

# Kinase Mutations as Predictive Biomarkers in Hematolymphoid Malignancies

## Introduction

Targeted therapeutics against kinases has been employed in the clinic for over a decade. Their use of ten times requires concomitant molecular diagnostics evaluating the mutational status of the targeted kinases. While kinase mutations function as diagnostic and prognostic biomarkers in hematolymphoid malignancies, in this review, we focus on kinase mutations with predictive utility and their relevance to molecular diagnostics.

## ABL Mutations in Philadelphia-Positive Leukemias

The development of the tyrosine kinase inhibitor (TKI) imatinib against the BCR-ABL fusion product in chronic myeloid leukemia (CML) was a watershed event that ushered in the era of targeted therapeutics in oncology [1,2].

Although CML patients show a high rate of response to imatinib [3] and other approved TKIs (nilotinib [4], dasatinib [5] and bosutinib [6]), primary and acquired resistance is observed [7,8]. One of the earliest documented mechanisms of resistance to TKIs is mutations in the ABL kinase domain [9]. ABL kinase domain mutations are now believed to be one of the more frequent causes of TKI resistance [8].

The T315I mutation was the first mutation reported in the context of TKI resistance [9]. To date, more than 90 mutations have been described, although it appears that 15 amino acid substitutions account for more than 85% of the mutations observed clinically [8]. Kinase domain mutations may be classified into two groups – those that alter amino acids directly in contact with the TKI; or those that prevent the oncoprotein from achieving the inactive conformational state required for TKI binding [10]. Several studies have examined the relationship between specific ABL kinase domain mutations and TKI response [11-13]. Table 1 provides a list of mutations that have been studied for their clinical significance. (The reader is referred to other references for the *in vitro* sensitivity data of the mutations [14]) Our local institutional data for the distribution of ABL kinase domain mutations is displayed in Figure 1.

Compound mutations (defined as two or more mutations in the same BCR-ABL molecule [17]) resulting in high-level resistance may arise due to sequential TKI treatment [18,19].

With regards to the implications of ABL kinase domain mutations for the molecular diagnostics laboratory, several issues are noteworthy. The first is that conventional Sanger sequencing has a detection limit of 10-20% and may not enable the detection of low-level mutations that might have an impact on therapeutic response and clinical outcome [20]. Several techniques have been employed to detect low-level mutations, including allele-specific oligonucleotide polymerase chain reaction (PCR) [21] and mass spectrometry [20].



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More recently, next-generation sequencing (NGS) platforms have been utilized for the detection of low-level mutations [22,23].

Second, the distinction between compound and polyclonal mutations is difficult to address by existing platforms (conventional Sanger sequencing or NGS) due to PCR-mediated recombination generating chimeric amplicons containing sequences from more than one allele [24]. A conceivable solution is single-molecule sequencing, which has not yet entered the clinical molecular diagnostic arena [25].

## BRAF Mutations in Hematolymphoid Neoplasms

BRAF, a serine/threonine kinase is an established oncogene and

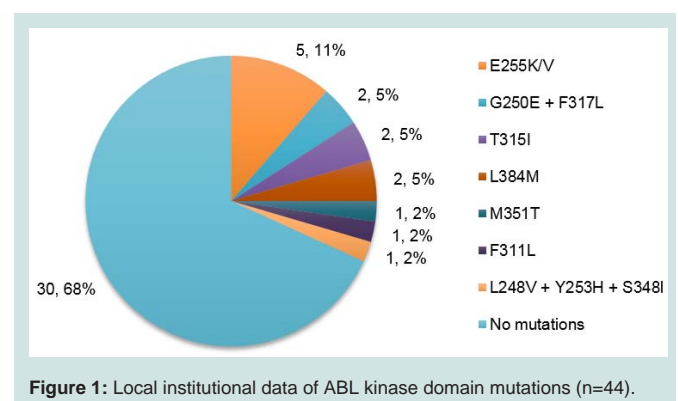


Figure 1: Local institutional data of ABL kinase domain mutations (n=44).

therapeutic target [26]. *BRAF* mutations were first reported in solid tumors in 2002 [27] and subsequently identified in hematolymphoid neoplasms (reviewed in Machnicki et al. [28]). The most common *BRAF* mutation across all tumor types is the V600E mutation [26]. Vemurafenib was the first small molecule inhibitor reported to display enhanced activity against the *BRAF* V600E mutant protein compared to the wild-type protein [26,29]; currently, both vemurafenib and dabrafenib are approved for the treatment of *BRAF* V600E-mutant melanomas [30,31].

To date, the companion diagnostic-therapeutic paradigm for *BRAF* inhibitors has been reported in hairy cell leukemia [32-39], Langerhans cell histiocytosis (LCH) [40,41] / Erdheim-Chester disease (ECD) [41-43] and multiple myeloma [44,45]. These are discussed in detail below.

The genetic drivers of hairy cell leukemia (HCL) were unknown until 2011, when Tiacci et al. performed whole-exome sequencing of an index case which led to the identification of the *BRAF* V600E

mutation and subsequently showed the mutation to be present in a larger cohort of HCL samples [46]. Approximately a year later, Dietrich et al. reported the first case of a patient with refractory *BRAF* V600E-mutant HCL who responded to vemurafenib monotherapy; similar reports of response to *BRAF* inhibitors have since followed [32-39].

Similar to HCL, the genetic events in LCH and ECD (both diseases of histiocytes), remained obscure until the identification of *BRAF* V600E mutations in LCH (reported in 2010 [47]) and ECD (reported in 2012 [48,49]). Subsequent studies have demonstrated response of *BRAF* V600E-mutant LCH and ECD patients to vemurafenib [40-43].

The existence of *BRAF* mutations in multiple myeloma was first reported in 2011 [50]. Around two years later, Andrulis et al. reported clinical response in a multiple myeloma patient with the *BRAF* V600E mutation treated with the mutation-specific inhibitor vemurafenib [44]. Other authors have reported similar findings [45]. This represents the first example of an effective kinase inhibitor against multiple myeloma.

**Table 1:** ABL kinase domain mutations and clinical significance.

Mutations	Clinical significance	References
L248V	Favorable response rate to dasatinib was seen in patients with the L248V mutation	[13]
G250E	Favorable response rate to dasatinib was seen in patients with the G250E mutation	[13]
Q252H	CCyR (complete cytogenetic response) rate to dasatinib appeared to be lower in patients with the Q252H mutation (caution is advised when interpreting these response rates because of the small number of patients)	[13]
Y253H	Dasatinib treatment was associated with favorable CCyR rates in patients with the Y253H mutation	[13]
Y253H	Patients with the Y253H mutation had less favorable responses [13% of patients with these mutations achieved MCyR (major cytogenetic response); none achieved CCyR]	[11]
E255K/V	Favorable response rate to dasatinib was seen in patients with the E255K/V mutations	[13]
E255K/V	Patients with the E255V/K mutations had less favorable responses (43% of patients with these mutations achieved MCyR; none achieved CCyR)	[11]
V299L	CCyR rates to dasatinib appeared to be lower in patients with the V299L mutation (caution is advised when interpreting these response rates because of the small number of patients)	[13]
T315I	Patients with the T315I mutation respond poorly to imatinib, dasatinib, nilotinib	[9,11,13,15,16]
F317L	CCyR rates to dasatinib appeared to be lower in patients with F317L (caution is advised when interpreting these response rates because of the small number of patients)	[13]
F317L	In all patients but one, resistance to dasatinib was invariably found to be associated with mutations at residue 315 and/or at residue 317	[15]
F359C/V	Dasatinib treatment was associated with favorable CCyR rates in patients with the F359C/V mutations	[13]
F359C/V	Patients with the F359C/V mutations had less favorable responses (9% of patients with these mutations achieved MCyR; none achieved CCyR)	[11]
L384M	CCyR rates to dasatinib appeared to be lower in patients with the L384M mutation (caution is advised when interpreting these response rates because of the small number of patients)	[13]

### BTK Mutations in B-Cell Malignancies

Bruton's tyrosine kinase (BTK) is a non-receptor tyrosine kinase that was first implicated in X-linked agammaglobulinemia [51]. Subsequent work demonstrated that BTK is an important player in many B-cell malignancies [52].

Ibrutinib [53], an irreversible BTK inhibitor that binds covalently to the C481 residue of BTK at its active site [54], has been approved for the treatment of relapsed or refractory mantle cell lymphoma (MCL) [55]. Phase 1 and 2 trials have showed that ibrutinib is also efficacious against chronic lymphocytic leukemia (CLL) [56,57].

Similar to what has been observed with *ABL* resistance mutations developing in response to TKI therapy, a BTK C481S mutation resulting in ibrutinib resistance [58] has been reported in both MCL [59] and CLL [60,61] patients.

### Conclusion

The *BRAF* inhibitors, vemurafenib, dabrafenib and BTK inhibitor, ibrutinib have only recently been employed clinically for the treatment of hematolymphoid neoplasms and experience with these agents is still limited. The decade-long accumulated wisdom with BCR-ABL inhibitors for the treatment of CML suggests that multiple resistance mutations against vemurafenib/dabrafenib and ibrutinib might similarly develop. In melanomas, *MEK1* and *MEK2* mutations have been identified as a possible resistance mechanism against *BRAF* inhibitors [62]. The results of further studies into resistance mutations are eagerly anticipated.

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