



ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ



ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ

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A Meta-Analysis of microRNA profiling data in Acute Myeloid Leukemia

Μετα-ανάλυση δεδομένων έκφρασης microRNA στην Οξεία Μυελογενή Λευχαιμία

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I. Abstract

Introduction: Acute Myeloid Leukemia (AML) is a heterogeneous disease characterized by the abnormal proliferation of myeloid leukemic blasts in the bone marrow. Leukemogenesis is a complex process that results from deregulation of several cellular processes. Several studies show that microRNA (miRNA) deregulation contributes to AML pathogenesis.

Objectives: A meta-analysis to decipher the AML miRNA deregulation compared to healthy controls and to investigate possible roles of significantly deregulated miRNAs.

Methods: A systematic literature database and Genome Expression Omnibus (GEO) database search was implemented with defined eligibility criteria for study inclusion. Study derived miRNA lists were prioritized and analyzed with the robust rank aggregation (RRA) method. Bonferroni correction was implemented. Significant miRNAs were further studied through target prediction analysis and pathway enrichment analysis.

Results: From the literature dataset 12 studies were included for analysis, as well as 7 studies from the GEO database. The RRA method implementation and Bonferroni correction resulted in a 5 miRNA (hsa-miR-9, hsa-miR-31, hsa-miR-139-5p, hsa-miR-145, hsa-miR-181a-3p) meta-signature. Subsequent, target prediction analysis resulted in multiple miRNA associated targets and enrichment analysis showed several distinct cellular processes.

Conclusion: A five miRNA meta-signature that reached statistical significance is found to be deregulated in AML compared to healthy controls.

Keywords: acute myeloid leukemia; microRNA; meta-analysis; systematic review; target prediction analysis; pathway enrichment analysis.

I. Περίληψη

Εισαγωγή: Η οξεία μυελογενής λευχαιμία (ΟΜΛ) αποτελεί μια εξαιρετικά ετερογενή νόσο που χαρακτηρίζεται από τον ανεξέλεγκτο πολλαπλασιασμό μυελικών βλαστών στον μυελό των οστών. Η λευχαιμογένεση αποτελεί μια πολύπλοκη διαδικασία που προκαλείται από την διαταραχή πολλαπλών κυτταρικών λειτουργιών. Πολλαπλές μελέτες δείχνουν ότι η απορρύθμιση πολλαπλών microRNA (miRNA) συνεισφέρει στην παθογένεση της ΟΜΛ.

Στόχοι: Η διενέργεια μετα-ανάλυσης για την αποσαφήνιση της απορρύθμισης των miRNA στην ΟΜΛ σε σχέση με υγιείς μάρτυρες και η μελέτη πιθανών βιολογικών λειτουργιών σημαντικά απορυθμισμένων miRNA.

Μέθοδοι: Διενεργήθηκε συστηματική ανασκόπηση βιβλιογραφικών και γενετικών (Gene Expression Omnibus- GEO) βάσεων δεδομένων με προκαθορισμένα κριτήρια συμπερίληψης. Οι λίστες miRNA που προέκυψαν κατηγοριοποιήθηκαν βάσει σημαντικότητας και αναλύθηκαν μέσω της μεθόδου robust rank aggregation (RRA). Στα αποτελέσματα εφαρμόστηκε διόρθωση Bonferroni. Στατιστικά σημαντικά miRNA αναλύθηκαν ακολούθως μέσω ανάλυσης πρόβλεψης στόχου και ανάλυσης εμπλουτισμού.

Αποτελέσματα: Από την βιβλιογραφική ανασκόπηση προέκυψαν 12 μελέτες για περαιτέρω ανάλυση, ενώ από την GEO 7 μελέτες. Η εφαρμογή της μεθόδου RRA και της διόρθωση Bonferroni ανέδειξε 5 miRNA (hsa-miR-9, hsa-miR-31, hsa-miR-139-5p, hsa-miR-145, hsa-miR-181a-3p) με στατιστικά αλλαγή στην έκφραση μεταξύ πασχόντων και μαρτύρων. Οι επακόλουθες αναλύσεις πρόβλεψης στόχου και εμπλουτισμού ανέδειξαν πολλαπλούς mRNA στόχους και πολλές διαφορετικές εμπλεκόμενες κυτταρικές λειτουργίες αντίστοιχα.

Συμπεράσματα: Πέντε miRNA φαίνεται πως έχουν στατιστικά σημαντική διαφορά στην έκφραση μεταξύ των ασθενών με ΟΜΛ και υγιών μαρτύρων.

Λέξεις-κλειδιά: οξεία μυελογενής λευχαιμία; microRNA; μετα-ανάλυση; συστηματική ανασκόπηση; ανάλυση πρόβλεψης στόχου; ανάλυση εμπλουτισμού.

II. Introduction

Acute Myeloid Leukemia (AML) represents a heterogenous group of myeloid neoplasms characterized by the abnormal proliferation and infiltration of the bone marrow (BM) by poorly differentiated clonal blast cells ⁽¹⁾. AML represents the most common acute leukemia in adults, with a median age at diagnosis of 65 years ⁽²⁾⁽³⁾, whereas in children and adolescents is rare ⁽⁴⁾. 5-year-survival in AML patients is low, with chemoresistance and disease relapse occurring frequently ⁽⁵⁾.

Regarding disease subtype classification, there exist two main classification systems which integrate molecular, cytogenetic, clinical, and morphological features: the World Health Organization (WHO) 5th edition of myeloid and histiocytic/dendritic neoplasms ⁽⁶⁾ along with the earlier published 4th edition ⁽⁷⁾ and the International Consensus Classification of myeloid neoplasms and acute leukemia ⁽⁸⁾. Earlier, classifications systems (such as the widely used French- American- British classification ⁽⁹⁾) relied more in morphology, maturation features and clinical characteristics. The published literature analyzed herein relies primarily on the 4th edition of the WHO classification and the FAB classification (*Table 1*).

FAB classification	Disease Name
M0	Undifferentiated acute myeloblastic leukemia
M1	Acute myeloblastic leukemia with minimal maturation
M2	Acute myeloblastic leukemia with maturation
M3	Acute promyelocytic leukemia
M4	Acute myelomonocytic leukemia
M4Eos	Acute myelomonocytic leukemia with eosinophilia
M5	Acute monocytic leukemia
M6	Acute erythroid leukemia
M7	Acute megakaryoblastic

Table 1: French- American- British (FAB) classification of AML.

In de novo AML, gene sequencing has revealed that AML cases harbor at least 10 mutations which can be broadly categorized in nine domains of cellular functions that affect leukemogenesis: DNA methylation, transcription factor (TF) fusions, tumor suppressors, nucleophosmin function, cell signaling, chromatin modification, myeloid TFs, cohesion complex and spliceosome complex ⁽¹⁰⁾. The most frequently mutated genes include: fms like tyrosine kinase 3 (FLT3) (28 percent), nucleophosmin1 (NPM1) (27 percent), DNA-methyltransferase 3A (DNMT3A) (26 percent), among others ⁽¹⁰⁾⁽¹¹⁾. Cytogenetic alterations also contribute greatly to the pathogenesis of AML, with the most common being: translocation t(15;17) (q24.1;q21.2) and variants (13 percent), trisomy 8 (10 percent), t(8;21)(q22;q22.1) and variants (7 percent), 11q23.3 rearrangements (6 percent), inversion inv(16)(p13.1q22)/t(16;16)(p13.1;q22) (5 percent) ⁽¹²⁾.

Leukemogenesis is a multistep process resulting in the leukemic transformation of a myeloid precursor cell. The two-hit hypothesis of leukemogenesis suggests that the leukemic clone arises from at least two genetic lesions ⁽¹³⁾: class I mutations which provide the clone with a

proliferative advantage (e.g., FLT3-ITD mutations) and class II mutations which halt differentiation (e.g., CEBPA mutations)⁽¹⁴⁾.

MicroRNAs (miRNAs) represent a class of small, non-coding RNAs with a length of 19~ 22 nucleotides, that play a major role in post-transcriptional gene regulation and as a result affect many cellular processes proliferation, differentiation, apoptosis and hematopoiesis⁽¹⁵⁾. Consequently, deregulated miRNA expression in AML might aid leukemic transformation through multiple pathways.

In recent years, an increasing number of studies have demonstrated that miRNAs in AML cases are differentially expressed in contrast to healthy individuals, along with an association between miRNA signature and AML subtype. However, no meta-analysis of published articles and public database data has been conducted to date.

III. Methods

Search strategy and study selection

A systematic database search was conducted for the identification of microRNA profiling studies in AML, in a two-level fashion. Firstly, a comprehensive literature database search was performed. From July 11th 2023 to July 29th 2023, the databases PubMed, Google Scholar, Science Direct were thoroughly researched using the terms: “Acute Myeloid Leukemia”, “AML”, “microRNA”, “miRNA”. The initial results were screened based on relevance and eligibility. After the initial screening the included studies were assessed for eligibility and relevance based on title and abstract. The included studies were assessed as full text for eligibility. All duplicates were removed. Secondly, a database search on the publicly available genomic databases “Gene Expression Omnibus (GEO)” was performed with a similar screening process. All database searches were conducted in accordance with the *PRISMA 2020 guidelines*⁽¹⁶⁾ and the resulting PRISMA flowchart was created using *PRISMA2020*⁽¹⁷⁾.

Eligibility criteria

Due to the inherent high degree of heterogeneity in these kinds of studies, a strict set of eligibility criteria was implemented: 1) Human studies, 2) Case- healthy control study design, 3) No prior treatment for AML at the time of sampling, 4) No review articles, 5) English language, 6) No cell line data, 7) No Tumor Cancer Genome Atlas (TCGA) data, 8) No preselected miRNA (when the preselected miRNA number was under three).

Data extraction

Lists of available microRNA lists were extracted, along with their expression values and significance values- if available. Furthermore, a set of data from each individual article- such as: author, publication year, number of miRNA probes, platform used, number of cases and controls- were extracted. Some studies produced lists of differentially expressed miRNAs, through comparison of disease subtype (e.g., FAB subtype or Cytogenetic lesion) with healthy controls. These lists were treated autonomously without pooling the study data into a single list.

Statistical analysis

All extracted data were processed using Microsoft Excel. Regarding literature database miRNA lists: individual miRNA names were standardized through the latest miRbase version and all expression data (e.g., fold change, or counts per million) were log 2 transformed (if this was not already done by the authors). MiRNA lists were then sorted and ranked based on the absolute expression value, in a descending order. This was a necessary preprocessing step before the implementation of the robust rank aggregation (RRA) method ⁽¹⁸⁾. The RRA algorithm assumes that imported data are ranked based on significance (i.e., differential expression change), and the data are then compared to a null model that assumes random order of input lists. The output lists contain p- values assigned to each element (miRNA). False positive results were dealt with the Bonferroni correction method. For the GEO databases, the GEO2R ⁽¹⁹⁾ and GEOexplorer ⁽²⁰⁾ online webtools were at first utilized to generate the lists of differentially expressed miRNAs. Then the top 200 miRNAs from each ranked miRNA list were also analyzed through the RRA algorithm. Graphs were created using IBM SPSS Statistics Data Editor version 29.0.0. Significance was set to a p- value of 0.05.

Target prediction and Enrichment analysis

The meta- signature miRNAs were selected for target prediction analysis by using TargetScan v.8 ⁽²¹⁾ and DIANA microT- CDS 2023 webserver ⁽²²⁾. The top five miRNA targets from each algorithm were selected based on context score (for TargetScan) and interaction scores (for microT- CDS). Furthermore, DIANA TarBase v8 ⁽²³⁾ was used for experimentally validated target identification. Gene targets that overlapped between the prediction algorithm results or were found to be also experimentally identified, were further analyzed through the Ensembl Database (Release 110) ⁽²⁴⁾.

Pathway identification of predicted miRNA targets was carried out through DIANA miRPath v4 ⁽²⁵⁾ using pathway union analysis, target selection from TarBase v8 and pathway selection from the Kyoto Encyclopedia of Genes and Genomes (KEGG), as well as Genome Ontology (GO).

When meta-signature miRNAs were not reported as mature miRNAs based on the latest nomenclature (e.g., hsa-miR-9 and not has-miR-5p or hsa-miR-3p) both mature miRNA strands were used for the target prediction and pathway enrichment analysis.

IV. Results

Study selection and data extraction

Database search initially resulted in 946 publications from literature databases and 185 publications from the GEO database with 12 studies and 7 studies ultimately meeting the inclusion criteria- through a rigorous process (Figure 1). Four studies ⁽³⁹⁾⁽⁴¹⁾⁽⁴²⁾⁽⁴⁴⁾ were deleted from the literature results due to overlap with the GEO database results.

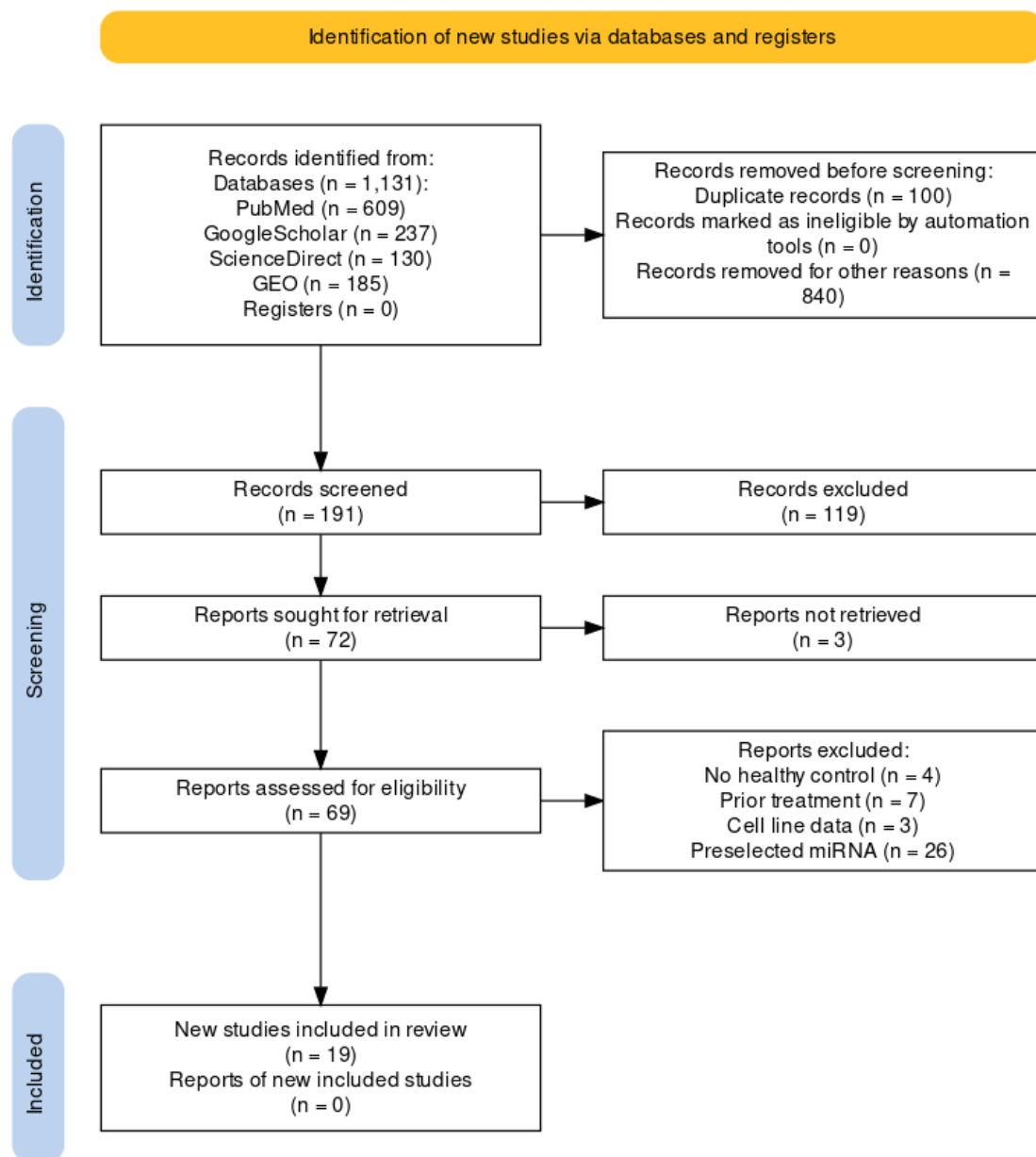


Figure 1: The resulting PRISMA flowchart.

Regarding the literature database results, five studies ⁽²⁸⁾⁽²⁹⁾⁽³⁰⁾⁽³⁵⁾⁽³⁶⁾ were not used in the meta-signature analysis due to limited data availability. Most studies used quantitative real time polymerase chain reaction (qRT-PCR) or a microarray method for differential miRNA expression identification. Average miRNA probe number was 368, with a range between 10 and 1488 miRNA probes- depending on the platform used. A total of 942 AML patients and 272 healthy controls were included. The majority of studies used FAB- classification or (cyto)genetic lesions as patient categorization and subsequent comparative analysis. Concerning the FAB

classification: 19 patients classified as M0, 43 as M1, 221 as M2, 121 as M3, 140 as M4, 156 as M5, 18 as M6, 7 as M7, 36 were characterized as not otherwise specified (NOS) and 71 patients were not categorized based on the FAB classification.

Apropos of the GEO database studies, most studies were conducted using either next generation sequencing or microarray. Average probe number was 792 (ranging from 559 to 851). A total of 133 AML patients and 74 controls were analyzed.

A summary of characteristics of the included studies can be found in Table 2 and Table 3.

Author	Year	Cases	Controls	miRNA	Platform	Age
Fayyad-Kazan et al (26)	2013	20	20	380	TaqMan Human MicroRNA Array A	Adult
Cammarata et al (27)	2010	29	3	365	TaqMan Custom Array	Adult
Memari et al (28)	2022	40	50	10	ABI157 PRISM 7500 real-time PCR System	N/A
Lutherborrow et al (29)	2011	27	8	1488	miRCURY LNA microRNA probe set	Adult
Isken et al (30)	2007	50	12	154	TaqMan master	Adult
Moussa Agha et al (31)	2020	31	11	380	TaqMan Human MicroRNA Array A	Adult
Zhi et al (32)	2013	140	135	25	Solexa sequencing analysis	Adult
Jongen-Lavrencic et al (33)	2008	215	4	260	TaqMan microRNA assay	Adult
Garzon et al (34)	2008	122	10	N/A	TaqMan miRNA assays, microarray assay	Adult
Daschkey et al (35)	2013	102	3	493	TaqMan miRNA Assays, miRXplore Microarray	Paediatric
Ding et al (36)	2018	156	10	N/A	N/A	Adult
Wang et al (37)	2012	10	6	123	miRCURY LNA arrays	Adult

Table 2: Literature dataset studies.

Author	Year	ID	Cases	Controls	Platform	Age
Vanhooren J et al (38)	2022	GSE196886	11	7	Illumina NextSeq500	Paediatric
Wieser R et al (39)	2013	GSE49665	52	13	miRCURY LNA arrays	Adult
Esa E et al (40)	2019	GSE142699	24	24	NanoString nCounter Human miRNA	Adult
Middleton F et al (41)	2019	GSE128079	10	9	Illumina NextSeq500	Adult
Abdelhammed S et al (42)	2021	GSE159028	12	12	Illumina NextSeq500	Adult
Link DC et al (43)	2010	GSE24222	1	1	Agilent- 021827 Human miRNA Microarray	Adult
Leoncini PP et al (44)	2022	GSE209871	23	8	nCounter Human v2 miRNA Expression Assay	Paediatric

Table 3: GEO dataset studies.

Meta- Analysis

A meta-analysis for significantly differential expressed miRNAs was conducted for each database (literature- derived studies and GEO- derived studies). From the literature database 24 miRNAs were found to be differentially expressed with an unadjusted p- value < 0.05. However, after Bonferroni correction only 4 miRNAs were found to be significantly deregulated (hsa-miR-9, hsa-miR-31, hsa-miR-139-5p, hsa-miR-145). The meta-signature of the GEO database revealed a four- miRNA signature that reached statistical significance with an unadjusted p- value. Not surprisingly, after the Bonferroni correction only one miRNA (hsa-miR-181a-3p) was of marginal statistical insignificance with an adjusted p- value of 0.055, nevertheless it was included for further analysis. Accumulatively, the resulting meta- analysis revealed a five- miRNA signature, that was further analyzed.

The most significantly deregulated miRNA was hsa-miR-9. In the literature databases it was reported in 3 out of 12 studies ⁽³¹⁾⁽³²⁾⁽³³⁾. Jongen-Lavrencic et al ⁽³³⁾ report a significant downregulation of hsa-miR-9 (with the exception of the 11q23 rearranged cohort that was upregulated). Zhi et al ⁽³²⁾ also report significant downregulation of hsa-miR-9. In contrast, Moussa-Agha et al ⁽³¹⁾ report an increase in miR-9 levels. Regarding the GEO dataset, hsa-miR-

9 was found to be downregulated in two studies (GSE159028⁽⁴²⁾ and GSE209871⁽⁴⁴⁾) without being statistically significant. Another differentially expressed miRNA of high significance (hsa-miR-31) presents a more uniform downregulated expression pattern among studies. In the literature dataset it is reported as significantly decreased in expression in three studies⁽²⁷⁾⁽³³⁾⁽³⁷⁾, as well as being reported as downregulated in the GEO study GSE142699⁽⁴⁰⁾ albeit with a p-value > 0.05. The remaining significant miRNAs (hsa-miR-5p, has-miR-145) and hsa-miR-181a-3p present with inconclusive reports regarding their regulation trend.

On a genome level, the deregulated meta-signature resides in different scattered genomic loci, apart from hsa-miR-9 and hsa-miR-181a-3p that are located in chromosome 1. The deregulated meta-signature p-values and chromosomal locations are found in *Table 4*.

microRNA	p-value	Adjusted p-value	Chromosome
hsa-miR-9	0.000002105326	0.001814791	1q22
hsa-miR-31	0.00001162547	0.010021155	9p21.3
hsa-miR-139-5p	0.00001162547	0.010021155	11q13.4
hsa-miR-145	0.00004713528	0.040630611	5q32
hsa-miR-181a-3p	0.00003944961	0.0553478	1q32

Table 4: The five miRNA meta-signature of AML.

Target prediction and enrichment analysis

The resulting meta-signature of miRNAs that reached statistical significance (along with hsa-miR-181a-3p) were further analyzed for target prediction and pathway enrichment analysis. A summary of target counts for each miRNA in each database can be found in *Figure 2*. MiR-9 (both hsa-miR-9-3p and hsa-miR-9-5p) has the most targets in all database searches, whereas the hsa-miR-181a-3p has the least. The top five gene targets for each individual miRNA predicted in TargetScan and DIANA microT-CDS, as well as the number of experimentally identified interactions through TarBase search, can be found in *Table 5*. Top gene targets that overlapped either between the prediction algorithm results or TarBase results were further analyzed with Ensembl Release 110. Four out of six protein coding genes recorded are transcription factors (*T-box transcription factor 1* (TBX1, ENSG00000184058), *POU class 2 homeobox 1* (POU2F1, ENSG00000143190), *one cut homeobox 2* (ONECUT2, ENSG00000119547), *Y-box binding protein 3* (YBX3, ENSG00000060138)) associated with blood/ lymphatic vessel development (TBX1), transcription regulation (POU2F1), organ morphogenesis/ cell fate and migration (ONECUT2) and GM-CSF promoter regulation (YBX3). Besides these, another gene target is *fascin actin-bundling protein 1* (FSCN1, ENSG00000075618) associated with actin filament assembly, cytoskeleton organization and cell motility. The sole experimental target of hsa-miR-181a-3p is *mitochondrial ribosomal protein S35* (MRPS35- ENSG00000061794) that is a functional component of mitochondrial translation.

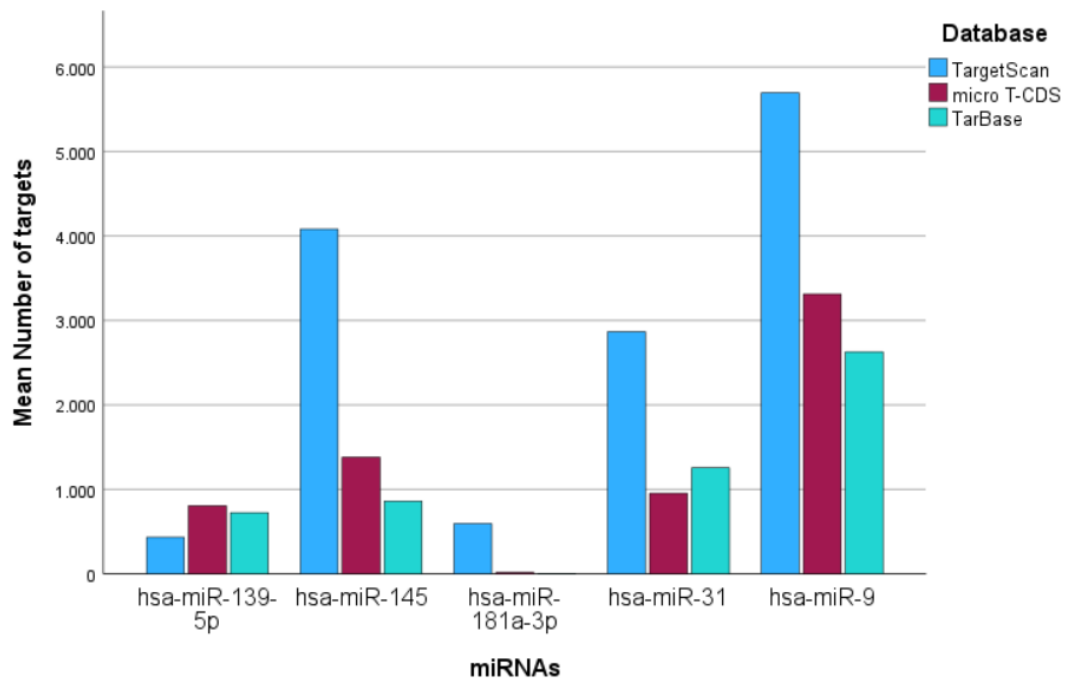


Figure 2: miRNA target count per miRNA and database.

microRNA	Database	Targets
hsa-miR-9	TargetScan (-3p)	ZNF99, ACTL6A, DSCC1, MZT1, NAA20
	TargetScan (-5p)	ONECUT2, YBX3, LYVE1, POU2F1, ONECUT1
	microT-CDS	ONECUT2, POU2F1, RIVF111, YBX3, MAGT1
	TarBase	2628 Interactions
hsa-miR-31	TargetScan (-3p)	POLR2K, ZMYM5, HAUS4, PCP4, RHOXF4
	TargetScan (-5p)	RNF144B, RSBN1, SH2D1A, AK4, PAX9
	microT-CDS	RSBN1, SSX2B, KRT36, VAT1L, MTAP
	TarBase	1255 Interactions
hsa-miR-139-5p	TargetScan	TBX1, H2AZ2, DPY30, ARL15, MORN4
	microT-CDS	PDE3A, CDH20, DCC, TBX1, PRDM16
	TarBase	724 Interactions (including TBX1)
hsa-miR-145	TargetScan (-3p)	DST, SLC30A5, SPINK13, SCT, COA1
	TargetScan (-5p)	CSRNP2, FSCN1, ABHD17C, MYO5A, FLI1
	microT-CDS	NMNAT3, SRGAP2, DAB2, FSCN1, TRIM2, FLI1
	TarBase	861 Interactions (including FSCN1)
hsa-miR181a-3p	TargetScan	NAP1L5, MAP3K5, ARL4A, AGPAT4, DEXI
	microT-CDS	RIBC1, TAL1, DDX41, TRAPPC4, DIMT1
	TarBase	1 Interaction (MRPS35)

Table 5: Top miRNA target genes per database.

Through miRPath v.4 pathway enrichment analysis was performed. Several pathways from GO and KEGG pathways were found to be significant. In the GO analysis, the most frequent associated pathways were protein binding related and nucleoplasm – cytoplasm compartment associated. In the KEGG analysis, cellular signaling pathways and cancer associated pathways were the most enriched. The top enriched pathways from GO and KEGG are reported in *Table 6*.

Term Name	Term Genes	miRNAs (n)	Merged FDR
GO			
protein binding	14297	7	4,4016E-137
nucleus	7177	7	1,8673E-113
cytosol	5614	6	8,00253E-92
cytoplasm	7391	5	2,93076E-89
nucleoplasm	4107	6	7,48813E-87
KEGG			
Neurotrophin signaling pathway	124	3	1,37569E-08
MAPK signaling pathway	329	3	1,01191E-07
Focal adhesion	213	2	1,01191E-07
Transcriptional misregulation in cancer	206	3	2,09337E-07
Salmonella infection	277	2	3,19031E-07

Table 6: Top GO compartments and KEGG pathways enriched by the meta- signature miRNA targets.

V. Conclusion

Through robust rank aggregation, 7 and 21 prioritized miRNA lists that were extracted from the GEO and Literature datasets respectively, ultimately identified a set of 4 significant deregulated miRNAs (hsa-miR-9, hsa-miR-31, hsa-miR-145, has-miR-139-5p) between AML patients and healthy controls from the literature dataset and one miRNA that was marginally not statistically significant from the GEO dataset (hsa-miR-181a-3p).

The trend of deregulation of each miRNA is not concise across reports of individual studies, apart from hsa-miR-31 being downregulated athwart different studies. The most significantly deregulated miRNA, hsa-miR-9, was found to have not a uniform trend of deregulation across studies. Jongen-Lavrencic et al ⁽³³⁾ and Zhi et al ⁽³²⁾ showed that hsa-miR-9 is downregulated compared to normal controls. However, in the subset of 11q23 rearranged (mixed lineage leukemia-(MLL-)) AML in the study by Jongen-Lavrencic et al ⁽³³⁾, hsa-miR-9 was significantly upregulated, a finding that is confirmed by Chen P et al ⁽⁴⁵⁾. Moreover, abnormally increased hsa-miR-9 expression might be a negative prognostic factor in AML ⁽⁴⁶⁾. Important predicted targets include transcription factors like ONECUT1 and 2, POU2F1 and YBX3 among others

(Table 5), signifying the pleiotropic effects of this miRNA in cellular function. The second most deregulated miRNA that showed consistent downregulation in AML patients across studies is hsa-miR-31. Interestingly, hsa-miR-31-5p is found to be repressed in human leukemic stem cells (LSCs), with its restoration eliminating LSCs and halting AML progression ⁽⁴⁷⁾. Regarding, hsa-miR-139-5p and hsa-miR-145 their expression levels in AML patients varied among different studies in both the GEO and Literature datasets. As far as has-miR-181a-3p is concerned, even though its deregulation resulted in an adjusted p- value of 0.055, it was included in further analyses. The deregulation signature is also found to be inconclusive between the included studies. However, its downregulation is associated with adverse outcome in cytogenetically normal (CN-) AML ⁽⁴⁷⁾.

AML is a highly heterogenous malignancy, evident by the different classifications and the diverse prognosis. Several of the included studies, compared different AML subtypes (either by FAB type, cytogenetic or molecular lesion) with healthy controls, that showed specific miRNA signatures can be associated with AML subtypes ⁽³⁵⁾⁽³⁴⁾⁽³³⁾. However, in the present study a subgroup meta- analysis of AML subtype specific meta- signature was not conducted.

The present study contains several limitations due to data availability, data preprocessing and the inherent heterogeneity of included studies due to different platforms utilized and different data processing methods. Nevertheless, the resulting miRNA meta-signature provides interesting starting points for further basic and clinical AML research.

VI. References

1. Döhner H, Weisdorf DJ, Bloomfield CD. Acute Myeloid Leukemia. *N Engl J Med*. 2015 Sep 17;373(12):1136-52. doi: 10.1056/NEJMra1406184. PMID: 26376137.
2. Siegel R, Miller K, Jemal A et al. Cancer statistics. CA: A Cancer Journal for Clinicians. 2017.
3. SEER Cancer Statistics Review (CSR) 1975-2014, Myeloid Leukemia
4. Dores GM, Devesa SS, Curtis RE, Linet MS, Morton LM. Acute leukemia incidence and patient survival among children and adults in the United States, 2001-2007. *Blood*. 2012 Jan 5;119(1):34-43. doi: 10.1182/blood-2011-04-347872. Epub 2011 Nov 15. PMID: 22086414; PMCID: PMC3251235.
5. Khoury JD, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia*. 2022 Jul;36(7):1703-1719. doi: 10.1038/s41375-022-01613-1. Epub 2022 Jun 22. PMID: 35732831; PMCID: PMC9252913.
6. Arber DA, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. *Blood*. 2022 Sep 15;140(11):1200-1228. doi: 10.1182/blood.2022015850. PMID: 35767897; PMCID: PMC9479031.
7. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, Sultan C. Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. *Ann Intern Med*. 1985 Oct;103(4):620-5. doi: 10.7326/0003-4819-103-4-620. PMID: 3862359.
8. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, Bloomfield CD, Cazzola M, Vardiman JW. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016 May 19;127(20):2391-405. doi: 10.1182/blood-2016-03-643544. Epub 2016 Apr 11. PMID: 27069254.
9. Shah A, Andersson TM, Rachet B, Björkholm M, Lambert PC. Survival and cure of acute myeloid leukaemia in England, 1971-2006: a population-based study. *Br J Haematol*. 2013 Aug;162(4):509-16. doi: 10.1111/bjh.12425. Epub 2013 Jun 21. PMID: 23786647.
10. Cancer Genome Atlas Research Network; Ley TJ, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013 May 30;368(22):2059-74. doi: 10.1056/NEJMoa1301689. Epub 2013 May 1. Erratum in: *N Engl J Med*. 2013 Jul 4;369(1):98. PMID: 23634996; PMCID: PMC3767041.
11. Stock W, Thirman MJ. Molecular genetics of acute myeloid leukemia. *UptoDate*, 2022.

12. Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, Wheatley K, Harrison CJ, Burnett AK; National Cancer Research Institute Adult Leukaemia Working Group. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood*. 2010 Jul 22;116(3):354-65. doi: 10.1182/blood-2009-11-254441. Epub 2010 Apr 12. PMID: 20385793.
13. Reilly JT. Pathogenesis of acute myeloid leukaemia and inv(16)(p13;q22): a paradigm for understanding leukaemogenesis? *Br J Haematol*. 2005 Jan;128(1):18-34. doi: 10.1111/j.1365-2141.2004.05236.x. PMID: 15606546.
14. Bachas C, Schuurhuis GJ, Hollink IH, Kwidama ZJ, Goemans BF, Zwaan CM, van den Heuvel-Eibrink MM, de Bont ES, Reinhardt D, Creutzig U, de Haas V, Assaraf YG, Kaspers GJ, Cloos J. High-frequency type I/II mutational shifts between diagnosis and relapse are associated with outcome in pediatric AML: implications for personalized medicine. *Blood*. 2010 Oct 14;116(15):2752-8. doi: 10.1182/blood-2010-03-276519. Epub 2010 Jun 30. PMID: 20592250.
15. Li M, Cui X, Guan H. MicroRNAs: pivotal regulators in acute myeloid leukemia. *Ann Hematol*. 2020 Mar;99(3):399-412. doi: 10.1007/s00277-019-03887-5. Epub 2020 Jan 13. PMID: 31932900.
16. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021 Mar 29;372:n71. doi: 10.1136/bmj.n71. PMID: 33782057; PMCID: PMC8005924.
17. Haddaway NR, Page MJ, Pritchard CC, McGuinness LA. *PRISMA2020: An R package and Shiny app for producing PRISMA 2020-compliant flow diagrams, with interactivity for optimised digital transparency and Open Synthesis*. *Campbell Syst Rev*. 2022 Mar 27;18(2):e1230. doi: 10.1002/cl2.1230. PMID: 36911350; PMCID: PMC8958186.
18. Raivo Kolde, Sven Laur, Priit Adler, Jaak Vilo, Robust rank aggregation for gene list integration and meta-analysis, *Bioinformatics*, Volume 28, Issue 4, February 2012, Pages 573–580, <https://doi.org/10.1093/bioinformatics/btr709>
19. GEO2R: <https://www.ncbi.nlm.nih.gov/geo/geo2r/>
20. Hunt GP, et al. GEOexplorer: a webserver for gene expression analysis and visualisation. *Nucleic Acids Res*. 2022 Jul 5;50(W1):W367-W374. doi: 10.1093/nar/gkac364. PMID: 35609980; PMCID: PMC9252785.
21. McGeary SE, Lin KS, Shi CY, Pham TM, Bisaria N, Kelley GM, Bartel DP. The biochemical basis of microRNA targeting efficacy. *Science*. 2019 Dec 20;366(6472):eaav1741. doi: 10.1126/science.aav1741. Epub 2019 Dec 5. PMID: 31806698; PMCID: PMC7051167.
22. Tastsoglou S, Alexiou A, Karagkouni D, Skoufos G, Zacharopoulou E, Hatzigeorgiou AG. DIANA-microT 2023: including predicted targets of virally encoded miRNAs. *Nucleic Acids Res*. 2023 Jul 5;51(W1):W148-W153. doi: 10.1093/nar/gkad283. PMID: 37094027; PMCID: PMC10320106.
23. Karagkouni D, Paraskevopoulou MD, Chatzopoulos S, Vlachos IS, Tastsoglou S, Kanellos I, Papadimitriou D, Kavakiotis I, Maniou S, Skoufos G, Vergoulis T, Dalamagas T, Hatzigeorgiou AG. DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA-gene interactions. *Nucleic Acids Res*. 2018 Jan 4;46(D1):D239-D245. doi: 10.1093/nar/gkx1141. PMID: 29156006; PMCID: PMC5753203.
24. Martin FJ et al. Ensembl 2023. *Nucleic Acids Res*. 2023 Jan 6;51(D1):D933-D941. doi: 10.1093/nar/gkac958. PMID: 36318249; PMCID: PMC9825606.
25. Tastsoglou S, Skoufos G, Miliotis M, Karagkouni D, Koutsoukos I, Karavangeli A, Kardaras FS, Hatzigeorgiou AG. DIANA-miRPath v4.0: expanding target-based miRNA functional analysis in cell-type and tissue contexts. *Nucleic Acids Res*. 2023 Jul 5;51(W1):W154-W159. doi: 10.1093/nar/gkad431. PMID: 37260078; PMCID: PMC10320185.
26. Fayyad-Kazan H, Bitar N, Najjar M, Lewalle P, Fayyad-Kazan M, Badran R, Hamade E, Daher A, Hussein N, ElDirani R, Berri F, Vanhamme L, Burny A, Martiat P, Rouas R, Badran B. Circulating miR-150 and miR-342 in plasma are novel potential biomarkers for acute myeloid leukemia. *J Transl Med*. 2013 Feb 7;11:31. doi: 10.1186/1479-5876-11-31. PMID: 23391324; PMCID: PMC3579719.
27. Cammarata G, Augugliaro L, Salemi D, Agueli C, La Rosa M, Dagnino L, Civiletto G, Messina F, Marfia A, Bica MG, Cascio L, Floridia PM, Mineo AM, Russo M, Fabbiano F, Santoro A. Differential expression of specific microRNA and their targets in acute myeloid leukemia. *Am J Hematol*. 2010 May;85(5):331-9. doi: 10.1002/ajh.21667. PMID: 20425795.
28. Memari F, Tavakolpour V, Mohajeri N, Poopak B, Fallah P, Alizadeh E, Kouhkan F, Zarghami N. Distinct power of bone marrow microRNA signatures and tumor suppressor genes for early detection of acute

- leukemia. Clin Transl Oncol. 2022 Jul;24(7):1372-1380. doi: 10.1007/s12094-022-02781-3. Epub 2022 Mar 5. PMID: 35247197.
29. Lutherborrow M, Bryant A, Jayaswal V, Agapiou D, Palma C, Yang YH, Ma DD. Expression profiling of cytogenetically normal acute myeloid leukemia identifies microRNAs that target genes involved in monocytic differentiation. Am J Hematol. 2011 Jan;86(1):2-11. doi: 10.1002/ajh.21864. PMID: 20981674.
 30. Isken F, Steffen B, Merk S, Dugas M, Markus B, Tidow N, Zühlsdorf M, Illmer T, Thiede C, Berdel WE, Serve H, Müller-Tidow C. Identification of acute myeloid leukaemia associated microRNA expression patterns. Br J Haematol. 2008 Jan;140(2):153-61. doi: 10.1111/j.1365-2141.2007.06915.x. PMID: 18173753.
 31. Moussa Agha D, Rouas R, Najar M, Bouhtit F, Naamane N, Fayyad-Kazan H, Bron D, Meuleman N, Lewalle P, Merimi M. Identification of Acute Myeloid Leukemia Bone Marrow Circulating MicroRNAs. Int J Mol Sci. 2020 Sep 25;21(19):7065. doi: 10.3390/ijms21197065. PMID: 32992819; PMCID: PMC7583041.
 32. Zhi F, Cao X, Xie X, Wang B, Dong W, Gu W, Ling Y, Wang R, Yang Y, Liu Y. Identification of circulating microRNAs as potential biomarkers for detecting acute myeloid leukemia. PLoS One. 2013;8(2):e56718. doi: 10.1371/journal.pone.0056718. Epub 2013 Feb 20. PMID: 23437222; PMCID: PMC3577716.
 33. Jongen-Lavrencic M, Sun SM, Dijkstra MK, Valk PJ, Löwenberg B. MicroRNA expression profiling in relation to the genetic heterogeneity of acute myeloid leukemia. Blood. 2008 May 15;111(10):5078-85. doi: 10.1182/blood-2008-01-133355. Epub 2008 Mar 12. PMID: 18337557.
 34. Garzon R, Volinia S, Liu CG, Fernandez-Cymering C, Palumbo T, Pichiorri F, Fabbri M, Coombes K, Alder H, Nakamura T, Flomenberg N, Marcucci G, Calin GA, Kornblau SM, Kantarjian H, Bloomfield CD, Andreeff M, Croce CM. MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. Blood. 2008 Mar 15;111(6):3183-9. doi: 10.1182/blood-2007-07-098749. Epub 2008 Jan 10. PMID: 18187662; PMCID: PMC2265455.
 35. Daschkey S, Röttgers S, Giri A, Bradtke J, Teigler-Schlegel A, Meister G, Borkhardt A, Landgraf P. MicroRNAs distinguish cytogenetic subgroups in pediatric AML and contribute to complex regulatory networks in AML-relevant pathways. PLoS One. 2013;8(2):e56334. doi: 10.1371/journal.pone.0056334. Epub 2013 Feb 13. PMID: 23418555; PMCID: PMC3572007.
 36. Ding C, Chen SN, Macleod RAF, Drexler HG, Nagel S, Wu DP, Sun AN, Dai HP. MiR-130a is aberrantly overexpressed in adult acute myeloid leukemia with t(8;21) and its suppression induces AML cell death. Ups J Med Sci. 2018 Mar;123(1):19-27. doi: 10.1080/03009734.2018.1440037. Epub 2018 Mar 1. PMID: 29493383; PMCID: PMC5901465.
 37. Wang F, Wang XS, Yang GH, Zhai PF, Xiao Z, Xia LY, Chen LR, Wang Y, Wang XZ, Bi LX, Liu N, Yu Y, Gao D, Huang BT, Wang J, Zhou DB, Gong JN, Zhao HL, Bi XH, Yu J, Zhang JW. miR-29a and miR-142-3p downregulation and diagnostic implication in human acute myeloid leukemia. Mol Biol Rep. 2012 Mar;39(3):2713-22. doi: 10.1007/s11033-011-1026-5. Epub 2011 Jun 16. PMID: 21678057.
 38. Vanhooren J, Van Camp L, Depreter B, de Jong M, Uyttebroeck A, Van Damme A, Dedeken L, Dresse MF, van der Werff Ten Bosch J, Hofmans M, Philippé J, De Moerloose B, Lammens T. Deciphering the Non-Coding RNA Landscape of Pediatric Acute Myeloid Leukemia. Cancers (Basel). 2022 Apr 22;14(9):2098. doi: 10.3390/cancers14092098. PMID: 35565228; PMCID: PMC9100904.
 39. Rommer A, Steinleitner K, Hackl H, Schneckenleithner C, Engelmann M, Scheideler M, Vlatkovic I, Kralovics R, Cerny-Reiterer S, Valent P, Sill H, Wieser R. Overexpression of primary microRNA 221/222 in acute myeloid leukemia. BMC Cancer. 2013 Jul 29;13:364. doi: 10.1186/1471-2407-13-364. PMID: 23895238; PMCID: PMC3733744.
 40. Ezalia Esa, et al. Construction of a microRNA-mRNA Regulatory Network in De Novo Cytogenetically Normal Acute Myeloid Leukemia Patients. Genetic Testing and Molecular Biomarkers 2021 25:3, 199-210
 41. Pandita A, Ramadas P, Poudel A, Saad N, Anand A, Basnet A, Wang D, Middleton F, Gilligan DM. Differential expression of miRNAs in acute myeloid leukemia quantified by Nextgen sequencing of whole blood samples. PLoS One. 2019 Mar 20;14(3):e0213078. doi: 10.1371/journal.pone.0213078. PMID: 30893351; PMCID: PMC6426230.
 42. Abdelhamed S, Butler JT, Jung S, Chen DW, Jenkins G, Gao L, Lim JY, Klco JM, Horton TM, Kurre P. Rational biomarker development for the early and minimally invasive monitoring of AML. Blood Adv. 2021 Nov 9;5(21):4515-4520. doi: 10.1182/bloodadvances.2021004621. PMID: 34587228; PMCID: PMC8579272.
 43. Ramsingh G, Koboldt DC, Trissal M, Chiappinelli KB, Wylie T, Koul S, Chang LW, Nagarajan R, Fehniger TA, Goodfellow P, Magrini V, Wilson RK, Ding L, Ley TJ, Mardis ER, Link DC. Complete characterization of the microRNAome in a patient with acute myeloid leukemia. Blood. 2010 Dec 9;116(24):5316-26. doi: 10.1182/blood-2010-05-285395. Epub 2010 Sep 28. PMID: 20876853; PMCID: PMC3012545.
 44. Leoncini PP, Vitullo P, Reddel S, Tocco V, Paganelli V, Stocchi F, Mariggio E, Massa M, Nigita G, Veneziano D, Fadda P, Scarpa M, Pigazzi M, Bertaina A, Rota R, Pagliara D, Merli P. MicroRNA profiling of paediatric AML with FLT-ITD or MLL-rearrangements: Expression signatures and *in vitro* modulation of miR-221-3p

- and miR-222-3p with BRD4/HATs inhibitors. *Oncol Rep.* 2022 Dec;48(6):221. doi: 10.3892/or.2022.8436. Epub 2022 Nov 2. PMID: 36321792; PMCID: PMC9647495.
45. Chen P, Price C, Li Z, Li Y, Cao D, Wiley A, He C, Gurbuxani S, Kunjamma RB, Huang H, Jiang X, Arnovitz S, Xu M, Hong GM, Elkahoun AG, Neilly MB, Wunderlich M, Larson RA, Le Beau MM, Mulloy JC, Liu PP, Rowley JD, Chen J. miR-9 is an essential oncogenic microRNA specifically overexpressed in mixed lineage leukemia-rearranged leukemia. *Proc Natl Acad Sci U S A.* 2013 Jul 9;110(28):11511-6. doi: 10.1073/pnas.1310144110. Epub 2013 Jun 24. PMID: 23798388; PMCID: PMC3710804.
 46. Maki K, Yamagata T, Sugita F, Nakamura Y, Sasaki K, Mitani K. Aberrant expression of MIR9 indicates poor prognosis in acute myeloid leukaemia. *Br J Haematol.* 2012 Jul;158(2):283-285. doi: 10.1111/j.1365-2141.2012.09118.x. Epub 2012 Apr 10. PMID: 22489576.
 47. Zhu B, Zhong W, Cao X, Pan G, Xu M, Zheng J, Chen H, Feng X, Luo C, Lu C, Xiao J, Lin W, Lai C, Li M, Du X, Yi Q, Yan D. Loss of miR-31-5p drives hematopoietic stem cell malignant transformation and restoration eliminates leukemia stem cells in mice. *Sci Transl Med.* 2022 Jan 26;14(629):eabh2548. doi: 10.1126/scitranslmed.abh2548. Epub 2022 Jan 26. PMID: 35080912.
 48. Schwind S et al. Prognostic significance of expression of a single microRNA, miR-181a, in cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *J Clin Oncol.* 2010 Dec 20;28(36):5257-64. doi: 10.1200/JCO.2010.29.2953. Epub 2010 Nov 15. PMID: 21079133; PMCID: PMC3018359.