Comment on Cortes et al, page 3207

Unraveling CML phase by phase

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Dasatinib induces hematologic and cytogenetic responses in both myeloid and lymphoid blastic phase of CML regardless of the presence of *BCR-ABL* kinase domain mutations, and these responses are durable at least in the short term.

Blastic transformation or blastic crisis of chronic myeloid leukemia (CML) is one of the most aggressive and intractable of leukemias, and it is therefore very encouraging to learn from the report by Cortes and colleagues published in this issue of *Blood* that dasatinib, one of the so-called second-generation tyrosine kinase (TK) inhibitors that includes also nilotinib, can induce major hematologic responses in about one third of patients treated previously with imatinib and complete cytogenetic responses in a similar proportion. These results are much better than might have been expected with classical cytotoxic drug combinations and shed some light on at least 4 questions that have fascinated CML aficionados over the years. First, do residual Ph-negative stem cells routinely survive in the marrow of patients after the onset of blastic transformation? The evidence here suggests that even in blastic transformation a significant number of presumably normal stem cells do survive and can reconstitute hematopoiesis if given the chance.

Second, what role does the *BCR-ABL* fusion gene play in causing or maintaining the blastic subclone? It might have been assumed that the *BCR-ABL* fusion gene set the scene for progression of leukemia to blastic phase but then became operationally irrelevant. This seems not to be the case. The fact that some patients respond to dasatinib even after failing imatinib suggests (but does not prove) that the *BCR-ABL* gene still plays some crucial cooperative role in maintaining the blastic phase (though studies that showed resumption of the capacity of Bcr-Abl in vitro to phosphorylate Crkl might have been definitive). This would mean either that dasatinib, at least in this setting, is generally a much more powerful *BCR-ABL* inhibitor than imatinib, perhaps because of its less stringent binding requirements, or that other activated TKs, such as *SRC* or other SRC family kinases, which are targeted by dasatinib but not by imatinib, have substituted for *BCR-ABL* in maintaining the transformed leukemia.[1]

Third, why do some of the responses seem to be durable, although the follow-up is still relatively short? If dasatinib-resistant subclones were already present when the drug was started, one would have expected to see resistance developing quite quickly; if, however, resistant subclones had not yet developed and dasatinib completely or near-completely suppressed the whole population in which they would have been expected to occur, then the drug should control the leukemia for some time. One hopes this proves to be the case.

And finally, what is the role of kinase domain mutations in "causing" resistance to TK inhibitors? An increasing body of evidence suggests that whereas some mutations are indubitably the cause of imatinib resistance, others seem merely to be epiphenomena.[2] Dasatinib actually controls almost all mutant subclones,[3,4] though not the notorious T315I. One might speculate therefore that it might for this reason be slightly more effective than imatinib for treating chronic-phase disease, but in "late" blastic phase disease resistance seems often to be due to mechanisms other than *BCR-ABL* kinase domain mutations. The challenge now must be to identify activated signal transduction pathways that maintain the blastic phenotype even when the *BCR-ABL* kinase remains fully suppressed.

The author declares no competing financial interests. ■

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Comment on Zou et al, page 3284

Inside-out, outside-in: what's the difference?

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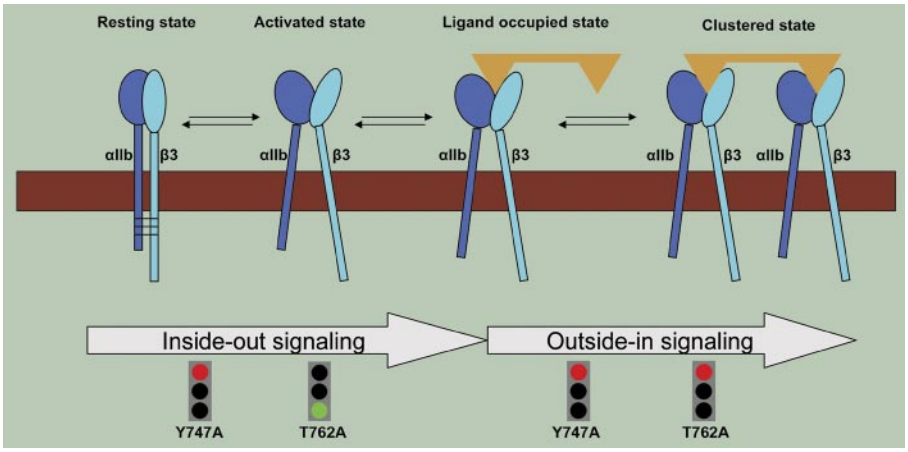
Zou and colleagues use retrovirus infection and fetal liver transplantation to engineer platelets in mice expressing αIIbβ3 and use point mutations to dissect inside-out and outside-in signaling in vivo.

As if integrin nomenclature were not complicated enough, with all its alphas and betas, we must now be cognizant of the subtleties of an additional layer of terminologies, "inside-out" and "outside-in" signaling. There is, of course, a logic to integrate these additional terms into our integrin vocabulary: they define distinct and biologically important aspects of integrin-mediated cellular responses. αIIbβ3 is the integrin that mediates platelet aggregation, and its ability to form a stable thrombus in vivo depends upon both inside-out and outside-in signaling. Engagement of fibrinogen or von Willebrand factor by the extracellular domain of αIIbβ3 is necessary for platelet aggregation and requires a conformational transition, the consequence of inside-out signaling. As platelets aggregate, occupied αIIbβ3 integrins cluster and trigger outside-in signaling that



Distinction between inside-out and outside-in signaling across integrin αIIbβ3.

stabilizes the aggregate and supports responses, including platelet spreading and clot retraction. Hence, inside-out and outside-in signaling constitute the 2 elements of the bidirectional signaling across αIIbβ3 (see figure) and represent potential antithrombotic therapeutic targets, and their dissection is indispensable to our understanding of platelet biology.

The inside-out and outside-in signaling pathways ultimately trace to cytoplasmic tails (CTs) of αIIbβ3, which serve as receivers and transmitters of bidirectional signaling within the receptor. While the CTs of the αIIb and β3 subunits are short, they are structurally complex and interact with numerous binding partners. Ordinarily, the contribution of specific structural elements or individual amino acids can be dissected by straightforward mutagenesis. However, platelets are anucleated; and alternative approaches have had to be invoked to examine inside-out and outside-in signaling. These approaches have included the following: (1) expression of αIIbβ3 in heterologous cells; (2) transfection of the primary megakaryocytes; (3) introduction of membrane-permeable peptides into platelets; and (4) generation of knock-in mice expressing mutant αIIbβ3. Each of these approaches has been used successfully to probe integrin signaling, but each approach has inherent limitations.

By infecting fetal liver cells from β3-deficient mice with retrovirus engineered to encode wild-type β3-subunit and transplanting these cells into irradiated recipients, Zou and colleagues show that these cells can successfully express αIIbβ3 and rescue both inside-out and outside-in signaling in blood platelets. This strategy is then used to introduce specific mutations into the mouse platelets. Inside-out signaling is evaluated by the capacity of the platelets to bind soluble fibrinogen, and outside-in signaling is assessed by platelet spreading. A single point mutation in the midregion of the β3 CT, β3Y747A, prevents restoration of both inside-out and outside-in signaling, while a point mutation at the extreme C-terminus of β3, β3T762A, results in selective loss of outside-in signaling. These results are generally consistent with those derived from the other approaches we outlined and fortify the conclusion that different sites within the β3 CT can mediate different aspects of the bidirectional signaling.

The experimental approach for in vivo expression in platelets described by Zou et al here and by others[1] allows analyses of αIIbβ3 activation in platelets in vivo and has high throughput potential. Fetal liver transplantation could undoubtedly be applied to other platelet proteins provided that a deficient mouse background is available. Low, variable, and unstable expression of the platelet protein may set limitations on this approach. Nonetheless, the Zou et al paper sets the precedent for a new strategy to dissect inside-out and outside-in signaling across αIIbβ3 in vivo.

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HEMATOPOIESIS

Comment on Raslova et al, page 3225, and comment on Macaulay et al, page 3260

MEGAprofiles provide big insights into platelet function

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This issue of *Blood* contains reports from 2 groups that have used microarray profiling of CD34$^+$-differentiated hematopoietic stem cells to dissect molecular mechanisms regulating megakaryocytopoiesis and/or for identification of functionally novel platelet receptors. Both reports provide new insights into proplatelet formation and platelet function.

Hematologists are intrigued by megakaryocytes (MKs), hematopoietic cells readily distinguishable by their abundant cytoplasm and large size (~ 35-160 μm), nuclear polyploidy (up to 64N), and relative paucity (0.02%-0.05% of total nucleated bone marrow cells). While discovery of the existence and hemostatic functions of blood platelets is generally assigned to Bizzozero based on pioneering observations made during the 1880s, linking megakaryocytes to platelet biogenesis is credited to James Wright in 1906, who made conclusions based on histomorphometric similarities of granular and cytoplasmic content between both cell types.[1] In the subsequent century, considerable insight has been achieved in identifying receptors and signaling pathways that regulate platelet and megakaryocyte biology. Nonetheless, the genetic machinery controlling megakaryocyte transition into the distinctive endomitotic switch that precedes the epiphany of cytoplasmic maturation, membrane demarcation, proplatelet formation, and platelet release remains incompletely