Long Non-coding RNAs in Myeloid Malignancies

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Acute myeloid leukemia (AML) represents 80% of adult leukemias and 15–20% of childhood leukemias. AML are characterized by the presence of 20% blasts or more in the bone marrow, or defining cytogenetic abnormalities. Laboratory diagnoses of myelodysplastic syndromes (MDS) depend on morphological changes based on dysplasia in peripheral blood and bone marrow, including peripheral blood smears, bone marrow aspirate smears, and bone marrow biopsies. As leukemic cells are not functional, the patient develops anemia, neutropenia, and thrombocytopenia, leading to fatigue, recurrent infections, and hemorrhage. The genetic background and associated mutations in AML blasts determine the clinical course of the disease. Over the last decade, non-coding RNAs transcripts that do not codify for proteins but play a role in regulation of functions have been shown to have multiple applications in the diagnosis, prognosis and therapeutic approach of various types of cancers, including myeloid malignancies. After a comprehensive review of current literature, we found reports of multiple long non-coding RNAs (lncRNAs) that can differentiate between AML types and how their exogenous modulation can dramatically change the behavior of AML cells. These lncRNAs include: H19, LINC00877, RP11-84C10, CRINDE, RP11848P1.3, ZNF667-AS1, AC111000.4-202, SFMBT2, LINC02082-201, MEG3, AC009495.2, PVT1, HOTTIP, SNHG5, and CCAT1. In addition, by performing an analysis on available AML data in The Cancer Genome Atlas (TCGA), we found 10 lncRNAs with significantly differential expression between patients in favorable, intermediate/normal, or poor cytogenetic risk categories. These are: DANCR, PRDM16-DT, SNHG6, OIP5-AS1, SNHG16, JPX, FTX, KCNQ1OT1, TP73-AS1, and GAS5. The identification of a molecular signature based on lncRNAs has the potential for have deep clinical significance, as it could potentially help better define the evolution from low-grade MDS to high-grade MDS to AML, changing the course of therapy. This would allow...
Hematopoiesis is the complex process of unidirectional and continuous formation and release of blood cells into circulation. Normal hematopoiesis is polyclonal and takes place in the hematogenous bone marrow (BM) (1–3). Pluripotent stem cells have the capacity to differentiate into multiple cell lineages (4–7), and this differentiation is irreversible during normal human physiology. The production of hematogenous BM is impressive, forming about $10^{10}$ erythrocytes and $10^8$–$10^9$ leukocytes every hour, regulated by numerous cytokines for the maintenance of lineages under normal parameters (8–10). When in need, production can increase up to seven times normal levels. Pluripotent stem cells differentiate into myeloid and lymphoid multipotent stem cells that further differentiate into cells oriented on a lineage, called colony forming units (CFU). This name depicts their capacity to produce colonies in vitro in the presence of growth factors, also called progenitors (11, 12).

All cells of the BM, including progenitors, precursors, mature cells and stromal cells, interact in a complex manner through cytokines, messenger RNAs (mRNAs), microRNAs, and other non-coding RNAs (ncRNAs) (13–16), by targeting genes responsible for cell proliferation, differentiation, methylation, and acquisition of resistance to therapy. Granulopoiesis is the process that gives rise to elements of the granulocyte series: segmented neutrophils, eosinophils, and basophils. Acute myeloid leukemia (AML) develops in different stages of granulopoiesis, each with a specific set of long non-coding RNAs (lncRNAs) (Figure 1). The progenitors of this series are the myeloid stem cell and the granulo-monocytic colony forming unit, which are not morphologically identifiable (17, 18).

Non-coding RNAs are small RNA molecules that are not translated into a protein (19), and have the potential of bringing new insights in the clinic for diagnosis, prognosis and therapy. DNA is a molecule composed of around 3 billion base pairs, out of which only 5–10% are transcribed, with protein-coding genes accounting for <2% of the human genome (20–22). Between 60% and 90% of the human genome is transcribed in non-coding transcripts, these transcripts being further divided into two categories depending on their primary role: either housekeeping or regulatory genes (23). Regulatory genes include microRNAs (miRNAs), circular RNAs and IncRNAs (24, 25) in regulation of gene transcription and translation, along with key cellular mechanisms such as differentiation, proliferation, or inflammation. In this manuscript, we aim to provide novel comprehensive insights on the role of IncRNAs in the pathogenesis of myeloid malignancies, illustrating their diagnostic and prognostic potential.

Myeloid precursors are formed by at least four generations of cells between granulo-monocytic CFUs and the mature elements of the series. Young precursors have azurophil granulations, also called primary granulations. Lineage-specific granulations (neutrophil, eosinophil, basophil) appear at the myelocyte stage. All lineages have the same maturation steps, with the only difference occurring in lineage specific granulations (26–29).

In normal conditions, the BM contains <5% blasts. When a mutation appears in a myeloid progenitor of the BM, the result is the development of a myelodysplastic syndrome (<20% blasts) or an acute leukemia (more than 20% BM blasts). Myelodysplastic syndromes (MDS) are clonal disorders of hematopoietic stem cells, characterized by cytopenia in the context of hyper or normocellular bone marrow, multilineage dysplasia, and frequent chromosomal abnormalities (30–33). The World Health Organization (WHO) classification based on morphology, cytochemistry, immunophenotype, cytogenetics, and clinical examination is described in Table 1 and Figure 2 (34–36).

**CYTOTOGENETICS IN THE CLINIC FOR MYELOID MALIGNANCIES**

Recurrent cytogenetic abnormalities are considered diagnostic for myelodysplastic syndrome, in the presence of cytopenia of unknown etiology and in the absence of myelodysplastic changes. Morphologic changes that are characteristic for myelodysplastic syndrome are described in Table 2 (37–39).

Depending on genetic background, MDS can either respond to standard therapies, or can evolve into an acute leukemia
Acute leukemias are caused by mutations in the unipotent or pluripotent stem cells from the bone marrow that yield an overproduction of immature cells and inhibition of normal hematopoiesis, resulting in anemia, thrombocytopenia, and neutropenia (42, 44–47). The type of malignant cell is defined according to the lineage, whether it is myeloid, lymphoid or mixed. Acute leukemias also occur when a hematopoietic stem cell acquires a sequence of mutations that confer an advantage of clonal proliferation (43, 48–52). The incidence of AML increases with age and represents 80% of adult leukemia (53) and 15–20% of childhood acute leukemic (54). Congenital leukemia is a rare disease that is acquired in the neonatal period (45). For AML, the symptomatology results from bone marrow infiltration by the malignant cell, which inhibits normal hematopoiesis. Leukemic cells are not functional; thus, the patient develops anemia, neutropenia, and thrombocytopenia, leading to fatigue, recurrent infections, and hemorrhage.

A common characteristic of acute leukemia is the hypercellularity of the bone marrow, with more than 20% blasts at the time of diagnosis. Blast subtype represents the principal diagnostic criteria between acute lymphoblastic leukemia and AML. WHO classification of AML divides them into four principal subtypes: AML with recurrent cytogenetic abnormalities, AML with dysplasia, AML associated with therapy and myelodysplastic syndrome, and the “not otherwise specified” AML (34).

Cytogenetics and molecular biology are of key importance in myeloid malignancies, as previously shown by several groups including ours (55–58). In AML with recurrent cytogenetic abnormalities, these very recurrent cytogenetics represent the most important trait, as their names clearly describes them (55–57). The best prognostics are associated with t(8;21) (q22;q22) and inv(16)(p13;q22), where a high percentage of patients achieve complete remission (58, 59). Patients with AML are routinely profiled for the presence of mutations in FLT3, NPM1, CEBPA, and, more recently, TP53 (59–61). According to the German-Austrian Acute Myeloid Leukemia Study Group, coordinated by Dohner et al., the genotype of mutant NPM1 without FLT3-ITD, the mutant CEBPA genotype, and younger age were each significantly associated with complete remission. The benefit of the transplant was limited to the subgroup of patients with the genotype FLT3-ITD or the genotype consisting of wild-type NPM1 and CEBPA without FLT3-ITD.

AML with dysplasia is associated with at least two dysplastic lineages and the leukemic clone develops following a previous MDS clone. Dyserythropoiesis is described as multinucleated erythroblasts, magaloblastosis, cytoplasmic vacuoles or karyorrhexis (62). The leukemic clone develops as an evolutionary hallmark of MDS and patients generally present with a reduction in the number of erythrocytes, neutrophils and thrombocytes (63, 64). Because of frequent pancytopenia, the prognosis is generally bad. Several predictors for transformation have been identified and include mutations of genes in growth signaling pathways (NRAS, KRAS, PTPN11, FLT3), mutations in genes more commonly observed in AML (NPM1, WT1, IDH2), and certain cytogenetic abnormalities (monosomy 7, complex karyotype, loss of 17p). Gene expression profiles that divide MDS into two major categories identify a progenitor gene signature subtype associated with a high risk of AML transformation (65).

Therapy-related MDS (t-MDS) and therapy-related AML (t-AML) are considered one entity in therapy-related myeloid
malignancies because of their similar pathogenesis, rapid progression from t-MDS to t-AML, and their equally poor prognosis. A small percentage of patients present with favorable risk fusion genes, whereas 50% have adverse cytogenetics. The most frequent molecular aberration in t-AML and t-MDS affects TP53 (33%). The selection of pre-existing treatment-resistant hematopoietic stem cell clones with the TP53 mutation has been shown as an important mechanism in the development of t-MNs and explains the high frequency of TP53 mutations in these patients (66, 67).

### CURRENT RESEARCH ON LONG NON-CODING RNAs IN MYELOID MALIGNANCIES

As previously mentioned, ncRNAs are transcripts that do not possess protein-coding activity, but regulate cell behavior through interactions established at the DNA, RNA, or protein levels. Due to their versatility, the ncRNAs are often proposed as diagnostic and prognostic tools (68, 69) or as part of combined therapy in cancer (70–72). The role of ncRNAs has been extensively analyzed during the past decade, with the focus being on miRNAs (73–77). LncRNAs are a class of transcripts with sequence lengths of more than 200 nucleotides (78, 79). They are involved in various molecular processes, such as chromatin interaction (80), modulating gene transcription through binding the promoter region (81), acting as a competing endogenous RNAs (ceRNAs) for microRNAs (82), interacting with the ribosome and thus interfering in translation as well as interacting with various proteins (83, 84) and determining cellular localization (84). Non-coding transcription across antisense strands of genes is a universal mechanism for both yeast and vertebrates. The antisense transcription stimulates nucleosome occupancy and acetylation of histone proteins, but does not always alter the transcription of protein-coding genes. Antisense transcription has a high level of histone turnover and makes genes more susceptible to changing signals due to a wide range of chromatic configuration. This is specific to eukaryotic cells and allows these cells to adapt to external environmental stress (85–87). Due to this supportive evidence, the role of lncRNAs in hematological malignancies is being investigated and numerous studies offer compelling evidence for the correlation between altered lncRNA expression and various clinicopathological characteristics of leukemia patients.

SBF2-AS1, DANCR, LINC00239, LINC00319, LINC00265, LEF1-AS1, and ZFAS1 are lncRNAs found to be overexpressed in AML (88–94). In vitro, the inhibition of SBF2-AS1 lncRNA causes leukemia cells to undergo apoptosis and cell cycle arrest. This lncRNA sponges the tumor suppressor miR-188-5p. The lncRNA DANCR is downregulated in undifferentiated hematopoietic progenitors, both malignant and BM-derived normal stem cells (89, 95, 96), the CD34-positive leukemic stem cells, as well as in normal BM-derived. However, for normal stem cells, siRNA-mediated knock out of DANCR does not affect normal hematopoiesis. In AML cells, DANCR silencing leads to impaired AML progression through inhibited self-renewal capacity and dormancy of leukemia cells. In vivo experiments also showed that the siRNA treatment leads to increased overall survival. This lncRNA acts by activating the c-Myc transcription factor through the WNT signaling pathway (89).

LINC00239 regulates chemoresistance to doxorubicin and exerts a protective effect against apoptotic cell death, promotes cell viability, cell cycle distribution, colony formation and migration. This lncRNA leads to mTOR/AKT activation (90). The overactivation of SOX61 in AML leads to increased cell proliferation and inhibited cell apoptosis in KG-1 cells and THP-1 cell (97). LINC00265 causes G0/G1 cell cycle arrest, decreased proliferative rates, apoptosis, and reduction of migratory capabilities and is involved in the phosphorylation of PI3K/AKT (92).

Some lncRNAs act as tumor suppressors in AML. H22954 is a novel lncRNA, downregulated in the bone marrow of AML patients. Its decreased expression is linked to a higher risk of

| TABLE 1 | Classification of myelodysplastic syndromes. |
|----------|-----------------------------------------------|
| Disease  | Peripheral blood | Bone marrow |
| ---------|------------------|-------------|
| Refractory cytopenia with unilineage dysplasia | Isolated cytopenia or bicytopenia | Unilineage dysplasia: ≥10% of the cells of one myeloid lineage |
| (refractory anemia, refractory neutropenia, refractory thrombocytopenia) | <1% blasts | <5% blasts |
| Refractory anemia with ring sideroblasts | Anemia | Dysplastic erythroid series |
| | 1% blasts | <5% blasts |
| Refractory cytopenia with multilineage dysplasia | Cytopenia | Dysplasia ≥10% cells in ≥2 myeloid lineages |
| | <1% blasts | <5% blasts |
| | <1 x 10^9/µl monocytes without Auer bodies | Without Auer bodies |
| Refractory cytopenia with multilineage dysplasia and ring sideroblasts | Cytopenia | Dysplasia ≥10% cells in ≥2 myeloid lineages |
| | <1% blasts | <5% blasts |
| | <1 x 10^9/µl monocytes without Auer bodies | Without Auer bodies |
| Refractory anemia with excess blasts 1 | Cytopenia | Uni or multilineage |
| | <5% blasts | dysplasia |
| | Without Auer bodies | 5–9% blasts |
| | ≥1 x 10^9/µl monocytes | Without Auer bodies |
| Refractory anemia with excess blasts 2 | Cytopenia | Uni or multilineage |
| | 5–19% blasts | dysplasia |
| | ≥Auer bodies | 10–19% blasts |
| | ≥1 x 10^9/µl monocytes | ≥Auer bodies |
| Unclassified myelodysplastic syndrome | Cytopenia | Dysplasia in <10% of the cells of one or more myeloid lineages associated with a cytogenetic abnormality |
| | <1% blasts | ≥5% blasts |
| Myelodysplastic syndrome associated with isolated 5q- | Anemia | Elevated megakaryocytes |
| | Usually normal or elevated thrombocytes | with hypolobulated nuclei |
| | <1% blasts | Unique cytogenetic abnormality: 5q- |
| | | Without Auer bodies |
relapse. H22954 expression inhibits AML cell proliferation in vitro and its overexpression leads to cell apoptosis. In vivo, it inhibits tumor growth in a mouse xenograft model as it interacts with the 3’UTR BCL2, a well-known antiapoptotic oncoprotein (98).

In AML with a monocytic/monoblastic phenotype, HOTTIP and LINC00152 are overexpressed specifically in the bone marrow microenvironment of patients with AML FAB classification M5. In vitro experiments have proven that the inhibition of LINC00152 leads to impaired proliferation of
leukemia cells and cell cycle arrest (99, 100). LINC00152 decreases the self-renewal capacity of leukemia cells (99), whereas HOTTIP acts by sponging the tumor suppressor miR-608 and the consequent overstimulation of the oncogene DDA1 (DET1- and DDB1-Associated Protein 1) (100). LINC00152 has a similar molecular activity, by sponging miR-193a and indirectly increasing the expression of Cyclin Dependent Kinase 9 (CDK9).

A subtype of AML is acute promyelocytic leukemia (APL), the former FAB M3 AML, with a particular genetic background and therapy protocol, based on the use of retinoic acid (101–103). In APL, the overexpression of ENST00000484765, ENST00000509010, and ENST00000416842 translates into a negative overall survival with a higher percentage of cancer stem cells present in the bone marrow, whereas the overexpression of ENST00000505646, Inc-SFMBT2-4 is translated into a better survival rate for the patients (104). Therefore, the expression analysis of these IncRNAs could be incorporated into current clinical practice (104).

TUG1 (105, 106), SNHG5 (107), LINC00926, LRRCC75A-AS1, FAM30A (108), IRAIN (109), ENSG00000260257, and ENSG00000236537 (110) can stratify AML patients into those with higher survival rates or lower survival rates, according to their expression level.

UCA1 is upregulated in the AML cell line HL-60, and its inhibition decreases cell viability, migration and invasion capacity (111), while surpassing chemoresistance (112). This IncRNA targets two tumor suppressor microRNAs, namely miR-126 in AML (111) and miR-125A in de novo AML (112).

H19 is a potential biomarker of AML response to therapy, being downregulated in CR. It has the ID2 gene as a potential downstream target. This IncRNA impairs apoptosis and promotes cell proliferation in AML (113). MALAT1 is a well-known IncRNA with oncogenic potential in solid cancers. In AML, a number of studies have reaffirmed its oncogenic role. MALAT1 correlated with the expression of NTRK3 gene can differentiate between patients carrying various mutations. For instance, patients with t(15;17) and the PML-RARA fusion gene have increased co-expression of MALAT1 and NTRK3; patients with t(8;21) exhibit an overexpression of MALAT1 and underexpression of NTRK3. As follows, in the clinical setting AML patients carrying the ETV6-NTRK3 gene fusion and associated MALAT1 overexpression can exhibit a better therapeutic response to NTRK3 inhibitor, AZ-23 (114).

HOTAIR is another oncogenic lncRNA with well-established roles in various tumors. The expression of HOTAIR is upregulated in AML cell lines (HL-60, K562) (115, 116) and in peripheral or bone marrow mononuclear cells from AML patients. It stimulates AML progression, by promoting a higher number of blast and stimulated cell proliferation (115–117).

ZEB1 is upregulated in AML cell lines, and its knockdown lowers cell proliferation, induces apoptosis and cell cycle arrest in the G0/S phase (118). CCDC26 is upregulated in AML cell line K562, where it stimulates cell division by impairing the translation of KIT proteins (119). Further details on the role of IncRNAs in leukemia are found in Table 3.

The search for IncRNAs biomarkers for AML can yield negative results. For instance, Sayad et al. have tried to analyze the expression level of FAS antisense RNA 1 (FAS-AS1) in the peripheral blood of AML patients vs. healthy controls. The study found no significant change in expression between the two groups, even though FAS has been found to play key roles in cellular apoptosis (120). In a study involving an Iranian population, it was proven that HOTAIR is not differentially expressed in the peripheral blood from healthy vs. AML patients (121). Several IncRNAs were reported to have both oncogenic and tumor suppressor roles in AML. On the one hand, MEG3 is upregulated in APL (122), whereas in AML cell lines, it was found to function as a tumor suppressor (123). On the other hand, TCGA analysis revealed that CRINDE is downregulated in APL (122) and upregulated in bone marrow stem cells (124), bone marrow samples and AML cell lines (125). This could be attributed to the fact that the AML cell lines were harvested from patients with other AML subtypes or due to differences in gene expression analysis.

### LONG NON-CODING RNAs IN MYELOID MALIGNANCIES—ANALYSIS OF TCGA DATA

In the present manuscript we also present a TCGA data analysis on myeloid malignancies. The TCGA data on the clinical characteristics of the patients and RNAseq counts was downloaded from the GDC TCGA Acute Myeloid Leukemia (LAML) cohort, which included 151 patients (https://xenabrowser.net/datapages/).

From the RNAcentral database (https://rnacentral.org/search?q=RNA), we downloaded the full set of human IncRNAs found in the Ensembl database. A total of 27,969 Ensemble ID codes for IncRNAs were retrieved and using a Venn diagram (http://bioinfogp.cnb.csic.es/tools/venny/), we identified the list of IncRNAs from the TCGA dataset, reaching a total of 275 IncRNAs. We ran a wide-range gene expression analysis with an online gene expression analysis tool based on the Jupyter Notebook in Python (126).

The RNASeq data showed an evenly distributed million reads per sample across the three analyzed cytogenetic risk groups: Figure 3 compares the expression level of IncRNAs in poor

### Table 2: Recurrent abnormalities in myelodysplastic syndromes.

| Unbalanced abnormalities | Balanced abnormalities |
|--------------------------|------------------------|
| −7 or del (7q)           | t(11;16)(q23;p13.3)     |
| −5 or del (5q)           | t(3;21)(q26.2;q22.1)    |
| i(17q) or t(17p)         | t(1;3)(p36.3;q21.1)     |
| −13 or del (13q)         | t(2;11)(p21;q23)        |
| Del (11q)                | inv(3)(p21q26.2)        |
| Del (12p) or t(12p)      | t(6;9)(p23;q34)         |
| Del (9q)                 |                        |
| Xq(13)                   |                        |
### TABLE 3 | Long non-coding RNAs in myeloid malignancies.

| Name of lncRNA | Expression | Targets | Type of sample | Effect | References |
|----------------|------------|---------|----------------|--------|------------|
| HOTTIP         | UP         | HOTTIP/microRNA-608/DDA1 axis | AML cell lines, bone marrow | Proliferation and cell cycle progression | (95) |
| HOTAIR         | UP         | N/A     | Bone marrow and peripheral blood mononuclear cells, AML cell line | Associated with higher white blood cell and BM blast counts, decreased overall survival, increased cell proliferation | (109, 110) |
|                 | UP         | miR-193a | Bone marrow mononuclear cells, AML cell line | Maintenance of the malignant phenotypes | (111) |
|                 | UP         | p15     | AML cell lines, umbilical cord blood, murine bone marrow progenitor cells | Decreases proliferation and colony of in AML CD34+ progenitor cells | (114) |
| SBF2-AS1       | UP         | miR-188-5p | AML cell lines | SBF2-AS1 inhibition induced AML cells apoptosis and arrested AML cells in G0/G1 phase | (83) |
| CCDC28         | UP         | Translation of KIT protein | AML cell line | Inhibition slows cell proliferation | (113) |
| PVT1           | UP         | N/A     | Bone marrow mononuclear cells | Differentiation between patients with a specific FAB class (M3) or cytogenetic risk | (115) |
| CCAT1          | UP         | miR-155 | Peripheral blood mononuclear cells, AML cell line | Repressed monocytic differentiation and promoted cell growth in AML M4 and M5 | (118) |
| DANCR          | UP         | c-MYC (and the whole WNT signaling pathway) | Bone marrow and peripheral blood samples | Impaired AML progression, through inhibited self-renewal capacity and dormancy of leukemia cells | (84) |
| LINC00239      | UP         | mTOR, AKT phosphorylation | AML cell lines | Regulates chemoresistance to doxorubicin and exerts a protective effect against apoptotic cell death, promotes cell viability, cell cycle distribution, colony formation and migration, knock out does not have significant therapeutic effect, but induced overexpression was much worse than the control | (85) |
| CYTORa         | Up         | miR-193a, CDK9 | Bone marrow samples, AML cell lines | Suppresses the proliferation, accelerates the apoptosis, and induces the cycle arrest of, decreased number of colonies | (94) |
| RP11-395P13.6-001 | UP   | N/A     | Bone marrow samples | Independently predicted poor OS, these are especially found in stem cells, AML-M3, depending on the risk factor, high risk vs. low risk group | (96) |
| AP001042.1     | LINC02082  | N/A     | AML cell lines, Bone marrow samples | Promoted cell proliferation and inhibited cell apoptosis in KG-1 cells and THP-1 cells, pediatric AML patients | (92) |
| LINC00319      | UP         | Stability of SIRT6 | AML cell lines | Its silencing represses the growth of AML cells | (96) |
| LINC00265      | UP         | Phosphorylation of PI3K and Akt | Bone marrow samples and serum, AML cell lines | G0/G1 cell cycle arrest, decreased proliferative rates, apoptosis, reduction of migratory capabilities | (87) |
| LEF1-AS1       | UP         | p21 and p27 | Bone marrow samples | Inhibited proliferation, less cell divisions, no difference in apoptosis levels | (88) |

(Continued)
TABLE 3 | Continued

| Name of lncRNA | Expression | Targets | Type of sample | Effect | References |
|---------------|------------|---------|----------------|--------|------------|
| PANDAR        | UP         | N/A     | Bone marrow mononuclear cells | Upregulation in non-M3-AML and cytogenetically normal AML | (88) |
| TUG1          | UP         | N/A     | Bone marrow samples and AML cell lines | Correlated with poor risk stratification, up-regulated especially in M1-AML patients, TUG inhibition decreases cell viability, increased apoptosis | (59) |
| CDKN2B-AS1b   | UP         | AdipoR1 | Bone marrow samples | Promotes cell senescence and apoptosis | (119) |
| SNHG5         | UP         | N/A     | Bone marrow samples and plasma | Poorer prognosis in AML (M4–M5) | (101) |
| LINC00926     | UP         | 322 potential mRNA targets | TCGA data | Favorable survival | (102) |
| SNHG29c       | UP         | miR-126 | AML cell lines | Knockdown inhibited cell viability, migration, and invasion, while stimulating apoptosis | (105) |
| UCA1          | UP         | miR-125A | AML cell lines | Increase of chemoresistance of pediatric AML Adriamycin-resistant | (106) |
| H19           | UP         | Potential downstream gene ID2 | TCGA and GEO, AML cell line, bone marrow samples | Significantly shorter OS, pro-proliferative and anti-apoptotic effects in leukemia cells | (107) |
| LINC00899     | UP         | miR19a/b | Bone marrow samples, AML cell line | Knockdown inhibited AML cell proliferation, colony formation in AML-M2 | (120) |
| RP11-3050.6   | UP         | Eomes   | TCGA data | LncRNA-mediated dysregulation of Eomes, blocking of NK cell differentiation | |
| RP11-222k16.2 | UP         | C/EBPβ | Bone marrow samples, AML cell line | Knockdown of C/EBPβ impairs ATRA-induced upregulation of NEAT1 in AML-M3 | (122) |
| MALAT1        | UP         | N/A     | Murine bone marrow samples | Expression specific for AML-M3 subtype | (123) |
| NEAT1         | UP         | C/EBPβ bind to the promoter of IncRNA NEAT1 | AML cell line | Knockdown of C/EBPβ impairs ATRA-induced upregulation of NEAT1 in AML-M3 | (122) |
| PILNA         | UP         | N/A     | Bone marrow mononuclear cells | Expresses in hematopoietic progenitors | (116) |
| LNC_177417    | UP         | N/A     | Bone marrow mononuclear cells | Expression specific for AML-M3 subtype | (123) |
| LNC_104449    | UP         | N/A     | Bone marrow mononuclear cells | Increases cell reproductive capacity | (124) |
| AC009495.2    | UP         | N/A     | Bone marrow mononuclear cells | Overexpression opposes cellular proliferation and promotes myeloid bias in vivo in AML with RUX translocation | (125) |
| XLOC_109948   | UP         | N/A     | Bone marrow mononuclear cells | Reduces overall survival, apoptosis resistance in NPM1-mutated AML | (126) |

(Continued)
TABLE 3 | Continued

| Name of lncRNA | Expression | Targets | Type of sample | Effect | References |
|----------------|------------|---------|----------------|--------|------------|
| AL035071.1     | UP         | Co-expression with MAPRE | Bone marrow mononuclear cell | Worse prognostic, shorter overall survival | (104) |
| RP11-732M18.3  | UP         | Co-expression with TULP4 | Bone marrow mononuclear cell | Worse prognostic, longer overall survival | (104) |
| MIR9-3HG       | UP         | N/A     | Overexpressed in BM stem cells vs. differentiated cells | Overexpressed in BM stem cells vs. differentiated cells | (104) |
| LINC00467      | UP         | N/A     | Overexpressed in AML cells vs. BM stem cells | Overexpressed in AML cells vs. BM stem cells | (104) |
| ZFAS1          | UP         | N/A     | AML cell lines | Inhibition leads to decreased cell proliferation, apoptosis induction, and cell cycle arrest | (112) |
| H22954<sup>d</sup> | DOWN     | BCL2, miR-5095, and miR-619-5p | AML cell lines, Bone marrow | Increased risk of relapse, H22954 expression inhibits AML cell proliferation in vitro, overexpression of this lncRNA leads to apoptosis, H22954 expression inhibits tumor growth in a mouse xenograft model, H22954 interacts with the BCL2 3′ UTR | (93) |
| LINC00504<sup>b</sup> | UP         | N/A     | TCGA data | Increased peripheral blast, Bone marrow blasts | (127) |
| CRNDE<sup>e</sup> | UP         | N/A     | Bone marrow samples and AML cell lines | Inhibition lowers proliferation and self-renewal capacity, while increasing apoptosis | (128) |
| LINC00877      | DOWN       | N/A     | TCGA data and bone marrow samples | Expression specific for AML-M3 subtype | (123) |
| RP11-84C10.2   | UP         | N/A     | TCGA data | Predicts better overall survival, these are especially found in stem, cells, AML-M3, depending on the risk factor, high risk vs. low risk group | (98) |
| RP11-84P1.3    | UP         | N/A     | TCGA data and bone marrow samples | Predicts better overall survival, these are especially found in stem, cells, AML-M3, depending on the risk factor, high risk vs. low risk group | (98) |
| ZNF667-AS1     | DOWN       | N/A     | Bone marrow samples | Predicts better overall survival, these are especially found in stem, cells, AML-M3, depending on the risk factor, high risk vs. low risk group | (98) |
| SFMBT2-4:1     | DOWN       | N/A     | Bone marrow samples | Poor prognostic factor for non-M3 AML | (103) |

Results from literature review.

<sup>a</sup> Also known as LINC00152.

<sup>b</sup> Also known as ANRIL.

<sup>c</sup> Also known as LPRC75A-AS1.

<sup>d</sup> De novo discovery. Uncharacterized LOC100998457.

<sup>e</sup> CRNDE gene can be transcribed into protein coding or non-coding transcripts, the lncRNA variant is also known under the name: LINC00180.

vs. normal risk category patients, Figure 4 depicts favorable vs. normal risk category, whereas Figure 5 depicts favorable vs. poor risk patients.

The heatmaps comparing the expression of lncRNAs between the poor vs. normal risk category, favorable vs. normal risk category, poor vs. favorable risk category, showed a heterogeneous distribution of lncRNA expression. By calculating the fold change between the peripheral blood gene expressions between each compared category, we were able to identify a total number of 10 lncRNAs that were significantly differentially expressed between each of the two analyzed groups.

Principal Component Analysis (PCA) revealed a significant clustering of lncRNA expression in the case of favorable vs. poor prognostic cytogenetic risk category (Figure 5). With the help of the online tool miRNet (https://www.mirnet.ca/miRNet/faces/home.xhtml), we were able to construct three lncRNA-microRNA interaction networks that have shown common ceRNA activity between the lncRNAs with differential expression between the analyzed groups. To better illustrate the difference in expression between the three groups, we developed violin plots in the Jupyter Notebook. PRDM16-DT has a highly statistically significant ($p < 0.001$) downregulated expression in the favorable risk category in comparison with normal or poor cytogenetic risk categories (Figure 6).

DANCR is upregulated in poor prognostic cytogenetic patients in comparison to the intermediate/normal risk category, while the difference between favorable and poor or normal risk is not significant (Figure 6).
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**FIGURE 3**

(A) Heat map comparing the expression level of lncRNAs in Poor vs. Normal risk category. (B) Library Size Analysis between TCGA samples belonging to Poor or Normal risk category. (C) Volcano plot analysis of lncRNA expression differentiating Poor vs. Normal risk category. The set FC was at 1 and p value at <0.05. (D) Table of lncRNAs with significant different expression between the Poor and Normal risk category, their fold change, average expression and p-value. (E) LncRNA-microRNA interaction network analyzing the common ceRNA activities of the lncRNAs with different expression between Poor or Normal risk category.

**FIGURE 4**

(A) Heat map comparing the expression level of lncRNAs in Favorable vs. Normal risk category. (B) Library Size Analysis between TCGA samples belonging to Favorable or Normal risk category. (C) Volcano plot analysis of lncRNA expression differentiating Favorable vs. Normal risk category. The set FC was at 1 and p value at <0.05. (D) Table of lncRNAs with significant different expression between the Favorable and Normal risk category, their fold change, average expression, and p-value. (E) LncRNA-microRNA interaction network analyzing the common ceRNA activities of the lncRNAs with different expression between Favorable or Normal risk category.
OIP5-AS1 is down-regulated in favorable risk categories in comparison with normal risk category \((p < 0.01)\). The difference in expression between the other two categories is not significant (Figure 6). SNHG16, JPX FTX, and KCNQ1OT1 are significantly upregulated in the cytogenetically favorable risk category. The difference in expression between the other two categories is not significant (Figure 7).

GAS5 can differentiate between favorable and poor risk categories, being overexpressed in patients with poor cytogenetic prognostic (Figure 8). TP73-AS1 is upregulated in poor cytogenetic risk category in comparison with favorable and normal risk categories \((p < 0.01)\) (Figure 8).

There is published data on analysis made in AML cell lines after long-term exposure to chemotherapeutics or bone marrow analysis post-treatment. The IncRNA named urothelial carcinoma-associated 1 (UCA1) is generally overexpressed in AML and it was proven that acquired chemoresistance to adriamycin in HL60 cells is correlated with increased expression of UCA1. In adriamycin-resistant AML cells UCA1 sponges miR-125a thus releasing hexokinase 2 (HK2) and hypoxia-inducible factor 1α (HIF-1α) from its control. The overexpression of UCA1 in post-treatment AML cells was confirmed by clinical analysis done on pediatric patients (112). Linc00239 is another IncRNA whose increased expression is related to a higher resistance to doxorubicin, as shown by the KG-1 and HL-60 AML cell lines. However, it is involved in the intrinsic resistance to chemotherapy and its expression in acquired chemoresistance was not evaluated (90). HOXA-AS2 is overexpressed in the bone marrow of patients who acquired adriamycin resistance. There are two downstream targets, miR-520c-3p and S100A4, that may explain the involvement of this IncRNA in chemoresistance. Still, more experimental data is needed (127). Correlations between IncRNAs and the clinical outcome of AML treatment are still not validated in an experimental setting. This is the case of IRAIN, a IncRNA whose low expression is correlated with a worse prognostic after chemotherapy by a higher relapse rate (109). The increased mannosylation (addition of a mannose glycoside) on the membrane proteins is a process through which the AML cells U937 acquire adriamycin resistance. ALG3 alpha-1,3-mannosyltransferase is an enzyme responsible for this process. In ADR/U937 cells, the IncRNA FTX is overexpressed. This IncRNA targets miR-342, thus decreasing its expression and it releases ALG3 mRNA from the RNA interference inhibition (128).

The most commonly course of action in the case of a combinatorial treatment that would involve a IncRNA and a chemotherapeutic would be first sensitizing the cells, with the help of the IncRNA therapeutic inhibition or overexpression, depending on its initial pathological expression level, and then cells are completely eliminated with the help of the chemotherapeutic. In adriamycin-resistant AML cells, if the IncRNA UCA1 is exogenously inhibited, the chemoresistance is impaired, thus the cells are killed by their exposure to adriamycin (112). The knockdown of HOXA-AS2 in adriamycin-resistant AML cells, and further exposure of cells to the same chemotherapeutic resulted in decreased cell viability in vitro and...
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FIGURE 6 | Violin plot of lncRNA with different expression between Normal, Favorable, and Poor risk category for PRDM16-DT, DANCR, OIP5-AS1, SNHG16 IncRNAs. ** represents statistically significant data. *** represents very highly statistically significant data.

In order to get a better view of the malignant function of the IncRNAs found to have different expression values between the included cytogenetic risk categories, we researched the literature to find the role of these IncRNAs in other malignancies, as well as their previously reported functions in AML (Table 4).

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The use of mouse models has the advantage of testing in vivo the findings initially reported in vitro, and potentially using these findings for therapeutic purposes, specifically new therapies for hematological malignancies with no cure other than allogeneic transplantation, as is the case with MDS currently.

ANRIL is a lncRNA which has been reported to reprogram glucose metabolism in AML cells in vitro through the induced expression of the Adiponectin receptor (AdipoR1). The in vivo experimental model, in which NOD-SCID mice were injected with either shRNA against ANRIL or scrambled shRNA, followed by flow cytometry analysis of their BM sample, validated the in vitro data and offered more reliability to the study (153). The same experimental design was used in order to prove the role of HOXA-AS2 overexpression and inhibition of the miR-520c-3p/S100A4 axis in the case of chemoresistance development in AML (127). HOTAIR is a lncRNA which was shown to decrease the in vivo tumor formation capacity through the demethylation of the promoter region in the case of the tumor suppressor gene HOXA5 (154). The in vitro silencing of the zinc finger antisense 1 (ZFAS1) lncRNA led to a higher percentage of AML apoptotic cells and a slower rate of proliferation of AML cells, while in vivo it resulted in the formation of smaller tumors, as compared to untreated mice (94).

We have previously reported the potential role of the lncRNA CCAT2 in the highly amplified 8q24.21 region, with implications for both myeloid malignancies and digestive cancers (55, 129, 155). Fosselfeder et al. describe CCAT2 as a lncRNA with
FIGURE 7 | Violin plot of lncRNA with different expression between Normal, Favorable, and Poor risk category for SNHG16, JPX, FTX, and KCNQ1OT1 lncRNAs. * represents statistically significant data. ** represents very highly statistically significant data.

FIGURE 8 | Violin plot of lncRNA with different expression between Normal, Favorable, and Poor risk category for TP73-AS1 and GASS lncRNAs.
### TABLE 4 | General and specific role of IncRNAs with differential expression between different cytogenetic risk categories in AML.

| IncRNAs  | Function in other malignancies | Function in AML | References          |
|----------|--------------------------------|-----------------|---------------------|
| DANCR    | Oncogenic role, promotes migration, invasion, proliferation | No record found | (129, 130)          |
| PRDM16-DT| Tumor suppressor role reported to be overexpressed in Flt3-itd mutation for AML | (102, 131, 132) |                     |
| SNHG6    | Oncogenic role, stimulates cancer cell growth, migration, invasion, cell autophagy | No record found | (133–135)           |
| OIP5-AS1 | Oncogenic role, promotes proliferation, maintenance of cell stemness | No record found | (136–138)           |
| SNHG16   | Oncogenic role, stimulates proliferation, migration, invasion | No record found | (139–141)           |
| JRX      | Tumor suppressor role contributes to MDR in AML-M5 | No record found | (142)               |
| FTX      | Oncogenic role, stimulates glycolysis, malignant cell proliferation, invasion and migration, poor prognostic | (143–145) |                     |
| KCNQ1OT1 | Oncogenic role, promotes malignant cell proliferation, chemoresistance | No record found | (146, 147)          |
| TP73-AS1 | Oncogenic role, promotes malignant cell proliferation, invasion and migration, poor prognostic | No record found | (149, 150)          |
| GASS     | Tumor suppressor role, inhibits cell proliferation, invasion, and promotes apoptosis | Mutations in this gene leads to worse prognostic | (151, 152)          |

Results from literature review.

Genomic localization at the chromosomal region 8q24 amplified in many malignancies (130). This was first described in colon cancer by Ling et al. (131) and later in breast adenocarcinoma by Redis et al. (129, 132). The 8q24 region is highly conserved and encompasses the MYC gene. In myeloid malignancies, a joint study between our institutions was initiated from the observation that CCAT2 is overexpressed in CD34+ cells in the bone marrow and in mononuclear cells from peripheral blood isolated from MDS patients, as compared with age-matched healthy individuals. We later discovered that the SNP rs6983267 overlapping CCAT2 is found in two allelic forms that contain either T or G and by using allele specific CCAT2 transgenic mice, confirming this data on patient samples from the US and Romania. In vivo, using transgenic mice after 7–9 months, both CCAT2-G mice and CCAT2-T mice had clinical symptoms of an underlying malignancy when compared to the wild-type siblings, having severe leukopenia and lymphocytopenia, moderate anemia and thrombocytosis (55). The CCAT2 role in cancer progression is valid for other types of malignancies and the amplified cancer-associated chromosome 8q24 modulates the Wnt target gene Myc (133), thus affecting the proliferative and self-renewal potential of cancer stem cells (134, 135). Even if this lncRNA was validated in several solid malignancies, including hepatocellular carcinoma, renal cancer and pancreatic cancer (136–138), it has yet to be properly validated in myeloid malignancies. Should it be, it holds great promise to explain the progression of a myelodysplastic syndrome into an acute leukemia.

One of the limitations of the manuscript is the insufficient information presented regarding the potential use of IncRNAs as therapeutics. Very little information has been published thus far regarding the topic, as the mechanisms that underlie their function have yet to be fully elucidated. One direction would be to use these molecules as potential targets. If a long non-coding RNA functions via cis regulatory mechanisms, it is extremely challenging to restore its function without expressing at the specific genomic locus (139). However, blocking of the function of long non-coding RNAs could be achieved by several strategies, with the most straightforward approach of downregulation with RNA interference (140). To regain the function of a lncRNA that is lost or downregulated in a myeloid malignancy, the simplest method is to supply with synthetic lncRNA molecules with same function (141). This could be achieved with a non-coding RNA mimic or with its expression vectors (142, 143). When transfected into cells, lncRNA mimics could be processed into a single-strand RNA molecule to target coding genes similar to the endogenous lncRNA, but viable experimental data has yet to be published.

Still, IncRNAs may represent interesting potential targets in AML therapy and monitoring, as they constitute most cellular transcripts and play pivotal roles in hematopoiesis (144, 145). For example, the lncRNA linc00239 (NR_026774.1), 662 nucleotides in length, is upregulated in AML patients, on malignant behaviors and chemosensitivity in AML cells. The presence of linc00239 increases chemoresistance to doxorubicin in AML cells partially by preventing doxorubicin-induced apoptotic cell death and is linked to the activation of the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway. Thus, the inhibition of PI3K/Akt/mTOR using 1 μM NVP-BEZ235 (BEZ) abolished the inhibitory effect of linc00239 on chemosensitivity and the preventative effect on doxorubicin-induced cell death (90). Their use could be implemented in risk stratification and prognosis in the clinic. Thus, in a clinical trial setting that enrolled 275 non-M3 AML patients in Taipei, Tsi et al. have shown that higher lncRNA scores were significantly associated with older age and adverse gene mutations. Further, the higher-score patients had shorter overall and disease-free survival than lower-score patients, which were also confirmed in both internal and external validation cohorts (TCGA database) (104). Multivariate analyses linked a lncRNA
score to an independent prognosticator in AML, irrespective of the risk based on the ELN classification. In the ELN intermediate-risk subgroup, lncRNA scoring system could well dichotomize the patients into two groups with distinct prognosis. Within the ELN intermediate-risk subgroup, they showed that allogeneic hematopoietic stem cell transplantation could provide better outcomes on patients with higher lncRNA scores. Through a bioinformatics approach, they identified high lncRNA scores correlated with leukemia/hematopoietic stem cell signatures. Thus, incorporation of lncRNA scoring system in the 2017 ELN classification can improve risk-stratification of AML patients and help in clinical decision-making.

CONCLUSION

There are several lncRNAs that have been implicated in several roles in AML. ANRIL, RP11-732M18.3, LNC_104449, CASC15, CCDC26, DANC1, LINC00467, MIR9-3HG, AL035071.1, AP001042.1, RP11-395P13.6-001, FAM30A, H22954, LEF1-AS1, LINC00152, LINC00239, LINC00265, LINC00519, LINC00504, LINC00899, LINC00926, LINC77417, LRRCC75A-AS1, MALAT1, NEAT1, PANDAR, PILOA, RP11-222K16.2, RP11-305O6.6, SBF2-AS1, TUG1, UCA1, ZFAS1 have potential oncogenic roles in AML. RP11-704M14.1, SFMBT2-4:1, IRAIN, TET2 function as tumor suppressors in AML.

There are two lncRNAs with both oncogenic and tumor suppressor roles in AML. MEG3 is upregulated in AML-M3, but in AML cell lines is downregulated. CRINDE is downregulated in AML-M3 and upregulated in AML bone marrow samples and cell lines.

There are lncRNAs with specific expressions for each FAB AML subtype. H19 is upregulated in M1-2 AML. LINC00877, RP11-84C10.2, RP11-848P1.3, ZNF667-AS1 are downregulated in M3 AML, and LINC02082-201, AC009495.2 are upregulated in M3 AML. HOTTIP, SNHG5, CCAT1 are upregulated specifically in M4-5 AML. CCAT1, PVT1, and XLOC_109948 are overexpressed in AML patients bearing risk mutations. HOTAIR is upregulated only in stem cells from bone marrow samples of AML patients.

Our original analysis of TCGA data on AML revealed that PRDM16-DT is downregulated, and DANC1 is upregulated in poor cytogenetic prognostic AML samples compared with normal cytogenetic risk. OIP5-AS1 is downregulated in both poor and favorable prognostic compared with normal cytogenetic risk. SNHG6 is underexpressed in poor prognostic compared with intermediate cytogenetic risk and overexpressed in poor prognostic compared with favorable prognostic. SNHG16, JXP, FTX, KCNQ1OT1 are upregulated in AML samples with favorable risk as compared with normal risk. GAS5 is overexpressed in poor prognostic compared with favorable prognostic, whereas TP73-AS1 is upregulated specifically in poor prognostic patients, being overexpressed in comparison with favorable and normal cytogenetic risk. These lncRNAs have the potential of becoming future biomarkers of cytogenetic risk, however they still need further validation on bone marrow samples, peripheral blood from another cohort, along with additional gene expression analysis methods.

The potential of lncRNAs in their use as biomarkers or therapeutic targets is still in its infancy. More knowledge has to be gained on their established molecular interactions, biological roles, standardization of isolation and gene expression analysis. As follows, more data is needed to fully introduce the idea of clinical uses of lncRNAs in the clinical evaluation or treatment of AML, especially in a large cohort non-interventional clinical trial that would be able to compare the sensitivity and specificity of currently applied diagnostic/prognostic methods.

AUTHOR CONTRIBUTIONS

A-AZ did the bioinformatics analysis. CT and A-AZ wrote the manuscript. IS critically revised the manuscript. GC and IB-N contributed to the design of the manuscript and supervised the project.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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