

Supplementary File Description: This file contains 2D amplitude plots of ddPCR signal by mutation for LOD determination, schematics about assay performance and R-based gate determination, and data from ddPCR of tumor tissue samples, matched CSF samples, correlations (MAF vs. clinical variables), and contingency plots for each cohort. It also contains tables detailing patient characteristics and a BRISQ report.

(if needed captions are included below)

Supplementary Figure 1. (a) Schematic depicting 2D amplitude differentiation between mutually exclusive C228T and C250T mutations. In our assay, the wildtype probe binds at the C228C locus. Since the sequence generated by the two mutations is identical (228 CCCCTTCCGG, 250 CCCCTTCCGG), our mutant probe (5'-FAM/CCC+C+T+T+CCGG/3IABkFQ/) can bind at either C250T or C228T loci. So, in the case of wildtype amplicon, only the wildtype probe binds at C228C locus. In the case of a C228T mutant, only the mutant probe can bind. In the case of C250T mutant, the wildtype probe binds at the C228C locus and the mutant probe binds at the C250T locus. This differential binding, along with mutually exclusive nature of the mutations in gliomas allows for differentiation on 2D amplitude plots of mutant probe fluorescence (FAM) against wildtype probe fluorescence (HEX). **(b)** 2D Amplitudes for Mutant Allele Frequencies, merging all replicates. Left (C250T) and Right (C228T). Panels on the left show 2D amplitude plot for a serial dilution of C250T A431 mutant DNA in a background of HBMVEC wildtype DNA at known mutant allele frequencies from 10% to 0.05%, along with a wildtype only blank (0%). $LoB = \text{mean}(\text{blank}) + 1.645(SD_{\text{blank}})$, and $LoD = LoB + 1.645(SD_{\text{lowest concentration sample}})$. LoD and LoB were used to calculate corresponding %MAF.

Supplementary Figure 2. R-based gating setting and detection of TERT Promoter Mutation in Plasma of Discovery, Validation and Multi-Institution Cohorts (a) Schematic depicting gating strategy and threshold determination using the training set (discovery and validation cohorts; n=83) **(b)** Schematic depicting blinded testing of validation set (multi-institution cohort; n=74) using gate and thresholds trained on discovery set.

Supplementary Figure 3. Tumor Tissue Analysis and Analysis of plasma TERT in Copies/mL. (a) gDNA was extracted from 21 TERT mutant and 4 TERT WT tumor tissue samples. 100 ng of tumor tissue gDNA was used as input for absolute quantification of copies of TERT mutant and TERT WT. Copies per 20 uL of TERT mutant and WT are plotted against Study ID, classified by SNaPSHOT/Pathology. **(b)** 4 replicates of 4 uL of cfDNA from matched plasma samples (21 TERT mutant and WT) and healthy control (10) was used as input for absolute quantification of TERT mutant and wildtype copies. Copies/mL were calculated using the formula described in Methods. Copies/mL for plasma samples are plotted against Study ID, classified by SNaPSHOT/Pathology as floating bars, with line at the mean copies/mL. **(d)** Samples are grouped as to depict concordance between tumor tissue and matched plasma. **(c)** Mean copies/mL of TERT mutant for plasma samples are plotted against SNaPSHOT/Pathology classification. Dotted line indicates threshold of 8.5 copies/mL, used to designate samples as mutant positive or negative.

Supplementary Figure 4. Correlations between **(a)** progression free survival, **(b)** overall survival, **(c)** tumor grade, **(d)** contrast enhancement, **(e)** type of TERT mutation, **(f)** tumor volume, **(g)** duration of disease, **(h)** age. Contingency tables are provided for **(i)** Discovery Sample Set 1 **(j)** Discovery Sample Set 2 **(k)** Multi-Institution Validation Cohort **(l)** Overall Combined Cohort. **(m)** 4 replicates of 4 uL of cfDNA from matched CSF samples (n=4; n=3 TERT mutant and n=1 WT) was used as input for absolute quantification of TERT mutant and wildtype copies. MAF is calculated using the formula described in Methods. Copies/mL for plasma samples are plotted against Study ID, classified by SNaPSHOT/Pathology as floating bars, with line at the mean copies/mL. **(n)** Contingency table for matched CSF plasma.

Supplementary Table 1. Patient Characteristics for Discovery Cohort 1. Tumor volume calculated by taking three measurements and using the following formula: $4\pi/3 * R1 * R2 * R3$. GBM = Glioblastoma, WT= Wildtype, N/A = Not Available, M=Male, F=Female

Supplementary Table 2. Patient Characteristics for Discovery Cohort 2. Tumor volume calculated by taking three measurements and using the following formula: $4\pi/3 * R1 * R2 * R3$. GBM = Glioblastoma, WT= Wildtype, N/A = Not Available, M=Male, F=Female

Supplementary Table 3. Patient Characteristics for Multi-Institution Cohort. Tumor volume calculated by taking three measurements and using the following formula: $4\pi/3 * R1 * R2 * R3$. GBM = Glioblastoma, WT= Wildtype, N/A = Not Available, M=Male, F=Female

Supplementary Table 4. BRISQ reporting guidelines for study cohort.