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REVIEW

Current knowledge about FLT3 gene mutations, exploring the isoforms, and protein importance in AML

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Abstract
Acute myeloid leukaemia (AML) is a complex haematological malignancy characterised by diverse genetic alterations leading to abnormal proliferation of myeloid precursor cells. One of the most significant genetic alterations in AML involves mutations in the FLT3 gene, which plays a critical role in haematopoiesis and haematopoietic homeostasis. This review explores the current understanding of FLT3 gene mutations and isoforms and the importance of the FLT3 protein in AML. FLT3 mutations, including internal tandem duplications (FLT3-ITD) and point mutations in the tyrosine kinase domain (FLT3-TKD), occur in 25–30% in AML and are associated with poor prognosis. FLT3-ITD mutations lead to constitutive activation of the FLT3 signalling pathway, promoting cell survival and proliferation. FLT3-TKD mutations affect the tyrosine kinase domain and affect AML prognosis in various ways. Furthermore, FLT3 isoforms, including shorter variants, contribute to the complexity of FLT3 biology. Additionally, nonpathological polymorphisms in FLT3 are being explored for their potential impact on AML prognosis and treatment response. This review also discusses the development of molecular treatments targeting FLT3, including first-generation and next-generation tyrosine kinase inhibitors, highlighting the challenges of resistance that often arise during therapy. The final chapter describes FLT3 protein domain rearrangements and their relevance to AML pathogenesis.

Keywords Acute myeloid leukaemia · Gene isoforms · Mutations · FLT3

Introduction and characterization of AML and its genetic landscape

One of the most severe haematological malignancies is acute myeloid leukaemia (AML), a heterogeneous clonal disorder characterised by the rapid proliferation of abnormal myeloid precursor cells in the bone marrow [1]. The development of this disease starts with accumulation of different classes of DNA mutations first in haematopoietic stem cells to form preleukaemic cells that eventually progress to AML [2]. AML can affect people of all ages but is more common in older adults. Fifty years ago, this disease was incurable but is now cured in 35 to 45% of adult patients. Patients aged 60 to 75 years have an enrichment of adverse karyotypic/molecular profiles and poor outcomes, with a median survival of ~2 years [3, 4]. On the other hand, younger patients with AML who are otherwise healthy and have favourable genetic profiles may have a greater chance of achieving complete remission with intensive chemotherapy. With a good response to treatment, 50 to 80% or more of patients may have relatively high five-year survival rates [5–7].

The new 5th WHO classification (starting in 2022) divides AML patients according to genetic abnormalities and defines AML by the WHO differentiation. The mutations in AML can be divided into groups according to the functional class of the mutated genes: (1) signalling and kinase pathway, (2) epigenetic modifiers, (3) nucleophosmin, (4) transcription factors, (5) tumour suppressors, (6) spliceosome complex and (7) cohesin complex [8, 9]. The

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identification and classification of mutations according to the present mutation status have essential impacts on patient prognosis and treatment choice. In the last decade, there has been an intensified focus on the fms-like tyrosine kinase 3 (FLT3) gene because its mutation seems to be crucial for AML, and approximately 30% of AML patients harbour some form of FLT3 mutation [10–12].

The main therapeutic approach used for approximately forty years is a combination of anthracycline and cytarabine. The treatment utilised a protocol known as "3 + 7," in which patients received a combination of anthracyclines for three days and cytarabine for seven days. Most recently, there is a new promise for patients in specific treatment in the form of inhibitors or cell therapy [13]. FLT3 mutations in AML have led to the development of numerous tyrosine kinase inhibitors (TKIs). The most effective FLT3 inhibitors include midostaurin, gilteritinib, crenolanib, sorafenib and quizartinib. However, patients often develop resistance, resulting in short and unsustainable responses [14].

Generally, mutations in AML can affect genes involved in signalling pathways, transcriptional regulation, epigenetic modifications, and cell cycle control. The identification of recurrent genetic alterations has allowed for the classification of AML into distinct subgroups, each with unique clinical and prognostic implications. Moreover, cytogenetic abnormalities, such as chromosomal translocations and rearrangements, are common in AML. These include the translocations t(8;21), inv(16)/t(16;16), and t(15;17), which result in fusion genes involving RUNX1-RUNX1T1, CBFB-MYH11, and PML-RARA, respectively [15]. These fusion genes disrupt normal gene expression and contribute to leukaemogenesis. Other recurrent genetic alterations in AML include mutations in genes such as NPM1, DNMT3A, IDH1/2, TET2, RUNX1, and TP53. Furthermore, recent research has shown the increasing importance of mutations in genes involved in growth signalling pathways (NRAS, KRAS, and PTPN11) [16]. These mutations affect various cellular processes, including DNA methylation, chromatin remodelling, and transcriptional regulation. These mutations contribute to the dysregulation of haematopoietic stem and progenitor cells, leading to the development of AML. As mentioned above, one of the most common genetic alterations in AML involves the FLT3 gene.

The role of FLT3 in AML

FLT3 plays a critical role in haematopoiesis, and the crucial importance of FLT3 is the regulation of haematopoietic stem and progenitor cells and maintenance of haematopoietic homeostasis. FLT3 is expressed in haematopoietic stem cells and in granulocyte/macrophage progenitor cells [17]. Experimental data suggest that FLT3 may also act on early lymphoid precursors, causing their development and thereby promoting the development of various immune cells, such as T cells, B cells, and natural killer (NK) cells; however, the exact mechanism is not known [18, 19]. FLT3 expression on leukaemic blasts can indicate the presence of FLT3 in most patients with AML [20]. Despite the major importance of FLT3 alterations in association with AML, they can also be detected in other diseases, as approximately 5% of acute lymphoblastic leukaemia (ALL) patients and few chronic lymphoblastic leukaemia (CLL) patients also exhibit FLT3 mutations [21, 22].

FLT3 mutations, particularly internal tandem duplications (FLT3-ITD) and point mutations in the tyrosine kinase domain (FLT3-TKD), are game changers in AML, as they are both associated with a poor prognosis. FLT3 mutations result in constitutive activation of the FLT3 signalling pathway, promoting cell survival and proliferation.

FLT3 gene structure and function

FLT3, also known as CD135, was identified 30 years ago, and its location is on chromosome 13q12.2. It belongs to the class III receptor tyrosine kinase (RTK) family and is a member of the RTK family together with the KIT, FMS, and PDGF receptors. All these proteins share structural characteristics, such as 5 immunoglobulin-like domains in the extracellular region, a juxtamembrane (JM) domain, 2 kinase domains (TK1 and TK2) separated by a kinase insert (KI) domain, and a C-terminal domain in the intracellular region – Fig. 1A [12, 23, 24].

In 1993, the full-length human FLT3 gene was successfully cloned from two different libraries: one from a pre-B-cell library [25] and the other from a CD34 + haematopoietic stem cell-enriched library [26]. The human FLT3 gene consists of 24 exons with a total length of 3,848 base pairs. Notably, certain sections of exon 1 and exon 24 remain untranslated [27, 28]. The entire genomic DNA sequence encompassing the FLT3 gene spans more than 97 kilobases [25]. The translation initiation codon is found in the latter part of the first exon. The signal peptide, which is responsible for guiding the protein to its proper location, is encoded by the first two exons. The extracellular immunoglobulin-like domains (ECD), which are crucial for receptor function, are encoded by exons 3 to 12. Exon 13 encodes the transmembrane domain, which anchors the protein to the cell membrane, and exons 14 through 24 are responsible for encoding the intracellular regions, specifically exons 14 and 15 for the JM domain, exons 15–17 for the TK1 domain, and 18–23 exons for the TK2 domain with a short kinase insert between TK1 and TK2 [28, 29]. The FLT3 receptor

binds to its ligand, the FLT3 ligand (FL), which is produced by cells in the haematopoietic microenvironment. The interaction between FLT3 and FL triggers the activation of signalling pathways that regulate the growth and differentiation of haematopoietic cells. The interaction between the FLT3 receptor and FL leads to receptor dimerization and autophosphorylation of tyrosine residues in the intracellular domain [30–32]. The activated FLT3 receptor subsequently triggers a cascade of signalling pathways, including RAF/MEK/ERK, PI3K/AKT, and STAT pathways. Activated FLT3 (Fig. 1B) stimulates the proliferation of haematopoietic cells and blocks apoptotic processes with a possible triggering of hyperactivation leading to malignant proliferation [33, 34]. When the ligand is not present, the kinase activity of FLT3 is subject to negative modulation by tyrosine phosphatases, which catalyse the removal of phosphate groups from tyrosine residues within the unbound JM domain. This dephosphorylation event facilitates the JM domain to assume its autoinhibitory conformation, thereby suppressing the kinase activity of FLT3 [35, 36].

FLT3 mutations in AML

The emergence of FLT3 mutations in AML typically occurs at later stages of disease development, rather than being among the initial mutations. Additionally, an extra 10% of patients acquire these mutations during the progression of the disease of AML. They arise during clonal evolution, contributing to disease heterogeneity and progression. On top of that, FLT3 alternations are prevalent upon AML diagnosis, exerting a significant influence on disease characterization, progression, and clinical management [37–39]. The two main types of FLT3 mutations found in AML are internal tandem duplications (FLT3-ITD), which affect the JM domain and its close surroundings, and point mutations in TKD or JM domain. FTL3 mutations are not detectable during standard sample analysis, but the FLT3 receptor is overexpressed on the cell surface of cell blasts and can contribute to the survival and proliferation of leukaemic clones [40, 41].

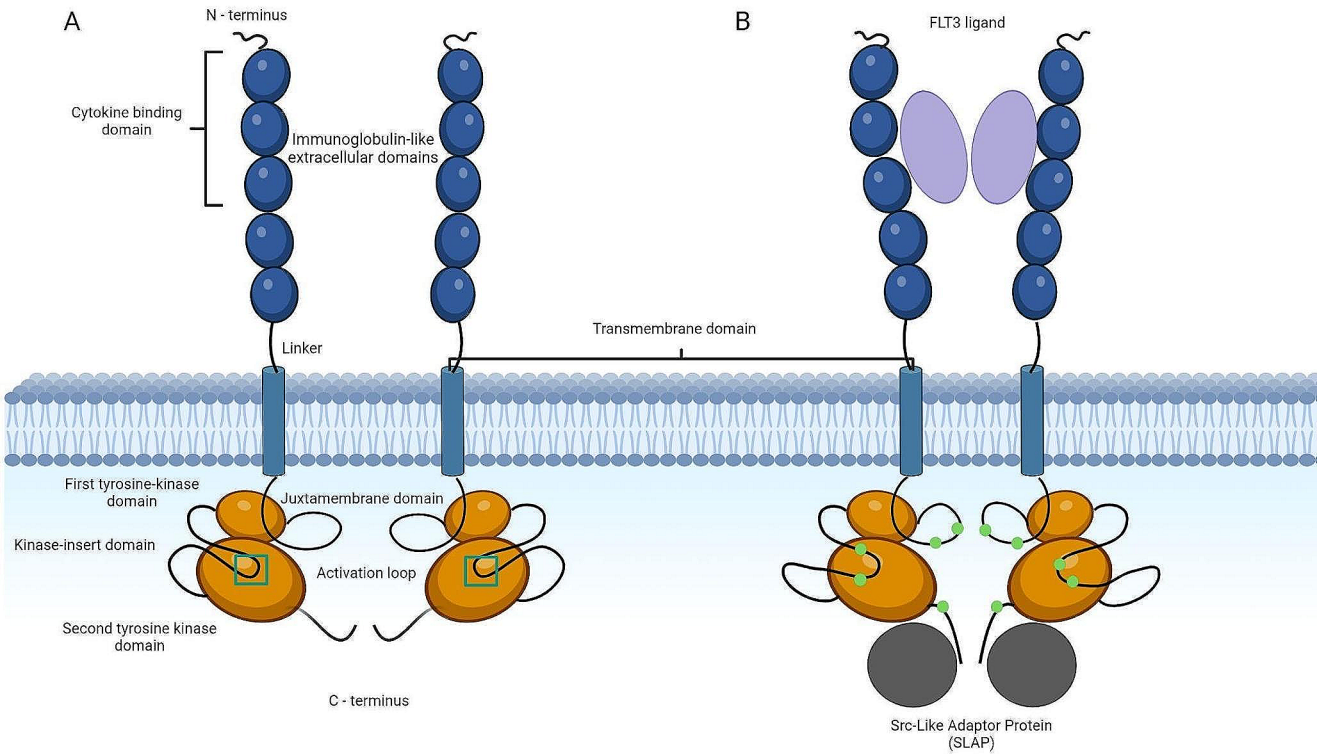

Fig. 1 FLT3 receptor **A**: In a nonactivated state lack tyrosine kinase activity and cannot initiate signalling pathways within the cell. **B**: Upon binding of a ligand such as the FLT3 ligand (FL), the two FLT3 receptors dimerize. This results in autophosphorylation of the tyrosine kinase domains within the receptor. The green spots represent the phosphorylated tyrosines. Src-like protein (SLAP) is shown binding to phosphorylated tyrosine residues on the intracellular domain of FLT3. This interaction serves as a regulatory mechanism to inhibit further signalling events downstream of FLT3 activation. The figure was created with BioRender.com

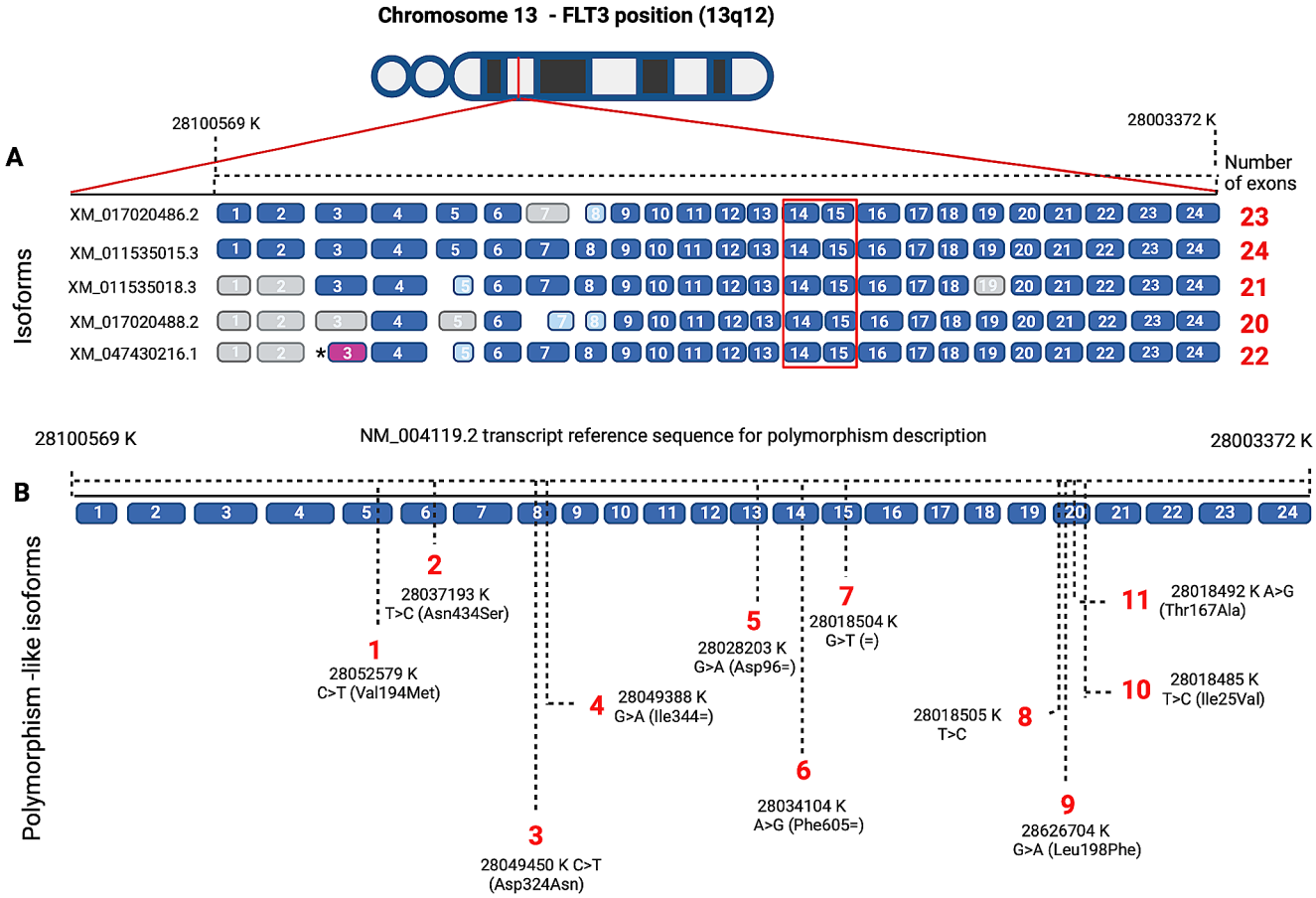

Fig. 2 Overview of isoforms and polymorphism-like isoforms of FLT3 gene. **A**: This scheme is alignment of NCBI annotated mRNA sequences of FLT3 gene with marked differences. Lacking exons are grey, shorter sequence of exon is in light blue and *purple is the same exon sequence on different positions compared to other isoforms. Exons 14 and 15 bounded by a red rectangle is the locus where ITD mutation is occurring. **B**: On this scheme are isoforms that are annotated polymorphism-like isoforms in the NCBI database and also in the LOVD database. Here are shown positions of 11 following listed isoforms and how this polymorphism affects amino acid type production: (1) FLT3(NM_004119.2):c.580G>A (p.(Val194Met); (2) FLT3(NM_004119.2):c.1301 A>G (p.N434S); (3) FLT3(NM_004119.2):c.970G>A (p.(Asp324Asn)); (4) FLT3(NM_004119.2):c.1032 C>T (p.I344=); 5.FLT3(NM_004119.3):c.288 C>T (p.D96=); 6.FLT3(NM_004119.2):c.1815T>C (p.F605=); 7. FLT3(NM_004119.2):c.615-15 C>A (p.(=); 8. FLT3(NM_004119.2):c.743-9 A>G; 9. FLT3(NM_004119.2):c.592 C>T (p.L198F); 10. FLT3(NM_004119.2):c.73 A>G (p.I25V); 11. FLT3(NM_004119.2):c.499 A>G (p.T167A). The figure was created with BioRender.com

FLT3 internal tandem duplication (FLT3-ITD) mutation

From a molecular point of view, the FLT3-ITD mutation involves the insertion of a tandem repeat in a segment of DNA within the JM domain of the FLT3 gene [42]. This tandem duplication leads to the production of a longer FLT3 protein with an extended JM domain. The duplicated segment contains the activation loop of the tyrosine kinase domain, resulting in constitutive activation of FLT3 kinase activity [43, 44]. It is detected in approximately 25–30% of AML patients [45]. The presence and allelic burden (the ratio of mutant to wild-type (WT) FLT3-ITD alleles) can vary among patients and could have some impact on the prognosis and therapeutic implications. However, according to the 2022 ELN AML recommendations, the FLT3-ITD allelic ratio is no longer considered in the risk classification [11]. Furthermore, the FLT3-ITD mutation is associated with an adverse prognosis in AML. Patients with FLT3-ITD mutations often have a higher relapse rate than do those without this mutation [46]. Currently, we can find multiple studies where it is mentioned that the size of the ITD can have clinical implications, and generally, AML patients with larger ITD mutations tend to have a poorer prognosis compared to those with shorter ITD mutations. On the other hand, there are multiple studies that prove that the size of the ITD mutation has no significant effect on treatment response or survival. The consensus remains that FLT3-ITD presence is a sign of poor prognosis for AML patients, and the relationship between ITD size and clinical outcomes is complex and can be influenced by other factors such as

the specific location of the mutation, the presence of other genetic abnormalities, and individual patient characteristics [47–49]. Recently, studies have also begun to appear about FLT3-VSI (very short in-frame insertions) and reveal interesting trends. Surprisingly, a nonsignificant trend for decreased survival was observed in patients with simple FLT3 VSIs compared with composite cases with combined VSI and ITD abnormalities [50]. A significant diagnostic marker for this mutation is also a higher leucocyte count (due to an increased production of blasts that is stimulated by FLT3) at the time of diagnosis [51, 52]. AML patients with FLT3-ITD mutations generally have shorter overall survival than patients with FLT3 wild-type mutations [44]. FLT3-ITD status is routinely evaluated in the diagnostic workup of AML patients and helps guide treatment decisions to optimise patient outcomes [53]. Approximately 30% of ITDs are localised within the TKD region. Patients carrying ITD mutations in the TKD (TKD-ITD) demonstrate a poorer prognosis for survival in contrast to those with JMD-ITD, the underlying cause of which remains elusive [54].

FLT3-ITD is often associated with other molecular and cytogenetic abnormalities in AML, such as NPM1 mutations, DNMT3A mutations, and adverse cytogenetics [55, 56]. The co-occurrence of FLT3-ITD and other molecular or cytogenetic abnormalities can affect the prognosis of AML patients [57]. The most common cooccurring mutation in patients with FLT3-ITD mutations was NPM1 mutation, and these patients had a more favourable prognosis than did those with FLT3-ITD mutations alone. This combination is associated with a higher rate of complete remission and improved overall survival [58]. Moreover, the presence of DNMT3A mutations combined with FLT3-ITD mutations can be associated with a less favourable prognosis. Patients with both mutations may have a higher risk of relapse and a poorer response to standard treatments [59]. The combination of FLT3-ITD and TP53 in AML patients indicates that the prognosis tends to be particularly unfavourable. This combination of mutations may lead to a more aggressive disease course, reduced response to standard therapies, and shorter overall survival [60].

FLT3-point mutations

The FLT3-TKD mutation is a determined genetic alteration that affects mainly the tyrosine kinase domain. In the JM domain of the FLT3 gene, point mutations and deletions may also be observed. However, due to the low frequency of the alterations in the JM domain, there is only limited information on molecular and clinical associations [61]. The D835Y/V substitution constitutes approximately 50% of FLT3-TKD mutations in which the aspartic acid at position 835 is typically replaced by valine or tyrosine. Other FLT3 mutations that occur less commonly in AML include N676K, Y842C, K663Q, V592A, N841I, N841K, S451F, Y572C, R834Q, V579A, and F594L [28, 62, 63]. These substitutions can impact the structure or activity of the FLT3 kinase. The prevalence of the FLT3-TKD mutation is lower than that of the FLT3-ITD mutation, which is found in approximately 5–10% of AML patients. The impact of the FLT3-TKD mutation on AML prognosis is not as clear as that of the FLT3-ITD mutation. Some studies suggest an adverse prognosis, while other reports indicate variable outcomes depending on additional genetic or molecular factors [64–66]. It is important to note that the specific FLT3-TKD mutation can vary among individual patients and may differ in terms of amino acid substitution. The specific effects of individual FLT3-TKD mutations on the biology and prognosis of AML are still being investigated. The identification of the FLT3-TKD mutation was performed as part of the diagnostic workup for AML [43, 44].

FLT3 gene isoforms, polymorphisms, and splicing aberrations

The isoforms represent variant forms of a gene or its protein, offering a nuanced perspective on genetic diversity and functional adaptability. The isoforms arise from alternative splicing, alternative transcription initiation or termination, or other mechanisms. These isoforms often have similar sequences but may differ in certain regions. In addition to the primary, full-length FLT3 receptor, several shorter isoforms that lack certain functional domains have been described. The exact physiological roles of these shorter isoforms are not completely understood [67, 68]. However, alternative splicing and the creation of various isoforms are common mechanisms that allow for the functional diversity of proteins, and it is likely that different FLT3 isoforms may have slightly different roles in cell signalling and haematopoiesis [69]. Aberrant splicing of the *FLT3* gene results in the formation of the common mutant isoforms *FLT3*-ITD and *FLT3*-TKD, the importance of which is mentioned above. Moreover, *FLT3* splicing results in other isoforms are less strongly associated with AML. One well-studied isoform is commonly named isoform 2 (XM_011535018.3), which lacks exon 19 and leads to the formation of a truncated and nonfunctional tyrosine kinase domain [70].

Previously, we introduced two isoforms of the FLT3 gene linked to AML disease, which are specifically associated with DNA mutations. Additionally, there are likely nonpathological variants, commonly referred to as polymorphisms, which are prevalent in the general population. Global databases about human genome variability currently

annotate twenty polymorphism-like isoforms, where eleven of them are annotated in NCBI database (Fig. 2) [71]. Although the importance of their function remains uncertain, ongoing studies are elucidating a more precise comprehension of the significance of these polymorphisms. Clinical trials have indicated the potential discovery of polymorphisms associated with improved prognosis in adult AML patients. However, these findings mostly fall below a statistically significant threshold [72]. In a yet unpublished study by Alsheikh et al., *in silico* analysis of various algorithms revealed 20 novel nonsynonymous single nucleotide polymorphisms (nsSNPs) in the FLT3 gene that are considered damaging and contribute to AML. Moreover, 12 SNPs in the 3'UTR were predicted to have 69 different functional classes. Among them, 31 alleles affected a conserved miRNA site, while 37 derived alleles created a new miRNA site [73]. As was shown for other genes, it could be expected that particular polymorphisms of FLT3 may alter or modify disease and treatment outcomes. It was observed that the DCK 201-T and CDA-79-A variants are connected to conventional treatment toxicity in AML. Patients with the variant allele of DCK 201-T or the wild-type allele of CDA-79-A exhibited a significantly elevated risk of liver impairment ($p = 0.014$ and < 0.001, respectively) and renal impairment ($p = 0.004$ and 0.002, respectively). Moreover, patients with the variant allele of DCK 360-G or the wild-type allele of CDA-79-A demonstrated a notably greater association with neurological toxicity ($p = 0.002$ and 0.025, respectively) [74–76].

Furthermore, alternative splicing aberrations on FLT3 refer to irregularities in gene expression that can result in different isoforms of the FLT3 receptor. Some of these isoforms may lack the ECD due to these aberrations. The ECD of FLT3 is essential for ligand binding and receptor dimerization, both of which are necessary for receptor activation. This leads to a loss of function of the receptor, resulting in impaired signalling. Alternatively, the absence of the ECD could result in constitutive activation of the receptor, leading to uncontrolled cell proliferation [77, 78].

FLT3 protein and domain rearrangements

The FLT3 gene in humans encodes a protein comprising 993 amino acids. More than half of the protein is taken up by the extracellular domain, within which the transmembrane region is positioned between amino acids 542 and 564 [28].

After translation, the FLT3 protein undergoes a number of posttranslational changes, including glycosylation, phosphorylation, and ubiquitination. The glycosylation process consists of two steps. The FLT3 protein is partially glycosylated in the endoplasmic reticulum, where it enters during translation, where it forms the immature receptor. The other and final glycosylation occurs in the Golgi complex. The mature receptor is formed and translocated to the cell surface. It has at least nine N-linked glycosylation sites [79]. The first one can be found after the signal peptide at N43. Ig-like domain 1 (D1) contains two domains, N^{100} and N^{151}. The second Ig-like domain (D2) contains no glycosylation site. The other Ig-like domains 3–5 each contain two glycosylation sites: D3 N^{306} and N^{323}, D4 N^{351} and N^{354}, and D5 N^{473} and N^{502} [79].

The FLT3 protein is phosphorylated at serine, threonine, and tyrosine residues. More than 10 tyrosine residues are phosphorylated upon ligand-induced activation of the receptor—the positions of known phosphorylation sites are Y^{572}, Y^{589}, Y^{591}, and Y^{599} in the JM domain and Y^{726}, Y^{768}, Y^{793}, Y^{842}, Y^{955}, and Y^{969} in the kinase domain. The intracellular domain contains these phosphorylation sites, which serve as docking sites for interactions with signalling proteins to advance the receptor signal throughout the cell [77]. However, the phosphorylation sites of serine and threonine in FLT3 have not been sufficiently investigated. Phosphorylation and its regulatory role have already been described for other related RTKs, and these proteins could also play important regulatory roles [80].

Protein domain rearrangements, specifically ITDs, are a significant subtype of FLT3 mutation and are characterised by the insertion of a variable number of amino acids within the juxtamembrane domain (JM domain) of the FLT3 receptor [81]. The exact length and sequence of the inserted segment can vary between patients, making the ITD mutations heterogeneous [82] (Fig. 3).

Possibilities of molecular treatment for FLT3 and its development

Over the last few years, there has been significant progress in studying the fundamental disease-causing mechanisms of acute myeloid leukaemia (AML). This research has greatly enhanced the understanding of the condition of AML patients. The key determinants of how AML responds to chemotherapy and its long-term prognosis are cytogenetic and molecular abnormalities. However, these factors not only aid in predicting outcomes but also hold promise as potential targets for therapeutic interventions [83, 84]. Due to its prevalent mutations in acute myeloid leukaemia (AML), FLT3 has emerged as a promising therapeutic target, resulting in the development of numerous inhibitors [85]. FLT3 inhibitors are categorised as first-generation or next-generation tyrosine kinase inhibitors (TKIs), primarily because of their potency and specificity for FLT3 and its downstream targets [86]. The initial group, which included

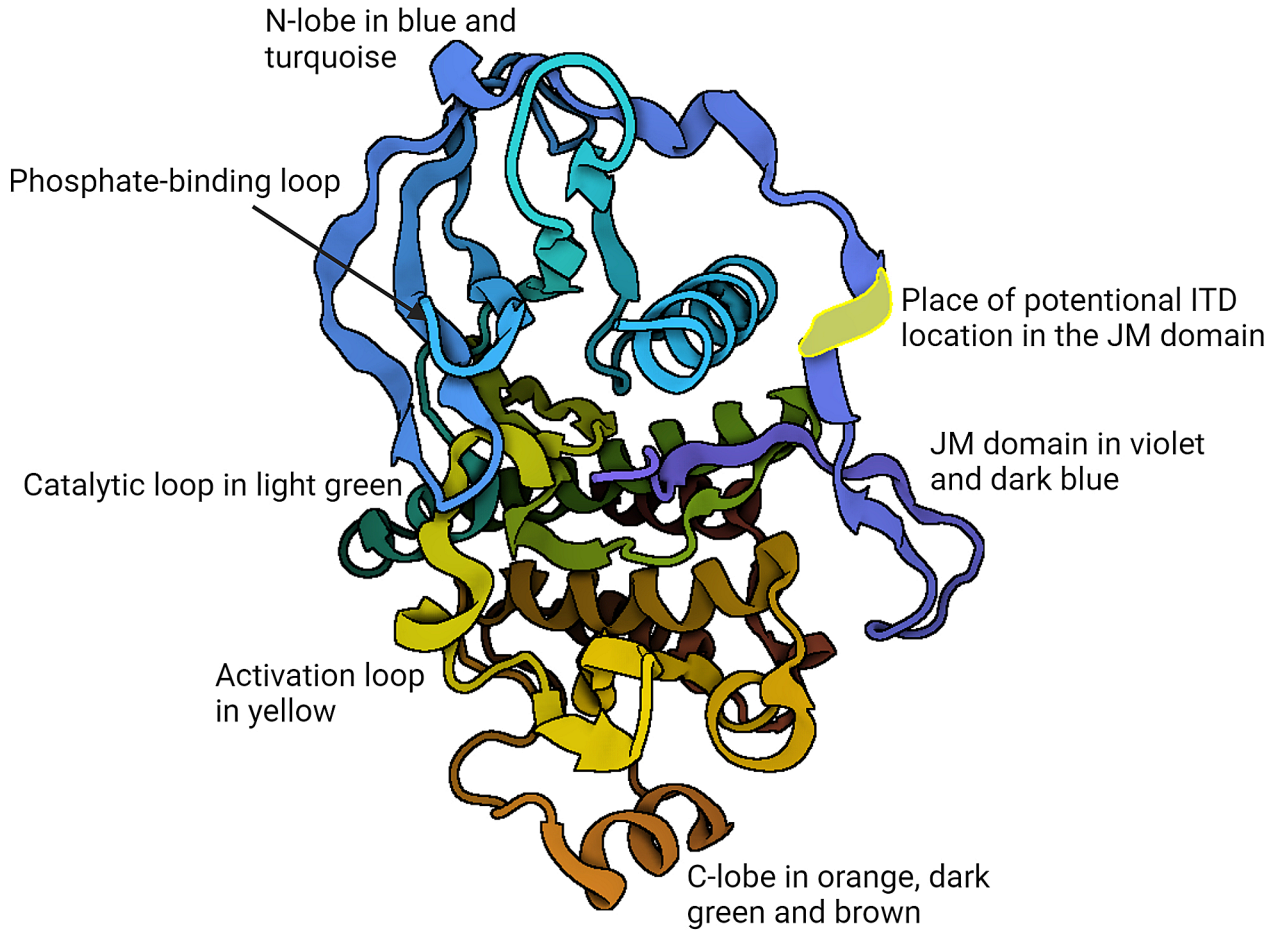

Fig. 3 Conformation of intracellular part of FLT3 – the native FLT3 kinase and juxtamembrane domains (the model was built using the Protein Builder tool available in BioRender.com from the PDB entries: 1RJB [35])

sunitinib, sorafenib, and midostaurin, exhibited relatively broad activity beyond FLT3, affecting other potential targets, such as KIT, PDGFR, VEGFR, RAS/RAF, and JAK2 kinases. While this versatility may contribute to heightened toxicity and clinical effectiveness in patients with non-FLT3-mutated acute myeloid leukaemia (AML), it leads to reduced efficacy in patients with FLT3-mutated AML with substantial allelic burden. In contrast, subsequent generations of inhibitors, such as quizartinib, crenolanib, and gilteritinib, exhibit enhanced FLT3 specificity and potency. These agents boast lower half-maximal inhibitory concentrations (IC50) and fewer side effects stemming from off-target interactions [87–89]. Furthermore, these FLT3 inhibitors fall into the Type I and Type II classifications, reflecting the distinct mechanisms through which they interact with the FLT3 receptor. Type I inhibitors bind the FLT3 receptor in both active and inactive conformation, either near the activation loop or the ATP-binding pocket, and are active against ITD and TKD mutations. Type II inhibitors bind the FLT3 receptor in the inactive conformation in a region adjacent to the ATP-binding domain [14].

Numerous FLT3 inhibitors, including midostaurin, sorafenib, quizartinib, and gilteritinib, have gained approval for cancer treatment. However, patients often develop resistance shortly after beginning FLT3 inhibitor therapy, leading to brief and unsustainable responses. This resistance arises from compensatory activation of FLT3 downstream pathways, safeguarding the bone marrow microenvironment, specific gene mutations, and activation of alternative proteins, collectively undermining the clinical impact of treatment [90, 91].

Among the most effective inhibitors are midostaurin, gilteritinib, crenolanib and quizartinib [14]; unfortunately, several mechanisms of resistance have already been identified [92]. The most commonly observed mechanism of resistance is secondary FLT3 mutation. While the FLT3 inhibitor initially targets and suppresses the primary FLT3 mutation (FLT3-ITD), AML cells can acquire additional mutations in the FLT3 gene that render the inhibitor less effective. These

secondary mutations can alter the structure of the FLT3 kinase domain and reduce inhibitor binding [93]. Type II inhibitors have no affinity for FLT3-TKD. Mutations in TKD can confer resistance by decreasing the binding affinity of type II inhibitors to the ATP binding site. For instance, the activation loop of the TKD has the most prevalent TKD mutation, residue D835, which may result in a decreased binding affinity for type II inhibitors. A mutation that affects the TKD residue F691 can potentially result in resistance to type I and type II inhibitors [94]. Furthermore, AML cells can activate alternative signalling pathways that bypass the need for FLT3 signalling. This process can involve the upregulation of other receptor tyrosine kinases or downstream signalling molecules, allowing cells to continue proliferating and surviving despite FLT3 inhibition [95]. The clonal evolution of the AML escape subclone or a pharmacokinetic factor can also play a significant role [96]. Their deeper description is beyond the scope of this review, but it does not detract from their importance.

Conclusion

In conclusion, gene mutations and isoforms of FLT3 in acute myeloid leukaemia (AML) play intricate and pivotal roles in AML pathogenesis [97]. FLT3 mutations, particularly internal tandem duplications (FLT3-ITD) and tyrosine kinase domain mutations (FLT3-TKD), are common in AML and are associated with various prognoses. FLT3-ITD mutations lead to constitutive activation of the FLT3 signalling pathway, promoting cell survival and proliferation, while the impact of FLT3-TKD mutations on AML prognosis varies [98]. While there is growing research into new FLT3 mutations, the significance of FLT3 expression levels both for wild-type patients, which are highly expressed in most acute leukaemias, and for patients with mutated FLT3 has received limited attention thus far [99, 100]. The existence of FLT3 isoforms adds to the complexity of FLT3 biology, and ongoing research is elucidating the specific roles of these isoforms in cell signalling and haematopoiesis [79]. Nonpathological polymorphisms in FLT3 are also being investigated for their potential impact on AML prognosis and treatment response [101]. The development of molecular treatments targeting FLT3, including both first-generation and next-generation tyrosine kinase inhibitors, holds promise for AML patients [102]. However, the challenge of resistance to these therapies remains a significant hurdle. The study of FLT3 protein domain rearrangements further deepens our understanding of AML pathogenesis [103]. Overall, comprehending the nuances of FLT3 mutations, isoforms, and protein function is essential for the development of targeted therapies and the improvement of outcomes for AML patients. As research in this field continues to evolve, novel insights and therapies may continue to emerge, offering new hope for those affected by this challenging disease.

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Declarations

Ethics approval and consent to participate Not applicable.

Competing interests The authors declare no competing interests.

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