



Targeting FLT-3 Mutations in Acute Myeloid Leukaemia: from Molecular Insights to Clinical Strategies

(FLT3-ITD / acute myeloid leukaemia / tyrosine kinase inhibitors / targeted therapy / gilteritinib / midostaurin)

MOHAMMADREZA AFSHARI, MARTINA ŘEZÁČOVÁ, DARINA MUTHNÁ

Department of Medical Biochemistry, Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, Czech Republic

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Corresponding author: Darina Muthná, Department of Medical Biochemistry Faculty of Medicine in Hradec Králové, Charles University, Šimkova 870, 500 03 Hradec Králové, Czech Republic; E-mail: muthnad@lfhk.cuni.cz

Abbreviations: ADP – adenosine diphosphate, AKT – protein kinase B, AML – acute myeloid leukaemia, ATP – adenosine triphosphate, AXL – AXL receptor tyrosine kinase, BIM – BCL-2-interacting mediator of cell death, CAR – chimeric antigen receptor, DFG – aspartate-phenylalanine-glycine (motif), EMA – European Medicines Agency, ERK – extracellular signal-regulated kinase, FDA – Food and Drug Administration, FLT-3 – FMS-like tyrosine kinase 3, FLT-3 L – FMS-like tyrosine kinase 3 ligand, GO – gemtuzumab ozogamicin, GRB2 – growth factor receptor-bound protein 2, GSK3 – glycogen synthase kinase 3, HSC – haematopoietic stem cell, ICC – international consensus classification, IDH – isocitrate dehydrogenase, ITD – internal tandem duplication, JAK – Janus kinase, MAPK – mitogen-activated protein kinase, MEK – MAPK/ERK kinase, MRD – minimal residual disease, mTOR – mammalian target of rapamycin, PD-1 – programmed cell death protein 1, PDGFR – platelet-derived growth factor receptor, PDK1 – phosphoinositide-dependent kinase 1, PI3K – phosphoinositide 3-kinase, PIP2 – phosphatidylinositol 4,5-bisphosphate, PIP3 – phosphatidylinositol (3,4,5)-trisphosphate, PROTAC – proteolysis-targeted chimeras, PTB – phosphotyrosine binding, PTPN11 – tyrosine-protein phosphatase non-receptor type 11, R/R – relapsed or refractory, SH2 – Src homology 2, SOS – son of sevenless, STAT – signal transducer and activator of transcription, TKD – tyrosine kinase domain, TKI – tyrosine kinase inhibitor, VEGFR – vascular endothelial growth factor receptor, WHO – World Health Organization, WT FLT-3 – wild-type FLT-3.

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Abstract. FMS-like tyrosine kinase 3 (FLT-3) mutations represent one of the most common genetic anomalies in acute myeloid leukaemia (AML), particularly in adults. The two most common types of mutations, internal tandem duplications (ITD) and tyrosine kinase domain (TKD) point mutations, facilitate uncontrolled cellular proliferation and unfavourable patient outcomes. These mutations are linked with a high relapse rate and shorter overall survival, highlighting the need for targeted therapies to be used. Recent advances in the discovery of new agents enabled incorporation of FLT-3 inhibitors into the frontline treatment regimen. First-generation inhibitors, such as midostaurin, provided the foundation for targeted therapy, while recently developed agents such as gilteritinib and quizartinib have shown more selectivity and demonstrated superior clinical efficiency and improved tolerability. This review discusses the significance of FLT-3 mutations, the evolution of targeted therapies, current treatment guidelines, and ongoing challenges such as resistance and high relapse rates. We also discuss the emerging combinations of therapies and novel agents currently in clinical trials that aim to overcome resistance and improve long-term outcomes for patients with FLT-3-mutated AML.

Introduction

Acute myeloid leukaemia (AML) is a genetically heterogeneous myeloid neoplasm characterized by rapid clonal expansion of undifferentiated myeloid precursor cells. Among the numerous genetic abnormalities identified in AML patients, mutations in FMS-like tyrosine kinase 3 (FLT-3) genes are among the most frequent in adult cases. These encompass internal tandem duplication (FLT-3 ITD) in juxtamembrane domain and point mutations in the tyrosine kinase domain (TKD), both of which would result in ligand-independent activation of FLT-3 receptor and activation of downstream signalling pathways leading to leukaemogenesis.

Identification of FLT-3 mutations has resulted in a paradigm shift in the treatment of AML, with small mole-

cule tyrosine kinase inhibitors (TKIs) being used concomitantly with conventional chemotherapy. In the past decade, the therapeutic landscape has evolved from the use of broad-spectrum TKIs to the development of more selective and potent inhibitors with fewer side effects. In parallel, our knowledge of resistance mechanisms and heterogeneity of AML has deepened, enabling more selective therapeutic strategies to be deployed according to the patients' genetic makeup. This review provides an updated overview of the biology of FLT-3 mutations, current and emerging targeted therapies, and future directions in the treatment of FLT-3-mutated AML.

AML

AML is a malignant disorder of haematopoietic stem cells (HSCs) (Döhner et al., 2015) characterized by rapid proliferation of undifferentiated myeloid blasts resulting in impairment of normal haematopoiesis. During the disease, the patients may suffer from many ailments related to immunosuppression and myelosuppression, such as severe infection, anaemia, thrombocytopenia and haemorrhage (National Cancer Institute, 2024).

In 2024, the National Cancer Institute of USA reported that 1 % of all new cancer cases and 1.8 % of all cancer deaths are due to AML (National Cancer Institute, 2024). Although AML affects individuals of all ages, two thirds of diagnoses occur in those over 55. Due to the aging population and the demographic changes in the next decades, the number of cases of AML is expected to rise exponentially.

AML is a heterogeneous disease with various subtypes resembling different stages of HSC differentiation (Grove and Vassiliou, 2014). Extensive research has increased our understanding of AML development, but no definitive hypothesis has been proved. The disease originates from normal HSCs that can self-renew and differentiate. This ability makes them susceptible to cancer development (Khoury et al., 2022). Impaired differentiation arises from genetic changes in HSCs. These changes can be caused by prior cancer treatments, such as exposure to topoisomerase II inhibitors, radiation, or alkylating agents (Sill et al., 2011), although most cases are due to chromosomal abnormalities or isolated gene mutations without clear causes (Pelcovits and Niroula, 2020).

Despite the limited number of leukaemic stem cells contributing to the development of AML, significant heterogeneity exists at the molecular, cytogenetic, phenotypic and clinical levels. Two main models have been proposed to explain this heterogeneity. The first model suggests that the diversity arises at various stages of stem cell differentiation, resulting in cells that display morphological characteristics resembling wild-type myeloid cells but still carrying molecular markers specific to AML. In contrast, the second model proposes that all leukaemic transformations originate in primitive multipotent stem cells, which generates heterogeneity through their capacity to acquire specific lineage-associated phenotypic markers. In this model, a stem cell may initially

express a certain surface antigen and subsequently acquire additional antigens while retaining the original one, resulting in formation of additional subtypes (Desai et al., 2022).

The molecular pathogenesis of AML has been extensively studied through cytogenetic analysis and genomic research, leading to the identification of recurrent structural variations in patients' chromosomes and gene mutations. These findings have been valuable for diagnosis and prognosis. Up to 50 % of AML patients exhibit normal karyotypes, meaning that they do not present detectable chromosomal abnormalities (Schlenk et al., 2008; Cancer Genome Atlas Research Network et al., 2013). Regarding the genetic alterations, patients often harbour multiple mutations, complicating treatment. The prevalent mutated genes are those for FLT-3 (28 %), NPM1 (27 %) and DNMT3A (26 %), which play crucial roles in signalling pathways, cellular regulation and DNA methylation (Cancer Genome Atlas Research Network et al., 2013).

FLT-3 mutations are the most common genetic alterations in AML, significantly impacting the signalling pathways that regulate apoptosis and cell proliferation. These mutations are classified into two subtypes: FLT-3 ITD and FLT-3 TKD. FLT-3 ITD is more prevalent, occurring in about 20 % of AML cases, whereas FLT-3 TKD is observed in approximately 7 % (Thiede et al., 2002).

Biomarkers for AML Patient Stratification

Stratification or grouping of AML has been attempted for a long time to categorize and evaluate the risk to patients. There are currently two widely used classification systems, the International Consensus Classification (ICC) of Myeloid Neoplasms and Acute Leukaemia (Arber et al., 2022) and the World Health Organization (WHO) 5th Edition Myeloid and Histiocytic/Dendritic Neoplasms (Khoury et al., 2022). The WHO 5th Edition is more widely used compared to the ICC due to it being more established, simple, reproducible and globally accepted by many haematological societies (Falini and Martelli, 2023).

This classification works first by dividing the AML into 12 groups according to well-defined genetic features, while cases without such genetic features are categorized according to the differentiation stage of leukaemic blasts into three categories (Swerdlow et al., 2017).

Although the FLT-3 mutation is a known genetic abnormality and is recognized as having a significant impact on the prognosis of AML, it has not yet been classified as a distinct subtype in the WHO 5th Edition classification. In 2022, the European Leukaemia Net published a guideline for risk stratification of AML. In the guideline, it is recommended to routinely test for mutation in genes such as *NPM1*, *CEBPA*, *FLT3* ITD, *TP53*, *RUNX1*, *ASXL1* and *BCR-ABL1*. The results from this test are essential for dividing patients into three categories of risk: favourable, intermediate and adverse (Döhner et al., 2022).

Mutations in NPM-1 promote cellular differentiation, which is associated with a more favourable prognosis and

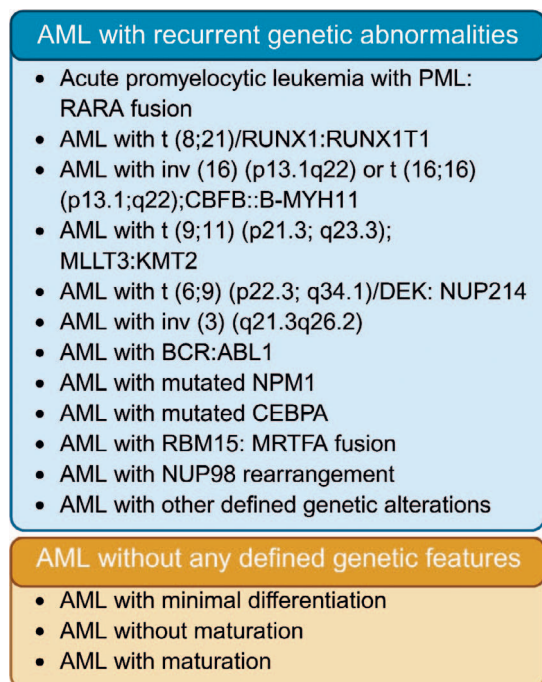


Fig. 1. WHO classification of AML. Created with BioRender (2025); <https://BioRender.com/lu8j30r>.

response to treatment with a lower risk of relapse. In contrast, FLT-3 ITD mutations drive increased proliferation of leukaemic cells, contributing to poorer outcomes. While previous guidelines incorporated the *FLT3* allelic ratio (ratio of mutated to wild-type alleles) to categorize risk, the 2022 ELN guidelines have simplified this categorization.

When NPM-1 is the lone mutation, AML is classified as favourable risk. However, under the updated guideline of 2022, the presence of FLT-3 ITD prioritizes the classification into intermediate risk group regardless of the allelic ratio. Consequently, while the presence of NPM-1 alone confers a favourable prognosis, the co-occurrence of FLT-3 ITD shifts the prognosis to intermediate risk categorization, neutralizing the favourable impact of NPM-1. In general, the presence of FLT-3 ITD places the patient in intermediate risk categorization, and addition of high-risk mutations would shift the prognosis toward the adverse risk. Risk categorization plays a crucial role in guiding treatment decisions during the course of therapy (Lachowiec et al., 2023).

Structure and Function of FLT-3

Molecular structure of FLT-3 kinase

FLT-3 is a transmembrane receptor belonging to the type III of receptor tyrosine kinases (Kikushige et al., 2008). Protein kinases are crucial in signal transduction throughout the cell. The human genome has more than 500 genes capable of producing several types of kinases. Out of them, 90 belong to a unique family called tyrosine kinases. There are two distinct groups of tyrosine ki-

nases found throughout the cell: those found in the cytoplasm and those found on the cell membrane (known as receptor tyrosine kinases). The main function of tyrosine kinases is to regulate cellular functions in an organism by using cell-to-cell signalling. These signals range from the initiation of differentiation to adhesion, motility, growth, or even death. Due to this wide range of functions in cells, tyrosine kinases have been linked to many human diseases such as cancer and diabetes (Robinson et al., 2000).

Tyrosine kinases achieve their function by selectively catalysing the phosphorylation of tyrosine residue in target proteins using ATP. This modification of proteins is an essential part of normal cellular communication and assists in maintaining homeostasis. Receptor tyrosine kinases are ligand dependent, meaning that an extracellular signal molecule should bind to the receptor to induce the enzyme activity (Paul and Mukhopadhyay, 2004).

In humans, the gene for FLT-3 ligand (FLT-3L) is located at chromosome 19q13.3–13.4. The FLT-3 ligand is available in two forms: membrane-bound and soluble. The membrane-associated ligand consists of 209 amino acids and can be cleaved by A disintegrin and metalloprotease 17, releasing a soluble version with 178 amino acids. This soluble ligand dimerizes to form an active molecule. Both forms function as growth factors with similar potency at binding to the FLT-3 receptor. The ligand is mainly produced by stromal fibroblasts of the haematopoietic bone marrow or by T lymphocytes (Drexler and Quentmeier, 2004). Working alongside other proteins, FLT-3L promotes differentiation, proliferation and development of various stem cells, myeloid, and lymphoid progenitor cells, specifically by binding to FLT-3 receptors (Peterlin et al., 2021).

The FLT-3 receptor, like other type III protein tyrosine kinase receptors, is made up of four regions consisting of an extracellular region responsible for ligand binding, a transmembrane region anchoring the receptor to the cell membrane, and an intracellular region including the juxtamembrane domain and two intracellular kinase domains interrupted by a kinase insert (Grafone et al., 2012; Kazi and Rönstrand, 2019a).

The extracellular region has five immunoglobulin-like domains (D1–D5). The D3 section can bind to dimerized ligands. The binding causes phosphorylation of juxtamembrane residues, which disturb the auto-inhibitory function of the kinase (Opatowsky et al., 2014) This leads to the phosphorylation of tyrosine residues in the intracellular domain, promoting the binding of additional proteins to the receptor and subsequent firing of downstream signalling pathways.

The intracellular region is organized into two main lobes: a smaller amino-terminal lobe and a larger carboxy-terminal lobe. The small lobe is made of five β -sheets in an antiparallel arrangement connected to an α C-helix. On the other hand, the large lobe is made up of multiple α -helices. Together, these lobes create a cleft-like structure that can function as an ATP-binding pocket, a site essential for catalytic activity (Huse and Kuriyan, 2002).

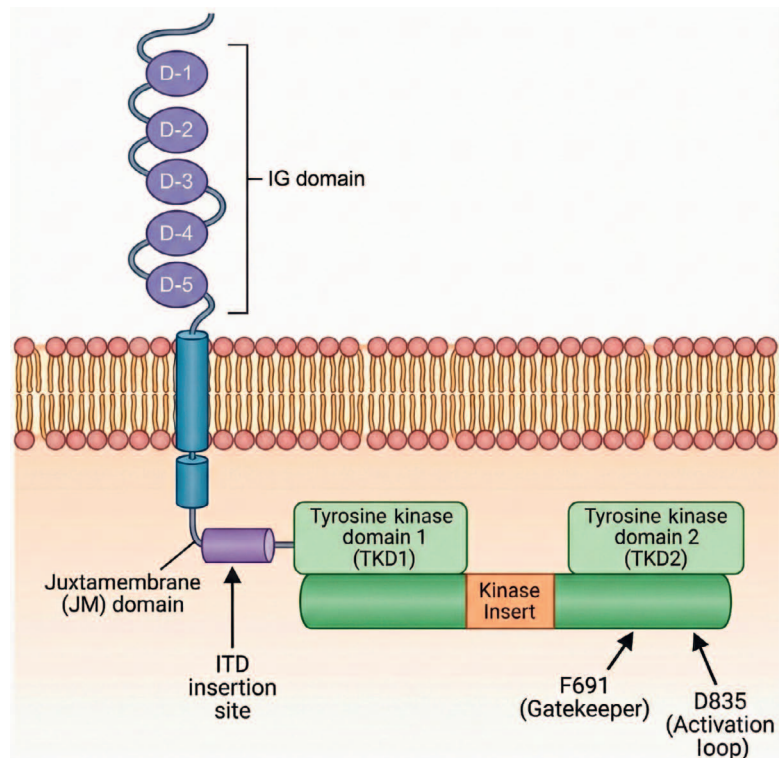


Fig. 2. Schematic representation of the FLT-3 receptor structure, showing the extracellular immunoglobulin-like (IG) domains (D-1 to D-5), the transmembrane region, and the intracellular juxtamembrane (JM) and tyrosine kinase domains (TKD1 and TKD2). The sites of common mutations, including the internal tandem duplication (ITD) insertion site and the F691 (gatekeeper) and D835 (activation loop) point mutations, are indicated. (Image generated using Google Gemini, 2025, gemini.google.com).

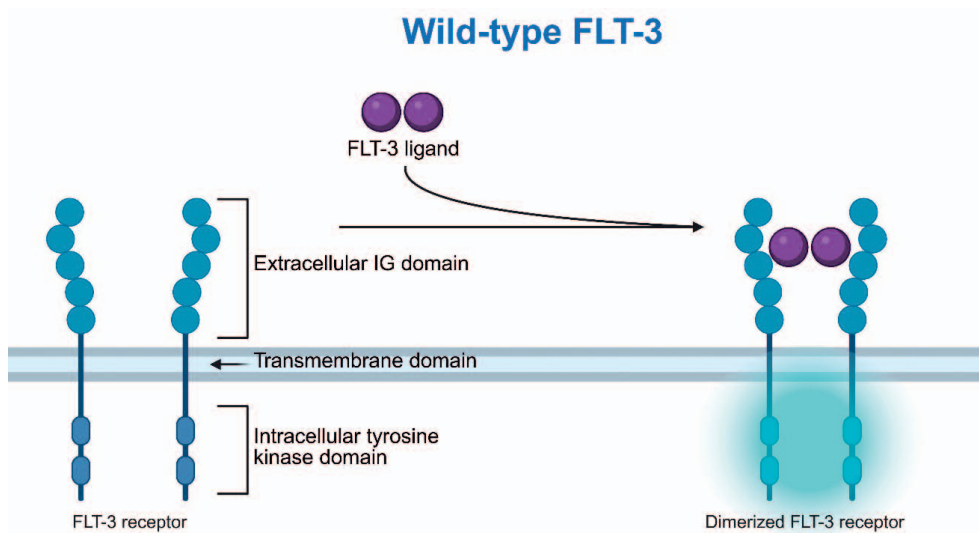


Fig. 3. Ligand-induced dimerization of wild-type FLT-3 receptor. Created with BioRender (2025); <https://BioRender.com/avjxh90>

The key structural features in the intracellular region contribute to FLT-3 functional adaptability. One of these features is the glycine-rich loop (GRL), which is located at the interface of the small lobe. It has an essential role in the binding of ATP, providing the necessary flexibility for both ATP binding and ADP release. This flexibility is

essential for enabling enzymatic activity, as the loop's conformation shifts during different stages of the catalytic cycle (Hanks and Hunter, 1995).

Another structural feature is the hinge region linking the small lobe to the large lobe, acting as a bridge. The hinge region supports the dynamic movement required

for the receptor's catalytic functions. Moreover, it helps position the ATP molecule within the binding pocket, aligning it for efficient phosphate transfer (Zhang et al., 2006).

A critical component of FLT-3 regulation is the activation loop (A-loop), a flexible segment within the kinase domain. This loop, which lies between the amino acid sequences of aspartate-phenylalanine-glycine (DFG) and alanine-proline-glutamate, plays an essential role in controlling the receptor's active and inactive conformations. In the active DFG-in state, the DFG aspartate residue is oriented toward the catalytic core, allowing access to magnesium ions necessary for ATP binding and catalysis. In contrast, during the DFG-out confirmation, the DFG motif reorients, moving the aspartate residue away from the active site, which leads to disruption of ATP binding and inhibits the kinase activity (Taylor and Kornev, 2011). The A-loop in the active state enables the binding of substrate proteins, facilitating downstream signalling. Within the A-loop, FLT-3 contains a tyrosine that can be phosphorylated, stabilizing the active form and enhancing the receptor's catalytic efficiency, although this phosphorylation is not strictly necessary for its activity.

The overall structural arrangement of the small and large lobes, along with the GRL, hinge, DFG sequence and A-loop, allows FLT-3 to adopt an active conformation essential for its signalling functions. Each domain within the kinase plays a defined yet interconnected role in maintaining the receptor's flexibility and stability, making it capable of responding precisely to external ligand signals and regulating downstream signalling cascades (Huse and Kuriyan, 2002).

Mutations in the *FLT3* gene can lead to structural anomalies in the FLT-3 receptor, primarily classified into two major subtypes: ITD and point mutations or deletions in TKD. Each mutation affects the structure of the receptor in the intracellular section and enables its activation through ligand-independent dimerization and phosphorylation (Kiyoi et al., 2020). The first of these mutations was discovered in 1996. It is presented by multiple repeated sequences limited to the juxtamembrane domain-coding sequence of the *FLT3* gene. As a result, this mutation renders the auto-inhibitory function of the receptor inactive and results in constitutive, ligand-independent activation of FLT-3 (Nakao et al., 1996; Griffith et al., 2004).

Thereafter, another mutation was observed at the D835 residue within the TKD coding sequence of the *FLT3* gene with an additional point mutation, deletion and insertion in the surrounding codons (Kiyoi et al., 2020). Later studies found that TKD mutations occur in the kinase domain of the FLT-3 receptor and specifically affect the activation loop. Mutations may result in conformational change and lead to increased signalling activity. The most common TKD mutation is at the D835 residue, with aspartic acid being replaced by another amino acid (e.g., D835Y, D835H); however, also other residues such as F691, I836, D839 and Y842 may be affected (Mohebbi et al., 2024).

Signalling pathways mediated by FLT-3

The presence of FLT-3 is indicative of its importance in haematopoiesis as it appears in early haematopoietic progenitors, but it is eventually down-regulated in more mature blood cells (Tsapogas et al., 2017).

The inactive receptor has a monomeric, unphosphorylated structure with dormant kinase moieties. Upon binding of FLT-3L, the receptor undergoes a conformational change intracellularly, allowing another FLT-3 receptor to bind to it and dimerize. After dimerization, the auto-inhibitory juxtamembrane domain is released from the kinase domain, allowing the kinase to access ATP. This change enables auto-phosphorylation of multiple tyrosine residues (Kazi and Rönnstrand, 2019b). The phosphorylated tyrosine residue provides an excellent docking site for a variety of signalling molecules, leading to their subsequent phosphorylation and activation of downstream signalling pathways (Kazi and Rönnstrand, 2019a). The activated FLT-3 receptor has a specialized docking site for adaptor proteins and signalling molecules, which contains SH2 or PTB domains (Wagner et al., 2013). Adaptor proteins such as GRB2 (Luttrell et al., 1996) and PTPN11 (Ruvolo, 2019) can both bind to the docking site and create signalling complexes with other proteins, e.g. GRB2 with SOS (Pierre et al., 2011).

One of the main pathways that is activated by FLT-3 is the Ras/Raf/MEK/ERK pathway (Moon and Ro, 2021). The triggering point of the cascade is SOS, an enzymatic factor essential for the conversion of inactive GDP-bound Ras to active GTP-bound Ras (Pierre et al., 2011). Activated Ras enables phosphorylation of Raf, which is followed by a series of downstream signalling phosphorylation steps and activation of kinases MEK and ERK. ERK can translocate to the nucleus to phosphorylate various transcription factors important for transcription of genes involved in the control of the cell cycle, differentiation, growth and cell senescence (Mendoza and Blenis, 2011; Degirmenci et al., 2020).

Additionally, FLT-3 can also activate the PI3K/AKT pathway. The signalling cascade starts with PI3K being activated by binding to GRB2 and brought into contact with the cell membrane, leading to phosphorylation of PIP2 present in its structure (Fruman et al., 2017). PIP2 is phosphorylated to PIP3, which binds and anchors AKT to the cell membrane to facilitate its activation by other kinases such as PDK1 (Alessi and Cohen, 1998; Fruman et al., 2017). AKT is cleaved from the cell membrane after activation and can phosphorylate a variety of other signalling molecules such as GSK3, BCL-2 and mTOR. These signalling molecules are responsible for physiological functions, including cell growth, metabolism, proliferation, protein synthesis and apoptosis (Arcaro and Guerreiro, 2007).

Furthermore, FLT-3 participates in the activation of the JAK/STAT pathway. The STAT protein binds to the phosphorylated tyrosine residue present on FLT-3 using its SH2 domain. Upon binding, JAK is recruited and forms a complex with FLT-3 molecules, enabling them

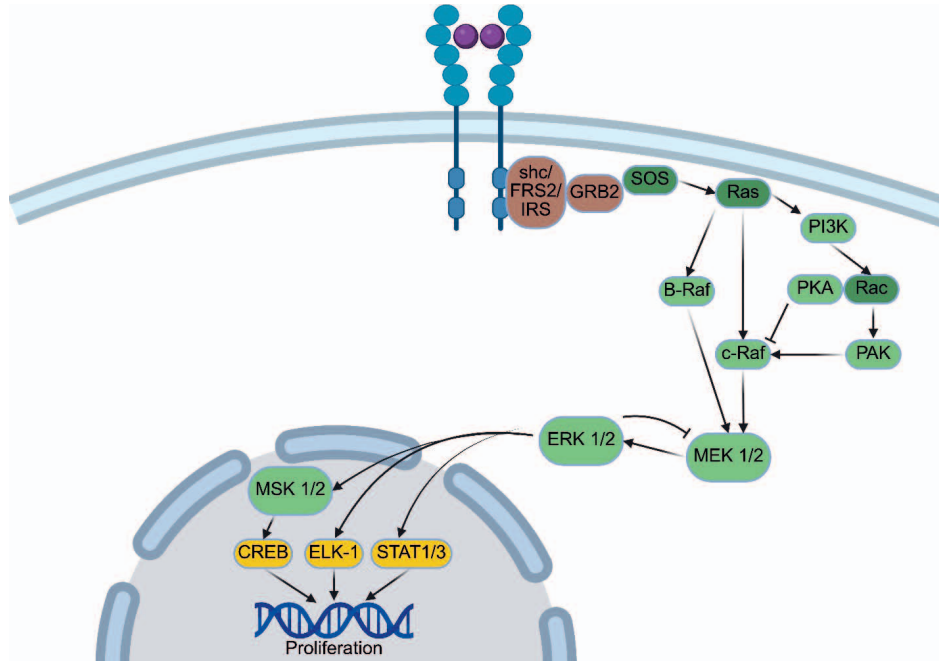


Fig. 4. Activation of the Ras/Raf/MEK/ERK pathway via FLT-3 receptor dimerization. Created with BioRender (2025); <https://BioRender.com/ytlk8y3>.

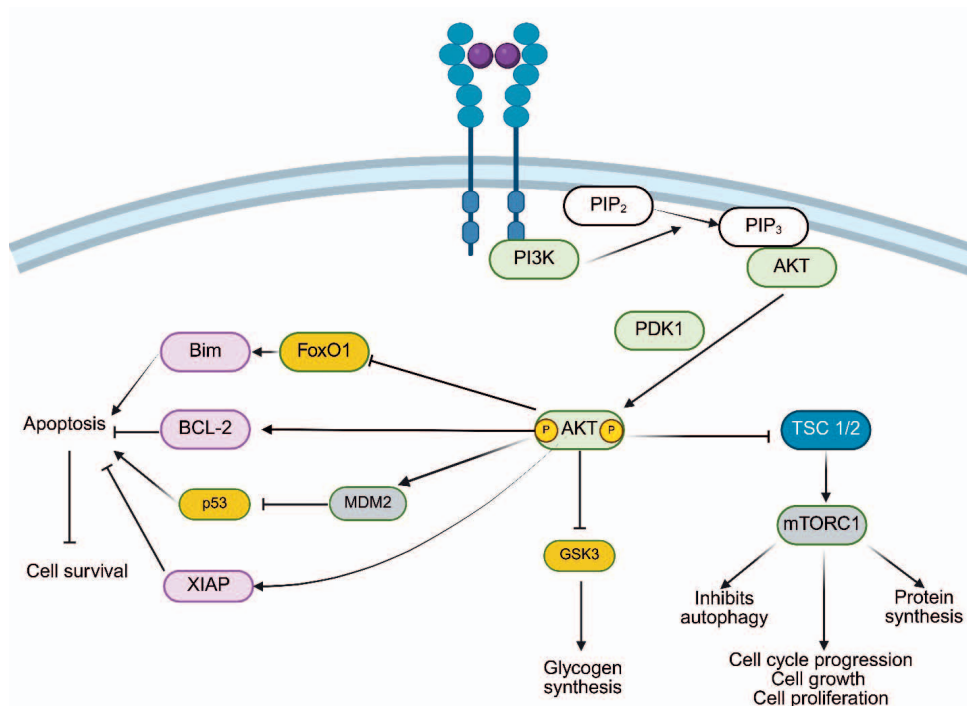


Fig. 5. FLT-3-mediated activation of the PI3K/AKT/mTOR pathway and its downstream effects on cells. Created with BioRender (2025); <https://BioRender.com/qwwqcoy>

to directly phosphorylate the STAT molecules attached to the tyrosine residues (Quintás-Cardama and Verstovsek, 2013). Phosphorylated STAT is released from the complex and dimerizes with other STAT proteins available in the membrane. The dimers move to the nucleus and attach to specific DNA sequences called STAT response ele-

ments, which up-regulate the target genes responsible for cell proliferation, survival, differentiation and immune responses (Schindler et al., 2007; Harrison, 2012).

Overall, FLT-3 is responsible for the activation and regulation of essential cellular functions such as cell proliferation, survival, differentiation and apoptosis using

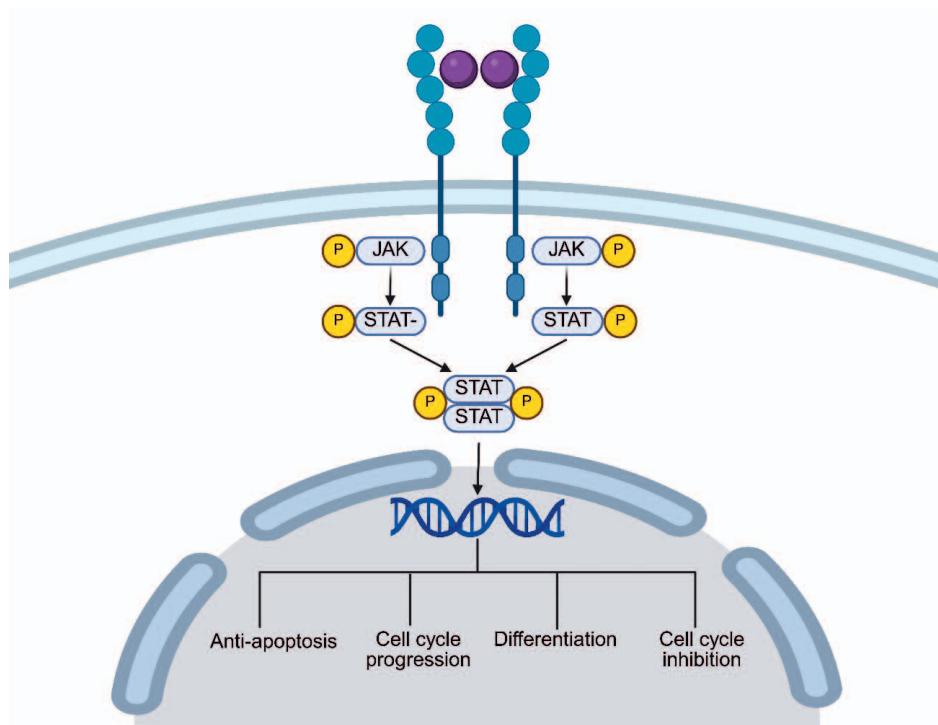


Fig. 6. FLT-3-mediated activation of the JAK/STAT pathway. Created with BioRender (2025); <https://BioRender.com/ap3uxb0>

various signalling pathways. This wide range of functions has made it an attractive target for cancer therapy development (Gutierrez-Camino et al., 2024).

Normal physiological roles of *FLT-3*

FLT-3, due to its ability to interact with various signalling pathways, plays a leading role in the physiological function of the cells such as HSC maintenance and self-renewal, homeostasis, tissue repair, cell differentiation and regulation of immune system (Gilliland and Griffin, 2002; Takahashi, 2011).

HSCs are responsible for providing the body with various blood cell types, each of which must be in the right amount and highly differentiated to meet the extensive demands of the human body. This equilibrium is achieved via FLT-3 signalling. When a ligand of FLT-3 binds to the receptor present on the cell membrane of HSCs, it activates the above-mentioned Ras/MAPK/ERK and PI3K/AKT pathways (Levis and Small, 2003). Cell functions such as cell survival, proliferation and self-renewal are regulated by these pathways, allowing haematopoietic homeostasis to be achieved. FLT-3 signalling maintains a balance between the number of dormant HSCs and proliferated HSCs, making sure that a continuous supply of an undifferentiated pool of HSCs is available while supplying the right number of differentiated cells to meet the organism's demand (Gilliland and Griffin, 2002).

FLT-3 not only regulates the number of differentiated cells but can also determine the differentiation of haematopoietic progenitor cells into various lineages such as

megakaryocytes, erythrocytes, neutrophils, eosinophils, basophils, dendritic cells and macrophages.

FLT-3-dependent JAK/STAT activation can target genes crucial for B-cell and T-cell development (Gilliland and Griffin, 2002; Zheng et al., 2004). In the case of dendritic cells, the PI3K/AKT and JAK/STAT pathways are activated to regulate their maturation and improve their antigen-capturing/antigen-presenting abilities, which is crucial for the proper immune response (Wodnar-Filipowicz, 2003).

Dysregulation of *FLT-3* in disease states

As previously mentioned, FLT-3 activation is ligand dependent. Upon binding of the ligand (whether soluble or membrane-bound) to FLT-3 receptors, the receptor tyrosine kinase is activated, which in turn initiates downstream signalling pathways. However, when FLT-3 is mutated (FLT-3 ITD or TKD), the receptor activation becomes ligand independent and permanent. This constant activation leads to persistent activity of signalling pathways, including JAK/STAT, Ras/MAPK/ERK and PI3K/AKT (Levis and Small, 2003; Zheng et al., 2004).

As described in previous paragraphs, all these pathways are involved in the inhibition of pro-apoptotic factors, promotion of cell survival, proliferation and differentiation.

Pathological activation of these signalling pathways results in cells becoming immortal and less differentiated, contributing to an abnormal number of undifferentiated cancer cells (Levis and Small, 2003; Stirewalt and Radich, 2003).

Targeting FLT-3 for AML Therapy

Conventional therapy for AML

Typical AML therapy begins with conventional standard induction therapy referred to as the “7+3” protocol. This involves continuous administration of cytarabine for seven days via intravenous infusion joined by anthracyclines, such as daunorubicin or doxorubicin, for the first three days. The combination of these chemotherapy agents is often associated with toxicities such as cytopenia, infections, and organ dysfunction and require hospitalization. In the case of successful treatment, the patient is required to undergo post-remission therapy, which includes consolidation with high-dose cytarabine or allogeneic stem cell transplantation. This is necessary due to the high chances of relapse (Döhner et al, 2022).

In CD33-positive patients, the combination of “7+3” therapy with gemtuzumab ozogamicin (GO) is an approved treatment strategy. GO is an antibody-drug conjugate which targets the transmembrane CD33 protein expressed on AML cells. Calicheamicin is the active component of the GO conjugate, capable of causing single- or double-strand DNA breaks. The DNA damage causes apoptosis by activation of ATM and ATR kinase pathways. For patients with relapsed or refractory (R/R) CD33⁺ AML, GO can serve as monotherapy (Swaminathan and Cortes, 2023).

FLT-3 inhibitors

The FLT-3 inhibitors are categorized either by type or by generation. These categorization systems help to determine better treatment and overcome challenges in

treating AML. The types of FLT-3 inhibitors are typically divided into type I and type II. The main difference between the type I and type II inhibitors is the way they bind to the FLT-3 receptors (Kiyoi et al., 2020).

FLT-3 receptors exist in two primary conformations defined by the position of the conserved DFG motif (Asp-Phe-Gly). When FLT-3 is activated, the DFG flips “inward” (DFG-in) with aspartate positioned to coordinate the binding of ATP to magnesium, while phenylalanine is tucked into the hydrophobic region and glycine becomes flexible for the loop opening. This conformation change enables substrate access and subsequent firing of the signalling pathway (Taylor and Kornev, 2011).

When inactive, the DFG motif flips “outward” (DFG-out) with phenylalanine extended away from the pocket, distorting the ATP binding site and blocking substrate binding. Type I inhibitors bind to the ATP-binding site and its vicinity and subsequently bind with molecules in both DFG-in and DFG-out conformations. In contrast, type II inhibitors require the DFG-out conformation. FLT3-TKD mutations preferentially stabilize the active DFG-in conformation, whereas FLT3-ITD allow the receptor to adopt both active and inactive conformations.

Consequently, type I inhibitors are active against both FLT3-TKD and FLT3-ITD, while type II inhibitors primarily inhibit FLT3-ITD and are ineffective against FLT3-TKD mutations that stabilize the active conformation and prevent transition to the inactive state (Eguchi et al., 2020; Alotaibi et al., 2021).

There are two generations of FLT-3 inhibitors. The first generation are multi-kinase inhibitors, meaning they target multiple kinases in addition to FLT-3. Usually, the earlier discovered medication belongs to this generation,

Table 1. Characteristics and activity profiles of common FLT-3 inhibitors in AML. This table summarizes the generation, binding type, regulatory approval status and efficacy against FLT3-ITD, and common resistance mutations (D835, F691L) for clinically relevant inhibitors.

Inhibitor	Generation/ Selectivity	Type (I/II)	Current approval (FDA/EMA)	Activity vs FLT-3 ITD	Activity vs D835 TKD	Activity vs F691L gatekeeper	References
Midostaurin	First generation, multikinase	I	FDA and EMA approved for newly diagnosed FLT-3-mutated AML	Active	Active, but less potent than newer type I inhibitors	Limited; F691L can mediate resistance	(Kennedy and Smith, 2020; Ge et al., 2022)
Gilteritinib	Second generation, relatively selective	I	FDA approved for R/R FLT-3-mutated AML; EMA approved	Active	Active against most D835 mutations	Reduced activity; F691L is a known resistance mutation	(Kennedy and Smith, 2020; Ge et al., 2022; Kiyoi et al., 2020)
Quizartinib	Second generation, FLT-3 selective	II	FDA and EMA approved for newly diagnosed FLT-3 ITD+ AML in combination therapy	Strong activity; designed for FLT-3 ITD	Poor/limited activity; D835 commonly causes resistance	Poor activity; F691L is a major resistance mechanism	(Ge et al., 2022; Negotei et al., 2023; Lin et al., 2024)
Crenolanib	Second generation, FLT-3 selective	I	Not yet FDA/EMA approved; in advanced clinical trials	Active	Active against D835 and other activation loop mutations	Activity reduced; F691L confers resistance, though less than with type II TKIs	(Kennedy and Smith, 2020; Ge et al., 2022)
Sorafenib	First generation, multikinase	II	Not approved specifically for AML (approved for solid tumours); used off label in FLT-3-mutated AML, including post-transplant	Active, especially against FLT-3 ITD	Limited; D835 often confers resistance	Poor activity; F691L markedly reduces sensitivity	(Kiyoi and Naoe, 2019; Kennedy and Smith, 2020; Ge et al., 2022)

which has more side effects due to off-target inhibitions (Ge et al., 2022). Conversely, the second generation of FLT-3 inhibitors are more specific and potent FLT-3 inhibitors. They have fewer side effects because they target FLT-3 more selectively (Larrosa-Garcia and Baer, 2017).

First generation of FLT-3 inhibitors

The first generation of drugs used for the treatment of FLT-3 varied in their ability to target solely the FLT-3, resulting in poor tolerability and efficacy. One of the first drugs to be studied for FLT-3 inhibition was lestaurtinib. It not only had activity against FLT-3 but also against JAK2 and Trk. Trials were conducted, but no significant response rates or survival were observed. Severe haematological and gastrointestinal side effects forced discontinuation of the drug in the treatment of FLT-3-mutated AML (Negotei et al., 2023).

Sorafenib emerged as a promising drug targeting multiple kinases, including c-KIT, platelet-derived growth factor receptor (PDGFR) and vascular endothelial growth factor receptor (VEGFR). However, as a type II inhibitor, it exhibited limited activity against FLT-3 mutations, such as FLT-3-D835, which restricted its efficacy. Consequently, sorafenib demonstrated improved outcomes when used in combination with another cytotoxic drug, such as a hypomethylating agent, rather than as monotherapy (Negotei et al., 2023).

Midostaurin was the first TKI of its generation capable of inhibiting both ITD and TKD mutations, with broad activity against VEGFR, protein kinase C, c-KIT and PDGFR- β , typical of type I inhibitors. The inhibitory effect disrupts receptor signalling, halts cell proliferation and induces apoptosis in AML cells with ITD and TKD mutations. Additionally, the drug is effective against cells over-expressing the wild-type forms of FLT-3 or PDGFR (Larson et al., 2021). Due to its qualities, midostaurin was approved by the Food and Drug Administration (FDA) (April 28, 2017) and European Medicines Agency (EMA) (September 18, 2017) as the first agent specifically targeting FLT-3 mutations (Larson et al., 2021; Negotei et al., 2023). It is approved only for AML patients who are FLT-3 mutation positive, and it is used in combination with “7+3” induction therapy; otherwise, it is not recommended as monotherapy. Like other TKIs, it is available in oral dosage form, which increases patient compliance (Levis, 2017; Stone et al., 2017; Wu et al., 2018).

Second generation of FLT-3 inhibitors

Gilteritinib is the second-generation FLT-3 inhibitor that not only targets FLT-3 but also AXL, an oncogenic tyrosine kinase, which is a well-known enzyme involved in instigating resistance against FLT-3 inhibitors. During clinical trials, gilteritinib was able to demonstrate better outcomes compared to conventional therapy in terms of median overall survival, event-free survival and complete remission percentage (Kayser and Levis, 2022; Negotei et al., 2023). Gilteritinib demonstrated better long-term survival in patients with positive FLT-3 ITD and those

with high FLT-3 ITD allelic ratio, a ratio reflecting the proportion of mutated to wild-type (WT) *FLT3* alleles, which is associated with higher disease burden and poor prognosis. Unfortunately, this effect was not observed in patients with the FLT-3 TKD subclass or patients with a low FLT-3 ITD allelic ratio (Negotei et al., 2023).

In 2020, gilteritinib received marketing authorization under the brand name of Xospata in the Czech Republic. Xospata is indicated as monotherapy for the treatment of adult patients with R/R AML who have FLT-3 mutation and is not recommended for use in newly diagnosed AML, AML without FLT-3 mutation, or as maintenance or consolidation therapy in the standard first-line setting.

Quizartinib is a second-generation TKI that was specifically designed to be used for AML patients with FLT-3 mutation. During the phase I trial, quizartinib demonstrated clinical activity against AML irrespective of the mutation status of the patient. However, patients with FLT-3 ITD status had a 53 % increase in overall response rate compared to 14 % of AML patients with WT FLT-3 (Mohebbi et al., 2024). Later phase II and III trials confirmed the efficacy of quizartinib as monotherapy against R/R FLT-3 ITD. In 2023, quizartinib received marketing authorization in the Czech Republic under the brand name Vanflyta. It is indicated for the treatment of AML patients who have a FLT-3 ITD mutation. Vanflyta is used in combination with standard “7+3” induction therapy, continued by cytarabine-based consolidation, and subsequently as maintenance monotherapy after consolidation chemotherapy.

Mechanism of resistance to FLT-3 inhibitors

Despite significant improvement in the selectivity of FLT-3 inhibitors that have increased potency and specificity while having fewer side effects, resistance remains a formidable challenge in the treatment of AML patients. The resistance can arise by different mechanisms and is characterized by two categories, primary (innate) and secondary (acquired).

Primary or innate resistance refers to a mechanism that is present prior to any exposure to FLT-3 TKIs. The innate resistance may arise from the protective role of the bone marrow microenvironment, which allows leukaemic blasts to evade the effects of the inhibitor. It is evident that while peripheral leukaemic blasts often respond well to FLT-3 inhibitors, their counterparts in the bone marrow are more resistant. This inconsistency may be partially attributed to the limited penetration of the inhibitors into the bone marrow environment and the presence of elevated levels of the CYP3A4 enzyme, which metabolizes FLT-3 inhibitors and decreases their efficacy (Alonso et al., 2015; Mohebbi et al., 2024).

Moreover, the bone marrow microenvironment can activate alternative pro-survival signalling pathways, enabling leukaemic cells to bypass FLT-3 inhibition. For example, in the case of resistance to quizartinib, the leukaemic cells demonstrated the ability to up-regulate fibroblast growth factor 2, which can activate fibroblast growth factor receptor 1, resulting in triggering the

MAPK pathway independently of FLT-3 (Mohebbi et al., 2024; Ruglioni et al., 2024).

Furthermore, most cases of FLT-3-mutated AML also express WT FLT-3, which, while sensitive to FLT-3L, shows relative resistance to FLT-3 inhibitors. This resistance is exacerbated during induction and consolidation therapy, when the bone marrow microenvironment experiences high levels of FLT-3L (Sato et al., 2011; Mohebbi et al., 2024).

The binding of FLT-3L to WT FLT-3 receptors can maintain the activity of signalling pathways such as FLT-3/MAPK, providing survival signals to leukaemic blasts despite the presence of FLT-3 inhibitors (Mohebbi et al., 2024; Ruglioni et al., 2024).

Older generations of TKIs, such as midostaurin and gilteritinib, are unable to bypass these mechanisms of resistance, leading to many relapsed patients. However, new agents such as quizartinib can target mutated FLT-3 in both the bone marrow microenvironment and the periphery, offering much better minimal residual disease (MRD) outcomes compared to previous therapeutic agents (Yang et al., 2014; Mohebbi et al., 2024).

Secondary resistance, on the other hand, involves acquired resistance that renders FLT-3 TKI ineffective. This can occur through secondary mutations in FLT-3, especially in the kinase domain (Eguchi et al., 2020; Mohebbi et al., 2024). The frequently targeted residues include F691, D835, I836, D839 and Y842.

Type I inhibitors, e.g. gilteritinib and crenolanib, remain active against some KD mutations, such as D835. However, prolonged use of these agents as monotherapy may lead to mutations at the F691 residue, which often leads to relapse (Ruglioni et al., 2024). Type II FLT-3 inhibitors interact poorly with most KD mutations and are vulnerable to resistance mechanisms (Mohebbi et al., 2024). However, some mutations, such as F691, confer resistance to both type mutations and are difficult to treat.

Importantly, clonal selection plays a significant role in both primary and secondary resistance. Pre-existing subclones harbouring mutations that can resist FLT-3 inhibitors may be present at low frequency even before beginning the treatment. They can rapidly expand under the selective pressure of FLT-3 inhibitor therapy and become the dominant cells and replace the FLT-3 TKI-sensitive cells in the clonal population (Eguchi et al., 2020; Mohebbi et al., 2024).

Overcoming resistance mechanisms

Overcoming resistance to FLT-3 TKIs has become a serious therapeutic challenge in recent years. The slow discovery of novel TKIs and the lack of alternative treatment have made it difficult to treat patients showing resistance. Addressing FLT-3 TKI resistance requires a multifaceted approach, one that includes understanding the underlying mechanism and developing novel drugs according to the strategic need (Desikan et al., 2022).

One strategy to overcome resistance is by simultaneous use of multiple drugs that not only inhibit the FLT-3 signalling pathway but also other pathways that can act

as compensatory or replacement such as PI3K/AKT and MAPK/ERK pathways. The combination therapy approach increases the anti-leukaemic activity of the treatment and mitigates the resistance development (Takahashi, 2023; Yang and Friedman, 2023).

Furthermore, there is strong interest in developing novel FLT-3 inhibitors that are more specific and potent. In July 2023, quizartinib was approved by FDA due to showing strong clinical potency compared to first-generation inhibitors such as midostaurin. By offering a new therapy option, the next generation of FLT-3 inhibitors offers hope to patients suffering from R/R AML (Erba et al., 2023).

One of the recent advancements in the treatment of R/R FLT-3-mutated AML involves combining FLT-3 TKI such as gilteritinib or quizartinib with venetoclax, a BCL-2 inhibitor. It is a beneficial combination because of the ability of FLT-3 ITD signalling to up-regulate anti-apoptotic proteins such as BCL-2 and MCL-1 to resist the effect of TKI therapy. Venetoclax monotherapy is similarly limited by adaptive resistance mechanisms involving activation of MAPK and PI3K signalling pathways, leading to increased MCL-1 expression. When combined, a synergistic effect is observed with TKI enhancing activation of BCL-2-interacting mediator of cell death (BIM), mitochondrial outer-membrane permeabilization and apoptosis induction, while venetoclax resensitizes leukaemic blasts resistant to TKI by inhibiting or neutralizing BCL-2 protection (Singh Mali et al., 2021; Zhu et al., 2021).

Clinical trials further validate this approach, particularly in triplet therapy combining hypomethylating agents, venetoclax and TKI together. A phase I/II study of azacitidine + venetoclax + gilteritinib (80 mg daily) showed for unfit FLT-3-mutated AML patients (median age 71) complete remission or complete remission with incomplete haematological recovery (CR/CRi) of 96 %, with 65 % achieving deep measurable MRD negativity and 18 months of relapse-free and overall survival rates of 71 % and 72 %, respectively (Short et al., 2024). A cohort of R/R FLT-3-mutated AML also showed promising results. In another recent similar phase I/II study of quizartinib + venetoclax + decitabine combination, a cohort of heavily pre-treated R/R FLT3-ITD AML showed high response rate to the treatment. Collectively, these results support triplet therapy as a promising strategy to deepen remissions and potentially bridge selected high-risk patients to transplant (Yilmaz et al., 2024).

In high-risk FLT-3-mutated cases, the use of allogeneic haematopoietic stem cell transplantation remains the cornerstone curative strategy despite the high relapse rate of 50 % in many cohorts. Relapse driven by persistent MRD and leukaemic clone re-emergence accounts for 80 % of post-transplant failure. This fact underscores the need for suppressing the residual FLT-3-mutated blasts during the vulnerable post-engraftment phase. This can be achieved by the use of FLT-3 inhibitors as post-transplant maintenance therapy. Maintenance with gilteritinib or sorafenib significantly reduces relapse incidence and

improves relapse-free survival, and trends towards better overall survival (Levis et al., 2024).

Another approach is to combine the use of TKIs with immunotherapy. Although this approach is relatively new, it has shown strong potential. By using this combination of therapies not only does the TKI inhibit leukaemogenesis, but the anti-leukaemic power of the immune system is harnessed, which simultaneously helps to eradicate any remaining cells that might show resistance. Combining checkpoint inhibitors targeting proteins such as programmed cell death protein 1 (PD-1) and chimeric antigen receptor (CAR) T-cell therapy has shown effectiveness in combination therapy (Ling et al., 2018; Petrazzuolo et al., 2022).

For better adoption of immunotherapy in AML treatment, it is essential to interpret and integrate complex genetic data, which requires a high level of multidisciplinary collaboration and infrastructure support. Also, the cost effectiveness and insurance reimbursement issues have furthered delayed the adoption procedure (Whiteside et al., 2016).

Current State of the Art in FLT-3 Targeted Therapy

Clinical trials

Currently, two active clinical trials are focusing on treating AML by inhibiting tyrosine kinase receptors using novel compounds. Both trials were identified through ClinicalTrials.gov and were selected for inclusion based on their focus on targeting tyrosine kinase receptors in AML patients and their status as active or recruiting trials.

The first trial (NCT04669067) is in phase Ib/II, evaluating the safety and efficacy of TL-895, which is a highly selective irreversible TKI, in combination with navtemadlin (KRT-232), a novel MDM2 inhibitor, for the treatment of adults with R/R FLT3-mutated AML. This combination aims to arrest the abnormal cell growth using TL-895, while navtemadlin promotes apoptosis by inhibiting MDM2, which suppresses the p53 protein. Eligible participants of the trial must have TP53 wild-type AML, be R/R to at least one prior therapy (including an FLT-3 inhibitor) and have adequate organ functions (Telios Pharma, Inc., 2023). The trial aims to provide additional treatment options to patients with FLT-3-mutated AML who have failed prior therapies. The study is expected to be completed by the end of 2025.

The second trial (NCT05947344) is currently in phase I evaluating the safety, tolerability, pharmacokinetics (PK) and efficacy of STI-8591. STI-8591 is a new TKI that has demonstrated excellent anti-leukaemic activity in FLT-3-mutated AML with drug resistance. The anti-leukaemic properties of STI-8591 are suspected to arrive from its ability to inhibit phosphorylation of mutated FLT-3 protein and deactivate its downstream targets. The study is estimated to finish by December of 2025 (Zhejiang ACEA Pharmaceutical Co. Ltd., 2025).

Personalized and targeted medicine approaches

In the past decades, the field of oncology has experienced rapid changes due to an increasing number of drugs available to treat various cancers, leading to increases in patient overall survival. The emergence of new genomic analysis technologies has deepened our understanding of cancer genomics and biology, which has enabled the use of personalized medicine, and extensive genetic profiling enabled oncologists to identify molecular anomalies and recognize specific mutations and selection of personalized therapies according to the patients' distinctive genetic profiling and need (Sargas et al., 2023). This is particularly desirable in malignancies with high variability such as AML (Testa et al., 2021; Grauman et al., 2023).

Traditionally, the treatment of AML was guided by factors such as age, level of physical functioning and chromosomal abnormalities. Nevertheless, this generalized approach often resulted in poor or variable outcomes, and in some cases, it caused significant toxicity to the patient. One notable advancement in personalized AML therapy is the development of agents specifically designed to target signalling pathways associated with leukaemogenesis. For example, mutations in genes mentioned above, e.g., *FLT3*, *IDH1* and *IDH2*, are common in AML and present poor responses to conventional treatment. However, they provide an attractive target for personalized treatment, such as the use of midostaurin and midoitinib to inhibit FLT-3 receptors, or ivosidenib and enasidenib targeting *IDH1/2* mutations. The use of these agents in patients with these specific mutations has remarkably improved efficacy and has led to improved outcomes and survivability (Roloff and Griffiths, 2018; Testa et al., 2021).

Furthermore, the concept of personalized medicine has expanded to include patients unfit for treatment using intensive chemotherapy. As detailed in section Overcoming resistance mechanisms, the use of triplet regimens represents a tailored therapeutic strategy that delivers high efficacy while considering the specific therapeutic needs of this patient population.

Although the use of small molecule inhibitors and the emerging triplet regimens have improved the management of many AML patients, the outcome remains poor for patients with R/R AML. Despite these advancements, resistance to targeted therapy frequently occurs, leaving patients with limited options other than conventional therapeutics such as high-dose cytarabine (Zeidner et al., 2021). The advancement in immunotherapy has presented clinicians with new avenues for personalized AML treatment. Immune checkpoint inhibitors such as pembrolizumab (Kwok et al., 2016) and nivolumab (Fulchiero and Jimeno, 2014) are used effectively to unleash the patient's immune system by inhibiting PD-1, which is elevated in AML, and enabling the evasion of leukaemic cells from the immune system (Zeidner et al., 2021).

The use of targeted protein degradation methods such as proteolysis-targeted chimaeras (PROTACS) has opened new possibilities in targeted therapy of FLT-3-

mutated AML. Unlike traditional inhibitors that block the protein function, PROTACS facilitates complete degradation of the targeted protein. This is utilized by leveraging the E3 ubiquitin-proteasome system (Hesham et al., 2024). With targeted FLT-3 degradation, all the signalling pathways related to it are shut down and AML loses its growth advantage and can undergo apoptosis or become more susceptible to conventional therapies (Hesham et al., 2024).

Additionally, another potentially innovative treatment of R/R AML is the use of CAR T-cell therapy. This treatment involves induction of the patient's T cells to recognize antigens present in leukaemic cells, leading to an immune response. Although the response to this treatment has been modest, the need for novel treatment for patients refractory to TKI- and venetoclax-based regimens has made it viable. The challenges facing the implementation of this treatment are the heterogeneous nature of the disease biology, the absence of a singular targetable antigen, and immune fatigue (Stern and Stern, 2021).

Moreover, introduction of screening methods such as MRD monitoring into the treatment has further helped with personalization of AML therapies. MRD refers to the small number of leukaemic cells that remain inside the body even after successful treatment, virtually undetectable with conventional screening methods. Techniques such as next-generation sequencing and multi-parameter flow cytometry allow clinicians to detect the smallest amount of MRD in the patient's body and enable them to use tailor-made treatment intensity according to the individual response to a drug. This approach allows identification of patients with a higher risk of relapse and facilitates timely intervention with targeted therapies, resulting in better treatment outcomes (Heuser et al., 2021).

The push towards personalizing the treatment of AML has advanced a long way, especially in the past decade, but there are still significant challenges ahead. One major challenge lies in expanding the novel drugs available to be used beyond the commonly mutated gene. Many patients suffer from mutations that are poorly understood, and available medications are unable to specifically target them (Döhner et al., 2022). Furthermore, the rapid development of resistance to current therapies has been a major concern that needs to be tackled by development of novel combination therapy strategies (Scholl et al., 2020). Despite all the challenges, personalized medicine offers an unparalleled opportunity to transform treatment and improve the patients' outcomes.

Conclusion

In conclusion, the FLT-3 mutation has emerged as an important therapeutic target in the treatment of AML. The inclusion of FLT-3 inhibitors in the therapeutic regimen has drastically improved the patients' outcomes, especially patients with FLT-3 ITD mutations. Nevertheless, resistance to monotherapy remains a clinical challenge. Understanding the molecular profile and structure of

FLT-3 receptor has helped to develop the next generation of inhibitors that are currently being tested. These new potential inhibitors offer promising strategies to overcome resistance and improve the patients' survival.

Ethical guidelines statement

This review article does not involve any original research with human participants or animals. However, all referenced studies involving human or animal subjects were conducted in accordance with the ethical standards of the responsible institutional and/or national research committees, and in compliance with relevant EU and international law and guidelines.

Conflict of interest

All authors declare that they have no conflicts of interest related to this work.

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