

S-9-3

## **Oncogenic FLT3 as an important target for acute myeloid leukemia: beyond ATRA and imatinib**

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Although traditional cytotoxic agents have been successful in inducing disease remission in nearly 80% of adult patients with acute leukemia, the majority of them eventually develop relapse. Clinical studies have taught us that dose- and/or combination-modification of cytotoxic chemotherapeutic regimen have little improved the prognosis. Against acute promyelocytic leukemia, however, we have a distinct success using all-trans retinoic acid in acute promyelocytic leukemia (APL). Pharmacological concentration of ATRA modulates the transcriptional function of PML-RARA and promotes the differentiation of APL cells. Clinically the combination of ATRA with chemotherapy has made a breakthrough in the prognosis of APL. Another success is imatinib, which is a kinase-inhibitor which target Abl, PDGFR and c-KIT at a nanomolar level. Imatinib now becomes a gold standard medicine for chronic myeloid leukemia in a chronic state. Recently the Japan Adult Leukemia Study Group reported that for patients with Ph-positive acute lymphoblastic leukemia, the combination of imatinib with chemotherapy rapidly induce high-quality complete remission. The characteristic common to the above two drugs are to target dominantly mutated oncoprotein associated with leukemogenesis. According to these lessons, we expect the oncogenic FLT3 as a new target for the treatment of acute myeloid leukemia.

FLT3 is a member of receptor tyrosine kinase class III and expressed on the cell surface of haematopoietic progenitors. The ligand of FLT3 (FL) is expressed as a membrane-bound or a soluble form by bone marrow stroma cells. Ex and in vivo

experiments have disclosed that FL-FLT3 interaction plays an important role in the maintenance, proliferation, and differentiation of immuno-hematological cells. We and others have shown that internal tandem duplications (ITD) of FLT3 are currently the most frequent single mutation described in AML (20-25%), and that kinase domain mutations (KDM) are also found in 5-7% of AML. These FLT3 mutations are associated with leukocytosis and poor prognosis of AML.

FLT3/ITD is ligand-independently dimerized and phosphorylated irrespective of the location and length of the ITD, and dominantly activated wild-type FLT3 expressed in the same cell. It has been shown to confer growth factor-independence on factor-dependent cell lines such as Ba/F3 and 32D cells, and to induce the constitutive activation of downstream signaling molecules such as signal transducer and activation of MAP kinase, Akt, SHC, Cbl, Vav and SHP. FLT3/ITD is likely to transmit not only constitutive but also additive signals such as STAT5a, implying an oncogenic function of FLT3/ITD such as anti-apoptosis and differentiation-block. Furthermore, FLT3/ITD but not wild-type FLT3 is stabilized with HSP90 chaperon complex, which may explain the sensitivity of FLT3/ITD-transformed cells to HSP90-inhibitors.

The KDMs, represented by D835Y, were found to cause the constitutive tyrosine phosphorylation of FLT3 and confer IL-3-independent growth on 32D cells, indicating a similar property to ITD. In the inactive form, the activation loop has been thought to block the access of ATP and the substrate to the kinase domain. Ligand-induced

activation leads to phosphorylation (Y842) within the loop causing an active configuration to be adopted and allowing kinase activity. The KDMs are thought to mimic the latter by interfering with the inhibitory effect of the loop, resulting in constitutive kinase activation. According to the 3D-model, D835 is found to be located near the key “hook” (Y572) within the JM domain. The KDMs are therefore similar to ITD mutations as to disrupting the auto-inhibitory mechanisms of FLT3. However, the dimerization is not needed for the constitutive activation in the case of KDMs, whereas the JM domain with ITD is involved in homo- and hetero-dimerization by itself. Further study is necessary to elucidate the biological difference between wild-type and mutant FLT3.

We also found that the high expression of wild-type FLT3 transcripts is observed in nearly 10% of AML and that it is an unfavorable prognostic factor for overall survival in AML patients without FLT3 mutation. Overexpressed wild-type FLT3 reveals autophosphorylation and has the high sensitivity to the FLT3 inhibitor as FLT3/ITD.

To date, several FLT3 kinase-inhibitors have entered clinical trials in the USA, while they are cross-inhibitory to other kinases in varying degrees. The early clinical studies targeting FLT3 for AML have indicated that reduction of peripheral blast cells and myelosuppression are observed in majority of patients, and that a partial response is achieved in some of them. Since these studies incorporated refractory AML patients to conventional chemotherapy, it seems not to be far unexpected that the response rates of FLT3-inhibitors were lower than those of imatinib to CML patients. On the other hand, these early reports have disclosed several problems (e.g. plasma concentration and adverse events), which should be carefully investigated at preclinical and clinical studies. We are now searching for FLT3-inhibitors from the view points of kinase specificity, effectiveness both to FLT3/ITD and D835-point mutation, excellent pharmaco-kinetics and dynamics and low toxicity. We believe that development of FLT3-specific small-molecule inhibitors

for use in combination with conventional chemotherapy will lead to an improved outcome of AML.

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