Rational for targeting the hedgehog signalling pathway in acute myeloid leukemia with FLT3 mutation

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The *FLT3* gene is frequently altered in acute myeloid leukemia (AML) and represents a hallmark of adverse prognosis (1). FLT3-ITD mutation induces constitutive tyrosine kinase activity of the receptor which is located in the endoplasmic reticulum (ER) where it activates STAT5. Clinical trials using FLT3 tyrosine kinase inhibitors (TKI) as monotherapy in AML patients with FLT3 mutation show clinical activity but failed to demonstrate long lasting remissions. Combining FLT3 TKI to vidaza, an hypomethylating agent, demonstrated improved activity and results from the ALLIANCE study which randomized the addition of midostaurin to standard chemotherapy at diagnosis showed a significant improvement in the 5-year overall survival in the combination therapy (2). However, the use of FLT3 TKI led to the identification of resistance mechanisms: (I) the overexpression of oncogenic kinases such as SYK, the AXL receptor tyrosine kinase and the PIM kinases; (II) the gradually increasing levels of FLT3-ligand occurring after repeated cycles of chemotherapy that impair the ability of TKI to inhibit FLT3-ITD activity; (III) the bone marrow microenvironment (BMM) which secretes molecules and generates cellular interactions reported as cytoprotective toward AML cells treated with TKI; (IV) the appearance of acquired mutations in the FLT3-ITD allele that frequently occur within the kinase domain and confer TKI resistance (3).

Understanding the intrinsic or extrinsic mechanisms that limit TKI efficacy is essential. In a recent paper, Lim *et al.* study the role of the highly conserved hedgehog (Hh) signalling pathway whose activation in FLT3-ITD leukemia may contribute to their development (4). The Hh pathway is complex and active in stem cells where it controls the properties of stem cells self-renewal. The canonical Hg signalling pathway is activated by three ligands that bind to a 12 trans-membrane receptor protein patched 1 (PTCH1), induce its internalization thus removing its repression of the 7 span trans-membrane protein smoothened (SMO protein), therefore allowing pathway activity. Following PTCH1 internalization, SMO interacts with the GLI zinc transcription factors GLI-2 and GLI-3 which accumulate as active forms in the nucleus and potentate the activity of positive regulators of the pathway among which are the serine/threonine kinase 36 (STK36) and the kinesin family member 7 (KIF7), finally leading to the transcription of target genes such as GLI-1, PTCH1, forkhead box protein M1 and WNT2a (5,6).

The role of the Hh signalling pathway in normal haematopoiesis is controversial but SMO knockout does not perturb steady-state normal haematopoiesis (7). In chronic myeloid leukemia (CML), the Hh signalling pathway is involved in the persistence and expansion of the CML stem-cells suggesting that SMO inhibitors + TKI could improve the molecular response (8). In AML, Hh signalling is activated in CD34+ leukemic cells that can express components of the pathway. GLI-1 expression correlates to reduced overall survival and microarrays analysis showed that GLI-2 is a negative prognostic indicator (9,10). Moreover, Hh pathway inhibition sensitizes leukemic cells to the effects of cytarabine and attenuates the leukemia-initiation potential of leukemic cells (11).

In their work, Lim *et al.* show, examining gene expression profiling, that FLT3-ITD AML over-express the *GLI-2* gene as well as its target gene Bcl-2, compared to wild type FLT3 AML and normal CD34+ haematopoietic cells (HC). Among FLT3-ITD AML, those expressing

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higher GLI-2 levels are associated to a shorter median overall survival. Lim *et al.* have generated a mice model allowing conditional expression of both FLT3-ITD and SmoM2, a constitutively active SMO mutant, in the haematopoietic system and they observe that, whereas GLI-1 and GLI-2 are not expressed in control mice, GLI-2 is expressed in SmoM2 mice, in FLT3-ITD mice and in FLT3-ITD/SmoM2 mice. In contrast, GLI-1 is expressed only by BM cells from SmoM2 and FLT3-ITD/SmoM2 mice but not FLT3-ITD mice. However, stimulation by the SHH, one of the three Hh ligand, induces GLI-1 expression in FLT3-ITD cells but not in normal BM cells suggesting that GLI-2 expression in FLT3-ITD cells allows these cells to response to Hh ligands. Whereas FLT3-ITD mice develop a chronic myeloproliferative disorder, SmoM2 mice have no alteration of peripheral blood cells and survive normally. In contrast, FLT3-ITD/SmoM2 mice have reduced survival and develop myeloid leukaemia with accumulation of immature myeloid cells, infiltration of extra-haematopoietic organs and cytopenia. In FLT3-ITD/SmoM2 mice, the accumulation of immature leukemic cells is explained by the expansion of a granulocyte/monocyte progenitor compartment (GMP) with increased proliferative potential, suggesting the Hh activation may control a transcriptional program leading to self-renewal. Interestingly, GMP cells from FLT3-ITD/SmoM2 mice compared to those from FLT3-ITD mice, express higher levels of genes activated during increased STAT5 signalling. In MOLM-14 cells expressing FLT3-ITD, a combination of the FLT3 TKI sorafenib and IPI-926, a SMO antagonist, further decreases STAT5 phosphorylation and also STAT5 expression compared to either sorafenib or IPI-926 alone. Overall, increased STAT5 activity generated by elevated Hh signalling downstream of FLT3-ITD could be implicated in disease acuity observed in FLT3-ITD/SmoM2 mice. It remained to demonstrate the potential to inhibit both pathways simultaneously. Lim *et al.* show that, in MV4-11 and MOLM-13 cells, interfering with the Hh signalling pathway using IPI-936 or LDE225 (another SMO inhibitor) or siRNA-mediated inhibition of SMO expression increase the inhibition of proliferation induced by sorafenib. Similar results were detected in primary samples from FLT3-ITD AML patients. Finally, in the FLT3-ITD/SmoM2 mice model, the association of sorafenib + IPI-926 cooperated to inhibit leukemic cell growth with some mice that did not die from leukemia.

This study and others have paved the way for the phase I studies actually ongoing with PF-04449913, an oral SMO inhibitor, alone or in combination with cytarabine or hypomethylating agents or vismodegib, another SMO antagonist actually tested in relapsed/refractory AML (NCT02073838). This approach may be particularly active in patients with FLT3-ITD mutations in association with TKI. Hh signalling can be targeted at many levels, from blocking Hh ligands to inhibiting the function of GLI (5,6). Interestingly, SMO inhibitors including vismodegib and IPI-926 have a rather good toxicity profile and show little or no haematopoietic toxicity which is obviously important in AML.

Some questions remain open: (I) is the level of GLI-2 expression in FLT3-ITD AML correlated to the efficiency of SMO inhibitors; (II) the effects of Hh inhibition in association with second generation FLT3 TKIs such as quizartinib and crenolanib need to be tested to make sure that they are identical; (III) the effects of SMO inhibitors will be correlated to the FLT3 allele burden as high allele burden reflects AML addiction to FLT3-ITD; (IV) Hh inhibition does not induce apoptosis but inhibits proliferation of leukemic cells, suggesting that even in association to TKI, long term control of leukemia will need the addition of chemotherapy; (V) because interactions of FLT3-ITD leukemic cells with the BMM induce their differentiation when treated with FLT3 TKI, the effect of such an interaction will be tested in the context of concomitant Hh inhibition; (VI) finally, it is necessary to determine in what other subtypes of AML Hh signalling inhibition could have a therapeutic interest and if Hh targeted inhibition can really help to eradicate the leukemic stem cells compartment (11).

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Footnote

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