi-miRNA signatures or integrated multi-omic panels. Finally, prospective interventional studies will be required to demonstrate that miRNA-guided

treatment stratification improves clinical outcomes compared with standard approaches, a necessary step before regulatory approval and widespread adoption can occur.

10. Conclusions

This study underscores the potential clinical utility of circulating miRNA profiles as complementary biomarkers in large B-cell lymphoma. Our findings highlight the biological and prognostic heterogeneity of LBCL and demonstrate that individual miRNAs exhibit distinct diagnostic and prognostic behaviors. miR-22-3p appears to function primarily as a disease-associated and subtype-linked biomarker, particularly enriched in non-GCB LBCL, without a clear impact on survival outcomes. In contrast, miR-155-3p emerged as a marker of disease aggressiveness, independently predicting inferior OS and reflecting tumor progression rather than disease initiation. Most notably, low miR-142-3p expression was consistently associated with inferior PFS and OS, and retained independent prognostic significance, suggesting a novel and clinically meaningful role for this miRNA in LBCL risk stratification. These molecular findings were observed alongside established clinical predictors, including high IPI score, B symptoms, hypoalbuminemia, hyponatremia, and treatment-related complications, emphasizing the multifactorial nature of prognosis in LBCL. Collectively, our results support the integration of circulating miRNA profiling with conventional clinical parameters to refine prognostic assessment and capture biologically relevant heterogeneity beyond traditional classifications. Validation of these findings in larger, multicenter cohorts, along with rigorous standardization and validation of miRNA measurement methodologies and analytical protocols, will be essential to establish the clinical applicability of miRNA-based biomarkers and to support their integration into multimodal prognostic models for personalized management of LBCL.

Περίληψη

Το Λέμφωμα από Μεγάλα Β κύτταρα (ΛΜΒΚ) παραμένει μια βιολογικά και κλινικά ετερογενής νόσος με σημαντικό ποσοστό ασθενών να παρουσιάζουν υποτροπή ή ανθεκτικότητα παρά τη χορήγηση τυπικών θεραπευτικών σχημάτων όπως το R-CHOP. Η πρόγνωση βασίζεται κυρίως στον Διεθνή Προγνωστικό Δείκτη (IPI) και σε βιολογικούς δείκτες (GCB/ABC υπότυπος, διπλή έκφραση MYC/BCL2). Ωστόσο, υπάρχει ανάγκη για νέους, μη επεμβατικούς και αξιόπιστους βιοδείκτες. Τα microRNAs (miRNAs) είναι ρυθμιστικά μόρια ~22 νουκλεοτιδίων που διαμορφώνουν τη γονιδιακή έκφραση σε μεταμεταγραφικό επίπεδο. Τα κυκλοφορούντα miRNAs έχουν προταθεί ως βιοδείκτες για τη διάγνωση και πρόγνωση στο ΛΜΒΚ.

Σκοπός της μελέτης: Σκοπός ήταν η αξιολόγηση των επιπέδων έκφρασης των miR-155-3p, miR-142-3p και miR-22-3p στον ορό κατά τη διάγνωση και η συσχέτισή τους με κλινικοεργαστηριακές παραμέτρους και την ολική επιβίωση (OS).

Ασθενείς και Μέθοδοι: Συλλέχθηκαν προοπτικά και αναλύθηκαν δείγματα ορού από 45 νεοδιαγνωσμένους ασθενείς με ΛΜΒΚ (εύρος ηλικίας 34–84 έτη, 26 άνδρες) που αντιμετωπίστηκαν στο κέντρο μας επί 4,5 έτη και από 26 υγιείς μάρτυρες. Όλοι οι ασθενείς έλαβαν ανοσοχημειοθεραπεία. Η απομόνωση RNA έγινε με miRNeasy Serum/Plasma Kit και η σύνθεση cDNA με το TaqMan Advanced miRNA cDNA Synthesis Kit (Applied Biosystems). Η qRT-PCR πραγματοποιήθηκε σε σύστημα LightCycler 480 με TaqMan™ Advanced miRNA assays για τα miR-155-3p (477926_mir), miR-142-3p (477910_mir) και miR-22-3p (477985_mir). Χρησιμοποιήθηκε εξωγενές spike-in Ath-miR-159 από Arabidopsis thaliana για ομαλοποίηση, ενώ κάθε δείγμα αναλύθηκε σε διπλότυπα. Οι 25η/75η εκατοστιαίες θέσεις των μαρτύρων ορίστηκαν ως κατώφλια υπο-/υπερέκφρασης. Οι συγκρίσεις έγιναν με τα τεστ Mann–Whitney U/χ^2, η OS αναλύθηκε με τη μέθοδο Kaplan-Meier και το log-rank test. Μεταβλητές με $p<0.05$ στη μονοπαραγοντική ανάλυση αξιολογήθηκαν περαιτέρω σε πολυπαραγοντική ανάλυση χρησιμοποιώντας το μοντέλο Cox proportional hazards, προκειμένου να εκτιμηθεί η ανεξάρτητη προγνωστική τους αξία (SPSS v29, Chicago, IL).

Αποτελέσματα: Η διάμεση ηλικία ήταν τα 67 έτη (58% άνδρες). Η διάμεση διάρκεια παρακολούθησης ήταν 26 μήνες. Συνολικά 13 ασθενείς απεβίωσαν μέχρι το τέλος καταγραφής της μελέτης. Τα επίπεδα των miR-155-3p και miR-142-3p δεν διέφεραν σημαντικά έναντι των μαρτύρων, ενώ το miR-22-3p ήταν αυξημένο στους ασθενείς

(p<0.001). Η χαμηλή έκφραση του miR-142 συσχετίστηκε με αυξημένη έκφραση του MYC (p=0.023) και χαμηλότερη συχνότητα του φαινοτύπου non-GCB (p=0.026), ενώ υψηλή έκφραση του miR-22 παρατηρήθηκε συχνότερα στον υποτύπο non-GCB (p=0.046). Στη μονοπαραγοντική ανάλυση, τα Β-συμπτώματα, η αλβουμίνη <3.5 g/dL, το IPI 3–5, η υπονατριαιμία, το ουδετεροπενικό εμπύρετο, η υπερέκφραση miR-155-3p ορού και η υποέκφραση miR-142-3p ορού συσχετίστηκαν με χειρότερη OS. Η πολυπαραγοντική ανάλυση επιβεβαίωσε την υπερέκφραση miR-155-3p (HR: 6.24, p=0.017) και την υποέκφραση miR-142-3p (HR: 7.52, p=0.01) ως ανεξάρτητους δυσμενείς προγνωστικούς δείκτες.

Συμπεράσματα: Τα αυξημένα επίπεδα miR-22-3p χαρακτηρίζουν τους ασθενείς με ΔΛΜΒΚ, χωρίς όμως να συσχετίζονται με την OS. Αντίθετα, η υπερέκφραση miR-155-3p και η υποέκφραση miR-142-3p αποτελούν ανεξάρτητους δυσμενείς προγνωστικούς παράγοντες για την OS, αναδεικνύοντας τη δυνητική χρησιμότητά τους ως βιοδείκτες στη διαστρωμάτωση κινδύνου. Τα ευρήματα, αν και προκαταρκτικά λόγω του μικρού μεγέθους του δείγματος, υποστηρίζουν την ανάγκη επιβεβαίωσης σε μεγαλύτερες πολυκεντρικές μελέτες και τη διερεύνηση της ενσωμάτωσής τους σε υφιστάμενα προγνωστικά συστήματα.

Abstract

Large B-cell lymphoma (LBCL) remains a biologically and clinically heterogeneous disease, with a substantial proportion of patients experiencing relapse or refractory disease despite standard immunochemotherapy regimens such as R-CHOP. Prognosis is primarily based on the International Prognostic Index (IPI) and selected biological markers, including cell-of-origin classification (GCB/ABC) and MYC/BCL2 double expression. Nevertheless, there is an ongoing need for novel, non-invasive, and reliable biomarkers. MicroRNAs (miRNAs) are small (~22-nucleotide) regulatory molecules that modulate gene expression at the post-transcriptional level, and circulating miRNAs have been proposed as potential diagnostic and prognostic biomarkers in LBCL.

Objective: The aim of this study was to evaluate serum expression levels of miR-155-3p, miR-142-3p, and miR-22-3p at diagnosis and to investigate their associations with clinicopathological parameters and overall survival (OS).

Patients and Methods: Serum samples were prospectively collected and analyzed from 45 newly diagnosed LBCL patients (age range 34–84 years; 26 males) treated at our center over a 4.5-year period, and from 26 healthy controls. All patients received immunochemotherapy. RNA isolation was performed using the miRNeasy Serum/Plasma Kit, and cDNA synthesis was performed using the TaqMan Advanced miRNA cDNA Synthesis Kit (Applied Biosystems). Quantitative RT-PCR was conducted on a LightCycler 480 platform using TaqMan™ Advanced miRNA assays for miR-155-3p, miR-142-3p, and miR-22-3p. Exogenous spike-in Ath-miR-159 from ***Arabidopsis thaliana*** was used for normalization, and all samples were analyzed in duplicate. The 25th and 75th percentiles of controls were used as cutoffs for low and high expression. Statistical analyses included Mann–Whitney U and χ^2 tests, Kaplan–Meier survival analysis with log-rank testing, and multivariable Cox proportional hazards modeling for variables with $p<0.05$ in univariable analysis (SPSS v29).

Results: The median patient age was 67 years (58% male), with a median follow-up of 26 months; 13 patients died during the study period. Serum miR-155-3p and miR-142-3p levels did not differ significantly between patients and controls, whereas miR-22-3p was significantly elevated in LBCL patients ($p<0.001$). Low miR-142 expression was associated with increased MYC expression ($p=0.023$) and a lower frequency of the non-GCB phenotype ($p=0.026$), while high miR-22 expression was more common in non-GCB cases ($p=0.046$). In

univariable analysis, B symptoms, serum albumin <3.5 g/dL, IPI score 3–5, hyponatremia, neutropenic fever, high serum miR-155-3p expression, and low serum miR-142-3p expression were associated with inferior OS. Multivariable analysis confirmed high miR-155-3p expression (HR 6.24, p=0.017) and low miR-142-3p expression (HR 7.52, p=0.01) as independent adverse prognostic factors for OS.

Conclusions: Elevated serum miR-22-3p levels characterize patients with LBCL but are not associated with overall survival. In contrast, high miR-155-3p expression and low miR-142-3p expression represent independent adverse prognostic factors for OS, highlighting their potential utility as circulating biomarkers for risk stratification. Although preliminary due to the limited sample size, these findings warrant validation in larger, multicenter studies and further exploration of their integration into existing prognostic models.

References

1. Alaggio R AC, Anagnostopoulos I, Attygalle AD,et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms . Leukemia. 2022;36(7):1720-1748.
2. Campo E JE, Cook JR, Quintanilla-Martinez L, et al . The International Consensus Classification of Mature Lymphoid Neoplasms: a report from the Clinical Advisory Committee. . Blood. 2022;140(11):1229-1253.
3. Siegel RL, Kratzer TB, Giaquinto AN, et al. Cancer statistics, 2025. CA Cancer J Clin. 2025;75(1):10-45.
4. Thieblemont C, Gomes Da Silva M, Leppä S, et al. Large B-cell lymphoma (LBCL): EHA Clinical Practice Guidelines for diagnosis, treatment, and follow-up. HemaSphere. 2025;9(9):e70207.
5. Kanas G, Ge W, Quek RGW, et al. Epidemiology of diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) in the United States and Western Europe: population-level projections for 2020-2025. Leuk Lymphoma. 2022;63(1):54-63.
6. Cerhan JR, Kricker A, Paltiel O, et al. Medical history, lifestyle, family history, and occupational risk factors for diffuse large B-cell lymphoma: the InterLymph Non-Hodgkin Lymphoma Subtypes Project. J Natl Cancer Inst Monogr. 2014;2014(48):15-25.
7. Teras LR DC, Cerhan JR, Morton LM, Jemal A, Flowers CR. . US lymphoid malignancy statistics by World Health Organization subtypes. . CA Cancer J Clin. 2016;66(6):443-459.
8. Chapuy B SC, Dunford AJ, Kim J, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. . Nat Med 2018:679-690.
9. Feugier P VHA, Sebban C, Solal-Celigny P, et al. Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: a study by the Groupe d'Etude des Lymphomes de l'Adulte. . J Clin Oncol. 2005;23(18):4117-4126.
10. Coiffier B SC. Diffuse large B-cell lymphoma: R-CHOP failure-what to do? . Hematology Am Soc Hematol Educ Program. 2016;1:366-378.
11. Coiffier B LE, Briere J, Herbrecht R, Tilly H, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med. 2002 Jan 24;346(4):235-42. doi: 10.1056/NEJMoa011795. PMID: 11807147. N Engl J Med. 2002;346(4):235-242.
12. Pfreundschuh M KE, Trümper L, Osterborg A,et al. MabThera International Trial (MInT) Group. CHOP-like chemotherapy with or without rituximab in young patients with good-prognosis diffuse large-B-cell lymphoma: 6-year results of an open-label randomised study of the MabThera International Trial (MInT) Group. . Lancet Oncol. 2011;11:1013-1022.
13. Tilly H, Morschhauser F, Sehn LH, et al. Polatuzumab Vedotin in Previously Untreated Diffuse Large B-Cell Lymphoma. N Engl J Med. 2022;386(4):351-363.
14. Parry EM RS, Okosun J. . DLBCL arising from indolent lymphomas: How are they different? . Semin Hematol. 2023;60(5):277-284.
15. Rosenwald A WG, Chan WC, Connors JM, et al. Lymphoma/Leukemia Molecular Profiling Project. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma.
. N Engl J Med. 2002;346(25):1937-1947.

16. Wright GW HD, Phelan JD, Coulibaly ZA, et al. A Probabilistic Classification Tool for Genetic Subtypes of Diffuse Large B Cell Lymphoma with Therapeutic Implications. Cancer Cell. 2020;37(4):551-568.
17. Schmitz R WG, Huang DW, Johnson CA, et al.. Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma.
. N Engl J Med. 2018;378(15):1396-1407.
18. Sehn LH SG. Diffuse Large B-Cell Lymphoma. . N Engl J Med. 2021;384(9):842-858.
19. Tavakkoli M, Barta SK. 2024 Update: Advances in the risk stratification and management of large B-cell lymphoma. Am J Hematol. 2023;98(11):1791-1805.
20. Howlett C, Snedecor SJ, Landsburg DJ, et al. Front-line, dose-escalated immunochemotherapy is associated with a significant progression-free survival advantage in patients with double-hit lymphomas: a systematic review and meta-analysis. Br J Haematol. 2015;170(4):504-514.
21. Project. IN-HsLPF. A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med. 1993;329(14):987-994.
22. Sehn LH BB, Chhanabhai M, Fitzgerald C, et al. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood. 2007;109(5):1857-1861.
23. Zhou Z SL, Rademaker AW, Gordon LI, et al. An enhanced International Prognostic Index (NCCN-IPI) for patients with diffuse large B-cell lymphoma treated in the rituximab era. . Blood. 2014;123(6):837-842.
24. Jelicic J J-JK, Bukumiric Z, Roost Clausen M, et. al.. Prognostic indices in diffuse large B-cell lymphoma: a population-based comparison and validation study of multiple models. . Blood Cancer J. 2023;13(1):157.
25. Ruppert AS DJ, Salles G, Wall A, et al. International prognostic indices in diffuse large B-cell lymphoma: a comparison of IPI, R-IPI, and NCCN-IPI. . Blood. 2020;135(23):2041-2048.
26. Mikhaeel NG HM, Eertink JJ, de Vet HCW, et al. Proposed New Dynamic Prognostic Index for Diffuse Large B-Cell Lymphoma: International Metabolic Prognostic Index. J Clin Oncol. 2022;40(21):2352-2360.
27. Kim H PK. Clinical Circulating Tumor DNA Testing for Precision Oncology. Cancer Res Treat. 2023;55(2):351-366.
28. Wu FT LL, Xu W, Li JY. . Circulating tumor DNA: clinical roles in diffuse large B cell lymphoma. 9. Ann Hematol. 2019;98(2):255-269.
29. Arzuaga-Mendez J P-FE, Lopez-Lopez E, Martin-Guerrero I, García-Ruiz JC, García-Orad A. . Cell-free DNA as a biomarker in diffuse large B-cell lymphoma: A systematic review. . Crit Rev Oncol Hematol. 2019;139:7-15.
30. Tavakkoli M BS. 2024 Update: Advances in the risk stratification and management of large B-cell lymphoma. . Am J Hematol. 2023;98(11):1791-1805.
31. Locke FL, Miklos DB, Jacobson CA, et al. Axicabtagene Ciloleucel as Second-Line Therapy for Large B-Cell Lymphoma. N Engl J Med. 2022;386(7):640-654.
32. Kamdar M, Solomon SR, Arnason J, et al. Lisocabtagene maraleucel versus standard of care with salvage chemotherapy followed by autologous stem cell transplantation as second-line treatment in patients with relapsed or refractory large B-cell lymphoma (TRANSFORM): results from an interim analysis of an open-label, randomised, phase 3 trial. Lancet. 2022;399(10343):2294-2308.

33. Ferreri AJM, Illerhaus G, Doorduijn JK, et al. Primary central nervous system lymphomas: EHA-ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol. 2024.
34. Lymphomas: Essentials for Clinicians. 3 ed. Lugano: ESMO Press; 2022.
35. Renaud L, Donzel M, Decroocq J, et al. Primary mediastinal B-cell lymphoma (PMBCL): The LYSA pragmatic guidelines. Eur J Cancer. 2025;220:115369.
36. Liu PP, Wang KF, Xia Y, et al. Racial patterns of patients with primary mediastinal large B-cell lymphoma: SEER analysis. Medicine (Baltimore). 2016;95(27):e4054.
37. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. Annu Rev Biochem. 2012;81:145-166.
38. Nemeth K, Bayraktar R, Ferracin M, et al. Non-coding RNAs in disease: from mechanisms to therapeutics. Nat Rev Genet. 2024;25(3):211-232.
39. Loganathan T, Doss CG. Non-coding RNAs in human health and disease: potential function as biomarkers and therapeutic targets. Funct Integr Genomics. 2023;23(1):33.
40. Fu XD. Non-coding RNA: a new frontier in regulatory biology. Natl Sci Rev. 2014;1(2):190-204.
41. Diamantopoulos MA, Boti MA, Sarri T, et al. Non-Coding RNAs in Health and Disease: From Biomarkers to Therapeutic Targets. LabMed. 2025;2(3):17.
42. Chen X, Fan S, Song E. Noncoding RNAs: New Players in Cancers. Adv Exp Med Biol. 2016;927:1-47.
43. Lee YS, Shibata Y, Malhotra A, et al. A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). Genes Dev. 2009;23(22):2639-2649.
44. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281-297.
45. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136(2):215-233.
46. H. L. MicroRNAs won the Nobel - will they ever be useful as medicines? . Nature. 2024.
47. Wightman B HI, Ruvkun G. . Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. Cell. 1993;75(5):855-862.
48. Lee RC FR, Ambros V. . The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;75(5):843-854.
49. Friedman RCF, K.K.; Burge, C.B.; Bartel, D.P. . Most mammalian mRNAs are conserved targets of microRNAs. . Genome Res. 2009;19(1):92-105.
50. Friedman RC, Farh KK, Burge CB, et al. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 2009;19(1):92-105.
51. Chen CZ, Lodish HF. MicroRNAs as regulators of mammalian hematopoiesis. Semin Immunol. 2005;17(2):155-165.
52. Saliminejad K, Khorram Khorshid HR, Soleymani Fard S, et al. An overview of microRNAs: Biology, functions, therapeutics, and analysis methods. 2019;234(5):5451-5465.
53. Calin GA, Dumitru CD, Shimizu M, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A. 2002;99(24):15524-15529.
54. Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer. 2006;6(4):259-269.
55. Garzon R, Calin GA, Croce CM. MicroRNAs in Cancer. Annu Rev Med. 2009;60:167-179.

56. Paul P, Chakraborty A, Sarkar D, et al. Interplay between miRNAs and human diseases. J Cell Physiol. 2018;233(3):2007-2018.
57. Markopoulos GS, Roupakia E, Tokamani M, et al. A step-by-step microRNA guide to cancer development and metastasis. Cell Oncol (Dordr). 2017;40(4):303-339.
58. Due H, Svendsen P, Bødker JS, et al. miR-155 as a Biomarker in B-Cell Malignancies. Biomed Res Int. 2016;2016:9513037.
59. Lee Y, Kim M, Han J, et al. MicroRNA genes are transcribed by RNA polymerase II. Embo j. 2004;23(20):4051-4060.
60. Lee Y, Ahn C, Han J, et al. The nuclear RNase III Drosha initiates microRNA processing. Nature. 2003;425(6956):415-419.
61. Nguyen TA, Park J, Dang TL, et al. Microprocessor depends on hemin to recognize the apical loop of primary microRNA. Nucleic Acids Res. 2018;46(11):5726-5736.
62. Lund E, Güttinger S, Calado A, et al. Nuclear export of microRNA precursors. Science. 2004;303(5654):95-98.
63. Chendrimada TP, Gregory RI, Kumaraswamy E, et al. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature. 2005;436(7051):740-744.
64. Westholm JO, Lai EC. Mirtrons: microRNA biogenesis via splicing. Biochimie. 2011;93(11):1897-1904.
65. Telonis AG, Magee R, Loher P, et al. Knowledge about the presence or absence of miRNA isoforms (isomiRs) can successfully discriminate amongst 32 TCGA cancer types. Nucleic Acids Res. 2017;45(6):2973-2985.
66. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69(3):89-95.
67. Califf RM. Biomarker definitions and their applications. Exp Biol Med (Maywood). 2018;243(3):213-221.
68. Oldenhuis CN, Oosting SF, Gietema JA, et al. Prognostic versus predictive value of biomarkers in oncology. Eur J Cancer. 2008;44(7):946-953.
69. Lone SN, Nisar S, Masoodi T, et al. Liquid biopsy: a step closer to transform diagnosis, prognosis and future of cancer treatments. Mol Cancer. 2022;21(1):79.
70. Muringampurath-John D, Jaye DL, Flowers CR, et al. Characteristics and outcomes of diffuse large B-cell lymphoma presenting in leukaemic phase. Br J Haematol. 2012;158(5):608-614.
71. Decruyenaere P, Offner F, Vandesompele J. Circulating RNA biomarkers in diffuse large B-cell lymphoma: a systematic review. Exp Hematol Oncol. 2021;10(1):13.
72. Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. Nat Rev Immunol. 2006;6(2):93-106.
73. Veryaskina YA, Titov SE, Kovynev IB, et al. MicroRNAs in the Myelodysplastic Syndrome. Acta Naturae. 2021;13(2):4-15.
74. Kotaki R, Koyama-Nasu R, Yamakawa N, et al. miRNAs in Normal and Malignant Hematopoiesis. Int J Mol Sci. 2017;18(7).
75. Londin E, Loher P, Telonis AG, et al. Analysis of 13 cell types reveals evidence for the expression of numerous novel primate- and tissue-specific microRNAs. Proc Natl Acad Sci U S A. 2015;112(10):E1106-1115.
76. Montagner S, Dehó L, Monticelli S. MicroRNAs in hematopoietic development. BMC Immunol. 2014;15:14.
77. Chen CZ, Li L, Lodish HF, et al. MicroRNAs modulate hematopoietic lineage differentiation. Science. 2004;303(5654):83-86.

78. Herrera-Merchan A, Cerrato C, Luengo G, et al. miR-33-mediated downregulation of p53 controls hematopoietic stem cell self-renewal. Cell Cycle. 2010;9(16):3277-3285.
79. Khalaj M, Woolthuis CM, Hu W, et al. miR-99 regulates normal and malignant hematopoietic stem cell self-renewal. J Exp Med. 2017;214(8):2453-2470.
80. Guo S, Lu J, Schlanger R, et al. MicroRNA miR-125a controls hematopoietic stem cell number. Proc Natl Acad Sci U S A. 2010;107(32):14229-14234.
81. Georgantas RW, 3rd, Hildreth R, Morisot S, et al. CD34+ hematopoietic stem-progenitor cell microRNA expression and function: a circuit diagram of differentiation control. Proc Natl Acad Sci U S A. 2007;104(8):2750-2755.
82. Bartel DP. Metazoan MicroRNAs. Cell. 2018;173(1):20-51.
83. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov. 2017;16(3):203-222.
84. Tagawa H, Ikeda S, Sawada K. Role of microRNA in the pathogenesis of malignant lymphoma. Cancer Sci. 2013;104(7):801-809.
85. Georgoulis V, Koumpis E, Hatzimichael E. The Role of Non-Coding RNAs in Myelodysplastic Neoplasms. Cancers (Basel). 2023;15(19).
86. Koumpis E, Georgoulis V, Papathanasiou K, et al. The Role of microRNA-155 as a Biomarker in Diffuse Large B-Cell Lymphoma. Biomedicines. 2024;12(12).
87. Peng Y, Croce CM. The role of MicroRNAs in human cancer. Signal Transduct Target Ther. 2016;1:15004.
88. Lu J, Guo S, Ebert BL, et al. MicroRNA-mediated control of cell fate in megakaryocyte-erythrocyte progenitors. Dev Cell. 2008;14(6):843-853.
89. Liu Z, Wang P, Yuan S, et al. LncRNA BC200/miR-150-5p/MYB positive feedback loop promotes the malignant proliferation of myelodysplastic syndrome. Cell Death Dis. 2022;13(2):126.
90. Ramsay RG, Barton AL, Gonda TJ. Targeting c-Myb expression in human disease. Expert Opin Ther Targets. 2003;7(2):235-248.
91. Liu W, Wu M, Huang Z, et al. c-myb hyperactivity leads to myeloid and lymphoid malignancies in zebrafish. Leukemia. 2017;31(1):222-233.
92. Kumar MS, Narla A, Nonami A, et al. Coordinate loss of a microRNA and protein-coding gene cooperate in the pathogenesis of 5q- syndrome. Blood. 2011;118(17):4666-4673.
93. Xiao C, Srinivasan L, Calado DP, et al. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. Nat Immunol. 2008;9(4):405-414.
94. Brinkmann K, Ng AP, de Graaf CA, et al. miR17~92 restrains pro-apoptotic BIM to ensure survival of haematopoietic stem and progenitor cells. Cell Death Differ. 2020;27(5):1475-1488.
95. Lam J, van den Bosch M, Wegrzyn J, et al. miR-143/145 differentially regulate hematopoietic stem and progenitor activity through suppression of canonical TGFβ signaling. Nat Commun. 2018;9(1):2418.
96. Arabanian LS, Fierro FA, Stölzel F, et al. MicroRNA-23a mediates post-transcriptional regulation of CXCL12 in bone marrow stromal cells. Haematologica. 2014;99(6):997-1005.
97. Li X, Xu F, Chang C, et al. Transcriptional regulation of miR-10a/b by TWIST-1 in myelodysplastic syndromes. Haematologica. 2013;98(3):414-419.
98. Coffre M, Koralov SB. miRNAs in B Cell Development and Lymphomagenesis. Trends Mol Med. 2017;23(8):721-736.

99. Gartenhaus KM-MaRB. Role of microRNA deregulation in the pathogenesis of diffuse large B-cell lymphoma (DLBCL). Leuk Res. 2013;37(11).
100. Mohammed Alsaadi MYK, Mahmood Hassan Dalhat, Salem Bahashwan,, Muhammad Uzair Khan AA, Hussein Almehdar and Ishtiaq Qadri Dysregulation of miRNAs in DLBCL: Causative Factor for Pathogenesis, Diagnosis and Prognosis. Diagnostics. 2021;11(1739).
101. Faraoni I AF, Cardone J, Bonmassar E. . miR-155 gene: a typical multifunctional microRNA. . Biochim Biophys Acta. 2009;1792(6):497-505.
102. Bondada MS YY, Nair V. . Multifunctional miR-155 Pathway in Avian Oncogenic Virus-Induced Neoplastic Diseases. Non-Coding RNA. 2019;5(1):24.
103. Thai TH CD, Casola S, Ansel KM, Xiao C, Xue Y, Murphy A, Frendewey D, Valenzuela D, Kutok JL, Schmidt-Supprian M, Rajewsky N, Yancopoulos G, Rao A, Rajewsky K. . Regulation of the germinal center response by microRNA-155.
. Science. 2007;316(5824):604-608.
104. Mattiske S SR, Neilsen PM, Callen DF. . The oncogenic role of miR-155 in breast cancer.
. Cancer Epidemiol Biomarkers Prev. 2012;21(8):1236-1243.
105. Costinean S, Zanesi N, Pekarsky Y, et al. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. Proc Natl Acad Sci U S A. 2006;103(18):7024-7029.
106. Markopoulos GS, Roupakia E, Tokamani M, et al. Roles of NF-κB Signaling in the Regulation of miRNAs Impacting on Inflammation in Cancer. Biomedicines. 2018;6(2).
107. Tili E, Michaille JJ, Cimino A, et al. Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock. J Immunol. 2007;179(8):5082-5089.
108. Sandhu SK, Volinia S, Costinean S, et al. miR-155 targets histone deacetylase 4 (HDAC4) and impairs transcriptional activity of B-cell lymphoma 6 (BCL6) in the Eμ-miR-155 transgenic mouse model. Proc Natl Acad Sci U S A. 2012;109(49):20047-20052.
109. Rai D, Kim SW, McKeller MR, et al. Targeting of SMAD5 links microRNA-155 to the TGF-beta pathway and lymphomagenesis. Proc Natl Acad Sci U S A. 2010;107(7):3111-3116.
110. Jiang D, Aguiar RC. MicroRNA-155 controls RB phosphorylation in normal and malignant B lymphocytes via the noncanonical TGF-β1/SMAD5 signaling module. Blood. 2014;123(1):86-93.
111. Kalkusova K, Taborska P, Stakheev D, et al. The Role of miR-155 in Antitumor Immunity. Cancers (Basel). 2022;14(21).
112. Bauer KM, Round JL, O'Connell RM. No small matter: emerging roles for exosomal miRNAs in the immune system. Febs j. 2022;289(14):4021-4037.
113. Alexander M, Hu R, Runtsch MC, et al. Exosome-delivered microRNAs modulate the inflammatory response to endotoxin. Nat Commun. 2015;6:7321.
114. Wang D, Wang X, Song Y, et al. Exosomal miR-146a-5p and miR-155-5p promote CXCL12/CXCR7-induced metastasis of colorectal cancer by crosstalk with cancer-associated fibroblasts. Cell Death Dis. 2022;13(4):380.
115. Veglia F, Sanseviero E, Gabrilovich DI. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. Nat Rev Immunol. 2021;21(8):485-498.
116. Ai L, Mu S, Wang Y, et al. Prognostic role of myeloid-derived suppressor cells in cancers: a systematic review and meta-analysis. BMC Cancer. 2018;18(1):1220.
117. Lu LF, Thai TH, Calado DP, et al. Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. Immunity. 2009;30(1):80-91.

118. Vigorito E, Perks KL, Abreu-Goodger C, et al. microRNA-155 regulates the generation of immunoglobulin class-switched plasma cells. Immunity. 2007;27(6):847-859.
119. Teng G, Hakimpour P, Landgraf P, et al. MicroRNA-155 is a negative regulator of activation-induced cytidine deaminase. Immunity. 2008;28(5):621-629.
120. Decruyenaere P OF, Vandesompele J. . Circulating RNA biomarkers in diffuse large B-cell lymphoma: a systematic review.
. Exp Hematol Oncol. 2021;10(1):13.
121. Due H SP, Bødker JS, Schmitz A, Bøgsted M, Johnsen HE, El-Galaly TC, Roug AS, Dybkær K. . miR-155 as a Biomarker in B-Cell Malignancies. . Biomed Res Int. 2016.
122. Lopez-Santillan M L-EA, Arzuaga-Mendez J, Lopez-Lopez E, Garcia-Orad A. Circulating miRNAs as biomarkers in diffuse large B-cell lymphoma: a systematic review.
. Oncotarget. 2018;9(32):22850-22861.
123. Huskova H KK, Karban J, Vargova J, Vargova K, Dusilkova N, Trneny M, Stopka T. . Oncogenic microRNA-155 and its target PU.1: an integrative gene expression study in six of the most prevalent lymphomas. Int J Hematol. 2015 Oct;102(4):441-50. doi: 10.1007/s12185-015-1847-4. Epub 2015 Aug 11. PMID: 26261072.
. Int J Hematol. 2015;102(4):441-450.
124. Zhong H XL, Zhong JH, Xiao F, Liu Q, Huang HH, Chen FY. . Clinical and prognostic significance of miR-155 and miR-146a expression levels in formalin-fixed/paraffin-embedded tissue of patients with diffuse large B-cell lymphoma. . Exp Ther Med. 2012;3(5):763-770.
125. Lawrie CH GS, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, Banham AH, Pezzella F, Boultwood J, Wainscoat JS, Hatton CS, Harris AL. . Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. . Br J Haematol. 2008;141(5):672-675.
126. Fang C ZD, Dong HJ, Zhou ZJ, Wang YH, Liu L, Fan L, Miao KR, Liu P, Xu W, Li JY. . Serum microRNAs are promising novel biomarkers for diffuse large B cell lymphoma. . Ann Hematol. 2012;91(4):553-559.
127. Yuan WXG, Y.X.; Na, W.N.; Chao, J.; Yang, X. . Circulating microRNA-125b and microRNA-130a expression profiles predict
chemoresistance to R-CHOP in diffuse large B-cell lymphoma patients. Oncol Lett. 2016;11:423-432.
128. Inada KO, Y.; Cho, Y.; Saito, H.; Iijima, T.; Hori, M.; Kojima, H. . Availability of Circulating MicroRNAs as a Biomarker for Early Diagnosis of Diffuse Large B-Cell Lymphoma.
. Open J Blood Dis. 2015:48-58.
129. Zheng Z SR, Zhao HJ, Fu D, Zhong HJ, Weng XQ, Qu B, Zhao Y, Wang L, Zhao WL. . MiR155 sensitized B-lymphoma cells to anti-PD-L1 antibody via PD-1/PD-L1-mediated lymphoma cell interaction with CD8+T cells. . Mol Cancer 2019;18(1):54.
130. Yuan WXG, Y.X.; Na, W.N.; Chao, J.; Yang, X. . Circulating microRNA-125b and microRNA-130a expression profiles predict chemoresistance to R-CHOP in diffuse large B-cell lymphoma patients. Oncol Lett. 2016;11:423-432.
131. Beheshti A SK, Vanderburg C, Ravi D, McDonald JT, Christie AL, Shigemori K, Jester H, Weinstock DM, Evens AM. . Identification of Circulating Serum Multi-MicroRNA Signatures in Human DLBCL Models. . Sci Rep. 2019;9(1):17261.
132. Dagan LN JX, Bhatt S, Cubedo E, Rajewsky K, Lossos IS. . miR-155 regulates HGAL expression and increases lymphoma cell motility.
. Blood. 2012;119(2):513-520.

133. Caramuta S LL, Ozata DM, Akçakaya P, et al. Role of microRNAs and microRNA machinery in the pathogenesis of diffuse large B-cell lymphoma. . Blood Cancer J. 2013;3(10):152.
134. Kalkusova K TP, Stakheev D, Smrz D. . The Role of miR-155 in Antitumor Immunity. Cancers (Basel). 2022;14(21):5414.
135. Iqbal J SY, Huang X, Liu Y, et al. Global microRNA expression profiling uncovers molecular markers for classification and prognosis in aggressive B-cell lymphoma. Blood. 2015;125(7):1137-1145.
136. Zhu FQ ZL, Tang N, Tang YP, et al. . MicroRNA-155 Downregulation Promotes Cell Cycle Arrest and Apoptosis in Diffuse Large B-Cell Lymphoma. . Oncol Res. 2016;24(6):415-427.
137. Due H SP, Bødker JS, Schmitz A, et al. miR-155 as a Biomarker in B-Cell Malignancies. Biomed Res Int. 2016;2016:9513037. doi: 10.1155/2016/9513037. Epub 2016 May 16. PMID: 27294145; PMCID: PMC4884835.
. 2016.
138. Ahmadvand M EM, Pashaiefar H, Yaghmaie M, et al. Over expression of circulating miR-155 predicts prognosis in diffuse large B-cell lymphoma. . Leuk Res. 2018;70:45-48.
139. Zhong H, Xu L, Zhong JH, et al. Clinical and prognostic significance of miR-155 and miR-146a expression levels in formalin-fixed/paraffin-embedded tissue of patients with diffuse large B-cell lymphoma. Exp Ther Med. 2012;3(5):763-770.
140. Iqbal J, Shen Y, Huang X, et al. Global microRNA expression profiling uncovers molecular markers for classification and prognosis in aggressive B-cell lymphoma. Blood. 2015;125(7):1137-1145.
141. Due H, Schönherz AA, Ryø L, et al. MicroRNA-155 controls vincristine sensitivity and predicts superior clinical outcome in diffuse large B-cell lymphoma. Blood Adv. 2019;3(7):1185-1196.
142. Yuan WX, Gui YX, Na WN, et al. Circulating microRNA-125b and microRNA-130a expression profiles predict chemoresistance to R-CHOP in diffuse large B-cell lymphoma patients. Oncol Lett. 2016;11(1):423-432.
143. Zare N, Haghjooy Javanmard S, Mehrzad V, et al. Evaluation of exosomal miR-155, let-7g and let-7i levels as a potential noninvasive biomarker among refractory/relapsed patients, responsive patients and patients receiving R-CHOP. Leuk Lymphoma. 2019;60(8):1877-1889.
144. Caivano A, La Rocca F, Simeon V, et al. MicroRNA-155 in serum-derived extracellular vesicles as a potential biomarker for hematologic malignancies - a short report. Cell Oncol (Dordr). 2017;40(1):97-103.
145. Anastasiadou E, Seto AG, Beatty X, et al. Cobomarsen, an Oligonucleotide Inhibitor of miR-155, Slows DLBCL Tumor Cell Growth In Vitro and In Vivo. Clin Cancer Res. 2021;27(4):1139-1149.
146. Vigorito E PK, Abreu-Goodger C, Bunting S, Xiang Z, Kohlhaas S, Das PP, Miska EA, Rodriguez A, Bradley A, Smith KG, Rada C, Enright AJ, Toellner KM, Maclennan IC, Turner M. . microRNA-155 regulates the generation of immunoglobulin class-switched plasma cells. . Immunity. 2007;27(6):847-859.
147. Chan JA KA, Kosik KS. . MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells.
. Cancer Res. 2005;65(14):6029-6033.

148. Xu LF WZ, Chen Y, Zhu QS, Hamidi S, Navab R. MicroRNA-21 (miR-21) regulates cellular proliferation, invasion, migration, and apoptosis by targeting PTEN, RECK and Bcl-2 in lung squamous carcinoma, Gejiu City, China.
. PLoS One. 2014;9(8):103698.
149. Chen L ZC, Wang T, You H, Yao R. . Curcumin Inhibits the Proliferation, Migration, Invasion, and Apoptosis of Diffuse Large B-Cell Lymphoma Cell Line by Regulating MiR-21/VHL Axis. . Yonsei Med J
. 2020;61(1):20-29.
150. Cui Q VF, Cristino AS, Salomon C, et al. Circulating cell-free miR-494 and miR-21 are disease response biomarkers associated with interim-positron emission tomography response in patients with diffuse large B-cell lymphoma.
. Oncotarget. 2018;9(78):34644-34657.
151. Wang Y LC. MicroRNA and cancer--focus on apoptosis. . J Cell Mol Med. 2009;13(1):12-23.
152. Ho KK MS, Lam EW. Many forks in the path: cycling with FoxO. Oncogene. 2008;27(16):2300-2311.
153. Zhao X GL, Pan H, Kan D, Majeski M, Adam SA, Unterman TG. Biochem J. Multiple elements regulate nuclear/cytoplasmic shuttling of FOXO1: characterization of phosphorylation- and 14-3-3-dependent and -independent mechanisms 2004;378(3):839-849.
154. Go H JJ, Kim PJ, Kim YG, et al. MicroRNA-21 plays an oncogenic role by targeting FOXO1 and activating the PI3K/AKT pathway in diffuse large B-cell lymphoma.
. Oncotarget. 2015;6(17):15035-15049.
155. Calin GA CC. MicroRNA signatures in human cancers.
. Nat Rev Cancer. 2006;6(11):857-866.
156. Wang WY ZH, Wang L, Ma YP, Gao F, Zhang SJ, Wang LC. . miR-21 expression predicts prognosis in hepatocellular carcinoma.
. Clin Res Hepatol Gastroenterol. 2014;38(6):715-719.
157. Baraniskin A KJ, Schlegel U, Chan A, et al. Identification of microRNAs in the cerebrospinal fluid as marker for primary diffuse large B-cell lymphoma of the central nervous system. . Blood. 2011;117(11):3140-3146.
158. Chen W WH, Chen H, Liu S,et al . Clinical significance and detection of microRNA-21 in serum of patients with diffuse large B-cell lymphoma in Chinese population.
. Eur J Haematol. 2014;92(5):407-412.
159. Mao X SY, Tang J. . Serum miR-21 is a diagnostic and prognostic marker of primary central nervous system lymphoma. . Neuro Sci. 2014;35(2):233-238.
160. Narducci MG AD, Picchio MC, Lazzeri C,et al. MicroRNA profiling reveals that miR-21, miR486 and miR-214 are upregulated
and involved in cell survival in Sezary syndrome. Cell Death Dis 2011; 2: e151.
. Cell Death Dis. 2011;2(e151).
161. Hermeking H. The miR-34 family in cancer and apoptosis. Cell Death Differ. 2010;17(2):193-199.
162. Chim CS, Wong KY, Qi Y, et al. Epigenetic inactivation of the miR-34a in hematological malignancies. Carcinogenesis. 2010;31(4):745-750.
163. Zhai L, Zhao Y, Liu Z, et al. mRNA expression profile analysis reveals a C-MYC/miR-34a pathway involved in the apoptosis of diffuse large B-cell lymphoma cells induced by Yiqichutan treatment. Exp Ther Med. 2020;20(3):2157-2165.

164. Fu J, Imani S, Wu MY, et al. MicroRNA-34 Family in Cancers: Role, Mechanism, and Therapeutic Potential. Cancers (Basel). 2023;15(19).
165. Balatti V, Tomasello L, Rassenti LZ, et al. miR-125a and miR-34a expression predicts Richter syndrome in chronic lymphocytic leukemia patients. Blood. 2018;132(20):2179-2182.
166. Asmar F, Hother C, Kulosman G, et al. Diffuse large B-cell lymphoma with combined TP53 mutation and MIR34A methylation: Another "double hit" lymphoma with very poor outcome? Oncotarget. 2014;5(7):1912-1925.
167. He M, Gao L, Zhang S, et al. Prognostic significance of miR-34a and its target proteins of FOXP1, p53, and BCL2 in gastric MALT lymphoma and DLBCL. Gastric Cancer. 2014;17(3):431-441.
168. Marques SC, Ranjbar B, Laursen MB, et al. High miR-34a expression improves response to doxorubicin in diffuse large B-cell lymphoma. Exp Hematol. 2016;44(4):238-246.e232.
169. Mogilyansky E, Rigoutsos I. The miR-17/92 cluster: a comprehensive update on its genomics, genetics, functions and increasingly important and numerous roles in health and disease. Cell Death Differ. 2013;20(12):1603-1614.
170. Dal Bo M, Bomben R, Hernández L, et al. The MYC/miR-17-92 axis in lymphoproliferative disorders: A common pathway with therapeutic potential. Oncotarget. 2015;6(23):19381-19392.
171. Ota A, Tagawa H, Karnan S, et al. Identification and characterization of a novel gene, C13orf25, as a target for 13q31-q32 amplification in malignant lymphoma. Cancer Res. 2004;64(9):3087-3095.
172. Fassina A, Marino F, Siri M, et al. The miR-17-92 microRNA cluster: a novel diagnostic tool in large B-cell malignancies. Lab Invest. 2012;92(11):1574-1582.
173. Alencar AJ, Malumbres R, Kozloski GA, et al. MicroRNAs are independent predictors of outcome in diffuse large B-cell lymphoma patients treated with R-CHOP. Clin Cancer Res. 2011;17(12):4125-4135.
174. Yan S, Jia C, Quan L, et al. Significance of the microRNA-17-92 gene cluster expressed in B-cell non-Hodgkin's lymphoma. Mol Med Rep. 2019;20(3):2459-2467.
175. Kwanhian W, Lenze D, Alles J, et al. MicroRNA-142 is mutated in about 20% of diffuse large B-cell lymphoma. Cancer Med. 2012;1(2):141-155.
176. Menegatti J, Nakel J, Stepanov YK, et al. Changes of Protein Expression after CRISPR/Cas9 Knockout of miRNA-142 in Cell Lines Derived from Diffuse Large B-Cell Lymphoma. Cancers (Basel). 2022;14(20).
177. Urbanek-Trzeciak MO, Galka-Marciniak P, Nawrocka PM, et al. Pan-cancer analysis of somatic mutations in miRNA genes. EBioMedicine. 2020;61:103051.
178. Lopez-Santillan M, Larrabeiti-Etxebarria A, Arzuaga-Mendez J, et al. Circulating miRNAs as biomarkers in diffuse large B-cell lymphoma: a systematic review. Oncotarget. 2018;9(32):22850-22861.
179. Larrabeiti-Etxebarria A, Lopez-Santillan M, Santos-Zorrozua B, et al. Systematic Review of the Potential of MicroRNAs in Diffuse Large B Cell Lymphoma. Cancers (Basel). 2019;11(2).
180. Alsaadi M, Khan, M., Dalhat, M., Bahashwan, S., Khan, M., Albar, A., Almehdar, H., & Qadri, I. . Dysregulation of miRNAs in DLBCL: Causative Factor for Pathogenesis, Diagnosis and Prognosis. . Diagnostics. 2021;11.

181. Rinaldi F, Marchesi F, Palombi F, et al. MiR-22, a serum predictor of poor outcome and therapy response in diffuse large B-cell lymphoma patients. Br J Haematol. 2021;195(3):399-404.
182. Marchesi F, Regazzo G, Palombi F, et al. Serum miR-22 as potential non-invasive predictor of poor clinical outcome in newly diagnosed, uniformly treated patients with diffuse large B-cell lymphoma: an explorative pilot study. J Exp Clin Cancer Res. 2018;37(1):95.
183. Zhu D, Fang C, He W, et al. MicroRNA-181a Inhibits Activated B-Cell-Like Diffuse Large B-Cell Lymphoma Progression by Repressing CARD11. J Oncol. 2019;2019:9832956.
184. Kozloski GA, Jiang X, Bunting KL, et al. MiR-181a Is a Master Regulator of the Nuclear Factor-κB Signaling Pathway in Diffuse Large B Cell Lymphoma. Blood. 2012;120(21):417-417.
185. Alsaadi M, Khan MY, Dalhat MH, et al. Dysregulation of miRNAs in DLBCL: Causative Factor for Pathogenesis, Diagnosis and Prognosis. Diagnostics (Basel). 2021;11(10).
186. Wang H, Wang A, Hu Z, et al. A Critical Role of miR-144 in Diffuse Large B-cell Lymphoma Proliferation and Invasion. Cancer Immunol Res. 2016;4(4):337-344.
187. Liu Y, Zhou X, Wang X. Targeting the tumor microenvironment in B-cell lymphoma: challenges and opportunities. J Hematol Oncol. 2021;14(1):125.
188. Casey SC, Amedei A, Aquilano K, et al. Cancer prevention and therapy through the modulation of the tumor microenvironment. Semin Cancer Biol. 2015;35 Suppl(Suppl):S199-s223.
189. Koumpis E, Papoudou-Bai A, Papathanasiou K, et al. Unraveling the Immune Microenvironment in Diffuse Large B-Cell Lymphoma: Prognostic and Potential Therapeutic Implications. Curr Issues Mol Biol. 2024;46(7):7048-7064.
190. Bejarano L, Jordão MJC, Joyce JA. Therapeutic Targeting of the Tumor Microenvironment. Cancer Discov. 2021;11(4):933-959.
191. Veglia F SE, Gabrilovich DI. . Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. Nat Rev Immunol. 2021;21(8):485-498.
192. Ai L MS, Wang Y, Wang H, Cai L, Li W, Hu Y. Prognostic role of myeloid-derived suppressor cells in cancers: a systematic review and meta-analysis. . Prognostic role of myeloid-derived suppressor cells in cancers: a systematic review and meta-analysis. BMC Cancer. 2018;18(1):1220.
193. Jabłońska E, Białopiotrowicz, E., Szydłowski, M., Prochorec-Sobieszek, M., Juszczyński, P., & Szumera-Ciećkiewicz, A. DEPTOR is a microRNA-155 target regulating migration and cytokine production in diffuse large B-cell lymphoma cells. Experimental Hematology. 2020.
194. CH. L. MicroRNAs and lymphomagenesis: a functional review.
. Br J Haematol. 2013;160(5):571-581.
195. Medina PP NM, Slack FJ. . OncomiR addiction in an in vivo model of microRNA-21-induced pre-B-cell lymphoma. Nature. 2010;467(7311):86-90.
196. Li J FR, Yang L, Tu W. miR-21 expression predicts prognosis in diffuse large B-cell lymphoma. 2015;8(11):15019–24
. Int J Clin Exp Pathol. 2015;8(11):15019-15024.
197. An G AC, Feng X, Wen K, Zhong M, Zhang L, Munshi NC, Qiu L, Tai YT, Anderson KC. . Osteoclasts promote immune suppressive microenvironment in multiple myeloma: therapeutic implication.
. Blood. 2016;128(12):1590-1603.

198. Wang X LC, Ju S, Wang Y, Wang H, Zhong R. . Myeloma cell adhesion to bone marrow stromal cells confers drug resistance by microRNA-21 up-regulation.
. Leuk Lymphoma. 2011;52(10):1991-1998.
199. Longley J, Foxall, R., Thirdborough, S., Beers, S., & Cragg, M. . MicroRNA manipulation of macrophage polarization in DLBCL to augment antibody immunotherapy. . Cancer Reasearch. 2024(2693).
200. Sun R, Zheng, Z., Wang, L., Cheng, S., Shi, Q., Qu, B., Fu, D., Leboeuf, C., Zhao, Y., Ye, J., Janin, A., & Zhao, W. . A novel prognostic model based on four circulating miRNA in diffuse large B-cell lymphoma: implications for the roles of MDSC and Th17 cells in lymphoma progression. . Molecular Oncology. 2020;15(246-261).
201. Zheng Z XP, Wang L, Zhao HJ, Weng XQ, Zhong HJ, Qu B, Xiong J, Zhao Y, Wang XF, Janin A, Zhao WL. J Exp Clin Cancer Res. 2017 Jun 21;36(1):82. doi: 10.1186/s13046-017-0551-z. PMID: 28637496; PMCID: PMC5480196. MiR21 sensitized B-lymphoma cells to ABT-199 via ICOS/ICOSL-mediated interaction of Treg cells with endothelial cells. . J Exp Clin Cancer Res 2017;36(1):82.
202. Wakabayashi I, Marumo M, Ekawa K, et al. Differences in serum and plasma levels of microRNAs and their time-course changes after blood collection. Pract Lab Med. 2024;39:e00376.
203. Sambrook JaDWR. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory. 2001.
204. Kubista M, et al. The real-time polymerase chain reaction. Molecular Aspects of Medicine. 2006;27(2):95-125.
205. J. B. Interpretation of qPCR curve shapes. MLO Med Lab Obs. 2015;47(6):38,40.
206. Aneja A, et al. Triple-FRET Technique for Energy Transfer Between conjugated polymer and TAMRA Dye with possible applications in medical diagnostics. Journal of Biological Physics. 2008;34(5):487-493.
207. Arya M, et al., . Basic principles of real-time quantitive PCR. Expert Review of Molecular DIagnostics. 2005;5(2):209-219.
208. McShane LM, Altman DG, Sauerbrei W, et al. Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst. 2005;97(16):1180-1184.
209. El-Daly SM, Gouhar SA, Abd Elmageed ZY. Circulating microRNAs as Reliable Tumor Biomarkers: Opportunities and Challenges Facing Clinical Application. J Pharmacol Exp Ther. 2023;384(1):35-51.
210. Kosaka N, Iguchi H, Ochiya T. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Sci. 2010;101(10):2087-2092.
211. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000;403(6769):503-511.
212. Lenz G, Wright G, Dave SS, et al. Stromal gene signatures in large-B-cell lymphomas. N Engl J Med. 2008;359(22):2313-2323.
213. Wang J, Li Y, Ding M, et al. Molecular mechanisms and clinical applications of miR-22 in regulating malignant progression in human cancer (Review). Int J Oncol. 2017;50(2):345-355.
214. Song SJ, Poliseno L, Song MS, et al. MicroRNA-antagonism regulates breast cancer stemness and metastasis via TET-family-dependent chromatin remodeling. Cell. 2013;154(2):311-324.

215. Quivoron C, Couronné L, Della Valle V, et al. TET2 inactivation results in pleiotropic hematopoietic abnormalities in mouse and is a recurrent event during human lymphomagenesis. Cancer Cell. 2011;20(1):25-38.
216. Cimmino L, Dawlaty MM, Ndiaye-Lobry D, et al. TET1 is a tumor suppressor of hematopoietic malignancy. Nat Immunol. 2015;16(6):653-662.
217. Marchesi F, Regazzo, G., Palombi, F., et al. Serum miR-22 as potential non-invasive predictor of poor clinical outcome in newly diagnosed, uniformly treated patients with diffuse large B-cell lymphoma: an explorative pilot study. . Journal of Experimental & Clinical Cancer Research. 2018;37.
218. Rinaldi F, Marchesi, F., Palombi, F., et al. (2021). MiR-22, a serum predictor of poor outcome and therapy response in diffuse large B-cell lymphoma patients. British Journal of Haematology, 195. . MiR-22, a serum predictor of poor outcome and therapy response in diffuse large B-cell lymphoma patients. British Journal of Haematology. 2021;195.
219. Lawrie CH, Soneji S, Marafioti T, et al. MicroRNA expression distinguishes between germinal center B cell-like and activated B cell-like subtypes of diffuse large B cell lymphoma. Int J Cancer. 2007;121(5):1156-1161.
220. Sun W, Shen W, Yang S, et al. miR-223 and miR-142 attenuate hematopoietic cell proliferation, and miR-223 positively regulates miR-142 through LMO2 isoforms and CEBP-β. Cell Res. 2010;20(10):1158-1169.
221. Kramer NJ, Wang WL, Reyes EY, et al. Altered lymphopoiesis and immunodeficiency in miR-142 null mice. Blood. 2015;125(24):3720-3730.
222. Huang W, Paul D, Calin GA, et al. miR-142: A Master Regulator in Hematological Malignancies and Therapeutic Opportunities. Cells. 2023;13(1).
223. Hezaveh K, Kloetgen A, Bernhart SH, et al. Alterations of microRNA and microRNA-regulated messenger RNA expression in germinal center B-cell lymphomas determined by integrative sequencing analysis. Haematologica. 2016;101(11):1380-1389.
224. Lawrie CH, Chi J, Taylor S, et al. Expression of microRNAs in diffuse large B cell lymphoma is associated with immunophenotype, survival and transformation from follicular lymphoma. J Cell Mol Med. 2009;13(7):1248-1260.
225. Wu C, Zhang Y, Yang F, et al. A comprehensive analysis of B symptoms reveals prognosis and heterogeneity across different sites of diffuse large B-cell lymphoma. Ann Hematol. 2025;104(11):5829-5840.
226. Armitage JO. Staging non-Hodgkin lymphoma. CA Cancer J Clin. 2005;55(6):368-376.
227. A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med. 1993;329(14):987-994.
228. Sehn LH, Berry B, Chhanabhai M, et al. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood. 2007;109(5):1857-1861.
229. Gupta D, Lis CG. Pretreatment serum albumin as a predictor of cancer survival: a systematic review of the epidemiological literature. Nutr J. 2010;9:69.
230. de Pádua Covas Lage LA, De Vita RN, de Oliveira Alves LB, et al. Predictors of Survival, Treatment Modalities, and Clinical Outcomes of Diffuse Large B-Cell Lymphoma in Patients Older Than 70 Years Still an Unmet Medical Need in 2024 Based on Real-World Evidence. Cancers (Basel). 2024;16(8).
231. Kaneko H, Shimura K, Yoshida M, et al. Serum Albumin Levels Strongly Predict Survival Outcome of Elderly Patients with Diffuse Large B-Cell Lymphoma Treated with Rituximab-Combined Chemotherapy. Int J Hematol Oncol Stem Cell Res. 2022;16(1):1-8.

232. Koumpis E, Florentin M, Hatzimichael E, et al. Hyponatremia in Patients with Hematologic Diseases. J Clin Med. 2020;9(11).
233. Castillo JJ, Glezerman IG, Boklage SH, et al. The occurrence of hyponatremia and its importance as a prognostic factor in a cross-section of cancer patients. BMC Cancer. 2016;16:564.
234. Pettengell R, Johnsen HE, Lugtenburg PJ, et al. Impact of febrile neutropenia on R-CHOP chemotherapy delivery and hospitalizations among patients with diffuse large B-cell lymphoma. Support Care Cancer. 2012;20(3):647-652.
235. Hirakawa T, Yamaguchi H, Yokose N, et al. Importance of maintaining the relative dose intensity of CHOP-like regimens combined with rituximab in patients with diffuse large B-cell lymphoma. Ann Hematol. 2010;89(9):897-904.