
eMethods
eFigure 1. Study flowchart
eFigure 2. Paired samples subjected to shipping

This supplementary material has been provided by the authors to give readers additional information about their work.
**eMethods**

**Droplet Digital PCR**

TaqMan PCR reaction mixtures were assembled from a 2× ddPCR Mastermix (Bio-Rad) custom 40× TaqMan probes/primers and 5 uL of isolated DNA. After droplet generation via an automated droplet generator, we performed PCR to endpoint as described below. Analysis of the ddPCR data was performed with QuantaSoft analysis software (Bio-Rad) that accompanied the droplet reader. Positive and negative clusters were set using the FAM and VIC thresholds based on the amplitude of positive controls that were run concomitantly with each assay.

**Droplet Digital PCR Materials**

Droplet digital PCR reagents were ordered from Bio-Rad. Primer/probe mix for EGFR T790M, EGFR L858R, EGFR exon deletion 19, and KRAS G12X and KRAS G13D were custom-made by Life Technologies. For EGFR del19 ddPCR assay, primer sequences are: forward, 5′-GTGAGAAAGTTAAAATCCCCGTC-3′, reverse, 5′-CACACAGCAGGAAGCGAACP-3′; probe sequences are: 5′-FAM-AGGAATTAAGAGAGAGACACATC-MGB-3′ (ex19 deletion hotspot probe), 5′-VIC-ATCGAGATTTCCTTGTTC-MGB-3′ (ex19 reference probe). For KRAS G12A assay, primer sequences are: forward, 5′-GCCTGCTGAAAATGACTGAATATAACT-3′, reverse, 5′-GCTGTATCGTCAAGGCACACTCTT-3′; probe sequences are: 5′-VIC-TTGGAGCTGTGCGATGTA-MGB-NFQ-3′, 5′-FAM-TTGGAGGTGTGCCTGG-TGGC–MGB-NFQ-3′. For KRAS G12C assay, primer sequences are: forward, 5′-GCCTGCTGAAAATGACTGAATATAACT-3′, reverse, 5′-GCTGTATCGTCAAGGCACACTCTT-3′; probe sequences are: 5′-VIC-TTGGAGCTGTGCGATGTA-MGB-NFQ-3′, 5′-FAM-TTGGAGGTGTGCCTGG-TGGC–MGB-NFQ-3′. For KRAS G12S assay, primer sequences are: forward, 5′-GCCTGCTGAAAATGACTGAATATAACT-3′, reverse, 5′-GCTGTATCGTCAAGGCACACTCTT-3′; probe sequences are: 5′-VIC-TTGGAGCTGTGCGATGTA-MGB-NFQ-3′, 5′-FAM-TTGGAGGTGTGCCTGG-TGGC–MGB-NFQ-3′. For KRAS G12V assay, primer sequences are: forward, 5′-GCCTGCTGAAAATGACTGAATATAACT-3′, reverse, 5′-GCTGTATCGTCAAGGCACACTCTT-3′; probe sequences are: 5′-VIC-TTGGAGCTGTGCGATGTA-MGB-NFQ-3′, 5′-FAM-TTGGAGGTGTGCCTGG-TGGC–MGB-NFQ-3′. For KRAS G12T assay, primer sequences are: forward, 5′-GCCTGCTGAAAATGACTGAATATAACT-3′, reverse, 5′-GCTGTATCGTCAAGGCACACTCTT-3′; probe sequences are: 5′-VIC-TTGGAGCTGTGCGATGTA-MGB-NFQ-3′, 5′-FAM-TTGGAGGTGTGCCTGG-TGGC–MGB-NFQ-3′. For KRAS G13D assay, primer sequences are: forward, 5′-GCCTGCTGAAAATGACTGAATATAACT-3′, reverse, 5′-GCTGTATCGTCAAGGCACACTCTT-3′; probe sequences are: 5′-VIC-TTGGAGCTGTGCGATGTA-MGB-NFQ-3′, 5′-FAM-TTGGAGGTGTGCCTGG-TGGC–MGB-NFQ-3′. For KRAS G13R assay, primer sequences are: forward, 5′-GCCTGCTGAAAATGACTGAATATAACT-3′, reverse, 5′-GCTGTATCGTCAAGGCACACTCTT-3′; probe sequences are: 5′-VIC-TTGGAGCTGTGCGATGTA-MGB-NFQ-3′, 5′-FAM-TTGGAGGTGTGCCTGG-TGGC–MGB-NFQ-3′. For KRAS G13S assay, primer sequences are: forward, 5′-GCCTGCTGAAAATGACTGAATATAACT-3′, reverse, 5′-GCTGTATCGTCAAGGCACACTCTT-3′; probe sequences are: 5′-VIC-TTGGAGCTGTGCGATGTA-MGB-NFQ-3′, 5′-FAM-TTGGAGGTGTGCCTGG-TGGC–MGB-NFQ-3′.

**EGFR L858R.** Standard curves were prepared using H1975. The L858R standard curve ranged from 1000 copies/uL to 25 copies/uL. L858R specific TaqMan probes/primers
(Bio-Rad) were used in the PCR reaction which had the following cycling conditions: 95 C x 10 min (1 cycle), 94 C x 30 s (40 cycles), 58 C x 1 min (40 cycles), and 10 C hold.

**EGFR exon 19 deletion.** Standard curves ranging from 2500 copies/uL to 25 copies/uL were prepared using A549 and PC9 genomic DNA as wild type and mutant respectively. Del19 specific TaqMan probes/primers (Bio-Rad) were used in the PCR reaction which had the following cycling conditions: 95 C x 10 min (1 cycle), 94 C x 30 s (40 cycles), 55 C x 1 min (40 cycles), and 10 C hold.

**EGFR T790M.** Standard curves were prepared using PC9 GR with concentrations ranging from 1000 copies/uL to 10 copies/uL. T790M specific TaqMan probes/primers (Bio-Rad) were used in the PCR reaction which had the following cycling conditions: 95 C x 10 min (1 cycle), 94 C x 30 s (40 cycles), 58 C x 1 min (40 cycles), and 10 C hold.

**KRAS G12X.** Standard curves were prepared using G12C and KRAS-wild type plasmids with concentrations ranging from 1000 copies/uL to 10 copies/uL for each KRAS variant. TaqMan probes/primers (Bio-Rad) were used in the PCR reaction which had the following cycling conditions: 95 C x 10 min (1 cycle), 94 C x 30 s (40 cycles), 60 C x 1 min (40 cycles), and 10 C hold.

Prespecified threshold for positive results were as follows:
- EGFR Exon 19 Del: >5 copies/mL
- EGFR L858R: >2 copies/mL
- EGFR T790M: >1 copy/mL
- KRAS G12X: >1 copy/mL
eFigure 1: Study flowchart. 180 patients with advanced NSCLC were consented to a prospective study of ddPCR-based plasma genotyping. 120 patients were newly diagnosed (cohort 1) and 60 patients had acquired resistance to EGFR kinase inhibitors (cohort 2). Plasma genotyping and tissue genotyping was successfully completed for 174 patients for **EGFR** exon 19/L858R (cohort 1 n=115, cohort 2 n=59), 54 patients for **EGFR** T790M (cohort 2 only) and 85 patients for **KRAS** (cohort 1 only). Patients were excluded from analysis if they did not complete the blood draw or tissue genotyping failed or was not performed.
eFigure 2: Paired samples subjected to shipping. The total DNA (PicoGreen) and mutant allele frequency does not differ between mailed EDTA tube on ice (A) or mailed Streck tube at room temperature (B) when compared with a paired sample drawn simultaneously from the same patient and processed immediately as per standard operating procedure. The outcome of plasma genotyping was identical between shipped draws and paired standard draws with respect to being positive or negative.