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Network Topology Defines Therapeutic Vulnerability and Resistance in FLT3-Mutant Acute Myeloid Leukemia

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Abstract

Background

FLT3-mutant acute myeloid leukemia (AML) is associated with aggressive disease biology, high relapse rates, and heterogeneous responses to chemotherapy and FLT3-targeted therapies. Although FLT3 mutations represent a key oncogenic driver, they coexist with multiple co-mutations affecting epigenetic regulation, transcription, and signaling pathways. This complexity suggests that clinical behavior may be better explained by the organization of gene interaction networks rather than individual genomic alterations.

Methods

We performed gene co-occurrence network analysis in patients with FLT3-mutant AML. Networks were constructed using weighted associations between recurrently mutated genes and analyzed across clinically relevant subgroups, including overall survival (> 18 vs < 18 months), response to induction chemotherapy (complete remission [CR] vs progressive disease [PD]), response to FLT3 inhibitor–based therapy, age at diagnosis (< 60 vs ≥ 60 years), and pre-transplant relapse. Network topology was assessed in terms of centrality, modularity, and connectivity.

Results

35 patients were included in the network analysis. An overall conserved core network was identified, centered on FLT3, RUNX1, TP53, and TET2, supported by epigenetic (DNMT3A, EZH2) and transcriptional (CEBPA, GATA2) modules. Clinical outcomes were associated with distinct network configurations. Favorable survival and response to chemotherapy were characterized by modular and hierarchically organized networks, incorporating differentiation and metabolic regulators such as CEBPA, IDH1, SF3B1, and CUX1. In contrast, poor survival and resistance were associated with highly interconnected and redundant networks, enriched in stemness and transcriptional regulators, including ASXL1, MECOM, GATA2, and ANKRD26. Response to FLT3 inhibitor–based therapy was associated with preservation of network complexity, whereas resistance was characterized by network contraction toward a minimal survival circuitry, with emergence of KIT as a bypass signaling node. Age-related analyses revealed more structured networks in younger patients, while older patients exhibited increased transcriptional and splicing complexity. Relapse was associated with network rewiring, including the emergence of CUX1, suggesting increased regulatory plasticity.

Conclusions

FLT3-mutant AML is a disease in which clinical behavior is determined by network architecture rather than single mutations. Modular networks are associated with therapeutic sensitivity, whereas redundant and rewired networks underlie resistance and relapse. These findings support the development of network-based biomarkers and therapeutic strategies to optimize treatment in FLT3-mutant AML.

Introduction

Acute myeloid leukemia (AML) is a biologically heterogeneous hematologic malignancy characterized by the accumulation of genetically and epigenetically altered myeloid precursors with impaired differentiation and uncontrolled proliferation. Among the most frequent and clinically relevant genomic alterations are mutations in the Fms-like tyrosine kinase 3 (FLT3) gene, which occur in approximately 25–30% of adult AML cases and are associated with adverse prognosis, high relapse rates, and increased leukemic burden[1, 2]. FLT3 mutations include internal tandem duplications (FLT3-ITD) and tyrosine kinase domain (FLT3-TKD) point mutations, both of which result in constitutive activation of downstream signaling pathways such as PI3K/AKT, RAS/MAPK, and STAT5, promoting leukemic cell survival and proliferation[3]. The therapeutic landscape of FLT3-mutant AML has evolved significantly over the past decade with the introduction of FLT3 inhibitors, including first-generation multi-kinase inhibitors such as midostaurin and more selective second-generation agents such as gilteritinib and quizartinib[4–6]. These therapies have improved response rates and survival outcomes across both newly diagnosed and relapsed/refractory settings. However, despite these advances, clinical outcomes remain highly variable, and most patients ultimately experience treatment resistance and disease relapse[7]. This variability reflects the intrinsic biological complexity of AML. FLT3 mutations rarely occur in isolation and are frequently accompanied by co-mutations in genes regulating epigenetic modification (DNMT3A, TET2, EZH2), transcription (RUNX1, CEBPA, GATA2), RNA splicing (SRSF2, SF3B1), and signal transduction (RAS pathway genes, KIT)[8, 9]. These co-occurring alterations interact in a non-linear manner, shaping leukemogenesis, influencing therapeutic response, and driving disease evolution. Increasing evidence suggests that resistance to both chemotherapy and FLT3 inhibitors arises not only from additional mutations but also from adaptive rewiring of signaling and transcriptional programs, including activation of alternative kinase pathways, expansion of leukemic stem cell populations, and transcriptional plasticity[8, 10, 11]. Traditional genomic approaches have largely focused on individual mutations or predefined pathways; however, such models fail to capture the complex, interconnected nature of oncogenic processes in AML. In recent years, network-based approaches have emerged as a powerful framework to study cancer biology at a systems level. By modeling genes as nodes and their interactions as edges, network analysis enables the identification of key regulatory hubs, pathway interdependencies, and emergent properties of tumor systems that are not apparent from single-gene analyses[12]. In AML, early applications of network analysis have demonstrated that co-occurrence patterns of genomic alterations reflect underlying biological programs, including differentiation states, epigenetic regulation, and signaling dependencies[11]. More recently, integrative studies combining genomic and functional data have shown that network topology—such as modularity, connectivity, and

centrality—can influence therapeutic vulnerability and resistance, suggesting that AML may exist in distinct “network states” with different clinical behaviors[13]. Within this context, FLT3-mutant AML represents an ideal model to investigate the role of network organization in cancer biology. The presence of a dominant oncogenic driver (FLT3) embedded within a complex mutational landscape provides a unique opportunity to study how signaling pathways interact with transcriptional and epigenetic programs, and how these interactions evolve under therapeutic pressure.

We hypothesized that FLT3-mutant AML is characterized by distinct network architectures that determine clinical outcome, response to therapy, and disease evolution, and that these architectures can be captured through gene co-occurrence network analysis. Specifically, we sought to identify core regulatory networks shared across patients, as well as context-specific network configurations associated with survival, response to chemotherapy, response to FLT3 inhibitors, age, and relapse. By applying a network-based analytical framework to a cohort of FLT3-mutant AML patients, we aimed to provide a systems-level understanding of disease biology, uncover mechanisms of resistance, and generate hypotheses for network-informed therapeutic strategies, including rational sequencing and combination approaches.

Materials and Methods

Mutational data were obtained from AML patients with confirmed *FLT-3* mutation treated at the Department of Laboratory and Hematological Sciences, Fondazione Policlinico Universitario Agostino Gemelli-IRCCS, Rome. Patient age, therapy and response, overall survival (OS), and pre-transplant relapse information were used to categorize groups of patients for each mutational network. Molecular and clinical data used for the network analysis were obtained from previously collected gene testing according and during clinical practice.

A patient-mutation matrix was generated to construct the mutational networks, where rows were linked to patients and columns were linked to all the unique genes where at least one mutation occurred in the patient population. Cells were assigned ‘1’ if the given patient had a mutation in the given gene. For each analysis, patients were assigned a group, and within each group, two genes would be co-occurring if a minimum threshold of patients within that group had a mutation in both genes. This minimum threshold is dynamic and depends on the size of the group.

Mutational networks (where nodes are mutated genes and edges display co-occurrences of those mutated genes) were constructed using Cytoscape (Version 3.10.2), as well as the calculation of each node’s *betweenness centrality*. Betweenness centrality was computed as the proportion of shortest paths between all node pairs that pass through a given node, reflecting its role as a bridge within the network. Nodes with higher betweenness centrality are interpreted as occupying more central bridging positions within the network. A lighter-colored node shows a lower betweenness centrality and a darker-colored node shows a higher betweenness centrality. Edge color represents the co-occurrence frequency of each mutated gene pair within the analyzed group, ranging from to dark-blue.

Results

Patients characteristics

Thirty-five consecutive patients newly diagnosed with FLT3-ITD mutated AML were included in the analysis. The median age was 59 years old (range 37–84) with a slight female predominance (M/F ratio was 14/21). The majority of patients (71%) presented with a normal karyotype at the time of diagnosis, and only two patients had a complex karyotype. First line therapy consisted of intensive chemotherapy without a FLT3-inhibitor in 10 patients (28%), and of a selective inhibitor combined with standard chemotherapy or given alone in 21 patients (60%). The remaining four patients (11%) received a different treatment. Complete remission was achieved in 30 patients (86%), while 5 patients (14%) were refractory to first line treatment. Second line treatment consisted of a second generation FLT3-inhibitor. Allogeneic hematopoietic stem cell transplantation was performed in 25 patients (71%). Median follow up was 774 days (range 108–1782).

A threshold of 18 months was used to divide patients between *short* and *long* overall survival, where eight patients were grouped as *short* OS and 27 patients were grouped as *long* OS. Therapy and response divided the patient cohort into 4 groups: *chemo & complete remission*, *chemo & resistant, chemo + FLT3-inhibitor & complete remission* and *chemo + FLT3-inhibitor & resistant*. These groups were populated with 7, 3, 19 and 2 patients respectively, where 4 patients were given alternative treatment. Age was divided into patients below and above 60 years old, with 23 patients in the former group, and 12 patients in the latter group. Finally, patients who underwent a transplant were put into two groups, depending on if relapse had occurred before transplant or not. Seven patients experienced relapse before transplant, while 26 patients did not, and two patients were not eligible for this category.

Global structure of FLT3-mutant gene co-occurrence networks

Gene co-occurrence network analysis in the overall FLT3-mutant AML population revealed a highly interconnected genomic architecture, with a subset of recurrently co-altered genes forming central hubs (edge weight ≥ 3; Fig. 1A). When the network was refined using a higher edge-weight threshold (≥ 5; Fig. 1B), a more compact and biologically cohesive subnetwork emerged, characterized by fewer nodes but stronger and more specific interactions. At lower stringency, the network was broadly distributed, with FLT3 positioned as a central node interconnected with key transcriptional, epigenetic, and signaling regulators, including RUNX1, TP53, TET2, DNMT3A, CEBPA, GATA2, EZH2, and KIT.

Within this configuration, RUNX1 and TP53 emerged as prominent central nodes, suggesting a critical role in network stability and disease aggressiveness, and displaying multiple high-weight connections with both epigenetic modifiers (TET2, DNMT3A, EZH2) and transcription factors (CEBPA, GATA2). This pattern suggests that FLT3 signaling is embedded within a transcriptional–epigenetic regulatory network, rather than functioning as an isolated oncogenic driver. In particular, the co-occurrence of FLT3 with TET2 and DNMT3A highlights a strong interaction between kinase signaling and epigenetic

dysregulation, while connections with CEBPA and GATA2 indicate integration with differentiation-related transcriptional programs, associated with myeloid differentiation and stem cell maintenance, while KIT represents a parallel kinase signaling node, potentially contributing to pathway redundancy.

Overall, increasing the stringency threshold resulted in a transition from a broad, highly connected network (weight ≥ 3) to a condensed hub-centric architecture (weight ≥ 5), in which FLT3, RUNX1, and TP53 constitute the core regulatory axis, supported by epigenetic (TET2, DNMT3A, EZH2) and transcriptional (CEBPA, GATA2) modules, with KIT acting as an auxiliary signaling component.

Network differences according to overall survival

To investigate the relationship between genomic architecture and clinical outcome, gene co-occurrence networks were stratified according to overall survival (OS > 18 months vs < 18 months), revealing distinct and biologically meaningful differences in network organization (Fig. 2A–B). Across both survival groups, TP53, RUNX1, and TET2 emerged as persistent core nodes, maintaining central positions within the network irrespective of outcome. Their consistent presence suggests that these genes represent foundational components of the FLT3-mutant leukemic network, integrating signaling, transcriptional regulation, and epigenetic control. However, marked differences were observed in the composition and topology of the surrounding network. In patients with OS > 18 months (Fig. 2A), the network was characterized by a more structured and modular organization, with additional involvement of genes associated with transcriptional regulation, RNA splicing, and metabolic/epigenetic control. In particular, CUX1, SRSF2, IDH1, and CEBPA were selectively enriched in this subgroup. These genes define a functionally coherent network centered on differentiation, RNA processing, and metabolic regulation, suggesting that favorable outcomes are associated with preservation of organized biological programs and controlled signaling hierarchy. Notably, the presence of CEBPA and IDH1 supports a model in which differentiation capacity and metabolic–epigenetic regulation may contribute to therapeutic sensitivity.

In contrast, patients with OS < 18 months (Fig. 2B) exhibited a less modular and more signaling-driven network, with the emergence of ANKRD26 and GATA2 as additional interacting nodes. The incorporation of these genes into the network suggests a shift toward altered stemness programs and dysregulated hematopoietic signaling. In this context, GATA2, a key regulator of hematopoietic stem cell maintenance, may contribute to enhanced self-renewal and resistance to therapy, while ANKRD26, previously associated with dysmegakaryopoiesis and predisposition to myeloid malignancies, may reflect underlying clonal instability or altered differentiation pathways. Importantly, the relative centrality of TP53 and RUNX1 appeared more pronounced in the poor-outcome network, with stronger and more numerous connections, suggesting that network dominance of stress-response and transcriptional deregulation pathways is associated with inferior survival. This is consistent with the known role of TP53 alterations in conferring genomic instability and resistance to therapy.

Overall, these findings indicate that while FLT3-mutant AML shares a common core network architecture, clinical outcome is determined by distinct network extensions and rewiring patterns. Favorable survival

is associated with integration of epigenetic, metabolic, and differentiation-related modules, whereas poor survival is characterized by expansion of stemness-associated and dysregulated signaling networks.

Network architecture and response to chemotherapy

To investigate the relationship between genomic architecture and response to induction chemotherapy, gene co-occurrence networks were compared between patients achieving complete remission (CR) and those with progressive disease (PD) (Fig. 3A–B). Across both groups, a set of genes remained consistently represented, including TET2, RUNX1, TP53, EZH2, and DNMT3A, defining a shared core network in FLT3-mutant AML. These genes likely represent foundational components of leukemogenesis, transcriptional regulation (RUNX1), and epigenetic control (TET2, DNMT3A, EZH2), as well as stress-response pathways (TP53). Despite this shared backbone, network topology differed markedly between responders and non-responders. In patients achieving complete remission (CR) (Fig. 3A), the network displayed a structured and relatively modular architecture, with defined connections centered on FLT3, RUNX1, and TP53, and supported by epigenetic regulators. The presence of IDH1 may reflect a more stable metabolic–epigenetic state, while SF3B1, a key splicing factor, may contribute to a more organized transcriptional landscape. Overall, this configuration is consistent with a hierarchically organized network, in which leukemic cells retain dependency on specific pathways, rendering them more susceptible to cytotoxic therapy.

In contrast, patients with progressive disease (PD) (Fig. 3B) exhibited a fully connected network topology, in which all nodes were interconnected, albeit with heterogeneous edge weights. This structure reflects a high degree of signaling redundancy and network plasticity, suggesting that leukemic cells are not dependent on a limited number of dominant hubs but rather exploit multiple parallel pathways. This PD-specific network was further characterized by the emergence of ASXL1, MECOM, ANKRD26, and GATA2, genes associated with epigenetic dysregulation, stemness, and transcriptional reprogramming. In particular: ASXL1 is linked to chromatin remodeling and adverse prognosis, MECOM (EVI1) is a well-established driver of aggressive AML and stem cell maintenance, ANKRD26 is associated with altered megakaryopoiesis and clonal instability, GATA2 regulates hematopoietic stem cell self-renewal and differentiation. The incorporation of these genes into a dense, highly interconnected network suggests that resistance is associated with expansion of stemness-related and epigenetically deregulated programs, enabling adaptation to chemotherapy-induced stress. Notably, while TP53 and RUNX1 remained central in both groups, their role appeared to differ according to network context. In CR, they participated in a coordinated, hierarchical structure, whereas in PD, they were embedded within a diffuse and redundant network, consistent with loss of functional dominance.

Overall, these findings indicate that response to chemotherapy in FLT3-mutant AML is not determined solely by the presence of specific mutations, but by the global organization of the underlying network. A modular, hub-centric architecture is associated with treatment sensitivity, whereas a fully connected and redundant network characterizes resistant disease.

FLT3 inhibitor–based therapy is associated with distinct network simplification in responders and signaling restriction in resistant disease

To evaluate the impact of FLT3-targeted therapy on network architecture, gene co-occurrence networks were compared between patients achieving complete remission (CR) and those with progressive disease (PD) following chemotherapy combined with midostaurin or gilteritinib (Fig. 4A–B). Across both groups, a conserved set of genes—RUNX1, TP53, TET2, and CEBPA—remained present, defining a shared core regulatory axis. These genes represent key functional domains, including transcriptional control (RUNX1, CEBPA), epigenetic regulation (TET2), and stress-response signaling (TP53), suggesting that FLT3-mutant AML retains a common biological backbone even under targeted therapeutic pressure. However, network topology and composition differed markedly between responders and non-responders. In patients achieving CR (Fig. 4A), the network exhibited a structured and moderately complex architecture, centered on FLT3 and interconnected with both transcriptional and epigenetic regulators. In addition to the shared core genes, EZH2, CUX1, CSF3R, DNMT3A, and ANKRD26 were selectively present in this subgroup. These genes define a multi-layered network integrating chromatin regulation (EZH2, DNMT3A), transcriptional modulation (CUX1), cytokine signaling (CSF3R), and clonal architecture (ANKRD26). The presence of these elements suggests that response to FLT3 inhibitor–based therapy is associated with preservation of a coordinated and hierarchically organized network, in which multiple biological processes remain functionally integrated but still dependent on dominant regulatory nodes.

In contrast, patients with PD (Fig. 4B) displayed a markedly simplified and restricted network, composed of fewer nodes and predominantly centered on FLT3, RUNX1, TP53, TET2, and CEBPA, with the notable addition of KIT, which was exclusively present in this group. This configuration suggests a collapse of network complexity into a reduced set of signaling and transcriptional dependencies, with KIT acting as a potential compensatory kinase pathway. The emergence of KIT in the resistant network supports the hypothesis that bypass signaling through alternative tyrosine kinases contributes to resistance to FLT3 inhibition, reinforcing pathway redundancy despite apparent network simplification. At the same time, the absence of broader epigenetic and regulatory components (e.g., EZH2, DNMT3A) indicates a loss of higher-order network organization, consistent with a shift toward dominant survival signaling pathways. Notably, while RUNX1 and TP53 remained central in both groups, their network context differed substantially. In CR, they were embedded within a diverse and interconnected regulatory network, whereas in PD they were part of a minimal, tightly constrained signaling module, suggesting a functional transition from coordinated regulation to stress-adaptive survival circuitry.

Overall, these findings indicate that response to FLT3 inhibitor–based therapy is associated with retention of network complexity and integration of epigenetic and transcriptional programs, whereas resistance is characterized by network contraction and reliance on alternative kinase signaling, particularly involving KIT.

Age at diagnosis is associated with distinct network composition and biological programs in FLT3-mutant AML

To assess the impact of age on the genomic architecture of FLT3-mutant AML, gene co-occurrence networks were stratified according to age at diagnosis (< 60 vs ≥ 60 years), revealing both a conserved core network and age-specific extensions (Fig. 5A–B). Across both age groups, a common set of genes was consistently observed, including TP53, RUNX1, TET2, KIT, DNMT3A, EZH2, GATA2, and ANKRD26, defining a shared backbone of FLT3-mutant disease biology. These genes integrate key functional domains, including transcriptional regulation (RUNX1, GATA2), epigenetic control (TET2, DNMT3A, EZH2), kinase signaling (FLT3/KIT), and clonal architecture (ANKRD26), suggesting that the fundamental network structure is preserved irrespective of age. However, age-dependent differences in network composition were evident, reflecting distinct biological programs. In patients aged < 60 years (Fig. 5A), the network was enriched for ASXL1, CSF3R, and CEBPA, which were selectively present in this subgroup. These genes are associated with chromatin remodeling (ASXL1), cytokine signaling (CSF3R), and myeloid differentiation (CEBPA). Their integration into the network suggests a more functionally organized and differentiation-oriented architecture, in which leukemic cells retain structured regulatory programs. In particular, the presence of CEBPA supports the maintenance of differentiation pathways, while CSF3R may reflect responsiveness to cytokine-driven signaling, collectively contributing to a more coherent and hierarchically regulated network.

In contrast, patients aged ≥ 60 years (Fig. 5B) exhibited selective inclusion of IDH1, CUX1, and SF3B1, genes involved in metabolic–epigenetic regulation (IDH1), transcriptional control (CUX1), and RNA splicing (SF3B1). This pattern indicates a shift toward increased transcriptional and post-transcriptional complexity, consistent with the known enrichment of splicing and epigenetic alterations in older AML populations. The presence of SF3B1 and CUX1 suggests disruption of RNA processing and transcriptional fidelity, while IDH1 may contribute to altered metabolic and epigenetic states. Notably, while the core nodes (TP53, RUNX1, TET2) remained central in both groups, the surrounding network in older patients appeared more heterogeneous and less modular, with broader but lower-weight interactions, consistent with increased clonal diversity and reduced dependency on dominant hubs. In contrast, younger patients maintained a more cohesive network structure, with clearer functional modules.

Overall, these findings indicate that age at diagnosis influences the peripheral architecture of the FLT3-mutant network, with younger patients characterized by differentiation- and signaling-oriented modules, and older patients by epigenetic, transcriptional, and splicing-related complexity.

Network features associated with transplant relapse

To explore the genomic architecture associated with disease recurrence, gene co-occurrence networks were compared between patients with no pre-transplant relapse and those who experienced relapse prior to transplant (Fig. 6A–B). Across both groups, a consistent set of genes was identified, including TP53, TET2, RUNX1, DNMT3A, EZH2, KIT, and CEBPA, defining a stable core network in FLT3-mutant AML. These genes encompass key biological processes, including transcriptional regulation (RUNX1, CEBPA), epigenetic control (TET2, DNMT3A, EZH2), kinase signaling (FLT3/KIT), and genomic stress

response (TP53), indicating that the fundamental leukemic framework is preserved regardless of relapse status. Despite this shared backbone, network topology and composition differed between the two groups, suggesting distinct biological states associated with disease stability versus recurrence. In patients without relapse (Fig. 6A), the network displayed a relatively organized and moderately connected architecture, with clear interactions among core nodes. The structure appeared cohesive and centered on key hubs, particularly RUNX1, TET2, TP53, and FLT3, suggesting a hierarchically regulated system in which leukemic cells remain dependent on dominant signaling and regulatory pathways. The integration of epigenetic regulators (DNMT3A, EZH2) and differentiation-associated factors (CEBPA) further supports the presence of a coordinated and functionally constrained network, potentially associated with better disease control.

In contrast, patients with relapse (Fig. 6B) exhibited a reorganized and more heterogeneous network, characterized by altered connectivity among core nodes and the emergence of CUX1 as a relapse-specific gene. The incorporation of CUX1, a transcription factor implicated in genomic instability and adverse-risk AML, suggests the activation of transcriptional dysregulation programs associated with disease progression. Within the relapse network, TP53 and TET2 maintained central roles, but their connections appeared more widely distributed, reflecting a less hierarchical and more adaptive network structure. The persistence of epigenetic regulators (DNMT3A, EZH2) alongside altered transcriptional inputs (CUX1) indicates a shift toward increased regulatory plasticity, which may facilitate clonal evolution and therapeutic escape. Notably, while KIT and CEBPA remained present in both groups, their relative positioning within the network appeared less dominant in the relapse setting, suggesting a reduced reliance on differentiation and canonical signaling pathways, and a greater dependence on transcriptional and epigenetic reprogramming.

Overall, these findings indicate that relapse in FLT3-mutant AML is associated with network reorganization rather than the loss of core components, with the addition of transcriptional instability nodes such as CUX1 marking a transition toward a more adaptive and therapy-resistant state.

Discussion

In this study, we provide a network-based characterization of FLT3-mutant AML, demonstrating that disease behavior is governed by dynamic network states rather than isolated genomic events. We identify a conserved core axis (FLT3–RUNX1–TP53–TET2) embedded within epigenetic and transcriptional modules, and show that clinical outcomes are driven by context-specific network rewiring. Our findings align with and extend recent evidence that FLT3-mutant AML is a highly plastic disease under therapeutic pressure. While FLT3 inhibitors such as gilteritinib and quizartinib have improved outcomes, resistance remains common and is increasingly recognized as a systems-level phenomenon involving pathway rewiring rather than single mutations[14, 15]. Recent genomic and longitudinal studies confirm that RAS/MAPK activation, transcriptional reprogramming, and clonal evolution are dominant mechanisms of resistance in FLT3-mutant AML[7, 15, 16]. A central observation from our analysis is that network modularity correlates with therapeutic sensitivity, whereas network

density and redundancy correlate with resistance. This is consistent with emerging concepts from cancer systems biology showing that robust, highly connected networks are less vulnerable to targeted perturbation, whereas hierarchically organized systems retain “Achilles heel” dependencies[12]. Recent clinical trials further support this interpretation. Combination strategies such as FLT3 inhibitors plus venetoclax and hypomethylating agents have demonstrated high response rates and deeper remissions, suggesting that simultaneous targeting of multiple network nodes can overcome redundancy[17]. Similarly, triplet regimens and MRD-guided strategies are being explored to prevent network adaptation and relapse[18, 19]. Our results provide a mechanistic framework for these observations. In chemotherapy-resistant patients, we observed fully connected networks enriched in ASXL1, MECOM, GATA2, and ANKRD26, reflecting expansion of stemness and transcriptional programs. These findings are consistent with recent data showing that leukemic stem cell–associated transcriptional states drive resistance and relapse in AML, particularly in FLT3-mutant disease[10]. In contrast, response to FLT3 inhibitor–based therapy was associated with retention of network complexity but preservation of hierarchical organization, whereas resistance was characterized by network contraction toward a minimal survival circuitry, with emergence of KIT as a bypass kinase. This observation is highly consistent with recent studies demonstrating that alternative tyrosine kinases and parallel signaling pathways mediate resistance to FLT3 inhibition, and that kinase switching or pathway compensation may inform sequencing strategies[15, 20, 21]. Importantly, recent data suggest that FLT3 inhibitor sequencing is not interchangeable, and that response depends on the specific resistance landscape and signaling context[21, 22]. Our network-based framework provides a conceptual basis for this, suggesting that network topology could serve as a predictive biomarker to guide sequencing decisions, particularly in identifying patients likely to benefit from type I versus type II inhibitors or combination approaches. Age-related differences further reinforce the role of network evolution over time, with older patients showing increased representation of splicing and transcriptional dysregulation (SF3B1, CUX1). This aligns with recent studies demonstrating that aging-associated AML is characterized by increased clonal complexity and epigenetic instability, contributing to inferior outcomes[23]. Finally, our analysis of relapse highlights that recurrence is driven by network rewiring rather than loss of core nodes, with emergence of transcriptional regulators such as CUX1, consistent with recent models of adaptive clonal evolution and transcriptional plasticity[11].

Clinical Perspective

These findings support a shift toward network-informed precision medicine in FLT3-mutant AML. Rather than relying solely on mutational status, therapeutic decisions could incorporate network modularity as predictor of chemotherapy sensitivity; network redundancy as predictor of resistance; bypass nodes (e.g., KIT) to guide combination or sequencing strategies; network rewiring as early indicator of relapse.

Ultimately, this paradigm may enable dynamic, adaptive treatment strategies aligned with disease evolution, improving FLT3 inhibitor sequencing; integration with venetoclax-based regimens; post-transplant maintenance strategies and long-term outcomes.

Declarations

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Ethic declaration

In accordance with the Declaration of Helsinki, this study involved exclusively the secondary analysis of previously collected, de-identified molecular data and limited clinical information obtained from an existing database. Because the investigators had no access to patient identifiers or to any re-identification key, the study was considered not to constitute research involving identifiable human participants and did not require formal IRB review under applicable institutional and national regulations.

Declaration of Competing Interest

MDR: scientific advisory board/consulting relationship/onoraria for Novartis, Pfizer, Sanofi Genzyme, AstraZeneca, Janssen, Daiichi Sankyo, Menarini, Recordati, Regeneron, Roche, Lilly, Amgen, Bayer, Qiagen, Guardant Health. R.D. reports receiving speaker bureau/advisor's fee from Ipsen, Novartis, Pfizer, Sanofi Genzyme, AstraZeneca, Janssen, Gilead, Lilly, Gilead, and EUSA Pharma. All other authors have declared no conflicts of interest.

Availability of data and materials

The molecular and genomic dataset supporting the conclusions of this article is available upon request to the corresponding author.

Authors contribution

M.D.R.: Conceptualization, methodology, investigation, clinical data interpretation, supervision, writing – original draft, writing – review and editing.

M.v.W.: Methodology, bioinformatic and network analysis, data visualization, formal analysis, writing – original draft, writing – review and editing.

F.F.: Investigation, data collection and curation, clinical and molecular data interpretation, writing – original draft, writing – review and editing.

T.M.: Investigation, molecular data generation and interpretation, data curation, writing – review and editing.

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Figures

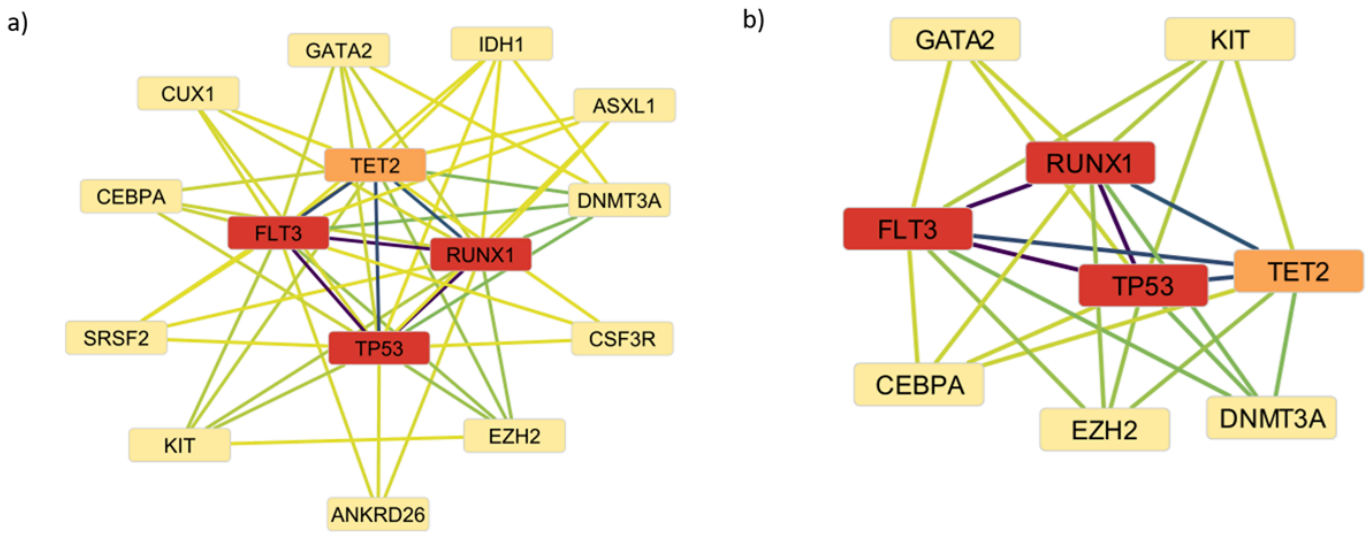
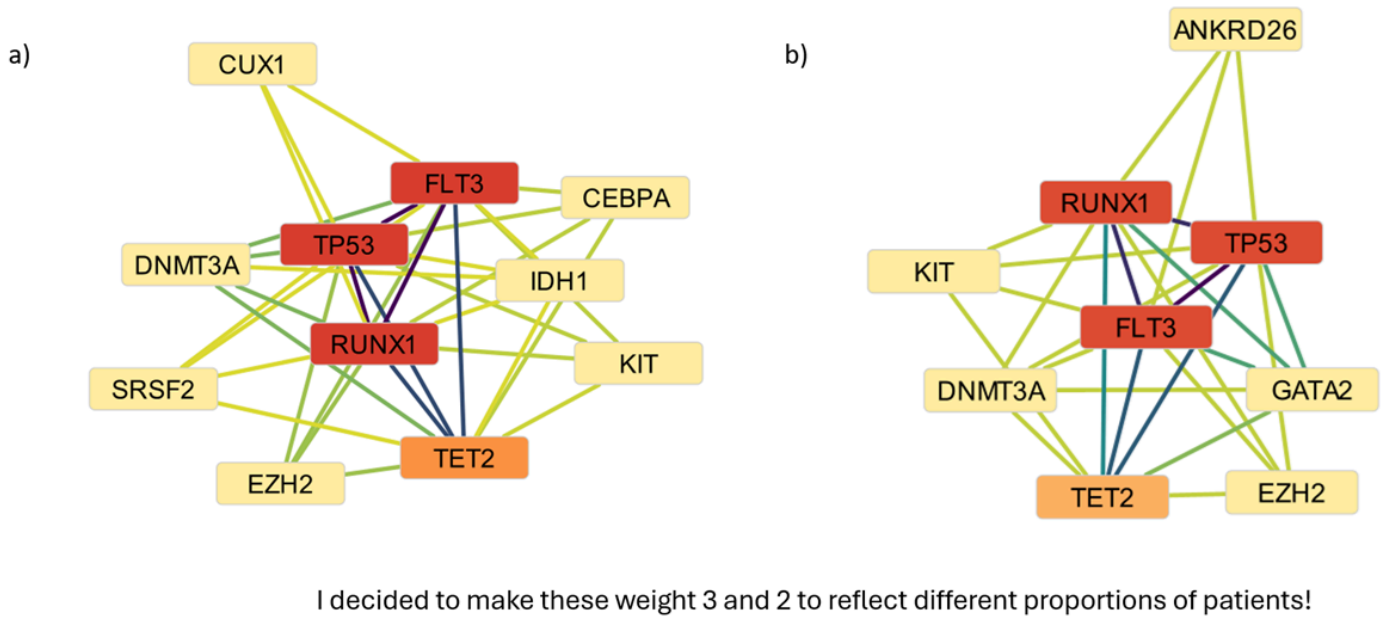


Figure 1

Gene co-occurrence network in the overall FLT-3 mutant population [weight 3 (a) and weight 5 (b)].



I decided to make these weight 3 and 2 to reflect different proportions of patients!

Figure 2

Gene co-occurrence network in FLT-3 mutant patients with (a) OS >18 months (weight 3) vs (b) <18 months (weight 2).

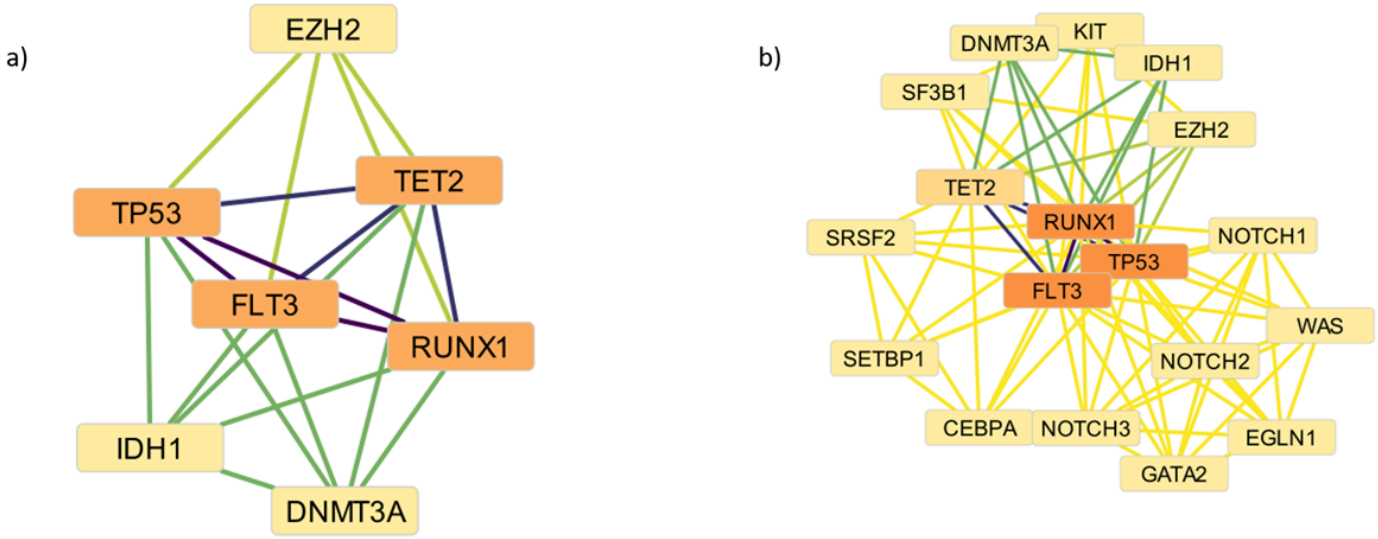


Figure 3

Gene co-occurrence network in FLT-3 mutant patients with CR (a) vs PD (b) to chemotherapy (weight=2).

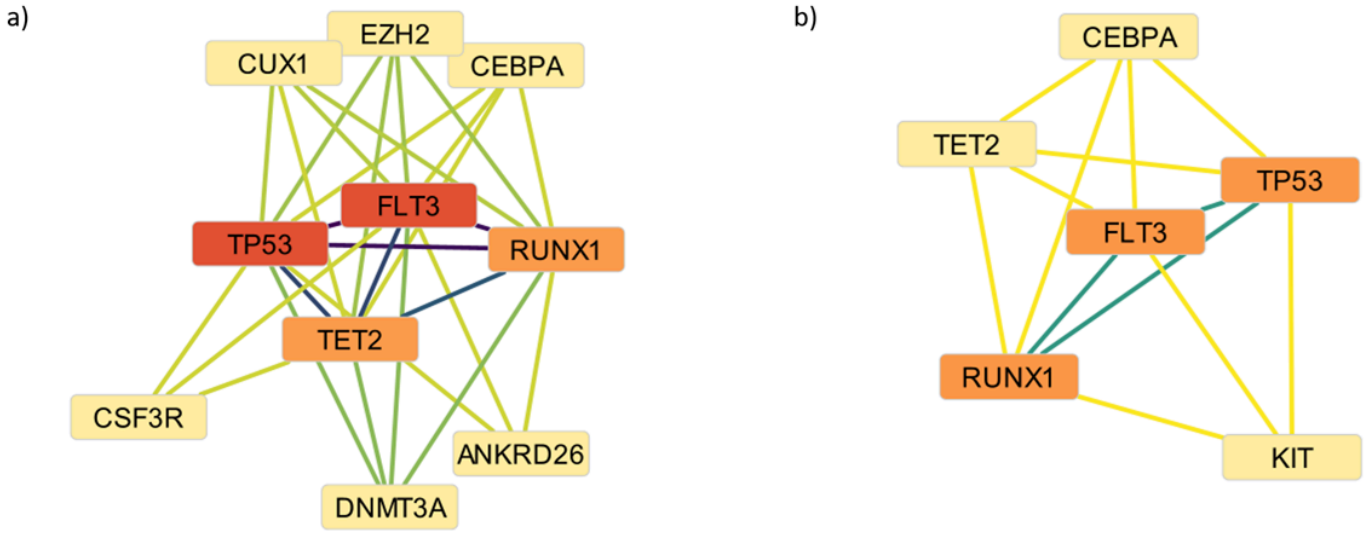


Figure 4

Gene co-occurrence network in FLT-3 mutant patients with CR (a, weight=3) vs PD (b, weight=1) to chemotherapy + midostaurin/gilteritinib.

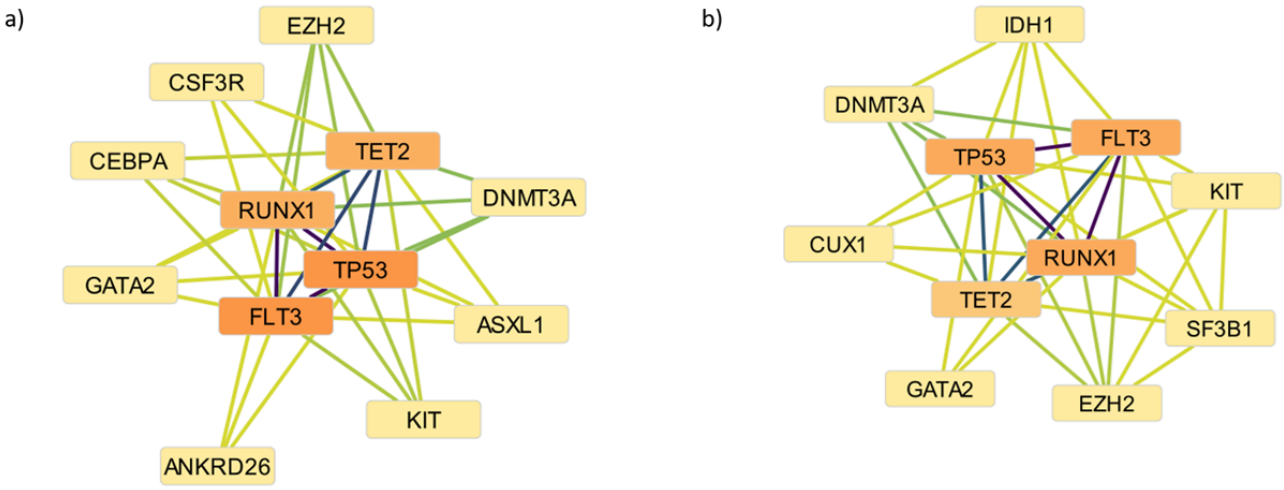


Figure 5

Gene co-occurrence network in FLT-3 mutant patients <60 (a, weight=3) vs >60 (b, weight=2) years.

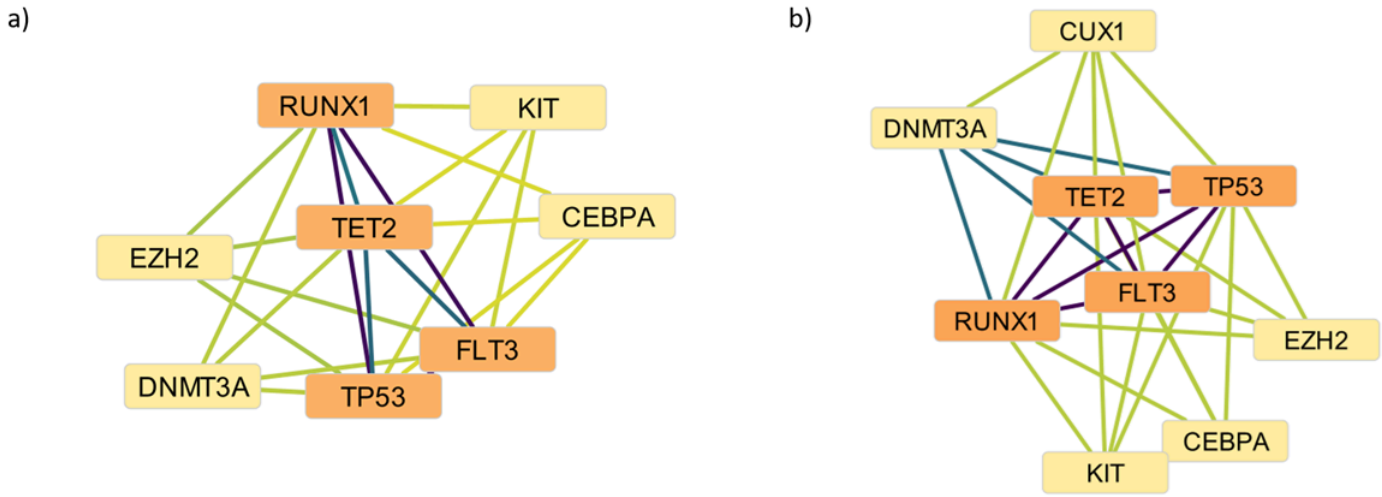


Figure 6

Gene co-occurrence network in FLT-3 mutant patients with no pre-transplant relapse (a, weight=3) vs relapse (b, weight=2).