Decoding the actionable potential of ribosome biogenesis in acute myeloid leukemia

Acute myeloid leukemia (AML) is a devastating hematological malignancy characterized by the rapid proliferation of abnormal myeloid progenitor cells in the bone marrow. AML progresses rapidly and has a high mortality rate, often within weeks or months if untreated. The disease primarily affects older adults, with a median age of 68 years, and has a poor prognosis, with a 5-year survival rate of <15% in patients older than 60 years at diagnosis.[1] Current standard treatment for AML typically involves intensive induction chemotherapy, often using the "7+3" regimen of cytarabine and an anthracycline, followed by consolidation therapy. While complete remission rates with this approach can reach 70-85% in patients with favorable genetics, overall outcomes remain suboptimal, particularly for older patients or those with high-risk features.[2] The limitations of current therapies, including treatment resistance and relapse, underscore the urgent need for novel therapeutic strategies. In recent years, the dysregulation of ribosomal biogenesis (RiBi) has emerged as a hallmark of cancer, including AML.[3,4] This intricate process, essential for assembling ribosomes and driving protein synthesis, plays a pivotal role in cellular proliferation and growth. Evidence suggests that cancer cells often exploit this mechanism through the development of specialized "onco-ribosomes," which promote oncogenic translation programs and contribute to metabolic rewiring.[5] The connection between RiBi and cancer progression sheds light on underlying disease mechanisms, offering promising avenues for targeted therapies and innovative treatment strategies. Recurrent somatic mutations in ribosomal proteins, found in 10-35% of multiple tumor types, including various hematological malignancies, highlight the significance of this process in oncogenesis. Additionally, disruptions in RiBi or translational control mediated by oncogenic factors can drive tumorigenesis.[5]

RiBi and translation are tightly controlled and influenced by the cellular microenvironment. Notably, the efficiency of RiBi varies across cell types, reflecting differences in functional demands. For instance, stem cells, such as hematopoietic stem cells (HSC), rely on low rates of protein synthesis to maintain metabolic homeostasis and self-renewal capacity.[5] Emerging research underscores that such tightly regulated translation is essential not only for HSC function but potentially for other types of somatic stem cells as well. As our understanding of RiBi deepens, it holds great potential to provide prognostic biomarkers and novel therapeutic targets. By optimizing our approach to cancer treatment, these insights may lead to significant improvements in patient outcomes.

In this study, we integrate transcriptomic data from multiple AML patient cohorts to build a RiBi-associated gene signature and define its prognostic and predictive value. Our findings uncover a RiBi-dependent vulnerability in AML that informs patient stratification and provides a rationale for personalized therapeutic interventions.

To find genes with actionable potential in AML, we collected bulk RNA-sequencing (RNA-seq) data from primary $CD34^+$ blasts acquired from AML adult patients and healthy donors corresponding to three different cohorts in accordance with the ethical regulations of the respective countries in which the studies were conducted[6-8] (ClinSeq, BEAT, FINN; Figure 1A). Our working pipeline which is outlined in Figure 1B, started with a differential expression (DE) analysis between 884 AML and 31 healthy donor RNA-seq samples. UMAP analysis showed a clear separation of the two groups (Figure 1C) following adjustment of the model for potential batch effects. We identified 6,427 deregulated genes (*P* adjusted [adj.]<0.05, log2 fold-change [FC] >0.5) that clustered in several Reactome pathways, exhibiting a substantial overrepresentation of terms related to RiBi and translation (Figure 1D).

Next, we subset the DE genes to filter only those corresponding to the gene ontology (GO) term RiBi and used the filtered outcome in a penalized Cox regression model with LASSO regularization that yielded a robust 31-gene signature significantly linked to overall survival in AML patients (Figure 1E-G). The gene signature was used to calculate a prognostic risk score (henceforth called RiBi score) for each patient by weighting the expression levels of the 31 genes with their corresponding β coefficients of the model (Figure 1H). The signature contains an equal amount of genes regulating early (e.g., *EXOSC2, PPAN, GEMIN4*) or late RiBi steps (e.g. *DDX56, NUDT16, SPOUT1*). Notably, 30% of the genes implicated in late RiBi events control mitochondrial RiBi (e.g., *RBFA, MRPS2, MTG1*) which may reflect the cumulative prognostic value in AML of two similar but spatially distinct processes. The signature contains also PRKDC, a central mediator of the DNA damage response with a distinct role in RiBi.[9] Based on this score, patients were stratified into low-risk (LR), moderate-risk (MR) and high-risk (HR) RiBi groups (Figure 1I).

Kaplan-Meier survival analysis, evaluated using the logrank test, revealed statistically significant differences in survival between the three groups, with RiBi status being inversely correlated to the 5-year survival of AML patients (Figure 2A). The prognostic value of the RiBi signature was further validated in the TCGA cohort,[10] reinforcing the ro-bustness of our analysis (Figure 2B, C). Cox proportional hazards regression analysis confirmed the independent prognostic

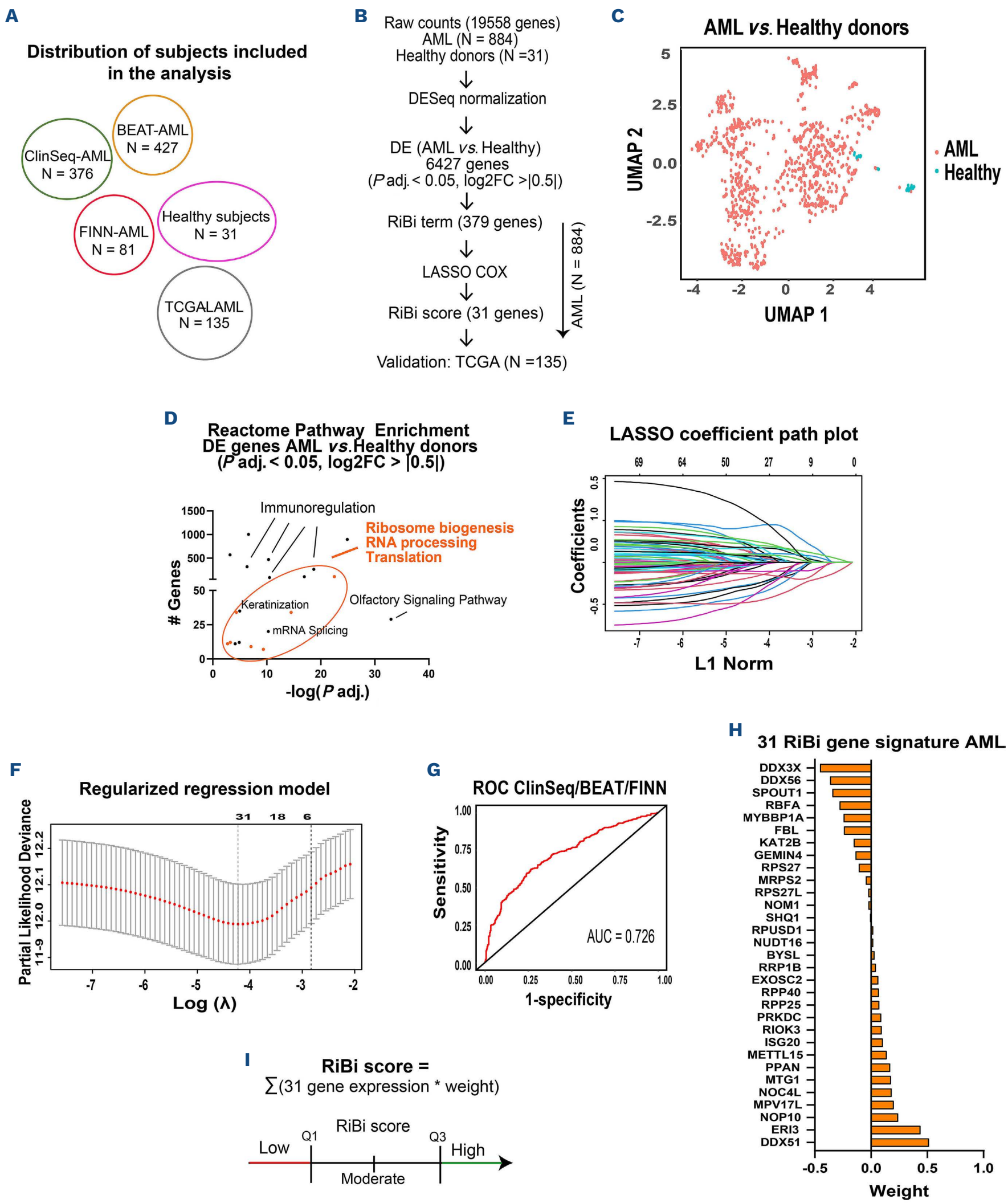

Figure 1. Development of an actionable ribosomal biogenesis signature in acute myeloid leukemia. (A) The sample size of the 3 cohorts used in the development of the 31-ribosomal biogenesis (RiBi) prognostic signature. (B) Graphical outline of the pipeline used for the detection and validation of a 31 RiBi gene prognostic signature in acute myeloid leukemia (AML). The 6,427 genes refer to differentially expressed (DE) genes between AML and healthy subjects fulfilling the criteria of *P* adjusted (*P* adj.<0.05, log2 fold-change [FC]>0.5. (C) UMAP of batch-corrected, normalized counts for AML patients and healthy donors from the cohorts presented in (A). (D) Reactome pathway analysis of DE genes between healthy individuals (N=31) and AML patients from 3 cohorts (ClinSeq, BEAT, FINN, N=884). The (y) axis shows the number of genes/Reactome terms, and the (x) axis shows the negative log-

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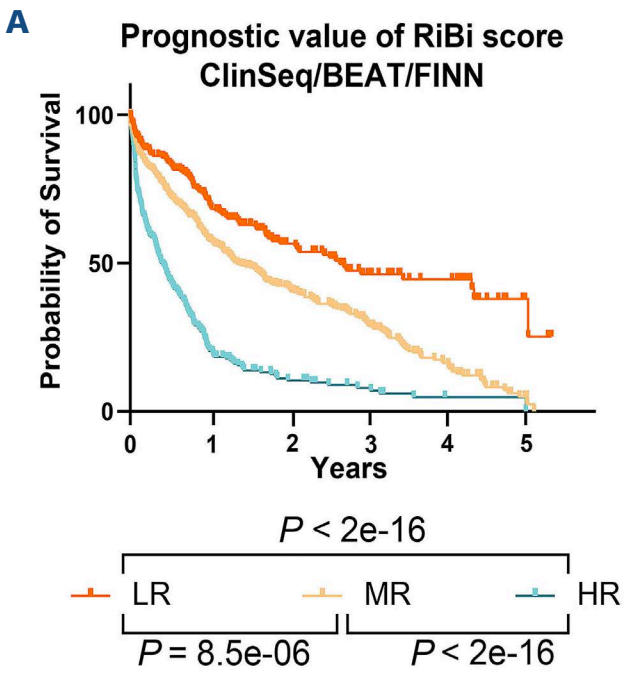
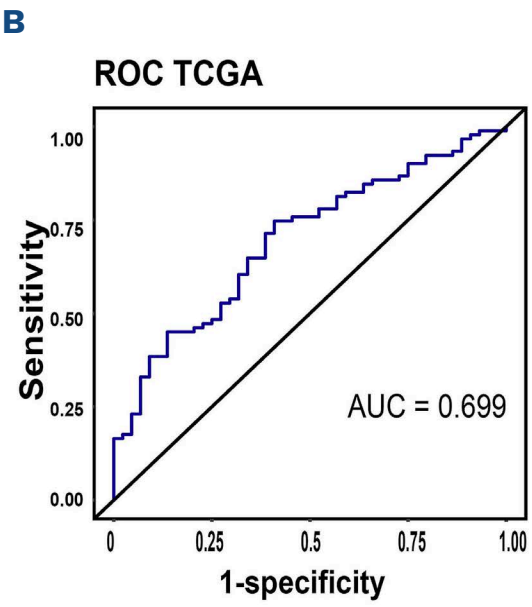
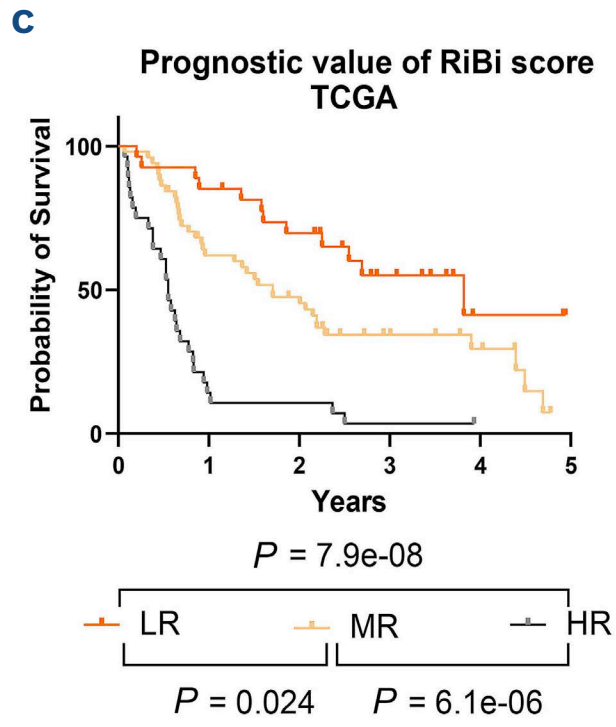
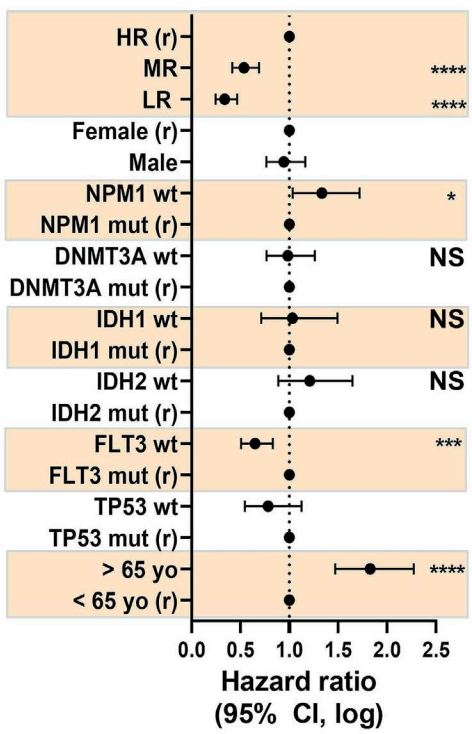
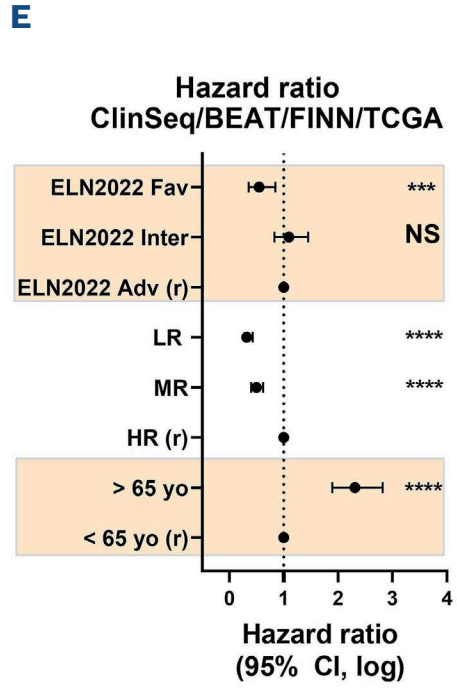
arithm of the adjusted *P* value for the enrichment test. Raw counts refer to bulk RNA-sequencing data. (E, F) Coefficient distribution for predictors (E) and cross-validation performance of the regularized Cox regression across different λ values (F) of the regression model used for the development of the gene signature. (G) ROC plot for the evaluation of the prediction model performance. (H) Barplot of the expression weights for the 31 RiBi prognostic genes in AML depicted on the left side of the plot. (I) Graphical illustration of the patient clustering method based on their RiBi score.

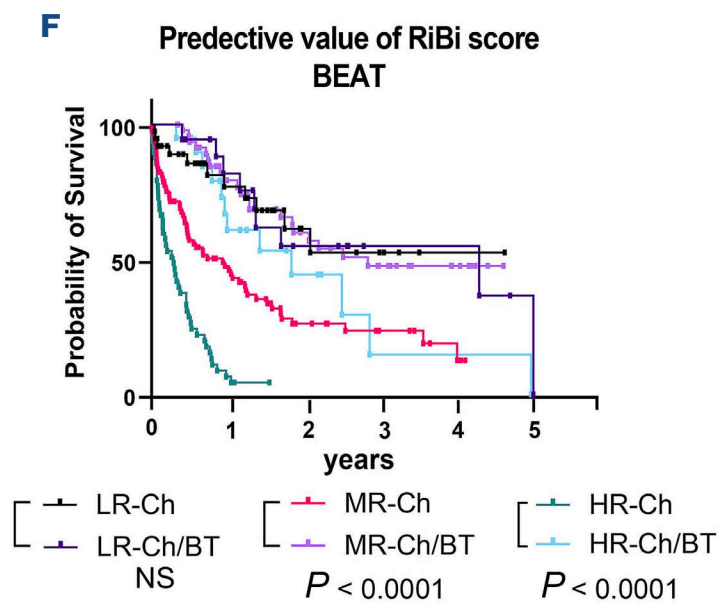


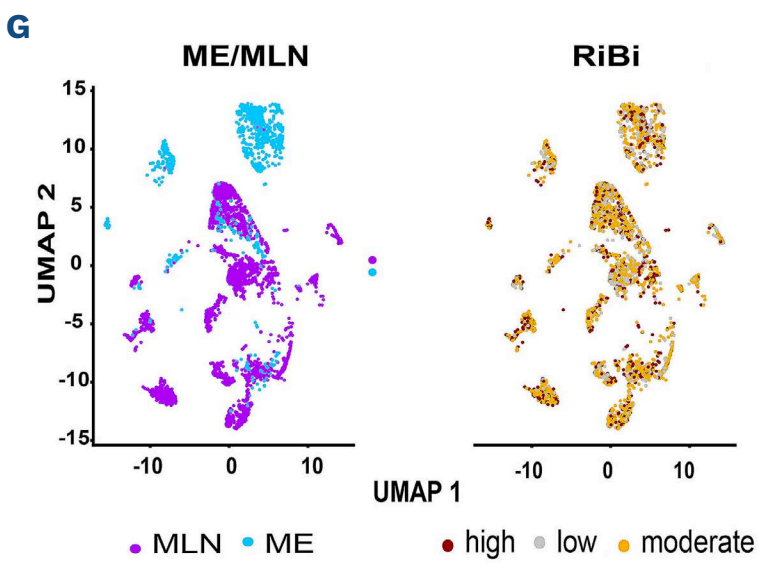












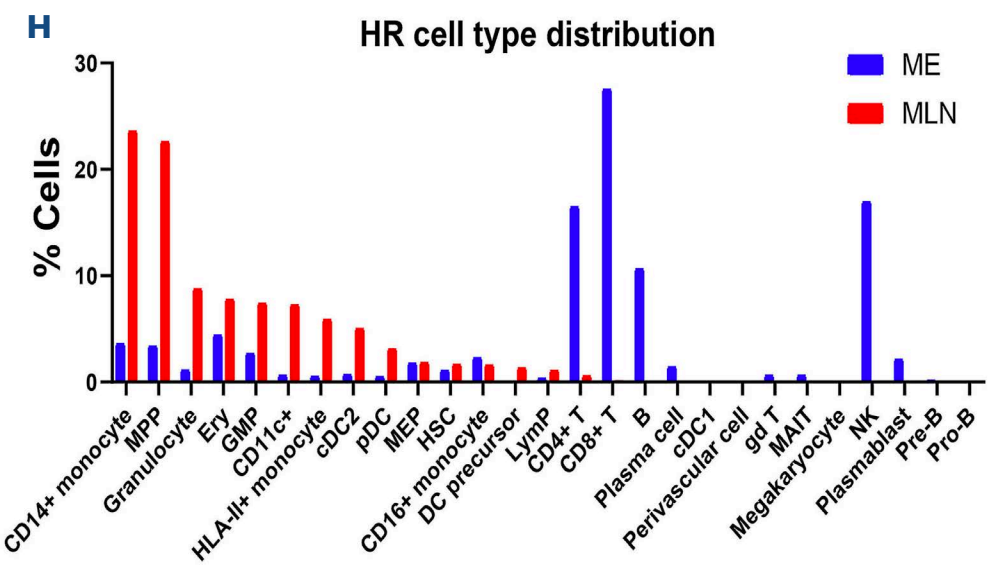


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Figure 2. Clinical value of the ribosomal biogenesis signature in acute myeloid leukemia. (A) Kaplan-Meier (KM) plot showing the prognosis of AML patients clustered in low-risk (LR), moderate-risk (MR) or high-risk (HR) ribosomal biogenesis (RiBi) groups. (B) ROC plot for the validation cohort (TCGA). (C) Survival plot for the 3 RiBi groups in the TCGA cohort. (D, E) Hazard ratio analysis of RiBi status and various actionable factors for AML prognosis (D) or the European Leukemia Network (ELN) 2022 genetic risk classification system (E). ****P<0.0001; ***P<0.001; *P <0.05; NS: not significant. (F) Survival KM plot of patients with different RiBi status that received either chemotherapy alone (Ch) or in combination with hemetopietic stem cell transplantation (HSCT) (BT) (BEAT-AML cohort). (G) UMAP plot of single-cell RNA-sequencing (scRNA-seq) data comparing the RiBi status on malignant (MLN) and non-malignant (non-MLN) cells of the tumor micro-environment (ME). (H) Distribution of different cell types following single-cell RNA-sequencing analysis of MLN and non-MLN cells of the tumor ME.[12] MPP: multipotent progenitors; HSC: hematopoietic stem cells; HR: hazard ratio; CI: confidence interval.

value of the RiBi status, even after adjusting for individual risk factors in AML such as *NPM1* and *TP53* mutations (Figure 2D), as well as after adjustment for the integrated European Leukemia Network (ELN) 2022 risk classification,[11] which incorporates cytogenetic and molecular features into a unified clinical framework (Figure 2E). These findings indicate that RiBi activity provides risk information beyond that captured by current clinical and molecular stratification models.

To assess the predictive value of our RiBi gene signature, we focused on the BEAT-AML[7]cohort that provides RNA-seq data from patients who received different treatments.[7] Our findings showed that patients with moderate to high RiBi, in contrast to those with low RiBi, had improved outcomes when treated with a combination of chemotherapy and HSC transplantation (HSCT) compared to chemotherapy alone, irrespective of the chemotherapeutic scheme that was followed (Figure 2F). This supports the potential role of the RiBi score as a stratification tool to reduce unnecessary transplant-related toxicity in low RiBi individuals. To further explore the role of RiBi in the tumor microenvironment, we analyzed single-cell RNA-seq data initially described in Lasry and colleagues.[12] This analysis revealed a shift in HR enrichment from mature immune cells (e.g., NK, $CD8^+$, $CD4^+$) in non-malignant samples to less differentiated progenitors and monocytic blasts (e.g., multipotent progenitors and $CD14^+$ monocytes) in malignant cells (Figure 2G, H). This redistribution suggests a metabolic reprogramming in leukemia cells that may promote stemness, immune evasion, and disease progression.[13] Overall, our comprehensive approach highlights the value of integrating bulk and single-cell data to develop clinically meaningful prognostic tools for AML, based on the expression of RiBi genes.

Despite significant advances in our understanding of AML, clinical outcomes remain dismal for many patients, particularly older adults and those with high-risk molecular features. Current prognostic models primarily rely on genetic and cytogenetic alterations, which, while informative, often fail to fully capture the disease's biological complexity or predict treatment response. Likewise, therapeutic regimens, including standard induction chemotherapy and targeted agents like FLT3 or IDH inhibitors, are frequently hindered by primary or acquired resistance, relapse, and lack of efficacy in patients with adverse-risk disease.[14] These limitations underscore an urgent need for new biomarkers that not only refine prognostic stratification but also inform therapeutic decision-making. In this context, our study introduces RiBi as a previously underexplored yet biologically critical determinant of AML progression and treatment vulnerability. Addressing this unmet clinical need, we propose RiBi as a novel and biologically grounded axis for AML classification. By quantifying RiBi activity, we uncovered a gene expression signature that not only refines risk stratification beyond conventional markers but also informs therapeutic responsiveness.

Through integrative analysis of bulk RNA-seq data from multiple AML patient cohorts, we identified a 31-gene RiBi signature that stratifies patients into LR, MR, and HR RiBi groups. This signature correlated inversely with long-term survival and retained independent prognostic value even when accounting for ELN 2022 risk classification and key genetic alterations, including *NPM1* and *TP53* mutations. The signature's prognostic strength was validated in an independent cohort, emphasizing its clinical robustness. Beyond prognosis, the signature also demonstrated predictive value, which is equally important for stratifying patients prior to therapeutic intervention to maximize clinical benefit. Single-cell transcriptomic data further revealed that high RiBi activity shifts from mature immune cells in healthy bone marrow to leukemic progenitors and blasts in AML, suggesting that elevated RiBi not only marks aggressive disease but also reflects a shift toward stem-like, immune-evasive cellular states.

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Contributions
Conceptualization by SM and DK. Methodology by SM, DCK, XZ, SP, and DDS. Investigation by all authors. Resources were provided by A-KÖF, SL, JB, and AL. Data curation by SM, DCK, and XZ. Bioinformatics by DK and XZ. Manuscript writing by SM and DCK. Manuscript review and editing by all authors. Visualization by SM, DCK, XZ, SP, and DDS. Supervision by A-KÖF, JB, and AL. Funding acquisition by A-KÖF, SL, JB, and AL.

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Data-sharing statement
The bioinformatics analysis performed in this study is deposited on GitHub (https://github.com/dimikanel/AML_RiBi).

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