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# The Effect of FLT3 Stimulators and their Role in Stem Cell Maintenance and Therapy

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**Abstract**

FMS-like tyrosine kinase 3 (FLT3) is important in the normal development of stem cells and the immune system. In acute myeloid leukemia (AML), there is an activating mutation of this tyrosine kinase gene. This mutation results in the survival and proliferation of leukemic blasts, which can result in an adverse prognosis. Consequently, FLT3- inhibition has become of interest for the treatment of myeloid leukemias such as AML. Tryosine kinase inhibitors (TKIs) are being developed and investigated for FLT3-mutated AML, and many are beginning to show efficient results. AML has a progressive low survival rate with the standard medical care being chemotherapy. AML patients have shown to have a mutation in the FLT3 gene in more than 30% of patient cases. Knowing this correlation, it is important to further investigate tyrosine kinase inhibitors use for treatment. This review summarizes information of what FMS-like tyrosine kinase 3 is and how it is important in the process of hematopoiesis. Then it will discuss the correlation of FLT3 mutation in patients with AML. Lastly, the advancement of treatment of AML using FLT3 inhibitors or TKIs will be discussed.

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**Keywords:** Flt3 inhibitors, Fms- like tyrosine kinase 3, acute myeloid leukemia, tyrosine kinase inhibitors

**Abbreviations and Symbols:**

Fms-Like Tyrosine Kinase 3 (FLT3)
Hematopoietic Progenitor Cell (HPC)
Hematopoietic Stem Cells (HSC).
Phosphoinositide 3 Kinase (P13k)
Acute Myeloid Leukemia (AML)
Granulocyte-Macrophages (GM)
Flt3-Internal Tandem Duplications (ITD)
Wild Type FLT3 (FLT3-WT)
B-Cell Acute Lymphoblastic Leukemia (B-ALL),
T-Cell Acute Lymphoblastic Leukemia (T-ALL)
Granulocyte Colony Stimulating Factor (G-CSF)
Hematopoietic Stem and Progenitor Cells (HSPC)
Mixed Lineage Leukemia (MLL)
Acute Lymphoblastic Leukemia (ALL)
Flt3 Ligand (FL)
Leukemic Stem Cells (LSC)
Myeloid Cell Leukemia-1 (MCL-1)
Flt3 Tyrosine Kinase Inhibitors (FLT3 TkIs)
5-azacytidine (AZA)
Heat Shock Proteins (Hsps)

## Introduction

The process by which hematopoietic stem cells and progenitors differentiate into blood cells of various lineages is called hematopoiesis. This process involves many interactions of transcription factors that influence the expression of downstream genes and they also facilitate differentiation and proliferation signals [1]. Cytokines also have a role of maintenance, expansion, and differentiation of hematopoietic stem cells in the bone marrow. The bone marrow comprises of hematopoietic stem cells and provides a microenvironment for them known as the stroma. This is where they undergo self-renewing and self-divisions of their stem cell pool. This will provide them an environment where they can differentiate into the mature cells they need to be to achieve their physiological function. During this process, cytokines help contribute to this differentiation of the cells [2]. Therefore, cytokines are important regulations in hematopoietic development. They provide a key role in the transfer of signaling to cells which affect their survival, proliferation, differentiation, and maturation. FMS-like tyrosine kinase (FLT3) is a typical example of a hematopoietic cytokine. However, this cytokine is often overexpressed in leukemias or it is mutated [3].

Accordingly, hematopoiesis can involve mutations in the regulatory genes that that are capable of promoting leukemogenesis [1]. Fms-like tyrosine kinase 3 has a critical role in the normal development of stem cells and the immune system [4]. FLT3 gene is the gene that encodes this tyrosine kinase receptor that controls survival, proliferation and differentiation of HSC. If this gene is mutated, then it causes deregulation of the balance between cell proliferation and cell differentiation [1]. FL is a cytokine that acts through FLT3 and has pleiotropic and potent effects on the development of HSCs and the immune system [2]. FLT3 is a cell surface receptor tyrosine kinase that is expressed by immature hematopoietic cells. This receptor collaborates with other growth factors, like cytokines, to produce proliferation of stem cells, progenitor cells, dendritic cells, and natural killer cells. It has been thought that FLT3 could be used as a therapeutic target for kinase inhibitors or other approaches for patients with mutations of this gene. This is because mutations of FLT3 have been commonly present in unfortunate diagnosis such as leukemia [4]. This tyrosine kinase was first found on a murine hematopoietic progenitor cell (HPC) but on humans it is restricted to CD34+ bone marrow cells which includes the hematopoietic stem cells (HSC) [5].

When a ligand binds to FLT3, dimerization of the receptor occurs. FLT3 then self-phosphorylates its tyrosine residues. This is shown in Figure 1, where if the tyrosine is not phosphorylated, they are in a closed conformation but if they are phosphorylated, they are in the active conformation and can proceed with the process. From this, adapter proteins are recruited and the Ras/MAP kinase or phosphoinositide 3 kinase (P13k) pathway is signaled. These pathways are vital in cell survival and growth. The ligand that binds to flt3 is important in promoting the survival and growth of hematopoietic progenitors. These progenitors include the ones in the myeloid and B lymphoid pathway. Since Flt3 is shown present in the CD34/CD33+ cells, it is shown to have influence in the formation of granulocyte-macrophages (GM) by human bone marrow. Flt3 also synergizes with other cytokines. Cytokines include interleukins, growth factors, colony forming units and transcription factors. Furthermore, Flt3 has been shown to have a wide array of functions and influences on cells belonging to the lymphoid and GM pathways [5]. FLT3 ligation provides growth-stimulation and antiapoptotic signals [6].

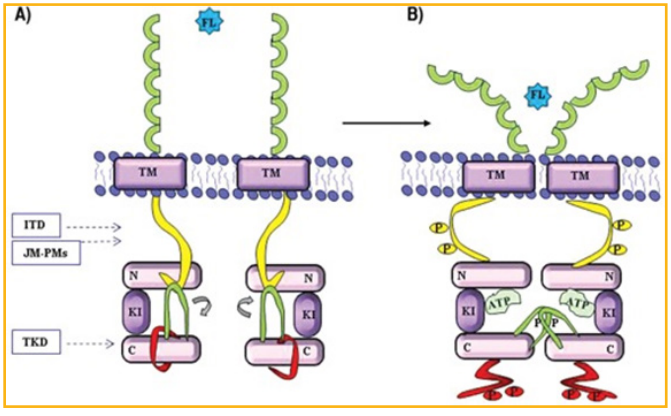


**Figure 1:** A. Inactive conformation of FLT3. B. Active conformation of FLT31.

In this paper, the role of FLT3 and its ligand on hematopoietic stem cells are discussed because of the interest in their clinical applications. Clinical applications include stem cell transplantation and cancer immunotherapy [2]. FLT3 is commonly mutated in acute leukemias. FLT3 internal tandem duplication mutation in acute myeloid leukemia (AML) have a poor prognosis in adult and pediatric patients. This discovered the use of FLT3 inhibitors to treat patients with acute leukemias. Many of these are still in preclinical development and some have entered clinical trials [7]. They will be discussed in this review as well as the overall importance of FLT3.

**Discussion**

A mutation of Flt3 is commonly found in AML [2]. It is also one of the most frequent genetic alterations in AML and indicates poor prognosis [6]. Since Flt3 plays a critical role in early hematopoietic development and regulates the growth and differentiation of CD34+ hematopoietic cells, a mutation in it can lead to severe problems. It is often recommended to receive allogeneic hematopoietic stem cell transplants for patients with Flt3 mutations even though sometimes this does not work for treatment either [8]. Allogenic transplant is the transplantation of stem cells from a matching donor. The other stem cell transplantation is autologous stem cell transplant which uses stem cells directly from the patient [9]. Patients where this transplant usually does not work are those with Flt3-internal tandem duplications (ITD) due in part because they have a higher rate of relapse. ITDs are always expressed in patients but they vary in length. These duplications are in the juxta membrane domain of the Flt3 in all subtypes of AML. They result in the constitutive activation of the receptor. Researchers do not know why but they believe this is due to the active tyrosine kinase site allowing access of ATP and substrates as a result of unfolding. Since therapeutic options are limited for those with this mutation, researchers are trying to find a way to prevent this by using Flt3 inhibitors as a maintenance therapy. They believe the inhibition of this Flt3 signaling could be an effective approach of treatment [2].

A report states how wild type FLT3 (FLT3-WT) is expressed in 93% of AML cases, almost 100% of B-cell acute lymphoblastic leukemia (B-ALL), 87% of T-cell acute lymphoblastic leukemia (T-ALL) and in a small percentage of chronic myeloid leukemia [10]. These high percentages of FLT3-WT in these diagnosis's show how the overexpression of FLT3 may result in the survival and proliferation of leukemic cells. 30% of mutations in FLT3 have been found in with AML [1].

ITD mutation results in the prevention of the association between the JM domain and the kinase domain as can be seen in Figure 1 for reference of where these are on the receptor. When this mutation prevents this association, the kinase will then undergo a conformational change and will be constantly activated. This will therefore block myeloid differentiation of hematopoietic progenitors since the normal signaling pathways will be disrupted. This report states the role of FLT3-ITD preventing Fox03-a mediated apoptosis. This then promote the survival and proliferation of AML cells. In addition, FLT3-ITD and their role in increased activation of downstream signaling which inhibits cellular phosphates which can lead to amplification of the proliferative and anti-apoptotic effects. Furthermore, FLT3-ITD causes significant increase in cell proliferation since there is a continuous activation of the receptor. This is involved in leukemic progression. This mutation is shown in Figure 2 on the right. The figure shows the presence of the ligand on FLT3-ITD will activate AKT phosphorylation and increased MAPK activation. On the left, shows the signaling pathways activation by FLT3-WT [1]. Patients with FLT3-ITD AML tend to be younger patients that have high white blood cell counts. These patients have a high risk of relapse if they achieve remission. This disease has improved in prognosis over the years because of the advances of allogeneic hematopoietic cell transplantation as well as FMS-like tyrosine kinase inhibitors [11].

Studies have shown that FLT3-ITD transformed cells injected into mice result in a leukemia-like syndrome. These FLT3-ITD transformed cells include Ba/F3 or 32D. This study also showed that the injection showed myeloproliferative disorder in the mice bone marrow cells. The FLT3 mutations result in the disruption of the kinase activity. These preclinical studies give researchers

a great idea on the responsibility of FLT3 and how they may be a viable therapeutic target for treatment of AML [12].

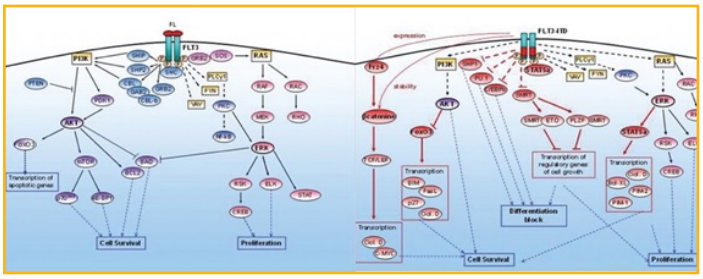

**Figure 2:** (Left) Signaling pathway of FLT3-WT (Right) Signaling pathway of FLT3-ITD1.

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Another study shows that FLT3 is highly expressed in mixed lineage leukemia (MLL) rearranged acute lymphoblastic leukemia [13]. Acute lymphoblastic leukemia (ALL) is in infants and is categorized by the rearrangements of the mixed lineage leukemia gene, drug resistance, and a poor treatment outcome. In this study, they show FLT3 expression in infants with MLL are higher compared to both infant and noninfant patients with ALL carrying MLL genes. The leukemic cells from infants with MLL are more sensitive to the FLT3 inhibitor than noninfant ALL cells. Their results found the use of flt3 inhibitors could be used in infants with MLL cells since they show with overexpression of FLT3. In the experiment, they assessed whether the level of expression of FLT3 was sufficient enough to self-phosphorylate and activate FLT3 in the absence of activating mutations. They examined several infant MLL and noninfant ALL patients with varying levels of FLT3 expression (Figure 3A). They also analyzed the patients expressing high levels of FLT3 were sensitive to the FLT3 Inhibitor drug, PKC412. Similarly, the patients with low level of FLT3 did not respond to the drug (Figure 3B). Furthermore, the study shows FLT3 overexpression in leukemic cells from infants with MLL rearranged ALL [13].

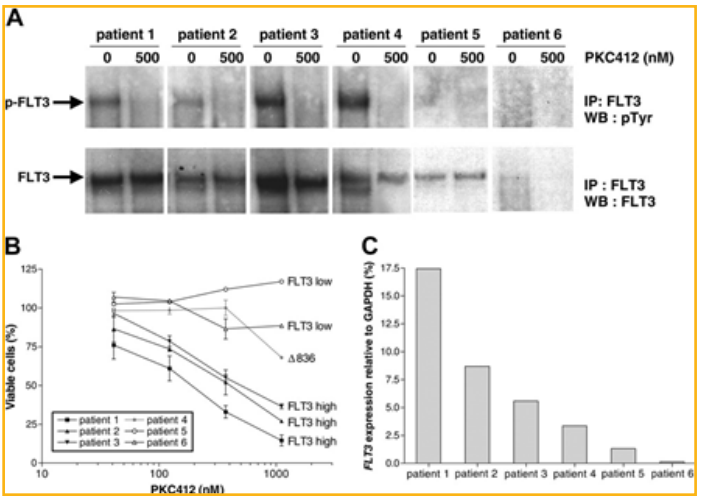


**Figure 3:** Observation of FLT3 phosphorylation in infant MLL patients with varying levels of FLT3 expression and noninfant ALL patients [13].

There have been a number of preclinical studies in vitro and in vivo that have shown clinical benefits of FL, that acts through FLT3. These are mostly done through stem cell transplantation and cancer immunotherapy. For stem cell transplantation, it is important to understand how stem cells work in order to use this therapy. The transfer of cells from the bone marrow into blood circulation is based on the administration of cytokines. Granulocyte colony stimulating factor (G-CSF) is an agent used to move many stem cells (CD34+) to be harvested and used for stem cell therapy. FL works like G-CSF through progenitors, but FL can also mobilize dendritic cell subsets. Since the FL can do this to the dendritic cells, the dendritic cells show a possible benefit of FL. The expansion of dendritic cells by FL can possibly be used for immune recovery in early post transplantation period. The downfall is that the reconstitution of a diversified T cell range does not start for at least six months and is impaired with age. This shows that FL to be used for immune recovery may be only used for selected patients. The interaction of these cells is important in immunological responses especially between DCs and NK cells. They are important in the

action of anti-tumor defense to prevent the relapses from stem cell therapy. This was shown in animal models. This study reports how FL was used to enhance protective immunity against pathogens in mice. They showed this could be due the FL's role in expanding DCs which captures microbial antigens. They believe this is important in reducing infections after stem cell therapy. The introduction of FL in patients after stem cell therapy could boost the hematopoietic and immune recovery to enhance antitumor immunity. In addition to stem cell therapy, there is also the studies of cancer immunotherapy. As stated above, the DCs are shown to induce antitumor immunity. FLs that expand these cells are what makes them a promising tool for treatment. This study reports a clinical trial of patients with advanced colon and lung carcinomas. Twelve patients were treated with a large number of autologous DCs after administration of FL. Two out of the twelve patients experience tumor regression and three of them experienced the disease stabilizing [2].

A study examined the effect of FLT3 on purified human CD34+ progenitor cells. They did this by cloning it. By adding FLT3 to irradiated long-term marrow cultures with CD34+ cells amplified both total and progenitor cell production. FLT3 showed to support maintenance of both GM and HPP progenitors in vitro. The study found that FLT3 has an important function in the regulation of multipotent and lineage committed hematopoietic progenitor cells. After comparing FLT3 and SLF, they conclude that they act similarly. FLT3 also synergizes with a combination of cytokines to induce the ex vivo expansion of bone marrow derived progenitor cells [14].

In one study, the expression of FLT3 within the hematopoietic stem cell and bone marrow in mice was investigated. They did this by using flow cytometry and quantitative reverse transcription polymerase chain reaction. First, they isolated the hematopoietic stem and progenitor cells (HSPC) from the mouse bone marrow. Since hematopoietic progenitor cells express high levels of FLT3 at the surface, they used the gating strategy to identify them. This part of the study confirmed the expression of FLT3 by cells within almost all HSPC populations, including hematopoietic stem cells. They confirmed this finding by flow cytometry as well but that it is most commonly found on cells with lymphoid potential. Leukemia with FLT3-activating mutations could have arisen from the FLT3 that is found in the bone marrow and in the primitive stem cell compartment. From Figure 4, it shows what this experiment found. How HSC has receptors which include FLT3 but as the stem cell differentiate, FLT3 is found in multiple lineages, strongly associating with lymphoid-GM potential. It is important to understand the expression of these receptors since it indicates the fate of cells [5].

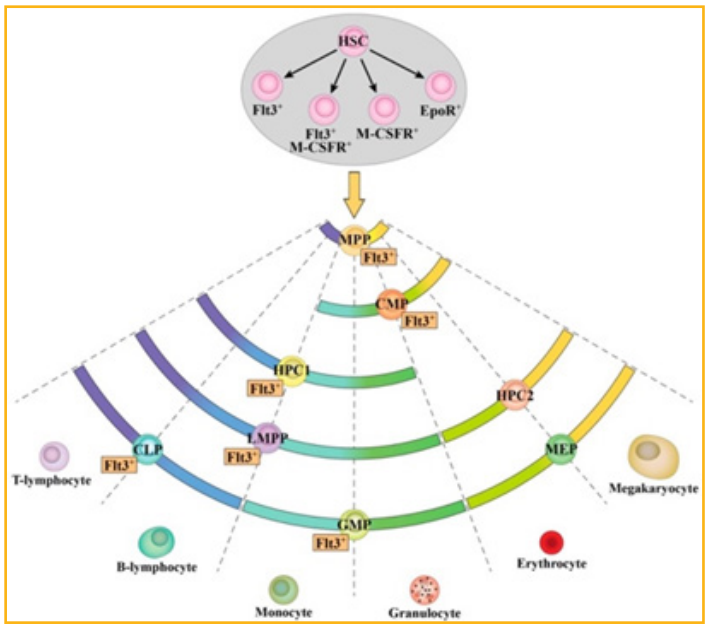


**Figure 4:** FLT3 expression in the murine HSPC cell line. This figure demonstrates the heterogeneity of HSPC and its expression of FLT3 [5].

In one study of the bone marrow of mice, it was shown that mice deficient in expression of FLT3 ligand showed deficient lymphopoiesis, preferentially affecting B cell development. It also showed the reduction of NK and DC. This agreed that FL could be important for the differentiation of stem cell lineages. In addition, FLT3 and its ligand are important for stem cell maintenance. They found that mice lacking expression of FL demonstrated reduced numbers of early B lymphoid progenitors. They also found in FL deficient mice, the reduction of early T cell progenitors as well15.

The focus of one review by [16], described the function

of the FLT3 receptor in the process of a therapy that would treat T cell-mediated autoimmune diseases. First, they explained FLT3 signaling and its role. A previous study found the role of FLT3 in DC development was found both in vitro and in vivo showing the treatment of progenitor cells or mice with FLT3L led to a proliferation of DCs. It was also shown that mice with a deficiency in FLT3 correlated with having a deficiency in DCs. This brought about more studies that focused on FLT3 being used as an immunostimulatory antitumor agent and then was found to produce anticancer effects. Since high levels of FLT3 are shown in leukemias, more studies showed the constitutive activation of it in leukemias because of a mutation of FLT3. Thus, treatment with FLT3 inhibitors has been of interest [16]. One study showed the treatment of a FLT3 inhibitor that inhibited type I interferon producing DCs. This in vivo treatment of mice resulted in a phenotype that was similar to FLT3 deficient mice. However, because of implications such as how the number of DCs returned to normal after therapy and the lack of effect on repopulation of bone marrow stem cells, showed more consideration needed to be done before clinical use [17]. Next, another study used an FLT3 inhibitor that showed similar results. They found the decrease in DC population which decreased an autoimmune response. There was no change in mature B and T cells. There was, however, a decrease in T-cell expansion. In the model that had multiple sclerosis, it was shown after treatment that the mouse showed significant improvement in the course of disease [18]. This study showed how inhibiting an ongoing T cell response via inhibition of DCs could prove to be effective, thus treating T cell mediated autoimmune diseases. This study supposes that targeting FLT3 would be the best approach in treating autoimmune disease because of its selectivity. This process would target a signaling pathway expressed in antigen-presenting cells, but not mature B or T cells. This process would allow targeting of the antigen presentation which would limit the event of targeting a wrong antigen that would be unknown or could mutate over time. Also, since mature B and T cells are not direct targets, this could decrease the chance of an auto-immune response that would destroy B and T cells [16]. As stated previously, dendritic cells can regulate and amplify immune responses. FLT3 stimulates the production and proliferation of these dendritic cells. In a preclinical study, mice were injected subcutaneously with FL for 8 days. They found by using flow cytometry, that mice treated showed an increase in cellularity in the spleen. They also compared FL DCs to the control DCs as stimulators of T-cell responses in an allogeneic mixed lymphocyte reaction. They found FL DCs were less effective stimulators than the control shown in Figure 5. Also shown in the figure is that the cytokines IL-2 and IFN-γ decreased in secretion during allogeneic T-cell responses for the FL DCs. Furthermore, after all of the data collected from this study, it was shown that the treatment of FL on mice reduced their ability to stimulate allogeneic T cells in mixed lymphocyte reaction even though DC numbers increased. This study further agrees with the others on the impaired allo-stimulatory capacity of DCs from bone marrow of FL treated mice. This study confirmed the important of the decrease in allo-stimulation caused by FL in vivo [19].

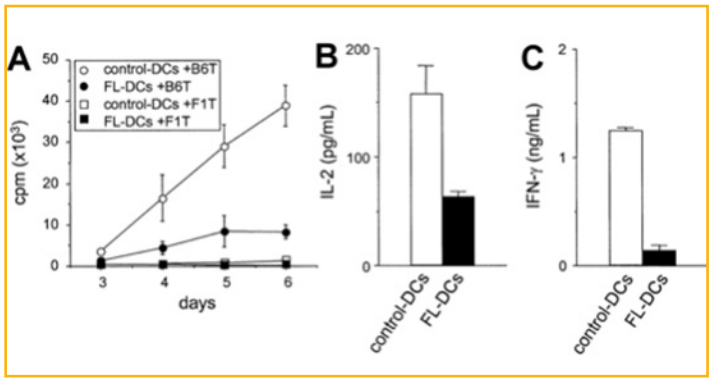


**Figure 5:** Comparing the data contained from control DCs and FL DC from mice [19].

Leukemic stem cells (LSC) are found in AML cells and they express high levels of myeloid cell leukemia-1 (MCL-1) even compared with normal hematopoietic cells. This suggest that AML LSCs rely on MCL-1 for survival. The AML LSCs express FLT3 and FLT3-ITD. Knowing this, this study analyzed the bone marrow of thirty adults with AML in Kyushu University Hospital. After stem and progenitor analysis of the cells in the bone marrow of these patients, they found among the thirty AML patients, that FLT3-ITD was detected in eleven of them. As shown in Figure 6, they showed the phenotypic characteristics of the bone marrow cells

in normal and AML. This study showed how AML LSCs expression high levels of FLT3 compared to normal hematopoietic stem cells [6].

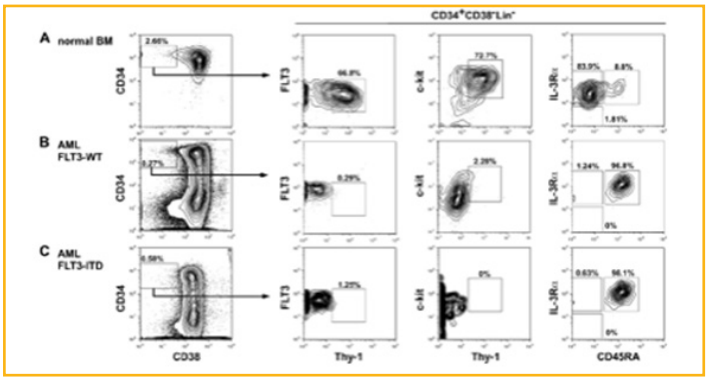


**Figure 6:** Phenotypic characteristics of bone marrow cells in A. (Normal bone marrow B.) AML-FTL3-Wild Type. C.) AML- FLT3-ITD [6].

**FMS-like Tyrosine kinase 3 (FLT3) Tyrosine kinase inhibitors (TKIs)**

A major advance in treatment of patients with FLT3 internal tandem duplication acute myeloid leukemia is TKIs. AML is a poor prognosis and many advances are being developed for treatment to replace the toxicity of chemotherapy [11]. Chemotherapy with or without allogeneic hematopoietic stem cell transplantation has limited efficacy in AML. It only has a cure rate of only 30-40% [20]. These advances include allogenic hematopoietic cell transplantation as well as tyrosine kinase inhibitor drugs. These drugs include, sorafenib, midostaurin, gilteritnib, quizartinib etc. Sorafenib is a multitargeted TKI. Midostraurin in 2017 was approved by the FDA as well as gilteritnib in 2018 for clinical use in FLT3 mutated AML [11]. Since FLT3 mutation are found in approximately one-third of patients with AML, FLT3 has become an attractive therapeutic target. FLT3 inhibitors are undergoing clinical evaluations to find the most efficient, selective, and potent compound against FLT3 mutation [21]. The reason for this is because of the greater potency and selectivity, the great efficacy in FLT3- mutated acute myelogenous leukemia (AML). This also promises less toxicity for the patient [22]. The way these FLT3 inhibitors work is by acting via competitive inhibition with adenosine triphosphate on the tyrosine kinase domain. Inhibition results in decreased autophosphorylation and its successive activation, thus they are labelled as first generation and second-generation inhibitors. The first generation are not as specific for FLT3 which include tandutnib (MLN518, CT53518), sunitnib, sorafenib, midostauin (PKC412), and lestaurtinib (CEP701). Next, second generation flt3 inhibiotrs are more selective and potent. These include quizartinib (AC220), crenolanib (CP-868-596), ponatinib, pacritinib (SB1518), and gliteritnib (ASP2215). Most of these drugs are undergoing trials in clinical settings and will be discussed below. Furthermore, past, ongoing, and planned trials will be discussed below for each of these inhibitors for treatment of patients with this disease.

A study examined six different FLT3 inhibitors for potency against mutant and wild type FLT3. They also examined them for cytotoxic effects against a series of primary blat samples from AML patients. They found that the FLT3 inhibition does not always induce cell death so some FLT3/ITD AML may not be continuously signaling FLT3. The six FLT3 inhibitors varied in their selectivity for FLT3 which influenced cytotoxic effect. Some were highly selective, intermediate selective, and less selective. From their data, they found a newly diagnose patient with AML may respond better to the less selective FLT3 inhibitor since their data suggest that response rate for diagnostic specimens is higher for them. This is shown in Figure 7,

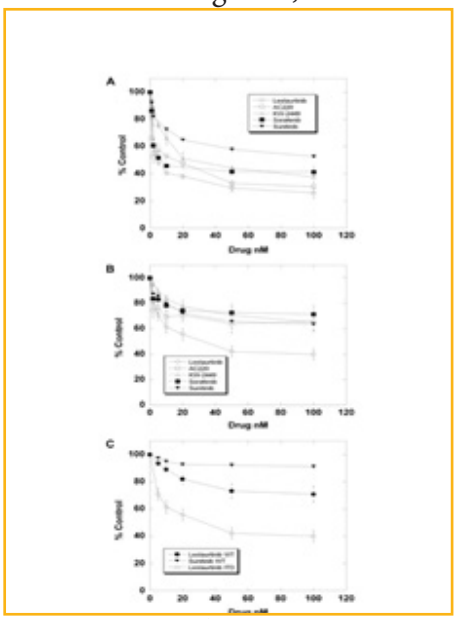


**Figure 7:** Cytotocicity assays of FLT3ITD primary AML samples [24]

where all of the inhibitors responses are shown. It shows, lestautnib, the less selective drugs shows a very modest cytotoxic effect against the wild type samples whereas sunitnib, highly selective inhibitor, shows no effect whatsoever. They found from their data that a high mutant allelic burden does correlated with cytotoxic response to selective flt3 inhibition. This means that only newly diagnosed AML patients with FLT3/ITD would respond to FLT3 inhibition therapy [24].

**FLT3 Inhibitor- Lestaurtinib**

Lestauritnib is a first generation agent that is relatively nonspecific for FLT3 and usually inhibit other class III receptor tyrosine kinases such as KIT and PDGFR [23]. A phase III study in 2017 investigated newly diagnosed AML patients. They did this through a randomized assessment through combination therapy of lestaurtinib to first-line chemotherapy. Therefore, they did it through intensive chemotherapy in combination with lestaurtinib starting two days after each chemotherapy. This study showed no overall clinical benefit after the administration of lestaurtinib in combination with chemotherapy. However, they did find lower rates of relapse and improved overall survival in the patients that achieved sustained levels of FLT3 inhibitory activity. Researchers of this study acknowledge during their study that second generation of more selective FLT3 inhibitors were designed that have the apparent capability of achieving more flt3 inhibition [25].

**FLT3 Inhibitor- Giliternib**

In a preclinical study, a FLT3 inhibitor named gilteritinib was examined. Gilteritnib is a pyrazinecarboxamide derivative that is being studied in AML clinical trials. This is because of its selectivity, potency, and activity against FLT3-activating mutations. This study compared gilteritnib to other flt3 tyrosine kinase inhibitors (FLT3 TKIs), which include midostaurin, sorafenib, quizartnib, and crenolanib. First, testing of the inhibitory activity of gilteritnib against different forms of FLT3 in leukemia cells was completed. This is summarized in Figure 8. It was shown that gilteritnib had similar levels of inhibition against mutations that sorafenib and quizartinib did. In addition, from this figure shows that gilteritnib may have the ability to modify the activity of FLT3 in AML. This is because of its activity against receptor tyrosine kinase Axl [26].

Furthermore, there are many obstacles researchers have to endure when trying to find the most efficient and beneficial FLT3 inhibitor to treat diseases such as AML. An obstacle includes how FLT3 inhibitors have shown to select against the activity of receptor c-kit. C-kit is important in the maintenance and process of hematopoiesis. When c-kit is not working properly, this can lead to marrow suppression. By using bone marrow from healthy donors, the comparison of gilteritnib and quizartnib was completed to show the difference in effects of c-kit. It was show that gilteritnib has an EC50 against wild-type c-kit of 102nM which is two orders of magnitude greater than that of mutant FLT3. It did not have much effect on normal hematopoiesis. These results are shown in Figure 9. This study shows the potential in gilteritnib and how compared to the other Flt3 inhibitors, it may be the most useful to use to further investigation [26].

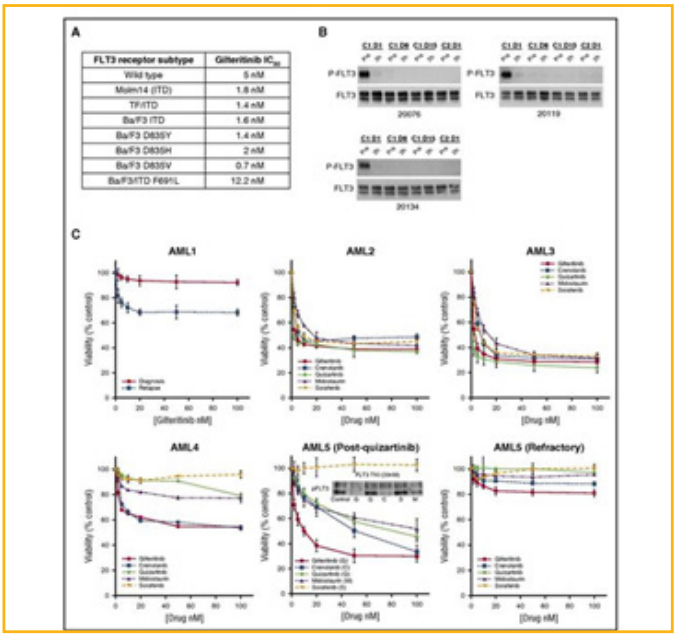


**Figure 8:** Anti-leukemic activity of gilteritnib [26].

However, there is a study known as the Admiral trial that discusses its results of its phase 3. They released results of patients with acute myeloid leukemia who received gilteritnib therapy in the phase 3 admiral trial [27].As discussed before, gilteritnib acts as an inhibitor of FLT3 and is known for its potency and selectivity. This drug became the first FLT3 inhibitor approved as a

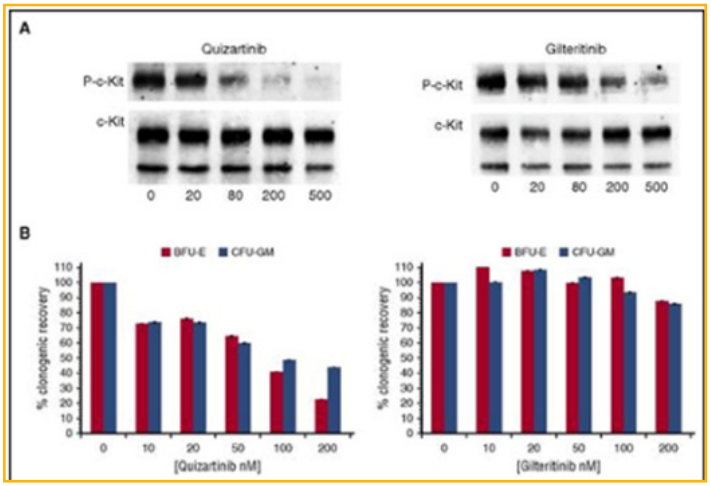


**Figure 9:** The comparison of quizartinib and gliteritnib, two flt3 inhibitors. This figure shows the comparison of both of their effects on the inhibition of c-Kit by comparing donated bone marrow from healthy patients [26].

single-agent therapy [28]. The results of the admiral showed emerging mutation at relapse in the patients that had FLT3-mutation. It was shown that the gliteritnib stimulated overall survival and resulted in higher remission rates compared to patients with chemotherapy. However, the patients given gliteritnib were shown to develop resistance after their initial response. In this trial, 247 patients were given gilteritnib and 75 of them relapsed during the study. In patients in this study that had FLT3mut+ R/R AML who relapsed on this treatment, Ras/MAPK pathway gene mutations and FLT3 F691L gatekeeper mutations showed most frequently. The presence of a RAS/MAPK pathway gene mutation did not benefit from this drug treatment which could possibly be because of fewer RAS/MAPK pathway gene mutations per patient at baseline than at relapse. The frequency of emergent FLT3 F691 gatekeeper mutations at relapse in patients who received 120-mg/day gilteritinib in the admiral study was similar to that observed in relapsed patients who received 20- to 450-mg/day gilteritinib [27]. In conclusion, the patients with R/R FLT3mut+ AML, gilteritnib resulted in overall longer survival and higher response rates compared to those with chemotherapy. In addition, the treatment had a favorable safety profile. These results are shown in Figure 10 showing the overall survival in patients treated with the flt3 inhibitor versus chemotherapy [28]. Based on the results of this trial, the FDA approved gilteritnib (Xospata) in November of 2018 for adults with FLT3-posistive acute myeloid leukemia (AML) in the relapsed or refractory setting [29].

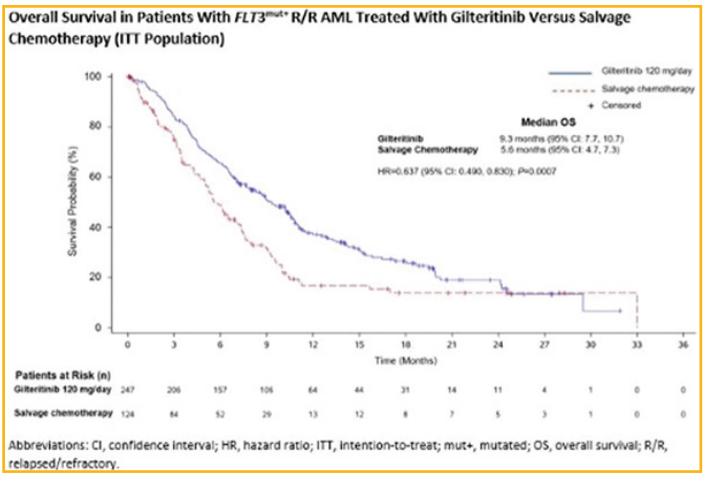


**Figure 10:** This figure represents patients with FLT3mut+ R/R AML treated with gilteritnib, a flt3 inhibitor, compared to patients treated with chemotherapy [30].

FLT3 inhibitors became to be a great potential for the treatment of patients with AML since 30% of these patients had a mutation in FLT3. This first FLT3 inhibitors were the tyrosine kinase inhibitors as discussed above. However, TKIs were originally developed for treatment of solid tumors. These drugs were then surprisingly found to have activity against FLT3. With further investigation of these drugs, there have been some that are designed for the specific purpose of treating patients with FLT3/ITD AML [31].

More recently, a study states that FLT3-mutated AML is changing after the approval of midostaurin for the frontline therapy and gilteritnib for relapsed or refractory patients. TKI drugs such as gilteritnib, quizartnib, and sorafenib, which are discussed above, have shown as positive randomized trials. This predicts further investigation of the use of FLT3 inhibitors. These are an important part of therapy for a large subset of high-risk patients such as those with AML [32].

In another study, the researchers believe that single agent TKI therapy is not curative and long-term survival remains low. They implement the idea of combination therapy. This would include agent that would induce apoptotic effects with would hopefully enhance

cytotoxicity against FLT3mut+ and wild type closes and potentially delay or prevent drug resistance. They combined venetoclax with a Flt3 tyrosine kinase inhibitor, gilteritnib as discussed before. This trial is in phase 1b clinical trial which means the safety and efficacy of the combination therapy for patients with relapsed or refractory AML is completed. The results showed that of the 15 patients treated in this clinical study, five of them had WT FLT3 and ten had mutant FLT3. The conclusions of the study were that combination therapy of venetoclax and gilteritnib showed to be well tolerated and showed 90% of patients had blast clearance. The trial showed that this therapy may be a highly effective treatment option for patients both with AML FLT3 mutation and those that have relapsed [30].

The effects of using FL, a immunomodulatory cytokine that acts through flt3, has shown important clinical use [2]. FLT3 inhibition is continuously evolving and leading to successes in the treatment of cancers. It is thought that the combination therapy of chemotherapy in addition of TKI would be a logical approach in the treatment of FLT3 mutated AML [31].

**FLT3-Inhibitor- Midostaurin (PKC412)**

Midostaurin is a first-generation FLT3 inhibitor, which are relatively nonspecific for FLT3 as stated above [23]. Researchers explained their phase 1B study of midostaurin with chemotherapy. This study was done in newly diagnosed adult patients with acute myeloid leukemia. They administered midostaurin to twenty-nine patients on three different dose schedules. For the first two dose schedules that includes receiving 100 mg midostaurin twice a day, 23 of the 29 patients failed to complete their planned therapy causing the discontinuation rate to be high. Adverse events occurred including nausea, vomiting, and diarrhea. Complete remission was achieved by 13 of the 29 patients. The third dose schedule includes patients receiving 50 mg of midostaurin twice a day with chemotherapy or after chemotherapy. The discontinuation rate was lower than the first. This combination therapy showed the midostraurin to be well tolerated in combination with chemotherapy. This study showed the potential of midostaurin with combination of chemotherapy shows improvements in the outcomes and overall survival of patients with FLT3-mutant AML. Although, this study did not account for the influence of stem cell transplantation or gene mutations other that FLT3. But this study did suggest the promising safety of the administration of these drugs and combination therapy for treatment for patients younger than 60 years [33]. Next, they conducted a phase 3 trial to confirm that the addition of midostaurin to chemotherapy would prolong overall survival in a population of 3277 patients from 18-59 years of age with newly diagnosed AML for FLT3 mutations. These patients were randomly selected to receive chemotherapy in addition to either midostaurin or placebo. The results showed that overall survival was significantly longer in the group that received the flt3 inhibitor rather than the placebo [34].

**FLT3-Inhibitor- Quizartinib**

Quizartinib is another FLT3 inhibitor that is active against both ITD mutant and wild type FLT3. In a phase 1 study of patients with AML it showed favorable results. Next, the Phase 2 study was completed in order to access the efficacy and safety of the drug administration. This include 333 patients in two cohorts. The first cohort was patients above the age of 60 with AML relapsed in less than a year. It was shown that 44% of these patients showed complete remission with quizartinib. However, some patients experienced fatigue, nausea, anemia, vomiting etc. The overall experience of the study showed that quizartinib monotherapy in patients show a high degree of activity. Many of the patients responded to the therapy and 8% were able to bridge to potentially curative hematopoietic stem cell transplantation [35].

Next, quizartinib was compared to the treatment of chemotherapy in relapsed or refractory FLT3-ITD AML patients. This was a randomized, controlled, phase 3 trial. It was shown that the treatment with the Flt3 inhibitor, quizartnib proved to have a survival benefit in comparison to chemotherapy. This study as well highlighted the potential of potent, selective Flt3 inhibitors and becoming a standard medical care for patients with the poor prognosis of AML [36].

**FLT3 Inhibitor- Sorafenib**

Sorafenib is a multikinase inhibitor that works to inhibit tumor growth as well as angiogenesis37. It does this

by inhibiting intracellular Raf kinases and cell surface kinase receptors. This drug has been evaluated in preclinical and clinical trials and has been approved by the U.S. Food and Drug Administration [38].

Researches discussed and examined the effects of Sorafenib, another FLT3 inhibitor, and its effects in older patients with untreated FLT3-ITD mutated AML. They did this by combining the sorafenib with 5-azacytidine. This treatment was for those that could not undergo chemotherapy because of age or fitness. The study comprised of twenty-seven patients with untreated AML and all of them had FTL3-ITD mutations. The study showed that the median survival of patients that received the combination therapy was 8.3 months of the entire group. There were three patients that were responding well so they went on to receive allogeneic stem cell transplantation. Those who responded had a higher median overall survival of 9.2 months (Figure 11). It was shown that twelve of the twenty-seven patients had secondary AML. Furthermore, the study showed that in older patients with FLT3-ITD mutated AML have worse results than do wild type FLT3 patients. However, patients with flt3-itd have slowing been improving in progress of outcomes. Sorafenib was discontinued because of its toxicity but when combined with AZA, it was well tolerated. This study had a small sample-size but their findings showed AZA in addition to sorafenib shows a great potential in treatment for elderly patients with FLT3-ITD AML. A larger sample size would be recommended [39].

Another study examined the effects of the combination therapy using Sorafenib, Cytarabine, and Idarubicin for initial therapy in younger patients with AML. First, they conducted a Phase I study to determine the efficacy and toxicity of the combination of these drugs. They treated patients with relapsed AML and chemotherapy to establish the feasibility of the combination. It was shown that sorafenib could be safety combined with chemotherapy. The results showed that the combination produced a higher complete remission rate as well as inhibition of FLT3 signaling [40]. This was the final report of a phase II study. This combination therapy was able to increase high complete response in patients with the FLT3 mutation and a one-year probability of survival of 74%. There were 62 patients involved in this study and 17 of them had the mutation of FLT3.

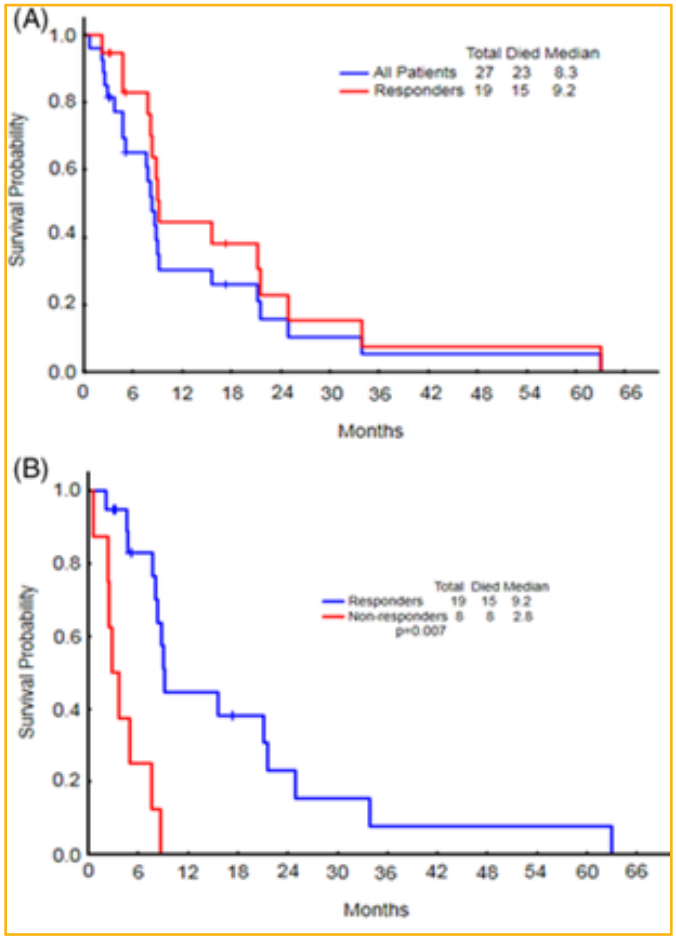


**Figure 11:** In the study, there were 27 patients that received combination therapy of sorafenib with 5-azacytidine (AZA). This figure shows the comparison of overall survival between all of the patients and the patients who responded. The median overall survival for all 27 patients was 8.3 months whereas the patients that were responders had a longer overall survival of 9.2 months [39].

Unlike other studies, this study showed significantly shorter overall survival for patients with FLT3 mutation. This could be due to the increased possibility of patients able to undergo allogeneic stem cell transplant as a result of sorafenib addition. The result of this study should be to better discern the potential role of sorafenib in the treatment of patients with FLT3-ITD AML [37].

In 2013, a Phase III study was directed on the administration of sorafenib to newly diagnosed AML elderly patients 60 years of age or older. They randomly

assigned patients to a placebo or sorafenib between chemotherapy cycles. They found no statistically significant improvement in event-free survival or overall survival in the arm treated with sorafenib. Essentially, they found more adverse effects that occurred in the treated arm. This study showed the lack of effects of sorafenib in elderly patients with AML, suggesting it was not beneficial [41].

A randomized, double-blind, placebo-controlled study was conducted to access the effects of sorafenib because of its potential to be an effective drug for the treatment of AML. Here, they conducted a Phase II trial with patients of the age 18-60 years old. This study found that the addition of sorafenib to chemotherapy increased toxicity but did show to have antileukemic efficacy. They advise to find a strategy to reduce the toxicity to determine a future role of this inhibitor in the treatment of this disease [42].

**FLT3 Inhibitor- Sunitinib**

Another first generation FLT3 inhibitor used is named Sunitinib. They explain how sunitinib was the first FLT3 inhibitor to be used in a clinical setting. It has showed to have equal efficacy against FLT3-itd mutation so it can be used in almost all patients with FLT3 mutation in contrast to quizartinib or sorafenib. This was used in a phase I/II trial is Here, they treated newly diagnosed AML elderly patients over the age of 60 with the presence of FLT3-ITD or tyrosine kinase domain. They found sunitinib added to chemotherapy showed respectable toleration and the complete remission rates and long-term outcome proved to be favorable in comparison to published studies. This showed the need for further investigation on this first generation FLT3 inhibitor in combination with chemotherapy [43].

**FLT3 Inhibitor- Crenolanib**

Crenolanib is a type I FLT3 tyrosine kinase inhibitor. This is a second-generation FLT3 inhibitor. This type I oral inhibitor inhibitors both FLT3-itd and FLT3-tkd mutations. However, this drug does not inhibit c-kit which allows for a full count recovery even when it is combined with chemotherapy. This study reports a phase II trial for the efficacy and the ability to tolerate the drug when it is combined with chemotherapy. They administered the drug in combination with cytarabine and anthracycline induction chemotherapy to patients with newly diagnosed AML. Their results displayed crenolanib to be safely combined at full doses with cytarabine/daunorubicin or cytarabine/idarubicin induction and chemotherapy. This study showed a 88% complete remission rate with full count recovery after one cycle of induction. This allowed allogenic stem cell therapy to be able to be administered on schedule. This trial continues but this phase showed encouraging results of anti-leukemic activity with no relapses of patient younger than 60 with flt3-mutant AML [44].

**Consequences of FLT3 Inhibitors**

When creating and exploring new treatment options, undesirable and unexpected results have the possibility of occurring. The unexpected results can be harmful and do the opposite of treating the patient. These need to be discussed and reviewed in order to prevent them from occurring again. For example, one study explains potential consequences of FLT3/ITD AML treatment. FLT3/AML is a disease that is shown to change between diagnosis and relapse. Leukemia cells become more drawn to FLT3 signaling after recurrence after chemotherapy. It has been shown that after the treatment of chemotherapy, this leads to a high level of FL in the plasma during recovery. Since FL acts through FLT3, when FLT3 in mutated, those high levels of FL from chemotherapy will maximize its activity in promoting the survival of leukemia blasts. This could be a large issue in the treatment of AML since 30% of them are shown to have the FLT3/ITD mutation. This leads to the questioning of chemotherapy promoting the relapse of FLT3/ITD AML. These findings can be used to predict clinical responses and help design treatments [45].

It is important to discuss potential consequences to FLT3 inhibitor therapy. These include cutaneous reactions in patients with AML which include leukemia cutis, sweet syndrome, infections, and treatment related effects. One study reports three cases of FLT3 inhibitor associated neutrophilic dermatoses. These patients had flt3mut+ AML and participated in clinical trials for treatment. First, there were seventy-seven patients in this clinical trial and only three of them developed confirmed cases of neutrophilic dermatosis. The first patient was in her 40s and relapsed with FLT3-ITD. She began to develop tender, red subcutaneous nodules

on her back and lower extremities. The second patient was a man in his seventies that developed postulonodules on his cheeks, ears, and scalp (Figure 12). Lastly, patient was a woman in her sixties that also developed tender, red eruptions on her neck and arms. All of these patients had to stop their treatments of the FLT3 inhibitors. These patients have had terminal differentiation of leukemia cells to neutrophils during their treatment resulting in neutrophilic dermatoses. This study discussed the importance of dermatologist awareness during the treatments with FLT3 inhibitors. This shows a complication of molecular-targeted therapy of AML and something to be aware of when proceeding with further investigation [46].

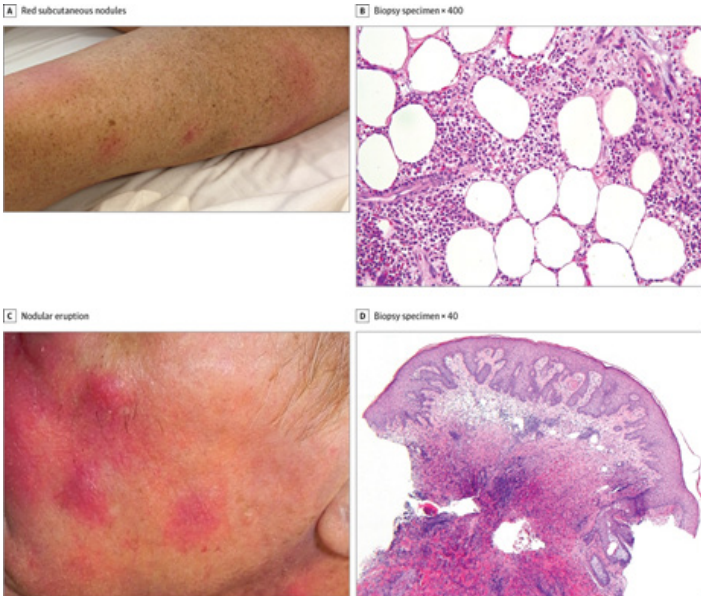


Figure 12: On the left shows the clinical findings in FLT3 inhibitor-associated neutrophilic dermatosis. This includes red subcutaneous nodules on the lower extremities of the patient and nodular eruption of the cheek. On the right-hand side, shows pathologic findings of the same thing confirming the diagnosis of neutrophilic dermatoses [46].

**Heat Shock Proteins (HSPs)**

Heat shock proteins (HSPs) are chaperone proteins that help proteins in the regulation of folding/unfolding. These proteins are expressed at low levels in normal physiological conditions but respond to stressful events at high levels. These proteins have shown to be a potential therapeutic target in AML. In a study, they took AML cells from 75 patients. The patients that had FLT3-ITD had an overrepresentation of HSP levels which showed that there was a strong dependence of HSPs in stabilizing FLT3-ITD encoded oncoproteins. This study showed that HSP90 inhibition mediates anti-leukemic effects through direct and indirect activity. They observed an increased frequency of FLT3 mutations in patients with high HSP levels. They believe from their observations that patients with FLT3 mutations have distinct HSP expression profile that supports leukemogenic effect of mutated FLT3. In conclusion, this study showed HSP90 inhibitors had a pro-apoptotic effect for most AML patients with FLT3-ITD [47].

**Summary & Conclusions**

In summary, the effect of FLT3 stimulators and their role in stem cell maintenance and therapy was described above. FLT3 is a receptor tyrosine kinase that is expressed by immature hematopoietic cells. This receptor is vital in the process of normal hematopoiesis as well as the immune system. However, patients with acute myeloid leukemia show to have a mutation in this receptor. The role of FLT3 in hematopoiesis has provided information of how FLT3-related leukemias, like acute myeloid leukemia, can be treated.

Once this receptor, FLT3, is mutated, it can dimerize, phosphorylate, and activate its kinase domains without the need for the ligand to bind. This is known as auto-phosphorylation. This results in the constitutive activation of FLT3. When this happens, downstream gene expression is disrupted and can be responsible for the development of leukemia. This is because normal FLT3 signaling has shown to increase the survival and growth of cells. However, the mutation of FLT3 signaling has shown the disruption in differentiation which results in the cell to transform into a leukemic blast, therefore, resulting in patients with leukemia. Patients with the FLT3 mutation have AML which has a very poor prognosis. Because of this, studies have brought about the idea of FLT3 inhibitors for a potential treatment of this disease for those who have that mutation. There have been positive randomized trials of the FLT3 inhibitor drugs that highlight the potential of this treatment in those patients. Many reports have been identified that shed light on the progess in this field. The only treatment for AML is chemotherapy

which includes many complications include relapse and toxicity. FLT3 inhibitors now emerge as an important part of therapy for high risk patients with AML that have no option of treatment.

The large amount of information on this topic discussed above has increased knowledge regarding the role and importance of FLT3 in the process of normal hematopoiesis. However, since most of the inhibitor drugs are still in the beginning of their research, more information and investigation is needed. In addition, the clinical significance of FLT3 signaling requires further investigation in its role in leukemia.

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