From Department of Medicine, Huddinge - MedH
Karolinska Institutet, Stockholm, Sweden

Biological impact and clinical relevance of long non-coding RNAs and post-transcriptional alterations in acute myeloid leukemia

Elisabetta Cozzi

Karolinska Institutet

Stockholm 2025

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2025

ISBN 978-91-8017-913-3
DOI https://doi.org/10.69622/30317671
Cover illustration: A figurative representation of lncRNAs identification and selection, depicted as a claw machine capturing a specific lncRNA molecule from a diverse pool of cellular components.

Biological Impact and clinical relevance of long non-coding RNAs and post-transcriptional alterations in acute myeloid leukemia

Thesis for Doctoral Degree (Ph.D.)

By

Elisabetta Cozzi

The thesis will be defended in public in room Gene (5108), Neo, Blickagången 16, 141 52, Flemingsberg, on Friday 23rd of January 2026, at 09:00.

Principal Supervisor:
Professor Sören Lehmann
Karolinska Institutet
Department of Medicine Huddinge
Center for Hematology and Regenerative Medicine

Co-supervisor(s):
Principal researcher Andreas Lennartsson
Karolinska Institutet
Department of Medicine Huddinge
Center for Hematology and Regenerative Medicine

Dr. Sophia Miliara
Karolinska Institutet
Department of Medicine Huddinge
Center for Hematology and Regenerative Medicine

Opponent:
Principal researcher Claudia Kutter
Karolinska Institutet
Department of Microbiology, Tumor and Cell biology
Division of SciLife at MTC

Examination Board:
Professor Sara Ek
Lund University
Department of Immunotechnology

Professor Andor Pivarcsi
Uppsala University
Department of Medical Biochemistry and Microbiology

Associate Professor Fredrik Johansson Swartling
Uppsala University
Department of Immunology, Genetics and Pathology

Popular science summary of the thesis

About 7–8% of our body is made up of blood, an extraordinary tissue that flows throughout the body and nourishes every organ. Blood is composed of approximately 55% of plasma, the liquid part, and 45% of a cellular fraction which comprise red blood cells, white blood cells, and platelets. Red blood cells, also recognized as erythrocytes, have the role to transport oxygen from the lungs to all tissues to then carry carbon dioxide back for exhalation. Platelets are essential for clot formation, while white blood cells, also named as leukocytes, serve as key players in immune defense. White blood cells are further divided into five main subgroups: neutrophils, eosinophils, basophils, monocytes, and lymphocytes, each with a specific role in protecting the body from infections and disease.

About 95% of blood cells are generated in the bone marrow through hematopoiesis, a process in which hematopoietic stem cells self-renew and can generate all the different types of blood cells mentioned above. Sometimes, however, this finely tuned system can go wrong. When these marrow cells acquire genetic mutations, they stop following the normal rules. The result is leukemia, a blood cancer in which abnormal cells multiply uncontrollably and pile up in the bone marrow and bloodstream. This overcrowding prevents healthy blood cells from forming properly, disrupting the body's normal functions and leading to serious health problems.

In this thesis, we focused on one particular subtype of leukemia called acute myeloid leukemia (AML). This disease affects hematopoietic stem cells and early myeloid progenitors, causing the abnormal accumulation of immature white blood cells. The term "acute" indicates that the disease progresses rapidly and requires urgent treatment. AML is the most common acute leukemia in adults, typically affecting older individuals. The standard therapy is chemotherapy, aimed at eliminating leukemic cells. However, many patients do not respond adequately, and in some cases, the disease may return, a situation known as relapse. For this reason, it is essential to deepen our understanding of AML and explore new therapeutic strategies.

Therefore, in the first two studies, we focused on better understanding the role exerted by a specific group of molecules, named long non-coding RNAs (lncRNAs), in AML. Proteins are often considered the main players in biology, as they carry out most of the vital functions within cells. Proteins are produced based on instructions contained in DNA, which are first copied into RNA and then translated

into proteins. However, not all RNA molecules encode proteins: some, called non-coding RNAs, still exert important roles in many biological processes. Among these, lncRNAs are RNA molecules longer than 200–500 nucleotides, capable of influencing gene expression in multiple ways. In cancers, including AML, these molecules can become dysregulated, disrupting their normal balance and contributing to the development or progression of the disease.

In **study I**, we performed genome-wide analyses comparing cells from AML patients with those from healthy donors and identified 136 lncRNAs that were expressed at different levels between the two groups. Among them, we discovered a new lncRNA, which we named myeloid and AML-associated intergenic lncRNA (MALNC). MALNC was found to be highly abundant in AML samples, and its expression was even higher in certain AML subgroups carrying specific genetic mutations, such as in acute promyelocytic leukemia (APL) patients with the *PML-RARA* fusion gene or in those with *NPM1*/*IDH2*^{R140} mutations. By analyzing patient data, we also observed that higher levels of MALNC were linked to better overall survival, independently of other known prognostic factors. However, functional experiments showed that *MALNC* mainly acts as an oncogene, particularly in APL. In fact, MALNC promotes leukemic cell growth, while its depletion makes cells more sensitive to differentiation treatments with ATRA all-trans retinoic acid (ATRA) and arsenic trioxide (ATO), two drugs commonly used in APL therapy. Finally, our results suggest that MALNC may be involved in several important signaling pathways, not only the retinoic acid pathway essential for the regulation of cell differentiation, but also those related to Rho GTPases, showing that it may exert a broader regulatory role in AML biology.

In **study II**, we used an advanced genetic approach called CRISPR interference (CRISPRi) to investigate the function of nearly 8,000 different lncRNAs in AML cells. By selectively switching off each lncRNA, we evaluated how their absence affected cell growth, differentiation, and response to venetoclax, a drug that targets the BCL-2 protein and is used in combination with low-dose chemotherapy for elderly patients or patients who cannot tolerate intensive chemotherapy treatments. Through this large-scale analysis, we identified 58 lncRNAs involved in cell proliferation, 4 in differentiation, and 23 in the response to venetoclax. Among these, we focused on one specific lncRNA named CATG00000106133.1. CATG00000106133.1 promotes leukemic cell proliferation and is expressed in specific AML subtypes, as well as in certain normal hematopoietic cell populations. In addition, gene editing experiments suggest that

CATG00000106133.1 may contribute to AML biology through its involvement in cytokine signaling and the regulation of immune response.

Finally, in **Study III**, we shifted our focus from non-coding RNAs to a protein called eukaryotic initiation factor 4A-3 (eIF4A3), which belongs to a family of enzymes known as helicases and is relevant in RNA metabolism at different levels. Using publicly available datasets, we discovered that eIF4A3 is dysregulated in AML context, and that its altered expression is linked to pathways that control how RNA is processed and translated into proteins, so-called post-transcriptional mechanisms. We also found that eIF4A3 is essential for AML cell survival, and that either reducing its expression or targeting it with specific inhibitors led to increased cell death. This highlights eIF4A3 as a promising therapeutic target for future AML treatments.

Together, these studies provide new insights into previously unexplored molecules, both long non-coding RNAs and proteins, that play important roles in AML biology. Understanding their functions not only improves our knowledge of how leukemia develops but also opens new opportunities to identify novel biomarkers and therapeutic targets to enhance disease management.

Divulgazione scientifica della tesi

Circa il 7–8% del nostro corpo è costituito dal sangue, un tessuto straordinario che scorre in tutto il corpo e irriga ogni organo. Il sangue è composto per circa il 55% da una parte liquida, chiamata plasma, e per il 45% restante da tre principali tipi di cellule: i globuli rossi, i globuli bianchi e le piastrine.

I globuli rossi, o eritrociti, trasportano l'ossigeno dai polmoni a tutti i tessuti per poi riportare l'anidride carbonica ai polmoni per essere espulsa. Le piastrine, invece, svolgono un ruolo fondamentale nella coagulazione del sangue, mentre i globuli bianchi, o leucociti, agiscono come difensori del nostro sistema immunitario. Questi ultimi si dividono in cinque principali sottogruppi: neutrofili, eosinofili, basofili, monociti e linfociti, ciascuno con una funzione specifica nella protezione dell'organismo da infezioni e malattie.

Circa il 95% delle cellule del sangue viene generato nel midollo osseo attraverso l'ematopoiesi, un processo in cui le cellule staminali ematopoietiche si auto-rinnovano e possono dare origine a tutti i diversi tipi di cellule descritti in precedenza. A volte, però, questo sistema finemente regolato può alterarsi. Infatti, quando le cellule del midollo acquisiscono mutazioni genetiche, deviano dal loro normale comportamento. Il risultato è la leucemia, un tipo di cancro del sangue in cui le cellule anomale si moltiplicano in modo incontrollato e si accumulano nel midollo osseo e nel sangue. Questo sovraffollamento impedisce la corretta formazione delle cellule del sangue sane, compromettendo il normale funzionamento dell'organismo e portando a gravi problemi di salute.

In questa tesi, ci siamo concentrati su un particolare sottotipo di leucemia chiamato leucemia mieloide acuta (LMA). Questa malattia colpisce le cellule staminali ematopoietiche e i precursori mieloidi precoci, portando all'accumulo anomalo di globuli bianchi immaturi. Il termine "acuta" indica che la malattia progredisce rapidamente e richiede un trattamento urgente. L'LMA è la leucemia acuta più comune negli adulti e colpisce tipicamente individui più anziani. La terapia standard è la chemioterapia, mirata all'eliminazione delle cellule leucemiche. Tuttavia, molti pazienti non rispondono in modo adeguato e, in alcuni casi, la malattia può ripresentarsi, situazione nota come recidiva. Per questo motivo, è essenziale approfondire la nostra comprensione della LMA ed esplorare nuove strategie terapeutiche.

Pertanto, nei primi due studi ci siamo concentrati sul migliorare la comprensione del ruolo di un gruppo specifico di molecole, chiamate RNA lunghi non codificanti (lncRNA), nella LMA. Le proteine sono spesso considerate le protagoniste principali della biologia, poiché svolgono la maggior parte delle funzioni vitali nelle cellule. Queste ultime vengono prodotte a partire dalle istruzioni contenute nel DNA, che vengono prima copiate in RNA e poi tradotte in proteine. Tuttavia, non tutti gli RNA codificano proteine: alcuni, detti RNA non codificanti, svolgono comunque ruoli regolatori in molti processi biologici. Tra questi, i lncRNA sono molecole lunghe più di 200–500 nucleotidi, capaci di influenzare l'espressione genica in modi diversi. Nei tumori, inclusa la LMA, queste molecole possono diventare deregolate, alterando il loro normale equilibrio e contribuendo allo sviluppo o alla progressione della malattia.

Nel **primo studio,** abbiamo eseguito analisi genomiche su larga scala confrontando le cellule di pazienti affetti da LMA con quelle di donatori sani, identificando 136 lncRNA espressi a livelli differenti tra i due gruppi. Tra questi, abbiamo scoperto un nuovo lncRNA, che abbiamo chiamato "myeloid and AML-associated intergenic lncRNA" o più brevemente MALNC. MALNC è risultato fortemente espresso nei campioni di LMA, con livelli ancora più elevati in alcuni sottogruppi di pazienti portatori di specifiche mutazioni genetiche, come nei casi di LPA (leucemia promielocitica acuta) con il gene di fusione *PML-RARA* o in pazienti con mutazioni in *NPM1/IDH2*^{R140}. L'analisi dei dati clinici ha inoltre rivelato che livelli più alti di MALNC erano associati a una migliore sopravvivenza complessiva, indipendentemente da altri fattori prognostici noti. Tuttavia, gli esperimenti funzionali hanno mostrato che MALNC agisce prevalentemente come un oncogene, in particolare nel contesto della LPA. Infatti, MALNC promuove la proliferazione delle cellule leucemiche, mentre la sua riduzione rende le cellule più sensibili ai trattamenti di differenziazione con ATRA (acido all-trans retinoico) e triossido di arsenico, due farmaci comunemente utilizzati nella terapia della LPA. Infine, i nostri risultati suggeriscono che MALNC possa essere coinvolto in diverse vie di segnalazione biologica rilevanti, non solo nella via dell'acido retinoico, essenziale per la regolazione della differenziazione cellulare, ma anche in quelle associate alle Rho GTPasi, indicando un possibile ruolo regolatore più ampio nella biologia della LMA.

Nel **secondo studio**, abbiamo utilizzato un approccio genetico avanzato chiamato CRISPR interference (CRISPRi) per studiare la funzione di quasi 8.000 diversi lncRNA nelle cellule di LMA. Spegnendo selettivamente ciascun lncRNA, abbiamo

valutato come la loro assenza influenzasse la crescita cellulare, la differenziazione e la risposta al venetoclax, un farmaco che agisce sulla proteina BCL-2 e viene impiegato in combinazione con chemioterapie a basso dosaggio nei pazienti che non possono tollerare trattamenti intensivi. Attraverso questa analisi su larga scala, abbiamo identificato 58 lncRNA coinvolti nella proliferazione cellulare, 4 nella differenziazione e 23 nella risposta al venetoclax. Tra questi, ci siamo focalizzati su uno specifico lncRNA: CATG00000106133.1. CATG00000106133.1 favorisce la proliferazione delle cellule leucemiche ed è espresso in specifici sottotipi di LMA, oltre che in alcune popolazioni di cellule ematopoietiche normali. I nostri risultati suggeriscono che CATG00000106133.1 possa contribuire alla biologia della LMA attraverso il suo coinvolgimento nella segnalazione delle citochine e nella regolazione della risposta immunitaria.

Infine, nel **terzo studio**, abbiamo spostato l'attenzione dagli RNA non codificanti a una proteina chiamata "eukaryotic initiation factor 4A-3" (eIF4A3), appartenente alla famiglia delle elicasi e convolta in diverse fasi del metabolismo dell'RNA. Utilizzando dataset pubblicamente disponibili, abbiamo scoperto che eIF4A3 è deregolata nel contesto della LMA e che la sua espressione alterata è associata a vie coinvolte nel processamento e nella traduzione dell'RNA, i cosiddetti meccanismi post-trascrizionali. Abbiamo inoltre osservato che eIF4A3 è essenziale per la sopravvivenza delle cellule di LMA, e che sia la riduzione della sua espressione sia l'inibizione mediante molecole specifiche portano a un aumento della morte cellulare. Questi risultati evidenziano eIF4A3 come un promettente bersaglio terapeutico per futuri trattamenti contro la LMA.

Complessivamente, questi studi forniscono nuove informazioni su molecole finora poco esplorate, sia RNA lunghi non codificanti che proteine, che svolgono ruoli chiave nella biologia della LMA. Capirne meglio le funzioni non solo arricchisce la nostra conoscenza della malattia, ma apre anche la possibilità di individuare nuovi biomarcatori e potenziali bersagli terapeutici utili per una gestione più efficace della LMA.

Abstract

Acute myeloid leukemia (AML) represents the most common acute leukemia in adults, marked by the abnormal expansion of myeloid progenitor cells that fail to properly differentiate. These immature cells accumulate in the bone marrow thereby distrupting normal hematopoiesis. Although advances in AML research have led to improvements in patient outcomes, the overall prognosis remains poor, particularly for relapsed and refractory cases. A deeper understanding of the molecular biology of AML is therefore urgently needed.

Therefore, in this thesis, we aim to clarify how long non-coding RNAs (lncRNAs) contribute to AML pathogenesis, considering their recognized involvement in various biological functions and their altered expression patterns across many cancers, including AML. Additionally, we examine post-transcriptional regulatory mechanisms, a layer of gene regulation that has received comparatively limited attention in AML research.

In **Study I**, using transcriptomic analyses of 7 AML patients and 5 normal bone marrow CD34+ samples, we identified 136 *de novo* lncRNAs that were differentially expressed in AML blasts. Among these, we characterized a novel transcript, which we named myeloid and AML-associated intergenic lncRNA (MALNC). *MALNC* was overexpressed in AML, particularly in patients harboring *PML-RARA* fusion or *NPM1/IDH2*^{R140} co-mutations, and higher expression was associated with better prognosis, independent of other established risk factors. Furthermore, *MALNC* knockout reduced AML cell growth and colony formation. In parallel, *MALNC*-depleted cells showed increased all-*trans* retinoic acid (ATRA)-induced differentiation and increased sensitivity to arsenic trioxide (ATO). To further characterize MALNC, transcriptomic analyses were performed in *MALNC* knockout clones both at baseline and following ATRA treatment, revealing modulation of genes involved in retinoic acid signaling. In addition, chromatin-binding experiments showed that MALNC interacted with genes related to the retinoic acid and Rho GTPase pathways.

In **Study II** we employed high-throughput CRISPR-interference (CRISPRi) screens to knock down 7,996 lncRNAs and investigate their roles in AML proliferation, differentiation, and response to venetoclax. The screens identified 58 lncRNAs involved in proliferation, 4 lncRNAs affecting differentiation, and 23 lncRNAs associated with venetoclax response. Among these, AC009299.3 emerged as a candidate linked to venetoclax resistance, with patient data further showing a

correlation between its expression and poor prognosis under standard chemotherapy treatment. In the proliferation screen, MIR17HG, CATG00000106133.1, and CATG00000056792.1 were identified as candidate lncRNAs promoting leukemic cell growth. Notably, CATG00000106133.1 was enriched in *de novo* and cytogenetically normal AML, strongly associated with *NPM1* and *IDH2_R140* mutations, and exhibited high expression in specific hematopoietic lineages. Functional validation through complete knockout of *CATG00000106133.1,* followed by transcriptomic profiling, revealed differential expression of genes involved in cytokine signaling and immune response pathways, providing insights into its role in AML biology.

In **study III**, we investigated genes involved in post-transcriptional mechanisms in AML. Using CRISPR knockout screens from the DepMap database, the exon junction complex helicase eIF4A3, a regulator of RNA polymerase I- and II-dependent processes previously linked to cancer progression, emerged as a top essential gene across 18 AML cell lines. Transcriptomic analyses revealed higher eIF4A3 expression in AML cell lines and patient samples compared to normal controls, with gene ontology analysis highlighting RNA metabolism and translation as key affected pathways. In addition, functional experiments showed that chemical inhibition or siRNA-mediated silencing of eIF4A3 induced AML cell death through impaired ribosome biogenesis and p53 activation, as well as through p53-independent mechanisms.

In summary, across these three studies, we identified novel lncRNAs and post-transcriptional regulators that expand our understanding of AML biology and drug response, pointing to their possible use as biomarkers or therapeutic targets to improve patient care.

List of scientific papers

I. Elisabetta Cozzi*, Anne Neddermeyer*, Xiangfu Zhong, Angelica Gamboa Cedeno, Dimitris C. Kanellis, Albin Österroos, My Björklund, Nona Struyf, Kasper Karlsson, Ying Qu, Alma Månsson, Tatjana Pandzic, Sofia Bengtzén, Christer Nilsson, Roland Fiskesund, Panagiotis Baliakas, Tom Erkers, Jiri Bartek, Olli-Pekka Kallioniemi, Hong Qian, Andreas Lennartsson, Sören Lehmann. **MALNC: a new muntant NPM1/IDH2^{R140} and PML-RARA-associated lncRNA with impact on AML cell proliferation, maturation and drug response.** *Cancer Gene Therapy*. (2025). https://doi.org/10.1038/s41417-025-00954-0.

II. Elisabetta Cozzi, Anne Neddermeyer, Sophia Miliara, Xiangfu Zhong, Tyler Weirick, Chung Chao Hon, Andreas Lennartsson, Sören Lehmann. **Identification of long non-coding RNAs involved in leukemogenesis and venetoclax response in acute myeloid leukemia through functional CRISPR-dCas9 interference screens.** *Manuscript*

III. Sophia Miliara, Elisabetta Cozzi, Xiangfu Zhong, Isaac Chan, Karl Ekwall, Sören Lehmann, Andreas Lennartsson, Jiri Bartek, Dimitris C. Kanellis. **The exon-junction complex helicase eIF4A3 holds therapeutic potential in acute myeloid leukemia**. *Leukemia*. 2024 Mar;38(3):663-666. https://doi.org/10.1038/s41375-023-02098-2.

*Equal contribution

Contents

List of abbreviations

AML	Acute myeloid leukemia
APL	Acute promyelocytic leukemia
ATO	Arsenic trioxide
ATRA	All-Trans retinoic acid
BCL-2	B-Cell Lymphoma 2
CAGE-seq	Cap analysis gene expression sequencing
CAT	CAGE-assisted transcriptome
cDNA	Complementary DNA
CEBPA	CCAAT/Enhancer-binding protein alpha
ChiRP	Chromatin isolation by RNA purification
CR	Complete remission
CRISPR	Clustered regularly interspaced short palindromic repeats
CRISPRi	CRISPR interference
CTSS	CAGE tag starting site
dCAs9-KRAB	Catalytically inactive Cas9 Kruppel-associated box
DE/DEGs	Differential expression/Differentially expressed genes
DDX	DEAD-box helicase
DNMT3A	DNA (cytosine-5)-methyltransferase 3 alpha
eIF4A3	Eukaryotic Initiation factor 4A-III
EJC	Exon junction complex
ELN	European leukemia net
FAB	French-American-British classification
FANTOM	Functional annotation of mammalian genome
FDA	U.S. Food and Drug Administration
FLT3	FMS-like tyrosine kinase 3
gRNA	Guide RNA
GO	Gene ontology

HOXA	Homeobox A cluster
HMAs	Hypomethylating agents
HSC	Hematopoietic stem cells
HSCT	Hematopoietic stem cell transplantation
IDH1/2	Isocitrate dehydrogenase 1 and 2
IDT	Internal tandem duplications
IRBC	Impaired ribosome biogenesis
IRF8	Interferon regulatory factor 8
KO	Knockout
LSC	Leukemic stem cell
lncRNA	Long non-coding RNA
MALNC	Myeloid and AML-associated intergenic lncRNA
miRNA	MicroRNA
mRNA	Messenger RNA
NBM	Normal bone marrow
ncRNA	Non coding RNA
NGS	Next-Generation sequencing
NPM1	Nucleophosmin 1
OS	Overall survival
PB	Peripheral blood
PCR	Polymerase chain reaction
PI	Propidium iodide
PML	Promyelocytic leukemia (gene)
RARA/RAR	Retinoic acid receptor alpha
RBP	RNA-binding Protein
RNA-seq	RNA sequencing
rRNA	Ribosomal RNA
RT-qPCR	Quantitative real-time PCR

RUNX1	Runt-related transcription factor 1
siRNA	Small Interfering RNA
TCGA	The cancer genome atlas
TP53	Tumor protein 53
TSS	Transcription start site
TTS	Transcription termination site
WHO	World health organization
WT	Wild-type
ZNF	Zinc finger

Introduction

In the literature review for this thesis, I present key concepts that frame and support my research, providing both background and context. The first two sections offer an overview of normal hematopoiesis and the leukemic landscape. Because all three projects in this thesis focus on a single leukemia subtype, the third section is devoted specifically to acute myeloid leukemia (AML). This section outlines the disease, from its etiology and pathogenesis to the various classification systems and the genetic alterations that disrupt normal cellular function. The AML section concludes with a comprehensive discussion of therapeutic approaches, covering both standard regimens and treatments specific to acute promyelocytic leukemia (APL) relevant to Study I, as well as venetoclax-based strategies examined in Study II. The fourth section shifts focus to long non-coding RNAs (lncRNAs), central to Studies I and II. It first describes their general features and biological functions, then examines their specific roles in AML, illustrating how selected lncRNAs influence disease biology and their potential clinical relevance. The fifth and final section provides an overview of post-transcriptional alterations in cancer and AML and highlights RNA helicases of relevance to Study III.

1 Background

1.1 Hematopoiesis

Hematopoiesis is the tightly regulated biological process that generates all blood cell lineages. In adults, this occurs primarily in the bone marrow and is initiated and maintained by hematopoietic stem cells (HSCs), rare, immature cells endowed with multipotency, self-renewal and long-lived capacity [1]. HSCs arise during embryonic development and can be broadly classified into long-term HSCs (LT-HSCs), which sustain lifelong hematopoiesis through extensive self-renewal, and short-term HSCs (ST-HSCs), which possess more limited self-renewal potential and act as transitional progenitors before committing to specific lineages [2].

Differentiation from HSCs to mature blood cells proceeds through a hierarchical cascade orchestrated by different growth factors and cytokines [3,4]. From HSCs, multipotent progenitors (MPPs) are generated, which then branch toward either the common myeloid progenitor (CMP) or common lymphoid progenitor (CLP) pathways.

CMPs give rise to the myeloid lineages, which include erythrocytes, granulocytes (neutrophils, eosinophils, basophils), monocytes, and megakaryocytes, the precursors of platelets. CLPs, on the other hand, differentiate into the lymphoid lineages, producing B lymphocytes, T lymphocytes, and natural killer cells.

1.2 Leukemia

The term "leukemia," derived from the two Greek words *leukos* (white) and *haima* (blood), was first introduced in 1847 [5]. It encompasses all neoplastic disorders in which hematopoietic cells at various maturation stages acquire genetic abnormalities that disrupt their normal maturation and differentiation processes [6]. As a result, immature progenitor cells, referred to as blasts, undergo uncontrolled proliferation and progressively accumulate in the bone marrow, peripheral circulation, and, in advanced stages, other organs. This expansion interferes with the production of healthy cells, leading to impaired oxygen transport, weakened immune defense, and defective hemostasis.

Different types of leukemia exist. Below is an overview of the main forms, classified according to the lineage of the affected cells and the aggressiveness or progression rate of the disease:

Acute leukemias: syndromes involving immature blood cells with a rapid progression of the disease

- Acute Lymphoblastic Leukemia (ALL): Expansion of lymphoid cells.
- Acute Myeloid Leukemia (AML): Expansion of myeloid cells.

Chronic leukemias: syndromes involving more mature blood cells and with a slower progression of the disease

- Chronic Lymphocytic Leukemia (CLL): Expansion of lymphoid cells.
- Chronic Myeloid Leukemia (CML): Expansion of myeloid cells.

1.3 Acute myeloid leukemia

AML is a hematopoietic disorder characterized by the uncontrolled proliferation and accumulation of myeloid progenitor cells in the bone marrow and peripheral blood [7–9]. The excessive growth of these immature myeloid cells impedes normal hematopoiesis, leading to bone marrow failure and a range of clinical complications [10]. AML is driven by the stepwise accumulation of genetic lesions, acquired at multiple and often temporally separated stages. A primary founder alteration (e.g., in *DNMT3A*, *TET2, IDH1*, *IDH2* or *ASXL*1) can occur in HSCs, leading to the formation of pre-leukemic HSCs [11]. These pre-leukemic cells acquire clonal and growth advantages but retain their ability to differentiate into mature blood cells. The acquisition of additional driver mutations (e.g., *NPM1, CEBPA*), either in pre-leukemic HSCs or in their committed progenitor derivatives, can promote the development of leukemic stem cells (LSCs), a small but highly significant subpopulation of self-renewing cells that lose normal differentiation capacity and can initiate and sustain the disease through the expansion of leukemic blasts. Further cooperating mutations (e.g., *FLT3-ITD*, *KRAS*, *NRAS*) can occur, promoting the development of subclones with distinct genetic combinations.

LSCs not only drive disease progression but also display strong resistance to conventional therapies, representing a major challenge in AML treatment and the main cause of relapse [12,13]. In the absence of clinical intervention, patients typically present with symptoms related to impaired hematopoiesis, including anemia-induced pallor and dyspnea, recurrent infections due to neutropenia, and an increased risk of hemorrhage caused by thrombocytopenia [14,15].

1.3.1 Epidemiology and etiology

Although AML can also occur in pediatric patients, it is the most common acute leukemia in adults [16]. More precisely, it accounts for 30% of all leukemias in adults, representing 0.6% of all tumors. The incidence ranges from 1.3 per 100,000 in individuals younger than 65 years to 12.2 cases per 100,000 in individuals over 65 years [17]. Despite advantages in treatment, AML remains a fatal disease for most of the patients, with a 5-year survival rate of approximately 23% following standard therapy [18].

A complex interplay of genetic and environmental factors influences the onset of AML. In most cases, AML arises as a *de novo* malignancy without a clearly identifiable cause. Known risk factors that can contribute to *de novo* AML development include exposure to certain environmental, physical and chemical agents, as well as pre-existing congenital disorders. Examples of such risk factors include cigarette smoking, benzene exposure, and high-dose ionizing radiation [19–21]. Additionally, children with Down syndrome have a 20-30% higher predisposition to developing AML [22]. Other inherited syndromes associated with an increased risk of AML include severe congenital neutropenia, Fanconi anemia, and Bloom syndrome.

AML can also manifest as a secondary malignancy (s-AML) in patients with a history of hematological disorder, such as myelodysplastic syndromes (MDS), myeloproliferative diseases, and chronic myeloid leukemia [17]. Furthermore, AML can arise as a consequence of prior chemotherapy or radiotherapy for a previous malignancy, particularly when treatment regimens include alkylating agents, topoisomerase II inhibitors, or platinum-based compounds [23]. This specific subset of AML is known as therapy-related AML (t-AML), and the onset can occur up to more than a decade after the initial treatment.

1.3.2 Classification

Over the years, various classification systems have been developed to better understand the pathogenesis of AML and to stratify patients according to prognosis and potential treatment strategies. Initially, AML was categorized using the French-American-British (FAB) classification, which grouped cases into eight subtypes (M0–M7) based primarily on the morphological features and differentiation status of leukemic blasts [24,25]. In 2001, the FAB system was replaced by the World Health Organization (WHO) classification, which began to integrate not only morphological and cytochemical features but also cytogenetic,

molecular, and clinical parameters [26]. Since then, advances in next-generation sequencing (NGS) and a deeper understanding of AML biology have prompted several revisions of the WHO classification, with five editions released to date, the latest being the revised 5th edition published in 2022 [27]. The WHO 2022 classification introduced a restructured hierarchical approach, prioritizing genetic features over purely morphological criteria. AML cases are stratified into two main categories:

- AML with defining genetic abnormalities
- AML defined by differentiation

Notably, within the AML category with defining genetic abnormalities, AML with myelodysplasia-related changes is included, along with a new subcategory for "other defined genetic alterations." This allows the incorporation of newly discovered or rare mutations that may, in the future, warrant classification as independent AML subtypes [28]. Additionally, separate entities include myeloid sarcoma and secondary myeloid neoplasms, which encompass AML arising after cytotoxic therapy or in the context of a germline predisposition.

In parallel with the release of the WHO 2022 update, a separate yet influential classification system was proposed, named the International Consensus Classification (ICC) [29]. While both classifications share many core principles, the ICC diverges in certain aspects:

- It introduces some distinct diagnostic entities and thresholds, particularly regarding genetic mutations.
- It places greater emphasis on *TP53* mutations, recognizing them as a defining feature in specific AML subgroups.
- It consolidates or redefines some borderline or provisional entities previously included in the WHO system.

A comparative summary of the key differences between the WHO 2022 and ICC 2022 classifications is provided in the table below (*Table 1*).

WHO 2022	ICC 2022
AML with defining genetic abnormalities • AML with *RUNX1::RUNX1T1* fusion • AML with *CBFB::MYH11* fusion • Acute promyelocytic leukaemia with *PML::RARA* fusion • AML with *KMT2A* rearrangement • AML with *DEK::NUP214* fusion • AML with *MECOM* rearrangement • AML with *RBM15::MRTFA* fusion • AML with *BCR::ABL1* fusion • AML with *NUP98* rearrangement • AML with *NPM1* mutation • AML with *CEBPA* mutation	**AML with recurrent genetic abnormalities** • AML with t(8;21)(q22;q22.1)/ *RUNX1::RUNX1T1* • AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB::MYH11* • Acute promyelocytic leukemia (APL) with t(15;17)(q24.1;q21.2)/ *PML::RARA*; APL with other RARA rearrangements • AML with t(9;11)(p21.3;q23.3)/ *MLLT3::KMT2A*; AML with other *KMT2A* rearrangements • AML with t(6;9)(p22.3;q34.1)/*DEK::NUP214* • AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2::MECOM* (EVI1); AML with other *MECOM* rearrangements • AML with *BCR::ABL1* fusion • AML with mutated NPM1 • AML with in-frame *bZIP CEBPA* mutations • AML with mutated *TP53*
AML defined by differentiation • AML with minimal differentiation • AML without maturation • AML with maturation • Acute myelomonocytic leukemia • Acute monoblastic/monocytic leukemia • Pure erythroid leukemia • Acute megakaryoblastic leukemia • Acute basophilic leukemia	**AML not otherwise specified** • AML with minimal differentiation • AML without maturation • AML with maturation • Acute myelomonocytic leukemia • Acute monoblastic/monocytic leukemia • Pure erythroid leukemia • Acute megakaryoblastic leukemia • Acute basophilic leukemia
AML with other (rare) defined genetic alterations	**AML with other rare recurring translocations**
AML, myelodysplasia-related	**AML with myelodisplasia-related gene mutations or cytogenetic abnormalities**
Myeloid sarcoma	**Myeloid sarcoma**

Table 1: Classification of AML according to WHO 2022 (left) and ICC 2022 (right).

1.3.3 Diagnosis

The diagnosis of acute myeloid leukemia (AML) is a multi-step process involving several complementary laboratory analyses [30]. Blood samples, bone marrow aspirates, and bone marrow biopsies are collected for diagnostic evaluation. These samples allow for the detection of blasts, assessment of their proportion, and evaluation of their degree of differentiation through complete blood cell count and differential count analyses as well as microscopic examination and

immunophenotyping by flow cytometry. In addition, cytogenetic analyses, including conventional karyotyping, fluorescence in situ hybridization (FISH), and molecular assays such as next generation sequencing (NGS)-based DNA gene panels or PCR-based tests, are performed to confirm the diagnosis, classify the disease into biological and prognostic AML subgroups, and guide therapeutic decisions.

According to diagnostic criteria, a blast count exceeding 20% in the bone marrow is generally sufficient to establish an AML diagnosis. However, the presence of specific recurrent genetic abnormalities can justify a diagnosis even with a lower blast percentage (≥10%), as recommended by the International Consensus Classification (ICC) 2022, or even without any threshold, as defined by the WHO 2022 classification [27]. Exceptions comprise AML with *BCR::ABL1* fusion, AML with *CEBPA* mutation, and other rare genetically defined subtypes, which still require a blast count of at least 20%. Moreover, NGS is increasingly employed, not only to identify somatic mutations but also to detect potential germline variants through sequencing of non-leukemic cells.

1.3.4 Prognostic factors and risk stratification

The prognosis of AML is determined by several clinical and biological factors assessed at the time of diagnosis [31].

Among clinical factors, patient-specific characteristics play a central role: age, performance status, and comorbidities significantly influence outcomes [31–35]. Patients over 60 generally have a less favorable prognosis, due both to a higher risk of treatment-related complications and reduced tolerance to intensive therapies. Overall health and functional capacity further shape treatment options, while additional comorbid conditions can complicate the administration of high-dose regimens. Disease history and clinical presentation also contribute to prognosis. Patients with *de novo* AML tend to experience a more favorable course compared with those with secondary AML, for whom achieving complete remission with standard chemotherapy is often more challenging. Certain AML-related complications may further worsen outcomes. Hyperleukocytosis, a condition characterized by markedly elevated white blood cell counts, can lead to leukostasis, impairing organ function and complicating treatment [36]. In contrast, extramedullary disease, affecting tissues such as the skin, gums, or central nervous system, often indicates a more aggressive disease course and can render treatment more complex and less effective [37].

Biological factors include genetic characterization, which is performed through cytogenetic analyses and molecular testing to predict therapy response and survival.

The European Leukemia Net (ELN) classification, introduced in 2017 [38] and refined subsequently in 2022 [27], provides a comprehensive framework for risk stratification for AML patients treated with intensive chemotherapy, dividing the disease into three categories, favorable, intermediate and adverse, based on specific chromosomal abnormalities and genetic mutations.

The favorable prognostic risk group includes cases of AML with nucleophosmin 1 (*NPM1*) mutations without FMS-Like tyrosine kinase 3 Internal tandem duplication (*FLT3-ITD*), as well as chromosomal abnormalities such as t(8;21), t(15;17), and inv(16). These cases generally exhibit a good rate of survival with standard chemotherapy. In contrast, the presence of *TP53* mutation, complex karyotypes, monosomy 5 or 7, t(6;9), and inv(3) is associated with poor treatment response, low rates of complete remission (CR) and a high risk of relapse. The intermediate-risk group primarily includes AML subtypes with *FLT3-ITD* mutations alone or in combination with *NPM1* mutations. It also encompasses AML with normal cytogenetics or other cytogenetic abnormalities not included in the other two groups [25]. In 2024, a new proposed ELN classification was introduced for those patients treated with less intensive therapies [39]. *Table 2* presents the 2022 ELN risk classification alongside the updated 2024 refinement specifically designed for patients ineligible for intensive chemotherapy.

Additionally, epigenetic factors, particularly DNA methylation patterns, have gained attention as a potential future tool in AML prognosis. Studies have demonstrated that DNA methylation signatures can be used to classify AML patients into prognostic risk groups, especially beneficial for cytogenetically normal AML patients who typically lack clear prognostic indicators [40,41].

Risk cathegory	ELN 2022 Intensive chemotherapy	ELN 2024 Less intensive treatment
Favorable	- t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1* - inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11* - Mutated *NPM1*wihout *FLT3*-ITD - bZIP in-frame mutated *CEBPA*	- Mutated *NPM1* (*FLT3*-ITD^{neg}, *NRAS*^{wt}, *KRAS*^{wt}, *TP53*^{wt}) - Mutated *IDH2* (*FLT3*-ITD^{neg}, *NRAS*^{wt}, *KRAS*^{wt}, *TP53*^{wt}) - Mutated *IDH1* (*TP53*^{wt}) - Mutated *DDX41* - Other cytogenetic and/or molecular abnormalities (*FLT3*-ITD^{neg}, *NRAS*^{wt}, *KRAS*^{wt}, *TP53*^{wt})
Intermediate	- Mutated *NPM1* with *FLT3*-ITD - Wild-type *NPM1* with *FLT3*-ITD (without adverse-risk genetic lesions) - t(9;11) (p21.3;q23.3)/*MLLT3*::*KMT2A* - Cytogenetic and/or molecular abnormalities not classified as favorable or adverse	- Other cytogenetic and molecular abnormalities (*FLT3*-ITD^{pos} and/or *NRAS*^{mut} and/or *KRAS*^{mut}; *TP53*^{wt})
Adverse	- t(6;9)(p23.3;q34.1)/*DEK*::*NUP214* - t(v;11q23.3)/*KMT2A*-rearranged - t(9;22)(q34.1;q11.2)/*BCR*::*ABL1* - t(8;16)(p11.2;p13.3)/*KAT6A*::*CREBBP* - inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2*, *MECOM(EVI1)* - t(3q26.2;v)/*MECOM*(*EVI1*)-rearranged - –5 or del(5q); –7; –17/abn(17p) - Complex karyotype, monosomal karyotype - Mutated *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, and/or *ZRSR2* - Mutated *TP53*	- Mutated *TP53*

Table 2: AML ELN 2022 prognostic classification based on intensive chemotherapy (left) and less intensive treatments (right).

1.3.5 Pathogenesis

AML is a highly heterogeneous disease driven by a wide range of genetic and molecular alterations. Approximately 50–55% of patients present with chromosomal abnormalities [42–44], while the remaining 45–50% are cytogenetically normal but carry somatic mutations in oncogenes, tumor suppressor, epigenetic regulators and/or other molecular factors. The pathogenesis of AML has traditionally been explained by the two-hit model, which proposes that leukemogenesis requires at least two cooperating types of mutations [45]: Class I mutations, which confer a proliferative or survival advantage (e.g., activating mutations in *FLT3* or *RAS*), and Class II mutations, which affect normal hematopoietic differentiation and promote leukemic transformation (e.g., *PML–RARA*, *CEBPA*, or *NPM1*). However, with the recent increase in knowledge in AML genetics, the mutational and molecular structure of AML has become more complex.

1.3.5.1 Genetic mutations

Genetic mutations can be classified by the affected gene function. They may impact signal transduction genes (*FLT3, KIT, RAS, JAK2, PTPN11*), transcription factors and co-activators (*NPM1, CEBPA, RUNX1*), spliceosome complexes (*SRSF2 and U2AF1*) or epigenetic regulators (*DNMT3A, TET2, IDH1/2, ASXL1, EZH2*), disrupting cellular homeostasis and contributing to oncogenic transformation. Alterations in tumor suppressor genes (*TP53, WT1, PHF6*) further impair genomic integrity, cell cycle control, and apoptosis, promoting leukemogenesis. Below are details on the most common and the most clinically relevant AML genetic mutations:

FMS-like tyrosine kinase 3 (FLT3) is a class III tyrosine kinase receptor involved in sustaining hematopoietic progenitors' survival and proliferation [46]. Internal tandem duplication (*ITD*) or mutations in the second tyrosine kinase domain (*TKD*) of *FLT3* occur in 25% and 5 to 10% of AML cases, respectively [47,48]. In both cases, the result of the mutation affecting the *FLT3* gene is a constitutive activation of this tyrosine kinase. As a consequence, the presence of *FLT3-ITD* mutation is linked to a poor prognosis in CN-AML, while the prognostic impact of *FLT3-TDK* mutations is still controversial.

CCAAT/enhancer-binding protein alpha (CEBPA) is a transcription factor involved in myeloid cell differentiation and proliferation regulation [49]. Mutations in *CEBPA*, occurring either as single (monoallelic) or double (biallelic) alterations, are found in approximately 10% of acute myeloid leukemia (AML) cases and are generally associated with a favorable prognosis. In particular, patients with in-frame *bZIP CEBPA* mutations, whether monoallelic or biallelic, exhibit high sensitivity to intensive chemotherapy, with over 90% achieving complete remission (CR) [50].

Nucleophosmin 1 (NPM1) is a protein involved in many biological processes within mammalian cells, among which the best described are ribosome biogenesis, chromatin remodeling, mitotic spindle assembly, DNA repair and apoptosis regulation [51]. Over the years, many reports have established a direct implication of NPM1 in cancers' pathogenesis, acting both as a proto-oncogene and a tumor suppressor, depending on the cell type and on the protein level [52]. *NPM1* mutations occur frequently in AML cells. More precisely, 35% of AML cases in adults show heterozygous mutation of *NPM1*, mostly localizing to exon 12 of the gene. In this region, over 50 molecular variants have been discovered so far, and

are all characterized by a 4 bp insertion between nucleotide 960 and 961 [53]. The most common mutation is mutation A, arising in about 80% of AML cases with mutant *NPM1,* where a TCTG duplication occurs at position 960. The result of this mutation is a frameshift at the C-terminal region of the protein, which causes the elimination of one or both tryptophans of the NOLS motif and, at the same time, the formation of a new Leucine rich Nuclear Export Signal (NES) motif. The consequence is the unfolding of the 3-helix structure of NPM1 C-terminal domain, thus decreasing the binding of the protein to the nucleolus [54]. The presence of the newly created NES motif increases NPM1 binding to the nuclear export protein, causing a trans localization of NPM1 mutated protein from the nucleus to the cytoplasm. One of the biological results of the accumulation of the protein in the cytoplasm is the aberrant recruitment of different NPM1 interactors to ectopic regions (nucleoplasm and cytoplasm), resulting in the alteration of their physiological function [55]. Alterations in NPM1 subcellular localization, therefore, affect multiple cellular pathways, including the regulation of *TP53*, *HOX* gene expression, and the maintenance of genomic stability. In addition, the presence of *NPM1c+* in AML cells induces not only the haploinsufficient expression of wild-type *NPM1* but also the formation of heterodimers between the mutant and wild-type form, with the subsequent alteration of NPM1's multifunctional activity in the nucleolus [55]. *NPM1* mutation alone associates with favorable outcomes with increased 5-year overall survival rates compared to other AML subtypes and enhanced response to chemotherapy. However, the occurrence of other genetic alterations, particularly the *FLT3-ITD* mutation, can significantly impact prognosis and treatment response. The presence of both *NPM1* and *FLT3-ITD* mutations is associated with a more aggressive disease course and poorer outcomes. Patients with this combination may experience higher relapse rates and reduced overall survival compared to those with NPM1 mutations alone.

***Isocitrate dehydrogenase 1 (IDH1)* and *isocitrate dehydrogenase (IDH2)* mutations**, which affect DNA methylation, occur in about 20% of AML cases and often associate with co-mutations in *NPM1* and *DNMT3A* [56]. The mutation consistently affects an arginine residue within the enzyme's active site, R132 in *IDH1*, and R140 or R172 in *IDH2*, occurring in approximately 80% and 20% of *IDH2*-mutated patients, respectively. In the presence of these alternations, the enzymes normally responsible for catalyzing the oxidative decarboxylation of isocitrate to α-ketoglutarate (a-KG), gain a neomorphic function that leads to the conversion of a-KG to the oncometabolite 2-hydroxyglutarate (2HG) [57]. This metabolite

inhibits TET2 and other α-ketoglutarate–dependent dioxygenases, resulting in a hypermethylated phenotype. These epigenetic alterations impair normal cell differentiation, thereby contributing to leukemogenesis [40]. The prognostic significance of *IDH* mutations is not fully clarified. However, *IDH1* mutations have been generally associated with a worse prognosis, whereas *IDH2* mutations may be linked to a favorable outcome. In addition, the co-occurrence of *IDH* mutations with *NPM1* mutations, in the absence of *FLT3-ITD*, has been associated with a better overall prognosis.

Tumor Protein 53 (TP53) mutations occur in approximately 5-15% of *de novo* AML cases, rising to 30% in therapy-related AML and up to 70% in patients with adverse chromosomal alterations, and are associated with an aggressive disease phenotype [48,58]. *TP53* encodes a crucial tumor suppressor protein that plays a critical role in sustaining genomic integrity by regulating fundamental cellular processes, such as DNA repair, cell cycle arrest, and apoptosis [59]. Therefore, patients harboring *TP53* mutations have a particularly poor prognosis, with low CR rates, high relapse rates, and inferior overall survival compared to *TP53*-wildtype AML [60,61]. In addition, resistance to standard cytarabine is often described, and the presence of complex karyotypes further limits therapeutic success [58,62].

1.3.5.2 Chromosomal alterations

Chromosomal alterations include translocations among chromosomes, resulting in gene rearrangements and/or gene fusions. One of the most common chromosomal abnormalities associated with a favorable outcome in AML is the t(8,21)(q22;22), where the *RUNX1-RUNX1T1* fusion transcript is generated due to a chromosomal rearrangement between the *RUNX1T1* gene, located on chromosome 8, and the RUNX1 gene located on chromosome 21 [63]. Presence of *RUNX1-RUNX1T1* fusion gene associates with a favorable prognosis in adults, but a poor prognosis in children. Another recurrent chromosomal alteration, observed in approximately 6-8% of *de novo* AML cases, is the inv(16)/t(16;16), which leads to the fusion *CGFB-MYH11* transcript. Generally, patients within this AML subtype have a good prognosis and respond well to the induction therapy [64]. Other common chromosomal abnormalities in AML are those affecting the long arm of chromosome 11 (11q), which leads to Mixed-Lineage Leukemia (*MLL*) translocations and poor prognosis, including translocations between chromosome 9 and chromosome 11 (t(9;11)) [65].

The **t(15;17) translocation** is the defining genetic hallmark of acute promyelocytic leukemia (APL), occurring in about 5–10% of all AML cases and in ~95% of APL patients [66]. This rearrangement fuses the promyelocytic leukemia (PML) gene on chromosome 15q24 with the retinoic acid receptor alpha (*RARA*) gene on chromosome 17q21 [67,68]. Under normal conditions, RARA, a member of the nuclear retinoic acid receptor (RARs) family, regulates hematopoietic stem cell development, expansion, and differentiation. In the absence of physiological retinoic acid (RA), RARA associates with retinoid X receptor (RXR) to form heterodimers that bind retinoic acid response elements (RAREs) in the chromatin, recruiting corepressor complexes and repressing transcription. This repression is normally relieved when physiological levels of RA are present, allowing the recruitment of coactivators with subsequent induction of RARA target genes. The *PML* gene, in contrast, encodes a phosphoprotein with tumor suppressor activity. PML forms nuclear bodies that promote cellular senescence and apoptosis, largely through p53 activation [69]. In APL, the *PML–RARA* fusion oncoprotein acts as a constitutive repressor of RARA target genes. Specifically, *PML–RARA*, through both self -oligomerization and heterodimerization with RXR, competes with wild-type RARA and promotes the recruitment of epigenetic repressors, such as DNMT3A and HDAC, to RAREs elements. This not only blocks RARA-mediated transcription and arrests granulocyte differentiation, but also disrupts PML nuclear bodies, impairing apoptosis and contributing to leukemogenesis [70].

In the past, and since the disease was first described in 1957, APL was considered as a fatal and highly malignant hematopoietic disease [71]. However, today, with the introduction of targeted therapies, the disease outcome has been redefined. A breakthrough in APL treatment occurred with the institution of a combinatory treatment of all-trans-retinoic acid (ATRA) and arsenic trioxide (ATO). ATRA-ATO treatment has led to 90% complete remission and 5-year survival [72,73], redefining APL as one of the most curable forms of AML. Further details on APL therapy are provided in the AML treatment section.

1.3.6 Treatments

AML treatment has undergone significant evolution in recent years to address the disease's heterogeneity. It consists of two main phases: induction therapy, which aims to achieve complete remission, defined as less than 5% blasts in the bone marrow along with the recovery of normal peripheral blood counts and post-remission therapy, which focuses on preventing relapse and reducing minimal residual disease (MRD) [6,74].

The choice of treatment in each phase is tailored to patient-specific characteristics, with age and fitness for intensive chemotherapy being key factors in the initial assessment. Specifically, fitness for intensive chemotherapy is evaluated based on the presence of comorbidities and the patient's performance status, ensuring that treatment intensity is adapted to individual tolerance levels. In recent years, novel therapeutic strategies have been explored to enhance treatment efficacy and selectively target distinct AML subtypes and biological futures. Integrating these targeted agents into AML therapy has shown promise in improving outcomes, both in intensively treated patients and particularly in those ineligibles for intensive chemotherapy.

1.3.6.1 De novo-AML < 75 years old and eligible for intensive chemotherapy

The standard induction treatment for younger and medically fit patients typically follows the 7+3 regimen, consisting of a 7-day continuous infusion of cytarabine (a deoxycytidine analog) combined with daunorubicin (an anthracycline antibiotic) administered for 3 days [75]. In Sweden, a variant of the 7+3 is used with higher doses of cytarabine given for 5 days, a so called 5+3 schedule. With this regimen, 60–85% of patients younger than 60–65 years achieve complete remission. However, to improve overall survival (OS) and reduce the risk of relapse, modifications to the standard induction therapy have been introduced in recent years. Among them, CPX-351, a liposomal formulation of daunorubicin and cytarabine, has been FDA and EMEA-approved for the treatment of therapy-related t-AML and AML with myelodysplasia-related changes (AML-MRC). Another important advancement is gemtuzumab ozogamicin (GO), an anti-CD33 monoclonal antibody conjugated to calicheamicin, which has been approved alongside intensive chemotherapy for cytogenetically favorable CD33-positive patients as core-binding factor AML [76]. Additionally, the development of FLT3 inhibitors has further refined treatment strategies for AML patients carrying *FLT3* mutations. In addition to standard chemotherapy, midostaurin or quizartinib (specific for *FLT3_ITD* mutations) can now be used in combination with the 7+3 regime, as both have demonstrated improved OS compared to intensive chemotherapy alone [77,78].

For the post-remission therapy, 2 different approaches can be chosen. Allogeneic hematopoietic stem cell transplantation (HSCT) is recommended for patients with intermediate- to high-risk AML, as it remains the most effective curative strategy. However, HSCT is associated with high morbidity and mortality risks, including graft-versus-host disease (GVHD), making its use highly selective. For patients

with favorable-risk AML, consolidation chemotherapy with high-dose cytarabine (HiDAC), with or without gemtuzumab ozogamicin, is commonly administered. Furthermore, in intermediate- to adverse-risk patients who are ineligible for transplantation, CC-486 (oral azacitidine) may be added to consolidation therapy to prolong remission and improve overall survival [79].

1.3.6.2 De novo-AML > 75 years old or unfit for intensive chemotherapy

Treatment of this subclass of AML patients primarily relies on hypomethylating agents (HMAs), like azacitidine/decitabine, in combination with venetoclax [80,81], a BCL-2 inhibitor, which will further be described in the next paragraph. For patients with *IDH1* mutations, ivosidenib, either as monotherapy [82] or in combination with azacitidine, has been approved as an effective targeted therapy. Specifically, the combination with azacitidine has been demonstrated in the phase III AGILE trial by promoting better event-free survival and higher OS compared to patients singularly treated with azacitidine [83]. Additionally, gilteritinib, a FLT3 inhibitor approved as monotherapy for relapsed/refractory AML [84], is currently under investigation in combination with HMAs, with or without venetoclax, as a potential treatment strategy. Preliminary studies suggest that this combination may further enhance response rates and prolong survival in patients with *FLT3* mutations who may not respond adequately to standard therapies.

In terms of post-remission therapy, treatment decisions are similar to those used for patients treated with intensive chemotherapy. HSCT remains the preferred option for patients with intermediate/adverse-risk features, offering the potential for long-term remission. However, for patients who are ineligible for transplantation or belong to the ELN favorable-risk subgroup, continuous maintenance therapy with HMAs + venetoclax or targeted agents is often recommended to prolong remission and delay disease progression.

1.3.6.3 Venetoclax: a BCL-2 inhibitor

Venetoclax (ABT199) is a selective inhibitor of BCL-2 [85] and has shown, since its introduction in clinical trials, promising therapeutic effects in elderly patients or those unfit for intensive induction chemotherapy [86]. The BCL-2 protein family regulates the intrinsic apoptotic pathway and includes pro-apoptotic BH3-only proteins (NOXA, PUMA, BIM, BID, BAD) and the effector proteins BAX and BAK, as well as anti-apoptotic members such as BCL-2, BCL-XL, MCL-1, BCL-W, and BFL-1/A1 [87]. Under physiological conditions, anti-apoptotic proteins such as BCL-2 bind and sequester BAX and BAK thereby blocking apoptosis. In response to

cellular stress, BH3-only proteins are upregulated, promoting both the release of BAX and BAK from anti-apoptotic proteins and their subsequent activation. Activated BAX and BAK then oligomerize on the mitochondrial outer membrane, leading to mitochondrial outer membrane permeabilization (MOMP) and the release of mitochondrial factors such as cytochrome c, ultimately triggering the caspase cascade. Venetoclax functions as a BH3 mimetic. By binding to the BH3-binding groove of BCL-2, it displaces pro-apoptotic proteins such as BAX and BIM, thereby promoting cell death. Since BCL-2 is frequently upregulated in hematopoietic malignancies, including AML, venetoclax restores apoptosis and counteracts leukemic cell survival and chemoresistance.

Because of the positive results achieved with venetoclax in Chronic lymphocytic leukemia (CLL) [88,89], the compound was subsequently evaluated for AML, initially as monotherapy in relapsed and refractory patients. Yet a phase 2 trial demonstrated only limited efficacy [90]. Building on these findings, later investigations shifted to combination regimens and reported markedly improved outcomes. In particular, high response rates and favorable tolerability were observed when venetoclax was combined with hypomethylating agents (HMAs) or low-dose cytarabine (LDAC) [85,91–93]. These data led to the 2018 FDA approval of Venetoclax, specifically in combination with an HMA or low doses cytarabine for the treatment of elderly patients with newly diagnosed AML or those ineligible for standard intensive chemotherapy.

Currently, several additional studies are underway to evaluate venetoclax in combination with standard chemotherapy for younger, fit AML patients, as well as triple-agent regimens incorporating azacitidine and targeted therapies for cases harboring specific mutations such as *FLT3, IDH1, IDH2, NPM1,* and *KMT2A* rearrangements [94].

Although venetoclax represents a promising option for AML, relapse and resistance frequently occur. These phenomena can result from intrinsic or adaptive resistance mechanisms, this latest often driven by the selection of resistant clones harboring specific mutations such as *FLT3-ITD, N/KRAS, PTPN11* and *TP53*. Resistance mechanisms may include upregulated expression of partner anti-apoptotic proteins such as MCL-1 or mutations in *BAX* which can alter its physiological expression and/or its mitochondrial localization [95,96]. Continued research is therefore necessary to better understand the activity of venetoclax and to optimize combination strategies that enhance its therapeutic effect.

1.3.6.4 APL patient treatment: ATRA and ATO combination

All-trans retinoic acid (ATRA) combined with arsenic trioxide (ATO) is the adopted standard therapy for acute promyelocytic leukemia (APL), particularly in standard-risk patients. ATRA was first shown to induce differentiation of APL cells *in vitro* [97,98] and its combination with anthracyclines significantly improved response rates and long-term survival compared with either treatment alone [99,100]. ATO demonstrated efficacy in relapsed and refractory APL [101], and, when combined with ATRA, provided high remission rates with lower toxicity than ATRA plus anthracycline-based therapy. Since 2013, ATRA+ATO has therefore become the standard of care [102,103], replacing traditional chemotherapy-based regimens. Mechanistically, ATRA, a vitamin A derivative, has a dual therapeutic effect in APL cells. On one side, pharmacological level of ATRA promote conformational changes in PML-RARA homodimer, leading to the release of transcriptional co-repressors. This favors the recruitment of transcription co-activator complexes and the activation of those genes involved in myeloid cell differentiation, such as *PU.1* and *C/EBP* [104–106]. On the other hand, ATRA can promote the degradation of PML-RARA oncoprotein through the activation of both proteasome and autophagy-related pathways [107–109]. ATO acts synergistically with ATRA. In fact, it binds to the PML portion of the PML-RARA protein, causing sumoylation, polyubiquitination and subsequent proteasomal degradation of this latest [110]. In addition, ATO directly targets PML to promote the restoration of nuclear bodies, inducing cell apoptosis. This synergy between ATRA and ATO has been shown to be highly effective, allowing for both APL cell differentiation induction and clearance of leukemic initiating cells through apoptotic mechanisms specifically induced by ATO. Despite this, resistance mechanisms to ATRA-ATO therapy can still occur, which are specifically observed in relapse patients. Resistance mechanisms include genetic mutations arising in the *RARA* ligand binding domain or mutations in the arsenic binding site of the *PML* gene [111–113]. Consequently, ongoing research aims to develop novel therapeutic strategies to overcome resistance and improve outcomes for all patients with APL.

1.4 The Human transcriptome: Focus on long non-coding RNAs

The human transcriptome, which encompasses all RNA molecules encoded by our genome, can be envisioned as an iceberg (Figure 1). The visible tip, representing only ~2% of all transcripts, corresponds to well-characterized protein-coding messenger RNAs. Hidden beneath the surface lies the remaining ~98%, which does not translate into proteins [114–116]. Beyond essential housekeeping RNAs, such as transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), and small nuclear RNAs (snRNAs), this vast submerged portion was long regarded as "junk RNA." However, advances in next-generation sequencing have revealed a diverse landscape of regulatory non-coding RNAs (ncRNAs), highlighting their critical roles in cellular processes and shifting the perception of the transcriptome's hidden depths.

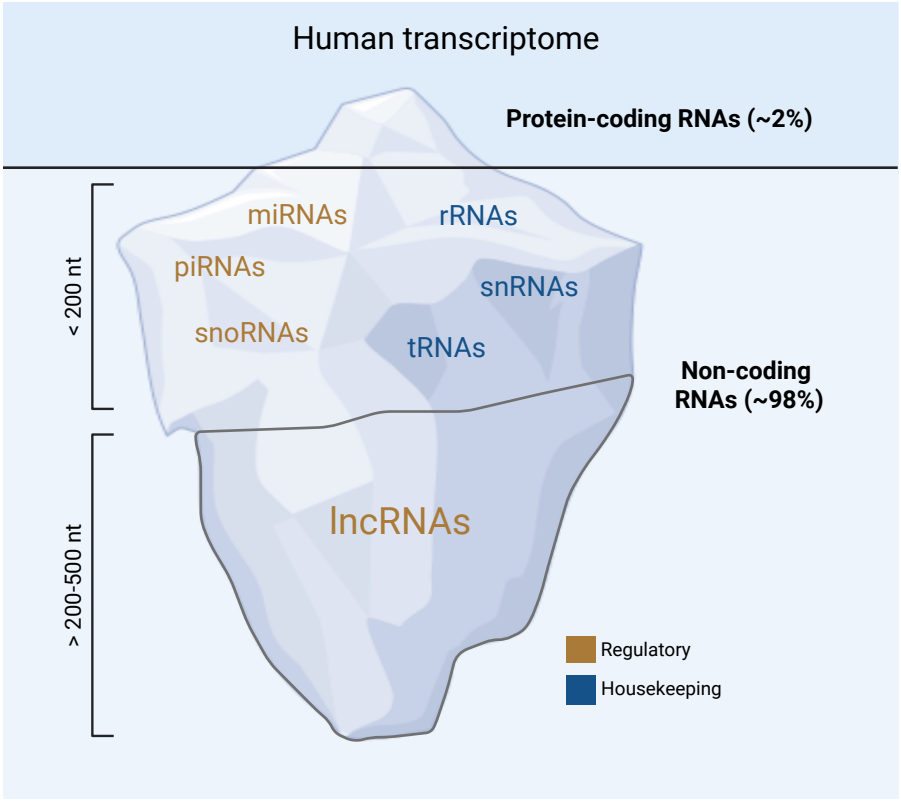

Figure 1. Representation of the human transcriptome as an iceberg. The visible tip of the iceberg illustrates protein-coding transcripts, while the larger portion below the water surface represents non-coding RNA transcripts. Non-coding RNAs can be categorized by length, greater or less than 200 nucleotides, and by function, distinguishing regulatory from housekeeping non-coding RNAs. Created with BioRender.com

A key distinction among regulatory ncRNAs can be made according to transcript length. Small ncRNAs, shorter than 200 nucleotides, include, as major categorizes, microRNAs (miRNAs), small nucleolar RNAs (snoRNAs), and PIWI-interacting RNAs (piRNAs). In contrast, long non-coding RNAs (lncRNAs) constitute a broad and heterogeneous group, originally defined as transcripts longer than 200 nucleotides. More recent definitions [117,118], however, suggest a threshold of 500 nucleotides to separate transcripts in the 200–500 nt range, which may retain limited protein-coding potential.

Although lncRNAs do not encode proteins and lack open reading frames (ORF), they share many processing features with messenger RNAs (mRNAs): they are transcribed by RNA polymerase II and undergo 5′ capping, 3′ polyadenylation, and canonical splicing to produce mature isoforms [119,120]. Unlike mRNAs, however, lncRNAs exhibit striking cell- and tissue-specific expression patterns [121–123], generally low abundance, poor evolutionary conservation, and extensive isoform diversity [124]. Functionally, lncRNAs can control a broad spectrum of biological processes, including cell differentiation, cell-cycle regulation, and development [125–128] by interacting with chromatin, proteins, and other RNA molecules, often forming secondary and tertiary structures [129,130]. These characteristics underscore both the complexity of lncRNA biology and its critical roles in gene regulation in health and disease.

The first lncRNAs were identified in the early 1990s with the discovery of H19 [131], an imprinted gene, and XIST, transcribed from the X-inactivation center and essential for X-chromosome inactivation [132]. Subsequent advances in arrays and next-generation sequencing technologies, including CAGE [133], enabled the systematic identification of thousands of lncRNAs [114,116,134,135]. Today, several lncRNA annotation databases exist, based on different strategies, including manual curation and/or automated transcriptome assembly. For example, the widely used GENCODE catalogue (v49) [136] includes over 34,800 human lncRNA genes and 189,000 transcripts, derived from the manual integration of cDNA and expressed sequence tag (EST) databases. In contrast, the FANTOM CAT [137] database integrates transcriptome assembly with short-read data from CAGE sequencing, providing one of the most comprehensive annotations of the human 5′ transcriptome, with 28,919 lncRNA genes. Finally, one of the most extensive lncRNA resources, the NONCODE (v6) database [138], integrates multiple datasets, both manually curated and transcriptome-based, and currently lists over 96,000 lncRNA genes and 173,000 transcripts.

1.4.1 Classification and function of long non-coding RNAs

According to their genomic location, lncRNAs can be classified into several categories [117,139]. The terms sense and antisense refer to lncRNAs transcribed from the same or opposite DNA strand, respectively, relative to a protein-coding gene. Intergenic lncRNAs are situated between two protein-coding genes, whereas intronic lncRNAs are located within the intronic regions of coding genes. Finally, Bidirectional lncRNAs are transcribed from the strand opposite to that of a coding gene and are typically found within 1 kilobase (kb) upstream of its promoter region.

Beyond these classes defined by proximity to protein-coding loci, some lncRNAs are distinguished by their association with other genomic regions or RNA elements. Enhancer lncRNAs, for instance, originate from enhancer regions and often orchestrate chromatin loops to modulate gene expression [140,141]. Pseudogene-derived lncRNAs [142] carry echoes of their ancestral genes, capable of fine-tuning the mRNA levels of their parental counterparts. Adding another layer of complexity, circular lncRNAs arise when precursor mRNAs undergo back-splicing [143,144], forming covalently closed loops that are remarkably stable and can act as molecular sponges or scaffolds.

lncRNAs exhibit remarkable diversity not only in their genomic location but also in their subcellular distribution. While most lncRNAs are retained in the nucleus [145–147], due to incomplete splicing, RNA–protein complex formation, or the presence of nuclear retention elements (NREs), many are also detected in the cytoplasm, either after nuclear export following transcription or as transcripts derived from mitochondrial DNA [148]. Moreover, lncRNAs can localize within specialized nuclear structures, such as nuclear bodies, or be released in extracellular vesicles like exosomes [149–151]. Notably, the subcellular localization of lncRNAs is closely linked to their functional roles, as illustrated in Figure 2.

Nuclear lncRNAs play key roles in the regulation of gene expression [119,139]. Depending on whether they act on the same chromosome or a different one, they are classified as cis- or trans-acting lncRNAs, respectively. Several nuclear lncRNAs function as molecular decoys, sequestering transcription factors or other regulatory molecules to modulate transcription. For example, PANDA plays a pivotal role in the DNA damage response by binding and sequestering the transcription factor NF-YA, thereby preventing p53-mediated apoptosis [152]. They can also act as guides, directing transcription factors or chromatin-

modifying complexes to specific promoters or genomic regions to regulate gene expression and chromatin accessibility. A well-characterized example is HOTAIR [153], which recruits the Polycomb Repressive Complex 2 (PRC2) to the *HOXD* locus, modulating developmental gene expression and chromatin state. Additionally, lncRNAs can serve as scaffolds, providing platforms for the assembly and recruitment of multiple chromatin-modifying complexes and cofactors. An illustrative example is TERC [154], a highly stable lncRNA that functions as a scaffold in the formation of the telomerase complex, essential for maintaining telomere integrity and cellular lifespan. Furthermore, some nuclear lncRNAs are associated with the formation and function of nuclear condensates: for example, NEAT1 is essential for paraspeckle assembly [155,156], while MALAT1 by localizing in nuclear speckles, is involved in pre-mRNA splicing [156–158], affecting the distribution and levels of serine- and arginine-rich (SR) proteins.

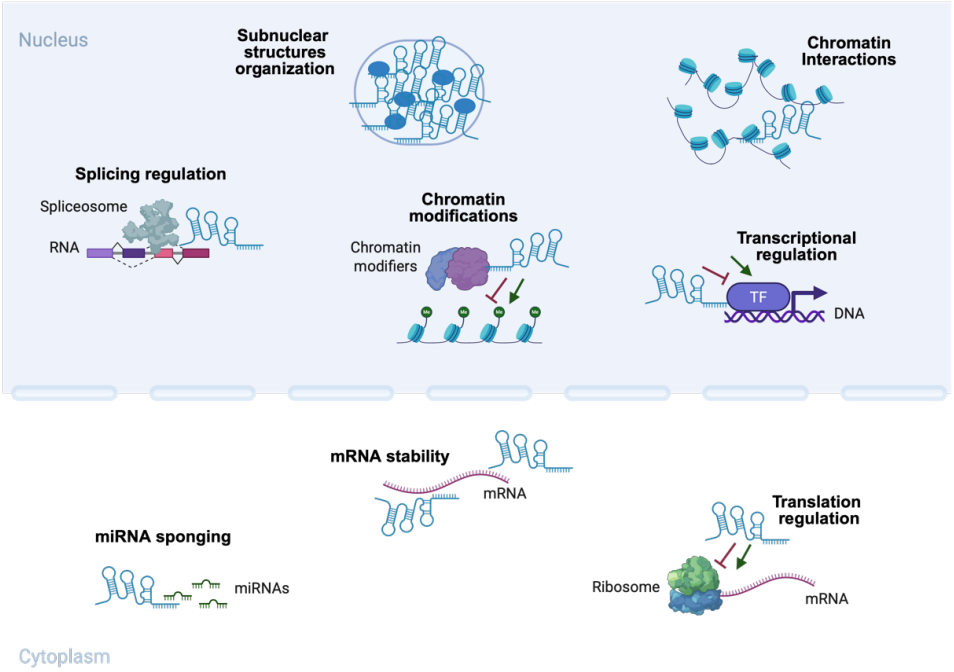

Figure 2. Schematic representation of lncRNA functions within the nucleus (top) and the cytoplasm (bottom). Created with BioRender.com

In contrast, cytoplasmic lncRNAs are primarily involved in post-transcriptional regulatory processes. lncRNAs as half-STAU1-binding site RNAs (1/2-sbsRNAs) [159], have been shown to induce mRNA decay through the presence of Alu sequences, which promote the recruitment of the RNA-binding protein STAU1, a

key factor in STAU1-mediated mRNA decay (SMD). Conversely, Terminal differentiation-induced ncRNA (TINCR) lncRNA has been shown to promote epidermal differentiation by interacting with STAU1 and base-pairing with target mRNAs via a 25-nucleotide "TINCR box," promoting the stabilization of these mRNAs rather than their degradation [160]. Another important cytoplasmic function of lncRNAs is their microRNA-sponging activity. Acting as competing endogenous RNAs (ceRNAs), they sequester microRNAs and prevent them from repressing their target mRNAs [161,162]. In cancer, for example, the pseudogene-derived lncRNA PTENP1, transcribed from the tumor-suppressor gene PTEN, acts as a sponge for miR-17, miR-19, and miR-26, ultimately enhancing PTEN protein levels and exerting a tumor-suppressive effect [163,164]. Beyond these roles, cytoplasmic lncRNAs can also regulate translation by influencing ribosome biogenesis and assembly [165–167], as well as by directly interacting with ribosomal proteins [168,169] or with translation initiation and elongation factors [170–172]. Furthermore, lncRNAs may influence post-translational modifications, such as ubiquitination [173], SUMOylation [174,175], or phosphorylation [176–178], impacting protein function and turnover.

1.4.2 Long non-coding RNAs in acute myeloid leukemia

Advances in high-throughput sequencing and integrative transcriptomic analyses have underscored the pivotal role of lncRNAs in promoting or inhibiting cancer development and progression [122,179,180]. Their dysregulation is often driven by a variety of genetic and epigenetic alterations, including single-nucleotide variants, copy number variations, and aberrant DNA methylation [180,181].

In AML, lncRNAs can function as either oncogenes or tumor suppressors by regulating fundamental cellular processes such as proliferation, apoptosis, differentiation, cell growth, and survival [182,183]. Aberrant lncRNA expression has also been shown to affect therapy-related pathways, contributing to the development of drug resistance mechanisms. These roles are not mutually exclusive, as a single lncRNA may exert multiple regulatory functions depending on the cellular context and subcellular localization.

For these reasons, lncRNAs have attracted significant clinical interest as potential therapeutic targets and biomarkers. Figure 3 summarizes the main functional roles and clinical implications of lncRNAs in AML.

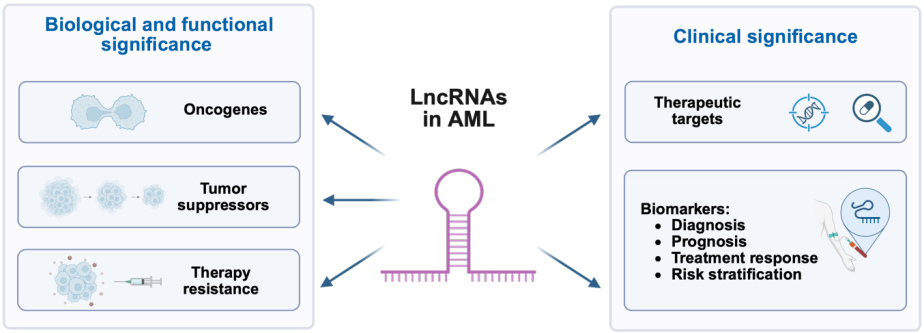

Figure 3. Schematic representation of lncRNA significance in acute myeloid leukemia (AML). Left: lncRNAs have biological and functional roles, acting as oncogenes, tumor suppressors, and promoting therapy resistance mechanisms. Right: lncRNAs can be clinically exploited as therapeutic targets or as biomarkers for prognosis, diagnosis, treatment response, and risk stratification. Created with BioRender.com

1.4.2.1 Biological and functional significance

Several lncRNAs with oncogenic functions have been described to be upregulated in AML. For instance, lncRNAs such as MALAT1, UCA1, SNHG1 and H19 have been reported to act as miRNA sponges, promoting AML proliferation by disrupting the miRNA-mediated regulation of oncogenic mRNAs [184–189]. A similar sponging activity has also been observed for ANRIL in HL60 cells [190], while in MOLM-13 cells, ANRIL was found to promote AML maintenance through dysregulation of leukemic cell metabolism [191]. The well-characterized lncRNA HOTAIR enhances leukemic cell growth by repressing *HOXA5* expression through interaction with DNMT3B [192]. Furthermore, HOTAIR can also influence *C-KIT* expression levels by acting as a miRNA sponge or epigenetically promoting *p15* silencing [193,194]. RUNXOR, an intragenic lncRNA of *RUNX1,* regulates *RUNX1* expression by binding to its promoter and enhancer regions [195]. In addition, the intergenic lncRNA PVT1 is specifically upregulated in APL cells, where it promotes promyelocyte proliferation by protecting MYC from degradation [196].

Conversely, IRAIN, MEG3, and NEAT1 are among the best-characterized lncRNAs with tumor-suppressor functions in AML. IRAIN is an antisense transcript of the insulin-like growth factor 1 receptor (IGF1R) gene and acts as a scaffold to promote long-range intrachromosomal promoter /enhancer loop formations. Low IRAIN expression in high-risk AML is linked to shorter overall survival, elevated white blood cell counts, and higher relapse rates [197,198]. MEG3 inhibits tumorigenesis through both p53-dependent and p53-independent mechanisms, promoting

apoptosis and reducing the expression of *BCL-2* and *MDM2* [199,200]. In AML, however, the MEG3 promoter is frequently hypermethylated, leading to diminished expression and loss of its anticancer activity [201]. NEAT1 is frequently overexpressed in solid tumors, where high levels correlate with poor overall survival [202]. In contrast, its expression is reduced in AML and APL. In APL cell lines, this downregulation is driven by the oncoprotein PML-RARα, whereas treatment with ATRA restores NEAT1 levels and its function as a repressor of leukemic cell proliferation [203–205]. Additionally, low cytoplasmic NEAT1 in AML has been associated with enhanced relapse and is thought to inhibit leukemogenesis through inactivation of the WNT signaling pathway [206]. Furthermore, other well-characterized lncRNAs, such as CAS15 and GAS5, are also downregulated in AML, modulating *SOX4* gene expression in AML with *RUNX1* translocation or p53 signaling, respectively [207,208].

In the context of AML, lncRNAs have also been recognized for their role in mediating therapy resistance, thereby influencing treatment response and clinical outcomes. Several long non-coding RNAs have been specifically implicated in resistance to the standard chemotherapeutic agent cytarabine. For instance, high expression of DANCR has been associated with reduced sensitivity to cytarabine, due to its involvement in the regulation of autophagy activation through the miR-874-3p/ATG16L1 axis [209]. Additionally, cytarabine sensitivity has also been shown to be influenced by HOTAIRM1 [210] and GAS6-AS2 [211], which contribute to resistance via modulation of the Wnt/beta-catenin and GAS6/TAM signaling pathways, respectively. Furthermore, other lncRNAs have been implicated in resistance to doxorubicin. Notably, XIST functions as a competing endogenous RNA for miR-29a, leading to increased *MYC* expression, enhanced cell viability, and decreased sensitivity to doxorubicin[212]. Similarly, MEG3 and UCA1 have been shown to promote doxorubicin resistance by sponging specific microRNAs, interfering with the regulation of *ALG9* expression [213] and the PI3K/AKT pathway [214], respectively. In addition, evidence of lncRNA involvement in non-standard treatments such as venetoclax has also been observed. For instance, SNHG1 promotes the recruitment of DNA methyltransferases DNMT1 and DNMT3B to the promoter of the tumor suppressor gene *ZCCHC10* [215], which is involved in p53-dependent apoptosis, thereby contributing to drug resistance.

1.4.2.2 Clinical relevance

From a clinical perspective, lncRNAs with well-defined roles in promoting or inhibiting AML pathogenesis and progression are under investigation as potential

therapeutic targets. While CRISPR gene editing, ribozymes, and aptamers are primarily used to explore lncRNA expression and function in preclinical studies, strategies such as antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), and small-molecule inhibitors have been investigated in various cancer types, often in combination with delivery systems such as nanoparticles and extracellular vesicles like exosomes, for their potential use in targeting lncRNAs in clinical settings. In the context of AML, a siRNA-loaded lipid nanoparticle targeting the overexpressed lncRNA LINC01257 has been explored *in vitro*, where it was shown to reduce KASUMI-1 cell proliferation without affecting healthy cells [216]. However, the translation of lncRNA-targeting therapies into clinical practice faces significant challenges [217]. Poor sequence conservation across species complicates the development of relevant animal models, while the often-low endogenous expression levels of lncRNAs require highly sensitive and efficient targeting methods. Additionally, the lack of effective, specific, and safe delivery systems for these therapeutic strategies remains a major hurdle, limiting their advancement beyond preclinical studies [217,218].

Nevertheless, beyond their potential as therapeutic targets, several long non-coding RNAs have also emerged as promising biomarkers with diverse clinical applications. Some lncRNAs have been identified as potential diagnostic and prognostic markers. For example, elevated levels of LINC00899 and PANDA have been described in acute myeloid leukemia and are associated with poorer overall survival [219,220]. Conversely, low levels of IRAIN have been shown in a cohort of 64 *de novo* non-M3 AML patients to correlate with worse prognosis compared to controls [197]. These findings highlight the potential use of these lncRNAs as prognostic markers to be incorporated into standard clinical routines. Furthermore, as previously highlighted, the expression of certain long non-coding RNAs is associated with drug resistance, underscoring their potential use as markers for treatment response. Therefore, incorporating the detection of expression levels for those lncRNAs with a well-defined correlation with treatment outcome into clinical routine could assist in guiding more informed therapeutic decisions, ultimately improving prognosis and reducing relapse rates. Finally, lncRNA-based expression signatures have demonstrated potential for risk stratification in AML. Analysis of multiple AML cohorts identified a four-lncRNA signature that can predict patient outcomes independently of age, sex, and ELN classification [197]. Similarly, in a cohort of 274 AML patients treated with chemotherapy, comprehensive lncRNA profiling revealed 33 lncRNAs significantly

associated with overall survival [221]. This set was refined into a robust four-lncRNA prognostic signature, capable of stratifying patients irrespective of their cytogenetic or mutational background.

Taken together, these findings underscore the multifaceted clinical relevance of lncRNAs in AML, not only as potential therapeutic targets but also as valuable biomarkers across diagnostic, prognostic, predictive, and risk stratification categories. Although their limited stability, low expression, and the lack of accessible and standardized detection methods continue to pose challenges, these promising applications highlight the need for further investigation into their utility in improving disease management and personalizing treatment strategies.

1.5 Post-transcriptional alterations: The role of DEAD-box helicases

While transcriptional alterations, such as those affecting the epigenetic machinery and transcription factors, are well established in cancer and AML, numerous post-transcriptional mechanisms, acting between messenger RNA (mRNA) synthesis and protein production, have also emerged as important regulators of gene expression in cancer, influencing cell fate and disease progression [222,223]. These include the dysregulation of non-coding RNAs as ribosomal and transfer RNAs, miRNAs, lncRNAs, and RNA-binding proteins (RBPs), all of which play crucial roles in mRNA metabolism, from RNA processing and splicing to dysregulation of mRNA decay and translation (Figure 4).

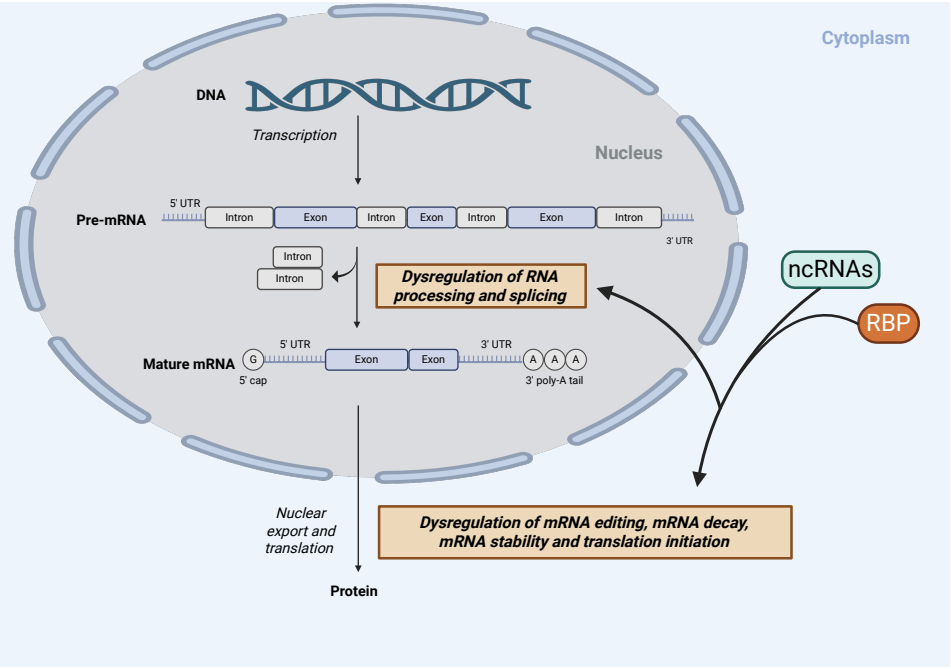

Figure 4. Schematic representation of post-transcriptional mechanisms that may be altered in tumorigenic conditions. Altered function of RNA-binding proteins and/or non-coding RNAs can affect pre-mRNA processing and splicing in the nucleus, while in the cytoplasm, spliced mRNAs can be impacted in terms of editing, stability, decay, and translation. Created with BioRender.com

Among the diverse families of RBPs involved in mRNA metabolism, DEAD-box helicases (DDX) represent a key group with specialized functions [224]. DEAD-box RNA helicases are RBPs characterized by a highly conserved Asp-Glu-Ala-Asp (D-E-A-D) motif and belong to superfamily 2 (SF2) of RNA helicases. Through ATP hydrolysis, DEAD-box proteins can bind RNA, form RNA-protein complexes, and

unwind duplex RNA, ultimately regulating mRNA translation by influencing alternative RNA splicing, ribosome assembly, RNA transport, RNA decay, RNA storage and translation initiation [225,226]. In the context of AML, DEAD-box helicase 5 (DDX5), for example, is essential for AML cell proliferation both *in vitro* and *in vivo* [227] while germline mutations in *DDX41* have been associated to higher risk of developing myeloid neoplasms such as AML, causing haplo-insufficient expression of the protein [228].

1.5.1 Eukaryotic Initiation factor 4A-III

Among DEAD-box proteins, the eukaryotic Initiation factor 4A-III (eIF4A3), also recognized as DDX48, [229] is ubiquitously expressed in the human body and has been found to exert an oncogenic function in several cancers [230–233]. eIF4A3 belongs to the eIf4A family, which also includes eIF4A1 and eIF4A2. While eIF4A1 and eIF4A2 are primarily involved in translation initiation processes [234], eIF4A3 forms to the core of the exon junction complex (EJC) [235] together with other 3 proteins: MAGOH, RBM8 (also known as Y14), and CASC3 (also known as MLN51). In the nucleus, the core of the EJC is assembled in a sequence independent manner roughly 24 nucleotides upstream of exon–exon junctions, where it acts as a scaffold for peripheral proteins involved in pre-mRNA splicing [236,237]. After splicing, the EJC core remains bound to the mRNA and, through dynamic interactions with other factors, regulates multiple post-transcriptional processes, including mRNA nuclear export, translation, and stability via nonsense-mediated mRNA decay (NMD) [236]. In addition to the role of eIf4A3 within the EJC, recent evidence [238] has underlined a critical role of this helicase also in ribosome biogenesis, promoting rRNA R-loop clearance within nucleolar compartments, and a role in the MDM2-p53 axis. Furthermore, transcriptomic analyses performed upon chemical inhibition of eIF4A3 have revealed defects in NMD affecting transcripts involved in cell cycle regulation, together with an impaired formation of RNA stress granules [239] .

In tumorigenic conditions, eIF4A3 is often upregulated and has been shown to have a prognostic value [240,241]. Although its role varies across tumor types, a recurring theme is its ability to promote cell proliferation, survival, and metastasis through aberrant expression and stabilization of RNA transcripts, including mRNAs but also circular RNAs, miRNA and long-non-coding RNAs. In glioblastoma, eIF4A3 has shown to increase the expression of the circular RNA circASAP1, which acts as an oncogene by activating the NRAS/MEK1/ERK1–2 signaling pathway [242]. In the same cancer type, eIF4A3 also stabilizes the lncRNA AGAP2-AS1, prolonging its

half-life and driving tumor progression [243]. In ovarian cancer, eIF4A3 promotes proliferation by binding to and stabilizing PDK4 mRNA [244], while in non-small cell lung cancer, eIF4A3 interacts with the lncRNA LINC00667, which enhances the stability of VEGFA mRNA, a key factor involved in angiogenesis and tumor growth [245].

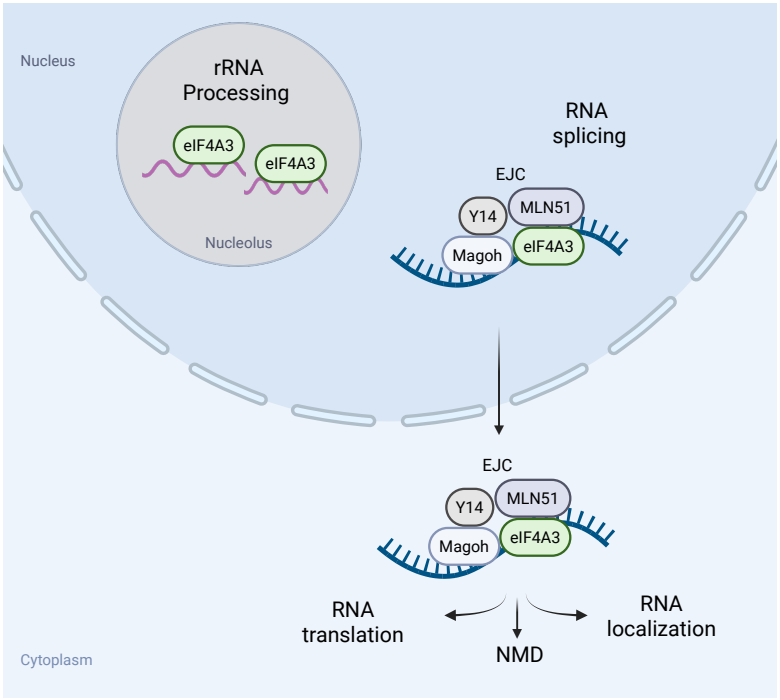

Figure 5. Schematic representation of eIF4A3 functions in different cellular compartments. In the nucleolus, eIF4A3 is involved in ribosome biogenesis by clearing R-loops from nascent rRNA. In the nucleoplasm, eIF4A3 forms the core complex of the exon junction complex (EJC) and promotes RNA splicing. In the cytoplasm, eIF4A3 participates in RNA translation, nonsense-mediated decay (NMD), and RNA localization. Created with BioRender.com

Although eIF4A3 has not been extensively investigated in hematopoietic malignancies, its central role in RNA metabolism and oncogenic processes suggests it may represent a clinically relevant factor. Preclinical studies have explored the therapeutic potential of eIF4A3 inhibition in solid tumors [246,247] highlighting its promise as a druggable target. Nevertheless, further research is required to define eIF4A3 specific functions across different cancer types and cellular contexts, including acute myeloid leukemia.

2 Research aims

STUDY I: MALNC: A NEW MUTANT NPM1/IDH2^{R140} AND PML-RARA ASSOCIATED LNCRNA WITH IMPACT ON AML CELL PROLIFERATION, MATURATION AND DRUG RESPONSE

- To identify novel differentially expressed lncRNAs in AML through comprehensive deep RNA sequencing analyses.
- To explore clinical and genetic associations of the most promising lncRNA with relevant AML subtypes and patient outcomes.
- To perform structural and functional characterization of the selected lncRNA candidate.
- To elucidate the molecular mechanisms underlying the activity of the lncRNA in AML pathogenesis.

STUDY II: IDENTIFICATION OF LONG NON-CODING RNAS INVOLVED IN LEUKEMOGENESIS AND VENETOCLAX SENSITIVITY OF ACUTE MYELOID LEUKEMIA THROUGH CRISPR-CAS9 INTERFERENCE SCREENS

- To identify, through CRISPR-dCas9 interference screens, novel long non-coding RNAs functionally implicated in AML, affecting proliferation, differentiation, and venetoclax response.
- To investigate the clinical significance of candidate lncRNAs in AML by expression profiling and correlation with clinical parameters.
- To biologically characterize selected lncRNAs using gene editing and *in vitro* assays.

STUDY III: THE EXON-JUNCTION COMPLEX HELICASE EIF4A3 HOLDS THERAPEUTIC POTENTIAL IN ACUTE MYELOID LEUKEMIA

- To explore essential genes in AML using publicly available CRISPR knockout screening datasets.
- To characterize the biological function and clinical significance of a selected gene using transcriptomic data from AML cell lines and primary patient samples.
- To evaluate whether the selected gene holds potential as a therapeutic target in AML.

3 Materials and methods

Specific materials and methods of particular relevance are described in the following section, with additional details provided in the corresponding units of Studies I–III.

Ethical considerations

The work presented in this thesis is based on AML cell lines and human samples from AML patients and healthy donors. All projects were ethically approved by the Stockholm board in accordance with the Declaration of Helsinki. Patient samples consisted of bone marrow aspirates collected during routine diagnostic procedures, while samples from healthy donors were obtained during scheduled appointments. All participants provided informed consent for the use of their biological material in research, in accordance with the Nuremberg Code and good research practices. They were fully informed about the study aims and procedures and had the capacity to decide voluntarily whether to participate. To ensure confidentiality, all samples were coded, and personal identifiers were stored separately, accessible only to a limited number of authorized personnel. This allowed patient data to be retrieved if a participant withdrew consent or if additional approval was required for future research, while maintaining anonymity for those handling the samples. In addition, in **Study I**, animal models were used to investigate the effect of lncRNA knockout on disease engraftment. All experiments were conducted under the ethical approval of the Swedish Board of Agriculture and adhered to the three principles of replacement, reduction, and refinement.

Cell lines

Different leukemic cell lines were used across the three projects, including HL-60, NB4, OCI-AML3, OCI-AML2, MOLM-13, Kasumi-1, K562, KG-1, THP-1, F36P, IMS-M2 and U937. All cell lines were cultured in RPMI 1640 medium supplemented with GlutaMAX, 10–20% heat-inactivated fetal bovine serum (FBS), and 1% penicillin-streptomycin. Cells were seeded at a density of 0.1–1 × 10^6 cells/mL and maintained at 37 °C in a humidified incubator with 5% CO_2.

Cohorts of primary AML and Normal Bone Marrow (NBM) samples

For all three studies, patient samples including molecular and clinical data were used to evaluate the expression of candidate lncRNAs and post-transcriptional molecules in primary cells, and to correlate their expression profile with clinical parameters. Primary samples from AML patients consisted of the mononuclear

cell fraction extracted from bone marrow using a Ficoll-Plaque density-gradient centrifugation, while samples from healthy donors were further enriched for $CD34^+$ cells using magnetic-activated cell sorting (MACS). Specifically, in **Studies I, II, and III**, the research group's own ClinSeq cohort [248,249], comprising 325 Swedish AML patient samples collected at diagnosis between 1999 and 2014, was analyzed. Clinical data for this cohort were obtained from the Swedish Acute Leukemia Registry and patient medical records, while transcriptomic and mutational data were obtained through RNA sequencing and targeted DNA panel sequencing [250]. In **Studies I and I**I, a parallel cohort, referred to as the Knut and Alice Wallenberg (KAW) cohort, was also included. This cohort comprised 103 AML patients as well as 11 healthy donors. In addition, in **Study I**, a smaller cohort consisting of 7 AML patients and 5 NBM samples was used for the discovery and identification of candidate lncRNAs through deep RNA sequencing, serving as a basis for further analyses.

Publicly available data from cell lines and primary cells

In **Study I**, to validate findings from the in-house ClinSeq cohort, data from the Cancer Genome Atlas (TCGA) AML cohort were used [251,252]. This cohort comprises clinical and transcriptomic data from 151 *de novo* AML patients diagnosed in the USA between 2002 and 2009. In **Study III**, to identify potential essential genes in AML and evaluate their expression levels, CRISPR screen knockout and RNA-seq data, performed on 18 AML cell lines, were retrieved from DepMap [253]. Additionally, RNA-Seq raw counts and clinical metadata from the BEAT AML cohort [254] were retrieved, comprising 476 AML patients and 23 healthy donors.

Real-time qPCR

To investigate gene expression at the single-gene level, real-time quantitative PCR (RT-qPCR) analyses were employed in **Studies I, II and III**. Total RNA was extracted from cells using spin-column–based methods and quantified with a spectrophotometer to assess yield and integrity, ensuring comparable input across samples. Following RNA to cDNA conversion using reverse transcriptase enzymes, RT-qPCR was performed. The TaqMan assay was preferentially used over SYBR Green because of its higher specificity and sensitivity, relevant for low-abundance transcripts such as lncRNAs. Relative quantification of gene expression was performed using the comparative CT method ($2^{-\Delta\Delta CT}$) [255]. Expression values were normalized to internal reference genes (*β-actin, GAPDH,*

or *TBP*), and the relative expression of each target gene was calculated against designated calibrator samples.

RNA sequencing

RNA sequencing (RNA-seq) is a next-generation sequencing technique that enables genome-wide profiling of a given transcriptome. In general, total RNA is purified, and its quality is assessed to ensure integrity and purity. Highly abundant RNA species, such as rRNAs, are usually removed to enrich for messenger and non-coding RNAs. The remaining RNA molecules are then fragmented and reverse-transcribed into complementary DNA (cDNA), which provides a more stable template for sequencing. Adapters are ligated to both ends of the cDNA fragments to generate a library that can be amplified and subsequently sequenced. Depending on the platform used, sequencing is performed through cyclic detection of incorporated nucleotides or other signal-based chemistries, producing millions of short reads that represent the original RNA molecules. The generated reads are then demultiplexed, quality-checked, and aligned to a reference genome to quantify transcript abundance and determine gene expression levels.

To detect genes with significant changes in expression between experimental groups, differential expression analyses were performed. Additionally, Gene Set Enrichment Analyses (GSEA) were conducted using Gene Ontology (GO) [256] and Reactome databases [257] to evaluate cellular pathways potentially impacted by the experimental perturbations. In this thesis, RNA-seq data from both publicly available sources and internal AML data were used to examine the expression of candidate lncRNAs in **Studies I and II**, as well as the expression of the *eIF4A3* helicase in **Study III**. Additionally, RNA-seq analyses were conducted in **Studies I and II** to evaluate differential gene expression in AML cell lines with knockouts of two lncRNAs of interest: *MALNC* and *CATG00000106133.1,* respectively. Similarly, in **Study III**, differential expression analysis was performed on AML patient samples stratified by high versus low EIF4A3 expression levels, as well as between AML and normal cells.

Cap Analysis of Gene Expression (CAGE) sequencing

CAGE sequencing is a technique developed at the RIKEN Institute in Japan to capture and analyze 5′-capped RNAs at a high-resolution level [258,259]. The method relies on biotinylation of the 7-methylguanosine cap structure of mature RNAs, enabling the selective pull-down of cDNAs, reverse-transcribed from these

capped transcripts, using streptavidin beads. Subsequently, a linker is added to the cDNA, and specific enzymes are used to shorten the cDNA fragments to 20-30 nt. These cDNA fragments, referred to as CAGE tags, are then amplified and sequenced, and the reads are mapped back to the genome. This process allows the identification of CAGE tag starting sites (CTSSs) at single-base resolution. Because RNA polymerase II often initiates transcription over a small region rather than a single nucleotide, CTSSs can be grouped into CAGE clusters using clustering algorithms. These Cage clusters define active transcription sites, and the number of CAGE tags within each cluster provides quantitative information on transcripts and isoform expression levels.

An evolved version of the classical CAGE protocol, known as HeliScope CAGE [260], has been extensively employed in the FANTOM5 project to map transcription start sites and discover novel mRNAs and lncRNAs across human tissues, including those targeted in the CRISPR interference screen of **Study II**. HeliScope CAGE is a more efficient technique, as it allows direct sequencing of first-strand cDNA without the use of linkers, enzymatic digestions, or PCR amplification, reducing the likelihood of technical artifacts. Furthermore, it requires less RNA input, enabling gene expression analysis even in small or restricted cell populations. HeliScope CAGE data were subsequently used in **Studies I and II** to assess lncRNA expression levels and transcription start sites localization.

CRISPR interference screen

In Study II, CRISPR interference (CRISPRi) [261] screens were conducted to identify lncRNAs involved in AML cell proliferation, differentiation, and response to the BCL2 inhibitor venetoclax. In the conventional CRISPR-Cas9 system, a single-guide RNA (sgRNA) directs the Cas9 nuclease to a specific genomic locus, where it introduces a double-strand break (DSB). Repair of this DSB predominantly occurs through the error-prone non-homologous end joining (NHEJ) pathway, often resulting in small insertions or deletions (indels) [262]. In protein-coding genes, such indels can cause frameshift mutations that disrupt the open reading frame (ORF), leading to loss of protein expression. However, because lncRNAs lack ORFs, the indels and frameshifts generated by traditional CRISPR-Cas9 approaches would not effectively disrupt their function. In contrast, CRISPRi employs a catalytically inactive Cas9 (dCas9), which can be fused to a Kruppel-associated box repressor domain (KRAB) [263]. dCas9 still binds DNA at sgRNA-specified sites but cannot cut the genome, while KRAB recruits chromatin-modifying complexes that deposit repressive histone marks. Together, they

silence genes by blocking RNA polymerase II access and establishing a repressive chromatin environment at promoter or proximal regulatory regions. Therefore, CRISPRi represents a more suitable strategy to achieve stable and specific transcriptional repression of lncRNAs.

As illustrated in Figure 6, 7,996 lncRNAs expressed across eight AML cell lines were identified through CAGE analysis from the FANTOM5 project [137]. A pooled gRNA library was then generated, containing six independent gRNAs designed within a region spanning 25 nucleotides upstream and 250 nucleotides downstream of the major CAGE peaks of each lncRNA. To minimize potential off-target effects, lncRNAs located within ±2 kb of a protein-coding gene transcriptional start site (TSS) were excluded from the library.

Following plasmid backbone vector cloning, the library was packaged into lentiviral particles and transduced into MOLM-13 AML cells, previously engineered to stably express dCas9-BFP-KRAB, in biological duplicates. Transduction was performed at low multiplicity of infection (MOI) to ensure that each cell received only one gRNA construct. In addition, to ensure sufficient library coverage, each replicate was maintained at a minimum of 80×10^6 cells.

After 4 days of puromycin selection to ensure stable integration, three independent CRISPR interference (CRISPRi) screens were performed:

- Proliferation screen: Cells were cultured for 21 population doublings, and samples were collected at time points 7 and 31 to assess the impact of lncRNA knockdown on cell growth.
- Differentiation screen: Cells were treated with either 10 nM ATRA or vehicle control (0.1% DMSO, v/v) for 72 hours.
- Drug response screen: Cells were treated with either 10 nM venetoclax or vehicle control (0.1% DMSO, v/v) for 48 hours.

For each screen, live cells positive for BFP (representing dCas9-expressing cells) were collected by flow cytometry at the specified time points. In the differentiation screen, $CD11b^+$ cells were additionally isolated from the ATRA-treated group. Next-generation sequencing (NGS) was then performed on extracted genomic DNA to quantify gRNA abundance under each screening condition. This allowed us to infer the relative contribution of each targeted lncRNA to the biological processes of interest, providing insights into their potential roles in AML pathogenesis and drug response.

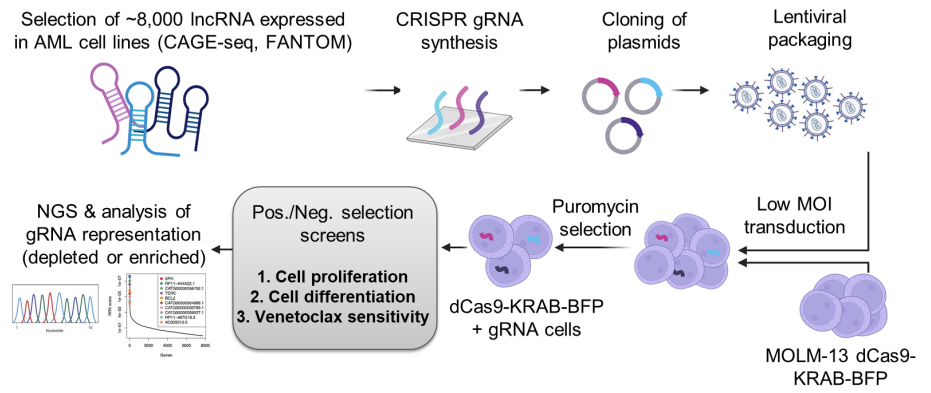

Figure 6. Workflow of the three CRISPR interference screens performed in study II. Created with BioRender.com

Paired guide RNA CRISPR- Cas9 Knockout

In **Studies I and II**, paired-guide RNA CRISPR-Cas9 knockout strategies were employed to disrupt the transcription of the targeted lncRNAs, *MALNC* and *CATGOOOOO106133.1*, respectively. Canonical CRISPR-Cas9 approaches typically use a single guide RNA (sgRNA) together with the Cas9 nuclease to induce small insertions or deletions (indels) [262]. However, as outlined earlier, this strategy is not optimal for lncRNAs, since partial truncations or indels may not fully abolish transcript function. To overcome this limitation, a double-knockout strategy was applied, in which two sgRNAs were designed to target sequences located upstream and downstream of lncRNA primary TSSs.

Knockout efficiency was verified in multiple steps. Initially, bulk cell populations were analyzed following genomic DNA extraction by PCR amplification across the targeted region. Successful deletions were identified by a band size shift on gel electrophoresis and confirmed by Sanger sequencing to ensure that the intended deletion had occurred. To obtain clonal cell populations, single-cell sorting was performed by flow cytometry. Individual clones were expanded and validated as described for the bulk populations. Heterozygous and homozygous deletions were distinguished by PCR genotyping using primers located both inside and outside the deleted region, and the absence of transcript expression was confirmed by RT-qPCR.

siRNA knockdown

RNA interference (RNAi) is a cellular mechanism in which RNA can trigger gene expression inhibition in a homology-dependent manner [264–266]. This process represents a key post-transcriptional regulatory pathway that modulates RNA stability and translation [267]. In mammals, RNAi is primarily mediated by microRNAs (miRNAs), which guide the RNA-induced silencing complex (RISC) to complementary messenger RNAs, resulting in translational repression or degradation. The same RNA interference machinery can also recognize exogenous double-stranded RNAs, a feature that can be exploited experimentally for gene silencing using short RNAs (shRNA) or small interfering RNAs (siRNAs). In the specific case of siRNA synthetic production, once introduced into the cell, siRNAs are incorporated into the multiprotein RISC complex, where Argonaute 2 (AGO2) mediates degradation of complementary target RNAs in the cytoplasm. In **Study III**, siRNA-mediated knockdown was performed to silence selected messenger RNAs. A SMARTpool ON-TARGET plus strategy was employed, consisting of four double-stranded siRNAs per target gene, each chemically modified to minimize off-target effects by reducing sense-strand incorporation into RISC and decreasing microRNA-like seed-based interactions [268–270]. The siRNAs were introduced into AML cell lines by electroporation, ensuring efficient intracellular delivery and robust depletion of the target transcripts for downstream functional analyses.

Chromatin Isolation by RNA Purification

Chromatin Isolation by RNA Purification (ChIRP) is a method developed in 2011 [271] to map RNA–protein–chromatin complexes. The technique uses biotinylated 20-nucleotide antisense probes complementary to the RNA of interest, enabling the pull-down of target RNA, together with its associated macromolecules (DNA and proteins), using streptavidin beads. Before hybridization, cells are crosslinked to stabilize RNA–chromatin interactions and then sonicated to fragment chromatin into 100–500 nt pieces. ChIRP employs two non-overlapping probe sets (“even” and “odd”) designed across the full length of the target RNA to perform parallel pulldowns, providing an internal control that helps distinguish specific from nonspecific interactions. This design also eliminates the need for prior knowledge of RNA accessibility. After reverse crosslinking, the isolated DNA can be analyzed by sequencing (ChIRP-seq) to identify genomic regions bound by the RNA, or the protein fraction can be analyzed by mass spectrometry (ChIRP-MS) to characterize RNA–RBP interactions. Additionally, the RNA itself can be recovered

to confirm efficient target RNA pulldown and to investigate potential RNA–RNA interactions occurring at the chromatin level. In **Study I**, ChIRP-seq analyses were performed to determine whether the lncRNA MALNC binds to chromatin and to explore its potential regulatory role at the DNA level.

Functional assays

In the work presented in this thesis, several functional assays were performed to investigate the role of selected lncRNAs and RNA helicases in specific biological processes, including cell viability, apoptosis, cell cycle regulation and cell differentiation.

In **Studies I, II, and III,** cell viability assays were conducted to evaluate the impact of lncRNA deletion or helicase perturbation on AML cell proliferation. Cell viability was assessed by measuring the metabolic activity of living cells. In Studies I and II, the WST-8 assay was used, while in Study III, the XTT assay was employed. Briefly, both methods rely on the addition of a tetrazolium-based reagent (either WST-8 or XTT), which is reduced by cellular dehydrogenase enzymes in metabolically active cells to produce a water-soluble colored formazan dye [272,273]. The absorbance of this dye, measured spectrophotometrically on a plate reader, is directly proportional to the number of viable cells.

In **Studies I and III**, apoptosis assays were performed to assess the impact of specific lncRNA deletions (Study I) or of *eIF4A3* and *TP53* knockdown (Study III) on cell death, distinguishing viable, early apoptotic, late apoptotic, and necrotic cells. Specifically, flow cytometry analyses were conducted following staining with Annexin V–FITC and propidium iodide (PI) [274]. Annexin V is a calcium-dependent protein that strongly associate with phosphatidylserine residues, which translocate from the inner to the outer layer of the plasma membrane during early apoptosis. In contrast, PI is a DNA-intercalating dye that penetrates only cells with compromised membrane integrity, thereby staining late apoptotic and necrotic cells. By evaluating the fluorescence emission of Annexin V conjugated to a fluorescent dye (in this case FITC) and PI, different cell populations can be distinguished: viable cells (Annexin V^-/PI^-), early apoptotic cells (Annexin V^+/PI^-), late apoptotic cells (Annexin V^+/PI^+), and necrotic cells (Annexin V^-/PI^+). In addition, in **Study III**, PI staining alone was used to discriminate live from dead unfixed cells, based on the selective uptake of the dye by cells with damaged membranes [275,276].

In **Study I**, PI was also employed to analyze cell cycle distribution. In this assay, cells were first fixed, allowing PI to intercalate into the DNA. The resulting fluorescence intensity is proportional to the DNA content, enabling discrimination of different cell cycle phases. Specifically, cells in the G_0/G_1 phase display a 2N DNA content, cells in the S phase show intermediate fluorescence due to ongoing DNA synthesis, and cells in the G_2/M phase exhibit a 4N DNA content [277].

In **Studies I and II**, cell differentiation upon ATRA treatment was assessed by flow cytometry analyses using CD11b, a granulocytic cell surface marker. In Study I, CD11b levels were also evaluated by flow cytometry analysis in APL cells following MALNC depletion. Under the same treatment conditions, RT-qPCR was performed to quantify CD11b and CD66d transcripts in *MALNC*-KO and *MALNC*-WT cells. CD66d, a member of the CEACAM (carcinoembryonic antigen-related cell adhesion molecule) family, was employed as an additional marker of granulocytic differentiation in Study I [278,279].

Animal studies

In **Study I**, we investigated whether depletion of the lncRNA MALNC affects the engraftment capacity and disease progression of AML cells in a mouse transplantation model. For this purpose, we used immunodeficient mice that transgenically express human cytokines relevant to myelopoiesis. These mice, known as NSG-SGM3 [280], are derived from the cross of NOD-scid gamma (NSG) mice [281–283], which carry a complete deletion of the interleukin-2Rγ (IL-2Rγ) gene and consequently lack functional T cells, B cells, and NK cells, with NOD/SCID-SGM3 (NSS) [284] mice that transgenically express human SCF (Stem Cell Factor), GM-CSF (Granulocyte-Macrophage Colony-Stimulating Factor), and IL-3. These cytokines enhance support for the human myeloid lineage, thereby improving the engraftment and functional differentiation of AML cells *in vivo*.

Briefly, sub-lethally irradiated NSG-SGM3 mice (9–10 weeks old) were transplanted with 1 × 10^6 NB4 *MALNC*-WT or NB4 *MALNC*-KO cells via tail vein injection, six hours post-irradiation (n = 4 per group). Animal welfare was monitored regularly, and blood sampling was initiated two weeks after transplantation. 21 days post-transplantation, when animals began to show signs of morbidity, all mice were sacrificed, and spleen, peripheral blood, and bone marrow were collected. Spleen weight was determined using an analytical balance, while blood and bone marrow parameters were analyzed using an automated cell counter (Sysmex XP-300) and flow cytometry. For flow cytometry, mCD45-PE

and hCD45-Pacific Blue antibodies were used to distinguish murine from human leukocytes, respectively, and propidium iodide (PI) was included to exclude dead cells. Gating was defined using fluorescence-minus-one (FMO) controls. The experimental workflow is summarized in Figure 7.

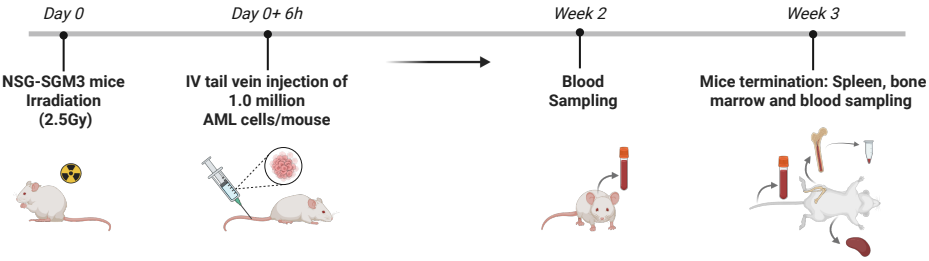

Figure 7. Schematic representation of *in vivo* experiments performed on NSG-SGM3 mice transplanted with NB4 *MALNC* wild-type and NB4 *MALNC* knockout cells. Created with BioRender.com

Statistical analysis

Statistical analyses for **studies I-III** were performed primarily using RStudio (v3.6.2 or later) and GraphPad Prism (v9.4.1 or later). Flow cytometry data were analyzed with FlowJo (v10.6.1–v10.8.0). Data distribution was evaluated for normality using the Shapiro–Wilk test. When the data followed a normal distribution, differences between two groups were analyzed with two-tailed Student's t-tests, while comparisons among three or more groups were performed through one- or two-way ANOVA analyses, followed by Dunnett's post-hoc test for multiple pairwise comparisons. For data not normally distributed, two groups' comparisons were carried out using the Mann–Whitney U test, and comparisons among three or more groups using the Kruskal–Wallis test, followed by Dunn's multiple comparison test. Associations between numerical variables were evaluated using Spearman's rank correlation (ρ). Overall survival (OS) was calculated from the date of diagnosis to either death or the most recent follow-up. Survival probabilities were estimated with the Kaplan–Meier method, and group differences were evaluated using the Mantel–Cox log-rank test. To determine the prognostic significance of candidate lncRNAs in **Studies I and II**, multivariate Cox proportional hazards regression analyses were conducted. A p-value or adjusted p value < 0.05 was considered statistically significant. Statistical significance is indicated in figures as follows: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

4 Results and discussion

4.1 Study I

4.1.1 Study rationale

As outlined in the literature review of this thesis, AML is a highly heterogeneous malignancy characterized by multiple, often co-occurring alterations at the genetic, epigenetic, and post-transcriptional levels. This complexity has driven research efforts toward the development of targeted therapies aimed at improving patient outcomes. Nevertheless, despite these advances, the overall survival rate for AML remains poor, and several unmet clinical needs persist. These limitations are largely due to an incomplete understanding of the molecular mechanisms underlying AML pathogenesis and progression. Given that the non-coding genome remains relatively underexplored in the context of AML, Study I was designed to use transcriptomic data from primary AML and normal bone marrow CD34+ cells to specifically investigate the role of lncRNAs in this disease. In fact, lncRNAs have been shown to function as both oncogenes and tumor suppressors in various cancers; however, their biological functions and clinical relevance in AML remain poorly defined. This study aimed to improve the identification and characterization of lncRNAs that may play key roles in AML biology and serve as potential therapeutic targets or biomarkers.

4.1.2 Results

In Study I, we initially performed deep RNA sequencing on a small discovery cohort, comprising 7 AML patients and 5 healthy bone marrow donors, to identify lncRNAs differentially expressed between leukemic and normal cells. From the 136 novel lncRNA candidate transcripts filtered in the initial analysis, we selected one currently only partially annotated lncRNA for further investigation, which we subsequently named MALNC (myeloid and AML-associated intergenic long non-coding RNA). This choice was driven by the observation that *MALNC* was consistently overexpressed in AML cells, not only in the initial discovery cohort but also in our larger internal ClinSeq cohort, suggesting potential disease relevance.

To explore the full length and transcript structure of MALNC, we employed PrimeWalks, RACE (Rapid Amplification of cDNA Ends) and CAGE sequencing analyses, which revealed that *MALNC* is a long transcript, spanning a region of more than 70 kilobases, with multiple isoforms transcribed from three mutually

exclusive TSSs. Polysome profiling and in silico analyses confirmed the absence of coding potential, supporting its classification as a non-coding RNA. Subcellular fractionation experiments showed that MALNC localizes to both the nucleus and cytoplasm, indicating possible multifunctionality and complex regulatory roles.

Subsequently, we analyzed *MALNC* expression in both our internal ClinSeq AML cohort (n = 325) and the publicly available TCGA AML cohort (n = 151) [251] to investigate potential correlations with clinical and genetic parameters. In both cohorts, *MALNC* expression was significantly associated with APL patients and AML cases harboring co-occurring *NPM1* and $IDH2^{R140}$ mutations. Moreover, higher *MALNC* expression associated with improved overall survival, both when including and excluding APL patients, and this correlation remained significant, also independently of other established prognostic factors.

Given that *MALNC* showed the highest expression in the APL/AML subtype in primary cells and across different cell lines tested, with the promyelocytic cell lines NB4 (*PML–RARA* positive) and HL-60 (*PML–RARA* negative but APL-derived) exhibiting the highest levels, we decided to further investigate MALNC within this AML subtype. Upon induction of cell differentiation with ATRA, we observed in both HL60 and NB4 cells a marked decrease in MALNC levels, suggesting that its expression is downregulated during myeloid maturation. Similarly, under physiological conditions, *MALNC* expression peaked at the promyelocytic stage and progressively decreased during normal myeloid differentiation. The involvement of MALNC in myeloid cell differentiation was reinforced by CRISPR knockout experiments. At basal levels, RNA sequencing analysis revealed altered expression of genes implicated in the retinoic acid signaling pathway, such as *ZNF536 [285]* and *DHRS3* [286–288], following *MALNC* deletion. Consistently, upon ATRA treatment, *MALNC*-deficient cells exhibited upregulation of retinoic acid–responsive genes such as *RARB* [289] and enhanced expression and higher protein levels of differentiation markers CD11b and CD66d. These findings support a model in which *MALNC* loss impacts the retinoic acid pathway and sensitizes APL cells to ATRA, promoting differentiation at both the transcriptional and phenotypic levels. Furthermore, ChIRP-seq analysis demonstrated that MALNC binds directly to key retinoic acid pathway genes [109,285,290,291], including *RARA*, *STAT1*, *RXRA*, and *ZNF536* in APL cell lines. This finding helps explain, at least in part, how MALNC may regulate cell differentiation by directly interacting with critical regulatory loci.

The functional consequences of *MALNC* loss were further investigated using both *in vitro* and *in vivo* approaches. *In vitro*, *MALNC* depletion resulted in reduced cell viability in HL60 cells and impaired colony-forming capacity in both NB4 and HL60 cells. The effects of *MALNC* knockout were also evaluated *in vivo* through transplantation of NB4 *MALNC*-WT and NB4 *MALNC*-KO cells into immunodeficient NSG-SGM3 mice. Spleen size, assessed by weight, and the proportion of human $CD45^+$ peripheral blood (PB) cells were significantly higher in mice transplanted with NB4 *MALNC*-WT cells compared with those receiving NB4 *MALNC*-KO cells (Figure 8A–B). Moreover, mice in the NB4 *MALNC*-WT group displayed elevated peripheral white blood cell (WBC) counts, along with reduced red blood cell counts, hemoglobin levels, and platelet counts (Figure 8C). Taken together, these findings indicate that *MALNC* knockout impairs AML cell growth *in vivo*.

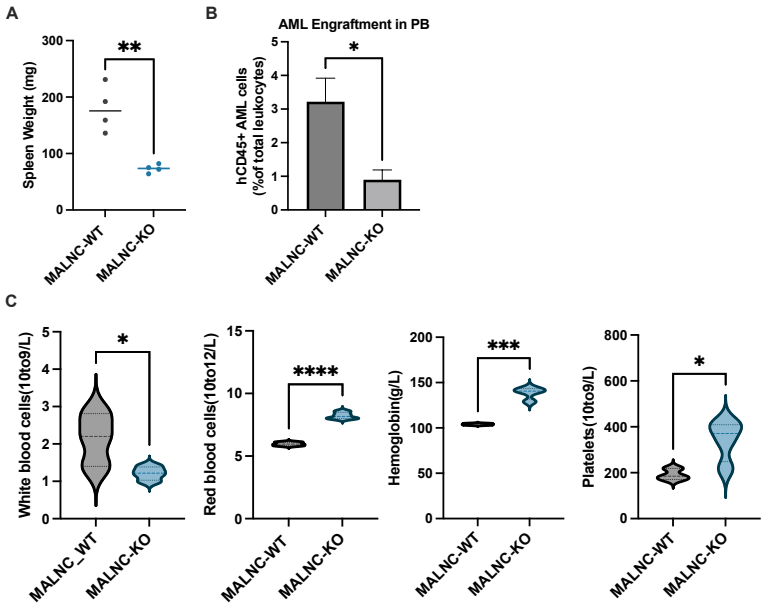

Figure 8. **(A)** Dot plot showing spleen weight of mice at 20 days post-transplantation with either NB4 *MALNC*-WT or NB4 *MALNC*-KO cells. **(B)** Bar graph showing the % of hCD45+ cells of total leukocytes in the Peripheral Blood (PB) of mice transplanted with NB4 *MALNC*-KO or *MALNC*-WT NB4 cells. **(C)** Mice hematological parameters at 20 days post-transplantation with either NB4 *MALNC*-WT or NB4 *MALNC* KO cells. Student's t-test was used for statistical analysis. Statistical significance is indicated as follows: P values * < 0.05; ** < 0.01; *** < 0.00.

Additionally, drug screening data showed that *MALNC* knockout cells were more sensitive to arsenic trioxide (ATO), a key therapeutic agent used in standard APL

treatment, suggesting that MALNC may also modulate drug sensitivity. Regarding its upstream regulation, analysis of ENCODE ChIP-seq data [292] comparing NB4 *MALNC* expressing cells and K562 non-expressing cells revealed MYC binding within the *MALNC* locus, indicating a potential direct or indirect role of MYC in the transcriptional regulation of *MALNC*.

4.1.3 Discussion

In **study I**, we reported the discovery of MALNC, a novel lncRNA overexpressed in AML. After characterizing its complex genomic locus and identifying several isoforms, we demonstrated that *MALNC* is specifically expressed in distinct AML subgroups, including APL and *NPM1/IDH2*^{R140} co-mutated cases, which together account for 10% and 5% of AML patients, respectively [27,293]. These findings reinforce accumulating evidence that lncRNAs are frequently associated with specific mutational backgrounds in AML [207,294–296]. In the case of MALNC, this expression pattern suggests a potentially shared transcriptional program between the two AML subtypes. This is further supported by previous observations that *NPM1/IDH2*^{R140} co-mutated AML displays an APL-like phenotype, characterized by the absence of *HLA-DR* expression, CD34 negativity, and high MPO levels[297,298]. In these two AML subgroups, MALNC may therefore exert a comparable functional role, an observation reminiscent of other lncRNAs such as CRNDE [299]. Notably, CRNDE, which is upregulated in APL cells, is also highly expressed in *NPM1* mutant AML, where in both contexts it contributes to a block in differentiation.

To further explore the functional role of MALNC, we focused on the APL subtype. In APL cell lines, *MALNC* expression decreased progressively during ATRA induced differentiation, a pattern mirrored in normal hematopoiesis, where its expression peaked in promyelocytes compared to earlier or later differentiation stages. This highlights that MALNC not only may serve as promyelocytic stage marker, but may also be involved in myeloid cell differentiation. Indeed, *MALNC* depletion not only showed reduced cell growth at baseline, but also enhanced differentiation upon ATRA treatment. Furthermore, under both basal conditions and ATRA-induced differentiation, several genes involved in the retinoic acid (RA) pathway showed altered expression. Some genes, such as *ZNF536*, were affected in both conditions, while others, including *RARB*, were mainly altered only after ATRA treatment. Moreover, 7 genes that were dysregulated in *MALNC*-KO cells, both at baseline and after ATRA treatment, were found to be directly bound by MALNC in NB4 cells. These results suggest that MALNC could play a stage-specific role in

aberrant myeloid differentiation. By influencing gene transcription, partly through its interaction with chromatin, MALNC affects cell proliferation and may contribute to the differentiation block characteristic of APL. Moreover, MALNC appears to regulate transcription both independently of external signals (such as ATRA) and in ways that become more pronounced after ATRA treatment, highlighting its heterogeneous and context-dependent activity. In addition, we further hypothesize that *MALNC* may be regulated upstream by MYC, as observed for other lncRNAs [300,301]. Supporting this, MYC ChIP-seq data from ENCODE show binding within the *MALNC* locus in cells expressing the lncRNA. This regulatory connection could also explain the decrease in MALNC levels upon ATRA treatment, as *MYC* is typically upregulated in leukemogenic contexts [302,303] but downregulated upon ATRA-induced differentiation [304–306].

From a clinical standpoint, MALNC may present diverse key features. On one hand, *MALNC* expression was found to correlate with better overall survival in AML patients, even after adjusting for favorable prognostic markers such as *PML-RARA* and *NPM1* [307,308]. This finding is somewhat counterintuitive, as MALNC appears to play a primarily oncogenic role, impairing differentiation and enhancing proliferation both *in vitro* and *in vivo*. However, such paradoxes are not uncommon in the AML context [12,309,310]. One possible explanation is that leukemic cells maintained in a more proliferative and less differentiated state may be more responsive to chemotherapy, thereby improving treatment outcomes. Therefore, MALNC holds potential as a broader prognostic marker in AML, possibly offering additional stratification value beyond currently established genetic indicators. In addition, the observed correlation between *MALNC* depletion and increased sensitivity to ATO in APL cell lines suggests that MALNC may serve as a marker for treatment response and/or a therapeutic target to enhance treatment efficacy. Considering that ATO, in combination with ATRA, represents the standard therapy for APL patients, it would be highly relevant to further investigate the mechanisms by which MALNC modulates response to this combinatorial treatment. Future *in vitro* and *in vivo* studies could help clarify whether MALNC could be used to assess treatment response or guide therapeutic strategies in APL patients.

4.2 Study II

4.2.1 Study rationale

As outlined in the rationale for Study I, lncRNAs have been shown to exert key roles in various cancers, including AML. The FANTOM5 project, using CAGE-seq data, identified more than 19,175 new functional lncRNAs in the human genome across 1,829 human cell types and tissues, and further generated an atlas of 27,919 lncRNAs expressed in normal and disease contexts [137]. This prompted us to further investigate the role of already annotated and newly discovered lncRNAs in the context of AML. To this end, we employed a CRISPR interference (CRISPRi) screen. CRISPRi is particularly suitable for studying lncRNAs because conventional CRISPR/Cas9 knockout approaches are often ineffective: the absence of open reading frames in lncRNAs means that small indels generally do not abolish their function. In contrast, CRISPRi represses transcription by targeting promoter regions. Moreover, the use of a CRISPR interference screen with a pooled sgRNA library enables the simultaneous and efficient repression of multiple lncRNA targets, allowing systematic assessment of their contribution to cellular phenotypes in a high-throughput manner.

This strategy has proven successful in uncovering novel lncRNAs involved in physiological processes [311–314] as well as in cancer [315–319]. However, only a few lncRNA-focused CRISPRi screens have been performed in the context of AML [320,321] and none have investigated their role under drug treatment. Therefore, this study aimed to identify lncRNAs involved in key biological processes relevant to leukemia progression, as well as to explore their potential roles in drug resistance, such as during venetoclax treatment, using CRISPRi screens.

4.2.2 Results

In **study II,** similar to study I, we investigated whether novel or previously unexplored lncRNAs could play a role in AML tumorigenesis. To this end, we performed three independent CRISPR–dCas9 interference screens, each targeting 7,996 lncRNAs identified through CAGE sequencing analysis from the FANTOM5 project. lncRNAs were targeted with barcoded gRNAs in the AML cell line MOLM-13 expressing dCas9-KRAB protein, and the abundance of each guide was subsequently assessed by next-generation sequencing (NGS) to evaluate the involvement of the targeted lncRNA in cell proliferation, differentiation, and response to the BCL-2 inhibitor venetoclax.

Candidate genes were filtered using two main criteria: (i) presence among the top 100 positive or negative hits according to the Robust Rank Aggregation (RRA) method, which assesses whether sgRNAs targeting a gene consistently rank among the most enriched or depleted guides, and (ii) overlap between the two biological replicates performed for each screen. Additionally, further filtering of candidate genes was conducted by considering the expression of candidate lncRNAs in primary cells and their correlation with clinical parameters.

In the differentiation screen, only 4 candidate genes were identified due to bottleneck effects and limited library coverage; therefore, this dataset was excluded from further analyses. The venetoclax response screen revealed 11 genes potentially involved in resistance and 12 associated with sensitivity. Among these latest, lncRNA AC009299.3 emerged as the most compelling candidate. This phenotype was validated with single-guide RNA perturbation, and subsequent clinical correlation analyses within our internal AML ClinSeq cohort (n=325) demonstrated that *AC009299.3* expression is higher in elderly patients, in intermediate and adverse ELN2017 subgroups, and in secondary AML cases. Importantly, its high expression correlated with worse prognosis in patients treated with standard chemotherapies.

The proliferation screen yielded the most robust results, identifying 47 candidate genes that may promote cell growth and 11 with potential inhibitory effects. Owing to the strength of these findings, the proliferation screen was prioritized for downstream analyses and became the focus of subsequent functional investigations. Among the candidates, three lncRNAs, MIR17HG, CATG00000106133.1, and CATG00000056792.1, were selected for further study based on their expression patterns and clinical associations. MIR17HG is a well-characterized lncRNA previously shown to drive cell proliferation in cancer settings [322–324], whereas CATG00000106133.1 and CATG00000056792.1 represent novel transcripts. In our internal KAW cohort (AML n = 103; $CD34^+$ n = 11), both novel lncRNAs showed elevated expression in AML samples compared with normal $CD34^+$ cells, consistent with the screen results. Clinical correlations were assessed within the internal ClinSeq AML cohort (n = 325), revealing notable associations: all three lncRNAs were linked to *NPM1* mutation; *MIR17HG* and *CATG00000056792.1* showed higher expression in female patients; and CATG00000106133.1 was enriched in *de novo* AML and cytogenetically normal cases.

Given that MIR17HG is already annotated and well characterized, and that *CATG00000056792.1* gene is located within the intronic region of *RUNX1*, implying that its targeting for functional studies or future therapeutic approaches could be challenging due to potential off-target effects, CATG00000106133.1 was selected for more in-depth investigation. Subcellular localization analysis revealed that CATG00000106133.1 is predominantly localized in the nucleus, suggesting that it may directly or indirectly influence gene transcription. Moreover, CAGE-seq data in normal cells demonstrated differential expression of *CATG00000106133.1* across hematopoietic differentiation stages, with the highest levels observed in basophils, natural killer cells, and monocytes. The functional role of CATG00000106133.1 was further explored by generating a double knockout in HL-60 cells of the first 5 and primary TSSs of *CATG00000106133.1*, as predicted from FANTOM5 CAGE clusters, thereby abolishing the transcription of all known transcript variants expressed in AML cell line. RNA sequencing of *CATG00000106133.1* deficient cells identified 1,406 differentially expressed genes (DEGs), including *HOXA11* and *HOXA10* from the HOXA cluster, which is well-established to drive leukemogenesis [325,326]. In addition, Gene Ontology (GO) and Reactome pathway analyses of these DEGs revealed significant enrichment for terms related to cytokine signaling and immune response. Interestingly, among the cytokine signaling–associated DEGs, *IRF8* was highlighted as a key candidate. Not only was *IRF8* downregulated upon *CATG00000106133.1* depletion, but it was also identified in motif enrichment analysis as one of the most enriched transcription factors located in proximity to the 5 primary TSSs of *CATG00000106133.1*, and as a top hit in the motifs common to the 1,406 DEGs. This dual evidence suggests that CATG00000106133.1 may modulate cytokine signaling pathways, potentially through a regulatory interplay with IRF8.

4.2.3 Discussion

In Study II, we identified several newly discovered and previously annotated lncRNAs with important roles in AML cell proliferation and drug response. Among these, AC009299.3 emerged as a key regulator of venetoclax sensitivity. Functional validation showed that *AC009299.3* knockdown reduced AML cell susceptibility to venetoclax, highlighting its potential role as a modulator of drug response. LncRNAs have previously been shown to influence drug responses in AML [184,210,214], including SNHG1, which promotes venetoclax resistance by epigenetically silencing *ZCCHC10 [215]*. However, our study is the first to identify a novel lncRNA, AC009299.3, that promotes AML sensitivity to venetoclax.

Interestingly, under standard chemotherapy conditions, high AC009299.3 expression correlated with poorer overall survival, suggesting that this lncRNA may mark biologically aggressive AML subtypes. Consistent with this, elevated *AC009299.3* expression was associated with secondary AML and with patients classified within the ELN intermediate or adverse risk groups [38]. Taken together, these findings raise the possibility that AC009299.3 could serve as a predictive biomarker to identify patients who may benefit more from venetoclax based regimens than from just conventional chemotherapy, thereby informing more personalized treatment strategies.

From the proliferation screen, three lncRNAs, MIR17HG, CATG00000106133.1, and CATG00000056792.1, were selected based on their expression profiles and clinical correlation analyses. Among them, we focused primarily on CATG00000106133.1, a previously uncharacterized lncRNA showing high expression in AML patient samples compared with $CD34^+$ normal cells. Its expression was also higher than that of *MIR17HG* and *CATG00000056792.1* in AML cells. RNA-seq analyses of *CATG00000106133.1* knockout clones revealed over 1,406 differentially expressed genes, including members of the *HOXA* cluster, such as *HOXA10* and *HOXA11*, which are well-established regulators of hematopoietic differentiation and AML pathogenesis [326–328] and often perturbed and associated with lncRNAs [329–331] in tumorigenic conditions. Pathway enrichment analyses (GO and Reactome) highlighted cytokine signaling and immune-related processes. Notably, IRF8, a transcription factor fundamental for monocytic and macrophage differentiation [332] and recognized as both an AML-biased dependency and an independent adverse prognostic factor [333,334], was downregulated upon *CATG00000106133.1* knockout. Moreover, IRF8 motifs were enriched within both the CATG00000106133.1 promoter and the promoters of differentially expressed genes. Taken together, these data suggest that CATG00000106133.1 and IRF8 might regulate each other, either directly or indirectly, in a feedback loop mechanism.

Overall, this study led to the identification of novel lncRNAs that regulate key cellular phenotypes and drug responses in AML. From the screening analyses, four candidates were selected and subsequently validated through functional experiments. These findings provide a strong foundation for mechanistic investigations, as understanding how these lncRNAs interact with other molecular players in AML may reveal novel biomarkers and potential therapeutic targets for future clinical intervention.

4.3 Study III

4.3.1 Study rationale

As outlined in the rationale for Studies I and II, the heterogeneity of AML, driven by a complex spectrum of genetic alterations, highlights the need to broaden our understanding of disease biology beyond the well-characterized mutations affecting signaling pathways, transcription factors, and epigenetic regulators. In particular, post-transcriptional alterations remain substantially underexplored, even though mutations in splicing factors such as *SRSF2, SF3B1* and *U2AF1* [335,336], as well as alterations in enzymes involved in mRNA N6-modification [337], clearly demonstrate the importance of post-transcriptional regulation in AML pathogenesis. In Study III, we therefore concentrate on essential genes in AML, with special attention to eIF4A3, a core helicase of the exon-junction complex (EJC). eIF4A3 orchestrates key post-transcriptional processes and has been implicated in tumor progression and poor prognosis in other cancers [238]. Based on these findings, we hypothesize that eIF4A3 may play a critical and therapeutically targetable role in AML pathogenesis.

4.3.2 Results

In Study III we firstly employed CRISPR-derived functional screening data obtained from the DepMap repository [253] to investigate essential genes in AML. Among the top 50 genes identified across 18 different AML cell lines, we observed a recurrent enrichment of genes involved in post-transcriptional mechanisms. This trend was further supported by Reactome pathway analysis, which revealed an enrichment of terms related to translation, ribosome biogenesis, splicing and nonsense-mediated decay. Among these top genes, the helicase eIF4A3 emerged as a prominent candidate due to its involvement in almost all the pathways just mentioned and its ranking as the most essential enzyme among members of the DEAD-box family, implicated in the treatment of both solid and hematological tumors [338]. Transcriptomic data from DepMap revealed significantly higher *eIF4A3* expression in AML cells compared to non-cancerous diploid cells. This pattern was also consistently observed in AML patient samples data, which showed markedly higher *eIF4A3* expression than normal $CD34^+$ cells. Additionally, differential gene expression analyses were conducted by comparing AML cell lines and patient samples to their respective normal controls, as well as patient samples with low versus high *eIF4A3* expression. Across all comparisons, Gene Ontology

(GO) analysis of differentially expressed genes consistently highlighted pathways related to RNA metabolism and translation.

Further characterization of eIF4A3 showed that siRNA-mediated knockdown of the enzyme induced AML cell death. A similar phenotype was observed also upon chemical inhibition of eIF4A3, while normal CD34+ and peripheral blood mononuclear cells were minimally affected. This effect on leukemic cell death, was partially dependent on p53 activation via the checkpoint triggered by impaired ribosome biogenesis (IRBC) [339]. In fact, the simultaneous silencing of *eIF4A3* and *TP53* reversed the effects on cell death in two out of the three AML cell lines tested, OCI-AML3 and IMS-M2, and rescued the upregulation of *CDKN1A* and *MDM2* in all three cell lines, an effect that was instead observed when *eIF4A3* was silenced alone. The involvement of eIF4A3 within the IRBC was further confirmed by the observation that co-silencing *eIF4A3* and *RPL5,* a core regulator of the IRBC (343), rescued the *eIF4A3*-mediated knockout effects on p53 in all cell lines tested. To explore why dual targeting of *eIF4A3* and *TP53* did not alter cell death levels compared with *eIF4A3* silencing alone in OCI-AML2, we investigated the expression of pro-apoptotic target genes. Interestingly, in OCI-AML2 cells, upregulation of the pro-apoptotic gene *BBC3* (PUMA) [340] upon *eIF4A3* knockdown occurred independently of *TP53* expression levels. These findings indicate that eIF4A3-induced apoptosis can occur through both p53-dependent and p53-independent mechanisms in AML context.

4.3.3 Discussion

In Study III, we highlighted the relevance of post-transcriptional mechanisms in the context of AML, focusing particularly on the dependence of AML cells on the helicase and EJC component eIF4A3. We demonstrated that *eIF4A3* is highly expressed in AML cell lines and patient samples, and that its elevated levels correlate with the dysregulation of genes involved in post-transcriptional regulation. These findings underscore the importance of further investigating genes involved in these mechanisms, such as eIF4A3, as potential novel therapeutic targets in AML. Moreover, both eIF4A3 depletion and chemical inhibition induced cell death in AML cell lines, which can occur either through p53 activation or independently of p53. This phenotype appears to result from nucleolar stress activation and impaired ribosome biogenesis caused by eIF4A3 loss, consistent with previous studies highlighting a central role for this helicase in ribosome biogenesis control [238]. In the p53-dependent pathway, eIF4A3 perturbation leads to 5sRNP complex (RPL5 together with RPL11 and 5s rRNA)

mediated sequestration of MDM2, resulting in p53 stabilization and subsequent cell death induction. However, we also observed evidence of p53-independent mechanisms: notably, the upregulation of the pro-apoptotic gene *BBC3* in OCI-AML2 cells following both *eIF4A3* knockdown and combined *eIF4A3* and *TP53* silencing suggests that apoptosis and PUMA upregulation can also be triggered through alternative mechanisms not directly involving p53 [341].

From a clinical point of view, the findings shown in this study related to cell death induction upon eIF4A3 perturbation may inform the development of novel targeted therapeutic strategies for patients with high levels of eIF4A3. As we also showed in this study, selective eIF4A3 inhibitors have already been developed, designed to interfere with eIF4A3 function within the EJC or its role in nonsense-mediated mRNA decay [246,342]. However, none of these compounds have yet been evaluated within *in vivo* experimental studies. Based on our results, it would therefore be of great interest to further investigate the effects of eIF4A3 inhibition in preclinical AML models, particularly in cases harboring mutant *TP53*, where targeting eIF4A3 could promote cell death independently of p53 status.

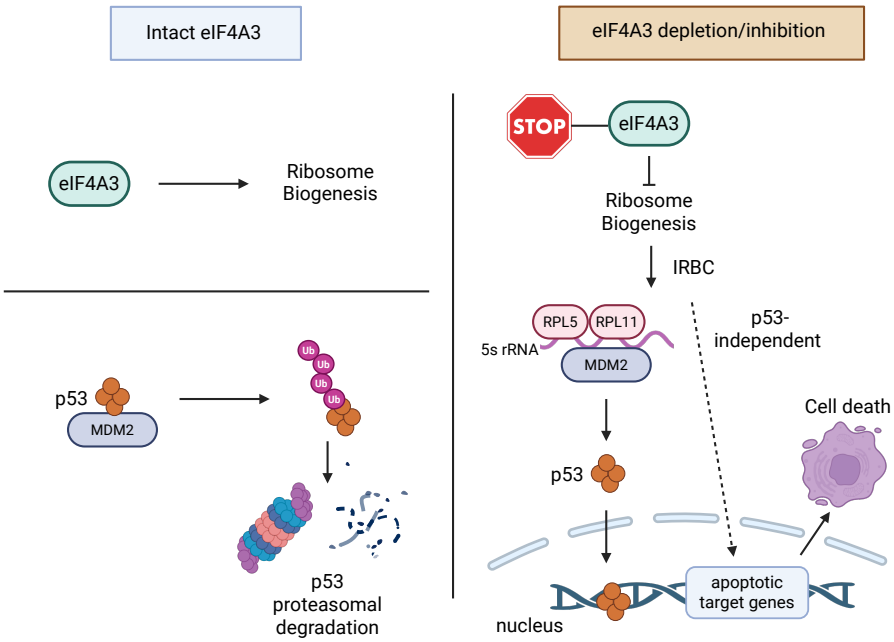

Figure 9. Schematic overview of AML cellular processes occurring before and after eIF4A3 perturbation. Created with BioRender.com

5 Conclusions and Points of prospective

Studies I and II of this thesis identified five novel lncRNAs associated with AML and highlighted their potential clinical relevance; however, substantial work remains before these molecules can be translated into clinical applications. Further mechanistic studies and validation in primary patient samples and animal models are essential to clarify their roles and therapeutic potential before entering in clinical trials. In fact, although long non-coding RNAs are attractive molecules due to their pronounced cell- and tissue-specific expression, their detectability and stability in body fluids, and the large number that remain unexplored, their poor conservation across species and the limited understanding of their precise biological functions present significant challenges. Notably, lncRNAs with potential as biomarkers for prognosis or predicting drug response require rigorous prospective validation in large and well-characterized patient cohorts. Their often-low abundance may also pose detection challenges in standard clinical settings. In addition, any therapeutic application of lncRNAs would require a clear understanding of their functions, including the specific roles of different isoforms, in both normal and disease contexts. Because lncRNAs can participate in multiple regulatory mechanisms, interacting with a broad spectrum of molecules, precise characterization is essential to minimize the risk of off-target effects.

Specifically, **in Study I**, we demonstrated that MALNC, in APL-specific context, appears to influence the expression of several genes, including genes involved in the retinoic acid pathway, and binds to chromatin, which may partially explain the dysregulation of these genes. However, further clarification is needed to understand how MALNC regulates gene expression, potentially through physical interactions with chromatin-modifying enzymes, transcription factors, or the PML-RARA fusion protein. Interestingly, ZNF536, a zinc finger protein associated with the regulation of the retinoic acid pathway [285,343], was found to be upregulated in APL cells lacking *MALNC* expression, both under basal conditions and upon ATRA treatment. Moreover, the genomic locus of *ZNF536* was shown to be bound by MALNC. These findings suggest that further investigation into the potential interplay between MALNC and ZNF536 could provide novel insights into the role of MALNC in APL biology. In parallel, since we show that MALNC also localizes to the cytoplasm, studies exploring its potential involvement in post-transcriptional mechanisms, such as affecting mRNA stability or acting as a miRNA sponge, may help clarify alternative or complementary functions of this lncRNA in APL gene regulation.

Therefore, further mechanistic studies, such as those described above, together with the findings presented in this thesis, could help clarify the biological function of MALNC, while also providing insights into its therapeutic potential. To confirm its role in APL pathogenesis, its involvement in cell proliferation, differentiation, and drug response should be validated in primary APL patient samples. These studies are crucial to determine whether the observations made in APL cell lines are consistent in more heterogeneous and clinically relevant contexts. *In vivo* investigations will also be essential to establish a clearer link between MALNC expression and sensitivity to anti-leukemic therapies, including ATO, which was shown *in vitro* to promote higher sensitivity upon *MALNC* depletion and the combinatorial ATO+ATRA treatment, and to evaluate its potential as a predictive marker for treatment response. Finally, although this study primarily focused on MALNC in the context of APL, it is also important to explore its role and clinical relevance in other AML subtypes, such as those harboring *NPM1*/*IDH2*^{R140} co-mutations, where *MALNC* was also found to be highly expressed, as well as in normal hematopoiesis, where it exhibits a distinct expression pattern with high levels at specific stages of differentiation.

In **Study II**, we identified one lncRNA, AC009299.3, with potential relevance to venetoclax sensitivity; however, its mechanism of action remains unclear. Additionally, further investigation of its expression in patient cohorts receiving venetoclax-based combination therapy could be essential to evaluate its potential as a biomarker of treatment response. Among the three lncRNAs identified as regulators of AML cell proliferation, we focused on CATG00000106133.1, which was found, both in the initial screen and through subsequent CRISPR-mediated knockout experiments, to promote cell proliferation. Although our RNA-seq data suggested a link between CATG00000106133.1, cytokine signaling, and immune response pathways, additional mechanistic studies are needed to clarify this connection. Interestingly, we identified IRF8 and IRF4 binding motifs within the CATG00000106133.1 promoter region, suggesting that these transcription factors, both members of the IRF family [344], may directly regulate its expression. Further IRF8/IRF4 perturbation and ChIP-seq experiments are needed to confirm this potential transcriptional regulation. Additionally, RNA-seq data also revealed reduced IRF8 mRNA levels upon CATG00000106133.1 knockout, indicating a possible feedback loop between the two molecules. Mechanistically, CATG00000106133.1 may regulate *IRF8* expression at multiple levels. At the transcriptional level, it could

influence the recruitment of transcription factors and/or chromatin modifiers at the *IRF8* promoter or other regulatory regions. Notably, AML proliferation has been shown to be promoted by IRF8 both *in vitro* and *in vivo* through the recruitment of ZMYND8 together with BRD4 to its enhancer regions [333]. Therefore, investigating a potential interaction between CATG00000106133.1 and these epigenetic regulators may be particularly relevant. Although CATG00000106133.1 is mainly nuclear, a small cytoplasmic fraction might regulate IRF8 post-transcriptionally. CATG00000106133.1 could affect IRF8 mRNA translation efficiency, but also act as a miRNA sponge, similar to linc-223, which has been shown to function as a ceRNA for miR-125-5p to promote IRF4 expression in AML cells [345]. Finally, since CAGE sequencing data suggested preferential expression of CATG00000106133.1 in certain healthy hematopoietic cell types, further investigation of its specificity and functional role in these contexts would be relevant. Beyond the lncRNAs we focused on, the proliferation and venetoclax screens identified 77 additional lncRNAs, representing novel candidates for future studies aimed at uncovering new mechanisms that regulate AML cell behavior.

Similar to the considerations outlined for lncRNAs, the use of helicase molecules as therapeutic targets in leukemic settings also requires further investigation. Specifically, our results from **Study III** position eIF4A3 as a promising target for AML therapy. Both genetic (siRNA) and pharmacological inhibition of eIF4A3 were shown to trigger cell death in AML cell lines. Additionally, chemical inhibition of eIF4A3 had minimal effects on normal CD34+ cells, underscoring its potential as selectively target in leukemic cells. However, a deeper understanding of eIF4A3's mechanisms of action in the leukemic context remains critical. Of particular importance will be to elucidate whether and how eIF4A3 depletion might affects other biological processes in leukemic context, and to clarify the mechanisms underlying p53-independent cell death. Moreover, future studies should determine whether *eIF4A3* loss induces cell death in AML models harboring mutant *TP53*, as demonstrating efficacy in this subclass would strengthen the translational relevance of targeting eIF4A3 in patients lacking functional p53. To translate these findings into a potential clinical setting, additional studies in patient-derived cells and relevant animal models are essential to validate the effects of *eIF4A3* depletion and to assess potential toxicity and safety. Finally, evaluating the synergistic effects of eIF4A3 inhibition in combination with current standard-of-care therapies, both *in vitro* and *in vivo*, will be crucial to establish its

therapeutic relevance as a novel or complementary strategy to improve AML outcome.

Overall, these three studies expand knowledge of lncRNAs and post-transcriptional regulation in AML, shed light on the disease's molecular heterogeneity, and provide a foundation for further mechanistic characterization of these molecules. Such work will be essential to define their biological roles in greater detail and explore their value as complementary prognostic markers and possible therapeutic targets.

6 Acknowledgements

This marks the culmination of a journey made possible by the support and contributions of many people.

First of all, I would like to express my sincere gratitude to **Prof. Sören Lehmann**, my main supervisor. Your trust, guidance, and steady support have been essential throughout this PhD. I am especially grateful for your calm and thoughtful approach, which has helped me navigate challenges with more confidence. In addition, your clinical perspective has taught me how to connect biological discoveries with their clinical relevance, always keeping in mind how scientific research can be translated into practical patient care.

I would also like to thank **Andreas Lennartsson**, my co-supervisor, who first introduced me to the world of AML during my master's thesis. I am deeply grateful for everything I have learned from you about epigenetics and molecular biology. Your optimism, encouragement, and insightful advice have been invaluable throughout my PhD journey.

A special thanks goes to **Sophia Miliara**, my co-supervisor. You are truly an inspiring scientist and person. From the day we met, you have shared your passion for research and taught me how to become a scientist, not only by mastering numerous laboratory techniques but also by cultivating critical thinking, curiosity, and perseverance. You have always been there to support me and help expand my scientific knowledge. Even after leaving academia, your advice and previous input have continued to be a reliable source of guidance and inspiration.

I am also very grateful to **Dimitris Kanellis**, my mentor, for guiding me through all the ups and downs of this PhD and for providing kind and practical advice along the way.

Many thanks to all present and past members of the Lehmann group. To **Xiangfu Zhong**, for your amazing contribution with complex bioinformatic analyses, and for always being available, with a smile, to help. To **Anne Neddermeyer**, for our extensive collaborations and for sharing your expertise on lncRNAs. To **Sofia Bengtzen** and **Anna Bohlin**, for always being there to assist with patient sample preparation, handling, and all the practical (and administrative) aspects of our work. To **Guoxiang Xie** for your valuable input during lab meetings and for the stimulating bioinformatic discussions. To **Angelica Gamboa-Cedeno and Albin Österroos**, thank you for helping with wet lab experiments and clinical data

analysis, respectively, and to **Liew-Littorin Markus** for the enjoyable and insightful discussions during lab meetings. I am also grateful and to my former colleagues **My Björklund, Ying Qu, and Christer Nilsson**. Although we had only a few occasions to meet, your contributions were important in shaping the story of some of the work presented in this thesis.

I would like to thank the members of the Lennartsson group, with whom I had the pleasure of collaborating throughout my PhD. Thanks to **Aonghus Naughton** for the great scientific discussions during joint meetings and the friendly conversations during social events. A warm thanks to **Anna Palau** for your generosity in sharing your expertise and for always being willing to help in the lab.

Hong Qian thank you for not only agreeing to chair my PhD thesis defense but also, together with **Alma Månsson**, for introducing me to animal studies. I greatly appreciated being welcomed like I would be one of your group members, learning all aspects of mouse handling and *in vivo* experiments, benefiting from your expertise, and knowing you were always available for any questions.

I would like to thank **Roland Fiskesund** for assisting with normal hematopoietic cell differentiation induction experiments and **Nona Struyf, Tom Erkers,** and **Olli-Pekka Kallioniemi** for your valuable contribution with drug screening data. I am also grateful to the **CRISPR Functional Genomics (CFG)** facility and the core facility for **Bioinformatics and Expression Analysis (BEA)** for supporting in the CRISPR screens project, as well as the **FANTOM Consortium** at the RIKEN Institute for providing key resources and data.

I am deeply grateful to the **patients and donors** whose trust and contribution made this research possible and to all the **corresponding authors** of the studies presented in this thesis.

Thanks to all past and present members of **HERM** for welcoming me from the very beginning. Joining in 2020, shortly after the pandemic, was not easy, but I found a supportive and inspiring environment, and many of you have become not only colleagues but also friends. Thanks to **Eva Hellström**, and now **Yenan Bryceson**, for leading a stimulating hematological environment, and to **Petter Höglund** for overseeing the entire MEDH department. I am also grateful to **Vanessa Lundin** for your valuable external scientific feedback, and to **Pedro Moura** for generously sharing your expertise in slide preparation. To **all other PIs** for your scientific contribution and advice during SAP seminars.

Thanks to the FACS facility, to **Belinda Pannagel** and **Narmadha Subramanian** for helping with long sorting sessions.

Thanks to **Anne-Sofie Johansson** and **Sara von Bahr Grebäck** for your help with administrative matters, and to **Annette Öster Fernström** for your invaluable support in organizing the dissertation and related documents.

Thanks to all the other members of HERM, mentioned here or not, past and present, for the years spent together: **Sadaf Fazeli, Teresa Mortera Blanco, Tessa Campbell, Heinrich Schlums, Jelve Nejati Zendegani, Sophia Hofmann, Katharina Kirchhof, Indira Barbosa, Alexandra Argyriou, Jonah Anderson, Elena Bonaiti, Runqing Zhang, Maaike Jongen, Yang Liu, Stan de Haan, and Sophia Hald.** Thanks to **Lars Gerullat, Anna Pumpe, Nikolaos Kyriakidis, Alexandra Helleux, Nicole Wild, Agneta Birgitta Andersson, Laura Sanz, and Filip Segerberg** for both the deep and fun conversations we shared during lunchtime. To **Jonas Their,** for always bringing positivity and happiness to the HERM corridors. To **Gloria Wu**, for sharing tips and tricks on APL cell culturing. To **Elory Leonard**, for the lovely conversations about Indonesia and the great food recommendations. To **Petar Mitev**, for your "What's up" every morning, your scientific support, and our random conversations about life and future perspectives. To **Caroline Eriksson, Sigrid Wahlen and Julian Fischbach,** for your friendship, for sharing tough moments, but also for celebrating big achievements together.

To the Italian friends I met at Flemingsberg campus, **Giulia Lorenzon, Elisa Saccon, Francesca Grassi** and **Luana Tomaipitinca,** thank you for our Italian chats, coffee breaks, and for always reminding me of home.

Thanks to my friends, both in Italy and in Stockholm, who have supported me, but mostly "sopportato" during these years of PhD:

To my childhood friend **Sara**, for your contagious laughter and for always making it feel as if nothing has changed, even though we now have our own lives and live in different cities. To **Federica**, my dear friend and psychologist of the heart (and in real life), I truly admire you for the amazing person you are. To **Gloria**, thank you for being such a wonderful friend, and to **Valentina**, for our unforgettable years of crazy study sessions in Trieste.

To **Roberta, Alessandro, Luca, Lisa, Kostas, Stefania, Christian, Elena, Jan, Chiara, Annarita, and Flavia,** meeting you here has been amazing. Thank you for

all the dinners, fika, and shared activities… You have been my second family during my years here in Sweden!

To **Marina**, you are such a strong and inspiring woman. I admire your elegance, which hides a determined and powerful soul. I'm so happy we met back in the days in Trieste. You have created such a beautiful family together with **Massimo** and **Santi**, and I hope we will share many more wonderful moments together.

To **Vanessa**, going out for that *samla* back in 2021 was one of the best decisions ever! You are the kindest and most generous person I have ever met, and I feel so grateful to have you, but also **Tim**, **Lova**, and **Olaf** in my life. I truly hope our Italian-Dutch friendship lasts forever.

To conclude, my deepest thanks go to the most important people in my life.

To **Lucio** and **Carla**, my aunts. You have always been on my side, since I was a child… I still remember the little encouragement poem you wrote for my very first day of school…and it's beautiful to think how far I've come since then. Thank you for always keeping me in your thoughts and for your endless love and care.

To **Eleonora**, my "little" sister, I feel so lucky to have you in my life. In recent years, the age gap between us has faded, and we've discovered an amazing friendship beneath our sisterhood. You inspire me with your creativity and originality, and I know you will reach important goals both academically and professionally in the future. I truly look forward to seeing where life will take us, sharing many more trips, adventures and special moments together.

To my parents, **Rita** and **Gianni**, your unconditional love has been my greatest gift. You've always placed Eleonora and me at the center of your world, raising our family in the best way one could ask for, with love and solid values. Thank you for believing in me and supporting every step of my journey. I wouldn't be who I am today without you. Even if I don't say it often, I love you deeply and am forever grateful for everything you've done.

Finally, but certainly not least, to **Luca**, my other half and the person I've shared my life with for the past nine years. You have been my "rock" throughout this PhD journey, and as we often say…you deserve a PhD yourself for all the support you've given me! There are not enough words to describe how much you mean to me, and I can't wait to spend many more years of life together.

7 Declaration about the use of generative AI

No AI tools were used in the writing of the “kappa”/comprehensive summary of this thesis.

I take full responsibility of the content of the “kappa”/comprehensive summary of the thesis.

8 References

[1] Orkin SH, Zon LI. Hematopoiesis: An Evolving Paradigm for Stem Cell Biology. Cell 2008;132:631. https://doi.org/10.1016/J.CELL.2008.01.025.

[2] Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001 414:6859 2001;414:105–11. https://doi.org/10.1038/35102167.

[3] Kaushansky K. Lineage-Specific Hematopoietic Growth Factors. New England Journal of Medicine 2006;354:2034–45. https://doi.org/10.1056/NEJMRA052706.

[4] Orkin SH. Diversification of haematopoietic stem cells to specific lineages. Nature Reviews Genetics 2000 1:1 2000;1:57–64. https://doi.org/10.1038/35049577.

[5] Piccaluga PP, Piccaluga PP. Introductory Chapter: A Brief History of Acute Leukemias Treatment. Acute Leukemias 2021. https://doi.org/10.5772/INTECHOPEN.96439.

[6] Loke J, Kansagra A. Fast Facts: Leukemia. 2022.

[7] Estey E, Döhner H. Acute myeloid leukaemia. The Lancet 2006;368:1894–907. https://doi.org/10.1016/s0140-6736(06)69780-8.

[8] Fialkow PJ, Singer JW, Raskind WH, Adamson JW, Jacobson RJ, Bernstein ID, et al. Clonal development, stem-cell differentiation, and clinical remissions in acute nonlymphocytic leukemia. N Engl J Med 1987;317:468–73. https://doi.org/10.1056/NEJM198708203170802.

[9] Lowenberg B, Downing JR, Burnett A. Acute myeloid leukemia. N Engl J Med 1999;341:1051–62. https://doi.org/10.1056/NEJM199909303411407.

[10] Kaushansky Kenneth, Lichtman MA., Prchal JT., Levi Marcel, Burns LJ., Linch DC. Williams hematology 2021.

[11] Long NA, Golla U, Sharma A, Claxton DF. Acute Myeloid Leukemia Stem Cells: Origin, Characteristics, and Clinical Implications. Stem Cell Rev Rep 2022;18:1211–26. https://doi.org/10.1007/S12015-021-10308-6.

[12] Heath EM, Chan SM, Minden MD, Murphy T, Shlush LI, Schimmer AD. Biological and clinical consequences of NPM1 mutations in AML. Leukemia 2017;31:798–807. https://doi.org/10.1038/leu.2017.30.

[13] Pollyea DA, Jordan CT. Therapeutic targeting of acute myeloid leukemia stem cells. Blood 2017;129:1627–35. https://doi.org/10.1182/BLOOD-2016-10-696039,.

[14] Vakiti A, Mewawalla P, Wood SK. Acute Myeloid Leukemia (Nursing). StatPearls 2022.

[15] Döhner H, Weisdorf DJ, Bloomfield CD. Acute Myeloid Leukemia. New England Journal of Medicine 2015;373:1136–52. https://doi.org/10.1056/NEJMra1406184.

[16] Eriksson A, Lennartsson A, Lehmann S. Epigenetic aberrations in acute myeloid leukemia: Early key events during leukemogenesis. Exp Hematol 2015;43:609–24. https://doi.org/10.1016/J.EXPHEM.2015.05.009.

[17] De Kouchkovsky I, Abdul-Hay M. 'Acute myeloid leukemia: a comprehensive review and 2016 update.' Blood Cancer J 2016;6:e441–e441. https://doi.org/10.1038/bcj.2016.50.

[18] Meyers J, Yu Y, Kaye JA, Davis KL. Medicare Fee-for-Service Enrollees with Primary Acute Myeloid Leukemia: An Analysis of Treatment Patterns, Survival, and Healthcare Resource Utilization and Costs. Appl Health Econ Health Policy 2013;11:275–86. https://doi.org/10.1007/s40258-013-0032-2.

[19] Kane E V., Roman E, Cartwright R, Parker J, Morgan G. Tobacco and the risk of acute leukaemia in adults. Br J Cancer 1999;81:1228–33. https://doi.org/10.1038/sj.bjc.6690833.

[20] Travis LB, Li CY, Zhang ZN, Li DG, Yin SN, Chow WH, et al. Hematopoietic malignancies and related disorders among benzene-exposed workers in China. Leuk Lymphoma 1994;14:91–102. https://doi.org/10.3109/10428199409049654.

[21] Gale RP. Radiation and leukaemia: Which leukaemias and what doses? Blood Rev 2023;58:101017. https://doi.org/10.1016/J.BLRE.2022.101017.

[22] Papakonstantinou E, Tragiannidis A, Ampatzidou M, Katzilakis N, Nikita M, Totikidis G, et al. Acute Leukemia in Children with Down Syndrome: A

Report from the Hellenic HESPHO Group. Hemato 2025, Vol 6, Page 8 2025;6:8. https://doi.org/10.3390/HEMATO6020008.

[23] Goel H, Rahul E, Gupta I, Chopra A, Ranjan A, Gupta AK, et al. Molecular and genomic landscapes in secondary & therapy related acute myeloid leukemia. Am J Blood Res 2021;11:472.

[24] Bennett JM, Catovsky D, Daniel M -T, Flandrin G, Galton DAG, Gralnick HR, et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol 1976;33:451–8. https://doi.org/10.1111/J.1365-2141.1976.TB03563.X.

[25] Saultz J, Garzon R. Acute Myeloid Leukemia: A Concise Review. J Clin Med 2016;5:33. https://doi.org/10.3390/jcm5030033.

[26] Jaffe ES, Harris NL, Stein H, Jaffe et al, Vardiman JW. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. IARC Press; 2001.

[27] Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood 2022;140:1345–77. https://doi.org/10.1182/BLOOD.2022016867,.

[28] Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia 2022 36:7 2022;36:1703–19. https://doi.org/10.1038/s41375-022-01613-1.

[29] Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood 2022;140:1200–28. https://doi.org/10.1182/BLOOD.2022015850.

[30] Della Porta MG, Martinelli G, Rambaldi A, Santoro A, Voso MT. A practical algorithm for acute myeloid leukaemia diagnosis following the updated 2022 classifications. Crit Rev Oncol Hematol 2024;198:104358. https://doi.org/10.1016/J.CRITREVONC.2024.104358.

[31] Liersch R, Müller-Tidow C, Berdel WE, Krug U. Prognostic factors for acute myeloid leukaemia in adults - biological significance and clinical use. Br J Haematol 2014;165:17–38. https://doi.org/10.1111/BJH.12750.

[32] Juliusson G, Antunovic P, Derolf Å, Lehmann S, Möllgård L, Stockelberg D, et al. Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. Blood 2009;113:4179–87. https://doi.org/10.1182/BLOOD-2008-07-172007.

[33] Appelbaum FR, Gundacker H, Head DR, Slovak ML, Willman CL, Godwin JE, et al. Age and acute myeloid leukemia. Blood 2006;107:3481–5. https://doi.org/10.1182/BLOOD-2005-09-3724.

[34] Giles FJ, Borthakur G, Ravandi F, Faderl S, Verstovsek S, Thomas D, et al. The haematopoietic cell transplantation comorbidity index score is predictive of early death and survival in patients over 60 years of age receiving induction therapy for acute myeloid leukaemia. Br J Haematol 2007;136:624–7. https://doi.org/10.1111/J.1365-2141.2006.06476.X;PAGEGROUP:STRING:PUBLICATION.

[35] Deschler B, Ihorst G, Platzbecker U, Germing U, Lübbert M. Development of a Frailty Score for Older Patients with Myelodysplastic Syndromes and Acute Myeloid Leukemia. Blood 2009;114:1775. https://doi.org/10.1182/BLOOD.V114.22.1775.1775.

[36] Bewersdorf JP, Zeidan AM. Hyperleukocytosis and Leukostasis in Acute Myeloid Leukemia: Can a Better Understanding of the Underlying Molecular Pathophysiology Lead to Novel Treatments? Cells 2020;9:2310. https://doi.org/10.3390/CELLS9102310.

[37] Bakst RL, Tallman MS, Douer D, Yahalom J. How I treat extramedullary acute myeloid leukemia. Blood 2011;118:3785–93. https://doi.org/10.1182/BLOOD-2011-04-347229.

[38] Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017;129:424–47. https://doi.org/10.1182/blood-2016-08-733196.

[39] Döhner H, DiNardo CD, Appelbaum FR, Craddock C, Dombret H, Ebert BL, et al. Genetic risk classification for adults with AML receiving less-intensive

therapies: the 2024 ELN recommendations. Blood 2024;144:2169–73. https://doi.org/10.1182/BLOOD.2024025409/2238249/BLOOD.2024025409.PDF.

[40] Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, et al. Leukemic IDH1 and IDH2 Mutations Result in a Hypermethylation Phenotype, Disrupt TET2 Function, and Impair Hematopoietic Differentiation. Cancer Cell 2010;18:553–67. https://doi.org/10.1016/J.CCR.2010.11.015.

[41] Garzon R, Volinia S, Papaioannou D, Nicolet D, Kohlschmidt J, Yan PS, et al. Expression and prognostic impact of lncRNAs in acute myeloid leukemia. Proc Natl Acad Sci U S A 2014;111:18679–84. https://doi.org/10.1073/PNAS.1422050112.

[42] Grimwade D, Hills RK, Moorman A V., Walker H, Chatters S, Goldstone AH, et al. 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;116:354–65. https://doi.org/10.1182/BLOOD-2009-11-254441.

[43] Mrózek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. Blood Rev 2004;18:115–36. https://doi.org/10.1016/S0268-960X(03)00040-7.

[44] Mrózek K, Heinonen K, Bloomfield CD. Clinical importance of cytogenetics in acute myeloid leukaemia. Best Pract Res Clin Haematol 2001;14:19–47. https://doi.org/10.1053/beha.2000.0114.

[45] Kelly LM, Gilliland DG. Genetics of myeloid leukemias. Annu Rev Genomics Hum Genet 2002;3:179–98. https://doi.org/10.1146/ANNUREV.GENOM.3.032802.115046/CITE/REFWORKS.

[46] Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. Blood 2002;100:1532–42. https://doi.org/10.1182/blood-2002-02-0492.

[47] Network CGAR, Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med 2013;368:2059–74. https://doi.org/10.1056/NEJMoa1301689.

[48] Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. N Engl J Med 2016;374:2209–21. https://doi.org/10.1056/NEJMOA1516192.

[49] Pabst T, Mueller BU, Zhang P, Radomska HS, Narravula S, Schnittger S, et al. Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-α (C/EBPα), in acute myeloid leukemia. Nat Genet 2001;27:263–70. https://doi.org/10.1038/85820;KWRD.

[50] Wakita S, Sakaguchi M, Oh I, Kako S, Toya T, Najima Y, et al. Prognostic impact of CEBPA bZIP domain mutation in acute myeloid leukemia. Blood Adv 2022;6:238–47. https://doi.org/10.1182/BLOODADVANCES.2021004292.

[51] Box JK, Paquet N, Adams MN, Boucher D, Bolderson E, O'Byrne KJ, et al. Nucleophosmin: from structure and function to disease development. BMC Mol Biol 2016;17. https://doi.org/10.1186/S12867-016-0073-9.

[52] Colombo E, Alcalay M, Pelicci PG. Nucleophosmin and its complex network: a possible therapeutic target in hematological diseases. Oncogene 2011;30:2595–609. https://doi.org/10.1038/onc.2010.646.

[53] Brunetti L, Gundry MC, Goodell MA. New insights into the biology of acute myeloid leukemia with mutated NPM1. Int J Hematol 2019;0:0. https://doi.org/10.1007/s12185-018-02578-7.

[54] Falini B, Bolli N, Liso A, Martelli MP, Mannucci R, Pileri S, et al. Altered nucleophosmin transport in acute myeloid leukaemia with mutated NPM1: molecular basis and clinical implications. Leukemia 2009 23:10 2009;23:1731–43. https://doi.org/10.1038/leu.2009.124.

[55] Falini B. Acute Myeloid Leukemia with Mutated Nucleophosmin (NPM1): Molecular, Pathological, and Clinical Features. Cancer Treat Res, vol. 145, 2009, p. 149–68. https://doi.org/10.1007/978-0-387-69259-3_9.

[56] Medeiros BC, Fathi AT, DiNardo CD, Pollyea DA, Chan SM, Swords R. Isocitrate dehydrogenase mutations in myeloid malignancies. Leukemia 2017;31:272–81. https://doi.org/10.1038/LEU.2016.275.

[57] Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Coller HA, et al. The Common Feature of Leukemia-Associated IDH1 and IDH2 Mutations Is a

Neomorphic Enzyme Activity Converting α-Ketoglutarate to 2-Hydroxyglutarate. Cancer Cell 2010;17:225–34. https://doi.org/10.1016/J.CCR.2010.01.020.

[58] Rücker FG, Schlenk RF, Bullinger L, Kayser S, Teleanu V, Kett H, et al. TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. Blood 2012;119:2114–21. https://doi.org/10.1182/BLOOD-2011-08-375758.

[59] Vousden KH, Lu X. Live or let die: The cell's response to p53. Nat Rev Cancer 2002;2:594–604. https://doi.org/10.1038/NRC864;KWRD.

[60] Haferlach C, Dicker F, Herholz H, Schnittger S, Kern W, Haferlach T. Mutations of the TP53 gene in acute myeloid leukemia are strongly associated with a complex aberrant karyotype. Leukemia 2008;22:1539–41. https://doi.org/10.1038/LEU.2008.143.

[61] Prokocimer M, Molchadsky A, Rotter V. Dysfunctional diversity of p53 proteins in adult acute myeloid leukemia: projections on diagnostic workup and therapy. Blood 2017;130:699–712. https://doi.org/10.1182/BLOOD-2017-02-763086.

[62] Bowen D, Groves MJ, Burnett AK, Patel Y, Allen C, Green C, et al. TP53 gene mutation is frequent in patients with acute myeloid leukemia and complex karyotype, and is associated with very poor prognosis. Leukemia 2009;23:203–6. https://doi.org/10.1038/LEU.2008.173.

[63] Pourrajab F, Zare-Khormizi MR, Hashemi AS, Hekmatimoghaddam S. Genetic Characterization and Risk Stratification of Acute Myeloid Leukemia. Cancer Manag Res 2020;12:2231–53. https://doi.org/10.2147/CMAR.S242479.

[64] Liu PP, Wijmenga C, Hajra A, Blake TB, Kelley CA, Adelstein RS, et al. Identification of the Chimeric Protein Product of the CBFB-MYHl l Fusion Gene in Inv(16) Leukemia Cells. Genes Chromosomes Cancer 1996;16:77–67. https://doi.org/10.1002/(SICI)1098-2264(199606)16:2.

[65] Kumar CC. Genetic Abnormalities and Challenges in the Treatment of Acute Myeloid Leukemia. Genes Cancer 2011;2:95. https://doi.org/10.1177/1947601911408076.

[66] Rowley JD, Golomb HM, Dougherty C. 15/17 TRANSLOCATION, A CONSISTENT CHROMOSOMAL CHANGE IN ACUTE PROMYELOCYTIC LEUKAEMIA. The Lancet 1977;309:549–50. https://doi.org/10.1016/S0140-6736(77)91415-5.

[67] De Thé H, Chomienne C, Lanotte M, Degos L, Dejean A. The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor α gene to a novel transcribed locus. Nature 1990;347:558–61. https://doi.org/10.1038/347558a0.

[68] Kakizuka A, Miller WH, Umesono K, Warrell RP, Frankel SR, Murty VVVS, et al. Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RARα with a novel putative transcription factor, PML. Cell 1991;66:663–74. https://doi.org/10.1016/0092-8674(91)90112-C.

[69] Noguera NI, Catalano G, Banella C, Divona M, Faraoni I, Ottone T, et al. Acute Promyelocytic Leukemia: Update on the Mechanisms of Leukemogenesis, Resistance and on Innovative Treatment Strategies. Cancers (Basel) 2019;11. https://doi.org/10.3390/CANCERS11101591.

[70] Zhou GB, Zhang J, Wang ZY, Chen SJ, Chen Z. Treatment of acute promyelocytic leukaemia with all-trans retinoic acid and arsenic trioxide: A paradigm of synergistic molecular targeting therapy. Philosophical Transactions of the Royal Society B: Biological Sciences 2007;362:959–71. https://doi.org/10.1098/RSTB.2007.2026.

[71] HILLESTAD LK. Acute Promyelocytc Leukemia. Acta Med Scand 1957;159:189–94. https://doi.org/10.1111/J.0954-6820.1957.TB00124.X.

[72] Jambrovics K, Uray IP, Keillor JW, Fésüs L, Balajthy Z. Benefits of Combined All-Trans Retinoic Acid and Arsenic Trioxide Treatment of Acute Promyelocytic Leukemia Cells and Further Enhancement by Inhibition of Atypically Expressed Transglutaminase 2. Cancers (Basel) 2020;12. https://doi.org/10.3390/CANCERS12030648.

[73] Wang ZY, Chen Z. Acute promyelocytic leukemia: from highly fatal to highly curable. Blood 2008;111:2505–15. https://doi.org/10.1182/BLOOD-2007-07-102798.

[74] Ferrara F, Schiffer CA. Acute myeloid leukaemia in adults. The Lancet 2013;381:484–95. https://doi.org/10.1016/S0140-6736(12)61727-9.

[75] Dombret H, Gardin C. An update of current treatments for adult acute myeloid leukemia. Blood 2016;127:53–61. https://doi.org/10.1182/BLOOD-2015-08-604520.

[76] Hills RK, Castaigne S, Appelbaum FR, Delaunay J, Petersdorf S, Othus M, et al. Addition of gemtuzumab ozogamicin to induction chemotherapy in adult patients with acute myeloid leukaemia: a meta-analysis of individual patient data from randomised controlled trials. Lancet Oncol 2014;15:986–96. https://doi.org/10.1016/S1470-2045(14)70281-5.

[77] Erba HP, Montesinos P, Kim HJ, Patkowska E, Vrhovac R, Žák P, et al. Quizartinib plus chemotherapy in newly diagnosed patients with FLT3-internal-tandem-duplication-positive acute myeloid leukaemia (QuANTUM-First): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2023;401:1571–83. https://doi.org/10.1016/S0140-6736(23)00464-6.

[78] Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S, Bloomfield CD, et al. Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. N Engl J Med 2017;377:454–64. https://doi.org/10.1056/NEJMOA1614359.

[79] Shimony S, Stahl M, Stone RM. Acute Myeloid Leukemia: 2025 Update on Diagnosis, Risk-Stratification, and Management. Am J Hematol 2025;0:1–32. https://doi.org/10.1002/AJH.27625.

[80] Wei AH, Panayiotidis P, Montesinos P, Laribi K, Ivanov V, Kim I, et al. 6-month follow-up of VIALE-C demonstrates improved and durable efficacy in patients with untreated AML ineligible for intensive chemotherapy. Blood Cancer Journal 2021 11:10 2021;11:1–8. https://doi.org/10.1038/s41408-021-00555-8.

[81] DiNardo CD, Jonas BA, Pullarkat V, Thirman MJ, Garcia JS, Wei AH, et al. Azacitidine and Venetoclax in Previously Untreated Acute Myeloid Leukemia. N Engl J Med 2020;383:617–29. https://doi.org/10.1056/NEJMOA2012971.

[82] Roboz GJ, DiNardo CD, Stein EM, de Botton S, Mims AS, Prince GT, et al. Ivosidenib induces deep durable remissions in patients with newly

diagnosed IDH1-mutant acute myeloid leukemia. Blood 2020;135:463–71. https://doi.org/10.1182/BLOOD.2019002140.

[83] Montesinos P, Recher C, Vives S, Zarzycka E, Wang J, Bertani G, et al. Ivosidenib and Azacitidine in IDH1-Mutated Acute Myeloid Leukemia. N Engl J Med 2022;386:1519–31. https://doi.org/10.1056/NEJMOA2117344.

[84] Perl AE, Martinelli G, Cortes JE, Neubauer A, Berman E, Paolini S, et al. Gilteritinib or Chemotherapy for Relapsed or Refractory FLT3-Mutated AML. N Engl J Med 2019;381:1728–40. https://doi.org/10.1056/NEJMOA1902688.

[85] Guerra VA, DiNardo C, Konopleva M. Venetoclax-based therapies for acute myeloid leukemia. Best Pract Res Clin Haematol 2019;32:145–53. https://doi.org/10.1016/j.beha.2019.05.008.

[86] Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nature Medicine 2013 19:2 2013;19:202–8. https://doi.org/10.1038/nm.3048.

[87] Chen HC, Kanai M, Inoue-Yamauchi A, Tu HC, Huang Y, Ren D, et al. An interconnected hierarchical model of cell death regulation by the BCL-2 family. Nat Cell Biol 2015;17:1270–81. https://doi.org/10.1038/NCB3236.

[88] Seymour JF, Kipps TJ, Eichhorst B, Hillmen P, D'Rozario J, Assouline S, et al. Venetoclax–Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia. New England Journal of Medicine 2018;378:1107–20. https://doi.org/10.1056/NEJMOA1713976.

[89] Stilgenbauer S, Eichhorst B, Schetelig J, Coutre S, Seymour JF, Munir T, et al. Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: a multicentre, open-label, phase 2 study. Lancet Oncol 2016;17:768–78. https://doi.org/10.1016/S1470-2045(16)30019-5.

[90] Konopleva M, Pollyea DA, Potluri J, Chyla B, Hogdal L, Busman T, et al. Efficacy and Biological Correlates of Response in a Phase II Study of Venetoclax Monotherapy in Patients with Acute Myelogenous Leukemia. Cancer Discov 2016;6:1106–17. https://doi.org/10.1158/2159-8290.CD-16-0313.

[91] DiNardo CD, Pratz K, Pullarkat V, Jonas BA, Arellano M, Becker PS, et al. Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. Blood 2019;133:7–17. https://doi.org/10.1182/BLOOD-2018-08-868752.

[92] Kwag D, Bang SY, Lee JH, Min GJ, Park S-S, Park S, et al. Venetoclax with Decitabine Versus Decitabine Monotherapy in Elderly Acute Myeloid Leukemia: A Propensity Score Matched Analysis. Blood 2022;140:3214–5. https://doi.org/10.1182/BLOOD-2022-171105.

[93] Wei AH, Strickland SA, Hou JZ, Fiedler W, Lin TL, Walter RB, et al. Venetoclax combined with low-dose cytarabine for previously untreated patients with acute myeloid leukemia: Results from a phase Ib/II study. Journal of Clinical Oncology 2019;37:1277–84. https://doi.org/10.1200/JCO.18.01600.

[94] Kantarjian H, Borthakur G, Daver N, DiNardo CD, Issa G, Jabbour E, et al. Current status and research directions in acute myeloid leukemia. Blood Cancer J 2024;14. https://doi.org/10.1038/S41408-024-01143-2.

[95] Condoluci A, Rossi D. Mechanisms of resistance to venetoclax. Blood 2022;140:2094–6. https://doi.org/10.1182/BLOOD.2022017341.

[96] Zhang H, Nakauchi Y, Köhnke T, Stafford M, Bottomly D, Thomas R, et al. Integrated analysis of patient samples identifies biomarkers for venetoclax efficacy and combination strategies in acute myeloid leukemia. Nat Cancer 2020;1:826–39. https://doi.org/10.1038/S43018-020-0103-X.

[97] Breitman TR, Selonick SE, Collins SJ. Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. Proceedings of the National Academy of Sciences 1980;77:2936–40. https://doi.org/10.1073/PNAS.77.5.2936.

[98] Breitman TR, Collins SJ, Keene BR. Terminal differentiation of human promyelocytic leukemic cells in primary culture in response to retinoic acid. Blood 1981;57:1000–4. https://doi.org/10.1182/BLOOD.V57.6.1000.1000.

[99] Avvisati G, Lo-Coco F, Paoloni FP, Petti MC, Diverio D, Vignetti M, et al. AIDA 0493 protocol for newly diagnosed acute promyelocytic leukemia: very long-term results and role of maintenance. Blood 2011;117:4716–25. https://doi.org/10.1182/BLOOD-2010-08-302950.

[100] Fenaux P, Castaigne S, Dombret H, Archimbaud E, Duarte M, Morel P, et al. All-Transretinoic Acid Followed by Intensive Chemotherapy Gives a High Complete Remission Rate and May Prolong Remissions in Newly Diagnosed Acute Promyelocytic Leukemia: A Pilot Study on 26 Cases. Blood 1992;80:2176–81. https://doi.org/10.1182/BLOOD.V80.9.2176.2176.

[101] Powell BL, Moser B, Stock W, Gallagher RE, Willman CL, Stone RM, et al. Arsenic trioxide improves event-free and overall survival for adults with acute promyelocytic leukemia: North American Leukemia Intergroup Study C9710. Blood 2010;116:3751–7. https://doi.org/10.1182/BLOOD-2010-02-269621.

[102] Lo-Coco F, Avvisati G, Vignetti M, Thiede C, Orlando SM, Iacobelli S, et al. Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. N Engl J Med 2013;369:111–21. https://doi.org/10.1056/NEJMOA1300874.

[103] Sanz MA, Fenaux P, Tallman MS, Estey EH, Löwenberg B, Naoe T, et al. Management of acute promyelocytic leukemia: updated recommendations from an expert panel of the European LeukemiaNet. Blood 2019;133:1630. https://doi.org/10.1182/BLOOD-2019-01-894980.

[104] Zhu J, Guo WM, Yao YY, Zhao WL, Pan L, Cai X, et al. Tissue factors on acute promyelocytic leukemia and endothelial cells are differently regulated by retinoic acid, arsenic trioxide and chemotherapeutic agents. Leukemia 1999;13:1062–70. https://doi.org/10.1038/SJ.LEU.2401448.

[105] Germain P, Iyer J, Zechel C, Gronemeyer H. Co-regulator recruitment and the mechanism of retinoic acid receptor synergy. Nature 2002;415:187–92. https://doi.org/10.1038/415187A.

[106] Orfali N, O'Donovan TR, Nyhan MJ, Britschgi A, Tschan MP, Cahill MR, et al. Induction of autophagy is a key component of all-trans-retinoic acid-induced differentiation in leukemia cells and a potential target for pharmacologic modulation. Exp Hematol 2015;43:781-793.e2. https://doi.org/10.1016/j.exphem.2015.04.012.

[107] Ablain J, Rice K, Soilihi H, De Reynies A, Minucci S, De Thé H. Activation of a promyelocytic leukemia-tumor protein 53 axis underlies acute promyelocytic leukemia cure. Nat Med 2014;20:167–74. https://doi.org/10.1038/NM.3441.

[108] Raelson J V., Nervi C, Rosenauer A, Benedetti L, Monczak Y, Pearson M, et al. The PML/RARα Oncoprotein Is a Direct Molecular Target of Retinoic Acid in Acute Promyelocytic Leukemia Cells. Blood 1996;88:2826–32. https://doi.org/10.1182/BLOOD.V88.8.2826.BLOODJOURNAL8882826.

[109] Zhu J, Gianni M, Kopf E, Honoré N, Chelbi-Alix M, Koken M, et al. Retinoic acid induces proteasome-dependent degradation of retinoic acid receptor alpha (RARalpha) and oncogenic RARalpha fusion proteins. Proc Natl Acad Sci U S A 1999;96:14807–12. https://doi.org/10.1073/PNAS.96.26.14807.

[110] Lallemand-Breitenbach V, Jeanne M, Benhenda S, Nasr R, Lei M, Peres L, et al. Arsenic degrades PML or PML-RARα through a SUMO-triggered RNF4/ ubiquitin-mediated pathway. Nat Cell Biol 2008;10:547–55. https://doi.org/10.1038/NCB1717.

[111] Goto E, Tomita A, Hayakawa F, Atsumi A, Kiyoi H, Naoe T. Missense mutations in PML-RARA are critical for the lack of responsiveness to arsenic trioxide treatment. Blood 2011;118:1600–9. https://doi.org/10.1182/BLOOD-2011-01-329433.

[112] Tomita A, Kiyoi H, Naoe T. Mechanisms of action and resistance to all-trans retinoic acid (ATRA) and arsenic trioxide (As2O3) in acute promyelocytic leukemia. Int J Hematol 2013;97:717–25. https://doi.org/10.1007/S12185-013-1354-4.

[113] Hattori H, Ishikawa Y, Kawashima N, Akashi A, Yamaguchi Y, Harada Y, et al. Identification of the novel deletion-type PML-RARA mutation associated with the retinoic acid resistance in acute promyelocytic leukemia. PLoS One 2018;13:e0204850. https://doi.org/10.1371/JOURNAL.PONE.0204850.

[114] Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, et al. Landscape of transcription in human cells. Nature 2012;489:101–8. https://doi.org/10.1038/NATURE11233.

[115] Katayama S, Tomaru Y, Kasukawa T, Waki K, Nakanishi M, Nakamura M, et al. Antisense transcription in the mammalian transcriptome. Science 2005;309:1564–6. https://doi.org/10.1126/SCIENCE.1112009.

[116] Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, et al. The Transcriptional Landscape of the Mammalian Genome. Science (1979) 2005;309:1559–63. https://doi.org/10.1126/SCIENCE.1112014.

[117] Mattick JS, Amaral PP, Carninci P, Carpenter S, Chang HY, Chen LL, et al. Long non-coding RNAs: definitions, functions, challenges and recommendations. Nat Rev Mol Cell Biol 2023;24:430–47. https://doi.org/10.1038/S41580-022-00566-8.

[118] Poliseno L, Lanza M, Pandolfi PP. Coding, or non-coding, that is the question. Cell Res 2024;34:609–29. https://doi.org/10.1038/S41422-024-00975-8.

[119] Statello L, Guo C-J, Chen L-L, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. Nat Rev Mol Cell Biol 2021;22:96–118. https://doi.org/10.1038/s41580-020-00315-9.

[120] Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet 2016;17:47–62. https://doi.org/10.1038/NRG.2015.10.

[121] Mercer TR, Dinger ME, Sunkin SM, Mehler MF, Mattick JS. Specific expression of long noncoding RNAs in the mouse brain. Proc Natl Acad Sci U S A 2008;105:716–21. https://doi.org/10.1073/PNAS.0706729105.

[122] Cabili M, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A, et al. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes Dev 2011;25:1915–27. https://doi.org/10.1101/GAD.17446611.

[123] Ravasi T, Suzuki H, Pang KC, Katayama S, Furuno M, Okunishi R, et al. Experimental validation of the regulated expression of large numbers of non-coding RNAs from the mouse genome. Genome Res 2006;16:11–9. https://doi.org/10.1101/GR.4200206.

[124] Ulitsky I, Bartel DP. lincRNAs: Genomics, Evolution, and Mechanisms. Cell 2013;154:26–46. https://doi.org/10.1016/J.CELL.2013.06.020.

[125] Fatica A, Bozzoni I. Long non-coding RNAs: New players in cell differentiation and development. Nat Rev Genet 2014;15:7–21. https://doi.org/10.1038/NRG3606.

[126] Kitagawa M, Kitagawa K, Kotake Y, Niida H, Ohhata T. Cell cycle regulation by long non-coding RNAs. Cellular and Molecular Life Sciences 2013;70:4785–94. https://doi.org/10.1007/S00018-013-1423-0.

[127] Jiang N, Zhang X, Gu X, Li X, Shang L. Progress in understanding the role of lncRNA in programmed cell death. Cell Death Discov 2021;7:1–11. https://doi.org/10.1038/S41420-021-00407-1.

[128] Rabajante JF, del Rosario RCH. Modeling long ncRNA-mediated regulation in the mammalian cell cycle. Methods in Molecular Biology 2019;1912:427–45. https://doi.org/10.1007/978-1-4939-8982-9_17.

[129] Brown JA, Bulkley D, Wang J, Valenstein ML, Yario TA, Steitz TA, et al. Structural insights into the stabilization of MALAT1 noncoding RNA by a bipartite triple helix. Nat Struct Mol Biol 2014;21:633–40. https://doi.org/10.1038/NSMB.2844.

[130] Uroda T, Anastasakou E, Rossi A, Teulon JM, Pellequer JL, Annibale P, et al. Conserved Pseudoknots in lncRNA MEG3 Are Essential for Stimulation of the p53 Pathway. Mol Cell 2019;75:982-995.e9. https://doi.org/10.1016/j.molcel.2019.07.025.

[131] Brannan CI, Dees EC, Ingram RS, Tilghman SM. The product of the H19 gene may function as an RNA. Mol Cell Biol 1990;10:28–36. https://doi.org/10.1128/MCB.10.1.28-36.1990.

[132] Brown CJ, Ballabio A, Rupert JL, Lafreniere RG, Grompe M, Tonlorenzi R, et al. A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. Nature 1991;349:38–44. https://doi.org/10.1038/349038A0.

[133] Takahashi H, Lassmann T, Murata M, Carninci P. 5′ end-centered expression profiling using Cap-analysis gene expression (CAGE) and next-generation sequencing. Nat Protoc 2012;7:542. https://doi.org/10.1038/NPROT.2012.005.

[134] Kapranov P, Drenkow J, Cheng J, Long J, Helt G, Dike S, et al. Examples of the complex architecture of the human transcriptome revealed by RACE and high-density tiling arrays. Genome Res 2005;15:987–97. https://doi.org/10.1101/GR.3455305.

[135] Bertone P, Stolc V, Royce TE, Rozowsky JS, Urban AE, Zhu X, et al. Global Identification of Human Transcribed Sequences with Genome Tiling Arrays. Science (1979) 2004;306:2242–6. https://doi.org/10.1126/SCIENCE.1103388.

[136] GENCODE - Human Release 49 n.d. https://www.gencodegenes.org/human/

[137] Hon C-C, Ramilowski JA, Harshbarger J, Bertin N, Rackham OJL, Gough J, et al. An atlas of human long non-coding RNAs with accurate 5′ ends. Nature 2017;543:199–204. https://doi.org/10.1038/nature21374.

[138] Zhao L, Wang J, Li Y, Song T, Wu Y, Fang S, et al. NONCODEV6: an updated database dedicated to long non-coding RNA annotation in both animals and plants. Nucleic Acids Res 2020;49:D165. https://doi.org/10.1093/NAR/GKAA1046.

[139] Ma L, Bajic VB, Zhang Z. On the classification of long non-coding RNAs. RNA Biol 2013;10:924–33. https://doi.org/10.4161/RNA.24604.

[140] Andersson R, Gebhard C, Miguel-Escalada I, Hoof I, Bornholdt J, Boyd M, et al. An atlas of active enhancers across human cell types and tissues. Nature 2014;507:455–61. https://doi.org/10.1038/NATURE12787.

[141] Kim TK, Hemberg M, Gray JM, Costa AM, Bear DM, Wu J, et al. Widespread transcription at neuronal activity-regulated enhancers. Nature 2010;465:182–7. https://doi.org/10.1038/NATURE09033..

[142] Jacq C, Miller JR, Brownlee GG. A pseudogene structure in 5S DNA of Xenopus laevis. Cell 1977;12:109–20. https://doi.org/10.1016/0092-8674(77)90189-1.

[143] Pisignano G, Michael DC, Visal TH, Pirlog R, Ladomery M, Calin GA. Going circular: history, present, and future of circRNAs in cancer. Oncogene 2023;42:2783–800. https://doi.org/10.1038/S41388-023-02780-W.

[144] Chen LL. The biogenesis and emerging roles of circular RNAs. Nat Rev Mol Cell Biol 2016;17:205–11. https://doi.org/10.1038/NRM.2015.32.

[145] Kaewsapsak P, Shechner DM, Mallard W, Rinn JL, Ting AY. Live-cell mapping of organelle-associated RNAs via proximity biotinylation combined with protein-RNA crosslinking. Elife 2017;6. https://doi.org/10.7554/ELIFE.29224.

[146] Fazal FM, Han S, Parker KR, Kaewsapsak P, Xu J, Boettiger AN, et al. Atlas of Subcellular RNA Localization Revealed by APEX-Seq. Cell 2019;178:473-490.e26. https://doi.org/10.1016/j.cell.2019.05.027.

[147] Cabili MN, Dunagin MC, McClanahan PD, Biaesch A, Padovan-Merhar O, Regev A, et al. Localization and abundance analysis of human lncRNAs at single-cell and single-molecule resolution. Genome Biol 2015;16:1–16. https://doi.org/10.1186/S13059-015-0586-4.

[148] Mercer TR, Neph S, Dinger ME, Crawford J, Smith MA, Shearwood AMJ, et al. The human mitochondrial transcriptome. Cell 2011;146:645–58. https://doi.org/10.1016/j.cell.2011.06.051.

[149] Chujo T, Yamazaki T, Hirose T. Architectural RNAs (arcRNAs): A class of long noncoding RNAs that function as the scaffold of nuclear bodies. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms 2016;1859:139–46. https://doi.org/10.1016/J.BBAGRM.2015.05.007.

[150] Ip JY, Nakagawa S. Long non-coding RNAs in nuclear bodies. Dev Growth Differ 2012;54:44–54. https://doi.org/10.1111/J.1440-169X.2011.01303.X.

[151] Dragomir M, Chen B, Calin GA. Exosomal lncRNAs as new players in cell-to-cell communication. Transl Cancer Res 2018;7:S243. https://doi.org/10.21037/TCR.2017.10.46.

[152] Hung T, Wang Y, Lin MF, Koegel AK, Kotake Y, Grant GD, et al. Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. Nat Genet 2011;43:621–9. https://doi.org/10.1038/NG.848.

[153] Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, et al. Functional Demarcation of Active and Silent Chromatin Domains in Human HOX Loci by Noncoding RNAs. Cell 2007;129:1311–23. https://doi.org/10.1016/j.cell.2007.05.022.

[154] Gala K, Khattar E. Long non-coding RNAs at work on telomeres: Functions and implications in cancer therapy. Cancer Lett 2021;502:120–32. https://doi.org/10.1016/j.canlet.2020.12.036.

[155] Clemson CM, Hutchinson JN, Sara SA, Ensminger AW, Fox AH, Chess A, et al. An Architectural Role for a Nuclear Noncoding RNA: NEAT1 RNA Is Essential

for the Structure of Paraspeckles. Mol Cell 2009;33:717–26. https://doi.org/10.1016/J.MOLCEL.2009.01.026.

[156] West JA, Mito M, Kurosaka S, Takumi T, Tanegashima C, Chujo T, et al. Structural, super-resolution microscopy analysis of paraspeckle nuclear body organization. Journal of Cell Biology 2016;214:817–30. https://doi.org/10.1083/JCB.201601071.

[157] Wilusz JE, JnBaptiste CK, Lu LY, Kuhn CD, Joshua-Tor L, Sharp PA. A triple helix stabilizes the 3′ ends of long noncoding RNAs that lack poly(A) tails. Genes Dev 2012;26:2392–407. https://doi.org/10.1101/GAD.204438.112.

[158] Hutchinson JN, Ensminger AW, Clemson CM, Lynch CR, Lawrence JB, Chess A. A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains. BMC Genomics 2007;8:1–16. https://doi.org/10.1186/1471-2164-8-39.

[159] Gong C, Maquat LE. LncRNAs transactivate STAU1-mediated mRNA decay by duplexing with 39 UTRs via Alu eleme. Nature 2011;470:284–90. https://doi.org/10.1038/NATURE09701.

[160] Kretz M, Siprashvili Z, Chu C, Webster DE, Zehnder A, Qu K, et al. Control of somatic tissue differentiation by the long non-coding RNA TINCR. Nature 2013;493:231–5. https://doi.org/10.1038/NATURE11661.

[161] Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. Nature 2014;505:344–52. https://doi.org/10.1038/NATURE12986.

[162] Thomson DW, Dinger ME. Endogenous microRNA sponges: Evidence and controversy. Nat Rev Genet 2016;17:272–83. https://doi.org/10.1038/NRG.2016.20.

[163] Poliseno L, Salmena L, Zhang J, Carver B, Haveman WJ, Pandolfi PP. A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. Nature 2010;465:1033. https://doi.org/10.1038/NATURE09144.

[164] Ghafouri-Fard S, Khoshbakht T, Hussen BM, Taheri M, Akbari Dilmaghani N. A review on the role of PTENP1 in human disorders with an especial focus

on tumor suppressor role of this lncRNA. Cancer Cell Int 2022;22:1–12. https://doi.org/10.1186/S12935-022-02625-8.

[165] Vendramin R, Verheyden Y, Ishikawa H, Goedert L, Nicolas E, Saraf K, et al. SAMMSON fosters cancer cell fitness by concertedly enhancing mitochondrial and cytosolic translation. Nat Struct Mol Biol 2018;25:1035–46. https://doi.org/10.1038/S41594-018-0143-4.

[166] Li D, Zhang J, Wang M, Li X, Gong H, Tang H, et al. Activity dependent LoNA regulates translation by coordinating rRNA transcription and methylation. Nat Commun 2018;9:1–14. https://doi.org/10.1038/S41467-018-04072-4.

[167] Hansji H, Leung EY, Baguley BC, Finlay GJ, Cameron-Smith D, Figueiredo VC, et al. ZFAS1: A long noncoding RNA associated with ribosomes in breast cancer cells. Biol Direct 2016;11:1–25. https://doi.org/10.1186/S13062-016-0165-Y.

[168] Chen L, Kong R, Wu C, Wang S, Liu Z, Liu S, et al. Circ-MALAT1 Functions as Both an mRNA Translation Brake and a microRNA Sponge to Promote Self-Renewal of Hepatocellular Cancer Stem Cells. Advanced Science 2020;7:2000890. https://doi.org/10.1002/ADVS.202000890.

[169] Shuai T, Khan MR, Zhang XD, Li J, Thorne RF, Wu M, et al. lncRNA TRMP-S directs dual mechanisms to regulate p27-mediated cellular senescence. Mol Ther Nucleic Acids 2021;24:971–85. https://doi.org/10.1016/J.OMTN.2021.04.004.

[170] Hu G, Lou Z, Gupta M. The Long Non-Coding RNA GAS5 Cooperates with the Eukaryotic Translation Initiation Factor 4E to Regulate c-Myc Translation. PLoS One 2014;9:e107016. https://doi.org/10.1371/JOURNAL.PONE.0107016.

[171] Ebralidze AK, Guibal FC, Steidl U, Zhang P, Lee S, Bartholdy B, et al. PU.1 expression is modulated by the balance of functional sense and antisense RNAs regulated by a shared cis-regulatory element. Genes Dev 2008;22:2085–92. https://doi.org/10.1101/GAD.1654808.

[172] Gumireddy K, Li A, Yan J, Setoyama T, Johannes GJ, Ørom UA, et al. Identification of a long non-coding RNA-associated RNP complex regulating metastasis at the translational step. EMBO J 2013;32:2672–84. https://doi.org/10.1038/EMBOJ.2013.188.

[173] Yoon JH, Abdelmohsen K, Kim J, Yang X, Martindale JL, Tominaga-Yamanaka K, et al. Scaffold function of long non-coding RNA HOTAIR in protein ubiquitination. Nat Commun 2013;4:1–14. https://doi.org/10.1038/NCOMMS3939.

[174] Liu C, Peng Z, Li P, Fu H, Feng J, Zhang Y, et al. lncRNA RMST Suppressed GBM Cell Mitophagy through Enhancing FUS SUMOylation. Mol Ther Nucleic Acids 2020;19:1198–208. https://doi.org/10.1016/J.OMTN.2020.01.008.

[175] Qin G, Tu X, Li H, Cao P, Chen X, Song J, et al. Long Noncoding RNA p53-Stabilizing and Activating RNA Promotes p53 Signaling by Inhibiting Heterogeneous Nuclear Ribonucleoprotein K deSUMOylation and Suppresses Hepatocellular Carcinoma. Hepatology 2020;71:112–29. https://doi.org/10.1002/HEP.30793.

[176] Liang X, Liu J, Liu X, Jin Y, Xu M, Han Z, et al. LINP1 represses unfolded protein response by directly inhibiting eIF2α phosphorylation to promote cutaneous squamous cell carcinoma. Exp Hematol Oncol 2023;12:1–24. https://doi.org/10.1186/S40164-023-00395-1.

[177] Sang L jie, Ju H qiang, Liu G ping, Tian T, Ma G lin, Lu Y xin, et al. LncRNA CamK-A Regulates Ca2+-Signaling-Mediated Tumor Microenvironment Remodeling. Mol Cell 2018;72:71-83.e7. https://doi.org/10.1016/J.MOLCEL.2018.08.014.

[178] Xu J, Lu Y, Liu Q, Xia A, Zhao J, Xu X, et al. Long noncoding RNA GMAN promotes hepatocellular carcinoma progression by interacting with eIF4B. Cancer Lett 2020;473:1–12. https://doi.org/10.1016/J.CANLET.2019.12.032.

[179] Lorenzi L, Avila Cobos F, Decock A, Everaert C, Helsmoortel H, Lefever S, et al. Long noncoding RNA expression profiling in cancer: Challenges and opportunities. Genes Chromosomes Cancer 2019;58:191–9. https://doi.org/10.1002/GCC.22709.

[180] Yan X, Hu Z, Feng Y, Hu X, Yuan J, Zhao SD, et al. Comprehensive Genomic Characterization of Long Non-coding RNAs across Human Cancers. Cancer Cell 2015;28:529–40. https://doi.org/10.1016/j.ccell.2015.09.006.

[181] Esposito R, Lanzós A, Uroda T, Ramnarayanan S, Büchi I, Polidori T, et al. Tumour mutations in long noncoding RNAs enhance cell fitness. Nature

Communications 2023 14:1 2023;14:1–21. https://doi.org/10.1038/s41467-023-39160-7.

[182] Gourvest M, Brousset P, Bousquet M. Long Noncoding RNAs in Acute Myeloid Leukemia: Functional Characterization and Clinical Relevance. Cancers (Basel) 2019;11:1638. https://doi.org/10.3390/cancers11111638.

[183] Ng M, Heckl D, Klusmann JH. The Regulatory Roles of Long Noncoding RNAs in Acute Myeloid Leukemia. Front Oncol 2019;9:570. https://doi.org/10.3389/FONC.2019.00570.

[184] Hu N, Chen L, Wang C, Zhao H. MALAT1 knockdown inhibits proliferation and enhances cytarabine chemosensitivity by upregulating miR-96 in acute myeloid leukemia cells. Biomedicine & Pharmacotherapy 2019;112:108720. https://doi.org/10.1016/j.biopha.2019.108720.

[185] Li C, Gao Q, Wang M, Xin H. LncRNA SNHG1 contributes to the regulation of acute myeloid leukemia cell growth by modulating miR-489-3p/SOX12/Wnt/β-catenin signaling. J Cell Physiol 2021;236:653–63. https://doi.org/10.1002/JCP.29892,.

[186] Li J, Wang M, Chen X. Long non-coding RNA UCA1 modulates cell proliferation and apoptosis by regulating miR-296-3p/Myc axis in acute myeloid leukemia. Cell Cycle 2020;19:1454–65. https://doi.org/10.1080/15384101.2020.1750814,.

[187] Sheng XF, Hong LL, Li H, Huang FY, Wen Q, Zhuang HF. Long non-coding RNA MALAT1 modulate cell migration, proliferation and apoptosis by sponging microRNA-146a to regulate CXCR4 expression in acute myeloid leukemia. Hematology (United Kingdom) 2021;26:43–52. https://doi.org/10.1080/16078454.2020.1867781,.

[188] Zhang T juan, Zhou J dong, Zhang W, Lin J, Ma J chun, Wen X mei, et al. H19 overexpression promotes leukemogenesis and predicts unfavorable prognosis in acute myeloid leukemia. Clin Epigenetics 2018;10:1–12. https://doi.org/10.1186/S13148-018-0486-Z.

[189] Zhao TT, Liu X. LncRNA-H19 inhibits apoptosis of acute myeloid leukemia cells via targeting miR-29a-3p. Eur Rev Med Pharmacol Sci 2019;23:224–31. https://doi.org/10.26355/EURREV_201908_18651,.

[190] Wang CH, Li QY, Nie L, Ma J, Yao CJ, Chen FP. LncRNA ANRIL promotes cell proliferation, migration and invasion during acute myeloid leukemia pathogenesis via negatively regulating miR-34a. Int J Biochem Cell Biol 2020;119:105666. https://doi.org/10.1016/J.BIOCEL.2019.105666.

[191] Sun LY, Li XJ, Sun YM, Huang W, Fang K, Han C, et al. LncRNA ANRIL regulates AML development through modulating the glucose metabolism pathway of AdipoR1/AMPK/SIRT1. Mol Cancer 2018;17. https://doi.org/10.1186/S12943-018-0879-9.

[192] Wu S, Zheng C, Chen S, Cai X, Shi Y, Lin B, et al. Overexpression of long non-coding RNA HOTAIR predicts a poor prognosis in patients with acute myeloid leukemia. Oncol Lett 2015;10:2410–4. https://doi.org/10.3892/OL.2015.3552.

[193] Xing C yun, Hu X qu, Xie F yan, Yu Z jie, Li H ying, Bin-Zhou, et al. Long non-coding RNA HOTAIR modulates c-KIT expression through sponging miR-193a in acute myeloid leukemia. FEBS Lett 2015;589:1981–7. https://doi.org/10.1016/J.FEBSLET.2015.04.061.

[194] Gao S, Zhou B, Li H, Huang X, Wu Y, Xing C, et al. Long noncoding RNA HOTAIR promotes the self-renewal of leukemia stem cells through epigenetic silencing of p15. Exp Hematol 2018;67:32-40.e3. https://doi.org/10.1016/j.exphem.2018.08.005.

[195] Wang H, Li W, Guo R, Sun J, Cui J, Wang G, et al. An intragenic long noncoding RNA interacts epigenetically with the RUNX1 promoter and enhancer chromatin DNA in hematopoietic malignancies. Int J Cancer 2014;135:2783–94. https://doi.org/10.1002/IJC.28922.

[196] El-Khazragy N, Elayat W, Matbouly S, Seliman S, Sami A, Safwat G, et al. The prognostic significance of the long non-coding RNAs "CCAT1, PVT1" in t(8;21) associated Acute Myeloid Leukemia. Gene 2019;707:172–7. https://doi.org/10.1016/J.GENE.2019.03.055.

[197] Pashaiefar H, Izadifard M, Yaghmaie M, Montazeri M, Gheisari E, Ahmadvand M, et al. Low Expression of Long Noncoding RNA IRAIN Is Associated with Poor Prognosis in Non-M3 Acute Myeloid Leukemia Patients. Genet Test Mol Biomarkers 2018;22:288–94.

https://doi.org/10.1089/GTMB.2017.0281/ASSET/IMAGES/LARGE/FIGURE3.JPEG.

[198] Sun J, Li W, Sun Y, Yu D, Wen X, Wang H, et al. A novel antisense long noncoding RNA within the IGF1R gene locus is imprinted in hematopoietic malignancies. Nucleic Acids Res 2014;42:9588–601. https://doi.org/10.1093/NAR/GKU549.

[199] Jia Y, He E, Peng J, Zhao M. Decrease of lncRNA MEG3 in Acute Myeloid Leukemia Affected the Expression of p53 and MDM2. Clin Lab 2023;69:1902–7. https://doi.org/10.7754/CLIN.LAB.2023.230301.

[200] Lyu Y, Lou J, Yang Y, Feng J, Hao Y, Huang S, et al. Dysfunction of the WT1-MEG3 signaling promotes AML leukemogenesis via p53-dependent and -independent pathways. Leukemia 2017;31:2543–51. https://doi.org/10.1038/LEU.2017.116.

[201] Sellers ZP, Bolkun L, Kloczko J, Wojtaszewska ML, Lewandowski K, Moniuszko M, et al. Increased methylation upstream of the MEG3 promotor is observed in acute myeloid leukemia patients with better overall survival. Clin Epigenetics 2019;11:1–15. https://doi.org/10.1186/S13148-019-0643-Z.

[202] Ghaforui-Fard S, Taheri M. Nuclear Enriched Abundant Transcript 1 (NEAT1): A long non-coding RNA with diverse functions in tumorigenesis. Biomedicine and Pharmacotherapy 2019;111:51–9. https://doi.org/10.1016/J.BIOPHA.2018.12.070.

[203] Tang D, Hu P, Zhu D, Luo Y, Chen M, Zhang G, et al. C/EBPα is indispensable for PML/RARα-mediated suppression of long non-coding RNA NEAT1 in acute promyelocytic leukemia cells. Aging 2021;13:13179–94. https://doi.org/10.18632/aging.203000.

[204] Tang D, Wang H, Jiang Y, Chen M, Zhang G, Wu S, et al. ATRA-induced NEAT1 upregulation promotes autophagy during APL cell granulocytic differentiation. PLoS One 2024;19. https://doi.org/10.1371/JOURNAL.PONE.0316109.

[205] Zeng C, Xu Y, Xu L, Yu X, Cheng J, Yang L, et al. Inhibition of long non-coding RNA NEAT1 impairs myeloid differentiation in acute promyelocytic leukemia cells. BMC Cancer 2014;14:693. https://doi.org/10.1186/1471-2407-14-693.

[206] Yan H, Wang Z, Sun Y, Hu L, Bu P. Cytoplasmic NEAT1 Suppresses AML Stem Cell Self-Renewal and Leukemogenesis through Inactivation of Wnt Signaling. Advanced Science 2021;8:2100914. https://doi.org/10.1002/ADVS.202100914.

[207] Fernando TR, Contreras JR, Zampini M, Rodriguez-Malave NI, Alberti MO, Anguiano J, et al. The lncRNA CASC15 regulates SOX4 expression in RUNX1-rearranged acute leukemia. Mol Cancer 2017;16. https://doi.org/10.1186/S12943-017-0692-X.

[208] Pavlovic D, Tosic N, Zukic B, Pravdic Z, Vukovic NS, Pavlovic S, et al. Expression Profiles of Long Non-Coding RNA GAS5 and MicroRNA-222 in Younger AML Patients. Diagnostics 2021;12:86. https://doi.org/10.3390/DIAGNOSTICS12010086.

[209] Zhang H, Liu L, Chen L, Liu H, Ren S, Tao Y. Long noncoding RNA DANCR confers cytarabine resistance in acute myeloid leukemia by activating autophagy via the miR-874-3P/ATG16L1 axis. Mol Oncol 2021;15:1203–16. https://doi.org/10.1002/1878-0261.12661.

[210] Chen L, Hu N, Wang C, Zhao H. HOTAIRM1 knockdown enhances cytarabine-induced cytotoxicity by suppression of glycolysis through the Wnt/β-catenin/PFKP pathway in acute myeloid leukemia cells. Arch Biochem Biophys 2020;680. https://doi.org/10.1016/j.abb.2019.108244.

[211] Bester AC, Lee JD, Chavez A, Lee YR, Nachmani D, Vora S, et al. An Integrated Genome-wide CRISPRa Approach to Functionalize lncRNAs in Drug Resistance. Cell 2018;173:649-664.e20. https://doi.org/10.1016/J.CELL.2018.03.052.

[212] Wang C, Li L, Li M, Wang W, Liu Y, Wang S. Silencing long non-coding RNA XIST suppresses drug resistance in acute myeloid leukemia through down-regulation of MYC by elevating microRNA-29a expression. Molecular Medicine 2020;26:1–11. https://doi.org/10.1186/S10020-020-00229-4.

[213] Yu Y, Kou D, Liu B, Huang Y, Li S, Qi Y, et al. LncRNA MEG3 contributes to drug resistance in acute myeloid leukemia by positively regulating ALG9 through sponging miR-155. Int J Lab Hematol 2020;42:464–72. https://doi.org/10.1111/ijlh.13225.

[214] Yao Q, Zhang L, Wang Y, Liu J, Yang L, Wang Y. LncRNA UCA1 elevates the resistance of human leukemia cells to daunorubicin by the PI3K/AKT pathway via sponging miR-613. Biosci Rep 2021;41. https://doi.org/10.1042/BSR20201389/228611.

[215] Zhou H, Zhang Q, Huang W, He C, Zhou C, Zhou J, et al. Epigenetic silencing of ZCCHC10 by the lncRNA SNHG1 promotes progression and venetoclax resistance of acute myeloid leukemia. Int J Oncol 2023;62. https://doi.org/10.3892/IJO.2023.5512.

[216] Connerty P, Moles E, de Bock CE, Jayatilleke N, Smith JL, Meshinchi S, et al. Development of siRNA-loaded lipid nanoparticles targeting long non-coding RNA LINC01257 as a novel and safe therapeutic approach for t(8;21) pediatric acute myeloid leukemia. Pharmaceutics 2021;13:1681. https://doi.org/10.3390/PHARMACEUTICS13101681/S1.

[217] Winkle M, El-Daly SM, Fabbri M, Calin GA. Noncoding RNA therapeutics — challenges and potential solutions. Nature Reviews Drug Discovery 2021 20:8 2021;20:629–51. https://doi.org/10.1038/s41573-021-00219-z.

[218] Connerty P, Lock RB. The tip of the iceberg—The roles of long noncoding RNAs in acute myeloid leukemia. Wiley Interdiscip Rev RNA 2023;14:e1796. https://doi.org/10.1002/WRNA.1796.

[219] Wang Y, Li Y, Song HQ, Sun GW. Long non-coding RNA LINC00899 as a novel serum biomarker for diagnosis and prognosis prediction of acute myeloid leukemia. Eur Rev Med Pharmacol Sci 2018;22:7364–70. https://doi.org/10.26355/EURREV_201811_16274.

[220] Yang L, Zhou JD, Zhang TJ, Ma JC, Xiao GF, Chen Q, et al. Overexpression of lncRNA PANDAR predicts adverse prognosis in acute myeloid leukemia. Cancer Manag Res 2018;10:4999–5007. https://doi.org/10.2147/CMAR.S180150.

[221] Mer AS, Lindberg J, Nilsson C, Klevebring D, Wang M, Grönberg H, et al. Expression levels of long non-coding RNAs are prognostic for AML outcome. J Hematol Oncol 2018;11:52. https://doi.org/10.1186/s13045-018-0596-2.

[222] Audic Y, Hartley RS. Post-transcriptional regulation in cancer. Biol Cell 2004;96:479–98. https://doi.org/10.1016/j.biolcel.2004.05.002.

[223] DeOcesano-Pereira C, Velloso FJ, Carreira ACO, Ribeiro CSP, Winnischofer SMB, Sogayar MC, et al. Post-Transcriptional Control of RNA Expression in Cancer. Gene Expression and Regulation in Mammalian Cells - Transcription From General Aspects 2018. https://doi.org/10.5772/INTECHOPEN.71861.

[224] Bourgeois CF, Mortreux F, Auboeuf D. The multiple functions of RNA helicases as drivers and regulators of gene expression. Nat Rev Mol Cell Biol 2016;17:426–38. https://doi.org/10.1038/NRM.2016.50.

[225] Fan J, Li Z, Pei L, Hou Y. Post-transcriptional regulation of DEAD-box RNA helicases in hematopoietic malignancies. Genes Dis 2024;11. https://doi.org/10.1016/J.GENDIS.2024.101252.

[226] Rocak S, Linder P. DEAD-box proteins: the driving forces behind RNA metabolism. Nature Reviews Molecular Cell Biology 2004 5:3 2004;5:232–41. https://doi.org/10.1038/nrm1335.

[227] Mazurek A, Park Y, Miething C, Wilkinson JE, Gillis J, Lowe SW, et al. Acquired dependence of acute myeloid leukemia on the DEAD-box RNA helicase DDX5. Cell Rep 2014;7:1887–99. https://doi.org/10.1016/j.celrep.2014.05.019.

[228] Polprasert C, Schulze I, Sekeres MA, Makishima H, Przychodzen B, Hosono N, et al. Inherited and Somatic Defects in DDX41 in Myeloid Neoplasms. Cancer Cell 2015;27:658–70. https://doi.org/10.1016/j.ccell.2015.03.017.

[229] Holzmann K, Gerner C, Pöltl A, Schäfer R, Obrist P, Ensinger C, et al. A human common nuclear matrix protein homologous to eukaryotic translation initiation factor 4A. Biochem Biophys Res Commun 2000;267:339–44. https://doi.org/10.1006/bbrc.1999.1973.

[230] Yang M, Hu H, Wu S, Ding J, Yin B, Huang B, et al. EIF4A3-regulated circ_0087429 can reverse EMT and inhibit the progression of cervical cancer via miR-5003-3p-dependent upregulation of OGN expression. Journal of Experimental and Clinical Cancer Research 2022;41:1–20. https://doi.org/10.1186/S13046-022-02368-4

[231] Liu Q, Dong H. EIF4A3-mediated hsa_circ_0088088 promotes the carcinogenesis of breast cancer by sponging miR-135-5p. J Biochem Mol Toxicol 2021;35:e22909. https://doi.org/10.1002/JBT.22909;WGROUP:STRING:PUBLICATION.

[232] López-Cánovas JL, Hermán-Sánchez N, Moreno-Montilla MT, Del Rio-Moreno M, Alors-Perez E, Sánchez-Frias ME, et al. Spliceosomal profiling identifies EIF4A3 as a novel oncogene in hepatocellular carcinoma acting through the modulation of FGFR4 splicing. Clin Transl Med 2022;12:e1102. https://doi.org/10.1002/CTM2.1102.

[233] Blázquez-Encinas R, Alors-Pérez E, Moreno-Montilla MT, García-Vioque V, Sánchez-Frías ME, Mafficini A, et al. The Exon Junction Complex component EIF4A3 plays a splicing-linked oncogenic role in pancreatic ductal adenocarcinoma. Cancer Gene Therapy 2024 31:11 2024;31:1646–57. https://doi.org/10.1038/s41417-024-00814-3.

[234] Xue C, Gu X, Li G, Bao Z, Li L. Expression and Functional Roles of Eukaryotic Initiation Factor 4A Family Proteins in Human Cancers. Front Cell Dev Biol 2021;9:711965. https://doi.org/10.3389/FCELL.2021.711965.

[235] Le Hir H, Izaurralde E, Maquat LE, Moore MJ. The spliceosome deposits multiple proteins 20-24 nucleotides upstream of mRNA exon-exon junctions. EMBO J 2000;19:6860–9. https://doi.org/10.1093/EMBOJ/19.24.6860.

[236] Shibuya T, Tange T, Sonenberg N, Moore MJ. eIF4AIII binds spliced mRNA in the exon junction complex and is essential for nonsense-mediated decay. Nature Structural & Molecular Biology 2004 11:4 2004;11:346–51. https://doi.org/10.1038/nsmb750.

[237] Tange T, Shibuya T, Jurica MS, Moore MJ. Biochemical analysis of the EJC reveals two new factors and a stable tetrameric protein core. RNA 2005;11:1869–83. https://doi.org/10.1261/RNA.2155905.

[238] Kanellis DC, Espinoza JA, Zisi A, Sakkas E, Bartkova J, Katsori AM, et al. The exon-junction complex helicase eIF4A3 controls cell fate via coordinated regulation of ribosome biogenesis and translational output. Sci Adv 2021;7:eabf7561. https://doi.org/10.1126/SCIADV.ABF7561.

[239] Mazloomian A, Araki S, Ohori M, El-Naggar AM, Yap D, Bashashati A, et al. Pharmacological systems analysis defines EIF4A3 functions in cell-cycle and RNA stress granule formation. Commun Biol 2019;2:1–15. https://doi.org/10.1038/S42003-019-0391-9.

[240] Lin Y, Zhang J, Cai J, Liang R, Chen G, Qin G, et al. Systematic Analysis of Gene Expression Alteration and Co-Expression Network of Eukaryotic Initiation Factor 4A-3 in Cancer. J Cancer 2018;9:4568–77. https://doi.org/10.7150/JCA.27655.

[241] Yan H, Zhang L, Li R. Identification of m6A suppressor EIF4A3 as a novel cancer prognostic and immunotherapy biomarker through bladder cancer clinical data validation and pan-cancer analysis. Sci Rep 2023;13:1–17. https://doi.org/10.1038/S41598-023-43500-4.

[242] Wei Y, Lu C, Zhou P, Zhao L, Lyu X, Yin J, et al. EIF4A3-induced circular RNA ASAP1 promotes tumorigenesis and temozolomide resistance of glioblastoma via NRAS/MEK1/ERK1-2 signaling. Neuro Oncol 2021;23:611–24. https://doi.org/10.1093/NEUONC/NOAA214.

[243] Xu Q, Zhao T, Han H, Fan J, Xie W. EIF4A3 stabilizes the expression of lncRNA AGAP2-AS1 to activate cancer-associated fibroblasts via MyD88/NF-κb signaling. Thorac Cancer 2023;14:450–61. https://doi.org/10.1111/1759-7714.14762.

[244] Bai SS, Yan LW, Liu CH. Promotive role of eukaryotic translation initiation factor 4A isoform 3 in ovarian cancer cell growth and aerobic glycolysis through the pyruvate dehydrogenase kinase 4 signaling. Kaohsiung J Med Sci 2023;39:478–88. https://doi.org/10.1002/KJM2.12690.

[245] Yang H, Yang W, Dai W, Ma Y, Zhang G. LINC00667 promotes the proliferation, migration, and pathological angiogenesis in non-small cell lung cancer through stabilizing VEGFA by EIF4A3. Cell Biol Int 2020;44:1671–80. https://doi.org/10.1002/CBIN.11361.

[246] Ito M, Tanaka T, Cary DR, Iwatani-Yoshihara M, Kamada Y, Kawamoto T, et al. Discovery of Novel 1,4-Diacylpiperazines as Selective and Cell-Active eIF4A3 Inhibitors. J Med Chem 2017;60:3335–51. https://doi.org/10.1021/ACS.JMEDCHEM.6B01904.

[247] Mizojiri R, Nakata D, Satoh Y, Morishita D, Shibata S, Iwatani-Yoshihara M, et al. Discovery of Novel 5-(Piperazine-1-carbonyl)pyridin-2(1 H)-one Derivatives as Orally eIF4A3-Selective Inhibitors. ACS Med Chem Lett 2017;8:1077–82. https://doi.org/10.1021/ACSMEDCHEMLETT.7B00283.

[248] Wang M, Lindberg J, Klevebring D, Nilsson C, Mer AS, Rantalainen M, et al. Validation of risk stratification models in acute myeloid leukemia using sequencing-based molecular profiling. Leukemia 2017;31:2029–36. https://doi.org/10.1038/leu.2017.48.

[249] Wang M, Lindberg J, Klevebring D, Nilsson C, Lehmann S, Grönberg H, et al. Development and Validation of a Novel RNA Sequencing-Based Prognostic Score for Acute Myeloid Leukemia. J Natl Cancer Inst 2018;110:1094–101. https://doi.org/10.1093/jnci/djy021.

[250] Juliusson G, Lazarevic V, Hörstedt A-S, Hagberg O, Höglund M, Group SALR. Acute myeloid leukemia in the real world: why population-based registries are needed. Blood 2012;119:3890–9. https://doi.org/10.1182/blood-2011-12-379008.

[251] Hutter C, Zenklusen JC. The Cancer Genome Atlas: Creating Lasting Value beyond Its Data. Cell 2018;173:283–5. https://doi.org/10.1016/j.cell.2018.03.042.

[252] Wang Z, Jensen MA, Zenklusen JC. A practical guide to The Cancer Genome Atlas (TCGA). Methods in Molecular Biology 2016;1418:111–41. https://doi.org/10.1007/978-1-4939-3578-9_6.

[253] Tsherniak A, Vazquez F, Montgomery PG, Weir BA, Kryukov G, Cowley GS, et al. Defining a Cancer Dependency Map. Cell 2017;170:564-576.e16. https://doi.org/10.1016/j.cell.2017.06.010.

[254] Tyner JW, Tognon CE, Bottomly D, Wilmot B, Kurtz SE, Savage SL, et al. Functional genomic landscape of acute myeloid leukaemia. Nature 2018;562:526–31. https://doi.org/10.1038/S41586-018-0623-Z.

[255] Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method. Methods 2001;25:402–8. https://doi.org/10.1006/meth.2001.1262.

[256] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 2000;25:25–9. https://doi.org/10.1038/75556.

[257] Croft D, O'Kelly G, Wu G, Haw R, Gillespie M, Matthews L, et al. Reactome: a database of reactions, pathways and biological processes. Nucleic Acids Res 2010;39:D691. https://doi.org/10.1093/NAR/GKQ1018.

[258] Shiraki T, Kondo S, Katayama S, Waki K, Kasukawa T, Kawaji H, et al. Cap analysis gene expression for high-throughput analysis of transcriptional starting point and identification of promoter usage. Proceedings of the National Academy of Sciences 2003;100:15776–81. https://doi.org/10.1073/PNAS.2136655100.

[259] Takahashi H, Kato S, Murata M, Carninci P. CAGE (cap analysis of gene expression): a protocol for the detection of promoter and transcriptional networks. Methods Mol Biol 2012;786:181–200. https://doi.org/10.1007/978-1-61779-292-2_11.

[260] Kanamori-Katayama M, Itoh M, Kawaji H, Lassmann T, Katayama S, Kojima M, et al. Unamplified cap analysis of gene expression on a single-molecule sequencer. Genome Res 2011;21:1150–9. https://doi.org/10.1101/GR.115469.110.

[261] Gilbert LA, Horlbeck MA, Adamson B, Villalta JE, Chen Y, Whitehead EH, et al. Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation. Cell 2014;159:647–61. https://doi.org/10.1016/j.cell.2014.09.029.

[262] Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 2012;337:816–21. https://doi.org/10.1126/SCIENCE.1225829.

[263] Margolin JF, Friedman JR, Meyer WKH, Vissing H, Thiesen HJ, Rauscher FJ. Krüppel-associated boxes are potent transcriptional repression domains. Proceedings of the National Academy of Sciences 1994;91:4509–13. https://doi.org/10.1073/PNAS.91.10.4509.

[264] Montgomery MK, Xu S, Fire A. RNA as a target of double-stranded RNA-mediated genetic interference in Caenorhabditis elegans. Proceedings of the National Academy of Sciences 1998;95:15502–7. https://doi.org/10.1073/PNAS.95.26.15502.

[265] Sharp PA. RNA interference—2001. Genes Dev 2001;15:485–90. https://doi.org/10.1101/GAD.880001.

[266] Nishikura K. A short primer on RNAi: RNA-directed RNA polymerase acts as a key catalyst. Cell 2001;107:415–8. https://doi.org/10.1016/S0092-8674(01)00581-5.

[267] Hu B, Zhong L, Weng Y, Peng L, Huang Y, Zhao Y, et al. Therapeutic siRNA: state of the art. Signal Transduct Target Ther 2020;5:1–25. https://doi.org/10.1038/S41392-020-0207-X.

[268] Birmingham A, Anderson EM, Reynolds A, Ilsley-Tyree D, Leake D, Fedorov Y, et al. 3′ UTR seed matches, but not overall identity, are associated with RNAi off-targets. Nat Methods 2006;3:199–204. https://doi.org/10.1038/NMETH854.

[269] Jackson AL, Burchard J, Leake D, Reynolds A, Schelter J, Guo J, et al. Position-specific chemical modification of siRNAs reduces "off-target" transcript silencing. RNA 2006;12:1197–205. https://doi.org/10.1261/RNA.30706.

[270] Anderson EM, Birmingham A, Baskerville S, Reynolds A, Maksimova E, Leake D, et al. Experimental validation of the importance of seed complement frequency to siRNA specificity. RNA 2008;14:853–61. https://doi.org/10.1261/RNA.704708.

[271] Chu C, Quinn J, Chang HY. Chromatin isolation by RNA purification (ChIRP). J Vis Exp 2012. https://doi.org/10.3791/3912.

[272] Cory AH, Owen TC, Barltrop JA, Cory JG. Use of an aqueous soluble tetrazolium/formazan assay for cell growth assays in culture. Cancer Commun 1991;3:207–12. https://doi.org/10.3727/095535491820873191.

[273] Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, Worzella TJ, et al. Cell Viability Assays. Assay Guidance Manual 2016.

[274] Vermes I, Haanen C, Steffens-Nakken H, Reutellingsperger C. A novel assay for apoptosis Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. J Immunol Methods 1995;184:39–51. https://doi.org/10.1016/0022-1759(95)00072-I.

[275] Riccardi C, Nicoletti I. Analysis of apoptosis by propidium iodide staining and flow cytometry. Nat Protoc 2006;1:1458–61. https://doi.org/10.1038/NPROT.2006.238.

[276] Crowley LC, Scott AP, Marfell BJ, Boughaba JA, Chojnowski G, Waterhouse NJ. Measuring Cell Death by Propidium Iodide Uptake and Flow Cytometry. Cold Spring Harb Protoc 2016;2016:647–51. https://doi.org/10.1101/PDB.PROT087163.

[277] Pozarowski P, Darzynkiewicz Z. Analysis of cell cycle by flow cytometry. Methods Mol Biol 2004;281:301–11. https://doi.org/10.1385/1-59259-811-0:301.

[278] Schmitter T, Agerer F, Peterson L, Münzner P, Hauck CR. Granulocyte CEACAM3 Is a Phagocytic Receptor of the Innate Immune System that Mediates Recognition and Elimination of Human-specific Pathogens. Journal of Experimental Medicine 2004;199:35–46. https://doi.org/10.1084/JEM.20030204.

[279] Adrian J, Bonsignore P, Hammer S, Frickey T, Hauck CR. Adaptation to Host-Specific Bacterial Pathogens Drives Rapid Evolution of a Human Innate Immune Receptor. Current Biology 2019;29:616-630.e5. https://doi.org/10.1016/j.cub.2019.01.058.

[280] Wunderlich M, Chou F-S, Mizukawa B, Link KA, Mulloy JC. A New Immunodeficient Mouse Strain, NOD/SCID IL2Rγ-/- SGM3, Promotes Enhanced Human Hematopoietic Cell Xenografts with a Robust T Cell Component. Blood 2009;114:3524–3524. https://doi.org/10.1182/BLOOD.V114.22.3524.3524.

[281] Shultz LD, Lyons BL, Burzenski LM, Gott B, Chen X, Chaleff S, et al. Human Lymphoid and Myeloid Cell Development in NOD/LtSz-scid IL2Rγnull Mice Engrafted with Mobilized Human Hemopoietic Stem Cells,. The Journal of Immunology 2005;174:6477–89. https://doi.org/10.4049/JIMMUNOL.174.10.6477.

[282] Ishikawa F, Yasukawa M, Lyons B, Yoshida S, Miyamoto T, Yoshimoto G, et al. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor γ chainnull mice. Blood 2005;106:1565–73. https://doi.org/10.1182/BLOOD-2005-02-0516.

[283] Ito M, Hiramatsu H, Kobayashi K, Suzue K, Kawahata M, Hioki K, et al. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for

engraftment of human cells. Blood 2002;100:3175–82. https://doi.org/10.1182/BLOOD-2001-12-0207.

[284] Nicolini FE, Cashman JD, Hogge DE, Humphries RK, Eaves CJ. NOD/SCID mice engineered to express human IL-3, GM-CSF and steel factor constitutively mobilize engrafted human progenitors and compromise human stem cell regeneration. Leukemia 2004;18:341–7. https://doi.org/10.1038/SJ.LEU.2403222.

[285] Qin Z, Ren F, Xu X, Ren Y, Li H, Wang Y, et al. ZNF536, a novel zinc finger protein specifically expressed in the brain, negatively regulates neuron differentiation by repressing retinoic acid-induced gene transcription. Mol Cell Biol 2009;29:3633–43. https://doi.org/10.1128/MCB.00362-09.

[286] Billings SE, Pierzchalski K, Butler Tjaden NE, Pang X-Y, Trainor PA, Kane MA, et al. The retinaldehyde reductase DHRS3 is essential for preventing the formation of excess retinoic acid during embryonic development. FASEB J 2013;27:4877–89. https://doi.org/10.1096/fj.13-227967.

[287] Zolfaghari R, Chen Q, Ross AC. DHRS3, a retinal reductase, is differentially regulated by retinoic acid and lipopolysaccharide-induced inflammation in THP-1 cells and rat liver. Am J Physiol Gastrointest Liver Physiol 2012;303:G578–88. https://doi.org/10.1152/ajpgi.00234.2012.

[288] Adams MK, Belyaeva O V, Wu L, Kedishvili NY. The retinaldehyde reductase activity of DHRS3 is reciprocally activated by retinol dehydrogenase 10 to control retinoid homeostasis. J Biol Chem 2014;289:14868–80. https://doi.org/10.1074/jbc.M114.552257.

[289] Albanesi J, Noguera NI, Banella C, Colangelo T, De Marinis E, Leone S, et al. Transcriptional and Metabolic Dissection of ATRA-Induced Granulocytic Differentiation in NB4 Acute Promyelocytic Leukemia Cells. Cells 2020;9:1–25. https://doi.org/10.3390/CELLS9112423.

[290] Gianni M, Terao M, Fortino I, Licalzi M, Viggiano V, Barbui T, et al. Stat1 Is Induced and Activated by All-Trans Retinoic Acid in Acute Promyelocytic Leukemia Cells dated. On ligand binding, both type I and type II IFN recep n.d.

[291] Leid M, Kastner P, Chambon P. Multiplicity generates diversity in the retinoic acid signalling pathways. Trends Biochem Sci 1992;17:427–33. https://doi.org/10.1016/0968-0004(92)90014-Z.

[292] Gertz J, Savic D, Varley KE, Partridge EC, Safi A, Jain P, et al. Distinct properties of cell-type-specific and shared transcription factor binding sites. Mol Cell 2013;52:25–36. https://doi.org/10.1016/j.molcel.2013.08.037.

[293] Grimwade D, Ivey A, Huntly BJP. Molecular landscape of acute myeloid leukemia in younger adults and its clinical relevance. Blood 2016;127:29–41. https://doi.org/10.1182/blood-2015-07-604496.

[294] Huang H-H, Chen F-Y, Chou W-C, Hou H-A, Ko B-S, Lin C-T, et al. Long non-coding RNA HOXB-AS3 promotes myeloid cell proliferation and its higher expression is an adverse prognostic marker in patients with acute myeloid leukemia and myelodysplastic syndrome. BMC Cancer 2019;19:617. https://doi.org/10.1186/s12885-019-5822-y.

[295] De Clara E, Gourvest M, Ma H, Vergez F, Tosolini M, Dejean S, et al. Long non-coding RNA expression profile in cytogenetically normal acute myeloid leukemia identifies a distinct signature and a new biomarker in NPM1-mutated patients. Haematologica 2017;102:1718–26. https://doi.org/10.3324/haematol.2017.171645.

[296] Zhang R, Huo C-H. Long Noncoding RNA SOCS2-AS Promotes Leukemogenesis in FLT3-ITD+ Acute Myeloid Leukemia Through miRNA-221. Onco Targets Ther 2020;13:2925–34. https://doi.org/10.2147/OTT.S222734.

[297] Mason EF, Kuo FC, Hasserjian RP, Seegmiller AC, Pozdnyakova O. A distinct immunophenotype identifies a subset of NPM1-mutated AML with TET2 or IDH1/2 mutations and improved outcome. Am J Hematol 2018;93:504–10. https://doi.org/10.1002/AJH.25018.

[298] Mason EF, Hasserjian RP, Aggarwal N, Seegmiller AC, Pozdnyakova O. Blast phenotype and comutations in acute myeloid leukemia with mutated NPM1 influence disease biology and outcome. Blood Adv 2019;3:3322. https://doi.org/10.1182/BLOODADVANCES.2019000328.

[299] Ma X, Zhang W, Zhao M, Li S, Jin W, Wang K. Oncogenic role of lncRNA CRNDE in acute promyelocytic leukemia and NPM1-mutant acute myeloid

leukemia. Cell Death Discov 2020;6:121. https://doi.org/10.1038/S41420-020-00359-Y.

[300] Carramusa L, Contino F, Ferro A, Minafra L, Perconti G, Giallongo A, et al. The PVT-1 oncogene is a Myc protein target that is overexpressed in transformed cells. J Cell Physiol 2007;213:511–8. https://doi.org/10.1002/JCP.21133.

[301] Wang C, Chen F, Fan Z, Yao C, Xiao L. lncRNA CCAT1/miR-490-3p/MAPK1/c-Myc positive feedback loop drives progression of acute myeloid leukaemia. J Biochem 2020;167:379–88. https://doi.org/10.1093/JB/MVZ107.

[302] Renneville A, Roumier C, Biggio V, Nibourel O, Boissel N, Fenaux P, et al. Cooperating gene mutations in acute myeloid leukemia: a review of the literature. Leukemia 2008;22:915–31. https://doi.org/10.1038/LEU.2008.19.

[303] Hoffman B, Amanullah A, Shafarenko M, Liebermann DA. The proto-oncogene c-myc in hematopoietic development and leukemogenesis. Oncogene 2002;21:3414–21. https://doi.org/10.1038/SJ.ONC.1205400.

[304] Jiang G, Albihn A, Tang T, Tian Z, Henriksson M. Role of Myc in differentiation and apoptosis in HL60 cells after exposure to arsenic trioxide or all-trans retinoic acid. Leuk Res 2008;32:297–307. https://doi.org/10.1016/J.LEUKRES.2007.06.021.

[305] Dimberg A, Bahram F, Karlberg I, Larsson LG, Nilsson K, Öberg F. Retinoic acid-induced cell cycle arrest of human myeloid cell lines is associated with sequential down-regulation of c-Myc and cyclin E and posttranscriptional up-regulation of p27(Kip1). Blood 2002;99:2199–206. https://doi.org/10.1182/BLOOD.V99.6.2199.

[306] Awwad RT, Do K, Stevenson H, Fu SW, Lo-Coco F, Costello M, et al. Overexpression of BP1, a homeobox gene, is associated with resistance to all-trans retinoic acid in acute promyelocytic leukemia cells. Ann Hematol 2008;87:195–203. https://doi.org/10.1007/S00277-007-0402-7.

[307] Cicconi L, Platzbecker U, Avvisati G, Paoloni F, Thiede C, Vignetti M, et al. Long-term results of all-trans retinoic acid and arsenic trioxide in non-high-risk acute promyelocytic leukemia: update of the APL0406 Italian-German randomized trial. Leukemia 2019;34:914–8. https://doi.org/10.1038/s41375-019-0589-3.

[308] Falini B, Sciabolacci S, Falini L, Brunetti L, Martelli MP. Diagnostic and therapeutic pitfalls in NPM1-mutated AML: notes from the field. Leukemia 2021;35:3113–26. https://doi.org/10.1038/s41375-021-01222-4.

[309] van Waalwijk van Doorn-Khosrovani SB, Erpelinck C, Meijer J, van Oosterhoud S, van Putten WLJ, Valk PJM, et al. Biallelic mutations in the CEBPA gene low CEBPA expression levels as prognostic markers in intermediate-risk AML. Hematology Journal 2003;4:31–40. https://doi.org/10.1038/SJ.THJ.6200216.

[310] Schnittger S, Kern W, Tschulik C, Weiss T, Dicker F, Falini B, et al. Minimal residual disease levels assessed by NPM1 mutation–specific RQ-PCR provide important prognostic information in AML. Blood 2009;114:2220–31. https://doi.org/10.1182/BLOOD-2009-03-213389.

[311] Liu SJ, Horlbeck MA, Cho SW, Birk HS, Malatesta M, He D, et al. CRISPRi-based genome-scale identification of functional long noncoding RNA loci in human cells. Science 2017;355. https://doi.org/10.1126/SCIENCE.AAH7111.

[312] Haswell JR, Mattioli K, Gerhardinger C, Maass PG, Foster DJ, Peinado P, et al. Genome-wide CRISPR interference screen identifies long non-coding RNA loci required for differentiation and pluripotency. PLoS One 2021;16:e0252848. https://doi.org/10.1371/JOURNAL.PONE.0252848.

[313] Liang WW, Müller S, Hart SK, Wessels HH, Méndez-Mancilla A, Sookdeo A, et al. Transcriptome-scale RNA-targeting CRISPR screens reveal essential lncRNAs in human cells. Cell 2024;187:7637-7654.e29. https://doi.org/10.1016/j.cell.2024.10.021.

[314] Flores-Arenas C, Malekos E, Montano C, Covarrubias S, Sudek L, Dempsey V, et al. CRISPRi screen uncovers lncRNA regulators of human monocyte growth. J Biol Chem 2025;301:110204. https://doi.org/10.1016/J.JBC.2025.110204.

[315] Petroulia S, Hockemeyer K, Tiwari S, Berico P, Shamloo S, Banijamali SE, et al. Uncovering Novel lncRNAs Linked to Melanoma Growth and Migration with CRISPR Inhibition Screening. Cancer Research Communications 2025;5:1102–18. https://doi.org/10.1158/2767-9764.CRC-24-0416.

[316] Kim G, Siprashvili Z, Yang X, Meyers JM, Ji A, Khavari PA, et al. In Vivo CRISPR Interference Screen Reveals Long Noncoding RNA Portfolio Crucial for

Cutaneous Squamous Cell Carcinoma Tumor Growth. Journal of Investigative Dermatology 2025;0. https://doi.org/10.1016/j.jid.2025.04.038.

[317] John Liu S, Malatesta M, Lien B V., Saha P, Thombare SS, Hong SJ, et al. CRISPRi-based radiation modifier screen identifies long non-coding RNA therapeutic targets in glioma. Genome Biol 2020;21:1–18. https://doi.org/10.1186/S13059-020-01995-4.

[318] Tsung K, Liu KQ, Han JS, Deshpande K, Doan T, Loh YHE, et al. CRISPRi screen of long non-coding RNAs identifies LINC03045 regulating glioblastoma invasion. PLoS Genet 2024;20:e1011314. https://doi.org/10.1371/JOURNAL.PGEN.1011314.

[319] Fan C, Cats D, Selle M, Khorosjutina O, Dhanjal S, Mei H, et al. SMAD3 and p300 complex scaffolding by long non-coding RNA LIMD1-AS1 promotes TGF-β-induced breast cancer cell plasticity. Nucleic Acids Res 2025;53:841. https://doi.org/10.1093/nar/gkaf841.

[320] Selkirk E, Patel R, Hoyle A, Lie-a-Ling M, Smith D, Swift J, et al. SGOL1-AS1 enhances cell survival in acute myeloid leukemia by maintaining pro-inflammatory signaling. Heliyon 2022;8. https://doi.org/10.1016/j.heliyon.2022.e11362.

[321] Ng M, Verboon L, Issa H, Bhayadia R, Vermunt MW, Winkler R, et al. Myeloid leukemia vulnerabilities embedded in long noncoding RNA locus MYNRL15. IScience 2023;26:107844. https://doi.org/10.1016/J.ISCI.2023.107844.

[322] Yan J, Deng YX, Cai YL, Cong WD. LncRNA MIR17HG promotes the proliferation, migration, and invasion of retinoblastoma cells by up-regulating HIF-1α expression via sponging miR-155-5p. Kaohsiung J Med Sci 2022;38:554–64. https://doi.org/10.1002/KJM2.12523.

[323] Morelli E, Fulciniti M, Samur MK, Ribeiro CF, Wert-Lamas L, Henninger JE, et al. A MIR17HG-derived long noncoding RNA provides an essential chromatin scaffold for protein interaction and myeloma growth. Blood 2023;141:391–405.https://doi.org/10.1182/BLOOD.2022016892/1922286/BLOOD.2022016892.PDF.

[324] Meng Y, Hao D, Huang Y, Jia S, Zhang J, He X, et al. Positive feedback loop SP1/MIR17HG/miR-130a-3p promotes osteosarcoma proliferation and

cisplatin resistance. Biochem Biophys Res Commun 2020;521:739–45. https://doi.org/10.1016/J.BBRC.2019.10.180.

[325] Braekeleer E De, Douet-Guilbert N, Basinko A, Bris MJ Le, Morel F, De Braekeleer M. Hox gene dysregulation in acute myeloid leukemia. Future Oncology 2014;10:475–95. https://doi.org/10.2217/FON.13.195;WGROUP:STRING:PUBLICATION.

[326] Bach C, Buhl S, Mueller D, García-Cuéllar MP, Maethner E, Slany RK. Leukemogenic transformation by HOXA cluster genes. Blood 2010;115:2910–8. https://doi.org/10.1182/BLOOD-2009-04-216606.

[327] Guo C, Ju QQ, Zhang CX, Gong M, Li ZL, Gao YY. Overexpression of HOXA10 is associated with unfavorable prognosis of acute myeloid leukemia. BMC Cancer 2020;20:1–12. https://doi.org/10.1186/S12885-020-07088-6/TABLES/3.

[328] Thorsteinsdottir U, Sauvageau G, Hough MR, Dragowska W, Lansdorp PM, Lawrence HJ, et al. Overexpression of HOXA10 in murine hematopoietic cells perturbs both myeloid and lymphoid differentiation and leads to acute myeloid leukemia. Mol Cell Biol 1997;17:495. https://doi.org/10.1128/MCB.17.1.495.

[329] Zhang X, Lian Z, Padden C, Gerstein MB, Rozowsky J, Snyder M, et al. A myelopoiesis-associated regulatory intergenic noncoding RNA transcript within the human HOXA cluster. Blood 2009;113:2526–34. https://doi.org/10.1182/blood-2008-06-162164.

[330] Wang SL, Huang Y, Su R, Yu YY. Silencing long non-coding RNA HOTAIR exerts anti-oncogenic effect on human acute myeloid leukemia via demethylation of HOXA5 by inhibiting Dnmt3b. Cancer Cell Int 2019;19:1–14. https://doi.org/10.1186/S12935-019-0808-Z.

[331] Díaz-Beyá M, Brunet S, Nomdedéu J, Pratcorona M, Cordeiro A, Gallardo D, et al. The lincRNA HOTAIRM1, located in the HOXA genomic region, is expressed in acute myeloid leukemia, impacts prognosis in patients in the intermediate-risk cytogenetic category, and is associated with a distinctive microRNA signature. Oncotarget 2015;6:31613–27. https://doi.org/10.18632/oncotarget.5148.

[332] Kurotaki D, Osato N, Nishiyama A, Yamamoto M, Ban T, Sato H, et al. Essential role of the IRF8-KLF4 transcription factor cascade in murine monocyte differentiation. Blood 2013;121:1839–49. https://doi.org/10.1182/BLOOD-2012-06-437863.

[333] Cao Z, Budinich KA, Huang H, Ren D, Lu B, Zhang Z, et al. ZMYND8-regulated IRF8 transcription axis is an acute myeloid leukemia dependency. Mol Cell 2021;81:3604-3622.e10. https://doi.org/10.1016/j.molcel.2021.07.018.

[334] Pogosova-Agadjanyan EL, Kopecky KJ, Ostronoff F, Appelbaum FR, Godwin J, Lee H, et al. The Prognostic Significance of IRF8 Transcripts in Adult Patients with Acute Myeloid Leukemia. PLoS One 2013;8. https://doi.org/10.1371/JOURNAL.PONE.0070812,.

[335] Bamopoulos SA, Batcha AMN, Jurinovic V, Rothenberg-Thurley M, Janke H, Ksienzyk B, et al. Clinical presentation and differential splicing of SRSF2, U2AF1 and SF3B1 mutations in patients with acute myeloid leukemia. Leukemia 2020 34:10 2020;34:2621–34. https://doi.org/10.1038/s41375-020-0839-4.

[336] Cho YU, Jang S, Seo EJ, Park CJ, Chi HS, Kim DY, et al. Preferential occurrence of spliceosome mutations in acute myeloid leukemia with preceding myelodysplastic syndrome and/or myelodysplasia morphology. Leuk Lymphoma 2015;56:2301–8. https://doi.org/10.3109/10428194.2014.995648.

[337] Vu LP, Pickering BF, Cheng Y, Zaccara S, Nguyen D, Minuesa G, et al. The N6-methyladenosine (m6A)-forming enzyme METTL3 controls myeloid differentiation of normal and leukemia cells. Nat Med 2017;23:1369. https://doi.org/10.1038/NM.4416.

[338] Arna AB, Patel H, Singh RS, Vizeacoumar FS, Kusalik A, Freywald A, et al. Synthetic lethal interactions of DEAD/H-box helicases as targets for cancer therapy. Front Oncol 2023;12. https://doi.org/10.3389/FONC.2022.1087989.

[339] Zisi A, Bartek J, Lindström MS. Targeting Ribosome Biogenesis in Cancer: Lessons Learned and Way Forward. Cancers (Basel) 2022;14. https://doi.org/10.3390/CANCERS14092126.

[340] Han JW, Flemington C, Houghton AB, Gu Z, Zambetti GP, Lutz RJ, et al. Expression of bbc3, a pro-apoptotic BH3-only gene, is regulated by

diverse cell death and survival signals. Proc Natl Acad Sci U S A 2001;98:11318–23. https://doi.org/10.1073/PNAS.201208798.

[341] Yu J, Zhang L. PUMA, a potent killer with or without p53. Oncogene 2008;27:S71. https://doi.org/10.1038/ONC.2009.45.

[342] Iwatani-Yoshihara M, Ito M, Ishibashi Y, Oki H, Tanaka T, Morishita D, et al. Discovery and Characterization of a Eukaryotic Initiation Factor 4A-3-Selective Inhibitor That Suppresses Nonsense-Mediated mRNA Decay. ACS Chem Biol 2017;12:1760–8. https://doi.org/10.1021/ACSCHEMBIO.7B00041.

[343] Kurishev AO, Abashkin DA, Karpov DS, Marilovtseva E V., Chaika YA, Semina E V., et al. Schizophrenia risk gene ZNF536 modulates retinoic acid response and neuronal gene networks in SH-SY5Y cells. Front Mol Neurosci 2025;18. https://doi.org/10.3389/FNMOL.2025.1671354.

[344] Taniguchi T, Ogasawara K, Takaoka A, Tanaka N. IRF family of transcription factors as regulators of host defense. Annu Rev Immunol 2001;19:623–55. https://doi.org/10.1146/ANNUREV.IMMUNOL.19.1.623.

[345] Mangiavacchi A, Sorci M, Masciarelli S, Larivera S, Legnini I, Iosue I, et al. The miR-223 host non-coding transcript linc-223 induces IRF4 expression in acute myeloid leukemia by acting as a competing endogenous RNA. Oncotarget 2016;7:60155. https://doi.org/10.18632/ONCOTARGET.11165.