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Selection of therapy: Rational Decisions Based on Molecular Events

Jamshid S. Khorashad, MD, PhD^c and Michael W.N. Deininger, MD, PhD^{a,b}

^aChief, Division of Hematology and Hematologic Malignancies, University of Utah, Huntsman Cancer Institute

^bM.M. Wintrobe Professor of Medicine, University of Utah, Huntsman Cancer Institute

^cPost Doctoral Research Fellow, University of Utah, Huntsman Cancer Institute

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INTRODUCTION

Chronic myeloid leukemia is a haematopoietic stem cell malignancy caused by BCR-ABL, a fusion protein derived from a reciprocal translocation between chromosome 9 and 22 [t(9;22)(q34;q11)], cytogenetically visible as the Philadelphia chromosome (Ph). BCR-ABL is a constitutively active tyrosine kinase that activates multiple signaling pathways, thereby promoting the expansion of myeloid cells. The clinical result is a myeloproliferative neoplasm characterized by an increase of neutrophils and their precursors. In the initial chronic phase (CP) cellular differentiation is largely intact, and the disease is easily managed with drug treatment. In the absence of effective therapy, CML progresses to an acute leukemia, termed blastic phase (BP), sometimes through an intermediary state, termed accelerated phase (AP).^[1] BP CML has a poor prognosis, with survival frequently measured in weeks. The realization that the kinase activity of BCR-ABL is central to CML pathogenesis led to the development of imatinib, a small molecule ATP-competitive tyrosine kinase inhibitor (TKI) as a rational molecularly targeted therapy. Subsequently the second-line TKIs, nilotinib and dasatinib were introduced, initially for the treatment of patients who failed imatinib and recently for the therapy of newly diagnosed patients.^[2] All these drugs inhibit BCR-ABL activity, thereby blocking the activation of downstream signals critical for proliferation and survival of CML cells.^[3] However, they are distinct by their differential potency against the primary target, activity profiles against BCR-ABL mutants and 'off-target' effects against kinases other than BCR-ABL. Third generation TKIs, like ponatinib and DC-2036, are in clinical development and will add additional diversity to the therapeutic space.

Corresponding author for proof and reprints: Michael Deininger, MD, PhD, Chief, Division of Hematology and Hematologic Malignancies, M.M. Wintrobe Professor of Medicine, University of Utah, Huntsman Cancer Institute, 2000 Circle of Hope, Room 4280, Salt Lake City, Utah 84112-5550, 801/581-6363 phone, 801/585-0900 fax, michael.deininger@hci.utah.edu.
Coauthor address: Jamshid Sorouri Khorashad, MD, PhD, Post Doctoral Research Fellow, University of Utah, Huntsman Cancer Institute, Deininger Lab, 2000 Circle of Hope, Room 4270, Salt Lake City, Utah 84112-5550, 801/587-4001 phone 801/585-0900, fax jamshid.khorashad@hci.utah.edu

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In this paper we will review to what extent molecular data can be used to rationalize therapeutic choices. Naturally, this discussion will include two categories of data. The first is markers that globally measure risk, but do not provide a molecular rationale for therapy selection. The second category is biomarkers with a causal link to a clinical phenotype, such as certain mutations of the BCR-ABL kinase domain. Despite considerable progress on both fronts, as of 2011, therapy selection is still mainly based on clinical criteria, and therefore we have decided to discuss molecular biomarkers in the context of available clinical prognostication tools. Given the extensive literature on the prognostic value of BCR-ABL transcripts and their dynamics during therapy we will focus on biomarkers that do not directly reflect disease burden as a surrogate of responsiveness to treatment, and refer the reader to excellent reviews of this topic.^[4]

RISK STRATIFICATION

Disease phase

The most important clinical tool to risk-stratify CML patients is the phase of disease. Irrespective of the type of therapy, results tend to be best in CP, intermediate in AP and poor in BP. The early studies of imatinib in patients who had failed prior interferon-alpha (IFN) based therapies impressively confirmed the previous experience with IFN, conventional cytotoxic agents like hydrea, and allogeneic stem cell transplant. Results from the International Randomized Study of Interferon and STI571 (IRIS) and other single-center or community-based studies uniformly show that imatinib is most effective in early CP, with higher rates of complete cytogenetic response (CCyR) and major molecular response (MMR) in newly diagnosed patients compared to patients treated in late CP, defined as treatment initiation later than one year from diagnosis or after failure of IFN (Table 1).^[5-7] Response rates and the durability of responses are much lower in AP/BP compared to the CP (early or late).^[8-12] Compared to imatinib, nilotinib and dasatinib were shown to result in higher rates of CCyR and MMR in newly diagnosed patients;^[13-17] however, similar to imatinib, response rates and durability were lower in the patients treated in late CP, AP or BP.^[18;19] There are two major messages from these data. Firstly, the fact that none of the approved TKIs are able to completely abolish the effects of longer disease duration or progression suggests that the mechanisms underlying failure overlap; secondly, the fact that patient populations that meet the criteria for CP have very different response rates is evidence that CP defined on clinical/morphological grounds must encompass a spectrum of disorders. Compared to CP, AP is even more heterogeneous and represents the most difficult category in terms of outcome prediction. As few data are available on the results of TKI therapy in patients who are diagnosed in AP, it is likely that this rather uncommon group will encompass some patients who are biologically in CP. From the perspective of the Developed World, where some 90% of patients are diagnosed in CP, the most important question is whether we can accurately identify high risk patients who are in CP by morphological criteria.

Clinical risk stratification in CP

The first clinical risk stratification tool for newly diagnosed CP patients was established by Joseph Sokal and colleagues in a cohort of patients treated with cytotoxic drugs.^[20] Age, spleen size, platelet count and the percentage of blast cells in the peripheral blood were identified as independent variables in multivariate analysis.^[20] Remarkably, the predictive value of the Sokal score was subsequently confirmed in patients treated with IFN, imatinib and nilotinib.^[7;14;21;22] The fact that Sokal score is predictive of response irrespective of therapy suggests that it identifies critical biological disease features. Whether this reflects a biological quality that is present *ab initio* or acquired during a preceding period of undiagnosed disease is unknown. Subsequent studies aimed at improving the Sokal score.

By including more parameters in the multivariate analysis, Hasford, et al, developed a new score (Euro score) comprised of blood eosinophil and basophil counts in addition to the factors used in the Sokal score. The Euro score was superior in predicting survival in patients treated with IFN patients.^[23] Recently, a new prognostic score (EUTOS, European Treatment and Outcome Study for CML) was reported for patients treated with imatinib. The EUTOS score is based only on the percentage of basophils in blood and on spleen size and was shown to be superior to both the Sokal and the Euro scores in its prognostic ability.^[24]

The Hammersmith group developed a score for early identification of responders to second line TKIs nilotinib and dasatinib.^[25;26] This prognostication system is based on three factors: previous cytogenetic response to imatinib; Sokal risk at diagnosis; and recurrent neutropenia during imatinib therapy.^[25] Other groups reported that patients with poor performance status and no previous cytogenetic response to imatinib therapy had a low likelihood of responding to second-generation TKIs and should be offered additional treatment options.^[27;28] These observations suggests that *in vivo* drug sensitivity, i.e., the response to initial therapy (i.e., a cytogenetic response) identifies a good risk group of patients, and that this good risk is – to some extent – carried over to salvage therapies. This is consistent with experience in oncology, where failure after an initial response is usually prognostically more favorable than a primary refractory disease.

Cytogenetic clonal evolution (CE) refers to the presence or development of karyotypic abnormalities in addition to the Philadelphia chromosome (Ph) (Table 2). In some studies its presence at diagnosis was associated with poor prognosis.^[29;30] In the pre-IFN era, CE was noted in 30% to 50% of the patients before blastic transformation, and was considered as diagnostic of an accelerated phase.^[31;32] However, some patients who developed cytogenetic CE on IFN therapy continued in CP for long periods, with the occasional disappearance of CE.^[33] In patients treated with imatinib in late CP after IFN failure, CE was not a significant prognostic factor for major cytogenetic response.^[34] Subsequent studies confirmed this initial finding, but identified CE as an independently poor prognostic factor for survival and relapse free survival in CP and AP CML.^[35;36] Why CE appears to impact overall and relapse free survival, but not cytogenetic response, remains unexplained. CE was also shown to be associated with detection of low level BCR-ABL kinase domain (KD) mutations in imatinib-naïve patients, suggesting that both may reflect underlying genetic instability.^[37] While, at this point, the prognostic significance of CE at the time of diagnosis remains somewhat controversial, there is consensus that acquisition of CE after start of imatinib is indicative of a such a high risk of relapse that it is now considered failure.^[38–40] Limited data are available in patients treated with second-line TKIs, but results are generally similar. Notably, the impact of specific abnormalities is variable: trisomy 8, chromosome 17, and complex abnormalities are associated with the worst outcome.^[41]

While the established risk scores based on standard diagnostic tests provide some information on the risk of CP patients, they are too imprecise to inform far-reaching clinical decisions. Additionally, they are mostly epiphenomenal and do not identify the molecular causes underlying the higher risk of treatment failure or progression to advanced phase. To overcome this shortcoming genome-wide approaches as well as functional assays have been explored to develop more precise prediction tools.

Genome-wide scanning

Several groups have embarked on genome-wide scanning approaches to identify biomarkers that predict CP response to TKI therapy. Various technology platforms were used, including gene expression profiling and array comparative genomic hybridization (CGH). In a retrospective study of patients treated with chemotherapy (mostly hydroxyurea) Yong, et al,

investigated the gene expression profile of CD34+ cells stored at diagnosis and identified several genes (CD7, proteinase 3, elastase) whose level of expression was correlated with the duration of chronic phase.^[42] Radich, et al, compared gene expression in CP, AP and BP bone marrow cells and observed phase specific expression patterns that distinguished between CP and AP/BP. In contrast, expression patterns in AP and BP were very similar, suggesting that CML is essentially a two-phase rather than three-phase disease.^[43] They also saw significant overlap between the gene expression patterns of patients with resistance to imatinib with that of patients in AP/BP and found that samples from patients in second CP after blastic transformation had essentially maintained the profile of BP, indicating that a morphological remission fails to turn back the biological clock' of the disease. In aggregate these findings indicate that the biological features of imatinib resistance and disease progression overlap and override morphological criteria. This notion was further supported by an independent study by McWeeney, et al, who studied the gene expression profiles of CD34+ cells from patients in CP prior to imatinib treatment and identified a gene expression classifier that correlated with subsequent cytogenetic response.^[44] In metanalysis they detected overlap between the expression profiles of imatinib non-responders with those of short CP in the Yong, et al, study, and BP in another study that compared gene expression profiles in CD34+ cells from patients with CP vs. myeloid BP, further supporting the association between TKI failure and progression to AP/BC.^[42;45;46] It is likely that not all of these prognostically important genes directly confer resistance. For example, myeloid differentiation genes like ELA2, MPO, CSTA, CSTG and PRTN3 are downregulated in prospective non-responders (McWeeney, et al.) and patients with a short CP (Yong, et al.), an expression pattern consistent with the differentiation block that characterizes transformation from CP to AP/BP. Whether this differentiation block itself is causal to resistance remains to be established. From the point of clinical utility it is desirable to limit the number of genes necessary to predict molecularly advanced disease. Toward this goal the Seattle group recently reported a refinement of their molecular prediction algorithm to a set of only six genes (NOB1, DDX47, IGSF2, LTB4R, SCARB1, and SLC25A3) that distinguish between CP and AP/BP.^[47] Validation of these data in independent studies will be required before this classifier is used clinically. Comparative genomic hybridization (CGH) or SNP arrays were used to investigate genomic aberrations at the DNA level in newly diagnosed patients and during the progression of disease from CP to BC.^[48-50] Recurrent cryptic losses on certain chromosomes in CML CD34+ cells were correlated with loss of response to imatinib.^[51] Perhaps due to rather small sample sizes and the heterogeneity amongst the various studies, no consistent pattern has emerged, as yet, and additional validation studies will be required.^[51-54]

MEASURING SENSITIVITY TO BCR-ABL INHIBITORS

A different approach to response prediction would be to establish correlations between target inhibition and clinical response. Several groups reported that the extent of *in vitro* inhibition of BCR-ABL by imatinib in primary cells from newly diagnosed CML patients is correlated with subsequent clinical response,^[55-58] while another study did not find such a correlation.^[59] The conflicting results could be due to the technical differences such as the source of investigated cells (fresh or cryopreserved CD34+ cells), different culture conditions and differences in drug concentrations and exposure times to imatinib. Another approach is to quantify the *in vivo* BCR-ABL kinase inhibition in the first month of imatinib therapy. In a prospective study the degree of BCR-ABL inhibition (measured using immunoblots to quantify CrkL phosphorylation as a surrogate marker), was shown to be an excellent predictor of cytogenetic and molecular response.^[60] As this study was based on analysis of mononuclear cells (MNC) at different time points following treatment initiation, it is possible that the reduced kinase activity reflected a relative reduction of leukemic cells rather than the inhibition of kinase activity. Another approach to predict *in vivo* sensitivity is

to measure TKI inhibition of primary cells cultured *ex vivo*. For example, there is a fairly good correlation between imatinib inhibition of colony formation and clinical response.^[61] Unfortunately, all of these assays are too cumbersome for routine purposes.

DRUG TRANSPORTERS

Imatinib is subject to active transport mechanisms and the expression of several drug transporters has been shown to correlate with response, although some of the data remain controversial. For example, while *in vitro* studies in cell lines have demonstrated that imatinib, nilotinib and dasatinib are all substrates of the ABCB1 and ABCG2 ion transporters, there is little evidence that these mechanisms are operational in primary CML progenitor cells.^[62-65] In contrast, there is a fairly large body of data supporting the role of hOCT1 in active transport of imatinib into the cells and in association with response to imatinib therapy.^[66;67] For example, White, et al, reported that higher hOCT1 activity at diagnosis in MNC was predictive of optimal long term outcome in CML patients treated with imatinib in CP.^[68;69] Unexpectedly, hOCT1 seems to be more important for imatinib transport in differentiated cells compared to CD34+ cells, where expression is low and not predictive of response.^[63] The reason for these counterintuitive observations remains unclear, but it is likely that some of the discrepancies in the published data reflect differences in the composition of the cells analyzed in the various studies.^[70;70] Another problem is the use of prazosin to block the hOCT1 channel for measurement of hOCT1 activity. Since prazosin may target other transporters, this assay could overemphasize the role of hOCT1 as the main transporter of imatinib. Consistent with this, *in vitro* studies found that imatinib is a poor substrate for hOCT1 and concluded that this transporter is unlikely to contribute substantially to the deposition and activity profile of imatinib. Nevertheless, prazosin sensitivity might provide a composite surrogate for the activity of several transporters that are relevant to the intracellular uptake and retention of imatinib.^[71] In addition to hOCT1 activity,^[69] high hOCT1 expression was also shown to predict response to imatinib.^[67;72;73] As of now hOCT1 activity is one of few biomarkers that might indeed influence therapeutic decisions, since higher doses of imatinib (600mg/day) have been shown to overcome the negative effect of low hOCT1 activity on response.^[69] Unfortunately, the predictive value of hOCT1 remains limited to imatinib as the active transport of imatinib through hOCT1 does not seem to have any role in transport of nilotinib or dasatinib.^[74;75]

MOLECULAR MARKERS IN SECOND-LINE THERAPY

It is estimated that approximately 20–40% of newly diagnosed CP patients will eventually require switching to dasatinib or nilotinib for intolerance or resistance.^[40;70;76–79] While the mechanisms governing primary resistance remain poorly understood, many patients with acquired imatinib resistance have missense mutations in the BCR-ABL KD that reduce TKI sensitivity to various degrees depending on the mutation type and specific TKI.^[80–84] Given that dasatinib and nilotinib may eventually replace imatinib in frontline therapy, the mutation spectrum in second-line therapy will change dramatically.^[13;14] T315I was the first BCR-ABL KD mutation detected in clinical samples^[85] and is the only mutant resistant to imatinib and both second line TKIs dasatinib and nilotinib.^[86] Many other mutants detected in clinical samples have been validated biochemically and exhibit various degrees of resistance to available TKIs, supporting clinical decisions in a subset of patients.

KD mutations in IM naïve patients

Several studies have analyzed KD mutations in pre-imatinib samples using highly sensitive techniques. However, there was not apparent correlation between the presence of mutations and response; if resistance developed the mutation initially present was not necessarily

present at the time of relapse. Therefore there is no role for detection of mutations at a very low level prior to treatment. Whether patients with advanced disease should be screened is a matter of debate; since most of these patients will have received TKI therapy, this will usually be part of a resistance workup.^[37;87–89] Importantly the situation in CML is different from Philadelphia positive acute lymphoblastic leukemia (ALL), where BCR-ABL KD mutations conferring high-level imatinib resistance are present in a substantial proportion of patients at diagnosis, eventually give rise to relapse.^[90]

BCR-ABL KD mutations in patients on imatinib

Mutations not only cause resistance to imatinib and loss of response, but are also shown to be associated with outcome, irrespective of their level of TKI resistance. Specifically, several studies showed that mutations of the ATP binding loop (p-loop) were associated with poor prognosis in patients on imatinib.^[83;91] One possible explanation for the poor clinical outcome of patients with p-loop mutation could be the increased transformation potency of p-loop mutants Y253F and E255K observed in the *in vitro* experiments.^[92] Less surprisingly, other studies found correlations between the level of imatinib resistance and outcome, reflecting the efficacy of available treatment.^[93] It is obvious that such correlations are valid only in the context of a specific TKI, with a specific activity profile.

The detection of mutations in patients who are responding to imatinib therapy is considered a “warning sign,” as it is associated with a higher risk of loss of response.^[93;94] For example, in a study of patients who had achieved CCyR, the detection of a KD mutation was the only significant predictor for loss of CCyR.^[93] Interestingly, detection of KD mutation at the time of resistance to imatinib predicts a higher likelihood of developing other KD mutations after starting second line TKIs.^[88;95] Higher genetic instability might be responsible for the ‘development’ of the secondary mutations, which may have existed before the start of salvage therapy, but were suppressed to low levels by the dominant mutant clone. Altogether, these data suggest that KD mutations measure several different parameters influencing outcome; those related to biochemical consequences at the target level (high vs. low level resistance mutations; hypo- or hypermorphic kinase alleles with increased or reduced transforming potency^[92;96]) and others that provide an indirect measurement of critical disease features, such as genomic instability.

KD mutations and selection of second line therapy

In vitro studies have provided information about the sensitivity of the different BCR-ABL KD mutants to second line TKIs which provide guidance for choosing the appropriate drug after imatinib failure.^[80;81] A study by the Adelaide group suggested that screening for KD mutations may provide useful clinical information in some 43% of cases.^[97] However the clinical sensitivity of the various mutants to the second line TKIs does not completely match the *in vitro* predictions. For example, the Y253H, E255K, and F359V/C mutants are sensitive to nilotinib *in vitro*, but largely resistant in clinical studies. Similarly, F317L and V299L are moderately resistant to dasatinib *in vitro*, but highly resistant *in vivo* (Table 3). Thus the use of *in vitro* data alone to guide the choice of TKI for patients can be misleading, as it does not take into account important *in vivo* variables such as protein binding, cell influx/efflux.^[98] The exception is the T315I mutation, which consistently confers resistance to all currently approved TKIs.^[97] With this in mind, the most important information from mutational analysis of the BCR-ABL kinase domain is whether T315I is present or not. Several studies showed that the impact of T315I on survival depended on the disease phase at the time of detection, suggesting that, while T315I is a highly resistant mutant, it is not by itself associated with a more aggressive biological phenotype.^[99] In support of this, T315I was shown to exhibit reduced kinase activity *in vitro*.^[94] Although it remains somewhat controversial whether T315I is indeed a loss-of-function mutant,^[92;100] it can explain why

the mutant clone can regress after discontinuation of therapy.^[101;102] Patients with a T315I mutation will not benefit from dasatinib or nilotinib and should be offered an experimental drug if they are in CP and allogeneic stem cell transplantation if they have progressed to AP/BP. The most promising experimental agent is ponatinib, a multi-targeted kinase inhibitor that is active against all BCR-ABL mutants tested, including T315I. *In vitro* mutagenesis screens failed to reveal any new single mutation liability, in contrast to second-line TKIs tested with the same experimental system.^[103] Results from a phase 1 study have shown considerable activity in CP patients who failed two or more TKIs, including imatinib. Interestingly, the rate of CCyR was higher in patients with T315I than in patients with other KD mutations or without mutations.^[104] Thus, effective treatment can turn a poor prognostic marker into a favourable one. A speculative explanation for this is that CML that escapes from TKI therapy through acquisition of T315I identifies itself as extremely dependent on BCR-ABL and as such extremely sensitive to restoration of BCR-ABL inhibition with ponatinib.

Prognostic biomarkers or scores at the start of second line TKI

While the type of KD mutation is a valuable molecular marker to predict response to 2nd-line TKIs, many patients do not have mutations, and the correlation with response/resistance is tight only toward the 'negative' side (T315I). However, other biomarkers may be able to capture the underlying risk biology. For example *EVI-1* expression at the point of imatinib failure has been shown to identify patients who are likely to fail on second line regimens and might be better served by early referral for allogeneic transplantation.^[105] As discussed earlier BCR-ABL KD mutations predict a higher risk of developing (or: unmasking) secondary mutations on second line TKI therapy.^[88;95] Further investigations are needed to provide information on what pattern of gene expression or molecular markers can be used as prognostic tools for patients who start dasatinib or nilotinib as the first line therapy or after imatinib failure.

FUTURE DIRECTIONS

An honest account of molecular biomarkers in CML is that the only tool with broad clinical impact in 2011 is screening for KD mutations. All other prognostication relies on routine clinical and laboratory measurements that define disease stage and conventional risk scores. One could even argue that biomarkers in CML will never see their prime time, given the high rates of CCyR and low risk of progression in newly diagnosed patients treated with second generation TKIs.^[13;14] However, for two reasons, this is an unlikely scenario. Firstly, imatinib will come off patent in 2015, and the question will arise whether we can identify a patient population that will do equally well with a generic drug that will be far less expensive than second generation TKIs. In an era of run-away health care costs, this will have major health-economic implications. Prospective studies will be critical to support a biomarker-based approach to initial therapy. Secondly, therapeutic goals will shift toward disease eradication and the ability of TKIs to annihilate the leukemic cell clone may be correlated with molecular disease features present at diagnosis. In support of this, a high Sokal risk score was highly predictive of disease recurrence in the Stop Imatinib (STIM) trial, where patients with a complete molecular response to imatinib discontinued therapy.^[106] Thus, there may be three categories of CP patients at diagnosis. A first group, probably a small minority, who will achieve sustained molecular negativity and can eventually discontinue therapy; a second group where this may be achieved by combining TKIs with another biochemical therapeutic modality; and a third group where only an immunological approach such as allogeneic transplant is capable of permanently suppressing or even eradicating the CML clone. Undoubtedly, to maximize the impact of biomarker studies, it will be important not only to develop molecular markers, but also

convert this knowledge into novel and rational targeted therapies. A long way to go, but the first steps have been taken.

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Table 1

Response to imatinib according to disease phase

Disease Phase		CCyR	Overall survival
Chronic phase (CP)	Newly diagnosed ^[7;21]	5 years ~ 78–82%	5 years ~ 83%–89%
	Late CP ^[6]	5 Years ~ 55%	5 Years ~77%
Accelerated Phase ^[8;10]		~ 17%	12 months ~ 74%
Blastic Phase ^[11;12]		~ 7%	12 months ~ 32%

Table 2

Common additional cytogenetic abnormalities in Ph+ positive cells. [107;108]

Abnormality	% of cases with additional cytogenetic changes
Trisomy 8	34
Second Philadelphia chromosome	30
Isochromosome 17	20
Trisomy 19	13
Loss of the y chromosome	8 (of males)
Trisomy 21	7
Trisomy 17	5
Monosomy 7	5

Table 3

Sensitivity of mutants to nilotinib and dasatinib

Nilotinib			Dasatinib		
In vitro		In vivo	In vitro	In vivo	
O'Hare ⁸¹	Redaelli ⁸⁰	Branford ⁹⁷	O'Hare	Radaelli	Branford
T315I [*]	T315I [*]	T315I	T315I/A [*]	T315I [*]	T315I/A
Y253H ^{***}	E255V [*]	Y253H	F317L ^{***}	V299L ^{**}	V299L
E255V ^{***}	E255K ^{**}	E255K/V	V299L ^{***}	E255K ^{**}	F317L/I/C/V
E255K ^{***}	F359V ^{**}	F359V/C	E255V ^{***}	L248V ^{**}	
F359V ^{***}	G250E ^{**}		F317L ^{***}	F317L ^{**}	
Y253F ^{***}	Y253F ^{***}		E255K ^{***}	G250E ^{**}	
Q252H ^{***}	H396R ^{***}		Q252H ^{***}	E255V ^{***}	
T315A ^{***}	L248V ^{***}			Q252H ^{***}	
V379I ^{***}	Q252H ^{***}			F486S ^{***}	
	H396P ^{***}			L384M ^{***}	
	L384M ^{***}				
	F317L ^{***}				
	E279K ^{***}				

Modified from Branford et al. [97]

* Highly resistant according to *in vitro* studies

** Resistant according to *in vitro* studies

*** Moderately resistant/sensitive according to *in vitro* studies