Rational drug design and resistance in chronic myelogenous leukemia

Helena Jover Escapa. Biomedical Sciences degree
Universitat Autònoma de Barcelona

Introduction

- Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm that accounts for 15% of all cases of leukemia and has an annual incidence of 1.5 cases per 100,000 individuals.
- CML is driven by the BCR-ABL chimeric gene product, a constitutively active tyrosine kinase. The fusion gene results from the reciprocal balanced translocation t(9;22) (q34;q11.2), cytogenetically detected as the Philadelphia (Ph) chromosome [Fig.1].
- The BCR-ABL oncoprotein possesses constitutive kinase activity that leads to the stimulation of cell-growth pathways and reduced apoptosis of CML cells. Examples of drivers implicated on the transduction pathways affected are RAS and MAP kinases.
- The natural course of CML consist on 3 phases: chronic phase, accelerated phase and a terminal blastic phase characterized by the typical acute leukemia symptoms.
- Before the era of selective BCR-ABL tyrosine kinase inhibitors (TKIs), the median survival at diagnosis in CML was 3–7 years. TKIs have revolutionized the treatment, natural history, and prognosis of CML. They have turned it in to the first cancer in which a medical treatment can return patients to a normal life expectancy.

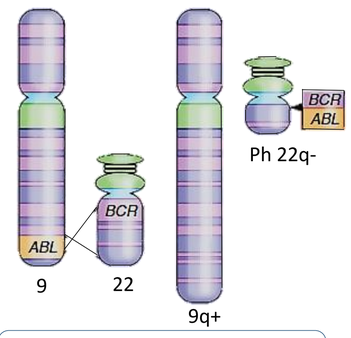


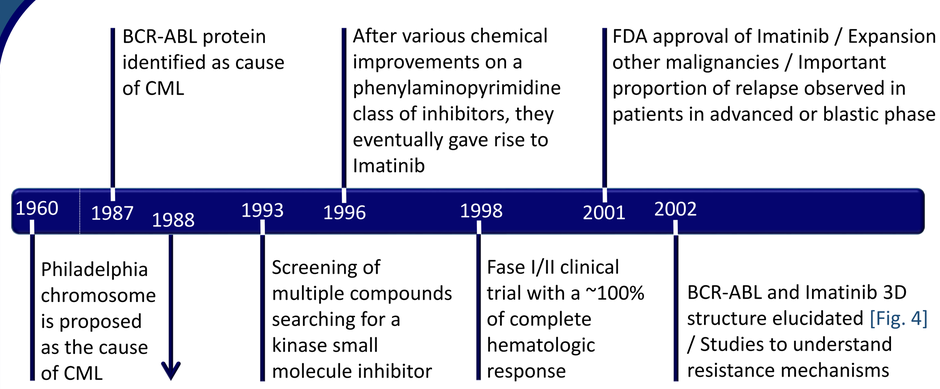
Figure 1. Translocation that creates de Philadelphia chromosome[1].

Objectives and methodology

The aim of this bibliographic revision is to describe the process of development of TKIs, specially emphasizing on the idea of rational anti-cancer drug design as the main strategy for overcoming resistance to therapy. I also pretend to review the actual situation of CML thanks to TKIs, an example of how biochemistry and molecular pathology can highly improve medicine.

Data has been obtained using mainly the searching engine *Pubmed*. Original articles of key publications of each progress in the field have been used, as well as recent reviews to understand the current situation.

From Philadelphia chromosome to Imatinib initial results



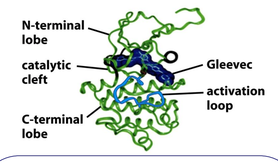


Figure 4. Imatinib targets the relatively well conserved **ATP-binding pocket** of the catalytic domain of Abl, but it can still achieve high specificity since it recognizes a distinctive **inactive conformation** of the activation loop of Abl, which is seen to differ from the inactive conformations of other kinases. These characteristics give both high specificity and affinity to Imatinib[2].

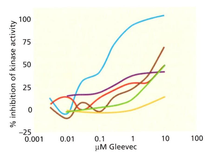


Figure 2. Responses of a group of six tyrosine kinase enzymes to Imatinib. The ability to inhibit c-Abl without affecting other enzymes is critical for therapy success[2].

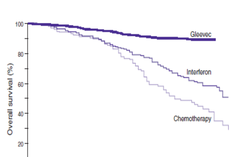
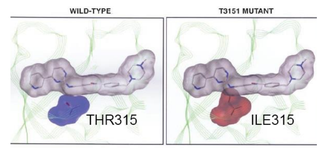


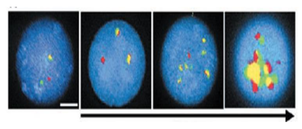
Figure 3. Overall survival in patients with CML treated with Imatinib, interferon or conventional chemotherapy[3].

Resistance mechanisms

First studies seeking for resistance mechanisms found out that relapse was **dependent on BCR-ABL**. These initial experiments revealed two resistance mechanisms:



Point mutations
After sequencing the region corresponding to the ATP binding pocket and the activation loop of the kinase domain of BCR-ABL of some CML patients, a single nucleotide change mutation (T315I) was seen to be among those that form part of a critical hydrogen bond with Imatinib[4].



Gene amplification
Multiple copies of the BCR-ABL gene were detected (by fluorescence in situ hybridization (FISH) in CML cells of patients that relapsed[4].

Further studies proved that 90% of CML patients who relapsed after responding to Imatinib had different kinase domain mutations.

To gain insight into the mutation mechanisms, they modelled each aminoacid substitution onto the crystal structure of the ABL kinase domain bound to Imatinib. This allowed the classification of mutations into two groups:

- ✓ Mutations that directly contact Imatinib (1-3).
- ✓ Mutations that prevent the conformation required for Imatinib-binding. These include mutations in the P loop region ATP phosphate binding loop (4-8) and in the vicinity of the activation loop (9-13).

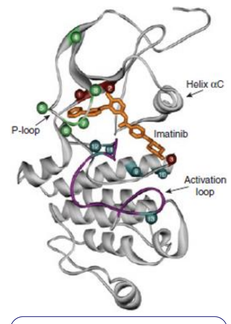
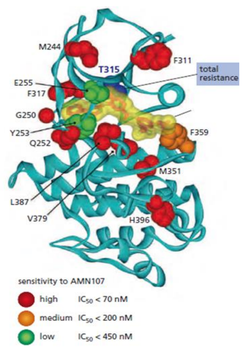


Figure 5. ABL kinase domain bound to Imatinib with 13 resistance mutations[5].

Overcoming resistance

- The complex array of mutations observed rendered difficult to envision a single second generation TKI active against all the mutations.
- A significant number of mutations prevent the kinase domain from achieving the closed conformation necessary for drug binding.

SOLUTION → to find inhibitors that bind Abl in the open configuration or with less stringent conformational requirements.



DASATINIB (2006*)	NILOTINIB (2007)	BOSUTINIB (2012)
• Dual-specific Src/Abl kinase • Binds BCR-ABL in both the active and inactive conformations [Fig. 8] • Active against 14/15 Imatinib-resistant BCR-ABL mutants • More than 300-fold potent than Imatinib	• Imatinib analogue, subtle differences account for greater potency [Fig. 7] • Active against 32/33 Imatinib-resistant BCR-ABL mutants [Fig. 6] • Less activity on the usual off-targets of Imatinib: c-Kit and PDGFR receptors.	• Dual-specific Src/Abl kinase • Activity against most Imatinib-resistant mutants of BCR-ABL • Minimal inhibitory activity against c-Kit and PDGF receptors

Year of FDA approval

Figure 6. Abl binded to Nilotinib. The locations of the amino acid substitutions of carried by the Imatinib-resistant BCR-ABL proteins are indicated in red, orange or green, and show different levels of sensibity to Nilotinib. T315I mutation is also resistant to Nilotinib[2].

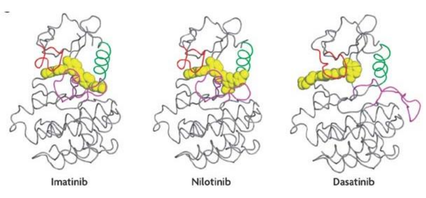


Figure 7. Binding modes of the Abl inhibitors Imatinib, Nilotinib and Dasatinib. Positions of the P-loop and activating loop vary according to whether the kinase is in an active conformation. The green helix is helix C, which often moves between the active and inactive states of kinases[6].

- Despite their efficacy and optimal safety profile, all these TKI are ineffective to the T315I mutation.
- T315 residue is located on the gatekeeper region of the ATP-binding site. It participates in a critical hydrogen bonding interaction required for high-affinity binding of the other TKIs. The T315I mutation alters the topology of the ATP-binding pocket causing a steric clash between the side chain of the isoleucine and the hydrogen from the drug.

SOLUTION → Structural-guided experiments of an inhibitor that accommodates into the T315I side chain thanks to a carbon-carbon triple bond linkage on its structure. It's the case of **Ponatinib**, a 500-fold potent than Imatinib approved by FDA in 2012.

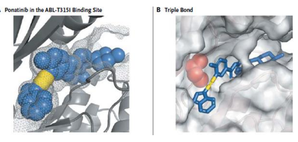
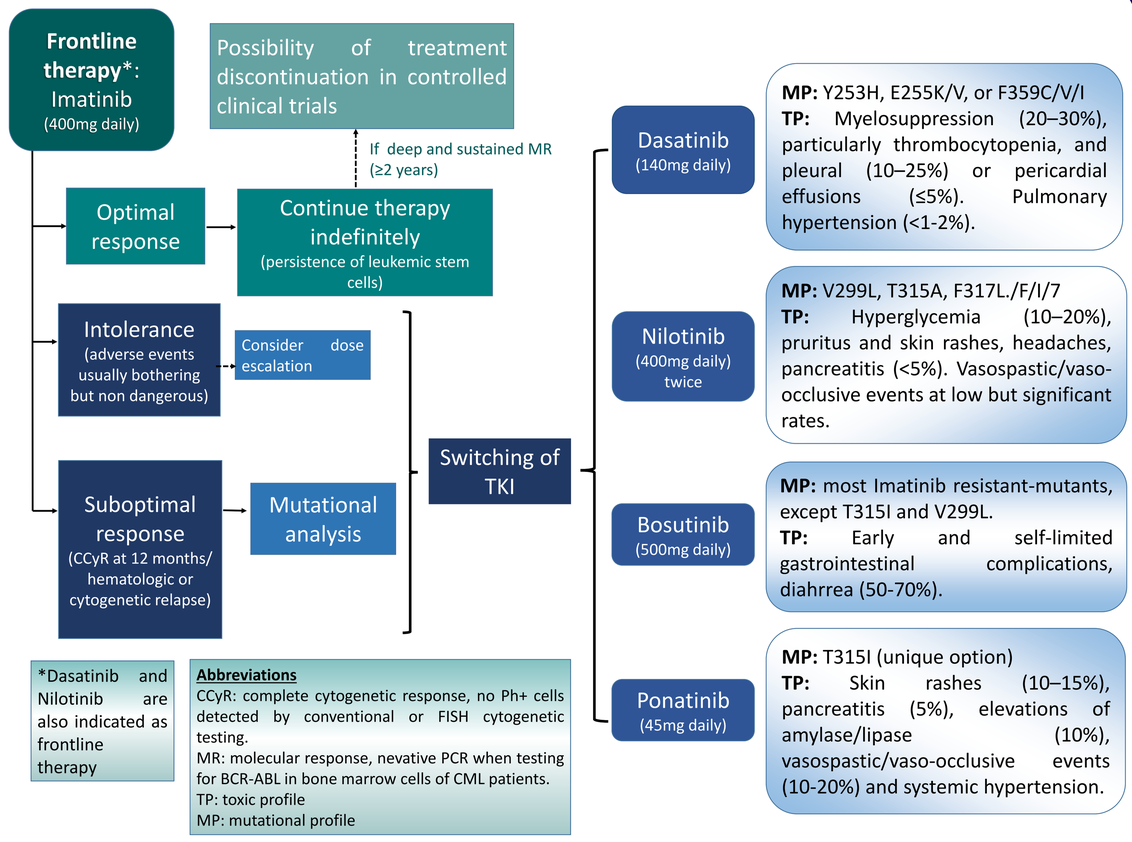


Figure 8. The triple bond is an unique structural feature of Ponatinib that allows to evade the mutant gatekeeper residue I315[7].

Current situation and treatment algorithm



- After 15 years of clinical use of Imatinib and thanks to the other TKI available, CML has become the first cancer in which a medical treatment can return patients to a normal life expectancy.
- In spite of superior data, since neither Dasatinib or Nilotinib have shown substantial amelioration in either overall survival (OS) or progression free survival (PFS) rates over Imatinib, it continues to represent the most commonly used TKI to treat CML frontline
- Moreover, owing to some important risks of second generation TKIs shown in the table, it may not be worth it to take them if there is no resistance or intolerance to Imatinib.

Conclusions

Once reviewed the development procedure of TKIs and their impact on CML patients, the following conclusions can be drawn:

- ✓ Imatinib and the rest of the TKIs approved for CML represent the first case of rational drug design against a human malignancy
- ✓ They also are an example of the problem that resistance supposes to cancer and a way of solving them
- ✓ Finally, CML is a pathology that can take profit from personalized medicine, owing to the different mutational profile of the different available drugs and the diversity profile of each one.

References

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