ORIGINAL RESEARCH

Ferroptosis-Related Gene Signature for Prognosis Prediction in Acute Myeloid Leukemia and Potential Therapeutic Options

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Background: Limited data were available to understand the significance of ferroptosis in leukemia prognosis, regardless of the genomic background.

Methods: RNA-seq data from 151 AML patients were analyzed from The Cancer Genome Atlas (TCGA) database, along with 70 healthy samples from the Genotype-Tissue Expression (GTEx) database. Ferroptosis-related genes (FRGs) features were constructed by multivariate COX regression analysis and risk scores were calculated for each sample and a novel prediction model was identified. The validation was carried out using data from 35 AML patients and 13 healthy controls in our cohort. Drug sensitivity analysis was conducted on various chemotherapeutic drugs.

Results: A signature of 10 FRGs was identified, as prognostic predictors for AML, and the risk scores were calculated to constructed the prognostic features of FRGs. Significantly lower overall survival was observed in the high-risk group. The predictive ability of these features for AML prognosis was confirmed using Cox regression analysis, ROC curves, and DCA. The prediction model performed well in our clinical practices, and had its potential superiority when comparing to classical NCCN risk stratification. Multiple chemotherapy drugs, including paclitaxel, dactinomycin, cisplatin, etc. had a lower IC50 in FRGs high-risk group than low-risk group.

Conclusion: The AML prognosis model based on FRGs accurately predicts AML prognosis and drug sensitivity, and the drugs identified worthy further investigation.

Keywords: ferroptosis, prediction model, TCGA, acute myeloid leukemia, drug sensitivity

Introduction

Acute myeloid leukemia (AML) stands as a principal type of adult acute leukemia, predominantly affecting the elderly.[1] The development of AML is attributed to various factors, including chromosomal abnormalities, gene mutations, and exposure to certain chemicals.[2] The 5-year survival rate hovers around 30%.[3,4] Presently, the most widely used and precise assessment of AML relies on cytogenetic distinctions, where patients harboring high-risk molecular mutations exhibit poorer prognoses.[5–7] Nevertheless, with the emergence and application of novel drugs like BCL-2 inhibitors and FLT3 inhibitors, many AML cases with high risks, such as *FLT3, P53, RUNX1* mutations, have experienced improved remission and prolonged survival.[8–12] To date, several innovative prognosis models are under development, enhancing our comprehension of leukemia's initiation and progression.[13,14]

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Ferroptosis, a prevalent and ancient form of cell demise driven by iron-dependent phospholipid peroxidation, is regulated by diverse cell metabolism-related signaling pathways.[15–17] Ferroptosis plays a crucial role in cancer cells, particularly those prone to metastasis, and its involvement in AML has been documented. Leukemic cells often display heightened iron intake and diminished iron expulsion, culminating in elevated intracellular iron levels, a vital component for proliferation and differentiation.[18,19] Currently, certain ferroptosis-related genes (FRGs) have been confirmed to participate in the survival of AML cells.[20,21] GPX4, a crucial protein involved in ferroptosis, exhibits heightened expression profiles across numerous subtypes of AML, and its high expression is consistently associated with poor prognosis in AML patients.[22,23] The inhibition of GPX4 is regarded as a promising therapeutic strategy, with the capability to specifically induce ferroptosis and thereby eradicate myeloid leukemia cells.[24] Therefore, targeting iron balance and instigating ferroptosis may offer fresh insights into AML therapy.

Due to the intricacy of gene mutations, a solitary gene mutation may inadequately explain disease prognosis. Consequently, polygenic interactions have been incorporated into disease prognosis prediction models, facilitating the development of new models that integrate information from multiple genes to more comprehensively reveal disease outcomes.[25,26] Previous scholars have identified some prognostic models based on ferroptosis-related genes, which have certain clinical value but not been systematically clinically validated and cannot adequately guide clinical work.[27,28] In this context, we integrated multi-gene information from The Cancer Genome Atlas (TCGA) database and The Genotype-Tissue Expression (GTEx) database to formulate a novel prediction model for FRGs to supplement this. And we also further validated the model's accuracy using clinical data from our center and additionally forecasted drug sensitivity to explore potential therapeutic options for AML patients. This comprehensive approach seeks to furnish valuable insights into disease prognosis and offer potential implications for clinical treatment. This study has been registered at *chictr.org.cn* as *# ChiCTR*******, and the technological roadmap is depicted in Figure 1.

Materials and Methods
Identification of the FRGs Prediction Model
Data Collection and Processing
RNA-seq data and corresponding clinical information of LAML patients were obtained from the TCGA database (https://www.cancer.gov/ccg/research/genome-sequencing/tcga).[29] This dataset included follow-up time, gender, age, race, French-American-British (FAB) classifications, cytogenetic risk, and white blood cell (WBC) count ($\times 10^9$/L).
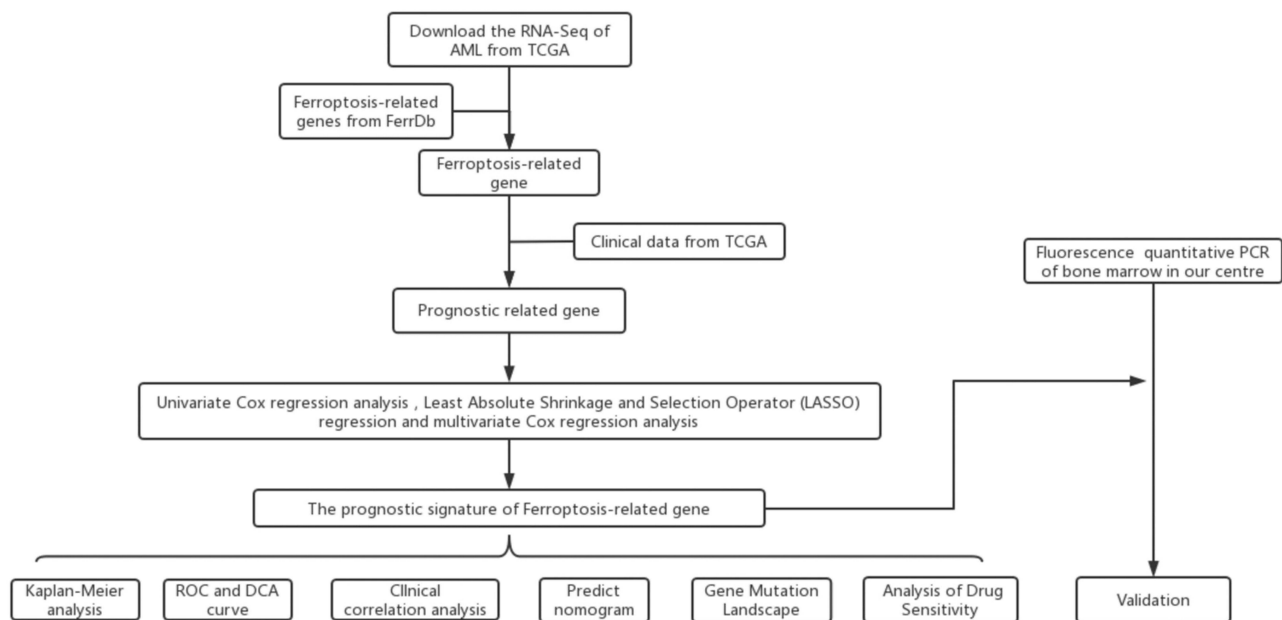

Figure 1 The technology roadmap of our study.

Additionally, RNA-seq data from healthy donors were downloaded from the GTEx database (https://gtexportal.org/). The set of ferroptosis-related genes (FRGs) was retrieved from the FerrDb database (http://www.zhounan.org/ferrdb/current/).[30] For further analysis, we normalized each microarray data, removed batch effects, and combined the data using the "sva" package. To identify differentially expressed FRGs, differential analysis was conducted using the "limma" package. Genes with significant differences in expression levels were selected based on the absolute value of $\log_2$ fold change (log2FC) > 1 and an adjusted P value < 0.05.

GO and KEGG Enrichment Analysis

The functions of up-regulated and down-regulated differentially expressed genes (DEGs) related to ferroptosis were examined. A P value of <0.05 was used as a threshold to screen for significant enrichment results. Gene Ontology (GO) enrichment analysis was employed to assess the biological pathways associated with the DEGs, with a focus on biological processes (BP), molecular functions (MF), and cellular components (CC) that were regulated by the differentially expressed FRGs. The BP, CC, and MF of TOP10 were visualized according to the Qvalue ranking. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was also performed using the "ggplot2" package in R. The TOP30 KEGG pathways were visualized according to the Qvalue ranking.

Confirmation of Prognostically Relevant FRGs

We only used data from AML patients to identify prognostic models. In subsequent analyses, we excluded 11 samples without survival information. The expression matrix of the FRGs was integrated with survival data, and the identification of genes closely associated with overall survival (OS) was carried out. Univariate Cox regression was employed to screen for FRGs associated with OS in AML patients. Subsequently, for a more refined selection, we leveraged the "glmnet" package in R, which implements Least Absolute Shrinkage and Selection Operator (LASSO) regression. This approach was further refined through 10-fold cross-validation to optimally tune the L1 penalty parameter, aiming to reduce the number of genes and thereby mitigate the risk of overfitting. A set of FRGs characteristics was constructed through multivariate COX regression analysis, and the risk score for each sample was calculated using the following formula:

$$Riskscore = \sum_{i=1}^{n} Coef\ RNAi \times Exp\ RNAi$$

where Coef RNAi is the ith FRG's coefficient and Exp RNAi is the ith FRG's expression. Risk scores were assessed for each AML patient, dividing them into low-risk (below the median) and high-risk (above the median) groups based on the median. The risk score was standardized using this formula: Risk score = (Risk score - Min) / (Max - Min). To comprehend the functional and expression-related relationships among individual FRGs, we employed STRING (https://string-db.org) with the confidence level = 0.400 and analyzed for pairwise correlation using spearman. Prognostic value assessment of the risk score was conducted using Kaplan-Meier analysis, univariate and multivariate COX regression analysis. A nomogram chart was developed based on prognostic characteristics to predict the OS of AML patients at 1 year, 3 years, and 5 years.

Comparison of Our Model with National Comprehensive Cancer Network (NCCN) Classical Model

We obtained AML RNA expression data, somatic mutation data, CNV files, and corresponding clinical and pathological information from the TCGA-LAML program. The "maftools" package was utilized to create Mutation Annotation Format (MAF) from the TCGA database for a deeper understanding of the somatic mutation landscape in AML patients. We categorized TCGA AML patients into poor/adverse, favorable, and intermediate groups following NCCN guidelines. Due to the limited number of patients in the favorable group, we conducted Kaplan-Meier analysis for patients in the favorable/intermediate group and poor/adverse group.

Validation of the Prediction Model
Clinical Data Sources

We conducted a retrospective study involving 35 AML patients and 13 healthy controls treated at the Hematology Department of ****** from September 2019 to September 2022. The diagnosis of AML was based on the Diagnosis and Therapeutic Effect Criteria for Hematological Diseases.[31] Inclusion criteria: Patients met the diagnostic criteria for newly

diagnosed AML. Exclusion criteria: Patients with diseases involving primary abnormal cell proliferation other than AML, such as myelodysplastic syndrome, chronic leukemia, etc.; patients with heterozygous and mixed leukemias; individuals with severe heart, brain, liver, kidney disease, and mental illness; Eastern Cooperative Oncology Group (ECOG) score > 2 points.

Real-Time Fluorescent Quantitative PCR for Bone Marrow FRGs

We employed real-time fluorescent PCR to assess the expression levels of FRGs in bone marrow samples, including ACSF2, CDO1, LPIN1, MYB, GPX4, MT1G, FH, DNAJB6, PSAT1, SOCS1, and ACTB mRNA. Total mRNA was extracted from cells using the one-step Trizol method following kit instructions. We extracted 2μg of total RNA from bone marrow cells, synthesized cDNA by sequentially adding 10 μL of 2 × ChamQ Universal SYBR qPCR Master Mix, 2 μL of Primer Mix (5μM), and 8 μL of Template cDNA and ddH2O. The reaction mixture was subjected to the following conditions: 37 °C for 15 min, then 85 °C for 5 s, and stored at 4 °C, then stored at −20 °C. Reaction conditions were as follows: 50 °C for 2 min (one cycle); 95 °C for 10 min (one cycle); followed by 40 cycles of 95 °C for 10s and 60 °C for 30s. Subsequently, 95 °C for 15s, 60 °C for 60s, and 95 °C for 15s were used. The data were calculated using the RQ=$2^{-\Delta\Delta Ct}$ method with ACTB as the internal reference. Primer sequences can be found in Table 1.

Validation of the FRGs Signature

Utilizing our prediction model, we categorized our AML patients into high-risk and low-risk groups. Kaplan-Meier analysis, COX regression, and Decision Curve Analysis (DCA) were used to validate the model.

Screening for Potentially Effective Drugs Based on Our Model

We retrieved data from the Genomics of Drug Sensitivity in Cancer (GDSC) database.[32] Construction of "ridge regression" to predict commonly used chemotherapeutic drugs IC50 based on GDSC cell line expression profile and TCGA gene expression profile, which were used to examine the clinical performance of chemotherapy in AML patients with varying risk score backgrounds.

Table 1 Primer Sequences for Ferroptosis-Related Genes

Gene	Primer
ACSF2	Forward: 5'-GAAGGACCTGGTGGTTGCTTA-3'
	Reverse: 5'-CAGTACCCTCGGATGCACAG-3'
CDO1	Forward: 5'-ATCAGCCATACGGAACCTGC-3'
	Reverse: 5'-CGAGCCCGAAGTTGCATTTG-3'
LPIN1	Forward: 5'-GGTGCATAGGGATAACTTCCTGA-3'
	Reverse: 5'-AGAAATCAATGGGGAATCTGTGGA-3'
MYB	Forward: 5'-TCCCAAGTCTGGAAAGCGTC-3'
	Reverse: 5'-GCACATCTGTTCGATTCGGG-3'
GPX4	Forward: 5'-AGCAAGATCTGCGTGAACGG-3'
	Reverse: 5'-TTCCACTTGATGGCATTTCCC-3'
MT1G	Forward: 5'-TTCCACGTGCACCCACTG-3'
	Reverse: 5'-GGAGCAGCAGCTCTTCTTG-3'
FH	Forward: 5'-TACCTGTGCATCCCAACGAT-3'
	Reverse: 5'-AACCACTAAATTCCTGCCCA-3'
DNAJB6	Forward: 5'-GCTGTCGGATGCTAAGAAACG-3'
	Reverse: 5'-GGTTACGGAATGTGAAGCCAAAT-3'
PSAT1	Forward: 5'-CATCTACGTCATGGGCTTGGTT-3'
	Reverse: 5'-GCCAGCTTCTGAACGTCTTC-3'
SOCS1	Forward: 5'-GTCCCCCTGGTTGTTGTAGC-3'
	Reverse: 5'-GAAGAGGTAGGAGGTGCGAG-3'
ACTB	Forward: 5'-GATTCCTATGTGGGCGACGA-3'
	Reverse: 5'-AGGTCTCAAACATGATCTGGGT-3'

Statistical Analysis

Statistical analysis was conducted using the Bioconductor software package in R×64 4.1.0. We assessed the sensitivity and specificity of the prognostic features in comparison to other clinical pathological features using receiver operating characteristic curves (ROC) and DCA. Logistic regression analysis and heatmaps were employed to analysis the relationship between clinical pathological features and FRGs. Kaplan-Meier survival analysis was used to evaluate the survival of AML patients based on ferroptosis features. Normally and non-normally distributed variables were analyzed by Unpaired Student's *t*-test and Wilcoxon test, respectively. A significance level of $P < 0.05$ was considered statistically significant in all analyses.

Results

Identification of FRGs Prediction Model for LAML

Enrichment Analysis of FRGs

A total of 221 samples, comprising 151 AML patients from the TCGA database and 70 normal donors from the GTEx database, were identified, and 184 DEGs related to ferroptosis were revealed through differential analysis (see Supplementary Table 1). GO enrichment analysis produced 2170 results, including 1712 BP enriched results primarily associated with cellular response to chemical stress, response to oxidative stress, and response to nutrient levels. There were 70 CC enriched results, primarily related to autophagosome, phagophore assembly site, and autolysosome, and 133 MF enriched results primarily related to functions like ubiquitin protein ligase binding, ubiquitin-like protein ligase binding, and RNA polymerase II-specific DNA-binding transcription factor binding (refer to Figure 2A and Supplementary Table 2). KEGG enrichment analysis resulted in the identification of 154 enriched pathways. Among them, Autophagy-animal, Ferroptosis, Mitophagy-animal, and Autophagy-other may play an important role in AML (refer to Figure 2B and Supplementary Table 3).

The Prognostic Features of 10 FRGs

In a univariate COX analysis, 30 genes (ACSF2, G6PD, PGD, LPCAT3, SLC1A5, CARS1, MAPK3, SOCS1, CDO1, MYB, LPIN1, IDH1, DNAJB6, BNIP3, DDIT4, SESN2, PSAT1, KLHL24, HSD17B11, AGPAT3, GPX4, HSPB1, HSF1, MT1G, FADS2, SRC, CDKN1A, ENPP2, FH, OTUB1) were found to be associated with the prognosis of AML (see Figure 3). Subsequently, through a multivariate COX analysis, 10 factors (ACSF2, SOCS1, CDO1, MYB, LPIN1, DNAJB6, PSAT1, GPX4, MT1G, and FH) were determined to be prognostic predictors for AML. Risk scores were calculated, and the prognostic features of FRGs were constructed using the formula: Risk score = −0.0234608484897763 * Expression of ACSF2 + 0.348749941214919 * Expression of CDO1 + 1.13835170050152 * Expression of LPIN1 + −0.10241309646517 * Expression of MYB + 0.353256404399872 * Expression of GPX4 + 0.828201566822827 * Expression of MT1G + −0.127737677888945 * Expression of FH + −0.871163009433076 * Expression of DNAJB6 + 1.39053870225325 * Expression of PSAT1 + −1.20437319972552 * Expression of SOCS1.

The Crosstalk Among 10 FRGs

Correlations among the 10 FRGs were shown in Protein-Protein Interaction Networks, with GPX4 and ACSF2 potentially being identified as key genes in sequencing according to degree (see Figure 4A). Correlation analysis indicated that the expressions of LPIN1 and ACSF2, GPX4 and ACSF2, PSAT1 and SOCS1, DNAJB6 and MYB, MT1G and PSAT1, MT1G and GPX4, FH and PSAT1, FH and MT1G were found to be positively correlated. Conversely, the expressions of CDO1 and ACSF2, MYB and SOCS1, GPX4 and MYB, GPX4 and DNAJB6, PSAT1 and LPIN1, MT1G and LPIN1, FH and DNAJB6 were found to be negatively correlated ($P < 0.05$, see Figure 4B).

The Risk score Serves as an Independent Prognostic Indicator for LAML Patients

Kaplan-Meier analysis revealed a significantly lower survival rate in the high-risk group compared to the low-risk group ($P < 0.001$, as shown in Figure 5A). A negative correlation between the risk score and the survival rate of AML patients was observed (Figure 5B). Subsequently, univariate and multivariate COX regression analyses were performed to assess whether clinical characteristics (Age, Gender, Race, WBC count ($\times 10^9$/L), FAB classifications, Cytogenetic
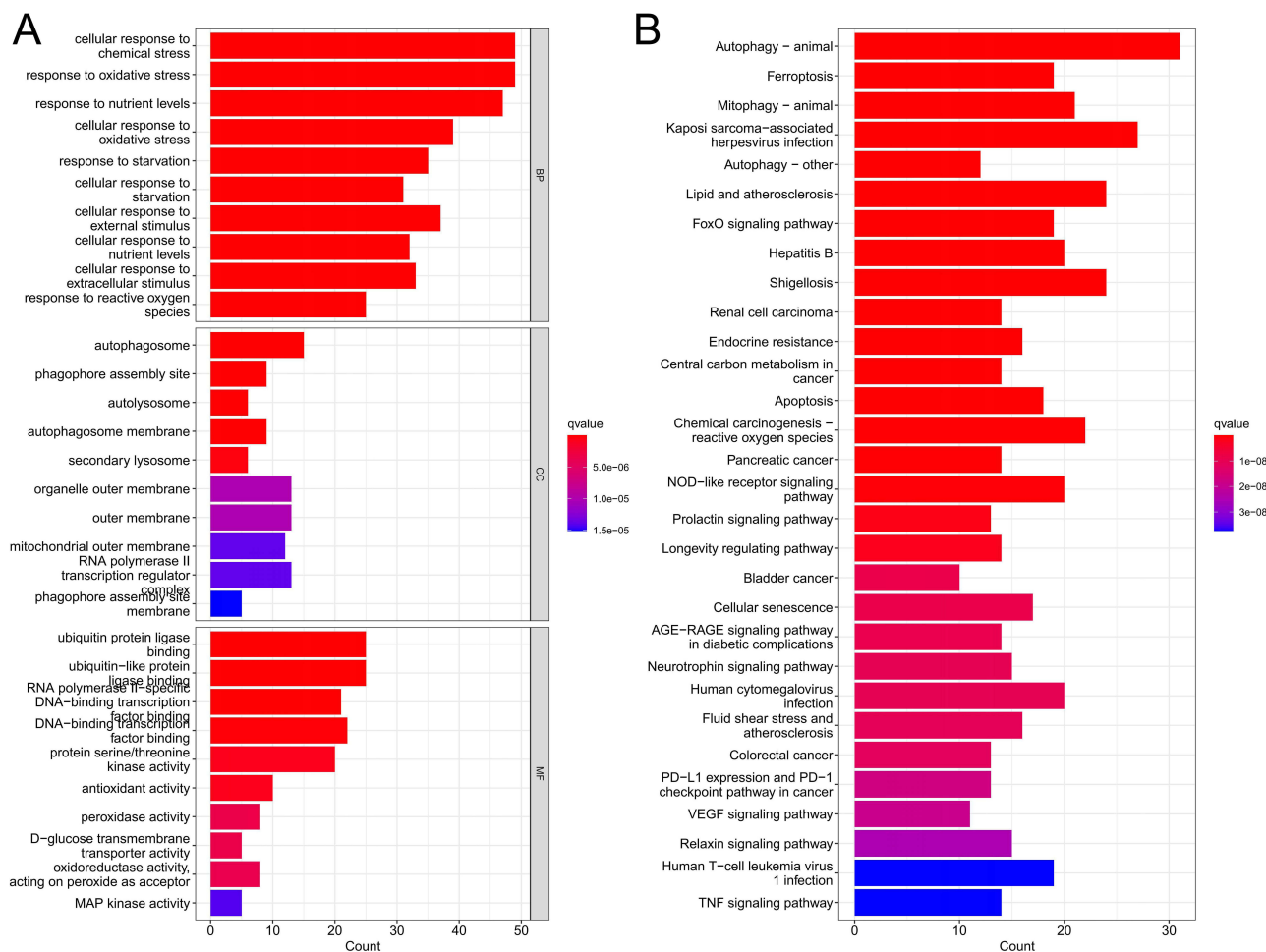
Figure 2 Enrichment analysis of ferroptosis-related genes. (**A**) GO enrichment analysis showed a total of 2170 results, including cellular response to chemical stress, response to oxidative stress, autophagosome, phagophore assembly site, ubiquitin protein ligase binding, ubiquitin-like protein ligase binding, etc. (**B**) GO enrichment analysis showed 154 enriched pathways, including Autophagy-animal, Ferroptosis, Mitophagy-animal, etc.

risk) and risk score were independent predictors of OS. The results of the univariate COX analysis indicated that FRGs risk features (Hazard Ratio (HR) = 1.018, 95% CI: 1.011–1.025), Age (HR = 1.049, 95% CI: 1.031–1.067), and Cytogenetic risk (HR = 0.568, 0.410–0.786) were all significant risk factors (Figure 5C). The results of the multivariate COX analysis showed that FRGs risk features (HR = 1.014, 95% CI: 1.006–1.021), Age (HR = 1.040, 95% CI: 1.023–1.058), and Cytogenetic risk (HR = 0.596, 0.412–0.864) were all significant predictors of OS (Figure 5D). The P values for age, cytogenetic risk, and risk score in univariate and multivariate COX analysis were all less than 0.05, indicating that they were independent predictors of OS in AML patients.

Furthermore, ROC and DCA were employed to evaluate the sensitivity and specificity of the prognostic features of AML clinicopathological characteristics. The results of the ROC showed that Age (AUC = 0.721, 95% CI: 0.624–0.818), Gender (AUC = 0.546, 95% CI: 0.455–0.637), Race (AUC = 0.522, 95% CI: 0.477–0.567), WBC count (AUC = 0.538, 95% CI: 0.431–0.645), FAB classifications (AUC = 0.604, 95% CI: 0.501–0.707), Cytogenetic risk (AUC = 0.354, 95% CI: 0.264–0.444). The area under the curve (AUC) for the FRGs was 0.722 (95% CI: 0.631–0.812) (Figure 5E), with AUCs of 0.722, 0.693, and 0.651 for 1, 2, and 3 years, respectively (Figure 5F), suggesting its capability to predict the prognosis of AML patients (Figure 5G). Additionally, the correlation heatmap of the prognostic features of FRGs with clinical and pathological features was analyzed (Figure 5H). By integrating clinical pathological characteristics, a nomogram chart of the prognostic features of FRGs was constructed, providing a more accurate and stable representation of the mutual relationships between variables and playing a certain role in the clinical management of AML patients, patients with high scores mean that they may have a shorter survival time (Figure 5I).
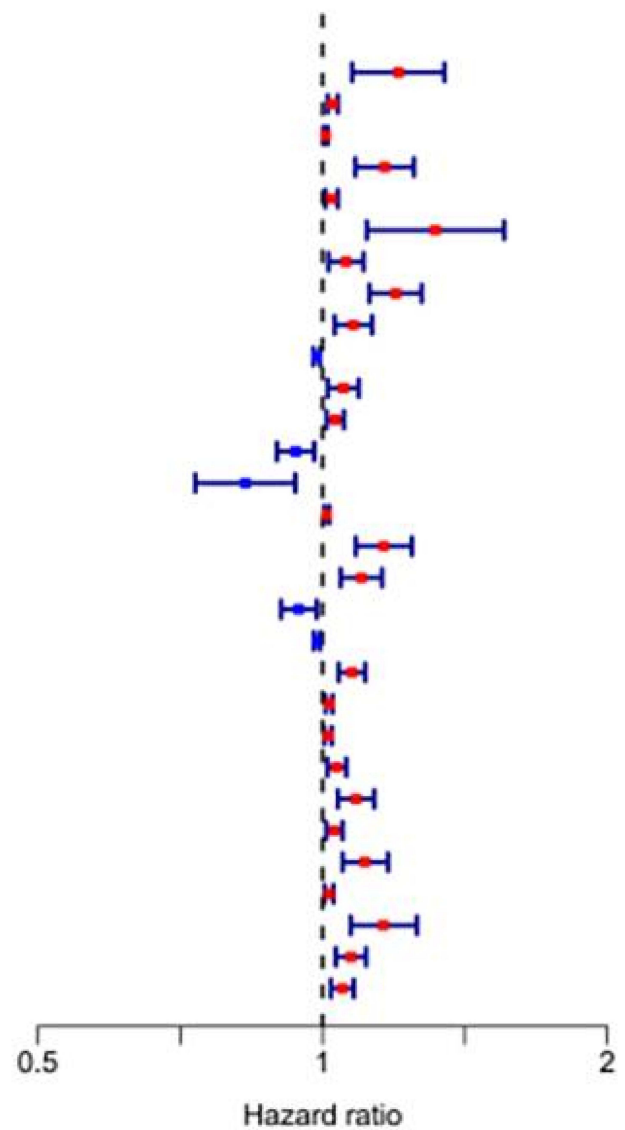
Figure 3 Univariate Cox analysis of ferroptosis-related genes.

Advantage and Limitation of the Risk Stratification Based on Genomic Mutation

Somatic mutation data from the TCGA-LAML dataset were analyzed, revealing that 66.43% (93 out of 140) of AML samples exhibited gene mutations, with 153 FRG mutations. Missense mutation was the most prevalent type, and single nucleotide polymorphism (SNP) was the most common variant type, with C>T being the most frequently observed single nucleotide variant (SNV). Among the mutated genes in AML, NPM1 exhibited the highest mutation frequency, followed by TTN, DNMT3A, IDH2, and TP53 (Figure 6A). Furthermore, a waterfall plot was utilized to depict the top 50 genes with the highest mutation rates, with TP53, FLT3, and KRAS mutations among the top 15 genes (Figure 6B). Based on molecular genetic changes, AML patients were categorized into poor/adverse and favorable/intermediate groups. Kaplan-Meier analysis demonstrated a lower survival rate in the poor/adverse group compared to the favorable/intermediate group ($P = 0.062$) (Figure 6C), though the difference was not statistically significant. Furthermore, DCA were employed to evaluate the sensitivity and specificity of the prognostic features of NCCN model and our model (Figure 6D).
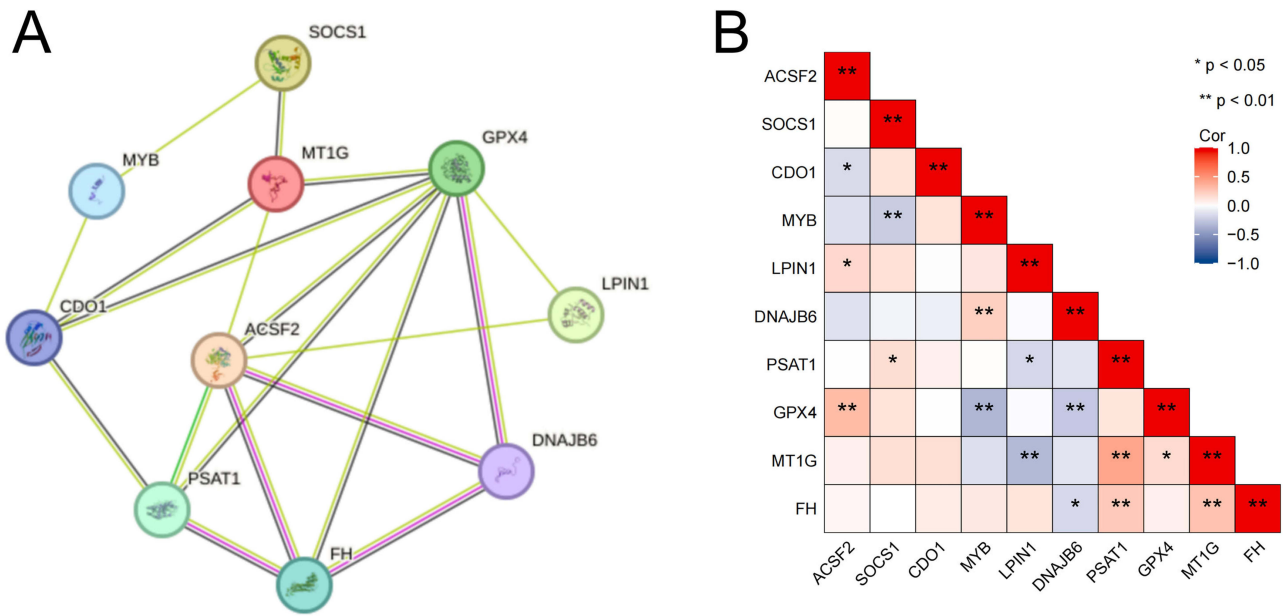
Figure 4 Protein-protein interaction networks (**A**) and correlation analysis (**B**) of 10 FRGs. **P < 0.01, *P < 0.05.
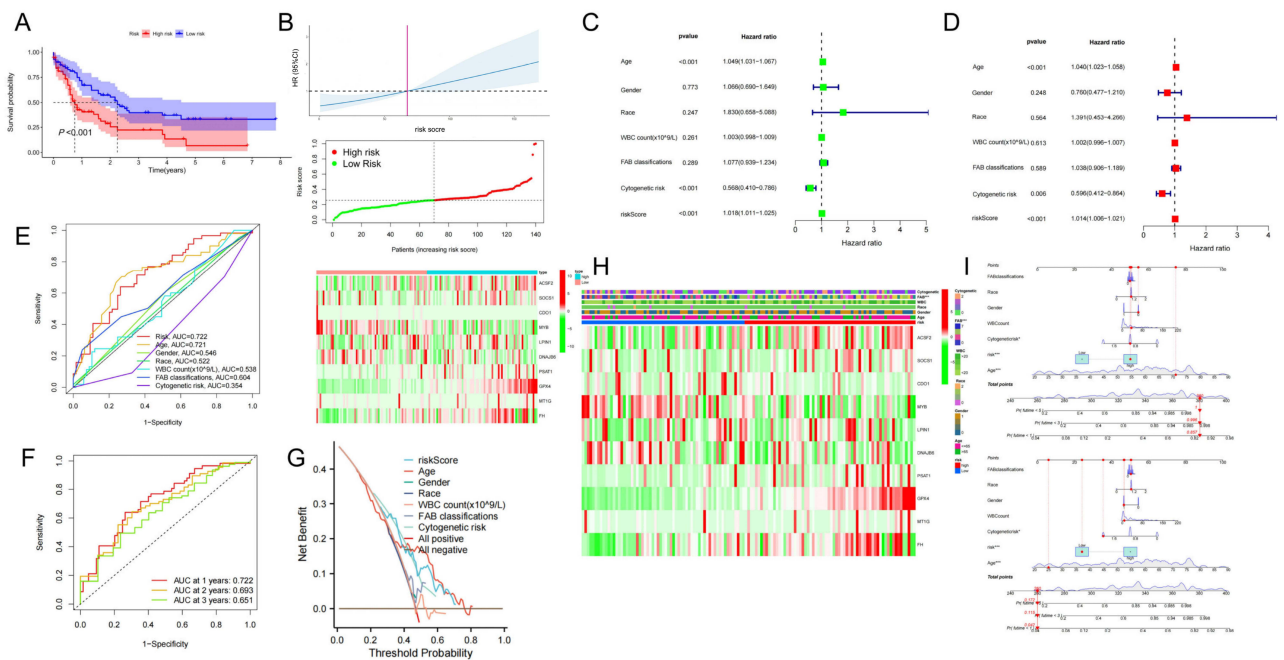

Figure 5 Development and validation of prognostic ferroptosis-related gene signature. (**A**) showed that patients in the high-risk group had a significantly lower survival rate compared to those in the low-risk group (P < 0.001). (**B**) showed the risk score, survival status and heatmap of patients in the two groups. Univariate (**C**) and multivariate (**D**) cox regression analyses showed that age, cytogenetic risk and risk score were independent predictors of OS in AML patients (P < 0.05). ROC analysis (**E**), time-dependent ROC analysis (**F**), DCA analysis (**G**) showed risk score can effectively predict the prognosis of AML patients. 5H showed the heatmaps of clinical information and gene characteristic. 5I Nomogram Diagram has a certain role in the clinical management of AML patients.

FRGs performed well in terms of prognostic accuracy based on our cohort samples.

Overall, 13 healthy controls (7 males, 53.85%) and 35 newly diagnosed AML patients (20 males, 57.14%) (including 4 patients diagnosed with AML-M1, 13 with AML-M2, 3 with AML-M3, 4 with AML-M4, and 11 with AML-NA) were included in this analysis (detail showed in Table 2). The enrolled AML patient cohort exhibited a median age of 58 years (ranging from 9.3 to 90 years) and the median follow-up was 6.9 months (ranging from 1 to 49.6 months).
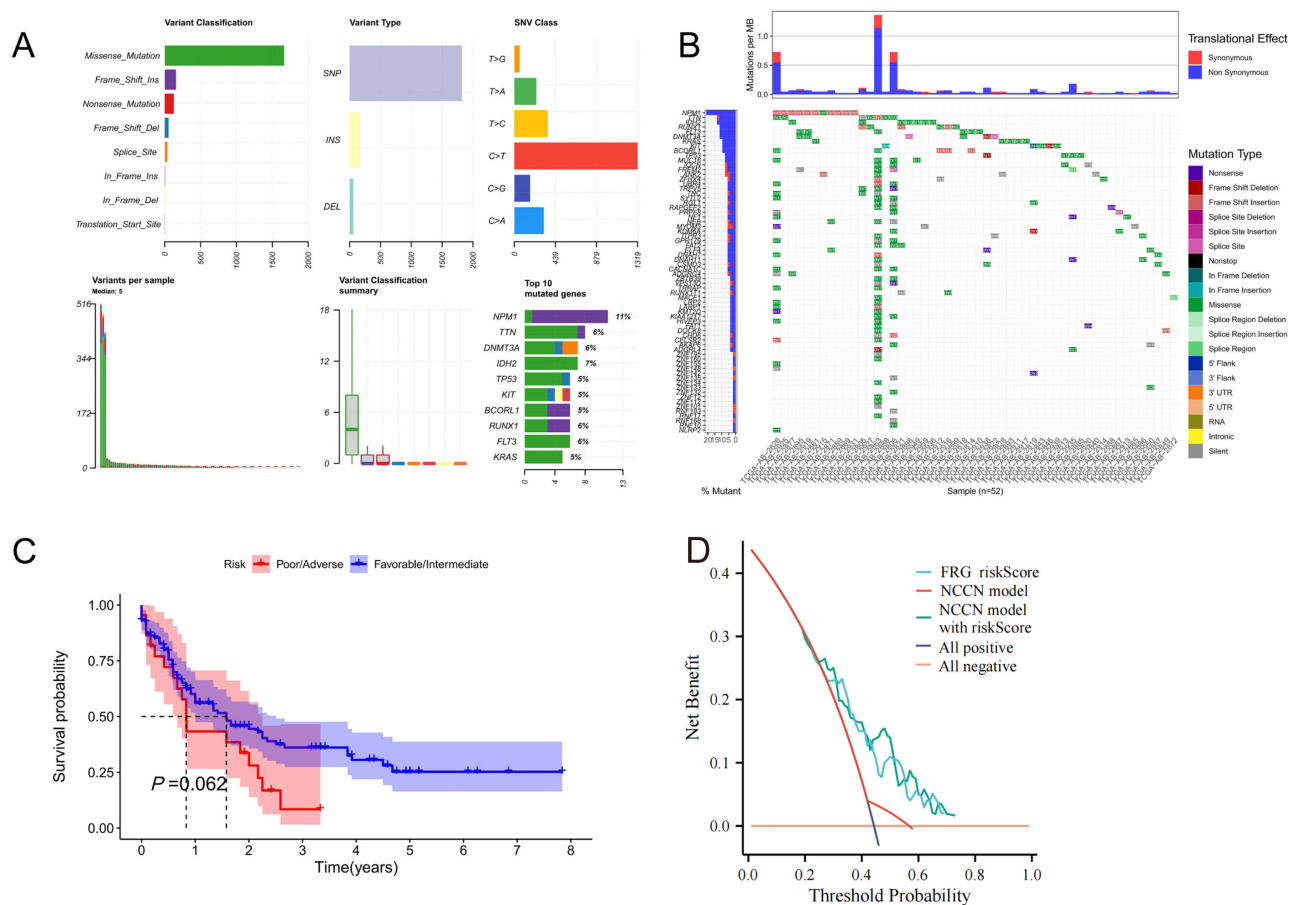
Figure 6 Gene mutation landscape and survival analysis of AML. (**A**) showed that AML patients has a high mutation rate. Gene mutation waterfall diagram (**B**) showed the top 50 genes with the highest mutation rates. Kaplan-Meier analysis showed (**C**) a lower survival rate in the poor/adverse group compared to the favorable/intermediate group (*P* = 0.062). DCA analysis (**D**) showed the sensitivity and specificity of the prognostic features of NCCN model and our model.

Fluorescence quantitative PCR was conducted on bone marrow samples from both AML patients and healthy donors, using ACTB as an internal control. The results indicated significant differences in the expression levels of ACSF2, LPIN1, MYB, GPX4, MT1G, FH, and PSAT1 between healthy individuals and AML patients (*P* < 0.05, as depicted in Figure 7A). Utilizing our prediction model, we stratified AML patients into high-risk and low-risk groups. Among these, 17 patients were included in the high-risk group and 18 patients were included in the low-risk group. Age, gender, diagnose, risk stratification by genetics (NCCN), and initial treatment, were not statistically different between the two groups (Supplementary Table 4).

Furthermore, Kaplan-Meier analysis unveiled that the high-risk group exhibited a markedly lower survival rate compared to the low-risk group (*P* < 0.01, as shown in Figure 7B). The results of the univariate COX analysis indicated that FRGs risk features (HR = 1.384, 95% CI: 1.153–1.660), remission after initial treatment (HR = 0.189, 95% CI: 0.055–0.656), and refractory / relapse (HR = 5.163, 1.364–19.553) were all significant risk factors (Figure 7C). The results of the multivariate COX analysis showed that FRGs risk features (HR = 1.497, 95% CI: 1.108–2.024), and refractory / relapse (HR = 13.178, 95% CI: 1.390–124.949) were all significant predictors for OS (Figure 7D). We observed an AUC of 0.763 for the FRGs in the ROC (Figure 7E), with AUCs of 0.763, 0.798, and 1.000 for 1, 2, and 3 years, respectively (Figure 7F), validating its capability to predict the prognosis of AML patients (Figure 7G).

Potential Treatment for AML Patients Based on Our Prognostic Model

Referring to prior reports,[7,33–37] we obtained 20 chemotherapy drugs potentially applicable for leukemia treatment (navitoclax, crizotinib, pinometostat, mitoxantrone, nilotinib, nelarabine, gefitinib, ibrutinib, paclitaxel, 5-fluorouracil,

Table 2 Clinical Characteristics of the AML Patients in Our Center

Patient #	Gender (F/M)	Age (years)	Diagnosis	Treatment	Risk stratification (NCCN)	Risk status (FRGs prognosis model)	Remission after initial treatment	Refractory/ Relapse within follow up	OS (months)	Outcome
1	F	65	AML	IA regimen	Poor/Adverse	Low	Yes	No	49.6	Alive
2	F	41	AML-M4	IDA; HAA regimen	Poor/Adverse	Low	No	Yes	31.1	Alive
3	M	55	AML	HAA regimen; IDA + HHT	Poor/Adverse	High	Yes	No	1.5	Dead
4	M	42	AML-M2a	HAA regimen; IA regimen	Favorable	Low	Yes	Yes	25.2	Alive
5	M	63	AML-M1	HA regimen	Poor/Adverse	Low	Yes	No	22.2	Alive
6	F	55	AML-M2	HA regimen	Poor/Adverse	High	Yes	No	21.2	Alive
7	M	62	AML-M2	HAA regimen; HA regimen	Poor/Adverse	Low	Yes	No	34.2	Alive
8	M	68	AML	AZA + HA regimen	Poor/Adverse	High	Yes	No	29.3	Alive
9	F	64	AML	AZA + HA regimen	Poor/Adverse	High	Yes	Yes	10.8	Dead
10	F	57	AML-M5	Ara-C+ VEN; AZA	Poor/Adverse	High	No	Yes	2.0	Dead
11	M	25	AML-M2a	IA regimen	Intermediate	Low	No	No	28.9	Alive
12	F	60	AML-M5b	VEN; AS+VEN	Poor/Adverse	Low	No	Yes	39.5	Dead
13	F	58	AML-M2a	HAA regimen; AZA + VEN	Poor/Adverse	High	No	Yes	6.9	Dead
14	M	96	AML-M5	AZA + VEN	N/A	High	Yes	Yes	4.8	Dead
15	F	64	AML	Ara-C	Poor/Adverse	High	No	No	1.5	Dead
16	F	74	AML-M2	AZA + VEN; VEN + HAA regimen	Poor/Adverse	High	Yes	No	2.5	Alive
17	F	55	AML	DA	Intermediate	High	Yes	Yes	19.7	Alive
18	M	18	AML-M2b	VEN + HAA regimen; FA regimen	Favorable	High	Yes	No	15.2	Alive
19	M	32	AML	IDA; G-CSF + HAD regimen	Intermediate	Low	Yes	No	14.4	Alive
20	M	58	AML-M1	HAA regimen	Poor/Adverse	High	No	Yes	1.0	Dead
21	M	87	AML-M5b	AZA + VEN	N/A	High	No	Yes	4.7	Alive
22	F	52	AML-M2	AZA + VEN	Intermediate	High	No	Yes	3.0	Dead
23	F	58	AML-M2a	AZA + VEN	Intermediate	Low	Yes	No	20.5	Alive
24	M	31	AML	IA regimen	Poor/Adverse	Low	Yes	No	13.2	Alive
25	M	61	AML -M4	AZA + VEN	Favorable	High	Yes	No	4.8	Alive
26	M	46	AML-M2a	IA regimen	Poor/Adverse	Low	Yes	No	5.9	Alive
27	F	26	AML-M1	Ara-C + VEN; HAE regimen	Intermediate	Low	Yes	No	10.9	Alive
28	M	9.3	AML-M2	HAG regimen; VEN + SOR + AZA	Poor/Adverse	High	No	Yes	5.9	Dead
29	M	35	AML-M2	AZA + VEN; IDA	Intermediate	Low	Yes	No	6.8	Dead
30	M	74	AML	AZA + VEN	Intermediate	High	Yes	No	13.8	Alive
31	M	59	AML-M1	AZA + VEN; HA	Intermediate	Low	Yes	No	3.5	Alive
32	M	58	AML-M4	AZA + VEN+HHT	Favorable	Low	Yes	No	3.1	Alive
33	F	64	AML	AZA + VEN	Poor/Adverse	Low	Yes	No	5.3	Alive
34	F	70	AML-M2a	AZA + VEN	Poor/Adverse	Low	Yes	No	4.1	Alive
35	M	57	AML	AZA + VEN	Poor/Adverse	Low	Yes	No	3.3	Alive

Notes: IA regimen: IDA + Ara-c; HAA regimen: HHT + Ara-C + ACM; HA regimen: HHT + Ara-C; FA regimen: Flu + Ara-C; HAD regimen: HHT + DA + Ara-C; HAE regimen: HHT + Ara-c + Eto; HAG regimen: HHT + Ara-C + G-CSF.
Abbreviations: AML, Acute Myelocytic Leukemia; DA, Daunorubicin; IDA, Idarubicin; Ara-c, Cytarabine; HHT, Homoharringtonine; ACM, Aclacinomycin; AZA, Azacytidine; VEN, Venetoclax; IDR, Idarubicin; AS, Arsenious acid; Flu, Fludarabine; G-CSF, Granulocyte colony-stimulating factor; Eto, Etoposide; SOR, Sorafenib; CR, complete response; PR, partial response; NR, No Response.
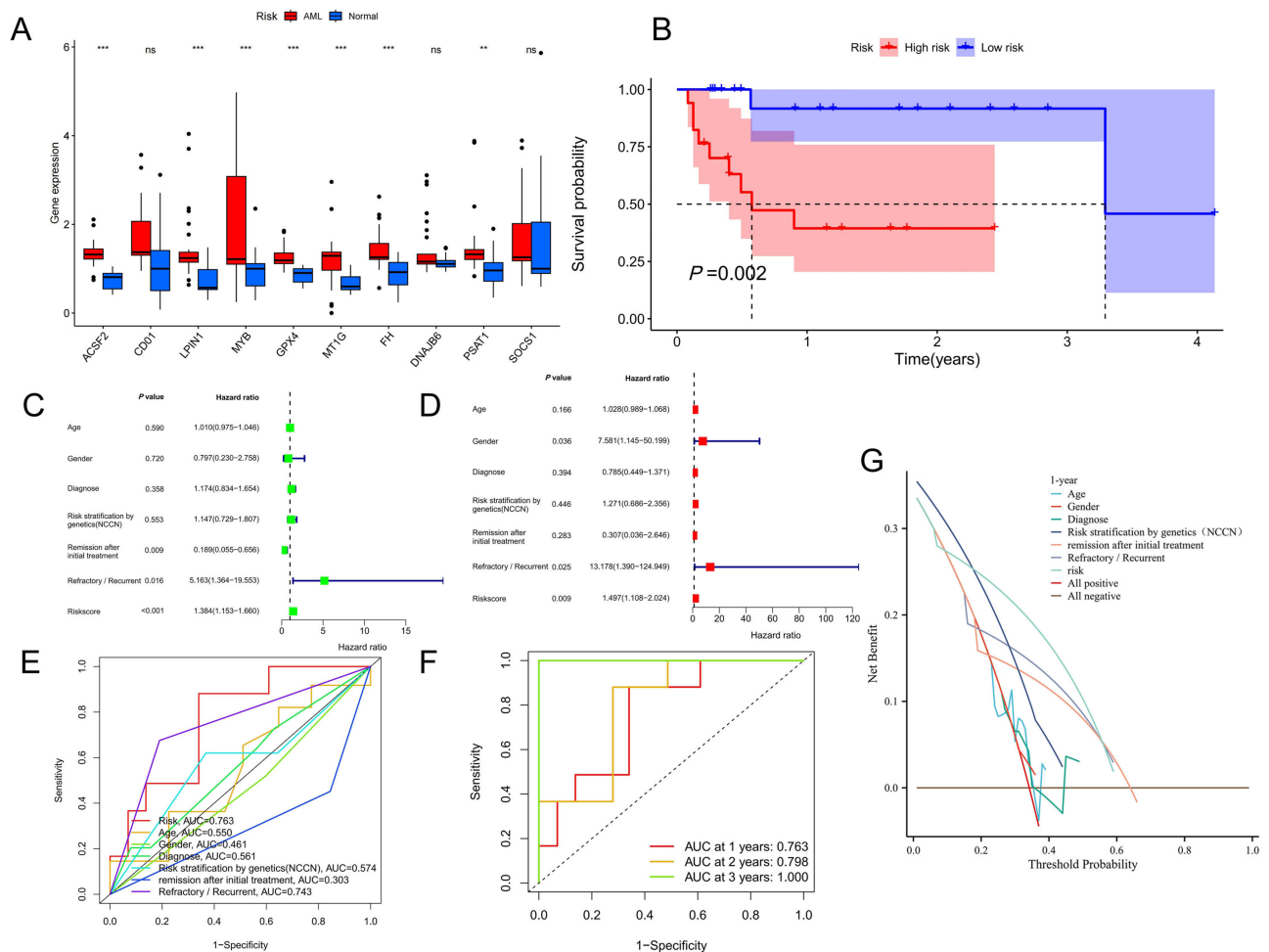
Figure 7 Clinical sample studies at our center. Real-time fluorescence quantitative PCR (**A**) on the bone marrow of AML patients and healthy donors. Kaplan-Meier analysis (**B**) showed the survival rate of the high-risk group was significantly lower than that of the low-risk group. Univariate (**C**) and multivariate (**D**) cox regression analyses, ROC analysis (**E**), time-dependent ROC analysis (**F**), and DCA analysis (**G**) validated the feasibility of FRGs prediction model. ***P < 0.001, **P < 0.01, ns: not significantly.

cisplatin, topotecan, vinorelbine, cyclophosphamide, vorinostat, camptothecin, dactinomycin, irinotecan, carmustine, cytarabine) from GDSC. Subsequently, we predicted their IC50 values in TCGA-LAML patients based on our prognostic model. Our findings revealed that the IC50 values of navitoclax (a BCL-2 inhibitor) in the high-risk group were significantly higher than those in the low-risk group. Additionally, paclitaxel (an autophagy inducer), 5-fluorouracil (an antipyrimidine agent), cisplatin (a mitotic inhibitor), topotecan (a topoisomerase I inhibitor), vinorelbine (an antimitotic agent), camptothecin (a topoisomerase I inhibitor), dactinomycin (an autophagy activator), and irinotecan (a topoisomerase I inhibitor) exhibited significantly lower IC50 values in the high-risk group compared to the low-risk group (P < 0.05, Figure 8A-I).

Discussion

AML is a prevalent hematological malignancy with a dismal prognosis. Therefore, it's crucial to study early detection and treatment methods for AML. In the past, prognostic prediction models based on cytogenetic and molecular genetics have been quickly applied to clinical practice once developed.[38] However, the application of classical risk models are constrained by the fact that a certain proportion of patients do not exhibit alterations in cytogenetic and molecular genetics. With the advent and utilization of novel therapeutic agents, the prognosis of patients once deemed to have unfavorable outcomes.[39,40] Therefore, a new prognostic model is urgently needed to predict the prognosis of AML in the era of new drugs. Nowadays, researchers have developed different prognostic model for AML concluding
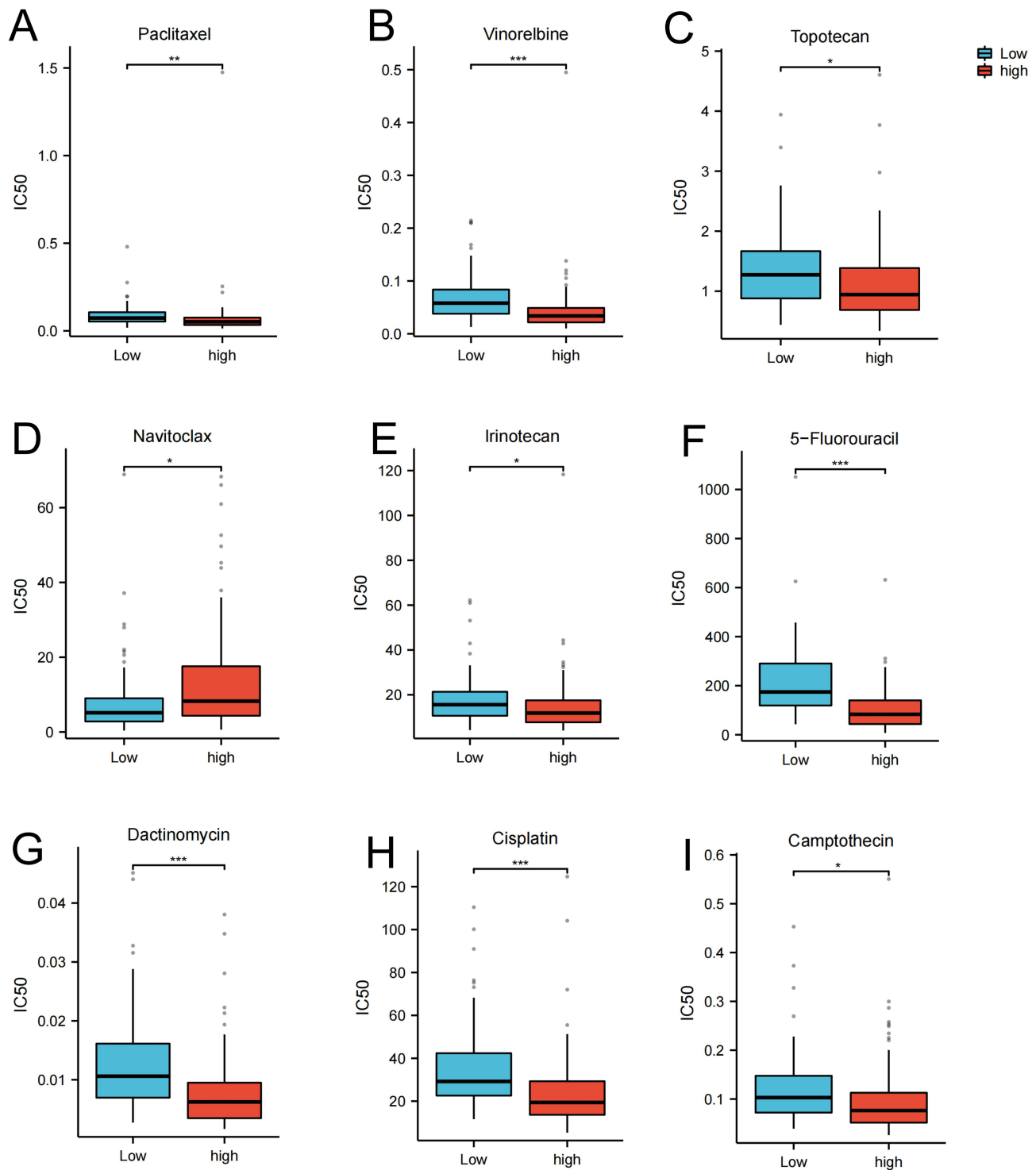
Figure 8 Drug sensitivity analysis of high-risk group and low-risk group. Navitocla (**D**) in the high-risk group were significantly higher than those in the low-risk group. Paclitaxel (**A**), vinorelbine (**B**), topotecan (**C**), irinotecan (**E**), 5-fluorouracil (**F**), dactinomycin (**G**), cisplatin (**H**), camptothecin (**I**) in the high-risk group were significantly lower than those in the low-risk group. ***FDR < 0.001, **FDR <0.01, *FDR< 0.05.

cuproptosis-related genes,[41] deconvoluted cell-type abundance,[42] hypoxia-related genes,[43] complement system-related genes.[44] Although these models show certain predictions, they all have some limitations. Ferroptosis plays an important role in the progression of AML. Research has shown that leukemia cells are more susceptible to the buildup of harmful ROS compared to other cancer cells, leading to irreparable peroxidative damage and

ferroptosis.[45,46] This unique form of cell death offers new possibilities for treating and predicting AML outcomes. Combining iron-based cell death triggers with chemotherapy can increase the effectiveness of the latter, improving the chances of remission.[19] Therefore, it is particularly important to analyze the genes of ferroptosis for the prognosis of AML and to develop flexible personalized treatment plans based on ferroptosis-related genes risk model.

In our study, we identified ten genes (ACSF2, SOCS1, CDO1, MYB, LPIN1, DNAJB6, PSAT1, GPX4, MT1G, FH) associated with ferroptosis as independent prognostic factors for AML. Using these ten genes, we developed a prognostic model that effectively predicts AML prognosis. Kaplan-Meier analysis demonstrated a significantly lower survival rate in the high-risk group compared to the low-risk group ($P < 0.001$). In fact, it was previously reported that eight ferroptosis-related genes were used to develop AML risk models, and the genes included in their models (including SOCS1 and FH) also share some similarities with our model.[47] However, this research have performed no validation of clinical samples, which reduced the credibility of this model. These findings suggest that these ferroptosis-related genes could provide valuable insights for future AML treatment and prognosis enhancement. Additionally, age and cytogenetic risk emerged as independent prognostic factors for OS in AML patients, consistent with previous research.[5,48–50] Moreover, our study revealed a high mutation rate in AML patients, with ferroptosis-related genes potentially playing a pivotal role in AML development. Traditionally, AML patients are classified into favorable, intermediate, and poor/adverse risk groups based on cytogenetic and molecular genetics profiles.[7] Using this classification, we further divided AML patients into poor/adverse and favorable/intermediate groups. We observed that the survival rate in the poor/adverse group was lower than that in the favorable/intermediate group, and our model ($P < 0.001$) outperformed the classical NCCN risk model ($P = 0.062$) in terms of survival prediction. Notably, the limited number of patients in the favorable group may have contributed to the lack of significant risk stratification using the classical NCCN risk model.

In our center's sample, significant differences were found in ACSF2, LPIN1, MYB, GPX4, MT1G, FH, and PSAT1 genes between healthy donors and AML patients ($P < 0.05$). Our study highlights GPX4 as a potential key gene for AML intervention through ferroptosis. Previous research has consistently shown upregulation of GPX4 in AML, with high expression correlating with poor prognosis.[51] Up to date, few studies address the relationship between ACSF2 and the prognosis of AML patients. Yao et al observed that the iron chelator deferoxamine can target ACSF2 to promote traumatic spinal cord recovery and thus may be promising as a new therapeutic avenue by targeting the ferroptosis-related gene ACSF2.[52] The LPIN1 gene is a member of the LPIN family, a key gene in the regulation of adipocyte differentiation and lipid metabolism. Down-regulated the expression of LPIN1 can effectively enhance the anticancer activity of novel HDAC/PI3K dual inhibitors.[53] AML cells rely on high levels of MYB expression, which play a downstream role in sustaining leukemia cells.[54] MT1G is a class of small molecule proteins with highly conserved structures that plays an important role in the pathogenesis of cancer, which reported to be associated with higher bleeding rates, early mortality events and negative outcome effects on overall survival in acute promyelogenous leukemia.[55,56] PSAT1 is one of the key enzymes in the serine synthesis pathway, and knockdown of PSAT1 leads to the inhibition of tumor cell proliferation, migration, and invasion in vitro.[57] Furthermore, mutations in FH were also found to be associated with some cancers. These proved our study with clinical coincidence.[58] Our study classified AML patients into high and low-risk groups, demonstrating significant differences in overall survival probability between them using Kaplan-Meier analysis. We further validated the reliability of our model for predicting patient prognosis through COX regression analysis, ROC and DCA. These findings underscore the specificity and robustness of our model, even with a smaller sample size, suggesting its potential superiority in distinguishing patient survival compared to traditional methods.

To identify potential therapeutic options for patients with diverse genetic backgrounds, we conducted an analysis of drug sensitivity. Our findings revealed notable differences in drug responses between high-risk and low-risk groups. Specifically, the high-risk group exhibited significantly higher IC50 values for navitoclax, a BCL-2 inhibitor, in contrast to the low-risk group. Conversely, paclitaxel, 5-fluorouracil, cisplatin, topotecan, vinorelbine, camptothecin, dactinomycin, and irinotecan displayed significantly lower IC50 values in the high-risk group compared to the low-risk group. Notably, conventional chemotherapeutics such as cytarabine and anthracycline (mitoxantrone) showed no discernible differences. Currently, the international standard induction regimen involves combining cytarabine with anthracycline, which continues to yield favorable outcomes in various contexts, solidifying its role in AML treatment.[59] Nevertheless, our exploration

suggests the potential for optimizing chemotherapy drugs based on individual genetic profiles. Prior research has high-lighted the significance of BCL-2 inhibitor treatment in refractory AML cases.[60] It has been effective in mitigating the adverse prognostic impact of mutations in splicing factor genes, as recognized in the European Leukemia Net 2022 risk stratification.[61] However, our model unveils a resistance to BCL-2 inhibitor (navitoclax) in the high-risk patient group. Therefore, caution is warranted when considering the utilization of BCL-2 inhibitors for this subgroup. Furthermore, our analysis revealed that the high-risk patient group exhibited heightened sensitivity to unconventional drugs such as paclitaxel, 5-fluorouracil, cisplatin, topotecan, vinorelbine, camptothecin, dactinomycin, and irinotecan. Research has demonstrated that nanomedicine loaded with paclitaxel can selectively target AML cells and induce ferroptosis, ultimately extending the survival of AML mice.[62] Chemotherapeutic agents like 5-fluorouracil and cisplatin, commonly used in treating solid tumors, have also exhibited anti-tumor effects through ferroptosis induction.[63,64] Additionally, dactinomycin and other drugs have shown potential as therapeutic alternatives for relapsed/refractory AML cases.[65–67] These findings align with our study, suggesting that a judicious combination of these unconventional drugs may offer a promising treatment approach for the high-risk group characterized by specific ferroptosis-related genes.

However, our study still has some limitations. The clinical significance of these FRGs should be confirmed through subsequent studies, as our research was constrained by a small sample size. As well as, the age range of patients in our study was relatively limited. It's crucial to acknowledge that the absence of data on complex karyotypes and the limited number of patients in the favorable group could introduce bias into the risk assessment based on the NCCN guidelines. Additionally, the drugs selected for this study should be considered as preliminary references for clinical treatment in diverse patient populations, and their efficacy should be further validated through subsequent clinical investigations.

In summary, we successfully identified and validated ten FRGs (ACSF2, SOCS1, CDO1, MYB, LPIN1, DNAJB6, PSAT1, GPX4, MT1G, FH) as potential prognostic indicators for AML patients. And a new AML prognosis prediction model based on these genes was constructed which showed favorable performance in AML patient stratification and prognosis prediction. Our study also provided potential therapeutic options for AML patients with diverse genetic backgrounds based on this prediction model, which could enhance the outlook for AML patients.

Data Sharing Statement

The data are available from the corresponding author upon a reasonable request.

Ethics Declarations

This study was reviewed and approved by the ethical committee of the First Affiliated Hospital of Zhejiang Chinese Medical University (Zhejiang Provincial Hospital of Chinese Medicine), with the approval number: NO.2023-KLS-243-01. All participants provided informed consent to participate in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Disclosure

The authors declare no conflicts of interest in this work.

References

1. Dohner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. *N Engl J Med*. 2015;373(12):1136–1152. doi:10.1056/NEJMra1406184
2. Pelcovits A, Niroula R. Acute myeloid leukemia: a review. *R I Med J (2013)*. 2020;103(3):38–40.
3. Kantarjian H, O'Brien S, Cortes J, et al. Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome. *Cancer*. 2006;106(5):1090–1098. doi:10.1002/cncr.21723
4. Shah A, Andersson TM, Rachet B, Bjorkholm M, Lambert PC. Survival and cure of acute myeloid leukaemia in England, 1971-2006: a population-based study. *Br J Haematol*. 2013;162(4):509–516. doi:10.1111/bjh.12425
5. Dohner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood*. 2010;115(3):453–474. doi:10.1182/blood-2009-07-235358
6. Mrozek K, Marcucci G, Nicolet D, et al. Prognostic significance of the European LeukemiaNet standardized system for reporting cytogenetic and molecular alterations in adults with acute myeloid leukemia. *J Clin Oncol*. 2012;30(36):4515–4523. doi:10.1200/JCO.2012.43.4738
7. Pollyea DA, Bixby D, Perl A, et al. NCCN guidelines insights: acute myeloid Leukemia, version 2.2021. *J Natl Compr Canc Netw*. 2021;19 (1):16–27. doi:10.6004/jnccn.2021.0002
8. Huemer F, Melchardt T, Jansko B, et al. Durable remissions with venetoclax monotherapy in secondary AML refractory to hypomethylating agents and high expression of BCL-2 and/or BIM. *Eur J Haematol*. 2019;102(5):437–441. doi:10.1111/ejh.13218
9. Mill CP, Fiskus W, DiNardo CD, et al. Effective therapy for AML with RUNX1 mutation by cotreatment with inhibitors of protein translation and BCL2. *Blood*. 2022;139(6):907–921. doi:10.1182/blood.2021013156
10. Daver N, Perl AE, Maly J, et al. Venetoclax plus gilteritinib for FLT3-mutated relapsed/refractory acute myeloid Leukemia. *J Clin Oncol*. 2022;40 (35):4048–4059. doi:10.1200/JCO.22.00602
11. Janssen M, Schmidt C, Bruch PM, et al. Venetoclax synergizes with gilteritinib in FLT3 wild-type high-risk acute myeloid leukemia by suppressing MCL-1. *Blood*. 2022;140(24):2594–2610. doi:10.1182/blood.2021014241
12. Xuan L, Wang Y, Yang K, et al. Sorafenib maintenance after allogeneic haemopoietic stem-cell transplantation in patients with FLT3-ITD acute myeloid leukaemia: long-term follow-up of an open-label, multicentre, randomised, Phase 3 trial. *Lancet Haematol*. 2023;10(8):e600–e611. doi:10.1016/S2352-3026(23)00117-5
13. Fu C, Kou R, Meng J, Jiang D, Zhong R, Dong M. Dong M: m6A genotypes and prognostic signature for assessing the prognosis of patients with acute myeloid leukemia. *BMC Med Genomics*. 2023;16(1):191. doi:10.1186/s12920-023-01629-1
14. Jones CL, Stevens BM, D'Alessandro A, et al. Inhibition of amino acid metabolism selectively targets human leukemia stem cells. *Cancer Cell*. 2018;34(5):724–740e724. doi:10.1016/j.ccell.2018.10.005
15. Distefano AM, Martin MV, Cordoba JP, et al. Heat stress induces ferroptosis-like cell death in plants. *J Cell Biol*. 2017;216(2):463–476. doi:10.1083/jcb.201605110
16. Shen Q, Liang M, Yang F, Deng YZ, Naqvi NI. Ferroptosis contributes to developmental cell death in rice blast. *New Phytol*. 2020;227 (6):1831–1846. doi:10.1111/nph.16636
17. Jiang X, Stockwell BR, Conrad M. Ferroptosis: mechanisms, biology and role in disease. *Nat Rev Mol Cell Biol*. 2021;22(4):266–282. doi:10.1038/s41580-020-00324-8
18. Grignano E, Birsen R, Chapuis N, Bouscary D. From iron chelation to overload as a therapeutic strategy to induce ferroptosis in Leukemic cells. *Front Oncol*. 2020;10:586530. doi:10.3389/fonc.2020.586530
19. Tang X, Wang Y, Zhu Y, Guo Y, Liu B. Basic mechanisms and novel potential therapeutic targets for ferroptosis in acute myeloid leukemia. *Ann Hematol*. 2023;102(8):1985–1999. doi:10.1007/s00277-023-05293-4
20. Birsen R, Larrue C, Decroocq J, et al. APR-246 induces early cell death by ferroptosis in acute myeloid leukemia. *Haematologica*. 2022;107 (2):403–416. doi:10.3324/haematol.2020.259531
21. Dong LH, Huang JJ, Zu P, et al. CircKDM4C upregulates P53 by sponging hsa-let-7b-5p to induce ferroptosis in acute myeloid leukemia. *Environ Toxicol: Int J*. 2021;36(7):1288–1302. doi:10.1002/tox.23126
22. Tothova Z, Kollipara R, Huntly BJ, et al. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell*. 2007;128(2):325–339. doi:10.1016/j.cell.2007.01.003
23. Wei J, Xie Q, Liu X, et al. Identification the prognostic value of glutathione peroxidases expression levels in acute myeloid leukemia. *Ann Transl Med*. 2020;8(11):678. doi:10.21037/atm-20-3296
24. Auberger P, Favreau C, Savy C, Jacquel A, Robert G. Emerging role of glutathione peroxidase 4 in myeloid cell lineage development and acute myeloid leukemia. *Cell Mol Biol Lett*. 2024;29(1):98. doi:10.1186/s11658-024-00613-6
25. Stiller S, Drukewitz S, Lehmann K, Hentschel J, Strehlow V. Clinical impact of polygenic risk score for breast cancer risk prediction in 382 individuals with hereditary breast and ovarian cancer syndrome. *Cancers*. 2023;15(15):3938. doi:10.3390/cancers15153938
26. Sullivan SM, Cole B, Lane J, et al. Predicted leukocyte telomere length and risk of myeloid neoplasms. *Hum Mol Genet*. 2023;32(20):2996–3005. doi:10.1093/hmg/ddad126
27. Wang J, Zhuo Z, Wang Y, et al. Identification and validation of a prognostic risk-scoring model based on ferroptosis-associated cluster in acute myeloid Leukemia. *Front Cell Dev Biol*. 2021;9:800267. doi:10.3389/fcell.2021.800267
28. Zheng Z, Wu W, Lin Z, et al. Identification of seven novel ferroptosis-related long non-coding RNA signatures as a diagnostic biomarker for acute myeloid leukemia. *BMC Med Genomics*. 2021;14(1):236. doi:10.1186/s12920-021-01085-9
29. Cancer Genome Atlas Research N, Weinstein JN, Collisson EA, et al. The cancer genome atlas pan-cancer analysis project. *Nat Genet*. 2013;45 (10):1113–1120. doi:10.1038/ng.2764
30. Zhou N, Bao J. FerrDb: a manually curated resource for regulators and markers of ferroptosis and ferroptosis-disease associations. *Database*. 2020;2020:baaa021. doi:10.1093/database/baaa021

31. Shen ZX. Diagnostic criteria and therapeutic principle of invasive fungal infection in hematological diseases or malignant tumors. *Zhonghua Nei Ke Za Zhi*. 2007;46(7):532–533.

32. Yang W, Soares J, Greninger P, et al. Genomics of Drug Sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells. *Nucleic Acids Res*. 2013;41(Database issue):D955–961. doi:10.1093/nar/gks1111

33. Kentsis A, Reed C, Rice KL, et al. Autocrine activation of the MET receptor tyrosine kinase in acute myeloid leukemia. *Nat Med*. 2012;18 (7):1118–1122. doi:10.1038/nm.2819

34. Campbell CT, Haladyna JN, Drubin DA, et al. Mechanisms of pinometostat (EPZ-5676) treatment-emergent resistance in MLL-rearranged Leukemia. *Mol Cancer Ther*. 2017;16(8):1669–1679. doi:10.1158/1535-7163.MCT-16-0693

35. DeAngelo DJ, Brunner AM, Werner L, et al. A Phase I study of lenalidomide plus chemotherapy with mitoxantrone, etoposide, and cytarabine for the reinduction of patients with acute myeloid leukemia. *Am J Hematol*. 2018;93(2):254–261. doi:10.1002/ajh.24968

36. Eletskaya BZ, Berzina MY, Fateev IV, et al. Enzymatic synthesis of 2-chloropurine arabinonucleosides with chiral amino acid amides at the C6 position and an evaluation of antiproliferative activity in vitro. *Int J Mol Sci*. 2023;24(7):6223. doi:10.3390/ijms24076223

37. Li Z, Zhang Y, Zhu C, et al. Folic acid modified lipid-bilayer coated mesoporous silica nanoparticles co-loading paclitaxel and tanshinone IIA for the treatment of acute promyelocytic leukemia. *Int J Pharm*. 2020;586:119576. doi:10.1016/j.ijpharm.2020.119576

38. O'Donnell MR, Abboud CN, Altman J, et al. NCCN clinical practice guidelines acute myeloid leukemia. *J Natl Compr Canc Netw*. 2012;10 (8):984–1021. doi:10.6004/jnccn.2012.0103

39. Carter JL, Hege K, Yang J, et al. Targeting multiple signaling pathways: the new approach to acute myeloid leukemia therapy. *Signal Transduct Target Ther*. 2020;5(1):288. doi:10.1038/s41392-020-00361-x

40. Short NJ, Daver N, Dinardo CD, et al. Azacitidine, venetoclax, and gilteritinib in newly diagnosed and relapsed or refractory FLT3-mutated AML. *J Clin Oncol*. 2024;42(13):1499–1508. doi:10.1200/JCO.23.01911

41. Wang X, Sun H, Dong Y, et al. Development and validation of a cuproptosis-related prognostic model for acute myeloid leukemia patients using machine learning with stacking. *Sci Rep*. 2024;14(1):2802. doi:10.1038/s41598-024-53306-7

42. Han DJ, Kim S, Lee SY, et al. Cellular abundance-based prognostic model associated with deregulated gene expression of leukemic stem cells in acute myeloid leukemia. *Front Cell Dev Biol*. 2024;12:1345660. doi:10.3389/fcell.2024.1345660

43. Liu X, Wang L, Kang Q, Feng C, Wang J. A hypoxia-related genes prognostic risk model, and mechanisms of hypoxia contributing to poor prognosis through immune microenvironment and drug resistance in acute myeloid leukemia. *Front Pharmacol*. 2024;15:1339465. doi:10.3389/fphar.2024.1339465

44. Liu C, Liu L. Identification and immunoassay of prognostic genes associated with the complement system in acute myeloid leukemia. *J Formos Med Assoc*. 2024;123(8):904–915. doi:10.1016/j.jfma.2024.01.024

45. Yang WS, SriRamaratnam R, Welsch ME, et al. Regulation of ferroptotic cancer cell death by GPX4. *Cell*. 2014;156(1–2):317–331. doi:10.1016/j.cell.2013.12.010

46. Lan H, Gao Y, Zhao Z, Mei Z, Wang F. Ferroptosis: redox imbalance and hematological tumorigenesis. *Front Oncol*. 2022;12:834681. doi:10.3389/fonc.2022.834681

47. Wu F, Xu G, Li G, et al. A prognostic model based on prognosis-related ferroptosis genes for patients with acute myeloid leukemia. *Front Mol Biosci*. 2023;10:1281141. doi:10.3389/fmolb.2023.1281141

48. Appelbaum FR, Gundacker H, Head DR, et al. Age and acute myeloid leukemia. *Blood*. 2006;107(9):3481–3485. doi:10.1182/blood-2005-09-3724

49. Elgarten CW, Aplenc R. Aplenc R: pediatric acute myeloid leukemia: updates on biology, risk stratification, and therapy. *Curr Opin Pediatr*. 2020;32(1):57–66. doi:10.1097/MOP.0000000000000855

50. Juliusson G, Antunovic P, Derolf A, 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(18):4179–4187. doi:10.1182/blood-2008-07-172007

51. Liu X, Zhong S, Qiu K, et al. Targeting NRF2 uncovered an intrinsic susceptibility of acute myeloid leukemia cells to ferroptosis. *Exp Hematol Oncol*. 2023;12(1):47. doi:10.1186/s40164-023-00411-4

52. Yao X, Zhang Y, Hao J, et al. Deferoxamine promotes recovery of traumatic spinal cord injury by inhibiting ferroptosis. *Neural Regen Res*. 2019;14 (3):532–541. doi:10.4103/1673-5374.245480

53. Imai H, Saijo K, Chikamatsu S, Kawamura Y, Ishioka C. LPIN1 downregulation enhances anticancer activity of the novel HDAC/PI3K dual inhibitor FK-A11. *Cancer Sci*. 2021;112(2):792–802. doi:10.1111/cas.14759

54. Klempnauer KH. C/EBPbeta cooperates with MYB to maintain the oncogenic program of AML cells. *Oncotarget*. 2023;14(1):174–177. doi:10.18632/oncotarget.28377

55. Jann JC, Streuer A, Hecht A, et al. RNA-sequencing of acute promyelocytic leukemia primary blasts reveals novel molecular biomarkers of early death events. *Leuk Lymphoma*. 2020;61(13):3066–3077. doi:10.1080/10428194.2020.1797006

56. Si M, Lang J. The roles of metallothioneins in carcinogenesis. *J Hematol Oncol*. 2018;11(1):107. doi:10.1186/s13045-018-0645-x

57. Wang M, Yue S, Yang Z. Downregulation of PSAT1 inhibits cell proliferation and migration in uterine corpus endometrial carcinoma. *Sci Rep*. 2023;13(1):4081. doi:10.1038/s41598-023-31325-0

58. Zavoshi S, Lu E, Boutros PC, et al. Fumarate hydratase variants and their association with paraganglioma/pheochromocytoma. *Urology*. 2023;176:106–114. doi:10.1016/j.urology.2022.11.053

59. Murphy T, Yee KWL. Cytarabine and daunorubicin for the treatment of acute myeloid leukemia. *Expert Opin Pharmacother*. 2017;18 (16):1765–1780. doi:10.1080/14656566.2017.1391216

60. Piccini M, Mannelli F, Coltro G. The role of venetoclax in relapsed/refractory acute myeloid leukemia: past, present, and future directions. *Bioengineering (Basel)*. 2023;10(5). doi:10.3390/bioengineering10050591

61. Senapati J, Urrutia S, Loghavi S, et al. Venetoclax abrogates the prognostic impact of splicing factor gene mutations in newly diagnosed acute myeloid Leukemia. *Blood*. 2023;142(19):1647–1657. doi:10.1182/blood.2023020649

62. Yu Y, Meng Y, Xu X, et al. A ferroptosis-inducing and leukemic cell-targeting drug nanocarrier formed by redox-responsive cysteine polymer for acute myeloid Leukemia Therapy. *ACS Nano*. 2023;17(4):3334–3345. doi:10.1021/acsnano.2c06313

63. Beretta GL. Ferroptosis-induced cardiotoxicity and antitumor drugs. *Curr Med Chem*. 2023. doi:10.2174/0929867331666230719124453

64. Li X, Yuan X, Fu H, et al. Konjac glucomannan, in combination with cisplatin, suppresses lymphoma malignant progression by inducing ferroptosis. *Anticancer Agents Med Chem*. 2023;23. doi:10.2174/1871520623666230529160837

65. Gionfriddo I, Brunetti L, Mezzasoma F, et al. Dactinomycin induces complete remission associated with nucleolar stress response in relapsed/refractory NPM1-mutated AML. *Leukemia*. 2021;35(9):2552–2562. doi:10.1038/s41375-021-01192-7

66. Minderman H, O'Loughlin KL, Smith PF, et al. Sequential administration of irinotecan and cytarabine in the treatment of relapsed and refractory acute myeloid leukemia. *Cancer Chemother Pharmacol*. 2006;57(1):73–83. doi:10.1007/s00280-005-0017-4

67. Ramaswamy K, Steinherz PG, Agrawal AK, et al. Clofarabine with topotecan, vinorelbine, and thiotepa reinduction regimen for children and young adults with relapsed AML. *Blood Adv*. 2022;6(8):2688–2694. doi:10.1182/bloodadvances.2021005753