Next-Generation Sequencing of the BCR-ABL1 Kinase Domain and Neighboring Domains Associated with Therapy Resistance

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Introduction

BCR-ABL1 mutation testing is recommended for CML and Ph+ ALL patients who fail first-line tyrosine kinase inhibitors (TKI) therapy or who have a suboptimal response to therapy. BCR-ABL1 mutations in the kinase domain (KD) of ABL1 account for at least 50-60% of all TKI resistant cases. New mutations such as T315I and T315L in the kinase domain of ABL1 outside of the KD have also been reported to lead to imatinib resistance.1 The current gold standard for BCR-ABL1 mutation detection is Sanger sequencing, which has an analytical sensitivity of >90%. Based on recent findings that mass spectrometry can identify low level BCR-ABL1 mutations that confer clinical resistance in patients sooner than Sanger sequencing,2 more sensitive methods for detecting BCR-ABL1 mutations should be clinically useful. Although commercial NGS cancer panels include ABL1 among the regions of interest, for clinical resistance ABL1 resistance mutations should be sequenced from BCR-ABL1 fusion transcripts instead of being sequenced from primary DNA as in the commercial panels, since we developed a fusion transcript-based BCR-ABL1 mutation assay on the scalable and cost-effective Ion Torrent platform that has 1% sensitivity and comprehensive coverage of the KD, regulatory domain, and the SH3/SH4 domains. This assay was designed to detect both the major and minor BCR-ABL1 gene products and can also detect the micro-BCR-ABL1 fusion protein accounting for over 99% of all CML and Ph+ ALL patients.

Materials and Methods

Reagent and long PCR workflow was performed to amplify BCR-ABL1 ex. 14, 16, and 18 fusion transcripts. The PCR products were enzymatically fragmented and ligated with Ion Torrent sequencing adaptors.3 Bar-coded libraries were quantified, pooled and amplified with the Onfocus system and sequenced with Ion Torrent PAQs. Sequencing data were analyzed with Torrent Suite v. 3.2.2 and the associated variant caller with variant frequencycaff tool adjusted to 1%. Variants derived from b2.2 were further processed and annotated with a proprietary analysis pipeline.

Results

Analysis: sensitivity - BCR-ABL1 18 fusion product from a cell line harboring the T315I mutation was cloned into the head of a wild type cell line with a final concentration of 2% (b2.2, 0.14% T315I). The 18 PCR product of T315I was amplified as 100% (Fig. 1A). The accuracy of the mutation frequency was demonstrated by the linear correlation between the expected and observed mutation frequency reported by Ion Torrent Variant caller (Fig. 1B). The sensitivity of detecting a low-level mutation was >95%.

Determination of phasing of compound mutations: The 18 PCR fragment (~300bp) sequencing shows identification of 18 or more mutations haplotype in BCR-ABL1. In sample MMD b2_9, mutation 18084 (CGaCG; 1.6% and 1.4% in T315I and sh353, but it is di with the adjacent silent mutation t18084G as shown in Table 2.

Conclusion

We demonstrated a highly sensitive NGS assay for deep sequencing of BCR-ABL1 resistance mutations with a LOD of 3% for single base substitutions, as shown by T315I sensitivity experiments, and the capability to detect complex mutations with deletions and insertions.

The assay determines the advantages of NGS over Sanger by detecting a greater number of resistance mutations within and outside of breakpoint regions and allowing for determination of the phasing status of compound mutations.

The mutation spectrum of BCR-ABL1 in CML patients is highly heterogeneous.

References


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