levels of surface complex expression. These results were most notable in large-sized platelet subpopulations. The implication is that the bleeding tendency in these disorders is secondary to abnormal *in vivo* platelet-vessel wall and platelet-platelet interactions caused by decreased levels of GPIb-V-IX. An interesting finding given the necessary interconnection between cytoskeletal components and membrane proteins. This suggested mechanism deserves to be explored in the future.

These interesting observations represent the first extension to exploring the altered downstream pathways which lead to bleeding dysfunction in the MYH9-related disorders. The shared pathway with BSS of decreased GPIb-V-IX complex seems to satisfy one question. However, these results do not explain the macrothrombocytopenia shared between all these disorders, including BSS. Therefore, as with any good result, more questions have arisen in this puzzle. Clearly, the challenges ahead are exciting.

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Imatinib: can one outwit chronic myeloid leukemia?

Imatinib mesylate (IM), or STI571, or Glivec, is a landmark medicine of which both the pharmaceutical industry and the hematology community can be justly proud. Indeed, it illustrates one of the few cases in which the precise knowledge of the molecular basis of a neoplastic condition has led to a deliberate search for a chemical that would target the crucial molecule, in this case the ABL protein tyrosine kinase (PTK). Within less than 20 years since the cloning of the BCR-ABL fusion gene,[1] we now have the results of highly significant clinical trials,[2,3] and a drug available in the pharmacy that can be prescribed to patients when appropriate. In this issue of Haematologica Marin *et al.* provide an authoritative review on the clinical use of IM in patients with chronic myeloid leukemia (CML).[4]

The analogy between chemotherapeutic agents that recognize a target specific to abnormal somatic cells and antibiotics that recognize a target specific to bacterial cells is evident. Since the introduction of penicillin or streptomycin, it took only a few years to observe that bacteria could *become resistant* to these agents. It took only a few more years to realize that this phenomenon was not due to some sort of adaptive process, but to selection by the antibiotic of bacterial mutants that already existed before exposure to the antibiotic itself.[5] In the case of IM, the initial clinical description has

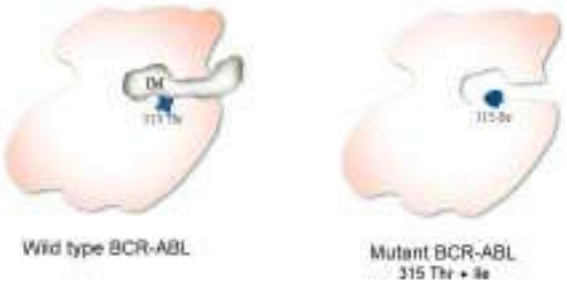

Figure 1. A common mechanism by which CML cells can be resistant to imatinib (IM). In the cartoon on the left, a space-filling model of the IM molecule is seen to fit in a *pocket* near the active center of the BCR-ABL protein: this strong binding causes a marked inhibition of the protein tyrosine kinase (PTK) activity of the BCR-ABL protein. The cartoon on the right depicts the BCR-ABL mutant protein found in several patients with CML who have become resistant to IM. As a result of a single nucleotide replacement, in this particular instance an isoleucine residue replaces a threonine residue in position 315, the isoleucine residue occupies more space, and thus impedes access to IM, which will no longer inhibit PTK.

Table 1. Point-mutations in the BCR-ABL gene associated with imatinib resistance in patients with CML or Ph+ acute lymphoblastic leukemia.

*Nucleotide change**	*Amino acid change°*	*Predicted/known to prevent imatinib*	*N° of cases (detected/tested) binding#*	*Refs.*
G749A	G250E	No	4/59	9,10
G756C	Q252H	Not known	6/31	10
T757C	Y253H	Yes	3/78	9,11
A758T	Y253F	Yes	1/50	11
G763A	E255K	Yes	18/130	9,13
A763T	E255V	Yes	1/50	11
C944T	T315I	Yes	16/118	9,11,13
C951G	F317L	Yes	1/28	9
T1052C	M351T	No	8/109	9,11

**Positions according to GenBank #M14752; °positions according to GenBank #AAB60394; §among patients who have developed resistance or have never responded to IM.*

again been similar: some 10% of patients become *resistant*.[6] A variety of mechanisms have been described. On one hand, there may be an extra Ph chromosome, or there may be a high level of BCR-ABL mRNA for other reasons:[7,8] in such cases, it is possible that one is dealing with a clone in which the level of PTK is too high to be inhibited effectively by the currently used dosage of IM. On the other hand, there may be additional chromosomal abnormalities other than the Ph one (as is often the case in the accelerated phase of CML), suggesting that one is dealing with a clone in which PTK inhibition is no longer sufficient to inhibit growth, because of lesion(s) that bypass the PTK-linked pathway of growth control. However, in a significant proportion of cases the molecular basis of IM-resistance is straightforward: a mutation in the BCR-ABL gene itself (see Figure 1), which compromises the close fit between the PTK active center and IM, crucial for the therapeutic action of the drug. This mechanism may account for some 50% of the IM-resistant cases, but it would not be surprising if this were an under-estimate, because detecting a somatic mutation in a heterogeneous cell population is not a technically trivial operation, especially if it is a point mutation, and more so if it is not yet known. Up till now, at least 9 different point mutations have been identified (see Table 1), but there might be more to come.

The development of resistance to IM in patients who have previously had a therapeutic response might tempt one to imagine that IM has induced a resistance-conferring mutation. A wealth of evidence from bacterial genetics and from somatic cell genetics[14,15] indicates that this is not, of course, the case: rather, mutations arise spontaneously, and mutant cells are selected by the agent to which the mutation confers resistance. There is no need or reason to invoke any other mechanism in the case of IM.[16,17] The probability of any mutation occurring depends mainly on (a) the number of cells at risk and (b) the mutation rate. Blagosklonny[16] has emphasized the role of genetic instability [i.e. an increase in (b)], and this certainly may be relevant in blast transformation and in the accelerated phase. However, it is important to note that genetic instability is not a must. In chronic phase CML the number of cells at risk can be very high (up to 10^{13}: of course the number of cells that have the potential to generate a mutant sub-clone will be much less), and therefore a mutant cell may turn up even with a normal mutation rate. Indeed, it was predictable that BCR-ABL mutations would be discovered even in patients who have never received IM, and this has now been confirmed.[8] Rare mutant somatic cells in our bodies are a bit like random noise that accompanies our existence in otherwise highly orchestrated multicellular organisms. With reference to other clonal blood disorders, rare PIG-A mutant cells have been found

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in normal people who do not have paroxysmal nocturnal hemoglobinuria.[18]

The next question is how to exploit maximally the proven efficacy of IM in the treatment of CML, despite its potential to select at the same time for CML cells that will eventually give IM-resistant disease. At the moment we can think of two ways. On one hand, it is not unconceivable that one can find PTK inhibitors that are still active on a wider range of mutants: *i.e.*, a cell that is IM-resistant could be sensitive to a second-generation IM.[16] Another alternative takes us back to the analogy with infectious diseases. Antibiotic-resistance is best avoided by using at least two drugs at the same time, and this approach is grounded on solid principles.[18] If the frequency of a mutant cell resistant to antibiotic A is, say, 10^{-9}, and the frequency of a mutant cell resistant to antibiotic B is the same, then the probability of a double-resistant cell (which must have two rare mutations at once) is as low as 10^{-18}. If we come back to CML, at the onset of treatment there may always be one cell that is already IM-resistant; there may always be one cell that is resistant to, say, cytosine arabinoside: but there may be none that is resistant to both. The use from the start of a drug combination instead of IM alone is already being contemplated for various reasons.[20-22] Although it may be regarded at first as unpleasant or inelegant, in our view this approach has intriguing possibilities. In fact, despite the great efficacy of IM in controlling CML, few would claim that it actually cures CML. It is still unclear whether IM simply blocks the proliferative advantage of Ph-positive cells,[23] rendering them similar to normal cells, or whether it indeed leads to the demise of all IM-sensitive cells.[24] In any case, if the rare IM-resistant cell was disposed of by another drug (and *vice versa*), then the combination could produce not just a remission, but a true pharmacologic cure of CML.

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Inside Haematologica: the impact of age on the outcome of allogeneic stem cell transplantation

Allogeneic stem cell transplantation is a fundamental therapeutic option for many patients with hematologic disorders. For instance, it is the only curative treatment for patients with non-malignant disorders such as thalassemia major[1,2] or paroxysmal nocturnal hemoglobinuria.[3,4] Its role might be sharply declining in chronic myeloid leukemia,[5-8] but new indications are constantly emerging.[9-15]

Allogeneic stem cell transplantation is remarkably effective and safe in children,[16-20] whereas transplant-related morbidity and mortality is directly related to age in adults and unacceptable in older patients undergoing unrelated-donor transplantation.[21,22] Attempts have been made to reduce them by adopting reduced-intensity conditioning regimens.[22-27]

In this journal's issue de la Cámara and co-workers[28] report data showing no significant difference in transplant-related mortality and morbidity between elderly patients (aged 55-59) and young adults (aged 20-40) receiving allogeneic stem cell transplantation at a single institution. As underlined by the authors, the outcome of elderly patients may be determined more by the severity of the underlying disorder than by age itself. Their data suggest that age alone (between 50 and 59) should not be an absolute barrier to conventional allogeneic stem cell transplantation from an HLA-identical sibling donor.

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