



# Battle at the Gates: Combating Mutant Kinases

## Introduction

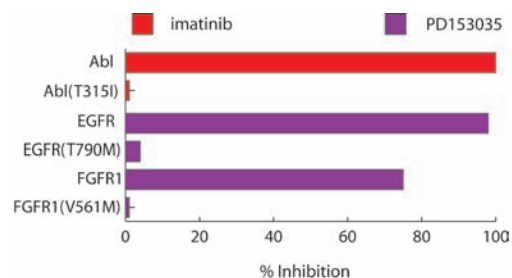
Molecular therapeutics that target protein kinases represent a major advance in the treatment of disease, particularly cancer. These drugs have proven successful in treating some cancer patients, particularly those with pathologies derived by oncogenic forms of tyrosine kinases. One of the first successful drugs developed against protein kinases is Gleevec® (imatinib), which inhibits the Abl and Kit tyrosine kinases. The initial success of Gleevec was tempered by patients who had responded initially to the drug but subsequently developed clinical resistance. The molecular basis of this resistance was soon identified—patients were shown to possess previously undetected mutant forms of Abl and Kit. Shortly thereafter during clinical studies of the EGF receptor (EGFR) inhibitors Iressa® (gefitinib) and Tarceva® (erlotinib), a similar phenomenon was observed, and mutant forms of EGFR resistant to the inhibitors were identified.

## Gatekeeper Mutants

It was soon realized that several of these drug resistant protein kinases achieved their resistance through similar mechanisms. One mutation was identified in Abl, Kit and EGFR: a mutation that changes a conserved threonine in the recess of the ATP-binding pocket to another amino acid. Remarkably, this mutation does not affect the

catalytic activity of the enzyme but interferes with the ability of the inhibitor to bind effectively, leaving the kinase active but resistant to these new drugs. This aptly named ‘gatekeeper’ residue appears to play a role in influencing the sensitivity of inhibitors in numerous protein kinases (Figure 1). However, not all protein kinase inhibitors developed are influenced by the amino acid at the gatekeeper position. This important realization implies testing various chemotypes for sensitivity to different gatekeeper sequences may be prudent to minimize the occurrence of this type of selective clinical resistance.

Figure 1: The Gatekeeper Effect

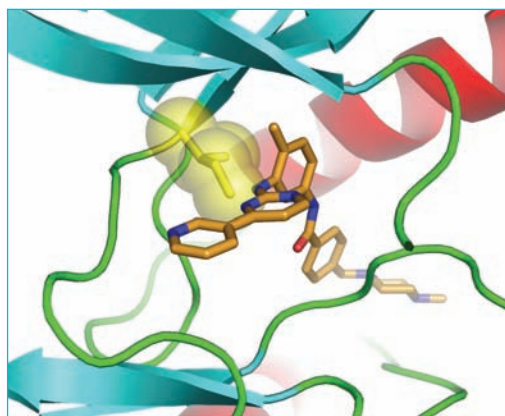


*Wild type and mutant kinases with alterations in the gatekeeper amino acid were tested for sensitivity to inhibition by small molecule inhibitors. The mutant kinases are substantially less sensitive to inhibition by the inhibitors tested.*

## Disease-Relevant Mutations

The availability of three-dimensional crystal structures of the protein kinases in complex with their respective inhibitors provides a glimpse of nature's defense mechanism (Figure 2). For example, the amino acid change identified in an imatinib-resistant form of Abl appears to sterically block the inhibitor from binding productively. Generally, the resulting amino acid substitutions at the gatekeeper appear to either sterically block inhibitor binding and/or interfere with a hydrogen bond interaction between protein and inhibitor. Once the mechanism of resistance was identified, the quest for rapid discovery of a second generation of inhibitors that were not susceptible to gatekeeper mutations was underway. To facilitate this search, Millipore has made available several disease-relevant mutant forms of protein kinases suitable for high-throughput screening and enzymology studies to understand the mechanism of action for new inhibitors.

Figure 2: Imatinib Resistance in Abl(T3151)



The crystal structure of Abl in complex with imatinib is shown with a modeled Ile residue (yellow) in place of Thr315 found in the wild type enzyme. The substitution sterically clashes with imatinib (shown in stick representation with carbon bonds colored orange).

Case study by Jeffrey Till, Ph.D. and Kimberly Owen.

### References

- Blencke *et al.* (2004) *Chemistry and Biology* **11**, 691-701.  
 Carter *et al.* (2005) *Proceedings of the National Academy of Sciences* **102**, 11011-11016.  
 Fabbro, D. and McCormick, F. *Protein Tyrosine Kinases: From Inhibitors to Drugs*. New Jersey: Humana Press, 2005.  
 Young *et al.* (2006) *Cancer Research* **66**, 1007-1014.

Kinase	Gatekeeper Residue	Cat. No.
cSrc	Thr	14-325
Abl	T3151	14-529
Abl (T3151)	Ile*	14-522
EGFR	Thr	14-531
EGFR (T790M)	Met*	14-725
EGFR (T790M, L858R)	Met*	14-721
c-Kit	Thr	14-559
SAPK2α	Thr	14-251
SAPK2α (T106M)	Met*	14-687
Tie2	Ile	14-540
KDR	Val	14-630
FGFR1	Val	14-582
FGFR1 (V561M)	Met*	14-734
JAK3	Met	14-629
IR	Met	14-466
IGF-1R	Met	14-465
TrkA	Phe	14-571
CDK1/cyclinB	Phe	14-450
CDK2/cyclinA	Phe	14-448

\*Gatekeeper positions of oncogenic kinases

## Millipore—the Kinase Leader

Millipore is committed to offering disease-relevant kinases and has the largest commercially available selection of inactive and mutant kinases, including a growing collection of gatekeeper mutants. As new drug resistant mutants are discovered, this important target class will undoubtedly aid in the development of successful second generation drugs.

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Lit. No. 1715-0706 Printed in U.S.A. 7/06

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