Supplemental Methods

Imaging analysis. All CT and ^{18}FDG PET-CT scans were prospectively interpreted during routine clinical evaluation by board-certified radiologists blinded to the plasma sequencing data and were independently interpreted by RECIST 1.1 criteria (1) using MINT Lesion (Mint Medical, Hamilton, NJ). PET Edge (MIM Software, Cleveland, OH) was used to calculate ^{18}FDG metabolic tumor volume (MTV) and SUVMax in all pre-treatment PET-CT scans. Primary clinical radiographic reports from post-treatment scans were classified as (i) showing no evidence of disease, (ii) recurrent/persistent disease, or (iii) equivocal findings due to inability to distinguish tumor from other processes. For all post-treatment scans, interpretations were compared to ctDNA results prior to or at the time of imaging (**Figure 2E**, **Supplemental Table S1**) .

Biological specimen collection and processing. 10-20 mL of peripheral blood was collected in K_2EDTA Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and processed within 3 hours of phlebotomy. Tubes were centrifuged at 1,800g for 10 minutes and plasma was frozen at -80° Celsius in 1-2 mL aliquots. A portion of the leukocyte-enriched plasma-depleted whole blood was also frozen at -80° Celsius in 1-2 mL aliquots and used for isolation of germline genomic DNA as previously described (2,3). For some patients, matched formalin-fixed paraffin embedded (FFPE) tumor tissue was available and DNA was isolated as previously described (2).

Library preparation and sequencing. DNA isolation, library preparation and sequencing were performed using the original version of CAPP-Seq for 5 patients and iDES-enhanced CAPP-Seq for 36 patients as previously described (2,3). We applied a 188 kb CAPP-Seq selector targeting 128 genes recurrently mutated in lung cancer (2,3). Briefly, we first identified genes of clinical importance (e.g. *KRAS*, *EGFR*, *ALK*, *ROS1*, *RET*, *BRAF*, etc.). We then supplemented this relatively small list of genes with a much larger number of regions from genes that were found to be recurrently mutated in TCGA whole exome analyses of NSCLC but which were not necessarily previously implicated in lung cancer biology. We used a custom bioinformatic approach that first maximizes the number of patients we would predict to be covered by at least 1 mutation and then in a second phase selects additional regions to maximize the number of mutations per patient (2). In order to meet our design size constraint of ~200 kb, some genes of potential interest such as RB1 were excluded. Both plasma and germline DNA (from PBMCs in plasma-depleted whole blood) were sequenced. We controlled for germline SNPs, subclonal variants and clonal hematopoiesis in pre-treatment plasma-derived cell-free DNA by sequencing DNA from peripheral blood mononuclear cells (PBMCs) at the matched time point and excluded variants based on read support in PBMCs using the CAPP-Seq adaptive variant-caller (2,3). Sequencing was performed on Illumina HiSeq 2500 or 4000 instruments (Illumina, San Diego, CA) using 2 x 100 or 2 x 150 paired-end reads with an 8-base indexing read.

Pre-treatment tumor genotyping. If pre-treatment tumor was available, it was used for genotyping (2). Otherwise, pre-treatment genotyping was performed noninvasively using CAPP-Seq with integrated digital error suppression (iDES) with minor modifications (3). Briefly, cell-free DNA sequencing reads were de-duplicated using molecular barcodes, background-polished to reduce stereotypical base substitution errors, and genotyping was performed to call single nucleotide variants (SNVs), insertions/deletions (indels) and fusions (4) from pre-treatment plasma using matched germline sample as background reference. Germline single nucleotide polymorphisms (SNPs) present within other samples run in the same sequencing lane were removed from consideration.

Classification of mutations. We compiled a list of putative driver genes that were previously identified by The Cancer Genome Atlas (TCGA) research network for lung adenocarcinoma (LUAC) and lung squamous cell carcinoma (LUSC) and by Rudin et al. and George et al. for small cell lung cancer (SCLC) (5-8). Nonsynonymous mutations targeted by CAPP-Seq that overlapped this list were defined as candidate drivers (**Supplemental Table S6**), while synonymous mutations or nonsynonymous mutations in other genes were considered candidate passengers (i.e. "other mutations"). We compared the observed frequencies of non-driver mutations in ctDNA within our cohort to their frequencies in NSCLC tumor sequencing within the genomic coordinates covered by our CAPP-Seq panel (**Supplemental Table S7**) (5,6).

Whole exome mutation burden analysis. To establish the relationship between the number of mutations observed in our CAPP-Seq panel to those present in the whole exome, we downloaded somatic variant calls from 1,178 whole exome NSCLC tumors sequenced by TCGA from http://gdc-portal.nci.nih.gov/legacy-archive (5,6). For noninvasively genotyped patients, we calculated the expected number of nonsynonymous mutations that would have been identified by CAPP-Seq on tumor tissue from the number of mutations identified in pre-treatment plasma, considering recovered haploid genome equivalents and the average fractional abundance of detected variants using the binomial distribution as previously described (2,3). CAPP-Seq versus WES mutation loads were compared using Pearson's correlation and linear regression (**Figure 4B**). Based on this analysis, the estimated mutation burden in the exome equals 41 times the number of nonsynonymous mutations detected in our CAPP-Seq selector. Using ROC analysis, this approach yielded an AUC of 0.90 for the classification of high mutation burden by CAPP-Seq when considering WES mutation levels exceeding 200 as the gold standard (9); similar performance was observed at other thresholds (e.g., >178 mutations (9), AUC=0.90; >300 mutations, AUC=0.92; >1000 mutations=0.97).

To validate the relationship between the number of mutations identified using CAPP-Seq and the predicted mutation burden in the exome we performed both whole exome sequencing and CAPP-Seq on tumor biopsy samples from 5 localized lung cancer patients. Library preparation was performed as previously described (2,3). For whole exome sequencing, tumor and germline (PBMC) sequencing libraries were enriched using a SeqCap EZ Human Exome Library v3.0 capture panel (Roche NimbleGen, Madison, WI) and sequenced to a median depth of ~100X. Indel realignment and base quality recalibration were performed using the Genome Analysis Toolkit v3.5 (default parameters), and SNVs were called using VarScan v2.4.1 (10,11). We required ≥3 supporting reads, ≥8 positional read depth in tumor and matched germline, ≥5% mutant AF in the tumor, <0.5% mutant AF in matched germline, and no overlap with the UCSC RepeatMasker track (12,13). We confirmed that no variants were SNPs in any other sample sequenced on the same lane, and removed SNPs previously catalogued by the Exome Aggregation Consortium (ExAC) at ≥0.001% population frequency.(14) We then compared the number of mutations identified in the whole exome to the number identified using CAPP-Seq for each of these 5 patients using Pearson's correlation and linear regression. This confirmed the predicted relationship described above using TCGA data, with non-synonymous mutation burden in the exome equaling 41 times the number of mutations covered by the CAPP-Seq panel (**Supplemental Figure S8**).

Response assessment and clinical endpoints. Freedom from progression (FFP) was defined as the time during which the patient was free from cancer progression, disease-specific survival (DSS) was defined as the time of lung cancer-related survival, and

overall survival (OS) accounted for death by any cause. Event-free survival (EFS) was measured to compare post-treatment disease surveillance by ctDNA vs. imaging, where the event was defined as post-treatment ctDNA detection or radiographic progression by RECIST 1.1 criteria (1), respectively. We tabulated lead-time of ctDNA MRD detection vs. imaging for each patient who experienced radiographic progression, and took the average of these values to calculate average lead-time of ctDNA MRD detection compared to imaging.

Survival analyses. Categorical time-to-event analyses of clinical endpoints including OS, DSS, FFP and EFS were conducted using the log-rank test to estimate *P*-values and Cox *exp(beta)* method to estimate hazard ratios, with results expressed as Kaplan-Meier plots. Since hazard ratios cannot be estimated reliably when there are no events in one of the two groups, we could not calculate them for some analyses (e.g. **Figure 2C**). The relationship of ctDNA as a continuous variable was assessed in Cox proportional hazards models with single or multiple covariates, with clinical outcome as the dependent variable, where the Wald test was used to assess the significance of covariates, and hazard ratios were calculated by *exp(beta)* method. The proportional hazards assumption was tested and confirmed for all Cox regression and Kaplan-Meier analyses by evaluating the Schoenfeld residuals. Survival analyses were performed using *R* v3.2.2 (http://www.r-project.org; 'survival' package) and Prism 7 (GraphPad Software, La Jolla, CA).

Other statistical analyses. To compare distributions of continuous variables we used the two-tailed student's t test, two-tailed student's t test with Welch's correction, Pearson's correlation, and linear regression, as appropriate. To assess performance of classification tests, we performed ROC analysis. To compare proportions between 2 populations, we used the Z test. To generate the ROC curve in **Figure 1D**, we performed CAPP-Seq monitoring of patient-specific mutation lists on pre-treatment plasma from 41 patients analyzed with CAPP-Seq (to evaluate sensitivity) and 54 healthy donors (to evaluate specificity) and for each case we calculated a previously described ctDNA detection index, which is analogous to the false positive rate (2,3). Statistical analyses were performed using *R* v3.2.2 (http://www.r-project.org) through the RStudio environment (Boston, MA) or Prism 7 (GraphPad Software, La Jolla, CA).

References for Supplemental Methods

1. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R*, et al.* New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer **2009**;45(2):228-47 doi 10.1016/j.ejca.2008.10.026.
2. Newman AM, Bratman SV, To J, Wynne JF, Eclov NC, Modlin LA*, et al.* An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med **2014**;20(5):548-54 doi 10.1038/nm.3519.
3. Newman AM, Lovejoy AF, Klass DM, Kurtz DM, Chabon JJ, Scherer F*, et al.* Integrated digital error suppression for improved detection of circulating tumor DNA. Nat Biotechnol **2016**;34(5):547-55 doi 10.1038/nbt.3520.
4. Newman AM, Bratman SV, Stehr H, Lee LJ, Liu CL, Diehn M*, et al.* FACTERA: a practical method for the discovery of genomic rearrangements at breakpoint resolution. Bioinformatics **2014**;30(23):3390-3 doi 10.1093/bioinformatics/btu549.
5. Cancer Genome Atlas Research N. Comprehensive genomic characterization of squamous cell lung cancers. Nature **2012**;489(7417):519-25 doi 10.1038/nature11404.

6. Cancer Genome Atlas Research N. Comprehensive molecular profiling of lung adenocarcinoma. Nature **2014**;511(7511):543-50 doi 10.1038/nature13385.
7. George J, Lim JS, Jang SJ, Cun Y, Ozretic L, Kong G*, et al.* Comprehensive genomic profiles of small cell lung cancer. Nature **2015**;524(7563):47-53 doi 10.1038/nature14664.
8. Rudin CM, Durinck S, Stawiski EW, Poirier JT, Modrusan Z, Shames DS*, et al.* Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer. Nat Genet **2012**;44(10):1111-6 doi 10.1038/ng.2405.
9. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ*, et al.* Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science **2015**;348(6230):124-8 doi 10.1126/science.aaa1348.
10. Koboldt DC, Chen K, Wylie T, Larson DE, McLellan MD, Mardis ER*, et al.* VarScan: variant detection in massively parallel sequencing of individual and pooled samples. Bioinformatics **2009**;25(17):2283-5 doi 10.1093/bioinformatics/btp373.
11. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L*, et al.* VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res **2012**;22(3):568-76 doi 10.1101/gr.129684.111.
12. Smit A, Hubley, R, Green, P. 2013-2015 <http://www.repeatmasker.org/> RepeatMasker Open-4.0.
13. Rosenbloom KR, Armstrong J, Barber GP, Casper J, Clawson H, Diekhans M*, et al.* The UCSC Genome Browser database: 2015 update. Nucleic Acids Res **2015**;43(Database issue):D670-81 doi 10.1093/nar/gku1177.
14. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T*, et al.* Analysis of protein-coding genetic variation in 60,706 humans. Nature **2016**;536(7616):285-91 doi 10.1038/nature19057.