Der9q deletions in CML: the next chapter in the story

The classic Philadelphia chromosome giving rise to Bcr-Abl is associated with a certain additional cytogenetic abnormality in a significant minority of patients. A number of groups have previously investigated the significance of large deletions adjacent to the translocation breakpoints in patients with chronic myeloid leukemia (CML). These deletions have been reported in between 10% to 15% of patients; they typically span the translocation breakpoint and often involve both chromosomes 9 and 22. The deletions can be many megabases in length and are presumably formed at the time of the Philadelphia translocation. Such deletions are associated with a poor prognosis in terms of shorter length of chronic phase, earlier disease transformation, and shorter survival. Previous analyses were largely confined to patients who had been treated with interferon-alpha (IFN-α), although interestingly the effect was still evident in patients treated by allogeneic transplantation. However, does the “poor-prognosis” effect still stand up in imatinib-treated patients?

In this edition of Blood, Huntly and colleagues (page 2205) present new data addressing the question of whether therapy with imatinib overrides the deletion effect. The answer seems to be that deletions still seem to confer a somewhat poorer prognosis in certain settings but that the effect is clearly “diluted” by the use of imatinib in comparison to groups of patients treated with IFN. For example, in 122 chronic-phase patients, significant differences in progression-free survival, but not overall survival, were seen among patients with (n = 15) and without (n = 107) deletions. In successive cohorts of analyzed patients it seems evident that as there is a greater proportion treated with imatinib so the prognostic impact of deletions diminishes.

Finally, why these deletions should confer a poor prognosis remains far from clear. The data presented in this article would suggest that the molecular abnormalities present in patients with deletions in some way cooperate with, but are not entirely dependent upon, Bcr-Abl. It would seem that this evolving story is far from complete: the prospective analysis of deletions in future clinical studies will not only allow confirmation of the initial observations of this study, but will also allow a long-term analysis of the true impact on survival. Furthermore, it is not inconceivable that elucidation of the molecular aberrations underlying this effect may allow further improvements in the therapy of CML in due course.

—Stephen G. O’Brien
University of Newcastle Medical School

Location matters... 

Molecular therapy of chronic myeloid leukemia (CML) since the advent of imatinib mesylate (Glivec, Gleevec) is a developing paradigm for oncology in general and for hematologic malignancies in particular. Our understanding of the basic biology of the disease, and the availability of high-throughput drug screens, arms us with small-molecule drugs that inhibit the key steps in the genesis, maintenance, and proliferation of the tumor. However, imatinib, at the vanguard, has shown that responses to single specific inhibitors alone may be short-lived and relapse is most often associated with a loss of drug binding caused by the exquisitely powerful selection of random cells carrying mutations in the Bcr-Abl tyrosine kinase domain that render them resistant to imatinib. Even as it becomes increasingly likely that imatinib does confer long-term benefit in the treatment of CML, the addition of other drugs will have an important role in improving clinical responses to imatinib used as a single agent or in managing resistance to this drug.

In addition to Bcr-Abl, other downstream proteins essential for transducing the oncogenic signal are good candidates for targeted therapy. For example, a key player is the oncoprotein Ras, known to be active only when tethered to the cytoplasmic membrane. This attachment depends on a specific posttranslational modification (called farnesylation, usually of the farnesyl transferase type) of Ras, although the exact mechanism underlying this location-dependent activity, and the cast of other proteins that behave similarly, is only partly understood. An important observation is that mutagenesis of one specific cysteine residue in oncogenic Ras, whereby farnesylation and localization to the cell membrane cannot occur, prevents transformation of fibroblasts via loss of Ras/MAPK (mitogen-activated protein kinase) signaling. Therefore, Ras signaling might represent an excellent target for intervention in many areas of oncology.

At present, clinical trials of inhibitors of the farnesyl transferases (known as FTIs) in CML are underway, and the in vitro data for these drugs are encouraging, even where responses to imatinib are poor. However, it appears that when Ras farnesylation is prevented, other lipid modifications such as geranylgeranylation may be possible, leading to restoration of Ras/MAPK signaling and the loss of the therapeutic effect.

In this issue, Kuroda and colleagues (page 2229) present in vitro and in vivo data describing the cytotoxic effects on...
CML cells of the third-generation bisphosphonate zoledronic acid (ZOL). ZOL is known to prevent both farnesylation and geranylgeranylation of Ras-related proteins and is thus potentially more efficient than conventional FTIs. Kuroda et al found that ZOL selectively inhibits the growth of CML cells in vitro and in vivo, at least partly via the induction of apoptosis, and is not effluxed by the P-glycoprotein pump. ZOL also combines well with imatinib exhibiting clear in vitro synergistic effects, a highly desirable interaction. Using a murine transplantation model, the authors showed that ZOL, like imatinib, prolongs survival of mice via the selective killing of the transplanted human leukemic cells, and this effect was even more significant when ZOL was combined with imatinib. A phase 1 clinical trial of ZOL has defined well-tolerated doses that are of the same relative magnitude as those used in the murine study. Perhaps most intriguingly, ZOL is known to rapidly localize to bone, raising the desirable prospect of a high local concentration of ZOL in the bone marrow, although this may reduce the efficacy against circulating leukemic cells.

The possible addition of ZOL to the armamentarium of drugs that can be effective against CML is a significant step forward. Many questions remain unanswered, however: Are the antiproliferative and pro-apoptotic effects of ZOL due solely to inhibition of Ras localization to the cell membrane or are other prenylated proteins also involved? Will the preferential concentration of ZOL in the bone marrow encourage proliferation of leukemic cells in ZOL-free extramedullary “sanctuaries”? And will ZOL be effective for killing imatinib-resistant CML cells? As previously shown for Bcr-Abl, the localization of a protein to the “correct” cellular compartment makes a world of difference to signal transduction and leukemogenesis.

—Junia V. Melo and Alex J. Tipping
Imperial College, London, UK


RNAi meets imatinib mesylate (STI571)

RNA interference (RNAi) describes a highly conserved mechanism of sequence-specific posttranscriptional gene silencing triggered by double-stranded RNA (dsRNA). DsRNA in the form of small interfering RNA (siRNA) can trigger RNAi in mammalian cells without activation of the nonspecific interferon response. With different tools to initiate RNAi now available, an increasing number of reports use this process for functional genomics in several organisms. Such studies include human cells where RNAi can inhibit both normal and aberrant gene expression arising from viral infection or cellular mutations.

One such mutation is the bcr-abl oncogene involved in leukemogenesis of chronic myeloid leukemia (CML) and a subset of acute lymphoblastic leukemia (ALL). Bcr-Abl tyrosine kinase activity is required for cellular transformation and can be inhibited by imatinib mesylate (STI571), which now represents an effective strategy to treat bcr-abl–positive leukemias. However, imatinib mesylate resistance does occur and this point is addressed in the study by Wohlbold and colleagues (page 2236). The authors used repeated electroporation to deliver anti-bcr-abl siRNA into cell lines that express either wild-type or imatinib mesylate-resistant mutants of Bcr-Abl. They demonstrate that inhibition of bcr-abl gene expression by siRNA and that of Bcr-Abl tyrosine kinase activity by imatinib mesylate can cooperate to inhibit proliferation and survival of bcr-abl–positive cells. Both strategies are nonoverlapping but rather complementary since suitable siRNA can inhibit both wild-type and mutant bcr-abl gene expression, resulting in comparable reduction of cell survival. Finally, bcr-abl gene silencing may depend on intracellular siRNA levels, suggesting some pharmacologic aspects of siRNA in common with more conventional drugs, at least in this specific model.

While these results suggest that cellular oncogenes may effectively be targeted by application of RNAi, either alone or in combination with other therapeutics, a number of important challenges remain. The first and most obvious is the effective delivery of siRNAs to human cells in a clinical setting. Retroviral or lentiviral gene transfer of suitable expression cassettes allow stable intracellular transcription of RNAi triggers and represent an alternative to physico-chemical transfection procedures. Additionally, stable expression of RNAi triggers may overcome the transient nature of RNAi in mammalian cells after a single siRNA application. Furthermore, potential side effects of RNAi or of specific RNAi triggers such as off-target gene silencing have to be considered carefully. These aspects of RNAi will certainly be studied in the future and help to better define the therapeutic potential of RNAi-based gene silencing in hematopoietic cells.

—Matthias Eder and Michaela Scherr
Medizinische Hochschule Hannover