

FLT3 Inhibitors in the Treatment of AML

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What is known about the FLT3 enzyme and gene?

FLT3 is a gene that encodes an enzyme called a tyrosine kinase (TK), and there are approximately 90 genes in this class in the human genome. TKs are regulators of growth and proliferation of cells. Often, these enzymes will respond to exogenous signals that trigger cell growth and initiate a cascade that begins at the plasma membrane of the cell and ends in the nucleus, resulting in proliferation and enhanced survival of that particular cell.

In the blood system, this cascade is a normal homeostatic mechanism for regulating the growth of blood cells. For example, in the event of an infection the number of white blood cells (WBCs) needs to be increased. The TKs are often the mediators of the body's signal that triggers an increase in WBC production. However, one of the key components of this process is that once the infection is gone, the WBC count should return to normal levels, because the stimulus that switches on the TKs is no longer present.

In acute myeloid leukemia (AML), which remains very difficult to treat, it has been found that the *FLT3* gene is mutated in approximately 30% of cases. *FLT3* is a TK that signals the growth and proliferation of blood cells in the bone marrow; it is one of several TKs with this property. The *FLT3* mutation identified in AML causes this gene to be constitutively activated, meaning that it is constantly turned on and does not turn off, so proliferation and survival signals are sent to bone marrow cells continuously. This mutation is thought to be a major contributor to the development and progression of AML.

Is it possible to inhibit the FLT3 TK using molecularly targeted therapy?

There is proof of principle that this approach can work. Imatinib (Gleevec, Novartis) is a very effective treatment for chronic myelogenous leukemia (CML), which is caused by a different constitutively activated TK, which, in the case of CML, is ABL, not *FLT3*. Imatinib is a small molecule that inhibits the ABL kinase, resulting in the death of CML cells. The inhibition of ABL and subsequent cell death by imatinib explains why this agent is so effective in

treating CML. With AML, the hope is that small molecule inhibitors of mutant *FLT3* might, by analogy to imatinib in CML, be effective treatment for AML patients who harbor the *FLT3* mutation.

Why does FLT3 become mutated?

There is some understanding about what might cause genes like *FLT3* to become mutated. For example, some congenital disorders increase the likelihood of a mistake occurring during DNA replication. These mistakes may contribute to the development of leukemia or other cancers. However, most AMLs are not inherited or caused by congenital disorders; most are acquired and seem to occur out of the blue. Every day, the body produces 10 billion WBCs, which live for only approximately 6–8 hours, and thus there is a huge demand for the replication of these cells. Within each cell are approximately 3 billion base pairs of DNA. Replicating 10 billion WBCs per day with 3 billion base pairs each can overwhelm the fidelity of the DNA replication machinery. Many mistakes in bone marrow cell replication occur every day, but most often they are in places that do not lead to any further problems. However, if a certain type of mutation occurs in a critical location in the genome, such as the *FLT3* gene, then leukemia can develop.

Does the presence of the FLT3 mutation correlate with the severity of AML?

There are several types of mutations that can occur in *FLT3*, and it appears that all of these confer a poor prognosis in AML. This group of patients is particularly difficult to treat with conventional chemotherapy, and they often relapse after treatment. In addition, there are 2 *FLT3* gene copies present in our cells, one on each chromosome. Drs. Bloomfield and Caligiuri, and other groups, have found that if both copies of *FLT3* are mutated, the prognosis is worse than if just one is mutated.

Could you describe some of the preclinical research on FLT3 mutations in AML and FLT3 inhibitors?

The seminal finding that *FLT3* was mutated in AML cells was published by Nakao et al in 1997. This Japanese research

group found that *FLT3* mutations were present in AML cells and not in normal cells, but the physiologic consequence of the mutations in *FLT3* were not understood.

Over the subsequent years, several laboratories found that a number of different types of mutations in the *FLT3* gene activate the FLT3 TK. As a consequence, FLT3 sends signals to the cell for continuous growth and proliferation, and cannot be turned off. These important observations indicated that this signal might be critical to the growth of leukemia cells.

Our group and several others began to develop strategies to screen for compounds that could inhibit FLT3, focusing in particular on cell-based strains. Using cells that are transformed by *FLT3* mutations, it is possible to assay for compounds that will kill those cells specifically based on the expression of the mutated *FLT3*. Our group, which included Dr. Jim Griffin and Dr. Richard Stone of Harvard Medical School and the Dana-Farber Cancer Institute, worked primarily with 2 small-molecule compounds: PKC412 (Novartis) and MLN518 (Millennium). Other small-molecule compounds that have been under investigation include SU11248, initially developed by Sugen and now by Pfizer, and also CEP-701, developed by Dr. Don Small at Johns Hopkins University in collaboration with Cephalon. All 4 of these compounds are potent FLT3 inhibitors.

Preclinical studies for each of these compounds reported similar findings: the compounds each turn off the FLT3 signal; each is effective in killing leukemic cells containing mutated *FLT3* in a cell-based assay system. Such in vitro findings were followed by studies in mouse models, which have shown that FLT3 inhibitors appear to have appropriate pharmacokinetic properties, do not have undue toxicities, and are effective in treating mouse models of leukemia induced by mutated *FLT3*. These findings in part provided a preclinical platform that enabled FLT3 inhibitors to eventually move forward into phase I and II clinical trials in humans with AML.

Are there any anti-FLT3 monoclonal antibodies in development?

Yes, ImClone has developed an antibody that binds to the FLT3 receptor TK that has shown promising activity in early preclinical trials. It is possible that several strategies, including both small-molecule inhibitors and monoclonal antibodies, will prove to effectively inhibit FLT3.

What have clinical trials found thus far in regard to the various FLT3 inhibitors?

There is not yet any phase III data on the FLT3 inhibitors, although all of the small-molecule compounds mentioned above have been through some form of phase II testing. In the phase I trials, each of these compounds appeared to be safe, without severe toxicities, and were effective at inhibiting FLT3. Based on the phase I results, it could not be said whether one compound was more effective than another.

Similarly, the phase II data on the FLT3 inhibitors as single agents shows that each compound appears to be active, with an often remarkable reduction in the peripheral blood blast percentage. For reasons that are not fully understood, there is less of a reduction in leukemic cells in the bone marrow.

Therefore, only a small fraction of patients in phase II studies have achieved a complete remission with any of the FLT3 inhibitors currently under evaluation, and in many cases there is not a significant reduction in the bone marrow blasts. It appears as if the leukemic cells in the bone marrow are somehow protected, whereas leukemic cells in the peripheral blood seem to be more susceptible to inhibition.

Another important observation from clinical trials thus far is that even the most dramatic responses in the peripheral blood blasts are typically short-lived. The response duration may be in the range of a few days to 30–40 days, but ultimately the leukemic cells return.

It is worth noting that the patients treated in these clinical trials constitute the worst prognostic group. Most had relapsed AML with the *FLT3* mutation, which is known to confer a poor prognosis. It was not expected that FLT3 inhibitors given as monotherapy would be curative. The purpose of these trials was primarily to see if they showed promising activity in treating AML in a clinical setting.

Could you describe any side effects that were seen in these trials?

One might expect that FLT3 inhibitors would be associated with many side effects because they inhibit not only FLT3 but also a range of other TKs, different for each compound. These agents are selective but not perfectly specific. Imatinib is also a selective inhibitor, targeting ABL as well as 4 other TKs. Since FLT3 is important for the growth and control of normal cells, it is somewhat surprising to see such a modest side effect profile. Most of these compounds are extremely well tolerated. Each has certain side effects, but far less severe than those seen with the standard intensive chemotherapy, which is still the cornerstone of treatment for AML.

Why are the responses to FLT3 inhibitors so short-lived?

The short-lived response duration is incompletely understood right now. With imatinib, such resistance is often attributed to mutations in the ABL kinase itself. In the limited number of patients we have evaluated in regard to resistance, a similar resistance mutation has not been identified in FLT3. Resistance can also develop due to increased metabolism of the drug or due to the leukemic cells pumping the drug back out of the cell. P glycoprotein is responsible for resistance to certain types of chemotherapy; a similar mechanism could also be related to resistance to FLT3 inhibitors.

In what ways are the anti-FLT3 compounds currently in clinical development different from one another?

All of the small-molecule FLT3 inhibitors work by binding to the adenosine triphosphate (ATP) binding site. The TKs require ATP as a binding source to send their proliferation signals to the cell. The FLT3 inhibitors thus preclude TK activation by inhibiting the binding of ATP. All of the FLT3 inhibitors currently in clinical trials share this property.

However, although each of these compounds inhibits ATP binding to FLT3, they differ in their chemical structure.

These structural differences may eventually be very useful in the clinical development of FLT3 inhibitors, because if resistance develops to one compound, it may be possible to use one that is structurally different to overcome resistance. As an example, with the reverse transcriptase inhibitors used to treat human immunodeficiency virus, each one alone engenders rapid resistance, but when 2 or more are given together, the development of resistance can be prevented. It is hoped that if resistance to FLT3 inhibitors develops in AML, the FLT3 inhibitors can be used in combination or sequentially to circumvent the problem.

Are FLT3 inhibitors being evaluated in combination with chemotherapy in clinical trials?

Now that clinical trials have identified some activity in relapsed AML, it may be best to move FLT3 inhibitors to the upfront setting, in combination with intensive induction therapy. There are 2 trials studying this strategy, one with PKC412 and one with CEP-701. The proof of principle that this approach may be effective can be seen with all-trans retinoic acid (ATRA), which is used to treat acute promyelocytic leukemia. ATRA is effective for short periods of time, but patients eventually relapse. However, ATRA plus chemotherapy is curative for acute promyelocytic leukemia patients. It is hoped that a similar effect will be found when FLT3 inhibitors are given in combination with chemotherapy.

What are other future research directions with FLT3 inhibitors?

It is important to try to develop newer and better inhibitors. One strategy is to develop higher affinity inhibitors, which might be more potent. In addition, more understanding is needed about the mechanisms of resistance and how to circumvent this resistance. There is interest in combining FLT3 inhibitors with other molecularly targeted therapies in designs that are perhaps more sophisticated than simply using intensive chemotherapy.

Finally, it is important to remember that only 30% of AML patients have the *FLT3* mutation. For the other 70% of patients, it is very likely that there is another mutation controlling growth and proliferation of cells. We need to continue using genomic strategies such as high-throughput DNA sequencing in order to identify mutations in other TKs or related kinases in order to locate additional targets.

Is it easy to identify the 30% of AML patients with the *FLT3* mutation?

In a given patient, it is easy to identify a mutation in *FLT3* using DNA amplification and sequencing. In the context of a clinical trial, identifying patients with the *FLT3* muta-

tion can be challenging because patients need to be treated fairly soon after they present, but sequencing can take 24–48 hours. From a practical perspective, there is a certain amount of complexity in genotyping each patient.

Should all AML patients be considered for clinical trials?

It is important that all AML patients be considered for clinical trials, particularly those with the *FLT3* mutation because they are known to have a poorer prognosis, and investigators are actively working on improving treatment for these patients. Several centers around the country are conducting trials with 1 or more of the FLT3 inhibitors.

How has imatinib affected the course of drug development?

It seems that the paradigm that started with imatinib for CML will be broadly extrapolated to other blood-based cancers as well as solid-tumor diseases. Gastrointestinal stromal tumors are responsive to imatinib, and there are data showing that similar strategies targeting the TK epidermal growth factor receptor with small molecule inhibitors like gefitinib (Iressa, AstraZeneca) have activity in non-small-cell lung cancer, one of the most challenging cancers to treat. The notion that a cell can be killed by inhibiting its growth signals using a relatively nontoxic agent is very encouraging. Hopefully, this strategy will lead to improved treatments for a broad spectrum of cancer types. The success of imatinib has also resulted in increased interest in the pharmaceutical industry for developing novel TK inhibitors, and other molecularly targeted therapy for cancer.

Suggested Reading

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