Comment on Wray et al, page 1852

Decatenation: fixing your knots

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How tumor cells gain resistance to drugs is critically important to elucidate for developing better cancer therapy. In this issue of Blood, Wray and colleagues have identified a mechanism whereby acute leukemia cells use a stimulator of topoisomerase II activity to allow proliferation despite drug inhibition of this essential enzyme.

Topoisomerase II (Topo II) performs essential DNA topologic changes in proliferating eukaryotic cells, mostly notably the decatenation (unknotted) of the newly replicated sister DNA molecules, needed for chromatid separation in anaphase of mitosis (see figure).

Because inhibiting this enzyme selectively kills proliferating cells, Topo II is the target of widely used cancer drugs. Despite their prevalence as chemotherapeutics, resistance to such drugs arises, for example, through up-regulation of ABC transporters that pump the drugs out of cells. Understanding all of the mechanisms of drug resistance is of great importance for the design of improved cancer treatment strategies.

In this issue of Blood, Wray et al compared the effects of a Topo II inhibitor, ICRF-193, on normal human CD34⁺ hematopoietic cells and a number of acute leukemia cell lines, finding that the leukemia cells were able to proliferate. Like many cell types, the CD34⁺ cells arrested cell-cycle progression in the presence of ICRF-193. This normal cellular response is mediated by checkpoint controls that respond to perturbed Topo II by inducing cell-cycle arrest in G2 phase as well as in metaphase of mitosis. The Topo II checkpoints aim to prevent failed attempts at chromosome segregation when the cell has insufficient decatenatory activity. The leukemia cells lacked both the G2 phase and metaphase checkpoint responses and continued to proliferate regardless of ICRF-193 treatment.

There are several plausible explanations for this behavior that relate directly to Topo II: (1) a deficient checkpoint signal, allowing unperturbed mitotic progression despite the inability to decatenate DNA; (2) a mutation in Topo II such that ICRF-193 could not bind; (3) an elevated level of Topo II protein, which compensated for the presence of drug; (4) a stimulation of Topo II activity such that catenations were processed faster than ICRF-193 could act; and (5) a factor that interfered with drug-enzyme interaction. Wray et al took significant steps in their attempts to clarify this mechanism of drug resistance.

Based on previous work, they asked if Mtnase is required for ICRF-193 resistance in the leukemia cells. Metnase binds directly to Topo II and stimulates its ability to decatenate DNA in vitro. Assumming Metnase stimulates...
decatenation in vivo, the continued proliferation of the leukemia cells treated with ICRF-193 ought to be accompanied by relatively normal chromosome segregation. This would explain the lack of checkpoint induction. Remarkably, they found that depletion of Metnase from the leukemia cells rendered them sensitized to ICRF-193, as measured by an increased propensity to arrest in mitosis. This effect seemed to be specific to the metaphase Topo II checkpoint, since the cells did not accumulate in G2 phase. Most strikingly, the absence of Metnase similarly sensitized to an unrelated Topo II inhibitor, VP-16. The leukemia cells were able to proliferate in the presence of VP-16, but rapidly succumbed to apoptosis if Metnase was depleted before VP-16 treatment.

The significance of these data is that some leukemia cells may be killed by combination therapies that target Topo II and Metnase. But before this is possible, a detailed understanding of the mechanism of Metnase action is needed. One confusing aspect of the current state of knowledge is that Metnase stimulates Topo II–mediated decatenation in vitro in the presence of several different Topo II inhibitors, despite these drugs having varied modes of action and distinct binding sites on Topo II. This may rule out simple interference of drug binding. An alternative is that Metnase stimulates or somehow modifies the Topo II enzyme cycle such that it is resistant to drug action. Perhaps investigating whether Metnase can stimulate Topo II in the presence of all classes of inhibitor would be revealing.

A second key issue is the nature of the enhanced Metnase activity in leukemia cells. This is important because Metnase activity could help predict the efficacy of Topo II inhibition as a cancer treatment. Metnase protein levels were apparently not altered in the leukemia cells, although they did have in common a phosphorylated isoform not abundant in CD34− cells. It is possible that phosphorylation activates Metnase-stimulated decatenation. There also is evidence that methylation of Metnase regulates its activity.

Whatever the mechanism of Metnase action and the predictive power of Metnase activity toward stratifying appropriate cancer therapies, these initial studies warrant rigorous further investigation. In the long term, small molecule inhibitors that disrupt the Metnase–Topo II interaction could be valuable in the clinic.

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES
2. Clarke DJ, Vas AC, Andrews CA, Diaz-Martinez LA, Williams JH. A second key issue is the nature of the enhanced Metnase activity in leukemia cells. This is important because Metnase activity could help predict the efficacy of Topo II inhibition as a cancer treatment. Metnase protein levels were apparently not altered in the leukemia cells, although they did have in common a phosphorylated isoform not abundant in CD34− cells. It is possible that phosphorylation activates Metnase-stimulated decatenation. There also is evidence that methylation of Metnase regulates its activity.

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Comment on Gratacap et al, page 1884

The clot thickens (or not)

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In this issue of Blood, Gratacap and colleagues analyze the effects of the multikinase inhibitor dasatinib on platelets, helping to explain the occurrence of bleeding related to its use for CML patients resistant to or intolerant of imatinib.1 In addition, by virtue of careful experimentation using multiple functional platelet assays, these authors are paving the way for the development of kinase inhibitors as antithrombotic agents.

Dasatinib is one of the second generation BCR-ABL inhibitors, along with bosutinib and nilotinib,2,3 used to treat chronic myeloid leukemia (CML) patients resistant to or intolerant of imatinib. These ATP-competitive inhibitors share potent inhibition of BCR-ABL, but differ significantly in their spectrum of multikinase inhibition (see table).4,5 The fact that imatinib also inhibits the kinases c-kit and PDGF-R has been appreciated for some time and taken advantage of clinically. Both dasatinib and nilotinib inhibit c-kit and PDGF-R, while bosutinib does not. However, both dasatinib and bosutinib also inhibit src-family kinases (SFK). Although bleeding during dasatinib use had been reported in association with thrombocytopenia, Quintana-Cardama, Cortes, and colleagues noted dose-related internal and mucosal bleeding in the absence of thrombocytopenia.6,7 The bleeding could not be explained by concomitant use of antiplatelet or anticoagulant medications.

They went on to show defective platelet function in the PFA-100 assay to epinephrine/collagen, but not ADP/collagen, when dasatinib was present at a concentration of 400 nM. Aggregation of platelets from CML patients receiving dasatinib was abnormal in response to arachidonic acid and epinephrine, but normal with low- and high-dose ADP, ristocetin, and high-dose collagen. Of note, imatinib use resulted in decreased aggregation in response to arachidonic acid only, while neither nilotinib-treated nor bosutinib-treated patients showed abnormal aggregation to any agonist tested.

It is in this context that the contribution by Gratacap, Payrastre, and colleagues can be examined further. These authors studied human platelet activation by thrombin, ADP, collagen (low- and intermediate-dose), and thrombin/ADP agonist tested.

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Multikinase profile of the BCR-ABL inhibitors in CML treatment. — indicates no inhibition; +, moderate inhibition; ++, strong inhibition at clinically relevant concentrations; and nr, not reported.
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