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FLT3 inhibition as tailored therapy for acute myeloid leukemia

The clinical success of the specific tyrosine kinase inhibitor, STI571[1,2] (otherwise known as Glivec® or imatinib, Novartis Pharma), has fostered onco-hematologic research worldwide to develop new molecularly targeted forms of therapy. The target of imatinib is preferentially BCR-ABL, an intracellular oncogenic tyrosine kinase that shares several homologies with the class III receptor tyrosine kinase (RTK) family, whose members include the FLT3, KIT, FMS, and PDGF receptors.[3,4] Most of these RTKs are implicated, either in mutated or wild-type conformations, in the constitutive activation and proliferation of human leukemias, especially acute myeloid leukemia (AML).[3,5]

Particular interest has been aroused by the relatively high frequency of FLT3 receptor mutations found in AML. The FLT3 receptor has several structural domains, including 5 immunoglobulin-like domains in the extracellular regions, a juxtamembrane (JM) domain, 2 kinase domains (TK1 and TK2) separated by a kinase insert (KI) domain, and a C-terminal domain in intracellular regions.[4] Ligand binding to the RTK extracellular domain leads to receptor dimerization, stabilizes an open conformation of the catalytic domain (A-loop) for adenosine triphosphate (ATP) and substrate binding, and enables transphosphorylation of the A-loop. The subsequent phosphorylation of tyrosine residues accompanies RTK activation. Consequently, the FLT3 receptor leads to induction of fundamental intracellular signaling pathways, which in turn regulate both cell proliferation and apoptosis.[3]

The FLT3 receptor has been found to be frequently targeted in AML and somewhat less commonly in myelodysplastic syndromes (MDS) by two different types of genetic alteration.

Firstly, an internal tandem duplication (ITD) of the JM domain-coding sequence of the *FLT3* gene (FLT3/ITD) is found in 20% to 41% of adult and pediatric patients with *de novo* or secondary AML, as well as in about 3% of patients with MDS,[6-10] as shown also by Moreno *et al.*[11] in this issue of Haematologica. These mutations constitutively activate the receptor, and are strongly associated with hyperleukocytosis, poor response to therapy and dismal prognosis. Among the distinctive forms of AML, higher frequencies of FLT3 alterations have been detected in acute promyelocytic leukemia (APL)[12] and in AMLs with apparently normal karyotype.[6-11] The reasons underlying these associations are currently unclear. When transplanted in murine hematopoietic progenitors, the mutant FLT3 receptor causes cellular transformation and produces a myeloproliferative syndrome, even though this does not by itself appear sufficient to cause acute leukemia.[13,14] An additional length mutation affecting the tyrosine kinase domain in exon 20 has been recently described.[15]

Secondly, point mutations in the FLT3 receptor have been reported to occur in 3% to 8% of AML patients, mostly at a specific site in the gene (D835 and I836)[16] (Figure 1). Although not apparently associated with either leukocytosis or worse prognosis, these point mutations result in similar deregulatory activity on the receptor and cause its constitutive activation and tend to worsen disease-free survival. Furthermore, the point mutations occur independently of FLT3/ITD. Taken together, these observations indicate that *FLT3* currently appears to be the most frequently mutated gene and constitutively activated receptor in AML.

The FLT3 receptor as a candidate target for tailored therapy in acute myeloid leukemia

Following the remarkable success of STI571, several researchers have pointed to the FLT3 receptor, or its signal transduction pathway,[17] as a possible specific target for tailored therapy. Several tyrosine kinase inhibitors, which were not originally developed with FLT3 as the intended target, have been reported to inhibit the FLT3 receptor on AML cell lines or prima-

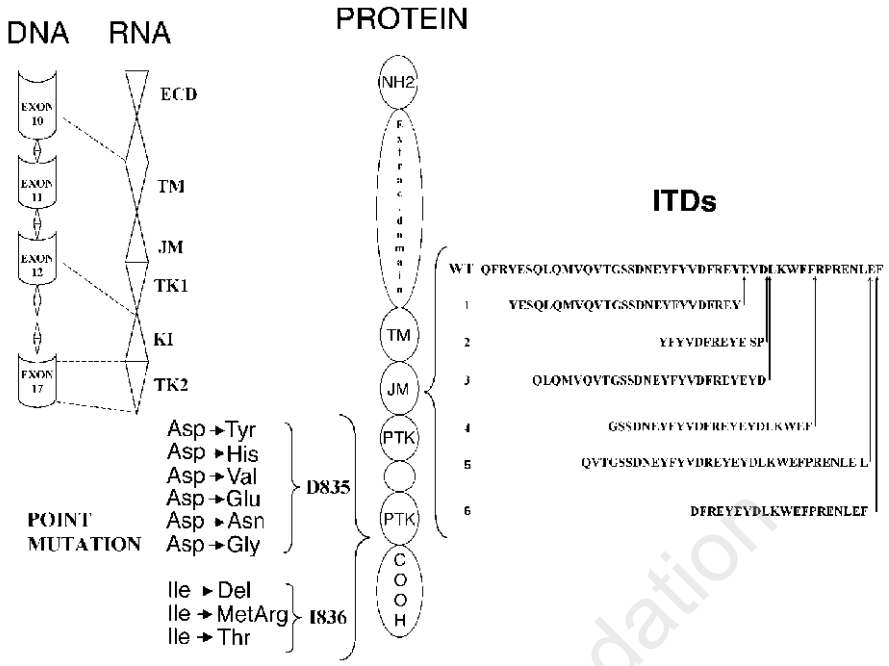

Figure 1. Schematic representation of the FLT3 receptor kinase domains with point mutations and some types of ITD. Schematic representation of the FLT3 receptor kinase domains: Genomic, mRNA and protein structure of the FLT3 receptor. Right side of the figure: DNA: schematic illustration of exons 10, 11, 12 and 17 of the FLT3 receptor gene. RNA, messenger RNA representation: extra cellular domain (ECD); transmembrane domain (TC); juxtamembrane domain (JM); tyrosine kinase domain (TK1); kinase insert (KI); tyrosine kinase domain2 (TK2); C terminal region (COOH). Protein: representation of protein domains of functional FLT3 receptor. The kinase domain is not drawn to scale. Middle of the figure: two types of activating mutations in FLT3 are associated with AML; the first type consists of ITDs of amino acids in the JM domain resulting in constitutive tyrosine kinase activation. WT, wild-type amino acid sequence of the region usually involved in ITDs;[1-6] some derivative ITDs sequences with the point of insertion (arrows). The second type of mutation consists of point mutations in the so-called *activation loop* of the second tyrosine kinase domain. Mutations at two specific residues, aspartic acid (D835) and/or isoleucine (I836), also result in constitutive FLT3 activation. Amino acid substitutions are depicted; arrows indicate the points of insertion of representative ITD sequences. *Modified from: Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. Blood 2002;100:1532-42.*

ry blast cells. These include the tyrphostin AG1295[18] or AG1296,[19] and herbimycin A (HA).[17,20,21]

Following on from their preliminary observations on the inhibitory activity of HA, Minani *et al*.[20] suggested that the mechanism behind the cytotoxicity of this ansamycin derivative, which is now known to target Hsp90, could be mediated by the inhibition of phosphorylation of ITD-FLT3. Exposure to another Hsp90 inhibiting HA, namely radicicol,[22] was able to dissociate ITD-FLT3 from the Hsp90 chaperone complex, activating blast cell apoptosis. More recently, inhibition of FLT3 has been reported in pre-clinical studies of other RTK inhibitors, namely CEP-701 or KT-5555,[23-25] PKC412,[26] and CT53518.[27] Moreover, in an interesting accompanying review that appeared in the same issue of Cancer Cell,[28] Sawyers reported a fourth inhibitor, SU11248,[29] that is now being evaluated in a clinical trial (in which we are directly involved). All four molecules have been designed to bind the receptor specifically and compete with ATP in its ATP-binding pocket. CEP-701 exerts an inhibitory action on the TrkA receptor tyrosine kinase.[23] The inhibitory activity of PKC412 extends to RTK other than FLT3, such as protein kinase C, VEGFR2, PDGFR, c-Kit, and FMS.[26]

Similarly, Millenium's compound, CT53518 and Sugen's SU11248 (the latter derived from the chemical research and development of SU5416)[28,29] both have further inhibitory effects on PDGFR and c-kit.[27]

With four FLT3 inhibitors heading into the clinic, is it possible to predict which patients stand to gain most benefit from these drugs?

Assuming that FLT3 is a suitable target for molecular therapy in AML, we can postulate that enrollment in clinical trials should be proposed for those patients with FLT3 receptor alterations in their leukemic cells. Furthermore, treatment could be extended to those AML patients refractory or resistant to conventional chemotherapy and/or with associated or concomitant mutated c-Kit and PDGFR translocation.[30-33] Based on pre-clinical studies, we can expect that AML patients with FLT3 alterations may show some clinical response.

Are all AML patients likely to respond to FLT3 inhibitors?

In CML Ph+ patients in chronic phase, the molecular target of imatinib is ABL, present either in its P210 form (as in the BCR-ABL fusion protein) or in its wild-type form. Furthermore, the BCR-ABL oncogene is absent from normal stem cells but present in its double isoform (b2-a2 and b3-a2, with very rare exceptions)[34,35] on the leukemic cells, offering a unique molecular target for imatinib's selectivity. On the other hand, FLT3 receptors carrying ITD exhibit more heterogeneous forms of mutation, giving rise to several individual structural forms of the receptor. It is likely that it will be difficult for the inhibitor drugs to recognize, bind and inhibit all these forms. Based on the different chemical formulations of the four inhibitors, and again learning from the ABL/imatinib interaction experience,[36] we can hypothesize that the different inhibitors are likely to show different combining activities with each form of the mutated FLT3 receptor. We may expect various degrees of clinical responsiveness among patients, simply based on the specific interactions of each inhibitor with variable forms of FLT3 ITD. We could also envisage peculiar, individual degrees of sensitivity to the same inhibitor among patients sharing the same molecular defect. In this respect, it is important to consider that, distinct from CML, AMLs comprise a spectrum of genetically heterogeneous diseases which may account for different sensitivities to the inhibitor.

Is it possible to predict resistance to these inhibitors? And could there be "intrinsic" resistance pre-dating the start of therapy?

It is now clear that resistance to imatinib is mostly due to point mutations in or around the ABL ATP binding pocket. Despite recent observations suggesting that *polyclonal* resistance to imatinib could be present prior to the initiation of therapy in a few CML patients,[37] in the majority of cases cellular resistance to imatinib is acquired during treatment. Multiple independent mutant clones seem to emerge during treatment with imatinib, along with the ABL point mutations that have been detected in relapsed cases,[37] and a clonal selection of these cells would confer refractoriness that could be defined as *acquired resistance*. On the other hand, the mutated FLT3 receptor form is present from the onset of disease in the majority of AML patients and ITD or point mutations are associated exclusively with the leukemic clone: either the ITD or the point mutation, or a combination of the two, may confer a proliferative advantage to the blast cell. If this is the case, resistance to FLT3 inhibitors could be present from the onset of disease (i.e. *intrinsic* resistance). Drug inhibitory pressure could then foster a clonal selection of cells with a second or further mutation, conferring further resistance (*acquired* resistance).

From the co-crystal structure of the ABL kinase bound to imatinib[36,38,39] it has been postulated that *acquired* drug resistance[35] depends on a reduced interaction between imatinib and the mutated ABL form, the latter being due to a single amino acid substitution. To overcome this obstacle, a mutated form of imatinib[36] and alternative inhibitors, such as PD173955,[36,40] have been designed. PD173955 is able to bind the ABL ATP pocket in the so-called *on* conformation, which is supposed to be more accessible to the drug. At present, however, there are no data on the co-crystal structure of the FLT3 receptor either in its wild-type conformation or in its mutated form. Furthermore, no information is available about the co-crystal structure of the FLT3 receptor associated with any of the inhibitors. In the absence of information regarding the structure whereby the receptor binds to the inhibitors, one can hardly speculate on *intrinsic resistance*.

In conclusion, unlike CML, AML with FLT3 ITD or point mutations are characterized by multiple genetic forms. This variety of forms hampers prediction of success rates for the majority of patients treated with any of the four new FLT receptor inhibitors. Only clinical trials will reveal whether this treatment can provide a viable option for such patients. On the other hand, extensive molecular characterization of FLT3 gene status will be required to determine whether a given alteration type is more susceptible to targeted inhibition. Our personal preference would be to include relapsed AML patients carrying FLT3 receptor ITD and/or point mutations in initial trials. Combined with extensive and multiparametric genetic characterization of enrolled cases, this would favor rapid identification of responders. Mechanism(s) of resistance could then be investigated and hopefully identified in subsequent studies.

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