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Multi-omics integration reveals immune hallmarks and biomarkers associated with FLT3 inhibitor sensitivity in FLT3-mutated AML

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Abstract

Acute myeloid leukemia (AML) is an aggressive hematologic malignancy characterized by poor clinical outcomes, frequently exacerbated by mutations in the FMS-like tyrosine kinase 3 (FLT3) gene. Although FLT3 inhibitors (FLT3i) have emerged as promising therapeutic agents, the absence of molecular biomarkers to predict FLT3i response remains a critical limitation in clinical practice. In this study, we performed a comprehensive multi-omics analysis integrating transcriptomic, proteomic, and pharmacogenomic data from the Beat AML cohort, the Cancer Cell Line Encyclopedia (CCLE), and the PXD023201 repository to elucidate the molecular consequences of FLT3 mutations in AML. Our analysis revealed significant differences in RNA and protein expression profiles between FLT3-mutant and wild-type AML cases, with a particularly striking association between FLT3 mutations and immune suppression. We further evaluated the drug sensitivity of FLT3-mutant patients to 3 FDA-approved FLT3i, gilteritinib, midostaurin, and quizartinib, and observed heightened sensitivity in FLT3-mutant cohorts, accompanied by the activation of immune-related pathways in treatment-responsive groups. These findings suggest a potential synergy between FLT3i efficacy and immune activation. Through rigorous bioinformatic analysis, we identified 3 candidate biomarkers: CD36, SASH1, and NIBAN2, associated with FLT3i sensitivity. These biomarkers were consistently upregulated in favorable prognostic subgroups and demonstrated strong correlations with immune activation pathways. The identification of CD36, SASH1, and NIBAN2 as predictive biomarkers offers a novel toolset for stratifying FLT3i response and prognosis.

Key Words: Acute myeloid leukemia; Drug sensitivity; FLT3 inhibitors; FLT3 mutations; Immunity; Multi-omics

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All data are available within the manuscript (Figs. 1–4).

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1. INTRODUCTION

Acute myeloid leukemia (AML) is a highly heterogeneous hematologic malignancy characterized by the clonal expansion of immature myeloid cells in the bone marrow, leading to impaired hematopoiesis and bone marrow failure.[1,2] Despite significant advancements in therapeutic strategies, including intensive chemotherapy and hematopoietic stem cell transplantation, the prognosis for AML patients remains suboptimal, with a 5-year overall survival (OS) rate of approximately 29%. This is particularly true for patients harboring high-risk genetic mutations, which are associated with worse clinical outcomes.[3] Among these mutations, internal tandem duplications in the FLT3 gene (FLT3-ITD) are the most prevalent, occurring in approximately 30% of AML cases, and are strongly correlated with poor prognosis due to increased relapse rates and resistance to conventional therapies.[4–8] FLT3-ITD mutations induce constitutive activation of FLT3 tyrosine kinase, resulting in aberrant signaling cascades that promote leukemic cell proliferation, survival, and resistance to apoptosis.[8,9] These findings underscore the critical need for targeted therapeutic strategies to address FLT3-driven oncogenic pathways.

The development of tyrosine kinase inhibitors (TKIs) targeting FLT3 has emerged as a promising therapeutic approach. Several FLT3 inhibitors (FLT3i), including midostaurin, gilteritinib, and quizartinib, have demonstrated efficacy as monotherapies or in combination with chemotherapy, leading to their approval for clinical use.[10] However, the emergence of resistance to FLT3i remains a significant barrier to achieving durable

remissions, and the molecular mechanisms underlying this resistance are not yet fully understood. A comprehensive elucidation of these mechanisms is essential to improve clinical outcomes and develop more effective therapeutic regimens. FLT3 is a class III receptor tyrosine kinase predominantly expressed in hematopoietic stem and progenitor cells. Upon binding to its ligand (FLT3-L), FLT3 activates downstream signaling pathways, including JAK-STAT, PI3K-AKT, MAPK-ERK, and Src family kinases, which collectively regulate critical cellular processes such as proliferation, survival, and migration.[11–13] Early studies on FLT3 primarily focused on genomic and transcriptomic analyses.[14,15] However, recent advances in multi-omics technologies, including proteomics, metabolomics, and epigenomics, have provided deeper insights into the complex role of FLT3 in leukemogenesis.[16–18] Despite these advancements, integrating multi-omics data to fully elucidate FLT3 signaling mechanisms and their contribution to therapy resistance remains a significant challenge.

To address these gaps, we employed a multi-omics approach leveraging the Beat AML database, a comprehensive resource that integrates genomic, transcriptomic, epigenetic, and clinical data from nearly 700 AML patients.[19] The Beat AML initiative was designed to enhance the understanding of AML pathogenesis through systematic analysis molecular abnormalities and identification of potential therapeutic targets. Additionally, we incorporated cell line data from the Cancer Cell Line Encyclopedia (CCLE) to complement clinical findings.[20] Both databases are anchored in transcriptomic profiling, enabling robust cross-validation of results. Furthermore, we utilized the clinical proteomics database PXD023201 to facilitate multi-omics integration and data mining across these diverse datasets.[21] Our analysis revealed that FLT3 mutations are associated with immune suppression, whereas FLT3i-sensitive cases exhibit immune activation. This suggests a potential synergistic interaction between FLT3 inhibition and immune system activation, highlighting the therapeutic potential of combining FLT3i with immunomodulatory agents. Finally, we identified a biomarker panel comprising three RNA molecules that demonstrate strong predictive value for FLT3i response and patient survival, offering a promising tool for personalized treatment strategies.

2. METHODS

2.1. Data source

The transcriptomic expression matrix (beataml_waves1to4_norm_exp_dbgap.txt), clinical metadata (beataml_wv1to4_clinical.xlsx), and sample annotation files (beataml_waves1to4_sample_mapping.xlsx) were retrieved from the BeatAML2.0 repository hosted on GitHub (biodev/beataml2.0_data: Data accompanying the BeatAML2.0 manuscript). FLT3i IC50 values were acquired from the Vizome platform (http://www.vizome.org/aml2/inhibitor/). Preprocessing of the expression matrix entailed the removal of quality control samples and pooled specimens, retaining only RNA-sequenced samples with unambiguous survival annotations. Clinical data processing involved stratification of patients harboring FLT3-TKD or FLT3-ITD mutations into a consolidated $FLT3^{mut}$ cohort. IC50 values were normalized using the transformation: normalized score = 1 – (IC50/10). Transcriptomic profiles of AML cell lines were derived from the CCLE RNA-seq count data (CCLE_RNAseq_genes_counts_20180929.gct; https://depmap.org/portal/data_page/?tab=allData). Cell lines were classified into $FLT3^{mut}$ and $FLT3^{wt}$ subgroups based on curated mutation annotations. Proteomic datasets were sourced from the clinical cohort described in Mann et al[21] (Data S1: "Imputed Proteome" matrix), with FLT3-mutant patients identified through systematic screening of "PanelSeq I" and "PanelSeq II" genomic panels.

2.2. Differential expression analysis

Differential expression analysis was conducted using R software (version 4.0.3). DERs and DEPs were identified using a linear model fitting approach in the R limma package[45] (version 3.60.6). For DERs, the threshold criteria were set at a P value <0.05 and a fold change >2, while DEPs were considered significant with a P value <0.05 and a fold change >1.2.

2.3. Enrichment analysis of biological processes for gene ontology

Enrichment analyses were performed using the Metascape platform[46] (http://metascape.org/gp/index.html#/main/step1) with default parameters. Upregulated and downregulated gene sets were analyzed separately. The analysis was conducted with the following parameters: a minimum overlap of 3 genes, a P value cutoff of 0.01, a minimum enrichment score of 1.5, and gene sets classified under the Gene Ontology (GO) Biological Processes category in the pathway classification.

2.4. GSEA and GSVA

GSEA and GSVA were performed using R software. GSEA was conducted with the clusterProfiler package[47] (version 4.12.6), using the following parameters: minGSSize = 5, maxGSSize = 1000, pvalueCutoff = 0.05, pAdjustMethod = "BH." GSVA was performed with the GSVA package[48] (version 1.52.3), with the settings: minSize = 5, maxSize = 5000, kcdf = "Gaussian." The gene sets used for GSEA and GSVA analyses were sourced from The Molecular Signatures Database (MSigDB) (h.all.v2024.1.Hs.symbols.gmt, https://www.gsea-msigdb.org/gsea/msigdb/human/collections.jsp).

2.5. Spearman correlation analysis

Spearman correlation analysis was performed using the "corr.test" function from the R "psych" package (version 2.4.12) to evaluate the relationship between the RNA expression levels of the eight candidate molecules and GSVA pathway scores. The Benjamini-Hochberg (BH) correction was applied to adjust P values for multiple comparisons.

2.6. Survival and interaction survival analysis

Survival curves were generated using the Kaplan–Meier method, and differences between the curves were assessed with the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CIs) for each multivariate survival analysis were estimated using the Cox proportional hazards model from the "survival" R package. This package was also used to identify proteins and RNAs interacting with FLT3i.

2.7. Visualization

Visualizations, including volcano plots, dot plots, box plots, and stacked bar charts, were created using the ggplot2 package (version 3.5.1). Heatmaps were generated with the pheatmap package (version 1.0.12), and Kaplan–Meier survival curves were visualized using the ggsurvplot function from the survminer package (version 0.5.0).

3. RESULTS

3.1. Multi-dimensional data integration reveals immune suppression in FLT3-mutant AML

FLT3 mutations, occurring in approximately 30% of AML cases, represent a key molecular driver associated with

adverse clinical outcomes.[8] To systematically characterize FLT3 mutation-driven molecular alterations, we performed a multi-omics integrative analysis incorporating transcriptomic (Beat AML database), proteomic (PXD023201 repository), and cell line pharmacogenomic (CCLE database) datasets. Rigorous quality control measures were applied to exclude pooled samples, quality control samples, and specimens with incomplete clinical annotations, resulting in a curated dataset of 647 AML samples with extensive molecular profiling. FLT3 mutation status was stratified using clinically relevant classifications, with FLT3-TKD variants incorporated into the mutant cohort due to their low prevalence. Drug sensitivity metrics were normalized using the Roy et al.[22] IC50 transformation protocol (1 – IC50/10), followed by tertile-based stratification into sensitive (upper third) and resistant (lower third) cohorts (**Fig. 1A**). Multi-platform differential expression analysis revealed conserved molecular signatures across biological hierarchies. Transcriptomic/proteomic profiling identified significant upregulation of GLI2 (Hedgehog pathway effector; fold change > 11.2, *P* value = 7.55e-41) and downregulation of ANK1 (erythropoiesis regulator; fold change < 0.42, *P* value = 0.0015) in FLT3-mutant cases (**Fig. 1B–D**), corroborating previous hematopoiesis studies.[23,24]

Gene Ontology Biological Process (GOBP) enrichment analysis revealed that the upregulated RNAs and proteins in FLT3-mutant samples were significantly enriched in mitochondrial-related biological processes, consistent with the findings reported by Shao et al.[25] Conversely, the downregulated molecules were predominantly associated with immune-related biological processes (**Fig. 1E and F**), in alignment with previous observations by van Galen et al.[15] Gene set enrichment analysis (GSEA) further corroborated the presence of immune dysregulation, demonstrating significant depletion in key immunological pathways including inflammatory response, NF-κB signaling, and interferon-γ-mediated signaling pathways (**Fig. 1G**). Through cross-dataset integration employing stringent concordance criteria (requiring differential expression in ≥1 dataset with consistent directional regulation across all other datasets), we identified 970 mutation-associated biomolecules (512 upregulated and 458 downregulated) with high confidence.

In summary, our comprehensive multi-omics analysis provides compelling evidence that immune suppression represents a fundamental pathological mechanism underlying the adverse clinical outcomes associated with FLT3 mutations in AML.

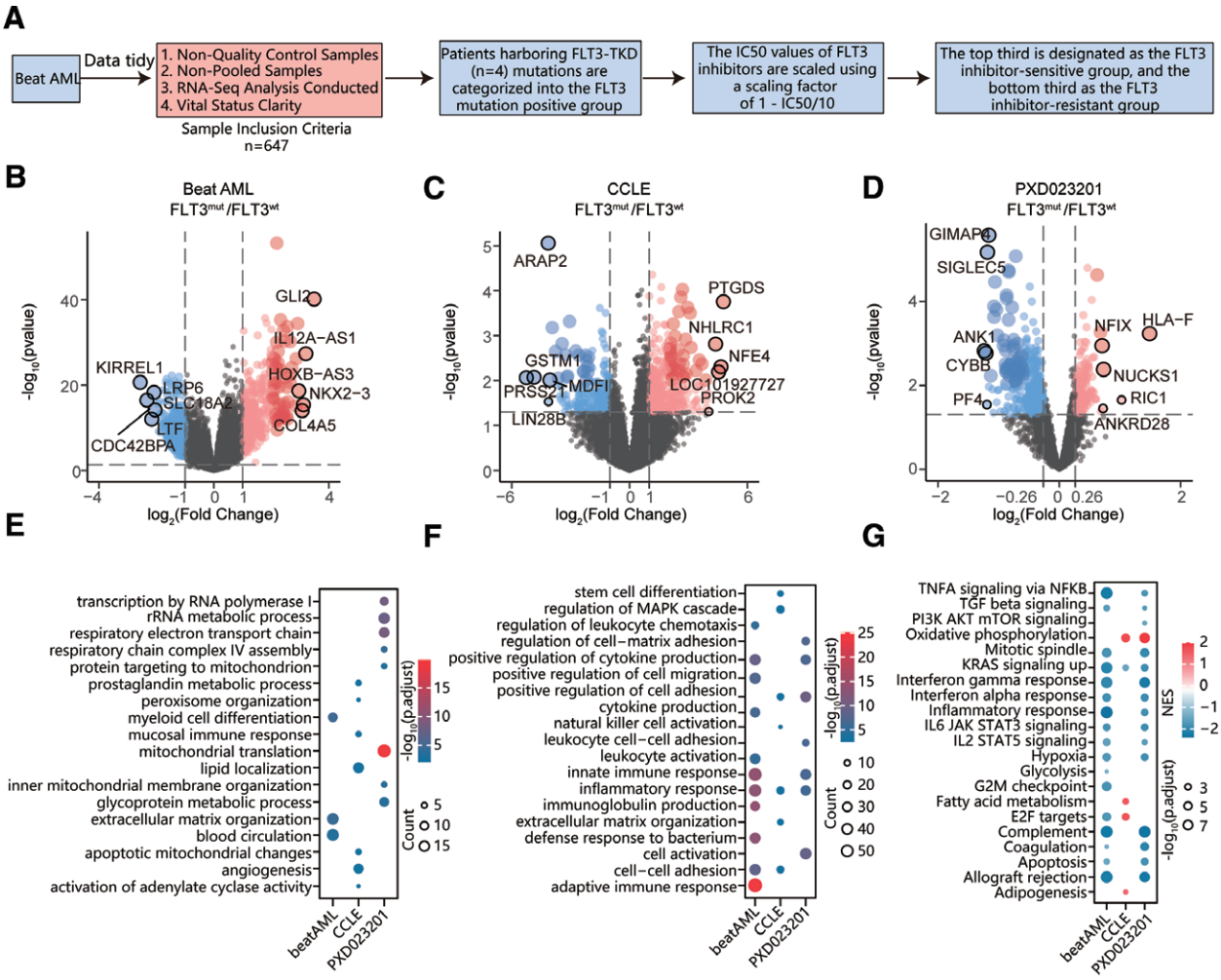

Figure 1. Multi-dimensional data integration reveals immune suppression in FLT3-mutant AML. (A) Workflow diagram of data cleaning for Beat AML. (B–D) Volcano plots of DERs and DEPs in patients with FLT3 mutations from 3 databases: Beat AML (B), CCLE (C), and PXD023201 (D). Significantly upregulated RNAs or proteins are marked in red, and downregulated ones are marked in blue. The dashed line represents the thresholds for statistical significance (*P* value) and fold change. (E) GOBP analysis of upregulated RNAs or proteins in FLT3-mutated patients across the 3 databases (Beat AML, CCLE, and PXD023201). (F) GOBP analysis of downregulated RNAs or proteins in FLT3-mutated patients. (G) Hallmark genesets enrichment analysis of all molecules in the Beat AML, CCLE, and PXD023201. AML = acute myeloid leukemia, CCLE = Cancer Cell Line Encyclopedia, FLT3 = FMS-like tyrosine kinase 3, DEP = differentially expressed proteins, DER = differentially expressed RNA, GOBP = gene ontology biological process.

3.2. Immune pathways and molecular mechanisms underlying resistance to FLT3i in FLT3-mutant AML

The U.S. Food and Drug Administration (FDA) has approved 3 FLT3i, gilteritinib, midostaurin, and quizartinib, for the treatment of FLT3-mutant AML. Although these agents have demonstrated improved clinical outcomes, the emergence of drug resistance remains a critical challenge, as it is strongly associated with significantly reduced OS. Elucidating the molecular mechanisms underlying FLT3i resistance and identifying novel therapeutic targets are therefore of paramount clinical importance. In this study, we systematically investigated the differential sensitivity of FLT3-mutant AML patients to these three FDA-approved FLT3i. Gilteritinib and midostaurin, classified as type I inhibitors, exert their effects by binding to the active conformation of the FLT3 receptor, thereby inhibiting its kinase activity. In contrast, quizartinib, a type II inhibitor, exhibits higher specificity by targeting the inactive conformation of FLT3, preventing receptor activation.

To evaluate patient responses, we leveraged data from the Beat AML clinical database, normalizing IC50 values and stratifying patients into sensitive and resistant cohorts for comparative analysis. Our findings revealed that FLT3-mutant patients displayed significantly greater sensitivity to FLT3i compared with their FLT3-wild-type counterpart (**Fig. 2A**). To delineate the molecular basis of resistance, we performed differential expression analysis between sensitive and resistant FLT3-mutant cases. Notably, due to the distinct mechanisms of action among the 3 FLT3i, the differentially expressed RNAs (DERs) exhibited considerable heterogeneity, as illustrated in the volcano plot (**Fig. 2B**). GOBP enrichment analysis demonstrated that upregulated molecules in the sensitive group were predominantly enriched in immune-related biological processes (**Fig. 2C**). Intriguingly, the observed immune suppression in FLT3-mutant patients (**Fig. 1F**) suggests that immune activation may synergistically enhance FLT3i efficacy. A striking observation was the divergent response profile of quizartinib compared to gilteritinib and midostaurin (**Fig. 2D**), likely attributable to its unique mechanism of action, as previously described by Schlaweck et al.[26] Furthermore, GSEA revealed that FLT3-mutant patients treated with gilteritinib and midostaurin exhibited significant activation of immune-related pathways (**Fig. 2E**), whereas FLT3-wild-type patients showed marked suppression of these pathways, with quizartinib displaying some exceptions (**Fig. 2F**). Given the substantial heterogeneity among the 3 FLT3i, we conducted a rigorous screening for DERs associated with FLT3i sensitivity, retaining only those molecules that demonstrated sensitivity to at least one inhibitor and consistent directional regulation across the other 2. This stringent filtering process identified 522 high-confidence DERs, comprising 286 upregulated and 236 downregulated molecules (**Fig. 2G and H**).

Collectively, these findings provide novel insights into the molecular mechanisms driving FLT3i resistance and underscore the critical role of immune-related pathways as potential therapeutic targets for overcoming resistance. This work lays the foundation for the development of innovative combination therapies to improve outcomes in FLT3-mutant AML.

3.3. Identification of immune and FLT3 signaling-related biomarkers for FLT3i sensitivity in AML

To identify DERs specifically associated with FLT3i sensitivity, we excluded previously identified molecules (DERs and differentially expressed proteins [DEPs] linked to FLT3 mutations) from subsequent analyses. This approach yielded 224 upregulated and 186 downregulated molecules (**Fig. 3A**). To refine our candidate selection, we performed Cox interaction analysis on these DERs to identify molecules interacting with FLT3i sensitivity. Candidates were required to interact with at least 2 of the 3 FLT3i, resulting in the identification of 7 upregulated DERs (CD36, SASH1, NIBAN2, TPPP3, HCAR3, AQP1, and LMNA) and 1 downregulated DER (ZNF208) (**Fig. 3B**). The expression patterns of these 8 molecules were consistent across treatments with all three FLT3i, with no significant differences observed between FLT3-mutant and FLT3-wild-type patients in the Beat AML, CCLE, and PXD023201 datasets (**Fig. 3C–F**), confirming the exclusion of FLT3 mutation-related biases.

Using interaction Cox analysis, we classified these molecules as favorable or risk factors based on their K values: $K < 0$ indicated favorable factors, while $K > 0$ denoted risk factors.[27] Volcano plot analysis revealed that the 7 upregulated molecules functioned as favorable factors, whereas the downregulated ZNF208 was identified as a risk factor (**Fig. 3G–I**). To further explore the functional relevance of these candidates, we performed gene set variation analysis (GSVA) on immune- and FLT3-related pathways from the Hallmark gene sets, calculating enrichment scores for each sample. This analysis revealed strong correlations between CD36, SASH1, and NIBAN2 and both immune and FLT3 signaling pathways, highlighting their potential as therapeutic targets to enhance FLT3i sensitivity (**Fig. 3J–L**).

Previous studies have implicated CD36 in cancer progression, metastasis, immune modulation, and therapy resistance.[28] CD36 promotes immune activation by enhancing macrophage function,[29] forms anti-tumor immune super-complexes, and inhibits tumor growth through TSP1-induced caspase activation.[30] SASH1, a putative tumor suppressor, inhibits tumor proliferation and invasion via the PI3K-AKT pathway,[31,32] while NIBAN2 modulates tumor progression through Ras pathway activation.[33,34] In conclusion, the identification of CD36, SASH1, and NIBAN2 as key regulators of immune activation and FLT3 signaling underscores their potential as therapeutic targets to overcome FLT3i resistance, offering new avenues for clinical intervention.

3.4. Favorable biomarker panel predicts FLT3i sensitivity and prognosis in AML

Given the clinical utility of biomarkers in prognostic prediction, we constructed a gene set comprising 3 favorable molecules: CD36, SASH1, and NIBAN2 termed "Favorable Factors," based on their association with immune status and FLT3 signaling. Patients were stratified into "Favorable Factors: High Score" and "Favorable Factors: Low Score" groups based on median GSVA scores. Interaction and multivariate Cox regression analyses, incorporating variables such as age, blast percentage, and FAB classification, demonstrated that a high score in this gene set robustly predicted FLT3i sensitivity (**Fig. 4A–C**). Specifically, the "Favorable Factors: High Score" group was associated with reduced risk in FLT3i-sensitive patients, whereas no significant correlation was observed in the FLT3i-resistant cohort.

Further analysis revealed that patients with high scores in this gene set exhibited significantly improved survival and prognosis, particularly within the FLT3i-sensitive group (**Fig. 4D–F**). These findings suggest that high expression of CD36, SASH1, and NIBAN2 not only predicts FLT3i sensitivity but also correlates with favorable clinical outcomes.

4. DISCUSSION

The pathogenesis of AML is driven by a complex interplay of genetic mutations, including those in FLT3, NPM1, and IDH1/2, which disrupt critical signaling pathways, leading to uncontrolled cell proliferation, impaired differentiation, and immune evasion.[8,35–38] Despite the approval of targeted therapies for FLT3-mutant AML, such as FLT3i, significant challenges remain, including high rates of relapse, the

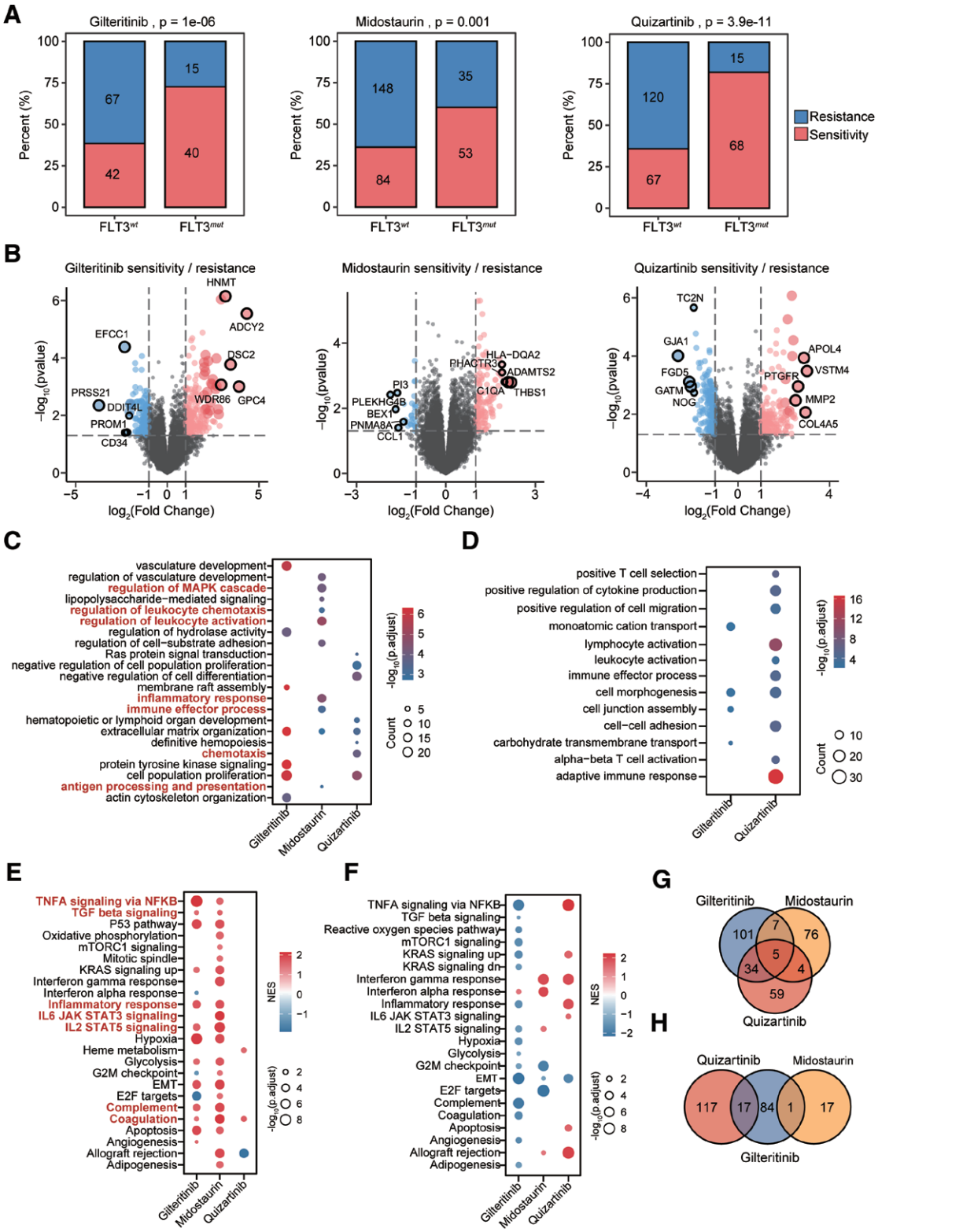

Figure 2. Immune pathways and molecular mechanisms underlying resistance to FLT3 inhibitors in FLT3-mutant AML. (A) Stacked bar plot showing the proportion of patients sensitive or resistant to gilteritinib, midostaurin, and quizartinib, grouped by FLT3-Mut and FLT3-WT. The numbers in each bar represent the number of patients. (B) Volcano plot of DERs between sensitive and resistant patients for the 3 FLT3 inhibitors (gilteritinib, midostaurin, and quizartinib). (C) GOBP analysis of upregulated DERs in sensitive patients for 3 FLT3 inhibitors. (D) GOBP analysis of downregulated genes in sensitive patients for 3 FLT3 inhibitors. No significant enrichment terms were found in the midostaurin group. (E–F) Enrichment analysis of Hallmark gene sets corresponding to 3 FLT3 inhibitors in FLT3-Mut (E) and non–FLT3-Mut (F) patients. (G–H) Venn diagram of upregulated (G) and downregulated (H) DERs in the sensitive groups for 3 FLT3 inhibitors (gilteritinib, midostaurin, and quizartinib). AML = acute myeloid leukemia, DEP = differentially expressed proteins, DER = differentially expressed RNA, FLT3-Mut = mutation FLT3 mutation, FLT3-WT = FLT3 wild-type, GOBP = gene ontology biological process.

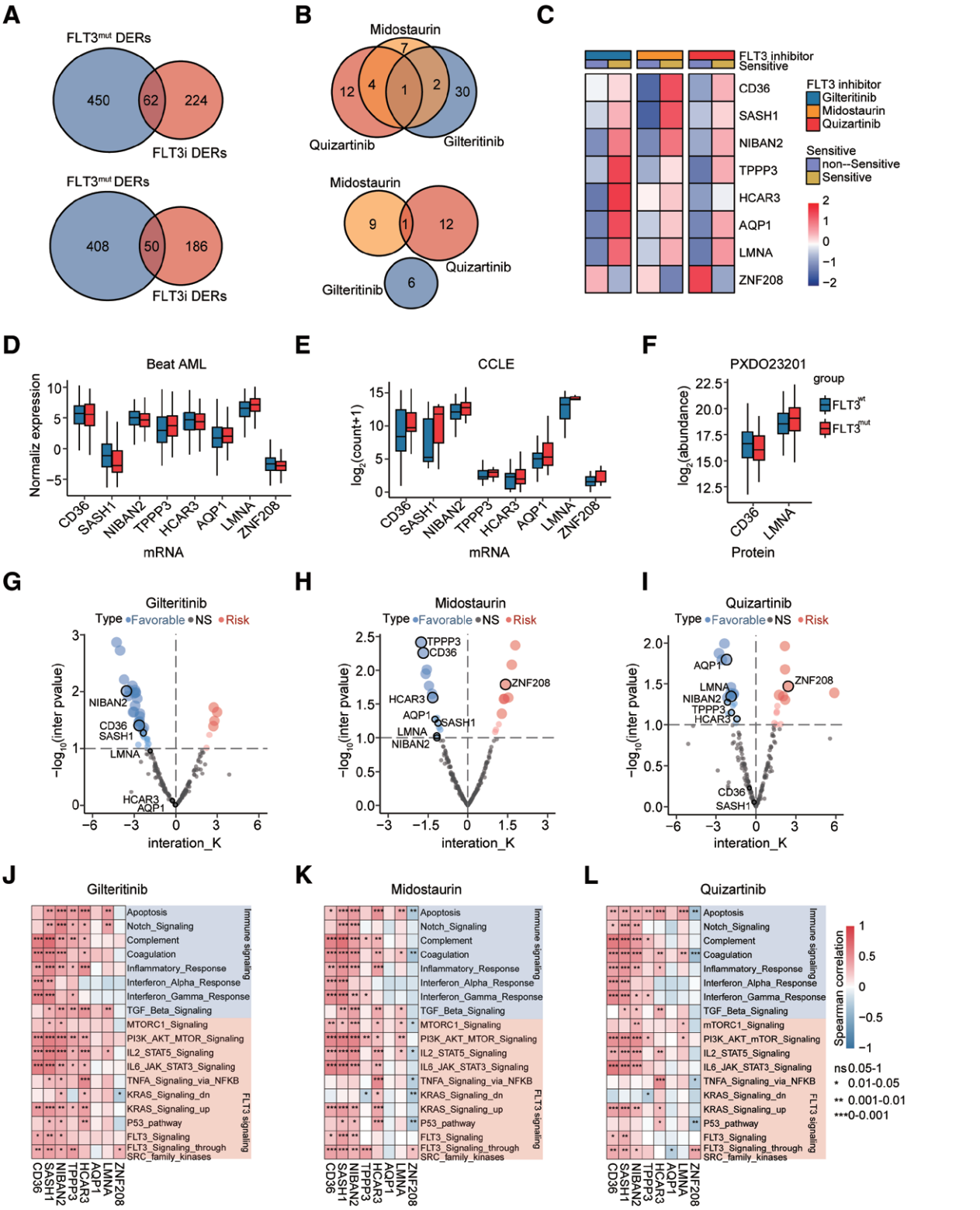

Figure 3. Identification of immune and FLT3 signaling-related biomarkers for FLT3 inhibitor sensitivity in AML. (A) Venn diagram of upregulated (top) and downregulated (bottom) DERs identified as unique molecules for FLT3 inhibitors (gilteritinib, midostaurin, and quizartinib) that are not caused by FLT3 mutations. (B) Venn diagram of upregulated (top) and downregulated (bottom) molecules in the interaction Cox analysis of the FLT3 inhibitor unique DERs. (C) Heatmap of the expression levels of 8 candidate targets with interaction effects across the 3 FLT3 inhibitors. (D–F) Boxplots of expression levels of 8 candidate targets across 3 databases (Beat AML, CCLE, and PXD023201). (G–I) Volcano plots of DERs interacting with FLT3 inhibitors. (J–L) Spearman correlations between the 8 candidate molecules and immune and FLT3 signaling pathways. AML = acute myeloid leukemia, CCLE = Cancer Cell Line Encyclopedia, DER = differentially expressed RNA.

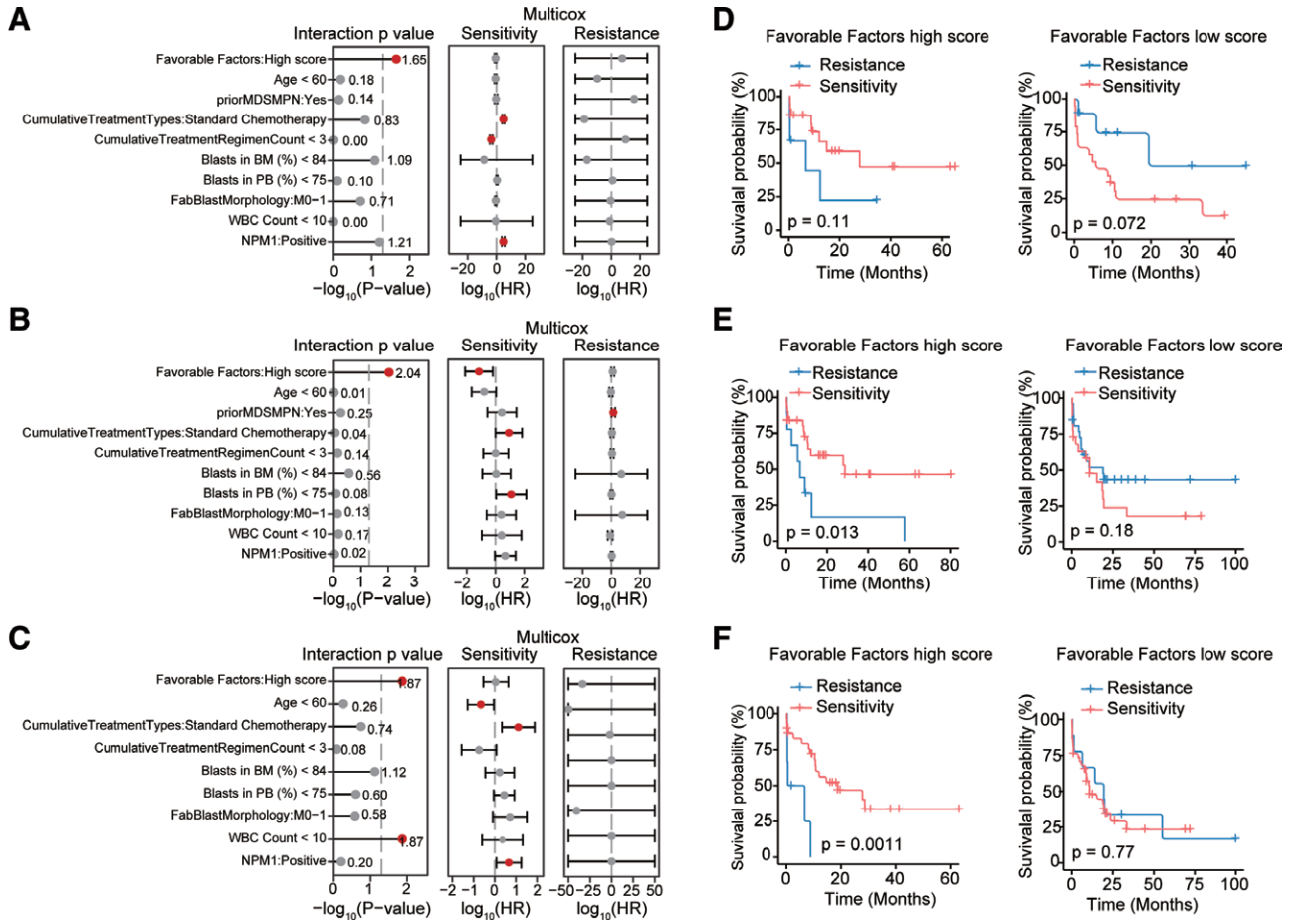

Figure 4. Favorable biomarker panel predicts FLT3 inhibitor sensitivity and prognosis in AML. (A–C) *P* values from Cox analysis of favorable factors and multiple clinical indicators interacting with gilteritinib (A), midostaurin (B), and quizartinib (C) sensitivity, as well as HRs derived from multivariable Cox regression. (D–F) Effect of "Favorable Factors: high or low scores" on overall survival in AML patients treated with gilteritinib (D), midostaurin (E), and quizartinib (F). AML = acute myeloid leukemia, HR = hazard ratios.

development of drug resistance, and poor prognosis, particularly among elderly patients. These limitations highlight the urgent need for novel therapeutic strategies to improve clinical outcomes.

In recent years, the role of the immune system in AML has garnered increasing attention. The efficacy of allogeneic stem cell transplantation, which relies on graft-versus-leukemia effects, underscores the importance of immune responsiveness in AML treatment.[39] Additionally, milder immunotherapeutic approaches, such as immune checkpoint inhibitors (ICIs), are being actively explored. Immune evasion mechanisms, particularly through the activation of immune checkpoints like PD-1/PD-L1, have been implicated in disease progression and resistance to conventional therapies.[40] ICIs, including nivolumab and pembrolizumab, have shown promise as adjunct therapies in specific AML populations, particularly when combined with chemotherapy or other targeted agents to overcome immune resistance.[41,42] Notably, the activation of innate immune stress response pathways, such as those involving IRAK1/4 complexes, has been linked to adaptive resistance in FLT3-mutant AML cells treated with FLT3i.[43] Furthermore, combining FLT3i like sorafenib with allogeneic immunity has demonstrated enhanced therapeutic responses, offering a potential strategy to circumvent resistance.[44]

This study employs an integrated multi-omics approach, combining clinical data with cell line databases, to investigate the molecular effects of FLT3 mutations across genomic, transcriptomic, and proteomic layers. By analyzing data from the Beat AML database, the CCLE, and the clinical proteomics database PXD023201, we identified significant differences in RNA and protein expression profiles between FLT3-mutant and FLT3-wild-type AML samples. Our findings reveal that FLT3 mutations not only disrupt mitochondrial function but also suppress immune-related biological processes, providing new insights into how FLT3 mutations drive AML progression by evading immune surveillance. Furthermore, we identified 3 key biomarkers: CD36, SASH1, and NIBAN2, that predict sensitivity to FLT3i. These biomarkers, which are associated with immune activation, offer potential tools for patient stratification and personalized treatment strategies. Incorporating these biomarkers into clinical practice could enable the optimization of treatment regimens based on individual immune profiles and drug sensitivity, potentially improving outcomes in FLT3-mutant AML, where immune suppression is strongly correlated with poor prognosis.

Despite these promising findings, several limitations of this study must be acknowledged. The relatively small sample size of the FLT3i-sensitive and resistant subgroups may limit statistical power, potentially obscuring significant effects and affecting the reliability of the results. Additionally, the generalizability of these findings to more heterogeneous patient populations remains uncertain, necessitating further validation in larger, multi-center cohorts. Future studies should focus on experimental validation using in vitro and in vivo models to confirm the clinical relevance of these biomarkers and their mechanistic roles in FLT3i sensitivity and resistance.

In conclusion, this study integrates multi-omics data to elucidate the mechanisms of immune suppression in FLT3-mutant AML and their relationship with FLT3i sensitivity. The identification of immune-related biomarkers, including CD36, SASH1, and NIBAN2, offers new perspectives for predicting FLT3i response and prognosis, paving the way for personalized treatment strategies. To translate these findings into clinical practice, the development of diagnostic tools, biomarker-based treatment stratification protocols, and well-designed clinical trials will be essential. A prospective clinical trial could stratify newly diagnosed FLT3-mutant AML patients based on biomarker expression levels, enabling direct comparisons of therapeutic outcomes. Combining FLT3i with immunomodulatory agents represents a promising approach, with long-term OS and progression-free survival (PFS) as primary endpoints. Such trials could also track dynamic changes in biomarker expression, treatment safety, and functional status, providing a comprehensive assessment of the clinical value of these interventions. This framework has the potential to support the development of personalized therapies for FLT3-mutant AML, ultimately improving patient outcomes and addressing the unmet need for effective treatments in this high-risk population.

5. CONCLUSION

This study provides a comprehensive multi-omics analysis of FLT3-mutant AML, revealing immune suppression and mitochondrial dysfunction as pivotal mechanisms driving poor clinical outcomes. Through the integration of transcriptomic, proteomic, and pharmacogenomic datasets, we identified CD36, SASH1, and NIBAN2 as key biomarkers associated with FLT3i sensitivity and favorable prognosis. These molecules are intricately linked to immune activation and FLT3 signaling, highlighting their potential as therapeutic targets to enhance treatment efficacy. Furthermore, the construction of the "Favorable Factors" gene set, comprising these biomarkers, establishes a robust predictive tool for FLT3i response and patient survival. These findings provide a foundation for the development of personalized therapeutic strategies in FLT3-mutant AML, offering new avenues for precision medicine in this high-risk patient population.

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