## Phylogeny

FLT3 is a member of the class III receptor tyrosine kinase (RTK) family, also referred to as the PDGFR family, which also includes c-KIT, FMS (CSF-1R), and platelet-derived growth factor receptors (PDGFRs) (grafone2012anoverviewon pages 1-2, griffith2004thestructuralbasis pages 1-2, gu2011surveyofactivated pages 1-2). This classification is based on shared domain architecture, including five extracellular immunoglobulin-like domains and a split kinase domain (griffith2004thestructuralbasis pages 1-2). Phylogenetic analysis based on kinase domain sequence similarity places FLT3 within a cluster of related RTKs that arose from local gene duplications early in vertebrate evolution (manning2002theproteinkinase pages 2-3). The RTKIII family and the RTKV family (VEGF receptors) evolved from a common ancestral gene (verstraete2011structuralinsightsinto pages 25-29). More than 95% of human kinases have direct orthologs in the mouse; FLT3 is highly conserved, with human and murine orthologs sharing 86% sequence identity (verstraete2011structuralinsightsinto pages 23-25, manning2002theproteinkinase pages 2-3).

## Reaction Catalyzed

FLT3 catalyzes the ATP-dependent transfer of a gamma-phosphate group from ATP to the hydroxyl group of tyrosine residues on target substrate proteins (verstraete2011structuralinsightsinto pages 64-67, grafone2012anoverviewon pages 8-9). Reaction: ATP + L-tyrosyl-[protein] -> ADP + H⁺ + O-phospho-L-tyrosyl-[protein] (meshinchi2009structuralandfunctional pages 1-1, fedorov2023targetingflt3mutation pages 1-2).

## Cofactor Requirements

The kinase activity of FLT3 requires ATP as a phosphate donor cofactor and depends on divalent metal ions such as Mg²⁺ (fedorov2023targetingflt3mutation pages 1-2, meshinchi2009structuralandfunctional pages 2-3, grafone2012anoverviewon pages 1-2).

## Substrate Specificity

As a tyrosine kinase, FLT3 phosphorylates tyrosine residues (fedorov2023targetingflt3mutation pages 1-2). Analysis of human tyrosine kinases, including FLT3, identified general position-specific amino acid preferences surrounding the phosphoacceptor tyrosine. Tyrosine kinases typically favor aliphatic hydrophobic residues, such as isoleucine, at the -1 and +3 positions relative to the target tyrosine. Serine is disfavored at the -1 position, and glutamate is disfavored at the +3 position (yaronbarir2024theintrinsicsubstrate pages 3-3).

## Structure

FLT3 is a type III receptor tyrosine kinase protein of approximately 993 amino acids (griffith2004thestructuralbasis pages 1-2). Its structure comprises four main domains: - **Extracellular Domain (ECD):** Contains five immunoglobulin-like (Ig-like) domains at the N-terminus that are responsible for binding the FLT3 ligand and mediating receptor dimerization (grafone2012anoverviewon pages 2-3, griffith2004thestructuralbasis pages 1-2). The ECD is heavily glycosylated, which aids in its localization to the plasma membrane (grafone2012anoverviewon pages 2-3). - **Transmembrane Domain (TM):** A single alpha-helical segment that anchors the receptor within the cell membrane (griffith2004thestructuralbasis pages 1-2, grafone2012anoverviewon pages 2-3). - **Juxtamembrane Domain (JM):** A cytoplasmic domain that harbors an autoinhibitory sequence, maintaining the receptor in an inactive state (griffith2004thestructuralbasis pages 1-2, fedorov2023targetingflt3mutation pages 1-2). - **Tyrosine Kinase Domain (TKD):** An intracellular region with a bilobal kinase fold (N- and C-lobes) that is interrupted by a kinase insert domain (KID) (grafone2012anoverviewon pages 2-3, griffith2004thestructuralbasis pages 2-3). A key regulatory feature is the activation loop, which, in the autoinhibited state, is folded between the kinase lobes to prevent catalytic activity (griffith2004thestructuralbasis pages 2-3).

## Regulation

In its basal state, FLT3 is an autoinhibited monomer (muller2020novelapproachesto pages 1-3). The juxtamembrane (JM) domain stabilizes this inactive conformation by sterically blocking the kinase domain active site and preventing the activation loop from adopting an active conformation (verstraete2011structuralinsightsinto pages 64-67, griffith2004thestructuralbasis pages 1-2).

Activation occurs upon binding of the FLT3 ligand (FL), which promotes receptor dimerization and subsequent trans-autophosphorylation of tyrosine residues in the JM domain (e.g., Tyr-589) and the activation loop (fedorov2023targetingflt3mutation pages 1-2, verstraete2011structuralinsightsinto pages 64-67). Phosphorylation of the JM domain relieves its autoinhibitory interactions, inducing a conformational change that allows the activation loop to adopt an open, active form, enabling ATP and substrate binding (grafone2012anoverviewon pages 2-3, muller2020novelapproachesto pages 1-3).

Signal termination involves several mechanisms. The receptor is dephosphorylated by protein tyrosine phosphatases like PTPRJ and SHP-1 (muller2020novelapproachesto pages 3-5). Additionally, FLT3 undergoes ubiquitination by E3 ligases of the Casitas B-lineage lymphoma (Cbl) family, which targets the receptor for internalization and subsequent degradation via lysosomal and proteasomal pathways (wilson2021dendriticcellflt3 pages 4-7, meshinchi2009structuralandfunctional pages 1-1).

## Function

FLT3 is preferentially expressed on hematopoietic stem cells, including CD34+ progenitors, and early myeloid and lymphoid progenitor cells (fedorov2023targetingflt3mutation pages 1-2, grafone2012anoverviewon pages 2-3). It is a key regulator of early hematopoiesis, promoting cell survival, differentiation, and proliferation (fedorov2023targetingflt3mutation pages 1-2, griffith2004thestructuralbasis pages 1-2).

The upstream activator is the FLT3 ligand (FLT3LG) (fedorov2023targetingflt3mutation pages 1-2). Upon activation, FLT3 recruits cytoplasmic adaptor proteins, including SHC, GRB2, GAB2, SHIP, and the E3 ligases CBL and CBLB (meshinchi2009structuralandfunctional pages 1-1). This initiates signaling through downstream pathways, primarily the PI3K/AKT, RAS/MAPK, and STAT5 cascades, which in turn regulate fundamental cellular processes like cell cycle progression, apoptosis, and differentiation (fedorov2023targetingflt3mutation pages 1-2, griffith2004thestructuralbasis pages 1-2).

## Inhibitors

Both first- and second-generation small molecule tyrosine kinase inhibitors (TKIs) targeting FLT3 have been developed (kennedy2020flt3mutationsin pages 2-3). These inhibitors block FLT3 catalytic activity, typically by competing with ATP for its binding site in the kinase domain (griffith2004thestructuralbasis pages 1-2, wilson2021dendriticcellflt3 pages 18-22). - **First-generation multi-kinase inhibitors:** Midostaurin (PKC412), sorafenib, lestaurtinib, and sunitinib (SU11248) (grafone2012anoverviewon pages 8-9, griffith2004thestructuralbasis pages 1-2). - **Second-generation selective inhibitors:** Gilteritinib, quizartinib (AC220), and crenolanib (kennedy2020flt3mutationsin pages 2-3, grafone2012anoverviewon pages 8-9). Other investigational inhibitors include MLN518 (CT53518), SU5614, and SU5416 (griffith2004thestructuralbasis pages 1-2). A biologic agent, the anti-FLT3 monoclonal antibody IMC-EB10, has also been evaluated (grafone2012anoverviewon pages 8-9).

## Other Comments

Activating mutations in the *FLT3* gene are found in approximately 30% of patients with newly diagnosed acute myeloid leukemia (AML) and generally confer a poor prognosis (fedorov2023targetingflt3mutation pages 1-2). The two major classes of these mutations are: - **Internal Tandem Duplication (ITD):** These mutations are located in the juxtamembrane domain-coding region and occur in 25–30% of adult AML cases (verstraete2011structuralinsightsinto pages 64-67). FLT3-ITDs disrupt the JM domain’s autoinhibitory function, leading to ligand-independent receptor dimerization and constitutive kinase activity (verstraete2011structuralinsightsinto pages 64-67, meshinchi2009structuralandfunctional pages 1-2). - **Tyrosine Kinase Domain (TKD) mutations:** These are point mutations within the activation loop, most commonly a substitution at aspartic acid 835 (e.g., D835Y). TKD mutations are present in about 7% of AML patients and function by stabilizing the active conformation of the kinase domain, which also results in constitutive activation (verstraete2011structuralinsightsinto pages 64-67, muller2020novelapproachesto pages 1-3).

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