

EXHIBIT 2-C – FINAL INVALIDITY CLAIM CHART
FOR U.S. PATENT NO. 11,530,454

Reference: Christopher Abbosh, et al. *Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution*. 545 Nature 446–451 (2017) (“Abbosh” NAT-NEO-00385073 and NAT-NEO-00385635).

U.S. Patent No. 11,530,454 Abbosh	
Claim	To the extent the preamble is construed to be limiting, Abbosh teaches a method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample. <i>See, e.g.,</i> Abbosh at NAT-NEO-00385073, NAT-NEO-00385074, NAT-NEO-00385075, NAT-NEO-00385076, NAT-NEO-00385077, NAT-NEO-00385078, NAT-NEO-00385081, NAT-NEO-00385082, NAT-NEO-00385085, NAT-NEO-00385086, NAT-NEO-00385090, NAT-NEO-00385091, NAT-NEO-00385093, NAT-NEO-00385635. For example, Abbosh teaches “The early detection of relapse following primary surgery for non-small-cell lung cancer and the characterization of emerging subclones, which seed metastatic sites, might offer new therapeutic approaches for limiting tumour recurrence. The ability to track the evolutionary dynamics of early-stage lung cancer non-invasively in circulating tumour DNA (ctDNA) has not yet been demonstrated. Here we use a tumour-specific phylogenetic approach to profile the ctDNA of the first 100 TRACERx (Tracking Non-Small-Cell Lung Cancer Evolution Through Therapy (Rx)) study participants, including one patient who was also recruited to the PEACE (Posthumous Evaluation of Advanced Cancer Environment) post-mortem study. We identify independent predictors of ctDNA release and analyse the tumour-volume detection limit. Through blinded profiling of postoperative plasma, we observe evidence of adjuvant chemotherapy resistance and identify patients who are very likely to experience recurrence of their lung cancer. Finally, we show that phylogenetic ctDNA profiling tracks the subclonal nature of lung cancer relapse and metastasis, providing a new approach for ctDNA-driven therapeutic studies.” Abbosh at NAT-NEO-00385073.

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Abbosh	
Claim	As another example, Abbosh teaches “Bespoke multiplex-PCR assay panels were synthesized for each patient, targeting clonal and subclonal single-nucleotide variants (SNVs) selected to track phylogenetic tumour branches in plasma (Fig. 1). SNV detection in plasma was established through a calling algorithm using negative control samples (see Methods).” Abbosh at NAT-NEO-00385073. As another example, Abbosh teaches: Figure 1 Phylogenetic ctDNA tracking. Overview of the study methodology. Multi-region sequencing of NSCLC was performed as part of the TRACERx study. PCR assay panels were designed on the basis of the phylogenetic analysis, targeting clonal and subclonal SNVs to facilitate non-invasive tracking of the patient-specific tumour phylogeny. Assay panels were combined into multiplex assay pools containing primers from up to 10 patients. Cell-free DNA (cfDNA) was extracted from pre- and postoperative plasma samples and multiplex-PCR was performed, followed by sequencing of the amplicons. Findings were integrated with M-seq exome data to track tumour evolution.

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Claim	Abbosh
	Abbosh at NAT-NEO-00385074. As another example, Abbosh teaches: “cfDNA extraction and quantification. Blood samples were collected in K2-EDTA tubes. Samples were processed within 2 h of collection by double centrifugation of the blood, first for 10 min at 1,000g, then the plasma for 10 min at 2000g. Plasma was stored in 1 ml aliquots at – 80 °C. Up to 5 ml of plasma per case was available for this study (range, 1–5 ml; median 5 ml). The entire volume of plasma was used for cfDNA extraction. cfDNA was extracted using the QIAamp Circulating Nucleic Acid kit (Qiagen) and eluted into 50 µl DNA Suspension Buffer (Sigma). Every cfDNA sample was analysed on the Bioanalyzer High Sensitivity (Agilent) and quantified by interpolation of the mononucleosomal peak height on a calibration curve prepared from a pure cfDNA sample that was quantified previously.” Abbosh at NAT-NEO-00385081.
1[a]. performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;	Abbosh teaches performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations. <i>See, e.g.,</i> Abbosh at NAT-NEO-00385073, NAT-NEO-00385074, NAT-NEO-00385076, NAT-NEO-00385081, NAT-NEO-00385082, NAT-NEO-00385093, NAT-NEO-00385635. For example, Abbosh teaches “Tissue-exome sequencing and processing. Whole-exome sequencing was performed on DNA purified from tumour tissue and normal blood as previously described, with the exception of CRUK0063_BR_T1-R1. This capture was performed according to the manufacturer’s 200 ng DNA protocol (Agilent). Annotated SNV calls from primary tumours are available in [ref. 9]. For this study, metastatic tissue biopsies from each of four patients (CRUK0035, CRUK0041, CRUK0044 and CRUK0063) and six metastatic samples acquired at post-mortem examination of CRUK0063 were obtained. Genomic DNA was purified from all tissue samples, and processed through the TRACERx bioinformatics pipeline as previously described. Annotated SNV calls are available in Supplementary Table 4.” Abbosh at NAT-NEO-00385081. As another example, Abbosh teaches “Processing and phylogenetic analysis of relapse and primary tumour multi-region whole-exome data. Biopsies from multiple regions from the primary tumour (n =

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Claim	327), metastatic biopsies (n = 4) and matching blood germline samples (n = 100) were subjected to multi-region whole exome sequencing and analysis including estimation of copy number, purity and ploidy, and phylogenetic tree construction as previously described. In brief, phylogenetic analysis was performed on the basis of the CCF determined for SNVs and clustered across tumour regions using a modified version of Pyclone into clusters with similar CCF values, filtered and processed as previously described. Mutation clusters are assumed to represent tumour subclones, either current or ancestral, and are used as input for construction of the phylogenetic relationship. Phylogenetic trees were primarily constructed using the published tool CITUP (0.1.0). However, in a small number of cases, including all relapse/autopsy cases, manual tree construction was required and performed as previously described⁹. Complete details of primary tumour tree construction can be found in [ref. 9]. Relapse tree construction was performed as follows. CRUK0063: clustering was performed twice, once across five primary tumour regions and once across five primary, one relapse, and six autopsy regions. To ensure consistency, when deriving a phylogenetic tree based on all tumour regions, for CCF clusters based on clustering, only the primary tumour regions were maintained for mutations not involved in metastatic relapse. A phylogenetic tree was constructed based on 17 mutation clusters. CRUK0035: clustering primary tumour regions with the relapse region revealed one cluster private to the relapse, and one cluster shared with the relapse and all other regions. CRUK0044: clustering primary tumour regions with the relapse region revealed a cluster private to the relapse, descended from a cluster private to region 1 in the primary tumour. CRUK0041: clustering primary tumour regions with the relapse region revealed cluster 4 as private to the relapse. This cluster must have evolved from cluster 3 only found in the relapse and in region 4. A private cluster 6 in region 4 must have evolved from cluster 4. However, this conflicts with clusters 2 and 5, found in the relapse and regions 1–3, but not region 4. This can be reconciled by assuming a polyclonal relapse, seeded primarily from regions 1–3, but with some contribution from cluster 3, private to region 4. Cluster data are available in Supplementary Table 4 under ‘PyClonePhyloCluster’. ” Abbosh at NAT-NEO-00385082. As another example, Abbosh teaches “The TRACERx study monitors the clonal evolution of NSCLC from diagnosis through to death. Using multi-region exome sequencing (M-seq)-derived tumour phylogenetic trees developed through prospective analysis of 100 patients from the TRACERx cohort, we conducted a phylogenetic approach to ctDNA profiling in early-stage NSCLC (Fig. 1). Bespoke

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Claim	multiplex-PCR assay panels were synthesized for each patient, targeting clonal and subclonal single-nucleotide variants (SNVs) selected to track phylogenetic tumour branches in plasma (Fig. 1).” Abbosh at NAT-NEO-00385073. As another example, Abbosh teaches: Figure 1 Phylogenetic ctDNA tracking. Overview of the study methodology: Multi-region sequencing of NSCLC was performed as part of the TRACERx study. PCR assay panels were designed on the basis of the phylogenetic analysis, targeting clonal and subclonal SNVs to facilitate non-invasive tracking of the patient-specific tumour phylogeny. Assay panels were combined into multiplex assay pools containing primers from up to 10 patients. Cell-free DNA (cfDNA) was extracted from pre- and postoperative plasma samples and multiplex-PCR was performed, followed by sequencing of the amplicons. Findings were integrated with M-seq exome data to track tumour evolution. Abbosh at NAT-NEO-00385074.

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Claim	Abbosh
	As another example, Abbosh teaches:

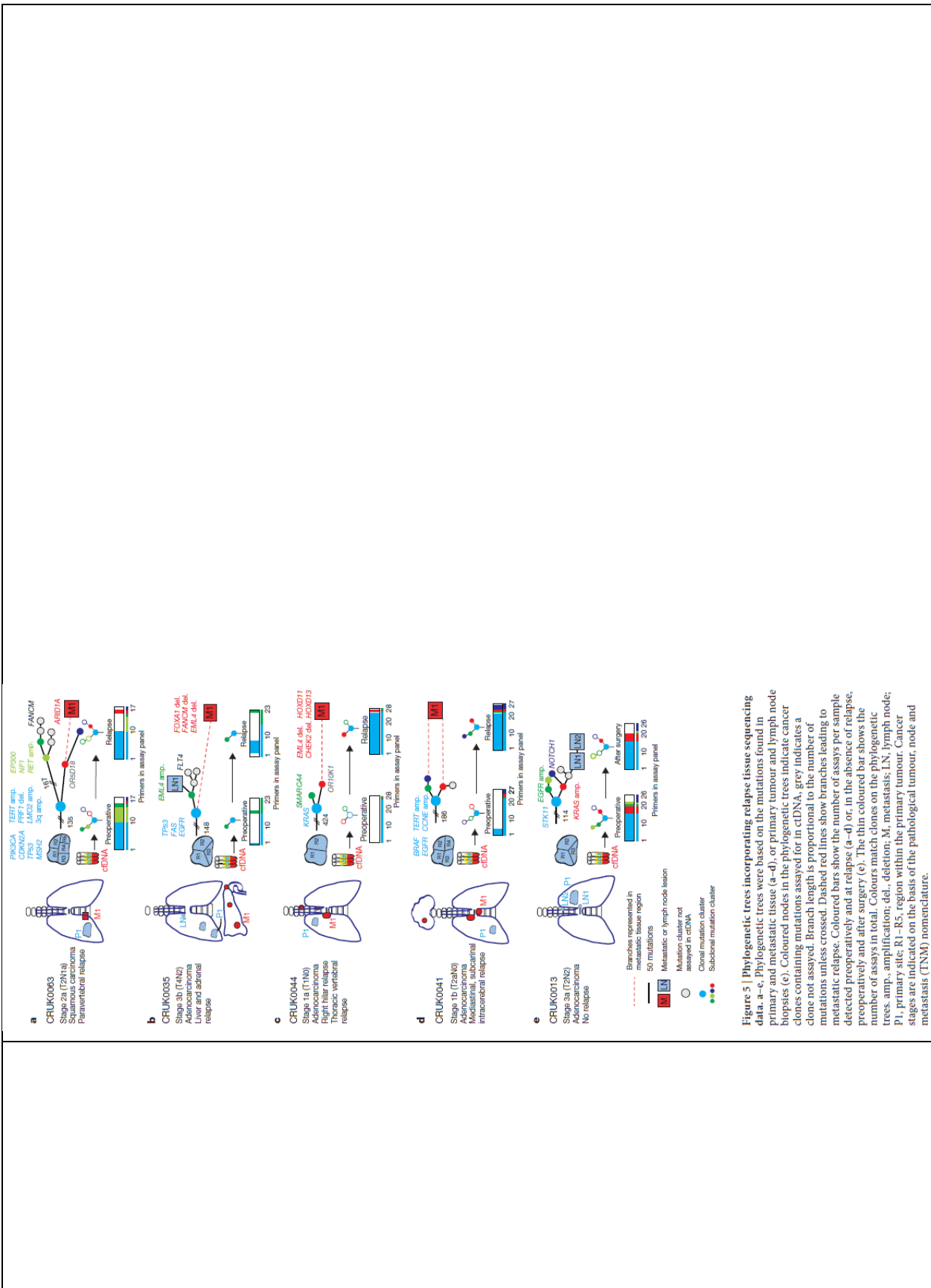


Figure 5 | Phylogenetic trees incorporating relapse tissue sequencing data. a–e, Phylogenetic trees were based on the mutations found in primary and metastatic tissue (a–d), or primary tumour and lymph node biopsies (e). Coloured nodes in the phylogenetic trees indicate cancer clones containing mutations assayed for in ctDNA, grey indicates a clone not assayed. Branch length is proportional to the number of mutations unless crossed. Dashed red lines show branches leading to metastatic relapse. Coloured bars show the number of assays per sample detected preoperatively and at relapse (a–d) or, in the absence of relapse, preoperatively and after surgery (e). The thin coloured bar shows the number of assays in total. Colours match clones on the phylogenetic trees. amp, amplification; del, deletion; M, metastasis; LN, lymph node; P1, primary site; R1–R5, region within the primary tumour. Cancer stages are indicated on the basis of the pathological tumour, node and metastasis (TNM) nomenclature.

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Claim	Abbosh
Abbosh at NAT-NEO-00385076.	
1[b]. performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume.	Abbosh teaches performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume. <i>See, e.g.,</i> Abbosh at NAT-NEO-00385073, NAT-NEO-00385074, NAT-NEO-00385075, NAT-NEO-00385081, NAT-NEO-00385085, NAT-NEO-00385635, Tables S5-S7. For example, Abbosh teaches “Therefore, we selected a threshold of two detected SNVs for calling a sample ctDNA-positive for validation within this study, to minimize type I errors when testing up to 30 tumour-specific SNVs per time point in a single patient.” Abbosh at NAT-NEO-00385073. As another example, Abbosh teaches “Samples from four patients could not be analysed (see cohort design in Extended Data Fig. 2a, patient characteristics in Extended Data Table 1a–c and Supplementary Table 1). Individual assay panels were designed to target a median of 18 SNVs (range, 10–22), comprising a median of 11 clonal SNVs (range, 2–20) and a median of 6 subclonal SNVs (range, 0–16) per patient (Extended Data Fig. 2b, e).” Abbosh at NAT-NEO-00385073. As another example, Abbosh teaches “A median of 18.5 SNVs (range, 12–20) were targeted by the LUSC assay panels, comprising a median of 8.5 clonal SNVs (range, 3–18) and a median of 9.5 subclonal SNVs (range, 2–17) (Extended Data Fig. 2d, e). A median of 28 SNVs (range, 25–30) were targeted by the LUAD assay panels, comprising a median of 20.5 clonal SNVs (range, 13–26) and a median of 6 subclonal SNVs (range, 0–13) (Extended Data Fig. 2d, e).” Abbosh at NAT-NEO-00385075. As another example, Abbosh teaches “cfDNA library preparation. Subsequently, 40 µl of cfDNA from each plasma sample, which is present as fragments of mononucleosomal and polynucleosomal length, was used as input into Library Prep using the Natera Library Prep kit; in two samples with extremely high cfDNA amounts, input was restricted to approximately 50,000 genome equivalents (165 ng). cfDNA was

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Claim	end-repaired and A-tailed. Natera custom adapters were ligated. The libraries were amplified for 15 cycles to plateau and then purified using Ampure beads following the manufacturer's protocol. The purified libraries were run on the LabChip. Successful libraries had a single peak at around 250 bp." Abbosh at NAT-NEO-00385081. As another example, Abbosh teaches "Analytical validation. Synthetic spikes representing twenty SNVs that were randomly selected from primer assay pool 1 were designed and synthesized (IDT) as 160-bp oligos with the respective SNV placed in the middle (position 80). These synthetic spikes were mixed at equimolar ratios and used to prepare a library. This library was titrated into a library prepared from mononucleosomal DNA (10,000 copies) from a normal cell line (AG16778 from Coriell). The library of 20 synthetic spikes was titrated into the mononucleosomal DNA library at 2.5%, 0.5%, 0.25%, 0.1%, 0.05% and 0% (each in triplicate), and 0.01%, 0.005% and 0.001% (each in quadruplicate). Because preparing spiked samples at such low levels is either subject to sampling noise (0.01% spikes into 10,000 genomic copies background is equivalent to one mutant copy), or is not possible (at levels less than 0.01%), samples were mixed as libraries. Following library mixing and sequencing, data was analysed to detect all the targets in assay pool 1 using the same parameters as used for the patient samples." Abbosh at NAT-NEO-00385081. In addition, to the extent Natera argues its Signatera product practices the '454 patent, Abbosh teaches this limitation. <i>See, e.g., Michael Metzker August 2, 2024 Opening Expert Report at Section (XII)(B).</i>
1[c]. sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA	Abbosh teaches sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads. <i>See, e.g.,</i> Abbosh at NAT-NEO-00385073, NAT-NEO-00385074, NAT-NEO-00385076, NAT-NEO-00385077, NAT-NEO-00385081, NAT-NEO-00385082. For example, Abbosh teaches "Here, we report a bespoke multiplex-PCR next-generation sequencing (NGS) approach to ctDNA profiling in the context of the prospective tumour evolutionary NSCLC

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Abbosh	
Claim from the sequence reads,	TRACERx study (https://clinicaltrials.gov/ct2/show/NCT01888601). We address determinants of ctDNA detection in early-stage NSCLC and investigate the ability of ctDNA to identify and genomically characterize postoperative NSCLC relapse within a tumour phylogenetic framework.” Abbosh at NAT-NEO-00385073. As another example, Abbosh teaches:

Figure 1 | Phylogenetic ctDNA tracking. Overview of the study methodology. Multi-region sequencing of NSCLC was performed as part of the TRACERx study. PCR assay panels were designed on the basis of the phylogenetic analysis, targeting clonal and subclonal SNVs to facilitate non-invasive tracking of the patient-specific tumour phylogeny. Assay panels were combined into multiplex assay pools containing primers from up to 10 patients. Cell-free DNA (cfDNA) was extracted from pre- and postoperative plasma samples and multiplex-PCR was performed, followed by sequencing of the amplicons. Findings were integrated with M-seq exome data to track tumour evolution.

Abbosh at NAT-NEO-00385074.

As another example, Abbosh teaches ‘Plasma SNV multiplex-PCR NGS workflow. The library material from each plasma sample was used as input into the multiplex-PCR (mPCR) using the relevant assay pool and an optimized plasma mPCR protocol. Optimal mPCR conditions were as previously described. Each

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Abbosh	
Claim	PCR assay pool was used to amplify the SNV targets from the 10 corresponding samples and 20 negative control Samples (plasma libraries prepared from healthy subjects; BioMed IRB 601-01 and E&I West Coast Board IRB00007807, study 13090-01A and 13090-04A). The mPCR products were barcoded in a separate PCR step. Each amplicon pool was sequenced on one Illumina HiSeq 2500 Rapid Run with 50 cycles paired-end reads using the Illumina Paired End v1 kit with an average target read depth of around 40,000 per assay.” Abbosh at NAT-NEO-00385081.

1[d]. wherein the sequencing has a depth of read of at least 50,000 per target locus.	Abbosh teaches a sequencing depth of read of at least 50,000 per target locus. See, e.g., Abbosh at NAT-NEO-00385084 and NAT-NEO-00385635. The figure is a box plot titled "Variants tested for in study". The y-axis is labeled "Depth of read" and ranges from 0 to 150,000 in increments of 50,000. There are two data series: "Not called" (represented by red boxes and points) and "Called" (represented by blue boxes and points). The "Not called" group has a median depth of approximately 50,000, with the box spanning from roughly 30,000 to 70,000. The "Called" group has a median depth of approximately 75,000, with the box spanning from roughly 50,000 to 100,000. Individual data points are overlaid on the boxes. A text label "P, wilcoxon test = 0.786" is present in the upper left area of the plot. Abbosh at NAT-NEO-00385084. In addition, to the extent Natera argues its Signatera product practices the '454 patent, Abbosh teaches this limitation. See, e.g., Michael Metzker August 2, 2024 Opening Expert Report at Section (XII)(B). For example, Signatera"focus[es] on patient-specific variants [and] enables ultra-deep sequencing (100,000X average depth of coverage) of each target." Signatera whitepaper (https://www.natera.com/wp-content/uploads/2020/11/Oncology-Clinical-A-personalized-tumor-informed-approach-to-detect-molecular-residual-disease-SGN_SR_WP.pdf) at 1. A POSA would also be motivated to modify Abbosh in view of the knowledge of a POSA as evidenced by the Signatera white paper to perform the method wherein the sequencing has a depth of read of at least 50,000 per target locus because higher read depth would improve sensitivity, and sequencing with depths greater than 50,000 was generally known to allow for improved detection of rare SNVs such as tumor-associated SNVs in cfDNA. See "General Knowledge of a POSA" section of NeoGenomics Invalidity
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Claim	Abbosh
	Contentions cover pleading. A POSA could do so by performing more sequencing using the sequencer instrument or involve more advanced sequencer models. A POSA would also have a reasonable expectation of success to modify Abbosh with the knowledge of a POSA as evidenced by the Signatera white paper in view of the sequencing depths obtained using Abbosh (Extended Data Figure 1c). Further evidence of motivation and reasonable expectation of success to increase the read depth to at least 50,000 per target locus is found in NEOGEN00275278–NEOGEN00275334 and NEOGEN00275270–NEOGEN00275277. <i>See, e.g.,</i> NEOGEN00275326 (describing tables plotting the “number of reads for each barcode according to which block of 48 barcodes it belongs to”) and NEOGEN00275329 (providing read counts of greater than 50,000 for some loci (e.g., in columns labeled FLD0200, FLD0217, FLD0233, FLD0234, FLD0235, FLD0236, FLD0237, FLD0238, and FLD0239).
8. The method of claim 1, wherein the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation.	Abbosh teaches the targeted multiplex amplification to amplify 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. <i>See, e.g.,</i> Abbosh at NAT-NEO-00385073, NAT-NEO-00385074, NAT-NEO-00385075, NAT-NEO-00385081, NAT-NEO-00385085. For example, Abbosh teaches “Therefore, we selected a threshold of two detected SNVs for calling a sample ctDNA-positive for validation within this study, to minimize type I errors when testing up to 30 tumour-specific SNVs per time point in a single patient.” Abbosh at NAT-NEO-00385073. <i>See also</i> claim 1[b] above.
10. The method of claim 1, wherein the method further comprises designing PCR primers or hybrid	Abbosh teaches designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample. <i>See, e.g.,</i> Abbosh at NAT-NEO-00385073, NAT-NEO-00385074, NAT-NEO-00385076, NAT-NEO-00385077, NAT-NEO-00385081.

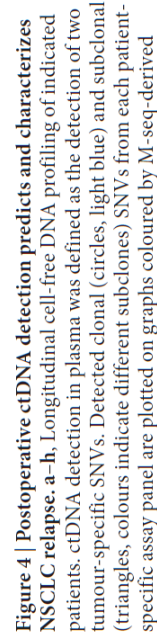
U.S. Patent No. 11,530,454 Abbosh	
Claim	capture probes targeting the plurality of SNV mutations identified in the tumor sample.
For example, Abbosh teaches: Figure 1 Phylogenetic ctDNA tracking. Overview of the study methodology. Multi-region sequencing of NSCLC was performed as part of the TRACERx study. PCR assay panels were designed on the basis of the phylogenetic analysis, targeting clonal and subclonal SNVs to facilitate non-invasive tracking of the patient-specific tumour phylogeny. Assay panels were combined into multiplex assay pools containing primers from up to 10 patients. Cell-free DNA (cfDNA) was extracted from pre- and postoperative plasma samples and multiplex-PCR was performed, followed by sequencing of the amplicons. Findings were integrated with M-seq exome data to track tumour evolution. Abbosh at NAT-NEO-00385074.	

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Abbosh	
Claim	
	As another example, Abbosh teaches “SNV assay design and optimization. Natera’s standard assay design pipeline was used to generate forward and reverse PCR primers for somatic SNVs detected in tumour samples. For every pair of primers, the probability of forming a primer-dimer was calculated and assays were combined into pools such that any primer combination in a pool is not predicted to form primer-dimers. For each patient, assays were prioritized such that (1) assays covering driver SNVs had highest priority and (2) there was uniform sampling of the phylogenetic tree. For the baseline cohort, 10 balanced pools were created, each containing on average 18 assays for 10 patients’ SNVs. For the longitudinal cohort, up to 10 extra assays were generated for samples. For patient CRUK0063 post-mortem analysis, new assays were designed on the basis of the M-seq of the metastatic biopsy retrieved on day 467 and of metastatic lesions collected post-mortem. A total of 103 new assays were designed compared with the 19 that were based on the primary tumour alone. Primer details are available in Supplementary Table 5 (baseline, preoperative cohort), Supplementary Table 6 (longitudinal cohort) and Supplementary Table 7 (extended longitudinal assays for CRUK0063). SNV assays were ordered from IDT. Each pool was optimized by running the multiplex-PCR and sequencing protocol using one plasma cfDNA library from a healthy subject. For optimization, PCR parameters (primer concentration and annealing temperature) that yielded the best percentage of on-target reads, depth-of-read uniformity (measured as the ratio of the 80th percentile to the 20th percentile), and number of drop-out assays (defined as assays with < 1,000 reads) were determined by sequencing. The PCR conditions that yield the best percentage of on-target reads, depth-of-read uniformity, and the lowest number of drop-outs were determined. For all pools, the optimal conditions were 10 nM primers and 60 °C or 62.5 °C annealing temperatures. Primer pairs contributing to dimer formation were removed from each final pool.” Abbosh at NAT-NEO-00385081.
12. The method of claim 1, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the SNV	Abbosh teaches detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. <i>See, e.g.,</i> Abbosh at NAT-NEO-00385073, NAT-NEO-00385075, NAT-NEO-00385076, NAT-NEO-00385077, NAT-NEO-00385078. For example, Abbosh teaches “The early detection of relapse following primary surgery for non-small-cell lung cancer and the characterization of emerging subclones, which seed metastatic sites, might offer

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Claim mutations detected in the cell-free DNA.	new therapeutic approaches for limiting tumour recurrence. The ability to track the evolutionary dynamics of early-stage lung cancer non-invasively in circulating tumour DNA (ctDNA) has not yet been demonstrated. Here we use a tumour-specific phylogenetic approach to profile the ctDNA of the first 100 TRACERx (Tracking Non-Small-Cell Lung Cancer Evolution Through Therapy (Rx)) study participants, including one patient who was also recruited to the PEACE (Posthumous Evaluation of Advanced Cancer Environment) post-mortem study. We identify independent predictors of ctDNA release and analyse the tumour-volume detection limit. Through blinded profiling of postoperative plasma, we observe evidence of adjuvant chemotherapy resistance and identify patients who are very likely to experience recurrence of their lung cancer. Finally, we show that phylogenetic ctDNA profiling tracks the subclonal nature of lung cancer relapse and metastasis, providing a new approach for ctDNA-driven therapeutic studies.” Abbosh at NAT-NEO-00385073, Abstract. As another example, Abbosh teaches “Lung cancer is the leading cause of cancer-related deaths. Metastatic non-small-cell lung cancer (NSCLC) cannot be cured with systemic chemotherapy, yet clinical studies have shown a 5% benefit of postoperative (adjuvant) chemotherapy on overall survival. This modest survival benefit may reflect a vulnerability of low-volume disease in the context of reduced intra-tumour heterogeneity. ctDNA detection in plasma following resection of breast and colorectal tumours has been shown to identify patients who are very likely to relapse postoperatively in advance of established clinical parameters. Identifying, monitoring and genomically characterizing residual disease following primary lung cancer surgery may improve outcomes in the adjuvant setting. This would create a therapeutic setting in which only patients who are very likely to have cancer recurrence would receive treatment and intervention could be directed to the evolving tumour subclone that seeds metastatic recurrence.” Abbosh at NAT-NEO-00385073.



tumour phylogenetic nodes. Mean clonal (blue) and mean subclonal (red) plasma VAF are indicated on graphs as connected lines. Preoperative and relapse M-seq-derived phylogenetic trees represented by ctDNA are illustrated above each graph. CTx, chemotherapy; RTx, radiotherapy; R, relapse. Lead time is indicated by the number of days in the panels.

Abbosh at NAT-NEO-00385075.

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Claim	
	As another example, Abbosh teaches “Detecting and characterizing NSCLC relapse. . . Patients were followed up every three months for the first two years following study enrolment and every six months thereafter with clinical assessment and chest radiographs. At least two SNVs were detected in 13 out of 14 (93%) patients with confirmed NSCLC relapse before, or at, clinical relapse (Fig. 4a–g and Extended Data Fig. 5). At least two SNVs were detected in 1 out of 10 (10%) patients (CRUK0013) with no clinical evidence of NSCLC relapse (Fig. 4h and Extended Data Fig. 6). Excluding a single case where no postoperative plasma was taken before clinical relapse (CRUK0041), the median interval between ctDNA detection and NSCLC relapse that was confirmed by CT imaging indicated by clinical and chest radiograph follow-up (lead time) was 70 days (range, 10–346 days). Of the 13 relapse cases, 4 exhibited lead times of more than six months (Fig. 4a–d). In two cases ctDNA detection preceded CT imaging inconclusive for NSCLC relapse by 157 days (CRUK0004, Fig. 4b) and 163 days (CRUK0045, Fig. 4d). ctDNA profiling reflected adjuvant chemotherapy resistance; CRUK0080, CRUK0004 and CRUK0062 had detectable ctDNA in plasma within 30 days of surgery. The number of detectable SNVs increased in all three cases despite adjuvant chemotherapy, with disease recurring within one year after surgery (Fig. 4a–c). In contrast, CRUK0013 had 20 SNVs detectable in ctDNA 72 h after surgery and 13 SNVs detectable before adjuvant chemoradiotherapy; 51 days after completion of adjuvant treatment and at postoperative days 457 and 667, no SNVs were detectable and the patient remains relapse-free 688 days post-surgery (Fig. 4h). ctDNA profiling detected relapse from the central nervous system; CRUK0029 had a PET scan performed 50 days before surgery demonstrating normal cerebral appearances. ctDNA remained detectable following surgery; 54 days postoperatively the patient was diagnosed with intracerebral metastasis; no extracranial disease was evident on CT imaging (Fig. 4e).” Abbosh at NAT-NEO-00385075-76.
14[pre]. A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more	To the extent the preamble is construed to be limiting, Abbosh teaches a method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample. See claim 1[pre] above.

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single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:	
14[a]. performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations; See claim 1[a] above.	Abbosh teaches performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations. See claim 1[a] above.
14[b]. performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons, wherein the target loci are amplified together in the same reaction volume. See claim 1[b] above.	Abbosh teaches performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons, wherein the target loci are amplified together in the same reaction volume. See claim 1[b] above.

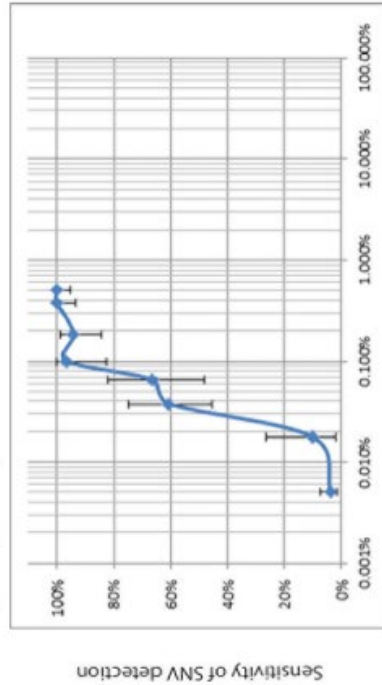
EXHIBIT 2-C: NEOGENOMICS' FINAL INVALIDITY CONTENTIONS CLAIM CHARTS
FOR U.S. PATENT NO. 11,530,454
(ABBOSH)

U.S. Patent No. 11,530,454	
Abbosh	
Claim	
are amplified together in the same reaction volume; and	
14[c]. sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads	Abbosh teaches sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads. See claim 1[c] above.
14[d]. wherein the method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus.	Abbosh teaches a method capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus. See, e.g., Abbosh at NAT-NEO-00385078, NAT-NEO-00385081, NAT-NEO-00385084. For example, Abbosh teaches Figure 1:

U.S. Patent No. 11,530,454

Abbosh

a Multiplex-PCR platform sensitivity and specificity



SNV spike input range	Eligible positive positions	True positive calls	Sensitivity	95% CI
>0.5	81	81	100.0%	95.6-100%
0.25-0.5%	54	54	100.0%	93.4-100%
0.1-0.25%	66	64	97.0%	89.5-99.6%
0.05-0.1%	51	43	84.3%	71.4-93.0%
0.01-0.05%	76	35	46.1%	34.6-57.9%
<=0.01%	212	9	4.2%	2-7.8%
Eligible negative positions		False positive calls	Specificity	95% CI
	5099	19	99.6%	99.4-99.8%

Abbosh at NAT-NEO-00385084.

Under Natera's interpretation of the claim, Abbosh teaches detection of an SNV because "once you have performed Step 1A and Step 1B and the first part of Step 1C and then you sequence, you will necessarily have identified the SNV if it exists." See Metzker deposition 2/11/2025 at 29:1-12.

EXHIBIT 2-C: NEOGENOMICS' FINAL INVALIDITY CONTENTIONS CLAIM CHARTS
FOR U.S. PATENT NO. 11,530,454
(ABBOSH)

U.S. Patent No. 11,530,454	
Abbosh	
Claim	
15. The method of claim 14, wherein the sequencing has a depth of read of at least 50,000 per target locus.	Abbosh teaches a method wherein the sequencing has a depth of read of at least 50,000 per target locus. See claim 1[d] above.
22. The method of claim 14, wherein the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation.	Abbosh teaches targeted multiplex amplification to amplify 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. See claim 8 above.
24. The method of claim 14, wherein the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample.	Abbosh teaches designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample. See claim 10 above.
26. The method of claim 14, wherein the method further comprises detecting	Abbosh teaches detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. See claim 12 above.

EXHIBIT 2-C: NEOGENOMICS' FINAL INVALIDITY CONTENTIONS CLAIM CHARTS
FOR U.S. PATENT NO. 11,530,454
(ABBOSH)

U.S. Patent No. 11,530,454	
Abbosh	
recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA.	
28. The method of claim 14, wherein the method is capable of detecting an SNV mutation that is present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus.	Abbosh teaches a method capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus. See claim 14[d] above.