

Tyrosine kinase Inhibitors in leukemia
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The clinical success of imatinib mesylate in treating patients with chronic myelogenous leukemia (CML) has prompted efforts to identify other cancers with mutated tyrosine kinases and also many efforts to develop new kinase inhibitors. At the present time, tyrosine kinase inhibitors targeted against Abl, PDGFR, KIT, EGFR, FLT3, and KDR clearly have clinical benefit in a variety of different types of cancer, including AML, gastrointestinal stromal cell tumors, lung cancers, and kidney cancers.

Since the most extensive experience with tyrosine kinase inhibitors is in patients with CML, it is likely that lessons learned about using imatinib in that disease will also be informative about other tumors and other kinase inhibitors. In particular, mechanisms of resistance to imatinib are likely to be observed with other tyrosine kinase inhibitors. Stable (early) phase patients are occasionally refractory to initial therapy with imatinib, although this is not uncommon in patients with more advanced disease. This type of resistance, termed primary resistance, is not well understood at this time. Patients who respond initially to imatinib, but later relapse with drug-resistant leukemic cells, are more commonly observed, particularly among patients with advanced disease. This type of resistance, termed secondary resistance, is typically associated with acquired mutations in the kinase domain of Bcr/Abl that reduce binding of imatinib. Occasionally, patients will have an amplification of Bcr/Abl or activation of another tyrosine kinase such as Src, as a mechanism of resistance. A third type of resistance is termed "molecular resistance," a situation which refers to the common situation where a stable phase CML patient has had a complete hematologic and cytogenetic response, almost always have a complete hematologic response, and most patients also have a complete cytogenetic response, Bcr/Abl is still detectable in the majority of patients by PCR.

More than 30 different mutations have been detected in the kinase domain of Bcr/Abl that confer complete or partial resistance to the drug in patients. Most of these point mutations are thought to either alter binding of imatinib to the kinase pocket, or cause the kinase to adopt an "activated" configuration, again resulting in reduced drug affinity. The degree of drug resistance varies from modest (2-3 fold) to high (>10 fold). The T315I mutation has been referred to as the gate keeper mutation, as it blocks binding of imatinib and other Abl kinase inhibitors (dasatinib, nilotinib) through steric hindrance with the drug. Interestingly, some patients with advanced disease have been found to have mutations in the kinase domain of Bcr/Abl, at low frequency, before receiving imatinib, supporting the notion that CML cells have some type of mutator phenotype.

There is increasing interest in defining the abnormalities in CML cells that lead to the accumulation of DNA damage, both in the form of point mutations that cause drug resistance, and other types of DNA damage that result in disease progression. In cell culture, CML cells rapidly develop new chromosome translocations, DNA deletions and amplification, and point mutations in random genes, and do so at a rate much higher than "normal." Recent studies from our lab and others has implicated the overproduction of reactive oxygen species (ROS), such as hydrogen peroxide and superoxide anion as being important contributors to ongoing DNA damage in CML cells. The signaling pathways that lead to production of ROS have been partially worked out and will be discussed. Similarly, mutants of Bcr/Abl have been

identified where kinase activity is normal, but production of ROS is diminished, and which are also dramatically less likely to cause DNA damage.

The phenomenon of molecular resistance is not well understood, and its clinical significance is also not clear. There are currently many patients remaining in cytogenetic remission and complete hematologic remission who remain PCR positive after 5-6 years of imatinib therapy. Studies have demonstrated that the putative stem cell population is PCR positive, and thus it is possible that this represents a population of cells that could still cause disease relapse in the future. However, the probability of relapse appears to go down over time, rather than up, challenging the notion that relapse is inevitable over time. Theories as to why these cells are not sensitive to imatinib will be discussed, as will hypothetical strategies to overcome their resistance. At the present time, it appears that CML stem cells are not dependent on Bcr/Abl, at least while they are quiescent, and that inhibition of Bcr/Abl kinase activity may be minimal for unknown reasons.

At the present time, several potent second generation Abl inhibitors are available and can be used to treat patients who are drug resistant. Clinical experience with dasatinib and nilotinib suggest that second remissions are commonly achieved in patients resistant to imatinib because of point mutations, with the exception of T315 mutants. Further, these remissions are clinically beneficial and may be long lasting. However, other mutations can occur that cause resistance to these new kinase inhibitors, and understanding the process of how and why CML cells develop new mutations is of increasing importance.