Identification of six hub genes and analysis of their correlation with drug sensitivity in acute myeloid leukemia through bioinformatics

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Background: Acute myeloid leukemia (AML) is a common hematopoietic malignancy and have unsatisfactory prognosis. Our study aimed to identify hub genes in AML and explore potential biomarkers through integrated bioinformatics.
Methods: Microarray datasets were analyzed to screen the differentially expressed genes (DEGs). Functional enrichment analysis was performed, and protein-protein interaction (PPI) network was generated by the STRING (11.0) database and Cytoscape (3.7.2) software. Hub genes were screened and verified through GEPIA2 and GEO microarray database. Sensitivity of AML cell lines with high expression of hub genes to the small-molecule drugs were identified using GSCA Lite.
Results: A total of 456 DEGs were identified and top 100 genes were screened out, of which six genes (*FLT3*, *PF4*, *CD163*, *MRC1*, *CSF2RB*, *PPBP*) were upregulated in AML and individually had a worse prognosis by the overall survival (OS) analysis. AML cell lines with *FLT3*-overexpression and *CSF2RB*-overexpression were sensitive to most small-molecule drugs, while, AML cells with *CD163*-overexpression were only sensitive to a few drugs. However, sensitivity to Erlotinib was correlated with high expression of *PF4* and *PPBP*.
Conclusions: In summary, *FLT3*, *PF4*, *CD163*, *MRC1*, *CSF2RB*, *PPBP* may be potential biomarkers and potential sensitive small-molecule drugs were correlated with overexpression of the biomarkers in AML.

Keywords: Acute myeloid leukemia (AML); differentially expressed genes (DEGs); survival; drug sensitivity

Introduction

Acute myeloid leukemia (AML) is the most malignant type of leukemia, with new cases increasing every year worldwide and as well as with age (1), which is a class of hematopoietic stem cells, malignant clonal diseases derived from progenitor cells. Abnormal primitive cells and partial primitive immature cells (leukemia cells) in bone marrow (BM) proliferate in large quantities and inhibit normal hematopoiesis, resulting in BM failure and rapid progress (2).

The prognosis of current AML patients is not optimistic, and only 40% of patients under the age of 60 survive more than 5 years. Although most patients can achieve remission after initial chemotherapy, patients who relapse after complete response rarely survive more than 5 years (3,4). Chemotherapy and hematopoietic stem cell transplantation (HSCT) have been the most important treatment for AML

in recent decades (5,6). Nevertheless, the drug resistance is increasingly hindering the therapeutic effect of AML patients, so that the quality of life of these patients is relatively poor. At the same time, recurrence remains the most common cause of HSCT failure in patients with AML, as a result, the prognosis for these patients remains poor (5). Moreover, there is increasing evidence that abnormal gene expression and mutated genes are involved in the carcinogenesis, progression and prognosis of AML. Therefore, there is an urgent need to find AML biomarkers and develop sensitive therapeutic drugs.

Over the past several decades, with the rapid rising of precision medicine, chip technology and bioinformatics analysis have been widely used to screen genetic changes at the genome level, which help us identify the differentially expressed genes (DEGs) associated with the carcinogenesis and progression of AML, and then find the precise sensitive chemotherapy drugs and improve patient outcomes. Consequently, in this study, in order to obtain reliable results, we downloaded two mRNA chip datasets (GSE9476 and GSE30029) from Gene Expression Omnibus (GEO) for analysis, and obtained the DEGs between AML patients and normal BM tissues. After a series of screening and validation, 456 DEGs were identified and six hub genes (*FLT3*, *PF4*, *CD163*, *MRC1*, *CSF2RB*, *PPBP*) were obtained. And then, the relationship between drug sensitivity and gene expression of the six hub genes was analyzed in AML cell lines. The study provides preliminary evidences for search the potential sensitive therapeutic drugs for AML.

We present the following article in accordance with the MDAR checklist (available at http://dx.doi.org/10.21037/tcr-20-2712).

Methods

Data processing for microarray data and DEGs

Two separate gene expression profiles [GSE9476 (7) and GSE30029 (8,9)] were chosen from GEO database (http://www.ncbi.nlm.nih.gov/geo/), and GSE9476 was based on the GPL96 platform, and GSE30029 was based on the GPL6947 platform. Then, the DEGs through GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) online analysis tool in NCBI were screened (10), and identified with fold change|FC| ≥1, and defined P value and false discovery rate (FDR) cutoffs of <0.05. Then, the Venn diagram was made through the web tool Bioinformatics & Systems Biology (http://bioinformatics. psb.ugent.be/ webtools/ Venn/).

Protein-protein interaction (PPI) network construction and hub gene identification

The PPI map was obtained by the Retrieval of Interacting Genes (STRING) database (https://string-db.org/), an online tool allowing users uploading the data of DEGs. It can be used to analyze the PPI information and to evaluate the interactive relationships among DEGs (11). The PPI pairs were set the combined score >0.4. These pairs were extracted and visualized for the PPI network using Cytoscape (3.7.2) software (www.cytoscape.org/). In Cytoscape, module screening was performed using the Molecular Complex Detection (MCODE) (scores >3 and nodes >4), and the degree of connection was computed using the Centralities for Cytoscape (Centiscape2.2) (12). Nodes with higher degree of connection were more essential for maintaining the stability of the entire network, usually, nodes with degree of connection ≥10 were considered to be core candidate genes.

GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

To evaluate the functional annotation and enrichment analysis of DEGs, the Database for Annotation, Visualization and Integrated Discovery (DAVID) tools (https://david.ncifcrf.gov/) were used (13,14). From the DAVID v6.8 online server, the functional annotation and enrichment analysis was performed by the Gene Ontology (GO). The biological process, molecular function, and cellular component are the three major components of gene function. KEGG collects large number of data about molecular-level information, biological pathways, chemical substances through high-throughput experimental technologies. The KEGG pathway was analyzed from the DAVID. P<0.05 and gene counts ≥10 were considered statistically significant.

Validation and screening of key genes using GEPIA2

The Gene Expression Profiling Interactive Analysis (GEPIA) is an online database of gene expression analysis, which based on tumor and normal samples from The Cancer Genome Atlas (TCGA; http://portal.gdc.cancer. gov/) and the Genotype-Tissue Expression (GTEx) (https:// www.gtexportal.org/) projects. GEPIA2 is an updated and enhanced version, which extends gene expression quantification from the gene level to the transcript level

and supports analysis of a specific cancer subtype and comparison between subtypes (15). The expression of hub genes between AML and normal BM tissues was verified by the database. Expression DIY (box plot) and survival analysis were performed. The parameters of the expression DIY (box plot) were set as follows: |log2FC| cutoff =1, P value cutoff =0.01, log scale = yes, jitter size =0.4, match TCGA normal and GTEx data. The parameters of the survival analysis were set as follows: overall survival (OS), group cutoff median, cutoff-high (%) =50, cutoff-low (%) =50, hazards ratio (HR) = yes, 95% confidence interval = yes, axis units months. According to the following screening criteria: the expression DIY (box plot) indicated that the gene was higher expressed in the AML sample than normal BM and peripheral monocytes, the survival analysis (OS) of the gene indicated a lower prognosis and must be statistically significant (P<0.05). The BloodSpot database (16) (http://servers.binf.ku.dk/bloodspot/) is a public microarray database for mRNA expression of BM and gene-centric blood cells. The correlation between the expression of key genes and the genetic characteristics of AML patients was analyzed using the BloodSpot database.

The relationship between genes expression and drug sensitivity in GSCA Lite

GSCA Lite (http://bioinfo.life.hust.edu.cn/web/GSCALite/) is a web-based platform for Gene Set Cancer Analysis, which is dynamic analysis and visualization of gene set in expression of malignant tumor genes and drug sensitivity correlation. The Drug Sensitivity Analysis of GSCA Lite has collected 481 small molecules from Cancer Therapeutics Response Portal (CTRP) (https://portals.broadinstitute.org/ctrp/) and 265 small molecules from Genomics of Drug Sensitivity in Cancer (GDSC) (https://www.cancerrxgene.org/) (17,18). Drug sensitivity and gene expression profiling data of cancer cell lines in GDSC and CTRP are integrated for investigation (19). The expression of each gene in the gene set is performed by Spearman correlation analysis with the small molecule/drug sensitivity (IC50). The Spearman correlation represents the gene expression correlates with the drug. The positive correlation means that the gene high expression is resistant to the drug, vise versa.

Statistical analysis

The results of this study were downloaded from the database according to the optimal statistical method selected by the database itself. Original chip data were downloaded from GEO databases for statistical analysis using unpaired sample *t*-test. The results were expressed as mean ± labeled standard deviation, and P<0.05 indicated that the results were statistically significant.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Results

Identification of DEGs based on clinical samples of GEO in AML

In our study, we selected two datasets: GSE9476 and GSE30029. GSE9476 contained 26 samples of AML patients and 20 samples of healthy volunteers, while GSE30029 contained 90 samples of AML patients and 31 samples of healthy volunteers. According to the criteria of P<0.05 and |log FC| ≥1, 2,058 DEGs were screened out in GSE9476 and 3653 DEGs were screened out in GSE30029 through comparing AML patient samples with healthy volunteers. We made a Venn diagram to show the association between the DEGs of GSE9476 and GSE30029. The 456 DEGs were the overlaps of the two datasets, as shown in *Figure 1A*.

KEGG and GO enrichment analysis, PPI network and key gene identification of DEGs

Among 456 DEGs, the PPI action network was shown in *Figure 1B*. Commonly, only connectivity degree ≥10 can be identified as potential core gene, accordingly, among 456 DEGs, 183 potential core genes which have been calculated by using Cytoscape (3.7.2) software met the condition of degree of connection ≥10 (*Figure 1C*). Then, the possible association among the183 DEGs was evaluated by STRING online tool. PPI network was shown in *Figure 1C*. Meanwhile, the top 100 genes of the degree of connection of these 183 genes were selected and shown in *Figure 1D* (Table S1). We found that the degree of tumor necrosis factor (TNF) was 106, which ranked first among these 100 genes. Then, the following genes were integrin subunit alpha M (ITGAM; degree =101), protein tyrosine phosphatase, receptor type C (PTPRC; degree =83), toll like receptor 2 (TLR2; degree =74), matrix metallopeptidase 9 (MMP9; degree =74) and so on. These 100 genes could be the core genes screened out in AML. The enrichment
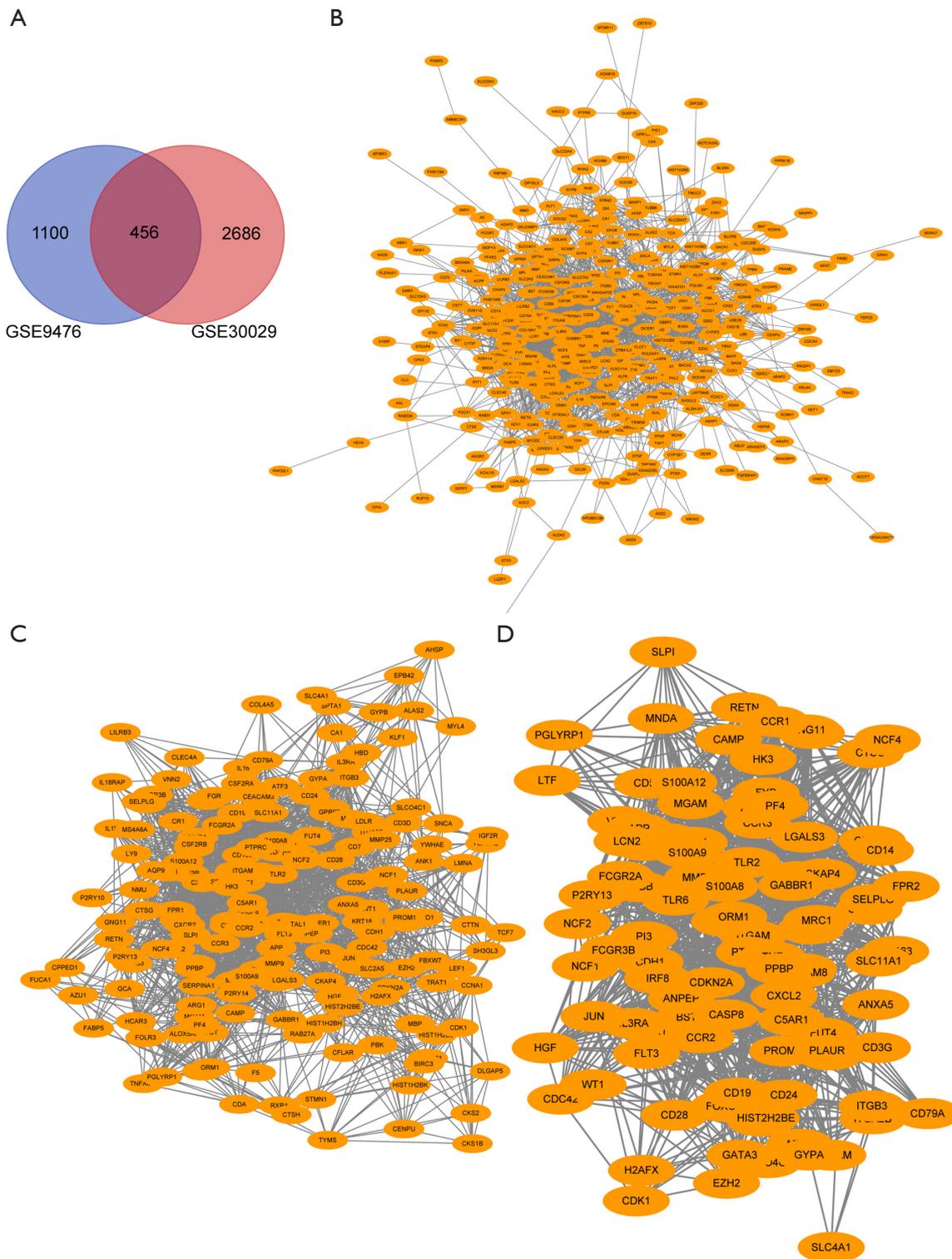
A

B

C

D

Figure 1 Venn diagram, PPI network of DEGs. (A) Venn diagram of the DEGs common to the two GEO datasets; (B) PPI network constructed with the 456 DEGs by the STRING; (C) 183 genes met the condition of degree ≥10, and visualization through the STRING and Cytoscape; (D)100 genes were visualized by the STRING and Cytoscape. PPI, protein-protein interaction; DEGs, differentially expressed genes; GEO, Gene Expression Omnibus. GDSC, Genomics of Drug Sensitivity in Cancer.
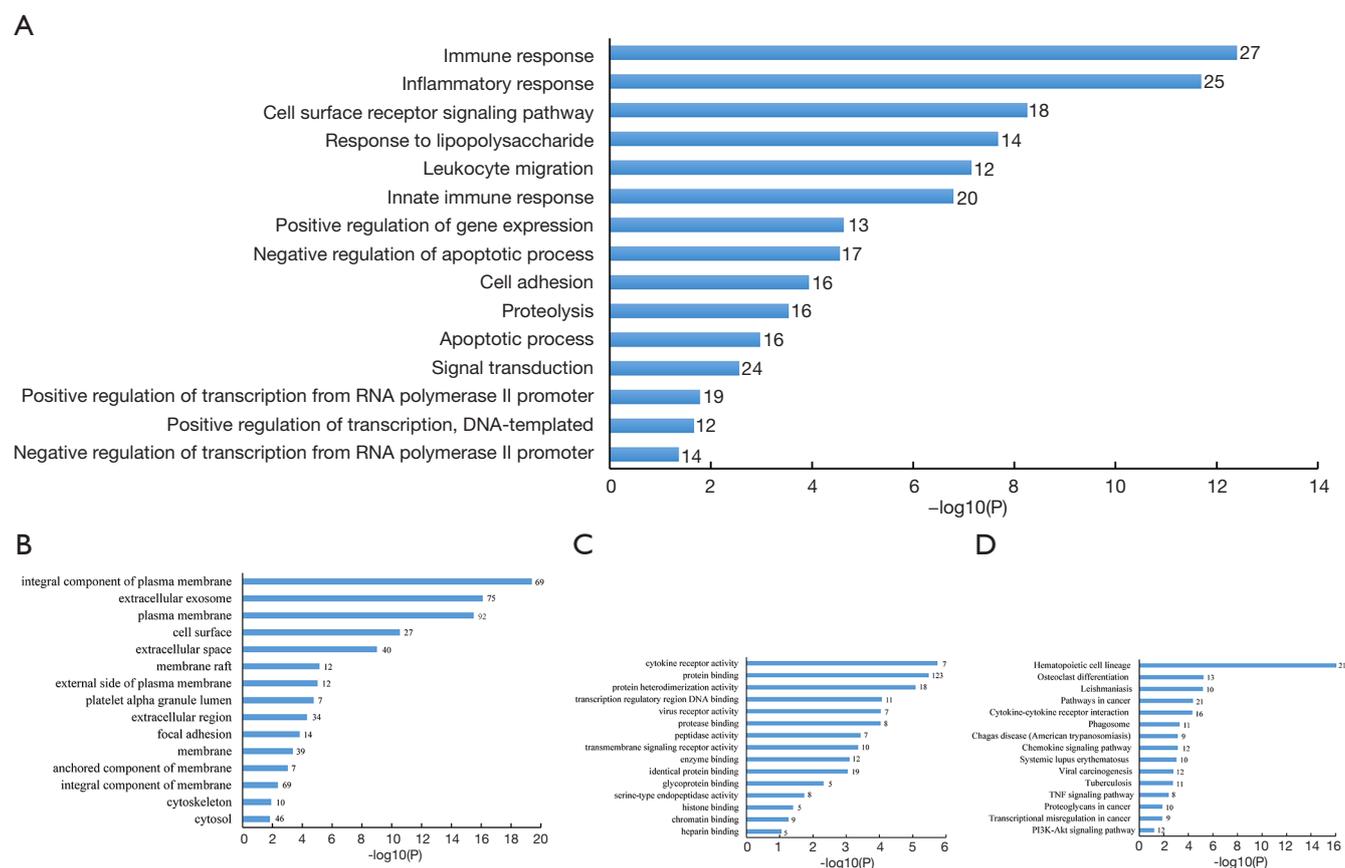
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A

B

C

D

Figure 2 The GO analysis and KEGG pathway analysis for the 183 DEGs. The blue bars were the enrichment terms, and the length of the bars was represented by the P value. At the same time, at the far right of each bar was the count of genes enriched in this particular process/pathway. (A) Top 15 GO terms in biological processes; (B) top 15 GO terms in cell component; (C) top 15 GO terms in molecular function; (D) top 15 enriched pathway of the 183 DEGs. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes.

analysis of gene function and pathway of these 183 DEGs were carried out by DAVID v6.8 online server, and the GO analysis and KEGG pathway analysis results were shown in (*Figure 2*). The most significant enrichment for DEGs in biological processes was in immune response with the enrichment count of 27 genes, which was the highest number of gene DEGs. The second is in inflammatory response (*Figure 2A*). The most significant enrichment for DEGs in cell component was in integral component of plasma membrane with the enrichment count of 69. The second was in extracellular exosome with gene enrichment number of 75 (*Figure 2B*). The most significant differentially expressed enrichment in molecular function was cytokine receptor activity, and the count of gene enrichment was 7. The second was protein binding, and the count of

gene enrichment was 123, which was the count of gene enrichment, and so on (*Figure 2C*). KEGG pathway analysis revealed that the most significant enrichment for DEGs was related to hematopoietic cell lineage, in which there was the enrichment count of 21 (*Figure 2D*).

The relationship between hub genes and prognosis of AML patients by GEPIA2

To further verify, screen and analyze the expression of the top 100 genes of DEGs in peripheral blood mononuclear and BM cells of AML patients and healthy volunteers, the expression DIY (box plot) and the survival analysis by GEPIA2 were performed. Accordingly, as shown in *Figure 3*, six higher expressed genes (*FLT3*, *PF4*, *CD163*,
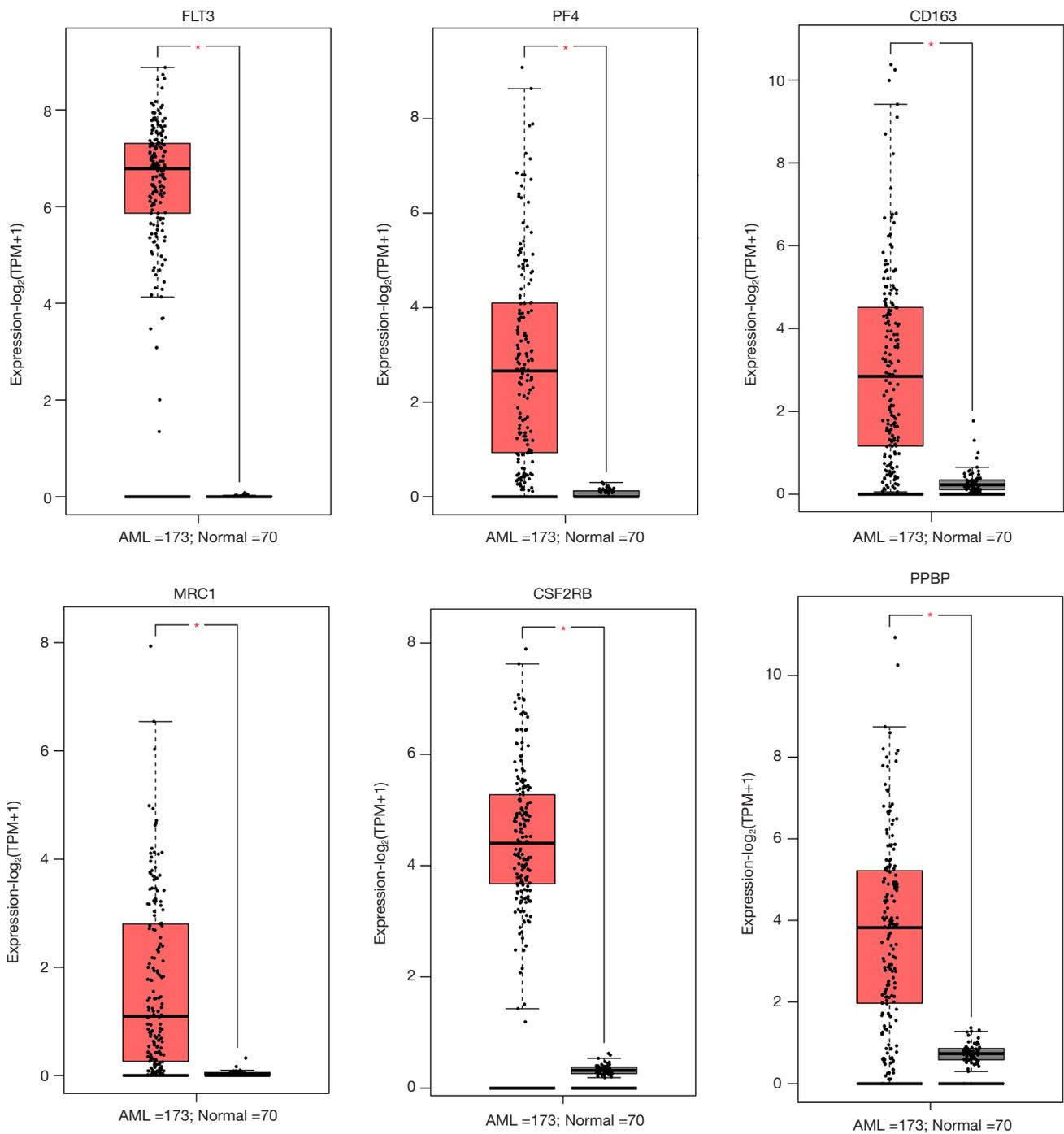
Figure 3 The expression levels of the six candidate genes in AML. The results are based on TCGA and GTEx data screening in AML (n=173) and normal control (n=70) from GEPIA2 database through the Expression DIY (box plot) (*, P<0.05), the red boxes are represented as the AML sample and the black boxes are represented as the normal tissue. *FLT3*, FMS-related tyrosine kinase 3; *PF4*, platelet factor 4; *CD163*, the macrophage scavenger receptor; *MRC1*, mannose receptor C-type 1; *CSF2RB*, colony stimulating factor 2 receptor subunit beta; *PPBP*, pro-platelet basic protein; AML, acute myeloid leukemia; TCGA, The Cancer Genome Atlas.

MRC1, *CSF2RB*, *PPBP*) were found in the AML compared with healthy volunteers (P<0.05). At the same time, as in *Figure 4*, the survival analysis (OS) indicated there was reverse relationship between the expression level of the above six genes and OS (P<0.05). The result suggests the six highly expressed genes might predict a worse prognosis in AML patients (P<0.05).

FLT3, PF4, CD163, MRC1, CSF2RB and PPBP may be the potential hub genes of AML

To further verify the differentially expression of the selected six candidate genes between AML patients and normal BM tissues, mRNA expression of *FLT3*, *PF4*, *CD163*, *MRC1*, *CSF2RB* and *PPBP* at mRNA levels comparing with AML samples and healthy volunteers of peripheral blood and/or BM mononuclear cells was performed through other microarray datasets in GEO database (*Figure 5*). We validated that they were higher expressed in AML samples obviously than control, which were statistically significant (P<0.05). In addition, as the recommended genetic classification of AML includes low-risk group, intermediate-risk group and high-risk group (20). In addition, the BloodSpot database was used to analyze the effect of hub genes expression and genetic abnormalities on risk stratification in AML patients (*Figure 6*). We found that high expression of these six hub genes in AML patients may indicate high-risk groups at the genetic stratification level.

The correlation of hub genes expression and drug sensitivity in AML cells by GSCA Lite

To analyze the correlation of six hub genes (*FLT3*, *PF4*, *CD163*, *MRC1*, *CSF2RB*, *PPBP*) expression in AML and the sensitivity of AML cells to the small-molecule drugs, we used the GDSC IC50 drug data from GSCA Lite database (*Figure 7*). We found that AML cell lines with *FLT3*-overexpression and *CSF2RB*-overexpression were sensitive to most small-molecule drugs which can also be used in malignancies including target drugs and non-target drugs, while AML cell lines with *CD163*-overexpression were only sensitive to CH5424802, Crizotinib, TAE684 and MPS1-IN-1. And Erlotinib (Tarceva) was sensitive for AML cell lines with *PF4*-overexpression and *PPBP*-overexpression. For example, looking up from the GDSC database, we discovery that Sorafenib (Nexavar), whose targets are PDGFR, KIT, VEGFR, RAF and FLT3. And in our study, we verified that its effect on AML cell lines with

FLT3-overexpression was sensitive, and AML cell lines with high *FLT3* expression were also sensitive to the other small molecule drugs such as all-trans retinoic acid (ATRA). In our analysis, AML cells with the overexpression *PF4* and *PPBP* displayed sensitivity to Erlotinib (Tarceva), whose drug target is EGFR from GDSC database. The sensitivity to methotrexate, which is an antimetabolite drug, is correlated with the high expression of *CSF2RB* in AML cell lines. Details of common small-molecule drug were shown in Table S2.

Discussion

AML is one of the most common and aggressive type of leukemia, and the treatment of AML is given priority to combination chemotherapy. The treatment of AML also has made progress in last decade, however, recurrence rates remained still high (3,4,6). So, searching for accurate molecular biomarkers is urgently needed. Microarray technology has enabled us to explore genetic changes in diseases and has been shown to be an effective way to identify novel biomarkers for human tumor including AML.

In the present study, 2 mRNA microarray datasets were analyzed to obtain DEGs between AML and healthy subjects of peripheral blood and BM samples. A total of 456 DEGs were identified among the 2 datasets. Then, we performed DAVID enrichment analysis on these 456 DEGs, GO and KEGG enrichment analyses were performed to explore interactions among the 456 DEGs. GO enrichment analysis revealed that changes in the most significant modules were mainly enriched in regulation of transcription from RNA polymerase II promoter, DNA-templated, cell proliferation and division. While, changes in KEGG were mainly enriched in viral carcinogenesis, transcriptional mis-regulation in cancer, cell cycle and p53 signaling pathway. Previous studies have reported that the dysregulation of transcription from RNA polymerase II promoter, cell proliferation, cell division and immune response play important roles in the carcinogenesis or progression of tumors (21-23). Similarly, transcriptional mis-regulation in cancer, cell cycle and p53 signaling pathway have been shown in previous studies to play a very important role in the occurrence and development of tumors (22,24). Therefore, our results suggest that the DEGs played an important role in AML tumorigenesis and progression, which is consistent with the previous studies.

Through a series of screening, analysis and verification, we screened 6 DEGs (*FLT3*, *PF4*, *CD163*, *MRC1*, *CSF2RB*,
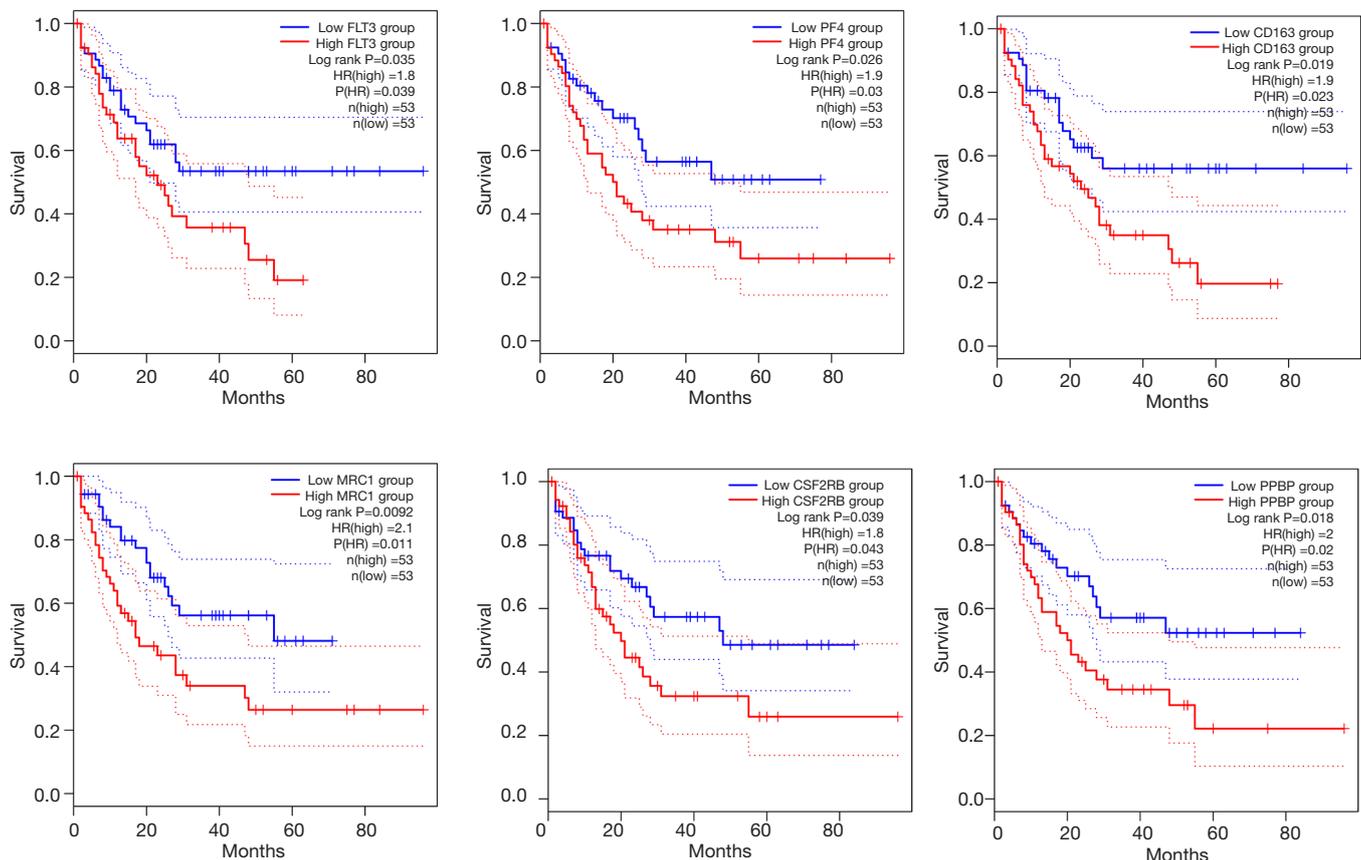
Figure 4 Kaplan-Meier survival curves of overall survival for the six candidate genes in AML. The survival curves are plotted using the GEPIA2 web server. Survival curves are represented as dotted lines, and the solid line represents the 95% confidence interval. The number of AML and normal bone marrow tissues (n) =53. The P values are calculated using log-rank statistics. *FLT3*, FMS-related tyrosine kinase 3; *PF4*, platelet factor 4; *CD163*, the macrophage scavenger receptor; *MRC1*, mannose receptor C-type 1; *CSF2RB*, colony stimulating factor 2 receptor subunit beta; *PPBP*, pro-platelet basic protein; AML, acute myeloid leukemia; HR, hazard ratio.

PPBP) as the hub genes. In BM and peripheral blood samples from AML patients, the expression of the hub genes was higher than healthy volunteers. In terms of the relationship between the expression of individual hub genes and patient prognosis, the OS rate of AML patients with higher expression of the above six hub genes was lower. Therefore, not only *FLT3*, *PF4*, *CD163*, *MRC1*, *CSF2RB* and *PPBP* may be AML oncogenes but also AML patients with higher expression of these six hub genes might have poor prognosis, as well as they might be potential biomarkers of AML. In addition, high expression of these six hub genes in AML patients may indicate high-risk groups at the genetic stratification level, which will provide a theoretical basis for the treatment of frontline doctors.

We found that AML cells with high expression of *FLT3*

and *CSF2RB* were sensitive to most small molecule drugs by bioinformatics analysis. While, AML patients with high expression of *CD163* were only sensitive to a few small molecule drugs. Only was Erlotinib (Tarceva) related with sensitive in AML cell lines with high *PF4* and *PPBP* expression. With traditional chemotherapy drugs, adaptive resistance existed in many AML patients, leading to poor prognosis for AML patients. There was a median survival of only 4 months for relapsed and/or refractory AML (25,26). So, it was urgent to find novel biomarkers and new therapeutic sensitive drugs for high-risk AML. In our study, the panel included *FLT3*, *PF4*, *CD163*, *MRC1*, *CSF2RB* and *PPBP* might be the novel biomarkers for target therapy in AML.

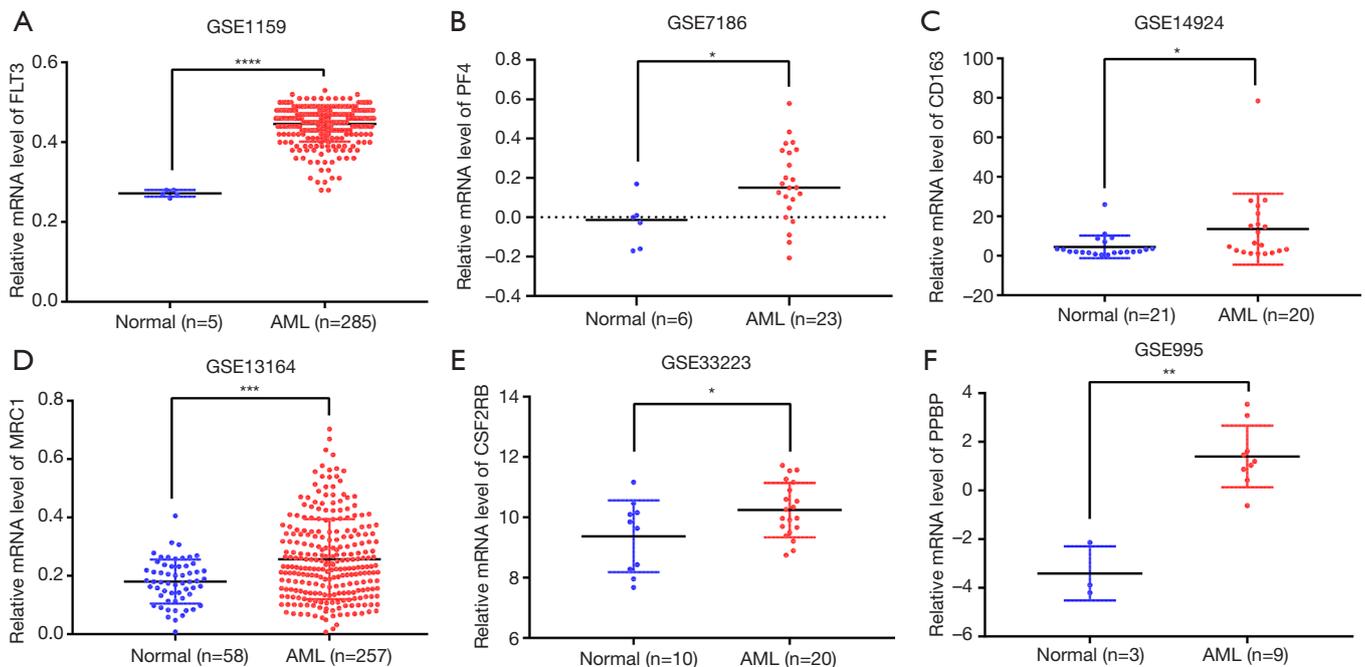*FLT3*, located in 13q12.2, which is an important cytokine

Figure 5 Differential expression of six candidate genes in AML samples and healthy volunteers was validated again in other data sets in GEO. The data was downloaded from GEO database, which was analyzed and visualized by GraphPad Prism 8.3.0. Non-paired sample *t*-test was used for statistical analysis. The relative mRNA expression of *FLT3*, *PF4*, *CD163*, *MRC1*, *CSF2RB*, *PPBP* in AML from GEO datasets: (A) *FLT3*; (B) *PF4*; (C) *CD163*; (D) *MRC1*; (E) *CSF2RB*; (F) *PPBP*. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ****, $P<0.0001$. *FLT3*, FMS-related tyrosine kinase 3; *PF4*, platelet factor 4; *CD163*, the macrophage scavenger receptor; *MRC1*, mannose receptor C-type 1; *CSF2RB*, colony stimulating factor 2 receptor subunit beta; *PPBP*, pro-platelet basic protein; AML, acute myeloid leukemia; GEO, Gene Expression Omnibus.

receptor, encodes a class III receptor tyrosine kinase and mainly expresses in the hematopoietic compartment and involved in the apoptosis, proliferation and differentiation of hematopoietic cells (26,27). *FLT3* mutations which occur in 30% of AML patients, may lead to the occurrence and development of acute leukemia, especially AML (28). In our study, the analysis from microarray datasets showed *FLT3* was upregulated in AML and the AML patients with the high expression of the *FLT3* gene had a worse prognosis. The result is in agreement with previous reports that high transcript levels of *FLT3* were associated with a worse prognosis in AML and pediatric AML patients (29,30). *FLT3* inhibitors (tyrosine kinase inhibitors) have been considered to be a target for anti-cancer drugs, which have been used in preclinical and clinical studies, such as sorafenib, lestaurtinib and sunitinib, and so on (26). We analyzed the association between the drug sensitivity of AML cells and the gene expression of these six hub genes using the GSCA Lite database. Our current results showed

that besides being sensitive to drugs targeting *FLT3*, AML patients with high *FLT3* expression were also sensitive to the other small molecule drugs such as ATRA. The studies of Ma *et al.* revealed combining ATRA and *FLT3* TKIs had highly synergistic drug activity to eliminate *FLT3*/internal tandem duplication (ITD)⁺ leukemia stem cells *in vitro* and *in vivo* (31). *PF4* (platelet factor 4, CXCL4, located in 4q13.3), encoding the protein of members of the CXC chemokine family. This protein is chemotactic for numerous other cell type and also functions as an inhibitor of hematopoiesis, angiogenesis and T-cell function. It is involved in regulating the differentiation of hematopoietic stem cells/progenitor cells, maintaining the survival and self-renewal of hematopoietic stem cells, and regulating the cardiac differentiation potential of induced pluripotent stem cells (32,33). Previous studies have shown that *PF4* is an endocrine factor, and its overexpression in tumors is associated with decreased OS of patients (34). The analysis of Chen *et al.* from the transcriptome database showed
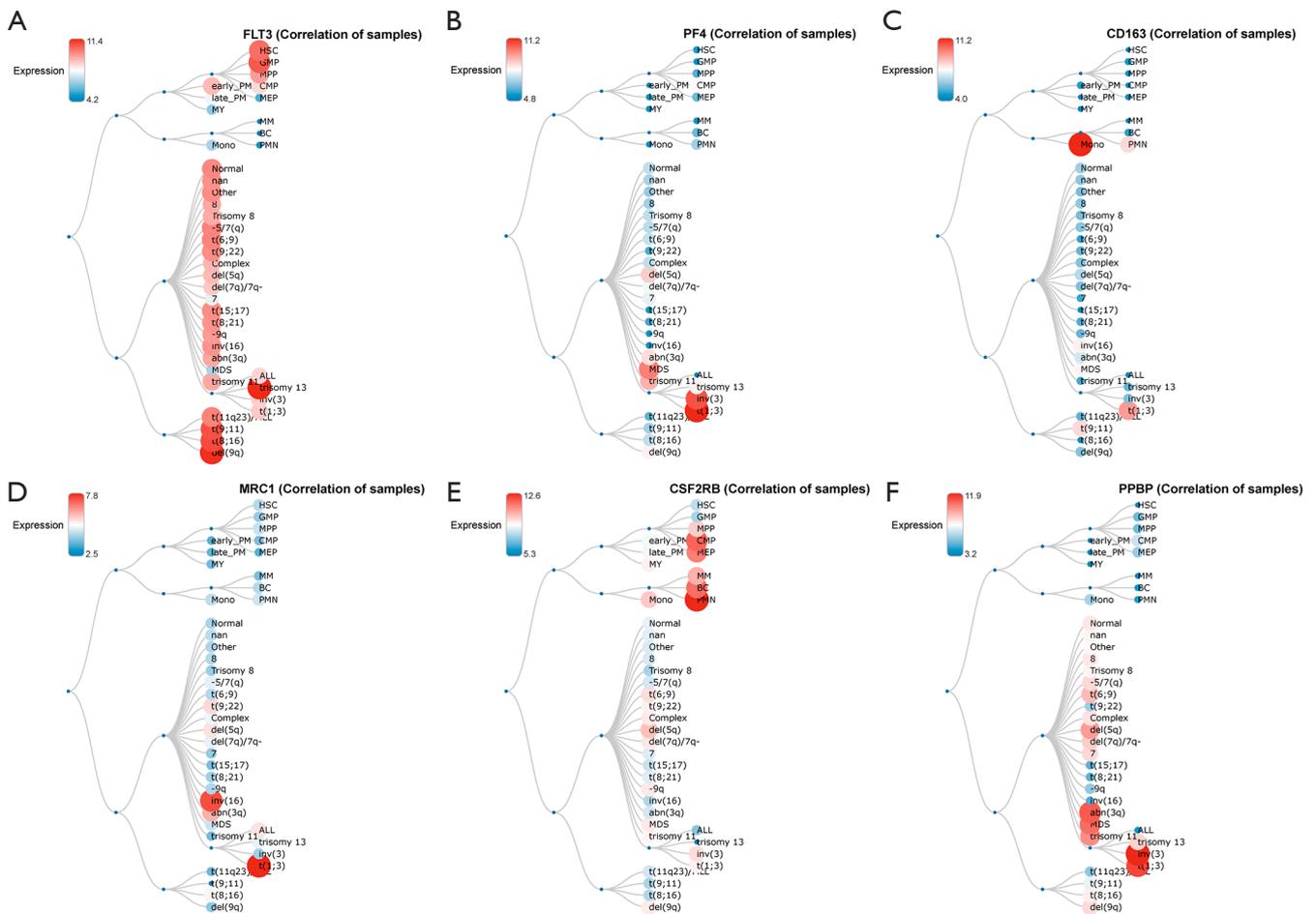
Figure 6 Relationship between expression of six hub genes and genetic characteristics of AML patients through BloodSpot database. (A) *FLT3*; (B) *PF4*; (C) *CD163*; (D) *MRC1*; (E) *CSF2RB*; (F) *PPBP*. *FLT3*, FMS-related tyrosine kinase 3; *PF4*, platelet factor 4; *CD163*, the macrophage scavenger receptor; *MRC1*, mannose receptor C-type 1; *CSF2RB*, colony stimulating factor 2 receptor subunit beta; *PPBP*, pro-platelet basic protein; AML, acute myeloid leukemia.

that the high expression of *PF4* was a risk factor for poor prognosis of patients (35). However, the study of Bai *et al.* indicated that PF4 protein was significantly decreased in in newly diagnosed AML group, compared with healthy control group (36). By database analysis, our results showed that the *PF4* transcription level of AML patients was higher than that of healthy controls and the OS rates of AML patients with high *PF4* expression were shorter compared with low expression of *PF4*. But further study is required to characterize the function of the gene involved in AML (34,35,37). *CD163* (the macrophage scavenger receptor, located in 13q12.2) encodes a protein that clears the scavenger receptor cysteine-rich (SRCR) superfamily. It functions as an acute phase-regulated receptor involved in

the clearance and endocytosis of hemoglobin/haptoglobin complexes by macrophages. Its overexpression is associated with associated with a poor prognosis in AML and the immune/inflammatory response, which is potential biomarker of myeloid leukemia cutis (38,39). At the same time, *CD163* is also involved in the development of multiple myeloma, meningioma, Hodgkin lymphoma and colorectal cancer (40-43). Additionally, *CD163* is regarded as a potential therapeutic target for cell-directed therapy on macrophages in some cancers, such as glioma, gastric cancer (44-46). In our study, *CD163* was identified to be significantly associated with AML prognosis by integrated bioinformatics analysis, which was consistent with the results of previous studies and would provide a preliminary
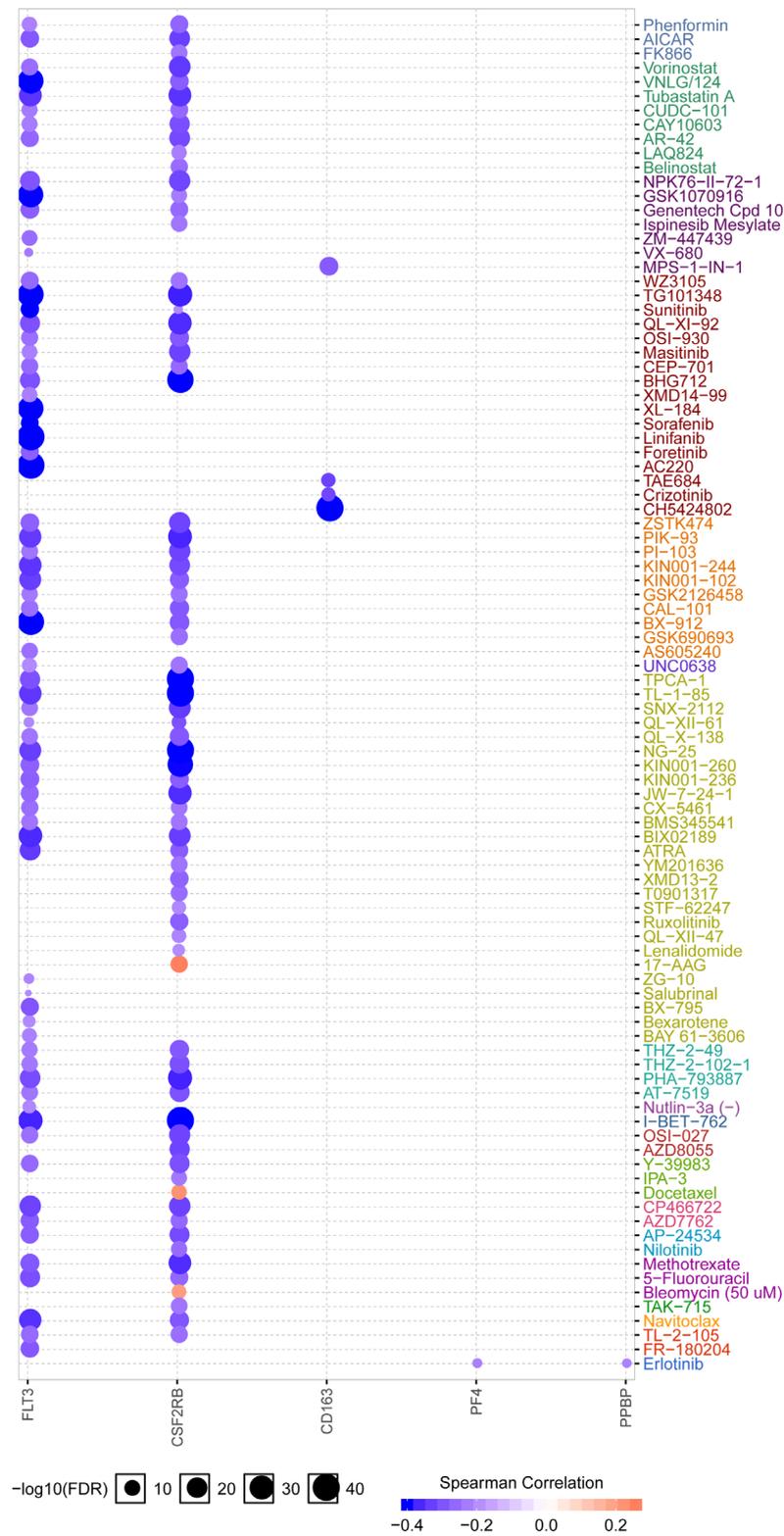
Figure 7 Correlation between drug sensitivity in AML and hub genes from GDSC database in GSCA Lite. AML, acute myeloid leukemia; GDSC, Genomics of Drug Sensitivity in Cancer.

basis for further development of targeted drugs for AML with high expression of *CD163*. *MRC1* (mannose receptor C-type 1, CD206, located in 10p12.33), a member of the C-type lectin receptor family, encodes the human mannose receptor (MR). It is involved in several biological processes, including cell-cell recognition, serum glycoprotein turnover, and neutralization of pathogens. *MRC1* mutations can cause activation and replication defects at DNA damage checkpoints, which are associated with tumorigenesis (47). *MRC1* can also regulate tumor microenvironment (48), the relationship between tumor recurrence and metastasis (49). In our study, we have preliminarily confirmed that *MRC1* is higher expressed in AML compared to healthy volunteers and is a potential biomarker for AML, which laid a solid foundation for our further development of targeted drugs targeting *MRC1* overexpression in AML. *CSF2RB* (colony-stimulating factor 2 receptor subunit beta, CD131) is located in 22q12.3. The protein encoded by *CSF2RB* is the common beta chain of the high affinity receptor for IL-3, IL-5 and CSF. It is a myeloid cytokine receptor, which is highly expressed in myeloid leukemia of Down syndrome (ML-DS), and *CSF2RB* variant A455D is an oncogenic hotspot mutation in ML-DS (50). The high expression of *CSF2RB* predicts the arrest of hematopoietic stem cell differentiation and *CSF2RB* was expressed at high levels in both mouse and human leukemia cells (51). In our study, we found *CSF2RB* was over-expressed and predicted worse prognosis in AML. *PPBP* (pro-platelet basic protein, CXCL7, located in 10p12.33) is a platelet-derived growth factor that belongs to the CXC chemokine family. It has been shown to stimulate various cellular processes including DNA synthesis, mitosis, glycolysis, intracellular cAMP accumulation, prostaglandin E2 secretion, and synthesis of hyaluronic acid and sulfated glycosaminoglycan. Previous studies have implicated serum levels of CXCL7 as potential prognostic markers in myelodysplastic syndromes and the overexpression of the gene in the marrow microenvironment contributed to induction of decitabine resistance in chronic myelomonocytic leukemia cells (52,53). In addition, *PPBP* is a biomarker in gastric cancer (54) and a predictive marker of sunitinib efficacy in clear cell renal cell carcinomas (55). The results of our analysis indicated AML patients with the high expression of *PPBP* gene had worse prognosis and AML cells with the overexpression *PPBP* displayed sensitivity to Erlotinib (Tarceva). Therefore, in present study, we thought *CSF2RB* and *PPBP* might also be a potential biomarker in AML.

Most of the six hub genes and their correlation with the sensitive drug we have derived from the study have been confirmed by previous studies to be associated with AML or cancer. But there are still several hub genes and their correlation with the sensitive drug have not been explored to provide a basis for guiding clinical medication, which may be the innovation of future research. Our study may not only provide potentially likely regulators of AML invasion and metastasis can serve as biomarkers in AML, also can give future researchers a broader perspective and more inspiration. But it still has limitation: (I) the analysis of drug sensitivity was based on the analysis of AML cell lines, which needs to be further verified in animal experiments; (II) the analysis of DEGs used in this study is based on the comprehensive analysis of clinical case samples, which needs to be further verified in animal experiments and other preclinical trial. Next, we will further verify it through animal experiments and other preclinical trial, which will be a significant and constructive study. Also, we will conduct further in-depth research on the expression and mechanism of these six hub genes in AML.

Conclusions

Our study was designed to identify DEGs that might be involved in the carcinogenesis or progression of AML. A total of 456 DEGs and six hub genes (*FLT3*, *PF4*, *CD163*, *MRC1*, *CSF2RB* and *PPBP*) were identified and might be regarded as promising biomarkers and potential sensitive drugs were identified for overexpression of the biomarkers in AML, and high expression of these six hub genes in AML patients may indicate high-risk groups at the genetic stratification level, which will provide a theoretical basis for the treatment of frontline doctors.

Acknowledgments

Footnote

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Table S1 Details of top 100 potential core genes

Degree	Gene symbol	Gene description
106	TNF	Tumor necrosis factor
101	ITGAM	Integrin subunit alpha M
83	PTPRC	Protein tyrosine phosphatase, receptor type C
74	TLR2	Toll like receptor 2
74	MMP9	Matrix metallopeptidase 9
63	CD44	CD44 molecule (Indian blood group)
59	JUN	Jun proto-oncogene, AP-1 transcription factor subunit
53	APP	Amyloid beta precursor protein
51	CDH1	Cadherin 1
50	CYBB	Cytochrome b-245 beta chain
49	FPR2	Formyl peptide receptor 2
49	LILRB2	Leukocyte immunoglobulin like receptor B2
48	FPR1	Formyl peptide receptor 1
46	FCGR2A	Fc fragment of IgG receptor IIa
46	CCR1	C-C motif chemokine receptor 1
46	MNDA	Myeloid cell nuclear differentiation antigen
43	CD19	CD19 molecule
43	CCR2	C-C motif chemokine receptor 2
41	CDK1	Cyclin dependent kinase 1
40	CEACAM8	Carcinoembryonic antigen related cell adhesion molecule 8
40	FGR	FGR proto-oncogene, Src family tyrosine kinase
40	ANXA5	Annexin A5
39	HGF	Hepatocyte growth factor
38	PPBP	Pro-platelet basic protein
38	CDKN2A	Cyclin dependent kinase inhibitor 2A
37	ITGA2B	Integrin subunit alpha 2b
37	CDC42	Cell division cycle 42
37	S100A12	S100 calcium binding protein A12
37	CD28	CD28 molecule
37	FLT3	FMS related tyrosine kinase 3
36	ITGB3	Integrin subunit beta 3
36	S100A9	S100 calcium binding protein A9
36	CXCR2	C-X-C motif chemokine receptor 2
36	GATA3	GATA binding protein 3
36	ARG1	Arginase 1
36	EZH2	Enhancer of zeste 2 polycomb repressive complex 2 subunit
35	PF4	Platelet factor 4
34	CXCL2	C-X-C motif chemokine ligand 2
34	CXCR1	C-X-C motif chemokine receptor 1
34	CAMP	Cathelicidin antimicrobial peptide
34	CD163	CD163 molecule
33	CD59	CD59 molecule
32	CD14	CD14 molecule
32	CTSG	Cathepsin G
32	C5AR1	Complement component 5a receptor 1
32	ANPEP	Alanyl aminopeptidase, membrane
32	FUT4	Fucosyltransferase 4
31	S100A8	S100 calcium binding protein A8
31	CCR3	C-C motif chemokine receptor 3
31	IRF8	Interferon regulatory factor 8
30	TLR6	Toll like receptor 6
30	PLAUR	Plasminogen activator, urokinase receptor
30	FCGR3B	Fc fragment of IgG receptor IIIb
29	CSF2RB	Colony stimulating factor 2 receptor beta common s
29	GGH	Gamma-glutamyl hydrolase
29	CSF3R	Colony stimulating factor 3 receptor
28	RETN	Resistin
28	CD24	CD24 molecule
28	GYPA	Glycophorin A (MNS blood group)
27	HIST2H2BE	Histone cluster 2, H2be
27	P2RY13	Purinergic receptor P2Y13
26	NCF2	Neutrophil cytosolic factor 2
26	IL3RA	Interleukin 3 receptor subunit alpha
26	MME	Membrane metalloendopeptidase
26	SPN	Sialophorin
25	LCN2	lipocalin 2
25	LTF	Lactotransferrin
25	FOXO1	Forkhead box O1
25	MGAM	Maltase-glucoamylase
25	PROM1	Prominin 1
24	CASP8	Caspase 8
24	H2AFX	H2A histone family member X
24	LGALS3	Lectin, galactoside binding soluble 3
23	NCF4	Neutrophil cytosolic factor 4
23	SLC11A1	Solute carrier family 11 member 1
23	HK3	Hexokinase 3
23	EPCAM	Epithelial cell adhesion molecule
23	SLC4A1	Solute carrier family 4 member 1 (Diego blood group)
22	ORM1	Orosomucoid 1
22	CKAP4	Cytoskeleton associated protein 4
22	IL1RN	Interleukin 1 receptor antagonist
21	NCF1	Neutrophil cytosolic factor 1
21	FYB	FYN binding protein
21	VNN2	Vanin 2
21	GABBR1	Gamma-aminobutyric acid type B receptor subunit 1
21	BST1	Bone marrow stromal cell antigen 1
21	MRC1	Mannose receptor, C type 1
21	WT1	Wilms tumor 1
21	PI3	Peptidase inhibitor 3
20	CD79A	CD79a molecule
20	PGLYRP1	Peptidoglycan recognition protein 1
20	SELPLG	Selectin P ligand
20	CEACAM1	Carcinoembryonic antigen related cell adhesion molecule 1
20	GNG11	G protein subunit gamma 11
20	NMU	Neuromedin U
20	SLCO4C1	Solute carrier organic anion transporter family member 4C1
20	CR1	Complement component 3b/4b receptor 1 (Knops blood group)
20	TNS1	Tensin 1
19	CD3G	CD3g molecule

Table S2 Details of common existed chemotherapy drugs

Compound	Synonyms	Drug targets	Drug targets pathway
Tubastatin A	–	HDAC1, HDAC6, HDAC8	Chromatin histone acetylation
VNLG/124	HDAC inhibitor XV	HDAC, RAR	Chromatin histone acetylation
GSK1070916	GSK-1070916	AURKA, AURKC	Mitosis
TG101348	TG101348, SAR302503	JAK2	Other, kinases
Sunitinib	Sunitinib Malate, SU-11248	PDGFR, KIT, FLT3, VEGFR, RET, CSF1R	RTK signaling
NVP-BHG712	BHG712	EPHB4	RTK signaling
Alectinib	CH5424802, Alecensa	ALK	RTK signaling
Sorafenib	Nexavar, 284461-73-0, BAY 43-9006	PDGFR, KIT, VEGFR, RAF	RTK signaling
Linifanib	ABT-869, ABT 869	VEGFR1, VEGFR2, VEGFR3, CSF1R, FLT3, KIT	RTK signaling
Quizartinib	AC220, AC 220, AC-220, Asp-2689	FLT3	RTK signaling
TAE684	NVP-TAE 684, TAE684, TAE-684	ALK	RTK signaling
ZSTK474	KIN001-167, ZSTK 474	PI3K (class 1)	PI3K/MTOR signaling
AICAR	N1-(b-D-ribofuranosyl)-5-aminoimidazole-4-carboxamide	AMPK agonist	Metabolism
Vorinostat	Zolinza, SAHA, suberanilohydroxamic acid, suberoylanilide hydroxamic acid, MK-0683	HDAC inhibitor Class I, IIa, IIb, IV	Chromatin histone acetylation
CAY10603	–	HDAC1, HDAC6	Chromatin histone acetylation
WZ3105	–	SRC, ROCK2, NTRK2, FLT3, IRAK1, others	Other
Masitinib	–	KIT, PDGFRA, PDGFRB	RTK signaling
BHG712	–	EPHB4	RTK signaling
Foretinib	GSK1363089, XL-880, EXEL-2880, GSK089	GSK1363089, XL-880, EXEL-2880, GSK089	RTK signaling
Quizartinib	AC220, AC 220, AC-220, Asp-2689	FLT3	RTK signaling
ZSTK474	KIN001-167, ZSTK-474, ZSTK 474	PI3K (class 1)	PI3K/MTOR signaling
Omipalisib	GSK2126458, GSK-2126458, EX-8678, GSK458	PI3K (class 1), MTORC1, MTORC2	PI3K/MTOR signaling
AS605240	KIN001-173, AS-605240	PI3Kgamma	PI3K/MTOR signaling
UNC0638	UNC-0638, UNC 0683	G9a and GLP methyltransferases	Chromatin histone methylation
Erlotinib	Tarceva, RG-1415, CP-358774, OSI-774, Ro-508231, R-1415	EGFR	EGFR signaling
BMS345541	BMS345541, IKK Inhibitor 3	IKK1, IKK2	Other, kinases
BIX02189	BIX 02189	MEK5, ERK5	ERK MAPK signaling
ATRA	Tretinoin, Vesanoid, Renova, Atralin, Tretin-X, Avita	Retinoic acid	Other
Salubrinal	EIF-2alpha Inhibitor	EIF2A	
Bexarotene	LG-100069, Targretin, Targret, Targrexin, Targretyn, Bexarotenum	Retinioic X receptor (RXR) agonist	Other
CP466722	CP-466722, CP 466722, 1080622-86-1	ATM	Genome integrity
AZD7762	AZD-7762, AZD 7762	CHEK1, CHEK2	Cell cycle
Methotrexate	Abitrexate, Amethopterin, Rheumatrex, Trexall, Folex	Antimetabolite	DNA replication
Navitoclax	ABT-263, ABT263, ABT 263	BCL2, BCL-XL, BCL-W	Apoptosis regulation
Vorinostat	Zolinza, SAHA, suberanilohydroxamic acid, suberoylanilide hydroxamic acid, MK-0683	Zolinza, SAHA, suberanilohydroxamic acid, suberoylanilide hydroxamic acid, MK-0683	Chromatin histone acetylation
Vincristine	–	–	Vincristine
Tozasertib	MK 0457, MK-0457, MK-045, VX-680 VX 680 VX-68	AURKA, AURKB, AURKC, others	Mitosis
Topotecan	–	–	DNA replication
Teniposide	Vumon	–	DNA replication
Sunitinib	Sutent, Sunitinib Malate, SU-11248	PDGFR, KIT, VEGFR, FLT3, RET, CSF1R	RTK signaling
Piperlongumine	Piplartine	Induces reactive oxygen species	Other
Panobinostat	Farydak, LBH589	HDAC	Chromatin histone acetylation
Paclitaxel	BMS-181339-01, Taxol, Onxol, Paxene, Praxel, Abraxane	Microtubule stabiliser	Mitosis
Mitomycin	Mytozytrex, NSC-26980, MMC, Mitosol, Mitozytrex	DNA crosslinker	DNA replication
GSK525762A	I-BET-762	BRD2, BRD3, BRD4	Chromatin other
Gemcitabine	Gemzar, LY-188011	Pyrimidine antimetabolite	DNA replication
Fluorouracil	5-FU	Antimetabolite (DNA & RNA)	Other
Etoposide	Etopophos, Vepesid, Eposin, VP-16	TOP2	DNA replication
Entinostat	MS-275	HDAC1, HDAC3	Chromatin histone acetylation
Dinaciclib	SCH727965, SCH 727965, SCH-727-965, MK-7965	CDK1, CDK2, CDK5, CDK9	Cell cycle
Crizotinib	Xalkori, PF2341066, PF-2341066, PF 2341066	MET, ALK, ROS1	RTK signaling
Bortezomib	PS-341, LDP-341, Velcade	Proteasome	Protein stability and degradation
Belinostat	PXD101, PXD-101	HDAC1	Chromatin histone acetylation
Phenformin	DBI	Biguanide agent	Other
FK866	Daporinad, APO866	NAMPT	Metabolism
LAQ824	Dacinostat	HDAC1	Chromatin histone acetylation
Genentech Cpd 10	–	AURKA, AURKB	Mitosis
Ispinesib Mesylate	SB-715992	KSP	Mitosis
GSK690693	GSK 690693, GSK-690693	AKT1, AKT2, AKT3	PI3K/MTOR signaling
Ruxolitinib	INCB-18424, Ruxolitinib Phosphate, Jakafi	JAK1, JAK2	Other, kinases
YM201636	YM-201636, YM 201636	PIKFYVE	PI3K/MTOR signaling
T0901317	TO-901317, TO901317	LXR, FXR	Other
Lenalidomide	CDC-501, CC-5013, Revlimid	CRBN	Protein stability and degradation
AZD8055	AZD-8055	MTORC1, MTORC2	PI3K/MTOR signaling
Docetaxel	RP-56976, Taxotere	Microtubule stabiliser	Mitosis
Nilotinib	Tasigna, AMN 107	ABL	ABL signaling
Bleomycin	–	dsDNA break induction	DNA replication