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ORIGINAL ARTICLE

Integrating resistance functions to predict response to induction chemotherapy in de novo acute myeloid leukemia

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Abstract

Objectives: This study explored resistance functions and their interactions in de novo AML treated with the “7 + 3” induction regimen.

Methods: We analyzed RNA-sequencing profiles of whole bone marrow samples from 52 de novo AML patients who completed the “7 + 3” regimen and stratified patients into CR (n = 35) and non-CR (n = 17) groups.

Results: A systematic gene set analysis revealed significant associations between chemoresistance and mTOR ($P < .001$), myc ($P < .001$), mitochondrial oxidative phosphorylation ($P < .001$), and stemness ($P = .002$). These functions were independent with regard to gene contents and activity scores. An integration of these four functions showed a prediction of chemoresistance (area under the receiver operating characteristic curve = 0.815) superior to that of each function alone. Moreover, our proposed seven-gene scoring system significantly correlated with the four-function model ($r = .97$; $P < .001$) to predict chemoresistance to the “7 + 3” regimen. On multivariate analysis, a seven-gene score of ≥−0.027 (hazard ratio: 11.18; 95% confidence interval: 2.06-60.65; $P = .005$) was an independent risk factor for induction failure.

Conclusions: Myc, OXPHOS, mTOR, and stemness were responsive for chemoresistance in AML. Treatments other than the “7 + 3” regimen need to be considered for de novo AML patients predicted to be refractory to the “7 + 3” regimen.

KEYWORDS
acute myeloid leukemia, chemosensitivity, induction chemotherapy, prediction model, resistance functions

1 | INTRODUCTION

Acute myeloid leukemia (AML) is a hematological malignancy characterized by clonal expansion of immature myeloid blasts by abnormal proliferation and differentiation of hematopoietic stem cells.[1] The incidence of AML is approximately 1.3 per 100 000 people. As one of the standard induction therapies against AML, the “7 + 3” (cytarabine 100-200 mg/m^2 for 7 days; idarubicin 12 mg/m^2 for 3 days) regimen can achieve a 70% complete response (CR) rate in patients newly diagnosed with de novo AML.[2] However, chemoresistance to induction chemotherapy remains one of the major obstacles in AML treatment. The outcome of patients with AML who are unable to achieve CR after their first induction chemotherapy is poor. Only <10% of patients with AML who failed to achieve CR after the induction chemotherapy showed long-term survival by further salvage chemotherapy or allogeneic hematopoietic stem cell transplantation.[3]

Identifying patients with AML who will fail to achieve CR by the “7 + 3” induction chemotherapy is important. Alternative induction

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regimens or clinical trials are reasonable treatment strategies for these patients. Old age, leukocytosis, and high-risk cytogenetic abnormalities are considered clinical features associated with failure of "7 + 3" induction therapies in AML.[4] In terms of cell function, cell quiescence, capability of DNA damage repair, and leukemic stem cell (LSC)-related leukemogenesis are closely associated with chemoresistance in patients with AML.[5-8] Notably, Farge et al[9] demonstrated a novel mechanism responsible for chemoresistance independent of LSCs. By using the patient-derived xenograft (PDX) model, increased mitochondrial mass and high mitochondrial oxidative phosphorylation (OXPHOS) status were observed in cytarabine-resistant AML cells. Furthermore, ATP-binding cassette (ABC) transporters are associated with chemoresistance in AML.[10] These findings suggest that chemoresistance is multifactorial in AML. Thus, a more comprehensive exploration of resistance functions in AML is required.

The present study aimed to systematically identify resistance functions and investigate their interactions in jointly determining chemoresistance. We used RNA sequencing to analyze whole bone marrow samples from 52 patients with de novo AML who completed a "7 + 3" or "7 + 3"-like induction chemotherapy. The patients were stratified into the CR group (n = 35) and the non-CR group (n = 17) according to success or failure in achieving CR under the first "7 + 3" or "7 + 3"-like regimen. Global expressional profiles were compared between the two groups and tested for functional enrichments. We further analyzed the interactions among different resistance functions by using a multifunction prediction model. Finally, we developed a targeted gene scoring system to predict whether patients with de novo AML could achieve CR under the "7 + 3" regimen in the current study.

2 | MATERIALS AND METHODS

2.1 | Patients

Medical records of 373 consecutive patients diagnosed with de novo AML from 2004 to 2017 were retrospectively reviewed. Because this study focused on the interactions between cellular functions for chemoresistance to the "7 + 3" regimen, only patients with AML who completed their first "7 + 3" or "7 + 3"-like regimen and had qualified RNA extracted from the bone marrow at initial diagnosis were enrolled. Patients who were < 20 years of age and who died early during the "7 + 3" regimen were excluded. Finally, a total of 52 patients were analyzed. These 52 patients were divided into the CR group (n = 35) and non-CR group (n = 17) according to their treatment response after the first "7 + 3" or "7 + 3"-like regimens. Table 1 shows the comparisons of demographic characteristics between both groups. Briefly, both groups did not show any significant differences in sex (P = .278), age (P = .785), initial leukocyte count (P = .810), French-American-British classification (P = .300), and percentage of blast cells in the bone marrow (P = .290). However, patients in the non-CR group had more high-risk cytogenetic features in accordance with the European Leukemia Network than patients in the CR group (P = .002). This study was approved by the Institutional Review Board of Taichung Veterans General Hospital and was in accordance with the current version of the Helsinki Declaration.

TABLE 1 Patient characteristics

Variable	Non-CR group (n = 17)	CR group (n = 35)	*P*-value
Sex			
Male	11	17	.278
Female	6	18	
Age, y (mean ± SD)	46.7 ± 13.9	45.5 ± 15.1	.785
Leukocytes, $10^3/\mu L$ (mean ± SD)	62.6 ± 60.1	58.3 ± 60.1	.810
Blasts in marrow, % (mean ± SD)	70.4 ± 15.9	65.0 ± 17.7	.290
FAB classification			
M0	1	0	.300
M1	1	1	
M2	12	25	
M3	0	0	
M4	2	6	
M5	0	3	
M6	1	0	
M7	0	0	
Cytogenetics[a]			
Favorable	0	5	.002
Intermediate	6	24	
Unfavorable	8	2	
Undetermined	3	4	

Note: *P*-values by *t* test for continuous variables and chi-squared test for categorical variables.
Abbreviations: CR, complete response; FAB, French-American-British; SD, standard deviation.
[a]Stratified according to European Leukemia Network.

2.2 | Study overview

Figure 1 shows the study flowchart. Briefly, we profiled gene expressions using RNA sequencing. After data processing and normalization, we utilized gene set enrichment analysis (GSEA) to systematically identify molecular signatures responsible for chemoresistance-related cellular functions. These molecular signatures were curated in the Molecular Signatures Database (MSigDB) collections. Representative functions were combined using a multiple regression model to determine whether the cellular functions jointly predicted chemoresistance to the "7 + 3" regimen. Finally, we proposed a targeted gene scoring system based on the multifunction prediction model.

2.3 | RNA-sequencing experiments

We extracted total RNA from whole bone marrow cells using TRIzol (Thermo Fisher Scientific, Inc). RNA samples were purified by using the RNeasy Mini Kit and DNase I (Qiagen). The mRNA contents were

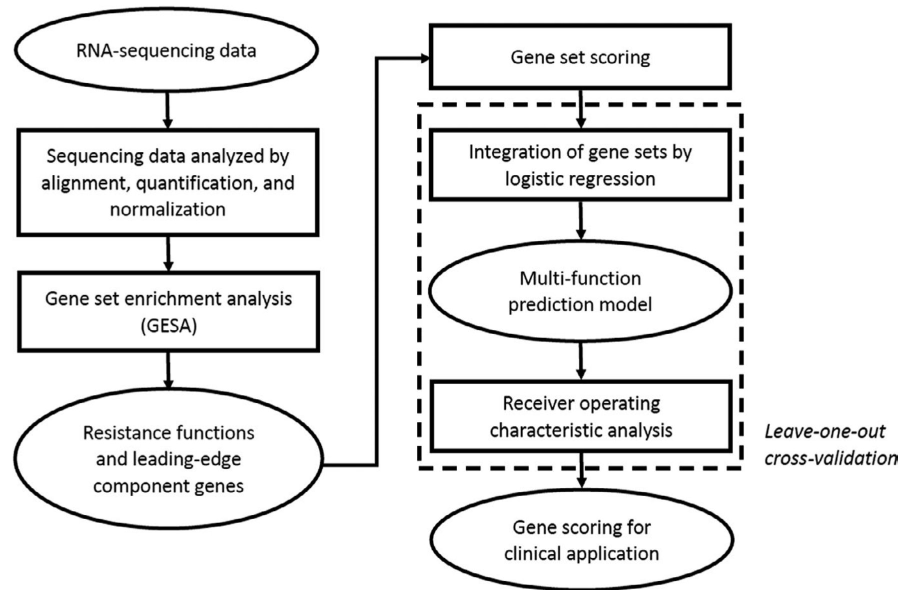

FIGURE 1 Study overview. Gene expressions were profiled using RNA sequencing. After data processing and normalization, gene set enrichment analysis (GSEA) was used to systematically identify molecular signatures responsible for chemoresistance-related cellular functions. These molecular signatures were curated in the Molecular Signatures Database (MSigDB) collections. Representative functions were combined using a multiple regression model to determine whether the cellular functions jointly predicted chemoresistance to the "7 + 3" induction chemotherapy. A seven-gene scoring system was also proposed based on the multifunction prediction model

enriched by using oligo(dT)-labeled magnetic beads, followed by fragmentation, conversion into cDNA, ligation of sequencing adaptors, and amplification. Quality-checked library products were subjected to 75-bp paired-end sequencing using a NextSeq 500 sequencer (Illumina Inc) with a throughput of approximately 20 million reads per sample.

We removed raw sequencing reads with low quality, containing adaptor sequences, or with high contents of unknown bases. Clean reads were aligned to the Ensembl GRCh37 human reference genome using TopHat2.[11] We used HTSeq software to count the mapped reads against Ensembl annotated genes (ENSG IDs).[12] Gene-level read counts were normalized and tested for differential expression between AML patients with and without CR using R package DESeq2.[13]

2.4 | Gene set enrichment analysis

We used GSEA software to identify cellular functions associated with "7 + 3" induction chemoresistance. Briefly, transcriptome-wide genes were ranked based on the significance of differential expression between the CR and non-CR groups. Predefined cellular functions, including curated pathways and gene signatures (C2 collection), gene ontologies (C5), oncogenic signatures (C6), and hallmark gene sets (H), were downloaded from the MSigDB[14] and tested for enrichment on either side of the ranked list. Statistical significance of the degree of enrichment was assessed using a 1000-time random permutation test. For each significant function, GSEA reported a core subset of genes, namely, leading-edge component genes, which accounted for the enrichment.

2.5 | Prediction model establishment by integrating resistance functions

We utilized a gene set scoring method to measure the resistance function activity represented by molecular signatures of interest. Briefly, log2-scaled z-values of leading-edge component genes were averaged in each patient. Scores of selected functions were z-transformed again to eliminate inter-cohort biases and were subjected to a multivariable logistic regression model with a binary outcome of the "7 + 3" regimen. A value of 0 was assigned for CR, and 1 was assigned for non-CR. For each patient, a prediction score was calculated using a simple average of gene set scores. A high score predicted refractoriness to the induction chemotherapy. An optimal cutoff of the multifunction score was obtained by using receiver operating characteristic (ROC) analysis.

2.6 | Performance assessment and validation of the prediction model

The overall performance of the proposed multifunction prediction model was assessed using the area under the ROC curve (AUROC). We conducted a permutation test to investigate the significance of AUROC achieved by the proposed model. Internal validation was conducted using leave-one-out cross-validation. In each round of leave-one-out cross-validation analysis, data from 51 patients were subjected to an ROC curve to determine an optimal cutoff. The remaining sample was tested by the same model. Each of the 52 patients was tested once. Accuracy, sensitivity, specificity, and *F*-score $\left(\frac{2\times \text{Precision} \times \text{Sensitivity}}{\text{Precision} + \text{Sensitivity}}\right)$ were calculated to assess the reliability of this proposed model.

2.7 | Statistical analysis

We used Student's *t* test and the chi-squared test to compare continuous and categorical variables between the CR and non-CR groups, respectively. Risk factors for non-CR were investigated using Cox proportional hazards regression, as quantified by hazard ratios (HRs) and accompanying 95% confidence intervals (CIs). Results were considered to be statistically significant when $P < .05$.

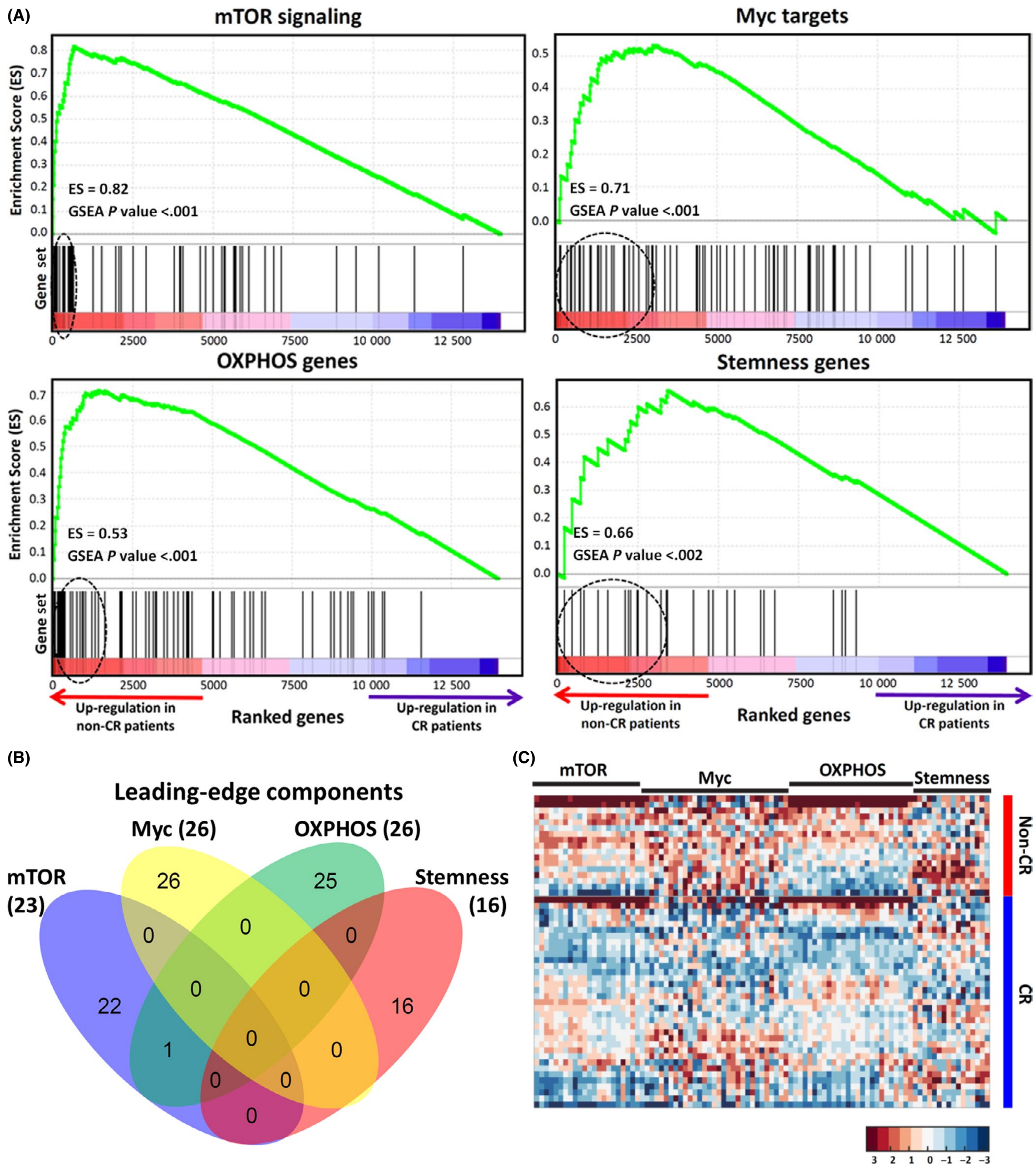

FIGURE 2 Resistance functions of induction chemotherapy. (A) Gene set enrichment analysis (GSEA) plots of selected molecular signatures. Myc, mTOR, oxidative phosphorylation (OXPHOS), and stemness accounted for about half of significant terms. All GSEA-adjusted *P*-values were ≤ .002, and all enrichment scores were ≥ 0.53. (B) Comparison of leading-edge components genes of the signatures. Although all four functions were associated with chemoresistance, the majority of their leading-edge component genes were independent. (C) Heat map of gene set scores of 52 patients with de novo AML. The four resistance functions categorized the gene expressions of patients with no complete response (non-CR) into two clusters [Colour figure can be viewed at wileyonlinelibrary.com]

3 | RESULTS

3.1 | Expressional and functional profiles associated with chemoresistance to the "7 + 3" regimen

After processing of RNA-sequencing data, the average throughput for the 52 bone marrow samples was 27.3 ± 4.0 million aligned paired-end reads (mean ± standard deviation [SD]). We used DESeq2 to normalize the gene expression profiles and test the expression difference between the CR and non-CR groups. We then analyzed approximately 8500 curated molecular signatures using GSEA for the enrichment in the transcriptome-wide profile of differential expression, identifying 28 resistance functions that significantly correlated with the "7 + 3" regimen (q value < .05). Remarkably, around half of the significant signatures were collectively related to mTOR signaling, mitochondrial OXPHOS, myc targets, and stem cell activities. We selected a mid-sized signature with balanced gene numbers to represent each category for subsequent analyses (all GSEA-adjusted P-values were ≤.002 and enrichment scores were ≥.53; Figure 2A).[15-18] To validate our results externally, we compared the expressional differences in the non-CR and CR groups from the cohort of Horibata et al[19] to our cohort. The overexpressed refractoriness signature from Horibata's cohort was also identified in our non-CR group (GSEA P < .001; Figure S1). However, the overexpressed signature of Horibata's CR group was not significantly higher in our CR group (GSEA P = .228; data not shown).

3.2 | Interactions among cellular functions for chemoresistance

Expression levels of leading-edge component genes of each signature were merged into a gene set score to measure resistance function

FIGURE 3 Performance of the four-function prediction model. (A) Receiver operating characteristic (ROC) curves of the four-function model and individual functions in predicting response to induction chemotherapy. (B) Prediction performance of the four-function model and individual functions. Although myc (0.82) had higher sensitivity than mTOR (0.71), oxidative phosphorylation (OXPHOS; 0.41), and stemness (0.71), it had lower specificity (77%) than OXPHOS (94%). However, with an accuracy of 0.85, the integration of the four functions demonstrated acceptable sensitivity (71%), without sacrificing specificity (91%). By Youden's index, the four-function model provided a better performance than each single pathway. (C) Association of the four-function score with chemoresistance. Dashed line represents cutoff (0.140) determined by ROC analysis. A high score is predictive of chemoresistance [Colour figure can be viewed at wileyonlinelibrary.com]

activity in each patient. Although the four functions (mTOR, myc, OXPHOS, and stemness) were all associated with chemoresistance, the majority of their leading-edge component genes were independent (Figure 2B and 2). Unsupervised hierarchical clustering in the non-CR group showed two major clusters in functions of OXPHOS and stemness (Figure 2C). Altogether, our data confirmed that chemoresistance to the "7 + 3" regimen was a multifactorial mechanism.

3.3 | Four-function prediction model for chemoresistance to the "7 + 3" regimen

We merged the scores of the four resistance functions (mTOR, myc, OXPHOS, and stemness) into a logistic regression model. The AUROC was 0.82 for the four-function model. It was 0.73, 0.81, 0.63, and 0.66 for mTOR, myc, OXPHOS, and stemness, respectively. The four-function model better predicted chemoresistance to the "7 + 3" regimen than each function alone (Figure 3A).

We further dissected the prediction power of the individual gene set and the integrated model. An optimal cutoff for each predictor was determined using ROC analysis. Although myc (82%) showed higher sensitivity than mTOR (71%), OXPHOS (41%), and stemness (71%), it had lower specificity (77%) than OXPHOS (94%; Figure 3B). However, with an accuracy of 0.85, the integration of the four functions exhibited an acceptable sensitivity (71%), without sacrificing specificity (91%; Figure 3B and 3). By the Youden index, which takes sensitivity and specificity into account simultaneously for superior model prediction,[20] the four-function model provided better performance than each single pathway (Figure 3B).

The four-function model was also examined internally by using leave-one-out cross-validation. After the validation, the accuracy, sensitivity, specificity, and F-score for the four-function model were 80.8%, 58.8%, 91.4%, and 66.7%, respectively, suggesting that this four-function model was reliable.

3.4 | Implementation of a seven-gene scoring system to predict CR achievement by the "7 + 3" regimen

We used a regression procedure to establish a gene scoring system to predict CR achievement by the "7 + 3" regimen. Briefly, we considered 72 leading-edge component genes of the four functions that had an average read count above the 50th percentile and that were detectable in all patients. A representative subset of these genes was identified by least absolute shrinkage and selection operator (LASSO) regression against the four-function score. The representative genes were then subjected to multiple linear regression against the score. Genes with an independent prediction power (multivariable $P < .05$) were weighted using corresponding regression coefficients and summed to a score. As a result, we developed a seven-gene scoring system. Among these seven genes, one, four, and two genes were the leading-edge component genes for mTOR, myc, and OXPHOS, respectively. The genes for stemness were not identified (Table 2). The seven-gene score significantly correlated with the four-function score ($\rho = .97$; $P < .001$; Figure 4A). A threshold at −0.027 was set for the seven-gene score by the cutoff point of the ROC curve (AUROC = 0.787; $P < .001$; Figure 4B). With this cutoff, the seven-gene scoring system could efficiently predict CR or non-CR in AML patients treated with the "7 + 3" regimen (Figure 4A).

3.5 | The seven-gene score as an independent predictive factor for "7 + 3" induction failure

We further investigated the risk factors for "7 + 3" induction failure. Table 3 shows the results. On univariate analysis, a seven-gene score of ≥−0.027 (HR: 10.18; 95% CI: 2.42-42.83; $P = .002$) and unfavorable cytogenetics (HR: 14.67; 95% CI: 2.64-81.57; $P = .002$) significantly increased the risk of "7 + 3" induction failure. Meanwhile, age ($P = .780$), sex ($P = .277$), and initial leukocyte count ($P = .770$) were not risk factors for "7 + 3" induction failure.

To validate the risk factors for "7 + 3" induction failure by univariate analyses, we conducted a multivariate analysis. Both a seven-gene score of ≥−0.027 (HR: 11.18; 95% CI: 2.06-60.65; $P = .005$) and unfavorable cytogenetics (HR: 16.43; 95% CI: 2.16-124.81; $P = .007$) were independent risk factors for "7 + 3" induction failure (Table 3). An integration model of a seven-gene score of ≥−0.027 and unfavorable cytogenetics to predict the induction failure was further performed. With a sensitivity of 50.0% and a specificity of 100%, the positive predictive value, negative predictive value, and accuracy of this intergraded model were 100%, 82.1%, and 84.8%, respectively.

TABLE 2 The seven-gene scoring model

Gene symbol	Gene name	Function category	Weight
CNOT7	CCR4-NOT transcription complex subunit 7	OXPHOS	0.11
DCUN1D4	Defective in cullin neddylation 1 domain containing 4	myc	−0.02
EXOSC2	Exosome component 2	myc	0.11
FKBP4	FKBP prolyl isomerase 4	myc	0.05
NDUFA8	NADH: ubiquinone oxidoreductase subunit A8	OXPHOS	0.10
PRDX4	Peroxiredoxin 4	myc	0.05
RPS27A	Ribosomal protein S27a	mTOR	0.28

Abbreviation: OXPHOX, oxidative phosphorylation.

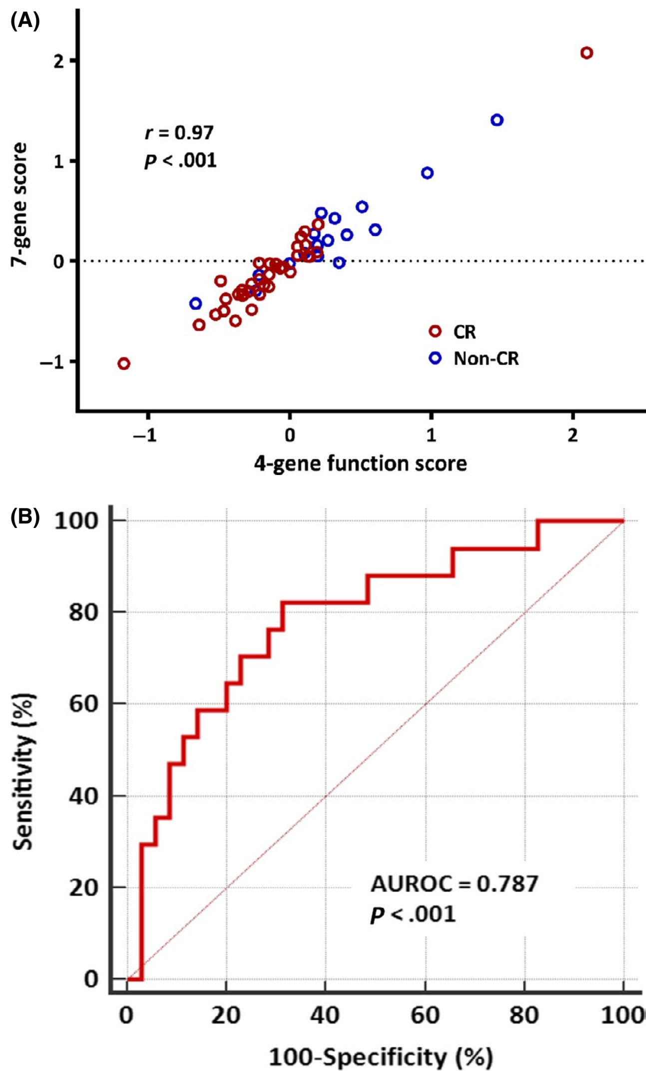

FIGURE 4 The seven-gene scoring system for potential clinical application. (A) Scatter plot of the seven-gene scores and original four-function scores. The cutoff for the seven-gene model and four-function model was −0.027 and 0.140, respectively. (B) The seven-gene scoring system achieved an acceptable performance (area under receiver operating characteristic [AUROC] = 0.787; P < .001) [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

In this study, we found that chemoresistance to the "7 + 3" induction regimen in de novo AML could be associated with mTOR, myc, OXPHOS, and stemness pathways. Among all possible molecules associated with chemoresistance, the family of ABC transporters is considered one of the most critical players of chemoresistance in AML.[10] However, studies regarding the application of ABC inhibitors did not often demonstrate a clinical benefit in patients with AML or myelodysplastic syndrome.[21,22] This result suggests that mechanisms other than ABC transporters must be involved in AML chemoresistance.[23] The function of LSCs is one example. The LSCs are associated with chemoresistance in PDX models.[24] A recent study by Ho et al[25] further demonstrated the evolution of LSC spectrums upon chemotherapy, which highlighted the role of LSCs in chemoresistance acquisition. However, another PDX study by Farge et al[9] revealed a different result, showing that quiescent LSCs or LSC gene signatures were not enriched in cytarabine-resistant AML cells. In contrast, high mitochondrial OXPHOS represented a hallmark of chemoresistance.[9]

To explore this issue, the current study proposed an integrated function model to predict chemoresistance in patients with de novo AML treated with the "7 + 3" induction regimen. Using transcriptome-wide expression profiling and systematic gene set enrichment analysis, we verified that chemoresistance to the "7 + 3" regimen was multifactorial. Not only mitochondrial OXPHOS and stemness but also mTOR and myc were independent functions participating in chemoresistance to the "7 + 3" regimen (Figure 2B and 2).

Although the precise role of mTOR signaling in chemoresistance is not fully understood, a phase I study has implied the feasibility of a combination of an mTOR inhibitor and chemotherapeutic agents for patients with refractory AML.[26] Recently, an in vitro study by Yang et al[27] also revealed that PI3K/Akt/mTOR pathway activation by long non-coding RNA linc00239 could be one of the mechanisms for chemoresistance against doxorubicin in AML. In terms of the role of myc on chemoresistance, myc may contribute to microenvironment-mediated drug resistance in AML by the protecting effect of mesenchymal stromal cells against leukemic cell apoptosis.[28] Furthermore, one in vitro study suggested that myc inhibition overcomes cytarabine resistance in AML.[29] From a cellular perspective, these results at least partially support our data that myc could play a crucial role in chemoresistance to the "7 + 3" regimen in patients with de novo AML (Figure 2C).

Our study also showed that the integrated function model could predict chemoresistance to the "7 + 3" regimen better than a single pathway in patients with AML. In the single-function context, myc might outperform mTOR, OXPHOS, and stemness (Figure 3A). However, the proposed four-function model provided superior prediction ability for chemoresistance by improving the prediction power. To increase the clinical application of the model, our study further established a seven-gene scoring system generated from the four-function model to predict "7 + 3" induction failure in AML. This targeted gene model not only correlated highly with the four-function model (r = .97; P < .001; Figure 4A), but also could largely decrease the turnaround time and cost of transcriptome-wide gene sequencing. Furthermore, multivariate analysis validated our target gene model, showing that the seven-gene score could be an independent variable for "7 + 3" induction failure in patients with de novo AML (Table 3; P = .005).

Because of the small number of patients, our study was unable to provide an independent validation cohort to verify the seven-gene score model, which is one of its significant limitations. To reduce this limitation, we used the data by Horibata et al[19] to validate our results externally, showing that the overexpressed refractoriness signature from Horibata's cohort was also identified in our non-CR group (GSEA P < .001; Figure S1). We also validated our results by the data from Brown et al.[30] The study by Brown et

TABLE 3 Risk factors for the "7 + 3" induction failure

	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
7-gene score						
≥−0.027 vs <−0.027	10.18	(2.42-42.83)	.002	11.18	(2.06-60.65)	.005
Cytogenetics[a]						
Unfavorable vs Non-unfavorable	14.67	(2.64-81.57)	.002	16.43	(2.16-124.81)	.007
Age, y	1.01	(0.97-1.05)	.780			
Sex (Female vs Male)	0.52	(0.16-1.70)	.277			
Leukocyte count						
≥10000/μL vs <10000/μL	0.81	(0.20-3.27)	.770			

Abbreviations: CI, confidence interval; CR, complete remission; HR, hazard ratio.
[a]Stratified according to European Leukemia Network.

al[30] showed that *SETBP1*, *ASXL1*, and *RELN* mutations are highly associated with primary chemoresistance in AML. However, the expressions of these three genes were not significantly different between the CR and non-CR groups in our study cohort (data not shown). Several reasons could explain this discrepancy. The study by Brown et al[30] was limited to cytogenetically normal AML patients. Besides, both DNA and RNA targeted genes were investigated. Moreover, only 23.4% (25/107) of patients in their cohort received the "7 + 3" regimen. Notably, compatible with the results of previous studies,[31,32] our data showed that unfavorable cytogenetics was one of the independent factors associated with induction failure of the "7 + 3" regimen. This result could indirectly verify the prediction of "7 + 3" induction failure by the seven-gene score model. Currently, we are conducting a prospective study to validate the efficacy of the seven-gene score model in patients newly diagnosed with AML.

In summary, our study demonstrated that myc, OXPHOS, mTOR, and stemness were responsive for chemoresistance in AML. We also proposed a four-function model to predict chemoresistance of patients with de novo AML to the "7 + 3" induction chemotherapy. We further provided a seven-gene scoring system generated from the four-function model for better clinical application. For patients with de novo AML predicted to be refractory to the "7 + 3" regimen, treatments other than the "7 + 3" regimen or clinical trials need to be considered for their induction therapy. Prospective studies with large numbers of patients are needed to validate our four-function model and seven-gene scoring system. By using this seven-gene scoring system, implementation of a targeted transcriptome panel by RNA sequencing or quantitative polymerase chain reaction in the routine setting could provide more clinical feasibility in the future.

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Yu-Chiao Chiu performed the research, analyzed, and interpreted the data. Tzu-Hung Hsiao designed the study and critically reviewed the manuscript. Jia-Rong Tsai analyzed the data. Li-Ju Wang performed the study. Tzu-Chieh Ho designed the study. Shih-Lan Hsu designed the study and reviewed the manuscript. Chieh-Lin Jerry Teng designed the study, interpreted the data, and wrote the paper. All authors gave final approval of the manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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