

# Construction of Immune-Related Prognostic Signatures in Acute Myeloid Leukemia

## Ruyi Xu

Bone Marrow Transplantation Center, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

## Yi Li

Bone Marrow Transplantation Center, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

## Yang Liu

Bone Marrow Transplantation Center, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

## Jianwei Qu

Bone Marrow Transplantation Center, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

## Wen Cao

Bone Marrow Transplantation Center, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

## Enfan Zhang

Bone Marrow Transplantation Center, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

## Jingsong He

Bone Marrow Transplantation Center, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

## Zhen Cai (✉ [caiz@zju.edu.cn](mailto:caiz@zju.edu.cn))

Zhejiang University School of Medicine First Affiliated Hospital <https://orcid.org/0000-0001-6026-3804>

---

## Research

**Keywords:** Acute Myeloid Leukemia, Tumor Microenvironment, Immune-associated genes, Prognostic signatures, Bioinformatics analysis

**Posted Date:** August 10th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-51235/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

**Background:** Acute Myeloid Leukemia (AML) is characterized as a type of hematological malignancy with poor survival. Accumulated evidence showed that dysregulated immune activities contribute to the pathogenesis of AML and accelerate the development of chemotherapy resistance. Thus, we aimed to construct prognostic signatures based on patients' immune features to sort out the high-risk group and to identify survival-related checkpoint molecules as potential therapeutic targets.

**Methods:** In the current study, we developed two prognostic signatures based on immune genes and infiltrated fraction of immune cells, respectively, using a least absolute shrinkage and selection operator model, and Cox regression analysis on 415 samples obtained from TCGA and GEO databases.

**Results:** We found the optimum strategy for predicting patients' survival is combined using these two prognostic immune-related signatures. Through our established signatures, we classified patients into Favorable Risk group and Poor Risk group, who showed significantly different OS and DFS. We further demonstrated the checkpoint molecules' profile in different risk groups.

**Conclusions:** we constructed a powerful prognostic tool here to help classify high-risk patients in early-stage, who may benefit from additional immune therapies by targeting identified checkpoint molecules.

## Background

Acute Myeloid Leukemia (AML), the most frequent acute leukemia in adults, is a type of hematological malignancy, characterized by proliferation and accumulation of abnormal hematopoietic progenitor cells in the bone marrow. These proliferated nonfunctional leukemia cells interfere with the normal hematopoietic system and then result in life-threatening symptoms such as immunodeficient, anemia, and thrombocytopenia [1, 2, 3]. Although considerable efforts have been devoted, very limited advances have been observed in AML treatment during the past few decades, with < 50% overall 5-year survival rate in AML patients. The most common clinical chemotherapy in AML, consisting of sequential courses of cytarabine and daunorubicin, showed a high rate of resistance and relapse [4].

Although the cause of leukemia hasn't reached an agreement, the proposal can be divided into three groups: (1) the instinct gene mutation and translocation of AML cells, including epigenetic dysregulation; (2) immune dysregulation; (3) bone marrow microenvironment dysregulation [5, 6]. With blossoming studies revealed that cancer immunity contributes to tumor pathogenesis, many immunotherapy approaches are introduced into AML clinical management and have been proved a promising outcome [7, 8, 9]. In contrast to solid tumors, AML cells express checkpoint inhibitor receptors and ligands, which can be potential targets in clinical therapies [10]. For better improving the outcome of AML patients, there is an urgent need for sorting out high-risk AML patients and providing them additional therapeutic schemes.

Therefore, in this study, we investigated 415 AML cases with survival data from two independent cohorts, The Cancer Genome Atlas (TCGA) and GSE 12417, and then used ImmPort database and CIBERSORT

method to generate two different immune-related prognostic signatures to predict the outcome of AML patients and sort out the most survival-related immune checkpoint molecules [11, 12]. These newly constructed signatures will help clarify AML patients into different prognosis and those high-risk patients may benefit from additional treatments targeting selected checkpoint molecules.

## Materials And Methods

### 2.1 Study population and immune gene sets

We obtained RNA expression and associated AML patients' clinical information from two public datasets, The Cancer Genome Atlas (TCGA, <https://gdc.nci.nih.gov>) and Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>). TCGA data consists of 173 cases while GSE12417 data contains 242 samples. GSE12417 data was obtained using the GPL96 platforms (Affymetrix Human Genome U133A Array, 163 samples), GPL97 platforms (Affymetrix Human Genome U133B Array, 163 samples) and GPL570 platforms (Affymetrix Human Genome U133 Plus 2.0 Array, 79 samples). The genes detected with more than one probe in GSE12417 were calculated by mean expression. The immune-related gene sets were obtained from the ImmPort database (<http://www.immport.org/>).

### 2.2 Immune genes related Signature Construction

We first matched RNA expression of TCGA samples with obtained immune-related gene sets under the standard that all genes' expression should  $> 0$  in at least half samples. Those low expressed genes which unmeet the standard were filtered out. Then we generated a univariate Cox proportional regression model to analyze the relevance between each immune gene and AML patients' overall survival time (OS). Those significant OS-related immune genes were then analyzed by the least absolute shrinkage and selection operator (LASSO) method to generate most survival predictive immune genes in AML patients. Next, we further conducted a stepwise Cox proportional hazards regression model on those LASSO selected genes and generated a risk score formula on basis of genes' expression and Cox regression coefficients: Risk Score = (gene1 expression \*gene1 coef) + (gene2 expression \*gene2 coef) +... (geneN expression \*geneN coef).

### 2.3 Immunotype Signature Construction

In this study, we used the CIBERSORT method to analyze the abundance of 22 types of tumor-infiltrating immune cells in the TCGA cohort. CIBERSORT is a bioinformatics tool that employs a deconvolution algorithm to quantify the cellular composition of infiltrated immune cells by RNA expression. Then the LASSO Cox regression model was conducted to determine the most OS-related immune cells in AML patients. The immunotype related prognostic formula was generated based on the LASSO model: Immunotype Index = Cell1 fraction  $\times$  Cell1 coef + Cell2 fraction  $\times$  Cell2 coef+...+ Cell5 fraction  $\times$  Cell5 coef. Finally, the GSE12417 cohort was used to validate the prognostic ability of the constructed immunotype signature.

## 2.4 Survival analysis

To assess the prognostic value of each constructed model, we plotted receiver operating characteristic (ROC) curves and obtained area under the curve (AUC) of ROC using SPSS software. The maximum Youden Index corresponded risk score was set as the cut-off value, with which patients were categorized as high risk or low risk. Then Kaplan-Meier plots were employed to show patients' Overall Survival (OS) and Disease-Free Survival (DFS) between different risk groups. The statistical significance was tested by the log-rank test.  $P < 0.05$  was considered a significant difference in all statistical settings.

## 2.5 Function enrichment analyses

We used Database for Annotation, Visualization and Integrated Discovery (DAVID, [http://david-ncifcrf.gov/](http://david.ncifcrf.gov/)) to analyze the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of AML significant OS-related immune genes.

# Results

## 1. Construction of immune genes signature

We firstly constructed an immune gene signature based on the TCGA cohort. We obtained all gene expression data and clinical information of 173 patients and summarized their clinicopathologic characteristics in Table 1. This TCGA cohort is comprised of 92 males and 81 females, whose median age is 58 years old. As for cytogenetic risk classification, 31 patients (18.5%) are identified as "Good", 101 patients (58.38) are "Intermediate" and 37 patients (21.395) are "Poor", and with three patients are "unclear". Among whom, 73 patients (42.2%) underwent different types of transplant therapy. The median overall survival time is 17.1 months, ranging from 0 to 118.1 months and 114 patients (65.9%) die during the follow-up.

Table 1  
Patient characteristics in TCGA cohort

Clinicopathologic characteristics	TCGA cohort, N = 173	
	Median or number	Range or percentage (%)
<b>Age (years)</b>	58	18–88
<b>Gender</b>		
Male	92	53.18%
Female	81	46.82%
<b>FAB</b>		
M0	16	9.25
M1	44	25.43
M2	38	21.97
M3	16	9.25
M4	34	19.65
M5	18	10.40
M6	2	1.16
M7	3	1.73
Unclear	2	1.16
<b>WBC</b>	17	0.4-297.4
<b>BM Blast (%)</b>	72	30–100
<b>PB Blast (%)</b>	39	0–98
<b>Cytogenetics Risk</b>		
Good	31	18.50
Intermediate	101	58.38
Poor	37	21.39
Unclear	3	1.73
<b>Transplant Type</b>		
Auto	6	3.47
Sib Allo	23	13.29
†BM: Bone Marrow; PB: peripheral Blood; DFS: disease free survival; OS: overall survival		

Clinicopathologic characteristics	TCGA cohort, N = 173	
	Median or number	Range or percentage (%)
MUD	29	16.76
Haplo	1	0.58
Auto, MUD	5	2.89
Auto, sib Allo	3	1.73
MUD, MUD	3	1.73
Sib Allo, Sib Allo	2	1.16
MUD, Auto, MUD	1	0.58
No	100	57.8
<b>DFS (Months)</b>	9.8	0-100.5
<b>DFS STATUS</b>		
Disease Free	90	52.02
Recurred/Progressed	81	46.82
Unclear	2	1.16
<b>OS (Months)</b>	17.1	0-118.1
<b>OS STATUS</b>		
Deceased	114	65.90
Living	59	34.10
†BM: Bone Marrow; PB: peripheral Blood; DFS: disease free survival; OS: overall survival		

For exploring the prognostic value of immune genes in AML patients, we employed a univariate Cox proportional regression model to assess the correlation of immune genes that are acquired from the ImmPort database, with OS in the TCGA cohort. Figure 1a shows the expression profile of 207 immune genes which were statistically significant related to OS. We then conducted GO and KEGG analysis to enrich those significant immune genes to related biological processes and pathways. Figure 1b and 1c showed that they primarily activate cytokine-cytokine receptor interaction and immune response. Then we used the LASSO Cox regression method to further select the most predictive immune genes in AML patients. With one SE above the minimum criteria in LASSO Cox regression, we got 15 genes in the model: ANGPTL3, CALCRL, CANX, CLEC11A, CRLF3, CSRP1, FAM19A5, FGF11, FGFR1, IL1RAP, MPO, PLXNB1, PPP3CB, SOCS1, WFIKKN1 (Fig. 1d, 1e).

## 2. Immune genes signature's predictive value on OS and DFS in the TCGA cohort

Next, we used a stepwise Cox proportional hazards regression to further assess the prognostic value of these 15 immune genes. Five genes were finally identified and a formula was derived on the basis of the five genes: Immune Risk = (CALCRL expression  $\times$  0.000398) - (CLEC11A expression  $\times$  0.000070) - (CRLF3 expression  $\times$  0.001053) - (PLXNB1 expression  $\times$  0.000757) + (SOCS1 expression  $\times$  0.003623). ROC curve was generated to evaluate the prognostic ability of this derived formula. As shown in Fig. 2a, the AUC index for the integrated model was 0.776 ( $p < 0.0001$ ). Then, the maximum Youden Index corresponded Immune Risk, -2.201, was set as the cut-off value. We classified patients of TCGA cohort into two groups: High Immune Risk (HIR), and Low Immune Risk (LIR). Kaplan-Meier survival analysis showed that the OS of patients in LIR was significantly longer than HIR ( $p < 0.001$ ) (Fig. 2b). In addition, LIR patients had longer Disease-Free-Survival (DFS) time ( $p < 0.001$ ) (Fig. 2c).

As shown in Fig. 2d, the mRNA expressions of five prognostic immune genes were significantly different ( $p < 0.001$ ) between HIR and LIR groups. We then further analyze the correlation between Immune Risk scores and FAB subtype (Fig. 2e) and cytogenetic risk (Fig. 2f). What's interesting is that the Immune Risk score was significantly positively associated with cytogenetic risk ( $p < 0.001$ ). Then we further evaluated the prognostic value of Immune Risk in different cytogenetic risk patients. Similarly, Kaplan-Meier survival analysis showed that patients in the LIR group have significantly longer OS than HIR, in which Immune Risk showed the best prognostic value in the "good" cytogenetic risk group ( $p < 0.001$ , Figure 2g).

### **3. Constructed Immune Risk signature poorly predicts the outcome of GSE cohort**

To validate the prognostic ability of Immune Risk, we calculated the Immune Risk score of each patient in the GSE12417 cohort (the detailed clinicopathologic characteristics was summarized in Table 2). With the same formula, patients were classified into the LIR group and HIR group base on the maximum Youden index (corresponded Immune Risk = -1.28). ROC curve was generated and as shown in Fig. 3a, the AUC index for the integrated model was only 0.52 with  $p = 0.6216$ . There was also no significant difference in OS between the two Immune Risk groups ( $p = 0.22$ ) (Fig. 3b). According to these results, the formula solely derived from survival-related immune genes exhibited the poor potential to predict patients' outcomes in the GSE cohort. Considering the immune system, which consists of various immune cells and factors, may play a complicated role in AML pathogenesis and disease progression, combined analyzing survival-related immune genes with AML microenvironment infiltrated immune cells may show greater prognostic ability.

Table 2  
Patient characteristics in GSE12417 cohort

Clinicopathologic characteristics	GSE 12417 cohort, N = 242	
	Median or number	Range or percentage (%)
Age (years)	60	17–85
<b>FAB</b>		
M0	6	2.49
M1	68	28.22
M2	79	32.78
M3	0	0
M4	53	21.99
M5	25	10.37
M6	9	3.73
M7	0	0
RAEB	2	0.83
<b>OS (Days)</b>	314	1-1518
<b>OS STATUS</b>		
Deceased	92	38.02
Living 150		61.98
		34.10
†OS: overall survival		

## 4. Construction Of Immunotype Related Signature

Then we used the CIBERSORT method to evaluate the fraction of immune cell types of AML patients in the TCGA cohort. The immune cells profile was summarized in Fig. 4a. Every patient showed different immunotypes, which can be regarded as an instinct feature of their tumor environment and may exert prognostic potentials. Next, we conducted a univariable Cox analysis to assess the relevance of immune cells fraction with patients' overall survival. As shown in Table 3, the five most relevant immune cell types are T cells CD4 memory activated, Eosinophils, Macrophages M2, Mast cell resting, and T cells regulatory (Tregs). Then we generated Immunotype Risk Index by the LASSO Cox regression method on the basis of the fraction of these immune cells. With one SE above the minimum criteria in LASSO Cox regression, the coefficients were calculated when  $\text{Log} \gamma = 2.375$  (Fig. 4b, 4c). A formula was generated based on the five infiltrated immune cells: Immunotype Risk Index =  $\text{Mast}_{\text{resting}} \times 35.36 - \text{T}_{\text{CD4 memory activated}} \times 463.51 - \text{Tregs} \times$

253.99-Macrophages<sub>M2</sub>×47.64-Eosinophils × 92.53. In addition, we compared the infiltrated immune cells between former classified HIR and LIR group, Fig. 4d showed a significant difference in the fraction of those five infiltrated immune cells.

Table 3  
Univariate Cox Regression Analysis of 22 immune phenotypes and overall survival with TCGA database

Covariate	<i>P</i>	Hazard Ratio	95% CI of Hazard Ratio
T cells CD4 memory activated	0.012	3.77E + 11	321.925 to 4.432E + 20
Eosinophils	0.048	33.986	1.035 to 1116.37
Macrophages M2	0.068	43.57	0.758 to 2505.885
Mast cells resting	0.074	0.194	0.032 to 1.172
T cells regulatory Tregs	0.097	33500.459	0.153 to 7.335E + 9
Mast cells activated	0.112	9.4172E-7	3.438E-14 to 25.795
B cells naive	0.187	5.206	0.448 to 60.458
T cells gamma delta	0.211	1.377E + 16	7.2936E-10 to 2.6004E41
T cells CD8	0.282	0.053	0.000246 to 11.232
T cells follicular helper	0.299	5046.650	0.001 to 4.922E + 10
Dendritic cells activated	0.335	7.4369E-19	9.9265E-56 to 5.5717E + 18
NK cells activated	0.347	43224625	5.2542E-9 to 3.556E + 23
Macrophages M0	0.411	3.6422E-11	4.8607E-36 to 2.7291E + 14
B cells memory	0.512	0.165	0.001 to 35.662
Plasma cells	0.583	0.404	0.016 to 10.268
Dendritic cells resting	0.62	2.3855E-77	0 to 2.682E + 226
T cells CD4 memory resting	0.721	0.650	0.061 to 6.899
Monocytes	0.759	1.13	0.517 to 2.47
NK cells resting	0.814	1.624	0.028 to 93.001
Macrophages M1	0.853	1165.582	5.3699E-30 to 2.53E + 35
T cells CD4 naive	0.917	0.567	0.000013 to 24673.124
Neutrophils	0.959	0.809	0.000251 to 2604.346

## 5. Immunotype Signature Associates With Aml's Os And Dfs

Next, ROC curves were employed to determine the predictive efficiency of the constructed immunotype model. As shown in Fig. 5a, the AUC index was 0.703 with  $p = 0.0001$ . Kaplan-Meier survival analysis showed patients in the Low Immunotype Risk (LITR) group have longer OS ( $p = 0.02$ ) and DFS ( $p = 0.047$ ) than High Immunotype Risk (HITR) group (Fig. 5b, 5c). Then we also validated the immunotype model in the GSE cohort. Figure 5d showed ROC curves which indicated that the AUC index was 0.6021 with  $p = 0.0079$ . Kaplan-Meier survival analysis was generated and showed patients in the LITR group lead significantly longer OS than the HITR group ( $p < 0.001$ , Fig. 5e). All the results indicated that the immunotype model possessed better prognostic ability than the former constructed Immune Risk model.

## 6. Combination analysis of immune genes signature and immunotype signature to maximum their predictive efficiency

As infiltrated immune cells and immune process activation all count for tumor pathogenesis, we wonder whether combine the two models can maximize their predictive abilities. First, we respectively calculated each patients' risk score according to the Immune Risk model and Immunotype Risk Index model. Then we selected patients who rated low risk in both models and labeled them as Favorable Risk. Meanwhile, we selected those who rated high risk in both models, and labeled them as Poor Risk in TCGA cohort and GSE cohort (Fig. 6a, 6d). Kaplan-Meier survival analysis was generated to assess the prognostic ability of the merged analysis model. As shown in Fig. 6b, 6c, and 6e, patients in Favorable Risk group lead significantly better outcomes than Poor Risk group on the basis of OS time and DFS time in both cohorts.

## Discussion

AML is a type of hematological malignancy with poor clinical outcome[15]. Although the therapy success depends on patients' age and leukemia type, the limited advances in treatment regime largely resulted in the unsatisfied outcome of AML patients[16]. AML patients benefit from these non-targeted cytotoxic drugs, but chemotherapy intolerance also is a big problem [17]. Besides, AML cells sheltered in the tumor microenvironment, where immune activities were dysregulated, resulting in cancer cells' self-renewal and developing drug-resistant [18, 19, 20, 21]. Thus, early identify high-risk patients and provide them additional immune targeting therapies may have promising results in clinical management. So, in this study, we constructed two different immune-based prognostic signatures to sort out high-risk patients and identified their most survival-related checkpoint molecules. Those dysregulated expressed immune genes may have therapeutic potential in AML clinical practice.

In the current study, we included 415 AML patients' mRNA expression and corresponded clinical information obtained from TCGA and GEO databases to construct prognostic predicting signatures. Firstly, based on the TCGA cohort, we constructed a five-immune genes survival signature, including CALCRL, CLEC11A, CRLF3, PLXNB1, and SOCS1. A majority of these genes are suggested to contribute to leukemia pathogenesis [22, 23]. For example, CALCRL expression was positively associated with the

engraftment capacity of primary AML patient samples in mice and higher SOCS1 expression was an independent poor prognostic factor for non-M3 and CN-AML patients [24, 25].

With this immune-genes related signature, we divided TCGA cohort patients into high risk and low risk groups, who showed significantly different OS and DFS. What's more, we found patients' Immune Risk score is significantly and positively associated with their cytogenetic risk classification. Under different cytogenetic risk conditions, the Immune Risk signature statistically correlated with patients' survival. However, when we validated this signature in the GEO cohort, ROC curves demonstrated this signature has very low predictive efficiency. And there is no survival difference between patients who were classified into different risk groups by the signature.

The above results indicated that analyzing AML patients' survival solely relies on dysregulated immune genes expression and subsequently impaired immune activation is not that convincing. The role immunity system played in the AML tumor microenvironment is too complicated to be simplified into different gene [26, 27, 28]. So, we took different infiltration of various immune cells into concern as many studies have reported that the fraction of infiltrated immune cells in the tumor microenvironment is so unique that could be regarded as patients' instinct feature to predict tumor progression [29, 30].

Thus, we used the CIBERSORT method to evaluate the fraction of infiltrated immune cells and generated an immunotype-related signature, which included five immune cells: T cells CD4 memory activated, Eosinophils, Macrophages M2, Mast cell resting and T cells regulatory (Tregs). With the immunotype-related signature, we classified patients into high risk and low risk. Different risk group patients, both in the TCGA cohort and the GEO cohort, showed significantly different outcomes.

For comprehensively understanding the role of immunity in predicting AML survival, we further classified patients into Favorable Risk and Poor Risk by combining analysis of dysregulated expressed immune genes and infiltrated immune cells. Patients' OS and DFS showed significant differences between Favorable Risk group and Poor Risk group, which indicates that the combined analysis of these two prognostic signatures optimized their predicting ability.

Finally, we compared the different expression of checkpoint molecules in Favorable Risk and Poor Risk groups. We found only CD274 and PDCD1 expression significantly correlated with AML patients' OS in the TCGA cohort, while in the GEO cohort, neither of those dysregulated expressed checkpoint molecules was associated with patients' survival. Based on the fact that the pathogenesis of AML is very complicated, we considered that combined analysis of different risk factors to predict patients' prognosis is more reliable and all those differently expressed checkpoint molecules may serve better as potential therapeutic targets.

Although predicting AML patients' survival with these constructed signatures is promising, there are also some limitations for implying them into clinical management. As those signatures are generated from two retrospective cohorts and enrolled amount is limited as well, larger prospective cohorts are needed to

validate the prognostic value of signatures. What's more, whether these identified checkpoint molecules could act as potential targets need to be further explored by mechanism studies.

## Conclusion

In summary, we generated two immune-associated signatures to predict the prognosis of AML patients and sort out associated checkpoint molecules as potential therapeutic targets. Our study provides new insight into the relevance between the immune profile and AML patients' survival. The constructed signatures help identify high-risk patients who may benefit from additional immune-based therapy.

## Abbreviations

AML

Acute Myeloid Leukemia; TCGA:The Cancer Genome Atlas; GEO:Gene Expression Omnibus; OS:Overall Survival; DFS:Disease Free Survival; LASSO:Least Absolute Shrinkage and Selection Operator; GO:Gene Ontology; KEGG:Kyoto Encyclopedia of Genes and Genomes; DAVID:Database for Annotation, Visualization and Integrated Discovery.

## Declarations

### Ethics approval and consent to participate

The data used in the study were obtained from public databases TCGA and GEO, therefore, ethical approval was not required.

### Consent for publication

Not applicable.

### Availability of data and material

The datasets supporting the conclusions of this article are available in TCGA at <https://gdc.nci.nih.gov> and GEO at <https://www.ncbi.nlm.nih.gov/geo/>. These data were derived from the following resources available in the public domain: GSE12417.

### Competing interests

The authors declare that they have no competing interests.

### Funding

This work was supported by the Funds for the National Natural Science Foundation of China (Grant No.81700201).

## Authors' Contribution

Z.C. initiated the study and supervised the project. RY.X. and Y.L. came up with the main conceptual ideas. RY.X. wrote the manuscript in consultation with JS.H., EF.Z., Y.L., JW.Q., W.C. participated in investigating related results. All authors contributed to the final manuscript.

## Acknowledgments

We thank the TCGA and GEO databases for the availability of the data.

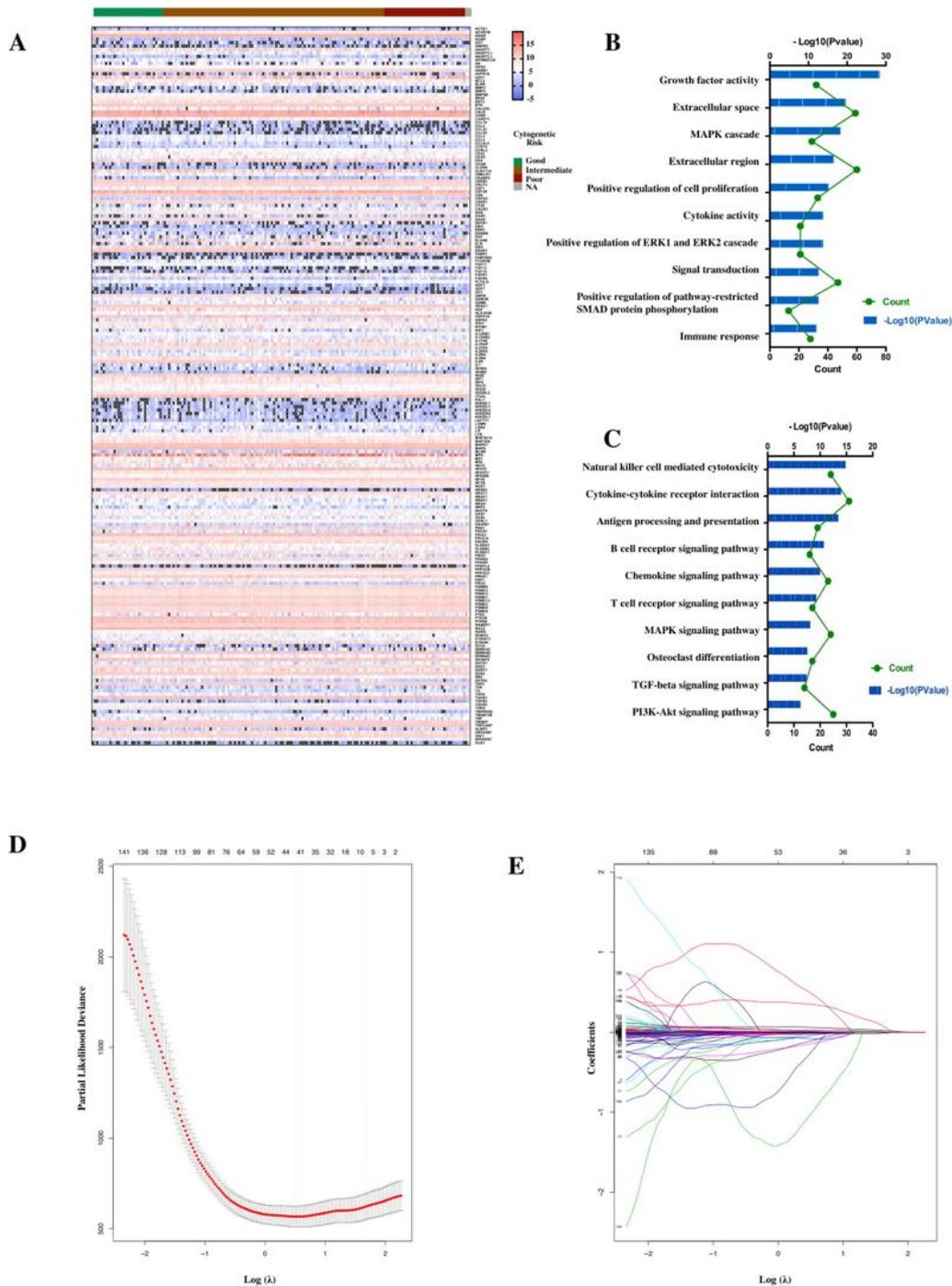
## References

1. Döhner H, Weisdorf DJ, Bloomfield CD. Acute Myeloid Leukemia. *N Engl J Med*. 2015;373(12):1136–52. Doi:10.1056/NEJMra1406184.
2. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N Engl J Med*. 2016;374(23):2209–21. Doi:10.1056/NEJMoa1516192.
3. De Kouchkovsky I, Abdul-Hay M. 'Acute myeloid leukemia: a comprehensive review and 2016 update'. *Blood Cancer J*. 2016;6(7):e441. doi:10.1038/bcj.2016.50.
4. Buckley SA, Kirtane K, Walter RB, Lee SJ, Lyman GH. Patient-reported outcomes in acute myeloid leukemia: Where are we now? *Blood Rev*. 2018;32(1):81–7. Doi:10.1016/j.blre.2017.08.010.
5. Deschler B, Lübbert M. Acute myeloid leukemia: epidemiology and etiology. *Cancer*. 2006;107(9):2099–107. Doi:10.1002/cncr.22233.
6. Shafat MS, Gnaneswaran B, Bowles KM, Rushworth SA. The bone marrow microenvironment - Home of the leukemic blasts. *Blood Rev*. 2017;31(5):277–86. Doi:10.1016/j.blre.2017.03.004.
7. Witkowski MT, Lasry A, Carroll WL, Aifantis I. Immune-Based Therapies in Acute Leukemia. *Trends Cancer*. 2019;5(10):604–18. Doi:10.1016/j.trecan.2019.07.009.
8. Liu Y, Bewersdorf JP, Stahl M, Zeidan AM. Immunotherapy in acute myeloid leukemia and myelodysplastic syndromes: The dawn of a new era? *Blood Rev*. 2019;34:67–83. Doi:10.1016/j.blre.2018.12.001.
9. Cancer Genome Atlas Research Network. Ley TJ, Miller C, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med* (2013) 368(22):2059–74. Doi:10.1056/NEJMoa1301689.
10. Daver N, Boddu P, Garcia-Manero G, et al. Hypomethylating agents in combination with immune checkpoint inhibitors in acute myeloid leukemia and myelodysplastic syndromes. *Leukemia*. 2018;32(5):1094–105. Doi:10.1038/s41375-018-0070-8.
11. ImmPort, toward repurposing of open access immunological assay data for translational and clinical research  
10.1038/sdata.2018.15

- Bhattacharya S, Dunn P, Thomas CG, et al. ImmPort, toward repurposing of open access immunological assay data for translational and clinical research. *Sci Data* (2018) 5:180015. Doi:10.1038/sdata.2018.15.
12. Newman A, Liu C, Green M, et al. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods*. 2015;12:453–7. Doi:10.1038/nmeth.3337.
  13. Lievens LA, Sterman DH, Cornelissen R, Aerts JG. Checkpoint Blockade in Lung Cancer and Mesothelioma. *Am J Respir Crit Care Med*. 2017;196(3):274–82. Doi:10.1164/rccm.201608-1755CI.
  14. Henriksen A, Dyhl-Polk A, Chen I, Nielsen D. Checkpoint inhibitors in pancreatic cancer. *Cancer Treat Rev*. 2019;78:17–30. Doi:10.1016/j.ctrv.2019.06.005.
  15. Komanduri KV, Levine RL. Diagnosis and Therapy of Acute Myeloid Leukemia in the Era of Molecular Risk Stratification. *Annu Rev Med*. 2016;67:59–72. Doi:10.1146/annurev-med-051914-021329.
  16. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424–47. Doi:10.1182/blood-2016-08-733196.
  17. Klepin HD. Elderly acute myeloid leukemia: assessing risk. *Curr Hematol Malig Rep*. 2015;10(2):118–25. Doi:10.1007/s11899-015-0257-2.
  18. Behrmann L, Wellbrock J, Fiedler W. Acute Myeloid Leukemia and the Bone Marrow Niche-Take a Closer Look. *Front Oncol*. 2018;8:444. Doi:10.3389/fonc.2018.00444.
  19. Kumar B, Garcia M, Weng L, et al. Acute myeloid leukemia transforms the bone marrow niche into a leukemia-permissive microenvironment through exosome secretion. *Leukemia*. 2018;32(3):575–87. Doi:10.1038/leu.2017.259.
  20. Wang A, Zhong H. Roles of the bone marrow niche in hematopoiesis, leukemogenesis, and chemotherapy resistance in acute myeloid leukemia. *Hematology*. 2018;23(10):729–39. Doi:10.1080/10245332.2018.1486064.
  21. Zhou HS, Carter BZ, Andreeff M. Bone marrow niche-mediated survival of leukemia stem cells in acute myeloid leukemia: Yin and Yang. *Cancer Biol Med*. 2016;13(2):248–59. Doi:10.20892/j.issn.2095-3941.2016.0023.
  22. 10.3390/cancers11121951  
Padella A, Simonetti G, Paciello G, et al Novel and Rare Fusion Transcripts Involving Transcription Factors and Tumor Suppressor Genes in Acute Myeloid Leukemia. *Cancers (Basel)* (2019) 11(12):1951. Doi: 10.3390/cancers11121951.
  23. Granziero L, Circosta P, Scielzo C, et al. CD100/Plexin-B1 interactions sustain proliferation and survival of normal and leukemic CD5 + B lymphocytes. *Blood*. 2003;101(5):1962–9. Doi:10.1182/blood-2002-05-1339.
  24. Angenendt L, Bormann E, Pabst C, et al. The neuropeptide receptor calcitonin receptor-like (CALCRL) is a potential therapeutic target in acute myeloid leukemia. *Leukemia*. 2019;33(12):2830–41. Doi:10.1038/s41375-019-0505-x.

25. Hou HA, Lu JW, Lin TY, et al. Clinico-biological significance of suppressor of cytokine signaling 1 expression in acute myeloid leukemia. *Blood Cancer J.* 2017;7(7):e588. Doi:10.1038/bcj.2017.67.
26. Vago L, Gojo I. Immune escape and immunotherapy of acute myeloid leukemia. *J Clin Invest.* 2020;130(4):1552–64. Doi:10.1172/JCI129204.
27. Li Z, Philip M, Ferrell PB. Alterations of T-cell-mediated immunity in acute myeloid leukemia. *Oncogene.* 2020;39(18):3611–9. Doi:10.1038/s41388-020-1239-y.
28. Ruggeri L, Capanni M, Tosti A, et al. Innate immunity against hematological malignancies. *Cytotherapy.* 2002;4(4):343–6. Doi:10.1080/146532402760271127.
29. Austin R, Smyth MJ, Lane SW. Harnessing the immune system in acute myeloid leukaemia. *Crit Rev Oncol Hematol.* 2016;103:62–77. Doi:10.1016/j.critrevonc.2016.04.020.
30. Yehudai-Resheff S, Attias-Turgeman S, Sabbah R, et al. Abnormal morphological and functional nature of bone marrow stromal cells provides preferential support for survival of acute myeloid leukemia cells. *Int J Cancer.* 2019;144(9):2279–89. Doi:10.1002/ijc.32063.

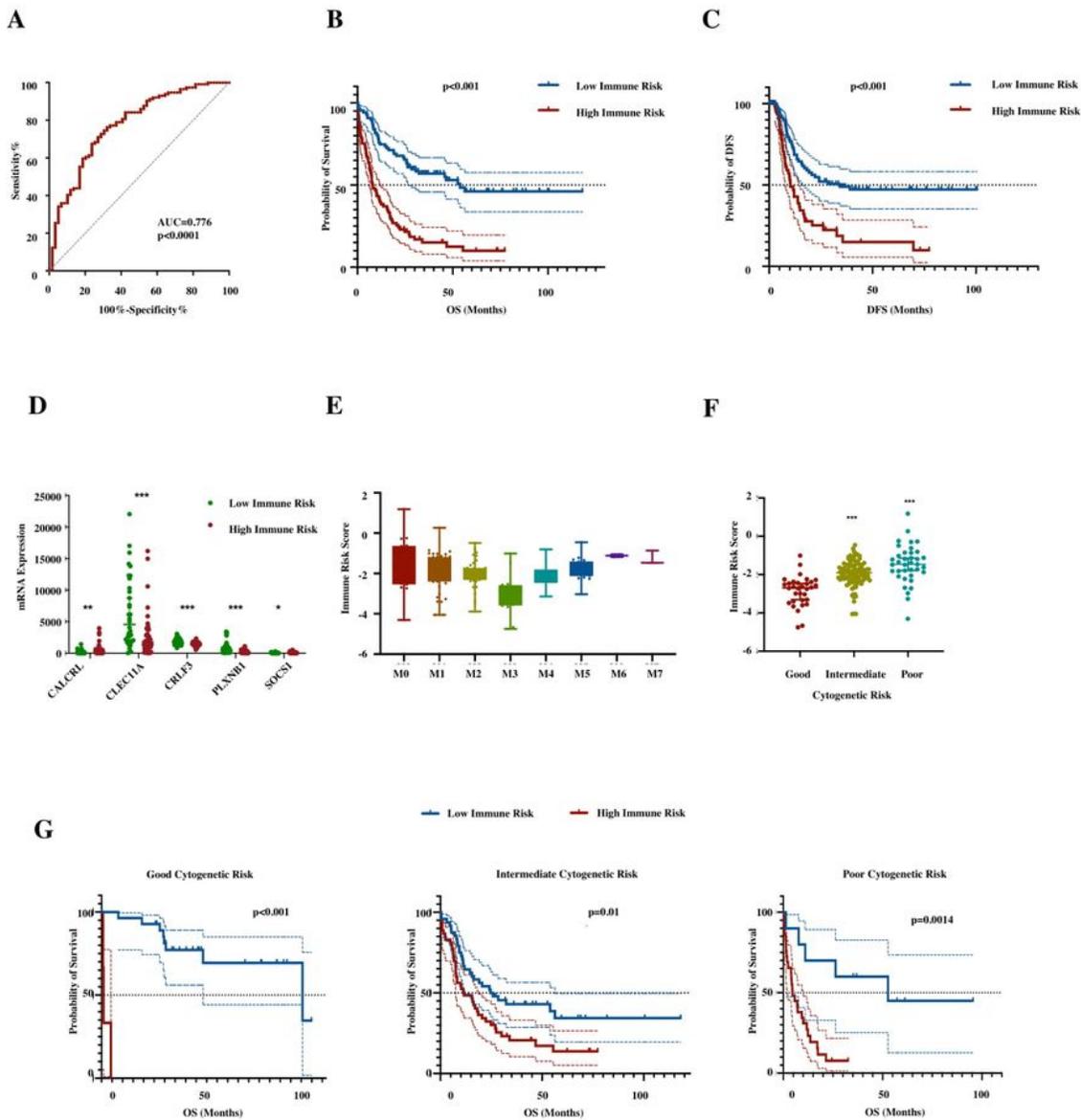
## Figures



**Figure 1**

**Figure 1**

Identification of survival-related immune genes in AML. (a) Heatmap of AML patients' OS associated immune genes. (b, c) Gene ontology (GO) enrichment and KEGG pathways analysis of identified survival-related immune genes. (d, e) LASSO coefficient profiles of 15 most significantly prognostic related immune genes. The optimal values of the penalty parameter  $\lambda$  were determined by 100-fold cross-validation.

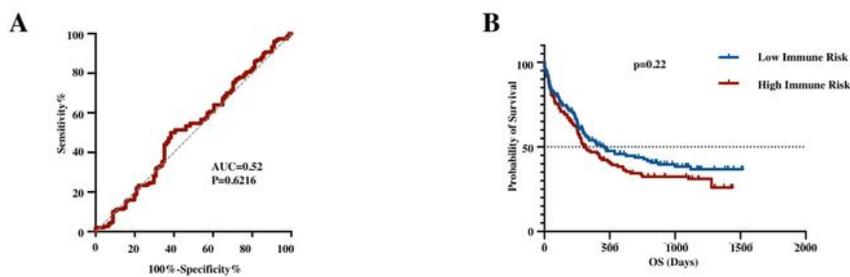


**Figure 2**

**Figure 2**

Constructed immune-genes signature well predicts patients' OS and DFS in the TCGA cohort. (a) ROC curve analysis of immune-genes signature. (b, c) Kaplan-Meier curves show patients' OS and Disease Free Survival (DFS) were significantly longer in the Low Immune Risk group. (d) mRNA expression of five signature involved immune genes. (e) Relationship between patients' Immune Risk score and FAB subtypes. (f) Relationship between patients' Immune Risk score and cytogenetic risk. (g) Kaplan-Meier

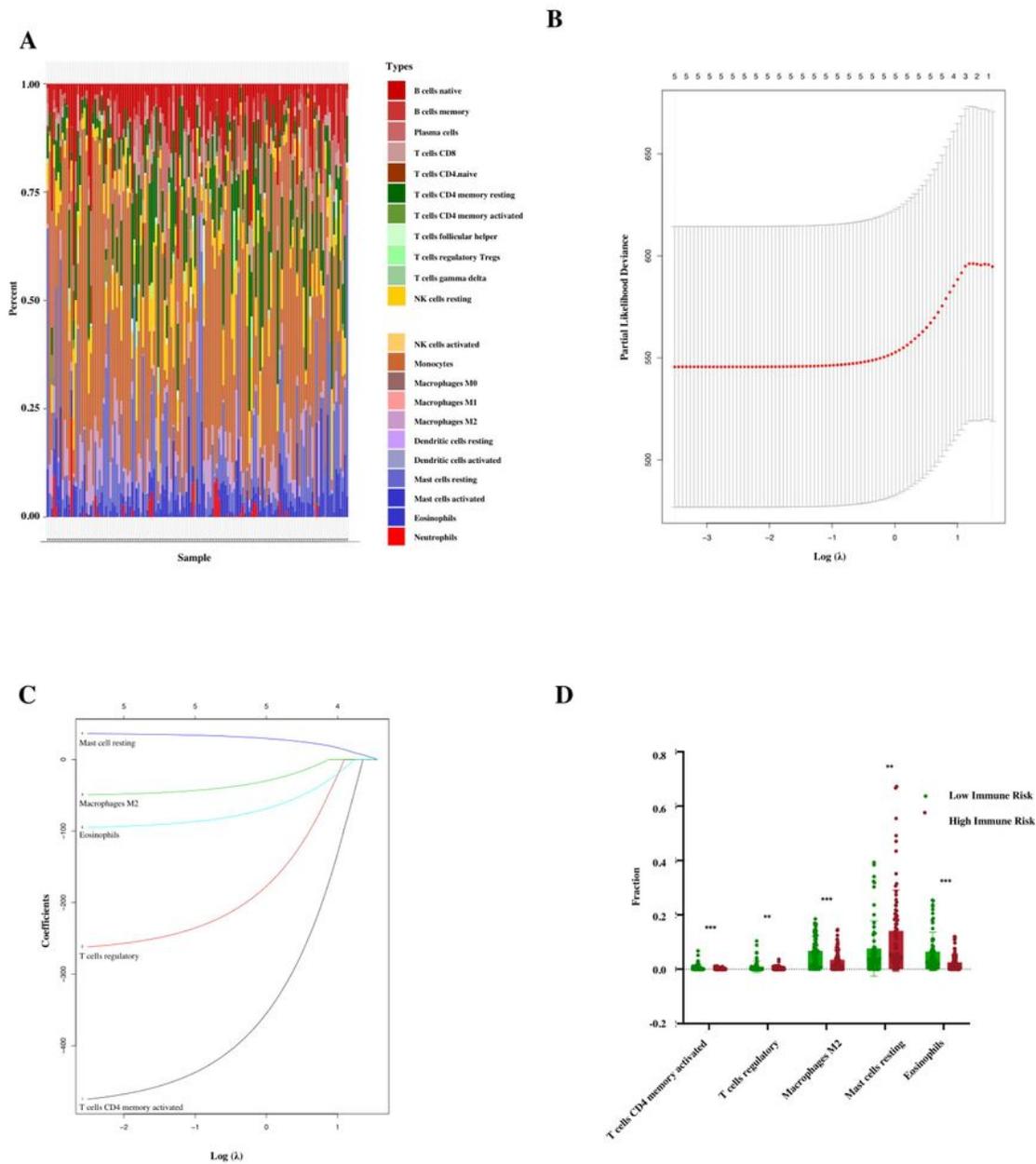
curves of OS in good, intermediate, and poor cytogenetic AML patients based on Immune Risk score. (Barplots show median  $\pm$  SEM; NS,  $p > 0.05$ ; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; and \*\*\*,  $p < 0.001$ )



**Figure 3**

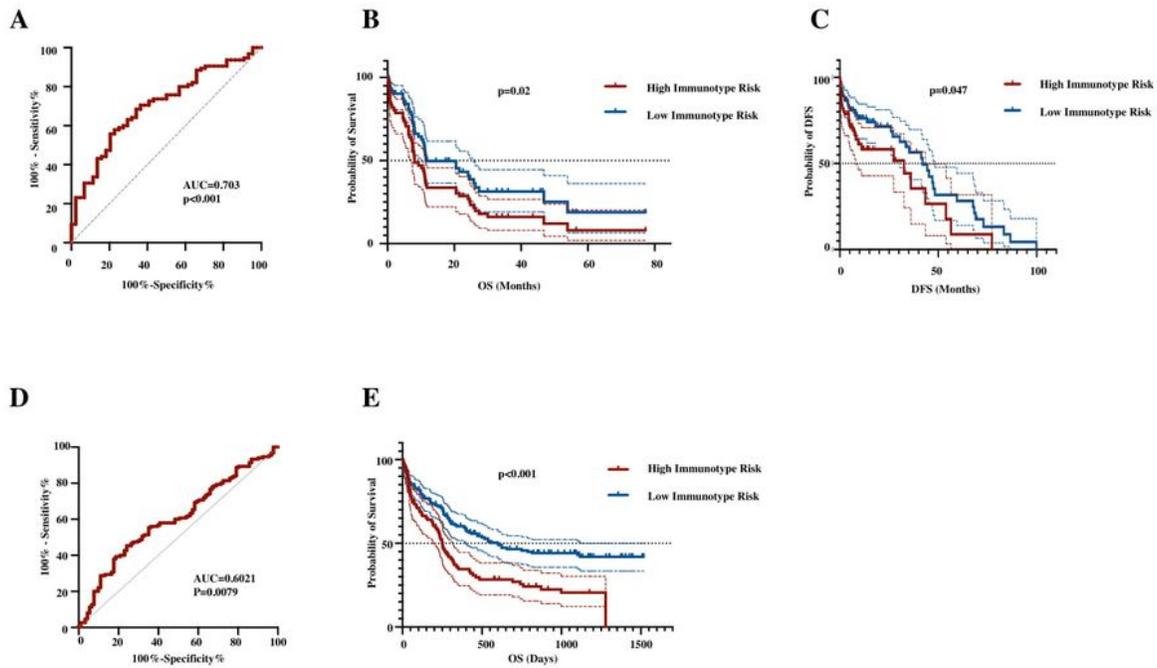
### Figure 3

Immune Risk signature poorly predicts the outcome of the GSE cohort. (a) ROC curve analysis of immune-genes signature. (B\b) Kaplan-Meier curves show there is no difference in OS between different risk group patients.



**Figure 4**

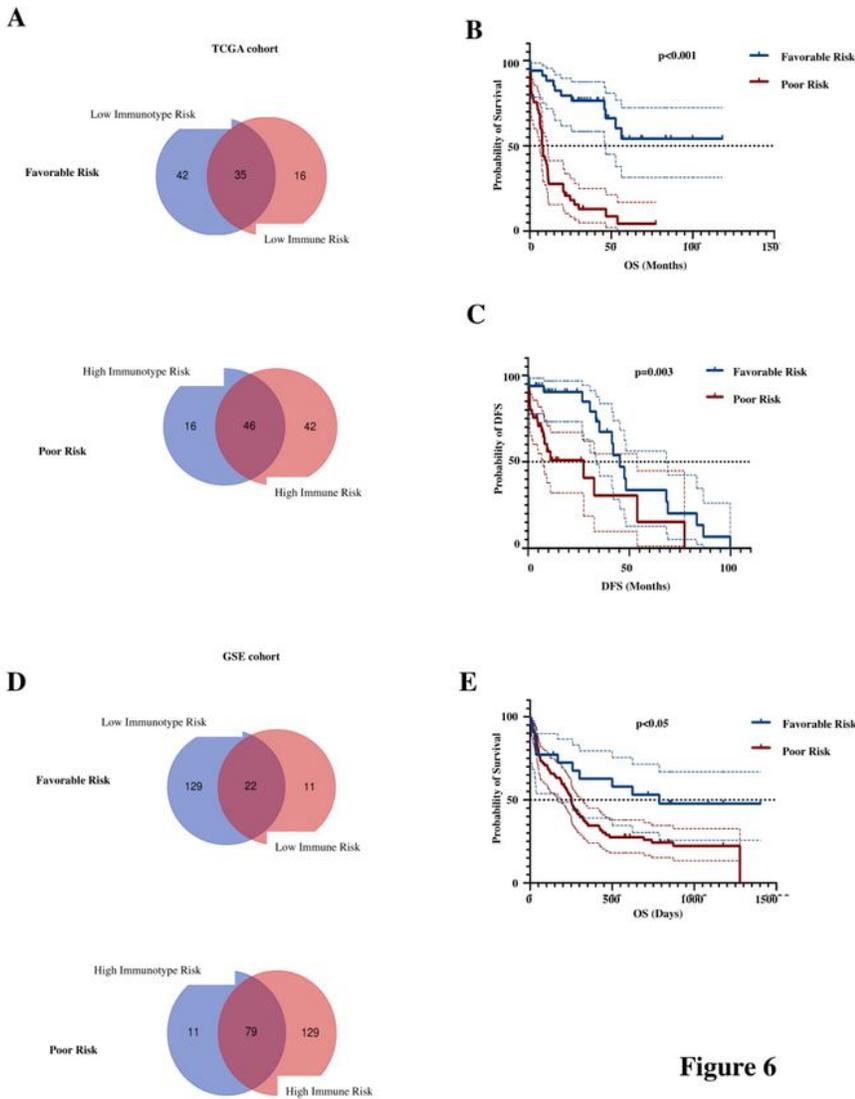
Construction of immunotype related signature. (a) Demonstration of 22 infiltrated immune cells fraction of TCGA cohort. (b) 100-fold cross-validation for tuning parameter selection in the LASSO model. (c) LASSO coefficient profiles of five infiltrated immune cells. (d) The fraction of five most OS-related infiltrated immune cells in different Immune Risk group. (Barplots show median  $\pm$  SEM; NS,  $p > 0.05$ ; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; and \*\*\*,  $p < 0.001$ )



**Figure 5**

**Figure 5**

Validation of Immunotype signature's prognostic ability in TCGA and GEO cohort. (a) ROC curve analysis of immunotype signature in the TCGA cohort. (b, c) Kaplan-Meier curves show in the TCGA cohort, patients' OS and DFS were significantly longer in the Low Immunotype Risk group. (d) ROC curve analysis of immunotype signature in the GEO cohort. (e) Kaplan-Meier curves show in the GEO cohort; patients' OS was significantly longer in the Low Immunotype Risk group.



**Figure 6**

**Figure 6**

The prognostic performance of combining two constructed signatures. (a) Venn diagrams sort out patients who were both identified as high-risk or low-risk by two signatures in the TCGA cohort. (b, c) Kaplan-Meier curves show in the TCGA cohort, patients' OS and DFS were significantly longer in the Favorable Risk group. (d) Venn diagrams sort out patients who were both identified as high-risk or low-

risk by two signatures in the GEO cohort. (e) Kaplan-Meier curves show in the GEO cohort; patients' OS was significantly longer in the Favorable Risk group.

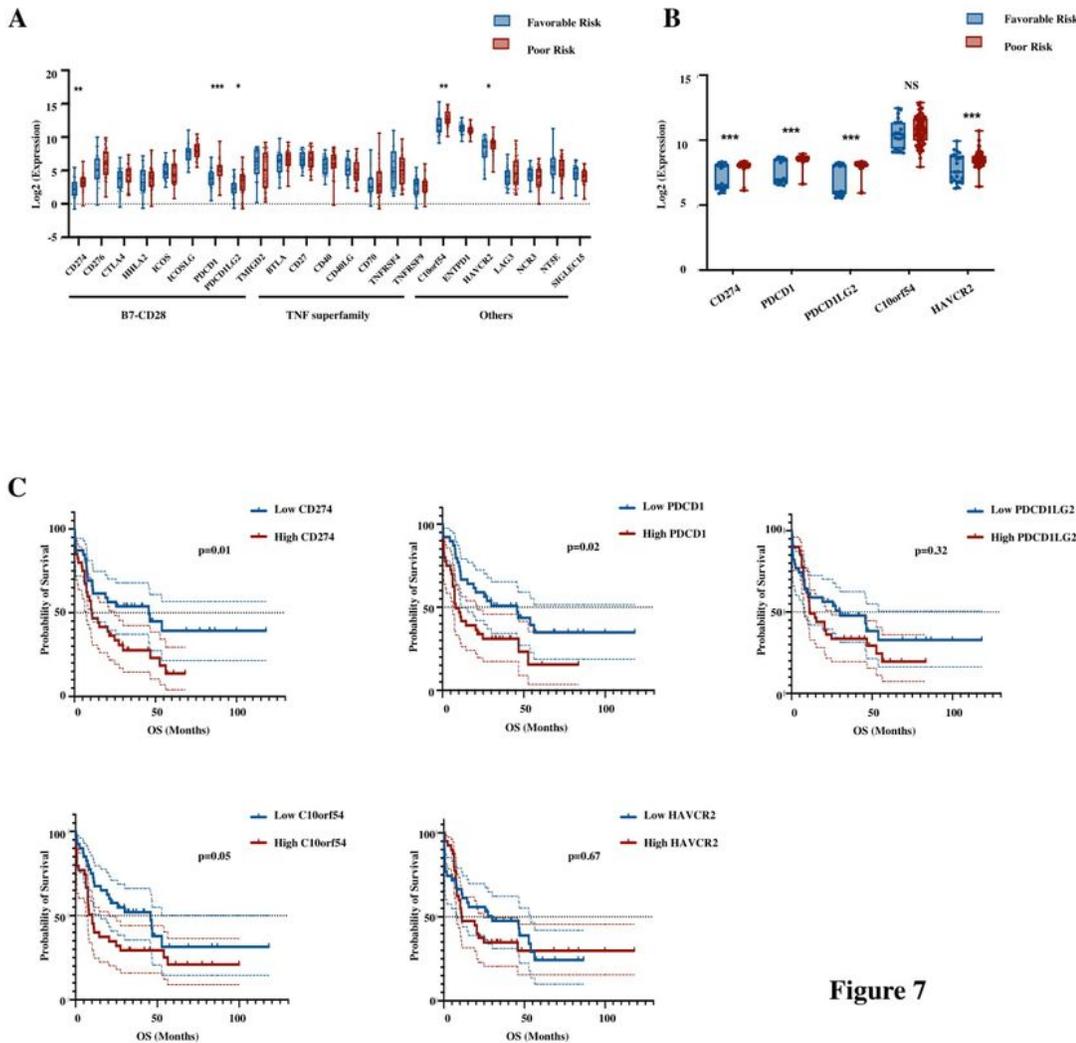


Figure 7

Figure 7

Checkpoint profile of different combination risk AML patients. (a) The mRNA expression of 23 common checkpoint molecules in Favorable Risk and Poor Risk groups of TCGA cohort. (b) The mRNA expression of most OS-related checkpoint molecules in Favorable Risk and Poor Risk groups of GEO cohort. (c)

Kaplan-Meier curves show the relationship between patients' OS and checkpoint molecules' expression in the TCGA cohort. (Barplots show median  $\pm$  SEM; NS,  $p>0.05$ ; \*,  $p<0.05$ ; \*\*,  $p<0.01$ ; and \*\*\*,  $p<0.001$ )