Supplemental Materials and Methods.

Circulating-free DNA Extraction

Whole venous blood (6-10 mL) was collected from patients into EDTA lavender capped vacutainer tubes (*Becton and Dickinson).* All patients gave consent for collection and genomic analysis of up to 50 mL of blood over a 3 month period on an IRB-approved protocol ongoing at the Dana Faber Cancer Institute. Whole blood was centrifuged for 10 min at 1200g and the plasma supernatant was further cleared by centrifugation for 10 min at 3000g. Cleared plasma was stored in cryostat tubes at -80C until use. Cell free DNA was isolated using the QIAmp Circulating Nucleic Acid Kit (Qiagen) according to the manufacturer's protocol. DNA was eluted in AVE buffer (100 uL) and stored at -80C until use.

Human *LINE* qPCR quantification

LINE-1 was quantified by real time PCR using a modified version developed by Rago et al. PCR was performed in a total 10μL using 1.9 μL sterile tissue culture grade double-distilled water (Invitrogen), 5 μL of Power SYBR® Green PCR Master Mix (Invitrogen), 0.4 μL of 10 mmol/L deoxynucleotide triphosphates (U.S. Biochemical), 0.6 μL of DMSO (Sigma), 0.8 uL of 50 mM MgCl2 and 0.2 μL of 100 mM forward primer FWD 5′-TCACTCAAAGCCGCTCAACTAC-3′ (Operon; desalted, 50 nmol scale), 0.2 μL of 100 mM reverse primer REV 5′-TCTGCCTTCATTTCGTTATGTACC-3′ (Operon; desalted, 50 nmol scale), 0.1 μL of Platinum taq DNA polymerase (Invitrogen), and 0.8 μL of purified patient derived cfDNA diluted 50 fold. The reaction was monitored on a StepOnePlus (Applied Biosystems) with the following cycling conditions: (94°C, 2 min) × 1, (94°C, 10 s; 67°C, 15 s; 70°C, 15 s) × 3, (94°C, 10 s; 64°C, 15 s; 70°C, 15 s) × 3, (94°C, 10 s; 61°C, 15 s; 70°C, 15 s) × 3, (94°C, 10 s; 59°C, 15 s; 70°C, 15 s) × 35. The threshold cycle number was determined using Applied Biosystems' analysis software. Standard curve was obtained by using a A549 cell line starting at 1000 pg/uL and serially diluted down tenfold for five points with an addition sixth point of 0 pg/uL (water). All samples and standard curves were run in triplicates.

PicoGreen total DNA quantification

Total DNA was quantified using a fluorescence absorbance PicoGreen assay (Invitrogen). Quant-iT™ PicoGreen® dsDNA Reagents and Kits (Invitrogen) were used according to manufacturer's protocol. Reaction was performed using 96 well, round bottom, non-treated plates (Costar). Five point standard curves ranging from 2000 pg/uL to 0.2 pg/uL with an additional sixth point of 0 pg/uL (water) were generated from A549 and PC9 derived genomic DNA) and run in triplicates. Patient derived cfDNA was diluted 100 fold in 1xTE buffer. 100 uL of diluted sample or neat standard was added to 100 uL of aqueous working solution of the Quant-iT™ PicoGreen®. Each sample was run in triplicate. Samples were excited at 480 nm and the fluorescence emission intensity was measured at 520 nm using EnSpire® Multimode Plate Reader (PerkinElmer).

Droplet Digital PCR Workflow

TaqMan PCR reaction mixtures were assembled from a 2× ddPCR Mastermix (Bio-Rad) and custom 40x TaqMan probes/primers made specific for each assay. Nineteen microliters of assembled ddPCR reaction mixture plus 6 uL of ultra pure distilled water was loaded into sample wells of an eight-channel disposable droplet generator cartridge (Bio-Rad). An additional seventy microliters of droplet generation oil (Bio-Rad) was loaded into the oil well for each channel. After droplet generation the cartridge was removed and manually transferred with a multichannel pipet to a 96-well PCR plate. The plate was heat-sealed, placed on a conventional thermal cycler, and amplified to the end-point. After PCR, the 96-well PCR plate was read on the QX-100 droplet reader (Bio-Rad). Analysis of the ddPCR data was performed with QuantaSoft analysis software (Bio-Rad) that accompanied the droplet reader.

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, *BRAF* V600E and *KRAS* G12C were custom-made by Life Technologies. The allele-specific MGB probes are labeled with either VIC or FAM at the 5' end and a nonfluorescent quencher (NFQ) at the 3' end. For EGFR L858R assay, primer sequences are: forward, 5'-GCAGCATGTCAAGATCACAGATT-3', reverse, 5'-CCTCCTTCTGCATGGTATTCTTTCT-3'; probe sequences are: 5'-VIC-AGTTTGGCCAGCCCAA-MGB-NFQ-3', 5'-FAM-AGTTTGGCCCGCCCAA-MGB-NFQ-3'. For EGFR del19 ddPCR assay, primer sequences are: forward, 5'-GTGAGAAAGTTAAAATTCCCGTC-3', reverse, 5'-CACACAGCAAAGCAGAAAC-3'; probe sequences are: 5'-VIC-ATCGAGGATTTCCTTGTTG-MGB-NFQ-3' (ex19 reference), 5'-FAM-AGGAATTAAGAGAAGCAACATC-MGB-NFQ-3' (ex19 deletion hotspot). For EGFR T790M assay, primer sequences are: forward, 5'-GCCTGCTGGGCATCTG-3', reverse, 5'-TCTTTGTGTTCCCGGACATAGTC-3'; probe sequences are: 5'-VIC-ATGAGCTGCGTGATGAG-MGB-NFQ-3', 5'-FAM-ATGAGCTGCATGATGAG-MGB-NFQ-3'. For KRAS G12C assay, primer sequences are: forward, 5'-GCCTGCTGAAAATGACTGAATATAAACT-3', reverse, 5'-GCTGTATCGTCAAGGCACTCTT-3'; probe sequences are: 5'-VIC-TTGGAGCTGGTGGCGTA-MGB-NFQ-3', 5'-FAM-TTGGAGCTTGTGGCGTA-MGB-NFQ-3'. For BRAF V600E assay, primer sequences are: forward, 5'-CATGAAGACCTCACAGTAAAAATAGGTGAT-3', reverse, 5'-TGGGACCCACTCCATCGA-3'; probe sequences are: 5'-VIC-CTAGCTACAGTGAAATC-MGB-NFQ-3', 5'-FAM-TAGCTACAGAGAAATC-MGB-NFQ-3'.

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

the following cycling conditions: 95 C x 10 min (1 cycle), 40 cycles of 94 C x 30 s and 55 C x 1 min, followed by 10 C hold.

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

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

***BRAF* V600E.** Standard curves were prepared using V600E and BRAF-wild type plasmids with concentrations ranging from 1000 copies/uL to 10 copies/uL. V600E specific TaqMan probes/primers (Life Technologies) were used in the PCR reaction which had the following cycling conditions: 95 C x 10 min (1 cycle), 40 cycles of 94 C x 30 s and 58 C x 1 min, followed by 10 C hold.

References:

Rago, Carlo, et al. "Serial assessment of human tumor burdens in mice by the analysis of circulating DNA." *Cancer research* 67.19 (2007): 9364-9370.