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Kinome expression profiling improves risk stratification and therapeutic targeting in myelodysplastic syndromes

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Abstract:
The human kinome, which comprises over five hundred kinases, plays a critical role in regulating numerous essential cellular functions. Although the dysregulation of kinases has been observed in various human cancers, the characterization and clinical implications of kinase expressions in myelodysplastic syndrome (MDS) have not been systematically investigated. In this study, we evaluated the kinome expression profiles of 341 adult patients with primary MDS and identified seven kinases (*PTK7, KIT, MAST4, NTRK1, PAK6, CAMK1D, and PRKCZ*) whose expression levels were highly predictive of compromised patient survival. We then constructed the KInase Stratification Score (KISS) by combining the weighted expressions of the seven kinases, and validated its prognostic significance in two external MDS cohorts. A higher KISS was associated with older age, higher peripheral blood and marrow blast percentages, higher Revised International Prognostic Scoring System (IPSS-R) risks, complex karyotype, and mutations in several adverse-risk genes in MDS, such as *ASXL1, EZH2, NPM1, RUNX1, STAG2, and TP53*. Multivariate analysis confirmed that a higher KISS was an independent unfavorable risk factor in MDS. Mechanistically, the KISS-high patients were enriched for genesets associated with hematopoietic and leukemic stem cell signatures. By investigating the Genomics of Drug Sensitivity in Cancer (GDSC) database, we identified axitinib and taselisib as candidate compounds that could potentially target the KISS-high myeloblasts. Altogether, our findings suggest that KISS holds the potential to improve the current prognostic scheme of MDS and inform novel therapeutic opportunities.

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Abstract

The human kinome, which comprises over five hundred kinases, plays a critical role in regulating numerous essential cellular functions. Although the dysregulation of kinases has been observed in various human cancers, the characterization and clinical implications of kinase expressions in myelodysplastic syndrome (MDS) have not been systematically investigated. In this study, we evaluated the kinome expression profiles of 341 adult patients with primary MDS and identified seven kinases (*PTK7, KIT, MAST4, NTRK1, PAK6, CAMK1D* and *PRKCZ*) whose expression levels were highly predictive of compromised patient survival. We then constructed the KInase Stratification Score (KISS) by combining the weighted expressions of the seven kinases, and validated its prognostic significance in two external MDS cohorts. A higher KISS was associated with older age, higher peripheral blood and marrow blast percentages, higher Revised International Prognostic Scoring System (IPSS-R) risks, complex karyotype, and mutations in several adverse-risk genes in MDS, such as *ASXL1, EZH2, NPM1, RUNX1, STAG2*, and *TP53*. Multivariate analysis confirmed that a higher KISS was an independent unfavorable risk factor in MDS. Mechanistically, the KISS-high patients were enriched for genesets associated with hematopoietic and leukemic stem cell signatures. By investigating the Genomics of Drug Sensitivity in Cancer (GDSC) database, we identified axitinib and taselisib as candidate compounds that could potentially target the KISS-high myeloblasts. Altogether, our findings suggest that KISS holds the potential to improve the current prognostic scheme of MDS and inform novel therapeutic opportunities.

Key Points:

- Through transcriptomic analysis, we identified seven kinases whose expressions were strongly predictive of compromised survival in MDS.
- The KInase Stratification Score (KISS) could improve risk-stratification and imply novel therapeutic opportunities in MDS.

Introduction

Myelodysplastic syndrome (MDS) is a heterogeneous constellation of myeloid neoplasms, originating from the clonal proliferation of malignant hematopoietic stem cells (HSC).[1] Although the initial clinical manifestations of MDS are usually characterized by ineffective hematopoiesis and peripheral blood (PB) cytopenias, the disease can eventually evolve into acute myeloid leukemia (AML) in about 30% of patients, and frequently becomes fatal.[1-3]

Currently the prognosis of newly-diagnosed MDS patients is most commonly evaluated with the revised International Prognostic Scoring System (IPSS-R),[4] however, it is observed that patients may still have variable clinical outcomes even if they are categorized within the same risk category.[5] As the genomic landscape of MDS becomes more elucidated with the advances in the sequencing technology, the IPSS-Molecular (IPSS-M) has recently been proposed to further fine-tune the risk stratification of MDS.[6,7] Nevertheless, it is both financially and computationally demanding to obtain the genomic information required by this more complex genetically inspired risk model. Endeavors to identify standardized molecular markers that could improve the outcome prediction for patients with myeloid neoplasms had been undertaken before as well. Most notably, Pellagatti et al. performed an integrative transcriptomic analysis of 125 MDS patients, and devised the gene expression profiling (GEP)-based Coxnet signature comprised 20 genes, for refining the risk classification in MDS.[8] Ng et al. first analyzed the global gene expression of AML patient derived leukemic stem cells (LSC) in the murine xenotransplantation models to identify prognostic biomarkers closely related to stemness, and then applied a statistical regression algorithm to generate the 17-gene LSC score (LSC17) that could improve risk stratification in patients of diverse AML subtypes.[9] Nevertheless, currently

no clinico-genomic or transcriptomic risk model could inform readily applicable treatment implications.

The treatment options for MDS have evolved substantially in recent years, in parallel with our deeper understanding of the pathophysiology of MDS. While hypomethylating agents (HMA) and allogeneic hematopoietic stem cell transplantation (allo-HSCT) remain the standard of care for high-risk MDS patients, venetoclax, the selective BCL2-inhibitor, has also been demonstrated to improve the response rates further.[10] However, relapses or progression to AML are still common, especially in those who are unfit for allo-HSCT. Therefore, there exists an unmet need for novel treatment strategies in MDS patients, especially those with high-risk disease.

Kinases are enzymes that catalyze the transfer of phosphate residues from phosphate-donors to target proteins, a biological process known as phosphorylation.[11] Collectively, the human kinome is composed of more than five hundred kinases, and comprises about 1.7% of the coding regions of our genome. The human kinome can be classified into nine typical and thirteen atypical families.[12] Kinases are critical players in various cellular processes, such as signal transduction, metabolism, proliferation, differentiation, and apoptosis.[13] Due to their functional versatility, more than 85% of the kinases are found to be dysregulated in human diseases,[13] and more than 70 small molecule kinase inhibitors have been approved by U.S. Food and Drug Administration (FDA) for therapeutic purposes.[14] However, none of the kinase inhibitors have received regulatory approval for the treatment of MDS yet.

In this study, we hypothesized that the aberrant expression of kinases could exert an impact on the clinical prognosis, and moreover, indicate novel treatment options in MDS patients. We first profiled the gene expressions of the human kinome to devise a highly prognostic kinase-

based risk score that could refine the risk-stratification of MDS, and further explored novel therapeutic possibilities by mining the well-curated Genomics of Drug Sensitivity in Cancer (GDSC) database.

Materials and Methods

Patients

From 1997 to 2019, 341 adult patients diagnosed with primary MDS according to the 2016 World Health Organization classification criteria,[15] and had adequate cryopreserved diagnostic bone marrow (BM) samples for DNA and RNA sequencing, at the National Taiwan University Hospital (NTUH) were included in this study. These patients were further annotated according to the 2022 International Consensus Classification (ICC) classification of myeloid neoplasms and acute leukemias after the release of the updated criteria.[16] Because allo-HSCT is a well-established disease course modifier in myeloid malignancies, including MDS, the 282 patients who did not receive allo-HSCT were designated as the NTUH-A cohort, while the other 59 patients who had received allo-HSCT were designated as the NTUH-B cohort. In addition, 19 healthy BM stem cell donors were recruited as healthy controls (HC). This study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of the NTUH. All participants provided written informed consent.

RNA sequencing (RNA-seq) and raw data pre-processing

RNA was extracted from the diagnostic BM samples (without CD34+ cell isolation), and library was constructed using the TruSeq Stranded mRNA Library Prep Kit (Illumina, San Diego, CA, USA) following the manufacturer's recommendations. The libraries were then sequenced on an Illumina NovaSeq 6000 with 150 bp paired-end read mode. Adapter sequences and low-quality bases in the raw sequencing data were removed using Cutadapt (v 3.0), and the clean reads were then aligned to the human reference genome GRCh38 using STAR (v2.7.6a) with two-pass mode.[17] The raw count of each gene was calculated according to the GENCODE v28 annotation and was converted into to transcripts per million (TPM) for further analysis.

Development of the KInase Stratification Score (KISS)

The overall workflow for survival modeling and establishment of the kinase-based risk score is illustrated in supplemental Figure 1A. A total of 517 kinase gene was extracted from the KinHub (http://www.kinhub.org/) database.[18] We excluded 125 kinases with low expression values (< 1 TPM) and then performed differential expression analysis between MDS patients and HCs to identify 61 over-expressed kinase in MDS (adjusted $P < 0.05$ and log2 fold-change > 0). For subsequent survival modelling, we transformed the original TPM values into the log2(TPM + 1) scale, where +1 term was to mitigate excessive variations of small values, and then performed z-transformation across all samples, so that the risk score calculated from different patient cohorts or different gene expression quantification methods (e.g., RNA-seq or microarray) would be more comparable. Next, univariate Cox proportional hazards regression was used to select 24 out of the 61 kinases that had significant impact on OS (hazard ratio (HR) > 1.0, adjusted $P < 0.05$) in NTUH-A cohort. To construct a parsimonious outcome prediction model, the least absolute shrinkage and selection operator (LASSO) Cox proportional hazards (PH)

regression modeling method was employed. We fitted the LASSO Cox regression model with 10-fold cross-validation to the 24 kinase genes (supplemental Figure 1B). Kinases with non-zero coefficients (N = 7) were then selected to construct the KISS, which was defined as the normalized gene expressions of component kinases weighted by their corresponding LASSO coefficients: (0.252 × *PTK7*) + (0.145 × *KIT*) + (0.144 × *MAST4*) + (0.072 × *NTRK1*) + (0.065 × *PAK6*) + (0.061 × *CAMK1D*) + (0.003 × *PRKCZ*). The expression levels of the seven kinases were significantly higher in MDS patients as compared to HCs (supplemental Figure 1C).

Additional details on description of materials and methods were presented in the supplemental Methods.

Results

Patient characteristics

The demographic and clinical characteristics of the overall 341 NTUH MDS patients had been described previously.[19] Briefly, the cohort consisted of 36.7% females and 63.3% males, with a median age of 68.3 years. 59 (17.3%) of the 341 patients received allo-HSCT as part of their treatment. In addition, 332 patients (97.4% of the cohort) had diagnostic cytogenetic information, and of these, 4.5%, 24.1%, 23.5%, 24.4% and 23.5% could be classified into very-low, low, intermediate, high and very-high risk categories in the IPSS-R scheme, respectively.

The KISS could risk-stratify patient outcomes across multiple MDS cohorts

Since dysregulation of kinases may play an important role in the pathogenesis and progression of MDS, we modeled the kinase expressions in MDS (detailed in the Materials and Methods section), and identified seven kinases, namely *PTK7, KIT, MAST4, NTRK1, PAK6, CAMK1D* and *PRKCZ*, whose expressions were significantly associated with compromised survival in MDS. Co-expression analysis of the seven members of KISS revealed that *PRKCZ* and *CAMK1D* formed a co-expression module, while *MATS4*, *PTK7*, *KIT*, *NTRK1*, and *PAK6* formed another one (Figure 1A). We further integrated the weighted sum of these seven kinase into the KISS prognostic score, and used the maximally selected log-rank statistics method to identify the optimal threshold dichotomizing the MDS patients into KISS-high and KISS-low (i.e., prognostically unfavorable vs. favorable) subgroups (Supplemental Figure 1D).

We first demonstrated that KISS-high patients had significantly shorter OS (Figure 1B, median 17.7 vs. 162.1 months, $P < 0.001$) and LFS (Figure 1C, median 10.3 vs. 118.1 months, $P < 0.001$) than KISS-low patients in the NTUH-A cohort. To assess the robustness of our scoring method, we also evaluated the prognostic capability of KISS in two external MDS cohorts. Using the same cutoff value as defined in the NTUH-A cohort, KISS-high patients consistently had significantly worse OS than KISS-low patients in both external validation cohorts, namely GSE114922 (Figure 1D, median OS 54.5 months vs. not reached (NR), $P = 0.036$) and GSE58831 (Figure 1E, median 54.5 months vs. NR, $P = 0.035$), demonstrating the robustness of our KISS for outcome prediction in MDS.

The KISS-high subgroup was associated with high-risk clinical and genetic features

The KISS, as a continuous variable, was significantly higher in the clinically defined high-risk MDS subclasses, such as MDS with excess blasts-1 (MDS-EB1) and MDS-EB2 (supplemental Figure 1E, Kruskal-Wallis $P < 0.001$). The KISS also appeared to elevate progressively ranging from IPSS-R very-low to very-high risk groups (supplemental Figure 1F, Kruskal-Wallis $P < 0.001$). The comparison of clinical characteristics and genetic alterations between the KISS-high and KISS-low patients of the NTUH-A cohort is presented in Table 1. (The annotation by the 2022 ICC classification is presented in supplemental Table 2) The KISS-high subgroup had more advanced age ($P = 0.020$), higher PB and BM (both $P < 0.001$) blast percentages, and lower platelet levels ($P = 0.003$) at diagnosis than the KISS-low subgroup. According to the 2016 WHO classification of MDS, the KISS-high subgroup had less frequent MDS with single lineage dysplasia (MDS-SLD, $P < 0.001$), MDS with multilineage dysplasia (MDS-MLD, $P < 0.001$), MDS with ring sideroblasts and single lineage dysplasia (MDS-RS-SLD, $P < 0.001$) or multilineage dysplasia (MDS-RS-MLD, $P = 0.001$) subtypes, but more frequent MDS-EB-1 and MDS-EB-2 (both $P < 0.001$) subtypes. According to the 2022 ICC classification, the KISS-high subgroup had lower proportions of MDS with mutated *SF3B1* ($P < 0.001$), MDS, not otherwise specified (NOS), with SLD ($P < 0.001$), and MDS, NOS, with MLD ($P < 0.001$), but higher proportions of MDS with mutated *TP53* ($P = 0.008$), MDS with EB ($P < 0.001$), MDS/AML with mutated *TP53* ($P = 0.003$), MDS/AML with myelodysplasia-related gene mutations or cytogenetics abnormalities ($P < 0.001$). In addition, patients in the KISS-high subgroup were more likely to harbor complex cytogenetics ($P < 0.001$) but had less frequent normal karyotype ($P < 0.001$). In terms of IPSS-R, the KISS-high subgroup was enriched for very-high and high risks (both $P < 0.001$), while inversely correlated with low ($P < 0.001$) and very-low ($P = 0.007$) risks. The KISS-high subgroup was significantly enriched for mutations in

ASXL1, *EZH2*, *NPM1*, *RUNX1*, *STAG2*, and *TP53* ($P < 0.001$, $P = 0.020$, $P = 0.027$, $P = 0.001$, $P = 0.029$, and $P < 0.001$, respectively), while the KISS-low subgroup had significantly higher frequency of *SF3B1* mutation ($P = 0.004$) (supplemental Table 3). Collectively, these results showed that the KISS was able to identify a subset of prognostically unfavorable patients with high-risk clinical and genetic features.

KISS as an independent prognostic factor for OS and LFS in MDS patients

Given that several conventional clinical factors also influence the survival of MDS patients, we conducted further subgroup analyses to explore the prognostic significance of KISS across various clinical scenarios. We found that the adverse prognostic impact of KISS on OS and LFS remained significant in both lower-risk (IPSS-R very-low, low, and intermediate risks merged; Figure 2A, median OS 45.1 vs. 162.1 months, $P = 0.001$; Figure 2B, median LFS 32.5 vs. 162.1 months, $P < 0.001$) and higher-risk (IPSS-R high and very-high risks merged; Figure 2C, median OS 13.0 vs. 85.2 months, $P = 0.009$; Figure 2D, median LFS 7.0 vs. 43.0 months, $P = 0.004$) MDS patient subsets. KISS was also able to stratify risk in MDS patients harboring either normal karyotype (supplemental Figure 2A) or complex cytogenetics (supplemental Figure 2B). Further, in the multivariate Cox PH regression analysis considering KISS and those statistically significant clinical variables and genetic mutations in the univariate Cox PH regression analysis, (supplemental Table 4), the KISS remained an independent adverse prognostic factor for OS (HR 1.692, 95% CI 1.151 - 2.488, $P = 0.008$) and LFS (HR 1.797, 95% CI 1.238 - 2.608, $P = 0.002$). (Table 4) Taken together, higher KISS predicted worse clinical outcomes not only in the overall MDS cohort, but also in various clinically relevant subgroups,

and this adverse prognostic impact appeared to be independent of other conventional risk factors, such as age, IPSS-R, and genetic mutations.

Additionally, to compare the prognostic performance of the KISS with other reported gene expression-based predictors in MDS, we calculated the Coxnet predictor and the LSC17 score in our MDS cohort.[8,9] Although the LSC17 score, composed of the weighted expression of 17 leukemic stem cell (LSC) related genes, was initially developed for risk determination in AML, as MDS is also a stem cell disease,[20,21] we reasoned that the LSC17 score may also play a role in MDS risk stratification. Indeed, both the Coxnet predictor and the LSC17 score could risk-stratify patients in the NTUH-A cohort (supplemental Figure 3A and 3B), and both scores were significantly higher in the KISS-high patients (Figure 2E and 2F, Wilcoxon rank-sum P = 0.044 and < 0.001, respectively). Nonetheless, we found that the KISS had superior predictive power for patient outcome than the Coxnet or LSC17 scores, assessed by the time-dependent ROC curves (Figure 2G).

KISS could help identify appropriate candidates for allo-HSCT

As we demonstrated the adverse prognostic impact of KISS on MDS patients, we were interested in whether the adverse prognostic impact of higher KISS could be mitigated by allo-HSCT. We therefore examined KISS-high patients in the entire MDS cohort (NTUH-A and NTUH-B) and found that allo-HSCT could significantly improve both OS and LFS in these high-risk patients (supplemental Figure 3C, median OS 37.7 vs. 17.7 months, P < 0.001; median LFS 32.9 vs. 10.3 months, P < 0.001). Conversely, patients categorized as KISS-low did not derive survival benefit from allo-HST (supplemental Fig. 3D). These findings suggested that KISS

could mitigate the adverse prognostic effect of the KISS-high status, and thus hold the potential for selecting MDS patients who would benefit from the allo-HSCT procedure.

Functional analysis revealed an enhanced stem cell signature in the KISS-high patients

To elucidate possible biological mechanisms underlying the adverse detrimental effect of higher KISS, we analyzed the RNA-seq data of BM samples in our MDS patient cohort (NTUH-A and NTUH-B merged). The differential expression analysis between the KISS-high vs. KISS-low patients was performed. The most significant overexpressed and underexpressed genes were illustrated in Figure 3A (the details of the top 300 DE genes were listed in supplemental Table 5). Notably, gene involved in the regulation of HSC proliferation and maintenance were significantly overexpressed in the KISS-high subgroup (FDR < 0.05), such as *CPXM1, PTK7, KIT, NYNRIN*, and *MSI2*.[9,22] The LSC17 score associated genes and *HOX* family genes were also significantly up-regulated in the KISS-high subgroup (Figure 3B and supplemental Figure 4A). The results of GSEA (detailed in supplemental Table 6) revealed that many of the pathways associated with HSC or LSC were positively enriched (Figure 3C),[23] while pathways encompassing genes down-regulated in HSC or LSC were negatively correlated. (supplemental Figure 4B) Together, the transcriptomic data analysis suggested that an enhanced stem cell transcriptional program may contribute to disease aggressiveness in the KISS-high patients.

Exploring novel therapeutic opportunities for the KISS-high patients

Several kinase inhibitors have been approved for the treatment of solid cancers, but not yet for MDS. Since we demonstrated that the KISS-high MDS patients had significantly worse

survival, we sought to investigate whether these patients would benefit from kinase inhibitor treatments. To this end, we queried the GDSC and CCLE datasets to model drug sensitivities with regard to the KISS.[24,25] Although the GDSC collection did not include MDS cell lines, because MDS and AML are closely related clonal myeloid diseases, and the diagnostic boundary for high-risk MDS and AML is gradually being blurred in recent years,[16] we selected the AML cell lines curated in the GDSC project as in vitro surrogates for drug response investigation.

The Pearson's correlation coefficients between drug sensitivity score (we used 1 – AUC as the surrogate for drug sensitivity) and KISS score across all AML cell lines were calculated. Intriguingly, we observed that kinase inhibitors were positively enriched at the top of the compound list, ordered by the descending Pearson correlation coefficients (Figure 4A and 4B), while the cytotoxic agents (such as paclitaxel, vinorelbine, etc.) were mostly at the tail the list, indicating that our analysis did prioritize the kinase inhibitors for the treatment of KISS-high cell lines. Importantly, we observed that axitinib and taselisib represented the most effective compounds (Pearson correlation coefficient > 0.6, supplemental Table 7) against KISS-high cell lines. (Figure 4C and 4D)

Discussion

MDS is a clonal stem cell disease, and our current understanding of its genomic landscape is dominated by genetic mutations in the epigenetic regulators and splicing machinery, while variants of the transcription factors, DNA repair proteins, and signaling pathways constitute a minor proportion.[26] The prognostic significance and therapeutic implications of kinome profiling in MDS have not been extensively explored. Although a wide array of kinase

inhibitors have been approved by the U.S. FDA for the treatment of several solid cancers and hematological malignancies,[27] while others are entering various phases of clinical trials, none of them have been approved specifically for MDS yet. Therefore, we reason that investigation of the kinome expression in MDS addresses this unmet medical need.

In this study, we discovered that the expression levels of seven kinases were significantly linked with the clinical outcomes of MDS patients through rigorous statistical modeling. We further integrated the weighted expression of these kinases into the KISS, a concise yet powerful risk score, and validated its prognostic impact in two external MDS cohorts. Even though the KISS-high subgroup was associated with distinct clinical characteristics and more frequent deleterious mutations in *ASXL1, EZH2, NPM1, RUNX1, STAG2* and *TP53*, our multivariate analysis attested the independent prognostic significance of the KISS. Notably, the KISS outperformed previously published Coxnet and LSC17 scores, [9,28] and held the ability to identify the prognostically unfavorable patients even within IPSS-R lower-risk or normal karyotype subgroups.

The components of the KISS include *PTK7, KIT, MAST4, NTRK1, PAK6, CAMK1D* and *PRKCZ*, which are all overexpressed in the MDS patients as compared to the HCs, and associated with inferior clinical outcomes. *PTK7* (protein tyrosine kinase 7) is an evolutionarily conserved transmembrane receptor tyrosine kinase (RTK), which was originally found to be involved in the canonical and noncanonical Wnt pathways of epithelial cells. More recently, *PTK7* was also found to be expressed by the HSPCs, with the highest expression level observed in the HSCs,[29] and its deficiency in the mouse model led to a diminished HSC pool.[30] *KIT* is a type-III RTK, which is strongly expressed in the HSPCs where it plays a role in the maintenance of balance between self-renewal and lineage commitment.[31,32] *MAST4* (microtubule associated

serine/threonine kinase family member 4) is widely expressed in the nervous system, especially in the cerebellar Purkinje cells and hippocampus, [33] and also determines the mesenchymal stromal cell (MSC) commitment toward the chondro-osteogenic fate by hampering the Sox9 transcriptional activity.[34] However, its role in hematopoiesis and HSC biology has been less reported. *NTRK1* (neurotrophic receptor tyrosine kinase 1) fusions have been implicated in a number of solid tumor malignancies,[35-38] representing an important therapeutic target.[39] In normal hematopoiesis, *NTRK1* is expressed at the highest levels in the common myeloid progenitors (CMP) and early monocytes, while in AML, *NTRK1* is overexpressed mainly in core-binding factor AML.[40,41] In *RUNX1::RUNX1T1* rearranged AML, BM stromal cells express nerve growth factor (NGF), which can bind to TRKA (encoded by the *NTRK1* gene), leading to leukemogenesis.[41] *PAK6* (p21-activated kinase 6) is implicated in tyrosine kinase inhibitor (TKI) resistance in chronic myeloid leukemia (CML) by interacting with the tumor suppressor miR-185.[42-44] *CAMK1D* (calcium/calmodulin dependent protein kinase ID) can network with the inhibitory leukocyte immunoglobulin-like receptor signaling pathway, and plays an essential role in AML development and maintenance.[45,46] *PRKCZ* (protein kinase C zeta) was found to be upregulated in MDS CD34+ BM cells, and participate in the thrombopoietin signaling axis and HSC self-renewal.[47,48] Overall, we provide evidence that the kinases selected by our KISS model are closely connected to the pathogenesis of myeloid malignancies. This is in parallel with the findings of our transcriptomic analysis that the KISS-high patients had overexpressed *HOX* genes and a positive enrichment for HSC and LSC gene signatures.

Having demonstrated that the KISS-high patients fared worse clinically, we were interested whether certain treatment modalities could reverse the adverse prognostic impact of higher KISS. We found that allo-HSCT could indeed improve survival in the KISS-high

subgroup; while on the contrary, the survival benefit of allo-HCST was not observed in the KISS-low subgroup, indicating that such intensive treatment option mainly benefits for those with truly high-risk diseases. Next, we attempted to search for compounds that could specifically target the KISS-high leukemic myeloblasts. By interrogating the GDSC project datasets, we identified axitinib and taselisib as our top hits. Axitinib is a selective inhibitor of vascular endothelial growth factor receptors (VEGFR) 1, 2, and 3. It can bind to the intracellular tyrosine kinase domains of VEGFRs and subsequently reduce the downstream phosphorylation of AKT and ERK1/2.[49,50] Although its first FDA approved indication was for the treatment of advanced renal cell carcinoma,[51] axitinib has also demonstrated antitumor activities in hematological malignancies. In the case of chronic myeloid leukemia (CML), axitinib can selectively target the *BCR::ABL1* fusion transcript that harbors the T315I gatekeeper mutation through a mutation-selective mechanism, and potently reduces the downstream phosphorylation targets in the leukemic cells.[52] Moreover, combining axitinib with sorafenib can also overcome the TKI resistance caused by the T315I, by inhibiting the Bcr-Abl/Grb2/Gab2 axis.[53] Although in a previous study by Giles et al., axitinib had only minimal activity in AML or MDS,[54] the sample size in this study was rather limited, and it is possible that only patient with higher KISS might derive greater treatment benefits. Taselisib is a PI3K inhibitor that potently inhibits the p110-alpha, delta, and gamma isoforms.[55] When combined with anti-microtubule chemotherapy, such as vinorelbine or paclitaxel, taselisib induced anti-proliferative, pro-apoptotic and anti-metastatic effects in human breast cancer cells, via the inhibition of downstream PI3K and MAPK pathway activities.[56] Furthermore, when combined with the BCL-2 inhibitor venetoclax, taselisib could effectively inhibit leukemic cell growth in AML cell lines or AML patient-derived xenograft

models.[57] Overall, our pharmaco-transcriptomic investigation provided evidence that axitinib and taselisib may be potential novel treatment options for KISS-high MDS patients.

We acknowledge there are some limitations in this study. First, our study population consisted of a retrospective MDS cohort, with non-homogeneous treatment courses that might have been influenced primarily by an individual patient's co-morbidities, quality-of-life expectations, as well as local reimbursement policies. Second, the patients included in this study were diagnosed with primary MDS, and from Asian ethnicity background, which needs to be considered when extrapolating the findings of this study to therapy-related MDS or other MDS patient populations. Third, although MDS and AML are closely related disease entities, the exact pathogenic mechanisms are not identical after all. Although we were only able to explore drug sensitivity data collected from the AML cell lines in the publicly accessible databases, we recognize that drug sensitivity experiments performed on MDS patient-derived BM hematopoietic cells or MDS-derived cell lines would reflect the real-life treatment efficacy in MDS patients more rigorously.

In conclusion, we performed the first extensive kinome expression analysis in MDS, and constructed the kinase-based risk score, KISS, that could not only robustly risk-stratify MDS patients but also raise the possibility of novel therapeutic approaches. We also provided evidence that the KISS can be externally validated in two additional MDS cohorts, profiled by different gene expression quantification techniques (both RNA-seq and microarray). The KISS can serve as an independent adverse prognostic factor in MDS, outcompeting the previously reported Coxnet and LSC17 signatures. Prospective validation in larger MDS cohorts is anticipated to further establish the applicability of KISS in a wider population of MDS patients.

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Author contributions

C-YY was responsible for data collection and management, statistical and bioinformatic analysis, result interpretation, literature research, and manuscript writing; C-CL and Y-HW were responsible for data management and statistical analysis; C-JK, C-HT, H-AH, and H-FT were responsible for data collection and management; C-LH and W-CC planned, designed, and coordinated the study over the entire period and wrote the manuscript. All authors read and approved the final manuscript.

None of the authors has a relevant conflict of interest.

Figure legends

Figure 1: Establishment of the KISS for MDS risk-stratification. (A) Pearson's correlation matrix of the seven kinases selected into the KISS model, and heatmap illustrating the normalized expression of the kinases, across the patients in the NTUH-A cohort. Patients with higher-risk MDS (EB1 and EB2) were notably clustered within the KISS-high subgroup. (B) OS and (C) LFS of the MDS patients in the NTUH-A cohort, stratified by the KISS. (D) OS of the MDS patients in the GSE114922 dataset, stratified by the KISS. (E) OS of the MDS patients in the GSE58831 dataset, stratified by the KISS.

Figure 2: The KISS can refine the current risk-stratification scheme for MDS. (A) OS and (B) LFS of the lower-risk IPSS-R (very-low, low and intermediate risks) MDS patients, stratified by the KISS. (C) OS and (D) LFS of the higher-risk IPSS-R (high and very-high risks) MDS patients, stratified by the KISS. (E) The Coxnet predictor and (F) LSC17 scores were significantly higher in the KISS-high subgroup. (G) The time-dependent ROC analysis of the KISS, Coxnet, and LSC17 scores demonstrated the superior prognostic performance of KISS.

Figure 3: Functional analysis of KISS-high vs. KISS-low MDS patients. (A) Heatmap illustrating the most significant DE genes (25 most up-regulated and 25 most down-regulated genes). (B) The LSC17 genes were significantly up-regulated in the KISS-high subgroup. (C) GSEA highlighted the enrichment of HSC and LSC-associated genesets in the KISS-high subgroup.

Figure 4: Pharmacogenomic investigation identified compounds that could specifically target the KISS-high myeloblasts. (A) Kinase inhibitors were positively enriched at the top of the compound list ordered by the Pearson correlation coefficients between the KISS and drug sensitivity. (B) Pearson's correlation of the KISS and drug sensitivity; a positive Pearson's correlation coefficient implies that a compound is more effective against the KISS-high AML cell lines. (C) The correlation between axitinib sensitivity and KISS. (D) The correlation between taselisib sensitivity and KISS.

Table 1. Clinical and laboratory characteristics according to the KISS strata.

Variable	KISS-high (n = 173)	KISS-low (n = 109)	*P* value
Gender			0.434
Female	59 (34.1)	43 (39.4)	
Male	114 (65.9)	66 (60.6)	
Age [a]	72.1 (17.8, 94.2)	67.6 (20.0, 94.2)	0.020
Laboratory data [a]			
WBC, X 10^9 /L	3.8 [0.3, 56.3]	4.3 [0.8, 25.5]	0.311
Hb, g/dL	8.4 [3.5, 15.3]	8.2 [4.2, 16.9]	0.535
Platelet, X 10^9 /L	75 [1, 721]	111 [3, 405]	0.003
PB blast (%)	1.0 [0, 18.0]	0 [0, 4.0]	<0.001
BM blast (%)	9.2 [0, 19.0]	2 [0, 17.8]	<0.001
2016 WHO classification			
MDS-SLD	5 (2.9)	26 (23.9)	<0.001
MDS-MLD	19 (11.0)	37 (33.9)	<0.001
MDS-RS-SLD	2 (1.2)	17 (15.6)	<0.001
MDS-RS-MLD	4 (2.3)	11 (10.1)	0.010
MDS-del(5q)	1 (0.6)	1 (0.9)	>0.999
MDS-EB1	55 (31.8)	7 (6.4)	<0.001
MDS-EB2	83 (48.0)	9 (8.3)	<0.001
MDS-U	4 (2.3)	1 (0.9)	0.689
IPSS-R			
Very low	3 (1.8)	10 (9.7)	0.007
Low	21 (12.4)	53 (51.5)	<0.001
Int	32 (18.8)	29 (28.2)	0.1
High	57 (33.5)	8 (7.8)	<0.001
Very high	57 (33.5)	3 (2.9)	<0.001
Karyotype			
Normal Karyotype	84 (49.4)	78 (75.7)	<0.001
Complex Karyotype[§]	35 (20.6)	1 (1.0)	<0.001

[a] Median [range].

Abbreviations: Hb, hemoglobin; MDS-SLD, MDS with single lineage dysplasia; MDS-MLD, MDS with multilineage dysplasia; MDS-RS-SLD, MDS with ring sideroblasts and single lineage dysplasia; MDS-RS-MLD, MDS with ring sideroblasts and multilineage dysplasia; MDS-EB, MDS with excess blasts; MDS-U, MDS, unclassifiable; IPSS-R, revised International Prognostic Scoring System.

453 **Table 2. Multivariable Cox analysis of the prognostic impact of KISS and other clinically**

Variable	OS				LFS			
	HR	95% CI Lower	95% CI Upper	P value	HR	95% CI Lower	95% CI Upper	P value
Age [a]	1.032	1.019	1.045	< 0.001	1.020	1.009	1.031	0.001
IPSS-R [b]	1.603	1.341	1.916	< 0.001	1.502	1.266	1.783	< 0.001
Mutation								
ASXL1	0.819	0.510	1.316	0.409	0.811	0.516	1.274	0.363
EZH2	2.754	1.450	5.233	0.002	1.835	0.977	3.447	0.059
RUNX1	1.066	0.692	1.641	0.772	1.036	0.679	1.581	0.870
SF3B1	0.561	0.302	1.041	0.067	0.560	0.311	1.010	0.054
SRSF2	1.084	0.632	1.861	0.769	1.294	0.778	2.150	0.321
STAG2	1.139	0.682	1.903	0.619	1.380	0.847	2.247	0.196
TET2	1.646	1.033	2.623	0.036	1.518	0.975	2.362	0.065
TP53	4.007	2.428	6.613	< 0.001	2.144	1.349	3.408	0.001
KISS [a]	1.692	1.151	2.488	0.008	1.797	1.238	2.608	0.002

454 **relevant variables.**

455 [a] As continuous variable.
456 [b] IPSS-R risk groups: Very low, low, intermediate, high, very high.
457 Abbreviations: CI, confidence interval; HR, hazard ratio.
458

459

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589

Figure 1

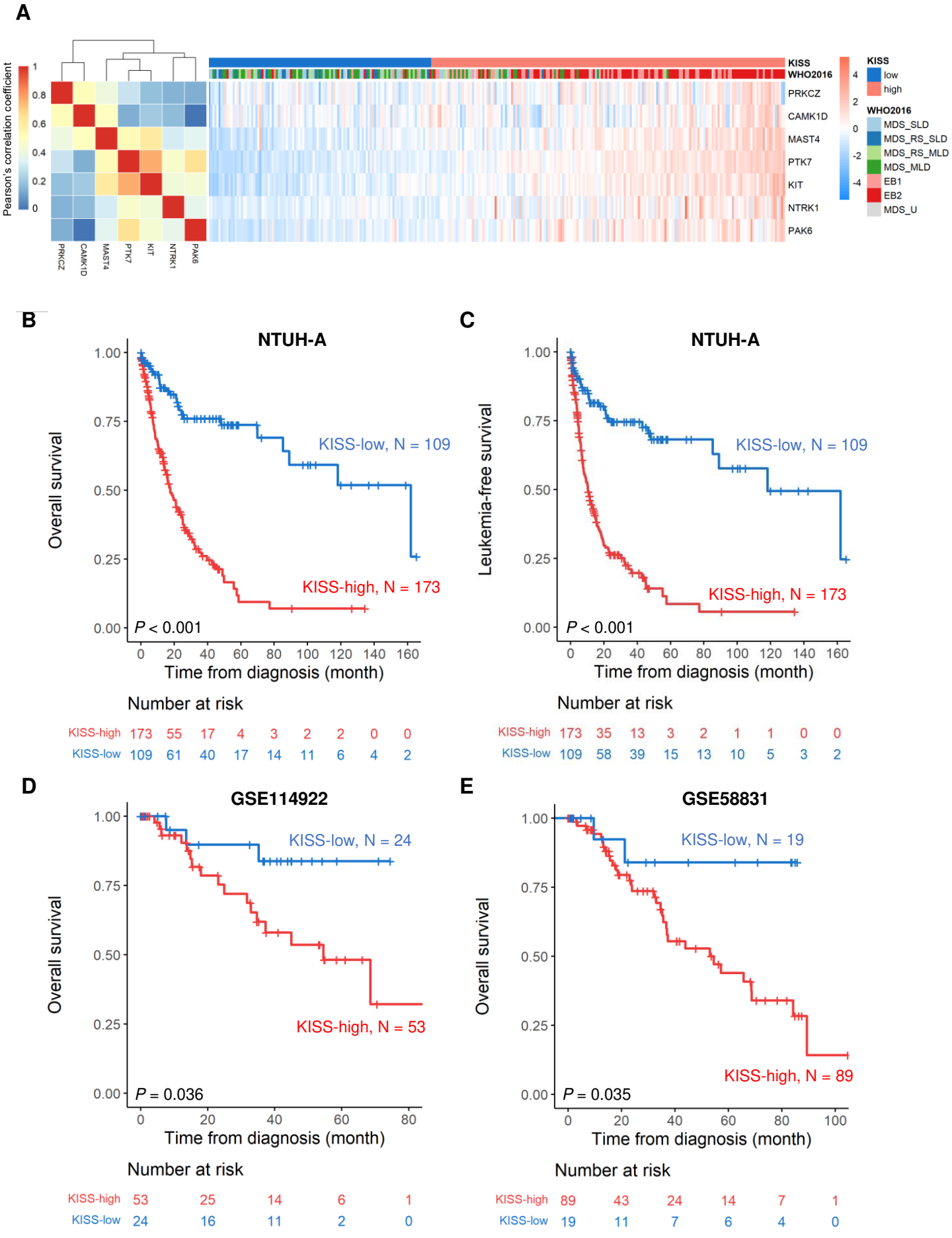

Figure 2

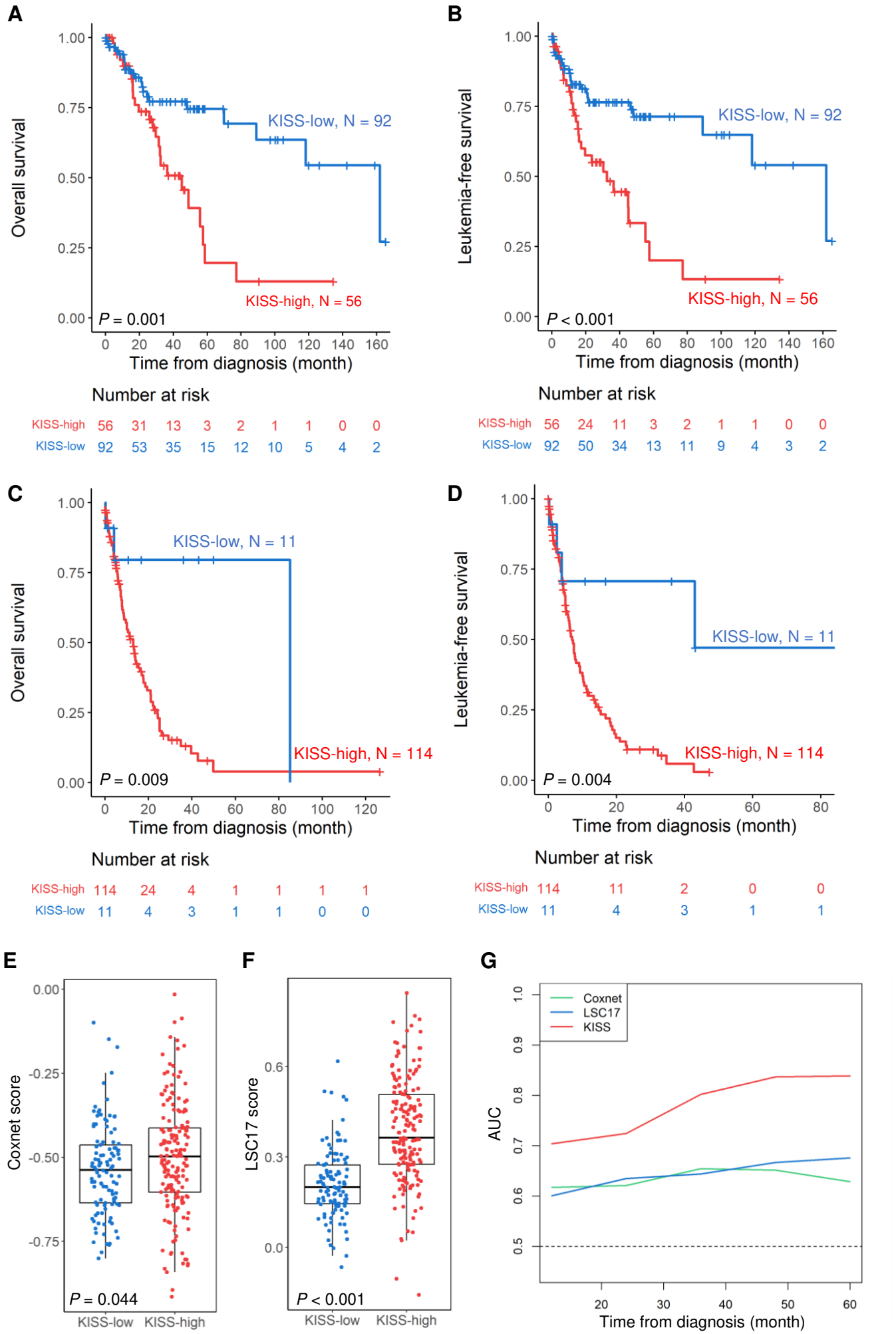

Figure 3

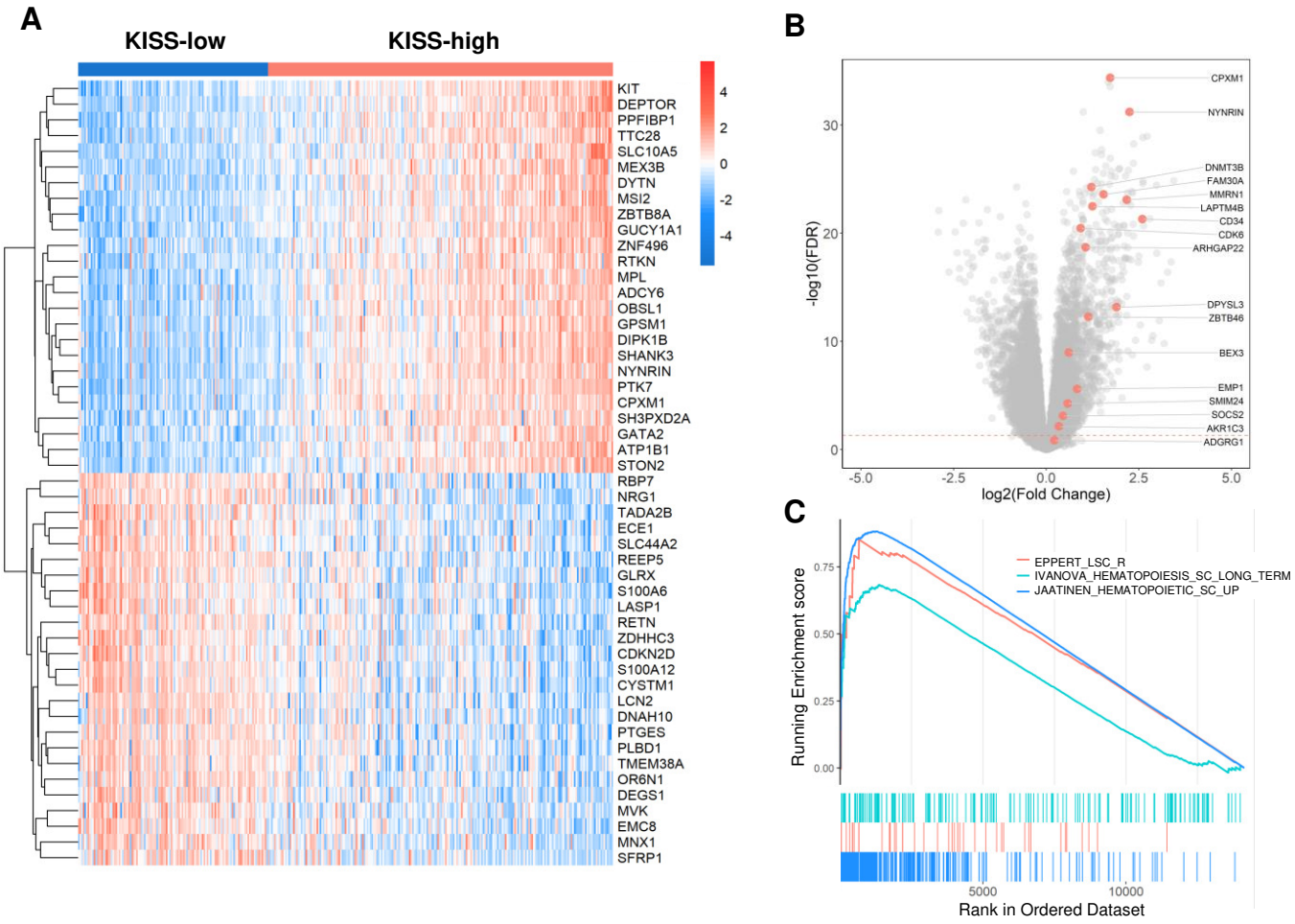

Figure 4