Next-Generation Medicine: Combining BCR-ABL and Hedgehog Targeted Therapies

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Running Title: Ponatinib and Vismodegib in BCR-ABL+ ALL

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Summary

Disease relapse remains a major cause of death in patients with BCR-ABL-positive leukemia despite advances in treatment with kinase inhibitors. Significant efforts are underway to target pathways that maintain leukemia stem cells. Targeting these pathways holds promise for definitive leukemia eradication or improvement of the effectiveness of currently available therapies.
Main Text

In this issue of Clinical Cancer Research, Katagiri and colleagues report their preclinical studies investigating the efficacy of combining the Hedgehog pathway antagonist, Vismodegib, and the ABL kinase inhibitor, Ponatinib, for targeting of BCR-ABL-positive leukemia cells. These drugs are currently FDA-approved for the treatment of basal cell carcinoma and BCR-ABL-positive leukemia, respectively. The scientific rationale for targeting of Hedgehog signaling in BCR-ABL-positive leukemia is derived from the work of two groups demonstrating a functional requirement for Hedgehog pathway activation in BCR-ABL-positive leukemia stem cells.

Leukemia stem cells have been functionally described as a subpopulation of cells that have limitless self-renewal capacity. In this model, a reservoir of such cells persists despite exposure to tyrosine kinase inhibitors and/or conventional chemotherapy agents that effectively kill the bulk of proliferating leukemia cells and induce deep molecular remissions. Recent studies have focused on characterizing the signaling pathways that impart cells with limitless self-renewal capacity with the premise that targeting these pathways may hold promise towards definitive eradication of leukemia. In myeloid leukemia, canonical Wnt, Hedgehog, Notch, and Forkhead pathways have emerged as functionally important pathways that regulate leukemia stem cell properties. The role of these pathways in lymphoid leukemia is under intense investigation.

Two groups have shown that Hedgehog activation is functionally important for BCR-ABL-positive leukemia stem cell properties. Dierks and colleagues showed that BCR-ABL-positive leukemia cells display increased Hedgehog activity through upregulation of Smo. Loss of Smo in BCR-ABL-positive leukemia stem cells reduces their capacity to expand and re-transplant in mice. Combination treatment with Cyclopamine and Nilotinib was more effective than Nilotinib alone for reducing disease burden and improving survival of mice in a bone marrow transplantation model. Zhao and colleagues reported overall similar findings—loss of Smo reduces the frequency of mice developing CML-like disease, while constitutive activation of Smo accelerates disease pathogenesis. The authors performed in vivo studies with Cyclopamine demonstrating that the Hedgehog pathway is a viable target. Inhibition of Hedgehog signaling impaired the development of CML disease and improved survival in this mouse model.

In this issue of Clinical Cancer Research, Katagiri and colleagues show that the combination of Vismodegib and Ponatinib enhances apoptosis of SK-9 cells, a BCR-ABL positive acute lymphoblastic leukemia cell line, compared with Ponatinib alone. Combination of these agents also improved survival of mice injected with BaF3 cells expressing various BCR-ABL kinase mutations compared with no treatment controls. There was a trend towards improved survival of mice treated with combination Vismodegib and Ponatinib versus Ponatinib alone (p=0.1005). Combination treatment reduced other markers of disease burden in vivo including CD19+ cells in blood, spleen weight, and BCR-ABL transcripts in bone marrow. These studies provide the first published work to combine Vismodegib and Ponatinib in preclinical animal models and to report the potential clinical efficacy of these drugs in combination.

The Hedgehog pathway has key functions during embryonic and fetal development. Activating mutations of the Hedgehog pathway and their role in tumorigenesis were first described in...
basal cell carcinomas and medulloblastomas. In adenocarcinomas, paracrine activation of Hedgehog signaling in stromal cells plays a role in promoting tumor growth. Three hedgehog ligands have been identified in humans. Lipid modification of Hedgehog ligands is essential for their stability in the extracellular matrix and for the establishment of signaling gradients. Binding of Hedgehog ligands to the Patched1 (PTCH1) cell surface receptor relieves tonic inhibition of Smoothened (Smo), a G protein-coupled-like receptor. Activation of Smo leads to suppression of kinases (e.g. GSK3β) that regulate proteolytic degradation and subcellular localization of GLI proteins (Gli1, Gli2, and Gli3), which are zinc-finger transcription factors that regulate Hedgehog-responsive genes that promote survival and proliferation (Figure 1). Recently, there has been intense interest in developing small molecule inhibitors targeting the Hedgehog pathway to treat various cancers. As the Hedgehog pathway is thought to have relatively limited functions in adult hematopoiesis, there is some belief that Hedgehog pathway inhibitors will preferentially kill leukemia cells that have aberrantly activated the pathway or have acquired a dependence on the pathway. This seems to be the case in studies with Smo inhibitors in BCR-ABL-positive leukemia.

The emergence of clonal variants expressing Abl mutations resistant to tyrosine kinase inhibitors often marks the clinical occurrence of disease relapse or disease progression. Many of these patients have leukemia cells that harbor the T315I mutation, which creates steric hindrance at the base of the ATP-binding pocket and precludes Imatinib, Nilotinib, and Dasatinib from binding. Ponatinib is the first FDA-approved drug effective against the T315I mutation. Combination treatment with Vismodegib and Ponatinib may be more effective than Ponatinib alone for several reasons. In leukemia cells without the T315I mutation, other tyrosine kinase inhibitors would also be expected to have synergistic killing. Inhibition of the Hedgehog pathway has been proposed to 1) promote differentiation of the leukemia stem cells leading to its increased sensitivity to tyrosine kinase inhibition; 2) disrupt the function of cells in the bone marrow niche that maintain leukemia stem cells; 3) impair long-term self-renewal capacity of leukemia stem cells; and 4) diminish survival signals in leukemia stem cells. Indeed, Katagiri and colleagues report that combined targeting of the Hedgehog and BCR-ABL pathways could potentially offer clinically meaningful responses in patients with chemotherapy-and tyrosine kinase inhibitor-resistant leukemia. This strategy will require evaluation in clinical trials.

The clinical efficacy of Vismodegib has been demonstrated in basal cell carcinomas and medulloblastomas, both malignancies where mutations that activate the Hedgehog pathway are known to drive disease initiation and progression. However, the clinical efficacy of Vismodegib in other types of cancers without a discrete mutation that activates the Hedgehog pathway remains uncertain. Furthermore, it is not clear whether Vismodegib would be more effective as frontline or maintenance therapy versus relapsed/refractory therapy. Additional preclinical models and early phase clinical trials are needed to address these questions. Interestingly, two groups have shown that Smo mRNA expression is associated with disease progression in chronic myelogenous leukemia. Increases in Smo transcript level preceded molecular relapse as determined by BCR-ABL PCR by several months in patients. Biologically this may reflect the expansion of the leukemia stem cell pool before hematologic evidence of
disease relapse or progression. If Smo transcript level is validated as a prognostic marker and standardized for clinical use, the rise in Smo transcript level could be a point in clinical management when Vismodegib is combined with a tyrosine kinase inhibitor before frank disease relapse or progression.

In summary, novel therapeutic approaches are necessary to improve the efficacy of currently available therapies and to prevent disease relapse and progression in BCR-ABL-positive leukemia. Hopefully some patients will experience disease control after relapse or be cured of their disease with these novel therapeutic approaches. Identifying Hedgehog pathway activation in BCR-ABL-positive leukemia has advanced this field substantially. As these findings progress into clinical trials of combination therapy, we will soon learn whether this strategy of combining Hedgehog pathway antagonists with other targeted therapies will have clinical impact in leukemia treatment.

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Figure 1. Model for synergy of Hedgehog and ABL inhibitors in BCR-ABL-positive leukemia.

A. Signaling through distinct cascades, both the Hedgehog and BCR-ABL signaling pathways can impact on leukemia cell survival in unperturbed leukemia cells.

B. Targeting Of BCR-ABL with Ponatinib or other ABL kinase inhibitors blocks leukemia cell survival and proliferation that is supported by BCR-ABL signaling and is sufficient to eliminate the bulk of leukemia cells, however, this single-agent therapy does not impact on cell survival, proliferation, or self-renewal signaling that is supported by Hedgehog.

C. Targeting Of Hedgehog with Vismodegib can block cell survival, proliferation, and self-renewal signaling that is supported by Hedgehog ligands in the bone marrow, however, this single-agent therapy does not impact on the leukemia-specific signals of cell survival and proliferation that is supported by BCR-ABL.

D. Combination therapy with Hedgehog (e.g. Vismodegib) and ABL kinase (e.g. Ponatinib) synergistically targets both pathways that collectively impact on leukemia cell survival, proliferation, and self-renewal, thereby providing a possible long-term eradication of leukemia cells.
References
Cell survival
proliferation
self-renewal

GLI
GSK3
BCR-ABL
Smoothened
Patched
Hh
STAT5
PI3K/AKT
MAPKs
etc.

ABL TKIs
e.g. Ponatinib

Vismodegib

Figure 1:
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