Two Detection Methods of Clinical KRAS G12C Mutation Detection for Companion Diagnostics

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INTRODUCTION

KRAS G12C is a common mutation in approximately 13% of lung adenocarcinoma, 3% of colorectal cancer and 2% of other solid tumors. The FDA-approved sotorelin, a KRAS G12C inhibitor, is targeted for the treatment of patients with KRAS G12C-mutated locally or metastatic non-small cell lung cancer (NSCLC). It is essential to detect the KRAS G12C mutation with a companion diagnostic before the drug is administered. We have developed two KRAS G12C mutation detection assays for qPCR or Sanger sequencing. Both QClamp® assays use our proprietary xeno nucleic acid (XNA) technology which specifically enhances the amplification of the target mutant DNA sequence while blocking amplification of the wildtype sequence, thus improving the sensitivity of the assays for qPCR (~1%) and Sanger sequencing (~15 to 20%) on 0.1% variant allele frequency. These and simple sensitive assays can be used for clinical research or validated in GLIA labs for KRAS G12C mutation detection for the purpose of companion diagnostic use. Although NGS methods are gaining traction in clinical settings, our methods are suitable for standard molecular diagnostics laboratory settings and do not add any additional steps to the qPCR or Sanger sequencing protocols. Our methods are especially valuable for labs that appreciate simple, economical, yet sensitive companion diagnostics assays for KRAS G12C mutation. QClamp® assays can be easily adapted to detect other single gene mutations with high sensitivity for companion diagnostics use or other mutation detection applications.

METHOD

**KRAS c.12 qPCR Assay**

The KRAS c.12 qPCR assay was performed in the presence or absence of G12 XNA at 8 different concentrations of the G12C mutant target sequence at 0.01, 0.1, 0.25, 0.5, 1.25, and 5% VAF were amplified by PCR in the presence or absence of XNA and scored by Sanger sequencing. The glycine codon (GGT) is identified in all the replicates for the KRAS G12 XNA, thus allowing the preferential amplification of the mutant variant by inhibiting amplification of the wildtype with and without XNA plotted in the graph.

**KRAS G12C Mutation Enrichment and Sanger Sequencing**

The KRAS G12 wildtype or G12C mutant target sequences at 0.01, 0.1, 0.25, 0.5, 1.25, and 5% VAF were amplified by PCR in the presence or absence of XNA and Sanger sequencing was performed by Sequetech.

RESULTS

**XNA increases KRAS c.12 qPCR sensitivity by inhibiting G12 wildtype amplification**

KRAS G12 XNA specifically binds the wildtype G12 sequence preventing amplification by DNA polymerase, therefore allowing preferential amplification of the G12C mutant target sequence in a linear dose-dependent manner. Amplification from the wildtype G12 sequence masks the mutant signal in the absence of XNA, and the G12C mutant is undetectable at 5% VAF. The qPCR samples were subsequently analyzed by Sanger sequencing.

**Conclusions**

KRAS G12 QClamp® qPCR and mutation enrichment for Sanger sequencing assays increase sensitivity for the low copy G12C mutant variant by inhibiting amplification of the wildtype with the G12 XNA, thus allowing the preferential amplification of the G12C mutant, hence increasing the signal-to-noise ratio. The KRAS G12 QClamp® CE and RUO assays are simple, reliable, and economical kits for mutation analysis in clinical specimens.

**REFERENCE**