Figure S1: Flow diagram, FFPET quality and tumor tissue sequencing characteristics

(A) Process flow diagram of analyzed samples from the LEMA trial.

(B) The distribution of FFPET DNA sample quality, as defined by a qPCR-based quality assay (Q-ratio). The Q-ratio corresponds roughly to the proportion of DNA in a sample that can be amplified. Q-ratios in this set ranged from 0.01 to 1 (mean 0.49; standard deviation 0.23).

(C) For all FFPET samples run, the input mass of DNA (as determined by a dsDNA fluorescent dye) is compared to the percent of on-target exonic positions that have at least 300x unique depth. The recommended minimum input mass for a given sample is 10 / Q-ratio + 10 ng of input. Post-sequencing, the quality check ensures 90% of panel positions have at least 300x deduped depth. Samples colored in blue had at least 10/Q+10 ng of input DNA into library prep, while those in red did not. 97% of samples (152/156) that met the input mass cutoff, met the depth cutoff, while 74% of samples (20/27) that did not meet the input mass cutoff, met the depth cutoff.

(D) The sample Q-ratio (x-axis) was plotted against the sample error rate, defined as the percentage of non-reference bases seen and excluding variants with greater than 5% AF. Lower quality samples showed higher error rates, as expected, and no sample with a Q-ratio above 0.15 showed an error rate greater than 0.05%.

Figure S2: Correlation between tissue TMB and tissue sample characteristics

(A) TMB in mutations per Mb are compared without use of paired germline data (i.e. only variants with AFs between 5% and 40% were counted) vs. with paired germline data (i.e. all variants present in tissue but not germline with AFs >5% counted). Data are colored by the mean AF from the paired germline data.

(B) Comparison of mean AF of variant calls versus tissue-germline TMB, no significant correlation is seen.

(C) WES TMB calculated with SNV variants only (x-axis) compared to tumor tissue panel TMB (y-axis) for a subset of samples from this study (n = 11).

(D) In-silico comparison of panel content and TMB results of the FDA-approved F1CDx panel (x-axis) and the tumor tissue panel (2.2 Mb) (y-axis). Both panels were intersected from WES variants to generate the correlation between panels using the described bioinformatics calculation method (Methods).

(E) TMB results shown per category of histology. LUAD: lung adenocarcinoma, LUSC: lung squamous cell carcinoma, LCNEC: large-cell neuroendocrine carcinoma and NOS: non-small cell lung cancer not otherwise specified. No significant difference was seen between the different histology types (ANOVA, p = 0.84).

(F) TMB results shown per category of NSCLC disease stage.

(G) TMB results shown split by PD-L1 expression. No significant differences are seen among the <1%, 1-50%, or >50% PD-L1 expression groups (Wilcoxon test).

(H) TMB results shown split by smoking status. Never smokers show significantly lower TMB than former smokers (p=0.031) or current smokers (p=0.047) (Wilcoxon test).

(I) TMB results compared to pack years of smoking history.

Figure S3: Correlation of tissue TMB with mutation status of lung cancer driver genes

TMB results are shown, split by the presence or absence of mutations in EGFR (A), BRAF (B), KRAS (C), TP53 (D), KEAP1 (E), and STK11 (F). Wt = wildtype tumors, the specified mutation is absent.

Figure S4: In-silico validation of the lung TMB panel

(A) The WES variants from the Rizvi et al. dataset are *in-silico* intersected with the tumor tissue panel for the 34 patients in the study. The TMB values intersected on the tumor tissue panel correlated with the original WES TMB values: tumor tissue panel TMB/Mb = 0.86 * WES TMB/Mb + 0.79, R-squared = 0.76 and Spearman's Rho = 0.74.

(B) Comparison of WES nonsynonymous TMB in the Rizvi et al. dataset, split in patients with durable clinical benefit (DCB, n=14) and no durable benefit (NDB; n=16), p-value = 0.017. The diamond symbol (♦) in the boxplot represents outliers.

(C) Kaplan-Meier curves showing progression free survival for patients from the Rizvi et al. dataset split by high vs. low TMB, using a WES cutoff of 10 mutations/Mb (HR=3.32, 95% CI: 0.87-7.99)

(D) Correlation of TMB results of the whole exome dataset from Rizvi et al. (x-axis) and theoretical TMB results of the Rizvi et al. dataset when only including the lung TMB panel regions (y-axis). In the WES dataset, a cutoff of 178 non-synonymous SNVs is equal to 5.6 mut/Mb and corresponds to 11.6 mut/Mb in the lung TMB panel.

(E) Comparison of nonsynonymous TMB from the Rizvi et al. dataset when applying the lung TMB panel, split in patients with durable clinical benefit (DCB; n=14) and no durable benefit (NDB; n=16), p-value = 0.002. The diamond symbol (♦) in the boxplot represents outliers.

(F) Kaplan-Meier curves showing progression free survival for patients from the Rizvi et al. dataset split by high vs. low TMB, using the WES cutoff as determined in Figure S4D (split by 5.6 nonsynonymous mutations/Mb; HR=4.2, 95% CI: 1.62-10.95).

(G) Kaplan-Meier curves showing progression free survival for patients from the Rizvi et al. dataset split by high vs. low TMB, using the lung TMB panel cutoff as converted from WES cutoff in Figure S4D (split by 11.6 mutations/Mb, HR=3.15, 95% CI: 1.36-7.28).

Figure S5: cfDNA sequencing characteristics

(A) Input mass of cfDNA compared with the median unique depth of each sample, colored by stage.

(B) and (C) Input mass of cfDNA compared with the percentage of on-target exonic positions that have at least 1000x (B) or 3000x (C) unique depth.

Figure S6: Correlation between tissue TMB and plasma TMB is impacted by input mass of cfDNA and plasma tumor burden

(A) Correlation of TMB between tissue (x-axis) and plasma (y-axis), colored by input mass of cfDNA in ng. Samples with less than 20 ng of cfDNA input show less correlation than those with more than 20 ng of input.

(B) Correlation metrics (R squared in red; Spearman's rho in blue) between tissue and plasma TMB using different cutoffs for AF. The tumor informed mean AF are calculated per plasma sample and categorized in seven cutoffs, from left to right: no cut-off, >0.01%, 0.03%, 0.1%, 0.3%, 1 and 3%. For each input mass, seven different variant AF cutoffs (applied to each reported variant detected in all plasma samples) were considered (from left to right: >0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 1%) as depicted in seven red and blue dots per mean tumor informed AF category.

Figure S7: Correlation between stage of disease, cfDNA input mass and tumor burden

(A) The mass of cfDNA extracted per mL of plasma, split by stage of disease.

(B) The tumor-informed mean AF per plasma sample, split by stage of disease.

Figure S8: Impact of cfDNA input mass, stage of disease and mean AF on bTMB and tTMB concordance

(A) Positive predictive value (PPV) for TMB high calls in plasma across different input mass cutoffs (>0, 10, 20, 30, 40, 50 ng) and stage cutoffs (any stage, II/III/IV, III/IV and only IV) are shown. PPV is shown on the y-axis, the input mass on the x-axis and stage is represented by the color of the bars. The number of samples assessed is listed above each bar.

(B) PPA for TMB high calls in plasma across different input mass cutoffs (>0, 10, 20, 30, 40, 50 ng) and tumor-informed mean AF cutoffs (any, >0.01%, >0.1%, >1%, mean AF). PPA is shown on the y-axis, the input mass on the x-axis and stage is represented by the color of the bars. The number of samples assessed is listed above each bar.

(C) Positive predictive value (PPV) for TMB high calls in plasma across different input mass cutoffs (>0, 10, 20, 30, 40, 50 ng) and tumor-informed mean AF cutoffs (any, >0.01%, >0.1%, >1%, mean AF). PPV is shown on the y-axis, the input mass on the x-axis and tumor-informed mean AF cutoffs are represented by color of the bars. The number of samples assessed is listed above each bar.