

**IN THE UNITED STATES DISTRICT COURT
FOR THE MIDDLE DISTRICT OF NORTH CAROLINA**

NATERA, INC.,	)	
	)	
Plaintiff,	)	
	)	
v.	)	
	)	C.A. No. 1:23-cv-629
NEOGENOMICS LABORATORIES,	)	
INC.,	)	
	)	
Defendant.	)	

**DECLARATION OF DR. MICHAEL L. METZKER IN SUPPORT OF
PLAINTIFF NATERA, INC.'S OPENING CLAIM CONSTRUCTION BRIEF**

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I, Michael Metzker, declare as follows:

1. I previously submitted two declarations in the above-captioned litigation in connection with Plaintiff Natera, Inc.'s ("Natera") motion for a preliminary injunction regarding NeoGenomics Laboratories, Inc.'s ("NeoGenomics") RaDaR™ test.¹ I incorporate those Declarations herein by reference, including information regarding my qualifications and expertise, including my curriculum vitae, as well as my understanding of relevant legal principles, the technology background, and U.S. Patent Nos. 11,530,454 (the "'454 Patent")² and 11,519,035 (the "'035 Patent")³ (collectively, the "Asserted Natera Patents").

2. I have been asked to provide my expert opinions as to the technical background and meaning of certain terms in the claims of the '454 Patent to one of ordinary skill in the art. I have been informed and understand that Natera has asserted Claims 1-28 of the '454 Patent against NeoGenomics (collectively, the "Asserted Natera Claims").

3. This Declaration is based on information currently available to me. I may continue my investigation and study as new information comes to light and circumstances

¹ See Doc. 17, *Declaration of Dr. Michael L. Metzker in Support of Plaintiff Natera, Inc.'s Motion for a Preliminary Injunction*, dated July 29, 2023 [hereinafter "Metzker Op. PI Decl."]; Doc. 145, *Reply Declaration of Dr. Michael Metzker*, dated Nov. 6, 2023 [hereinafter "Metzker Reply PI Decl."].

² See Doc. 1-1, NAT-NEO-00000001-00000222, Babiarz *et al.*, *Detecting mutations and ploidy in chromosomal segments*, U.S. Patent No. 11,530,454 (2022) (the "'454 Patent").

³ See Doc. 1-2, NAT-NEO-00000223-00000435, Rabinowitz *et al.*, *Methods for simultaneous amplification of target loci*, U.S. Patent No. 11,519,035 (2022) (the "'035 Patent").

evolve. Accordingly, I may supplement my opinions and/or provide rebuttal opinions and reserve the right to do so, including through testimony given at deposition, at any claim construction hearing, or at trial. I would be able to testify regarding the matters stated in this Declaration, if asked by the Court or by the parties' attorneys.

I. SUMMARY OF OPINIONS

4. In my opinion, the Asserted Natera Claims are not invalid for indefiniteness. A person of ordinary skill in the art ("POSA")⁴ would have understood the scope of the terms "clonal" (Claims 3, 5, 7, 17, 19, 21), "subclonal" (Claims 4, 5, 18, 19), "0.015% of the cell-free DNA comprising the SNV locus" (Claim 14) and "0.005% to 0.015% of the cell-free DNA comprising the SNV locus" (Claim 28) in the context of the Asserted Natera Claims with reasonable certainty.

II. BACKGROUND AND QUALIFICATIONS

5. I previously identified my background and qualifications in the Metzker Op. PI Decl.,⁵ and incorporate that discussion here.

6. As an expert in molecular biology, I am qualified to provide an opinion as to what one of ordinary skill in the art would have understood, known, or concluded as of the relevant timeframe, which is as of 2014, or several years prior to that.

⁴ Throughout this Declaration, I also refer to a POSA as one of ordinary skill in the art.

⁵ See Doc. 17 (Metzker Op. PI Decl.), ¶¶ 4-19, with one exception: I am no longer Chief Technology Officer at 454 Bio.

7. In formulating my opinions, I have relied upon my 30+ years of education, training, knowledge, and experience in the relevant art. But at all times, I have understood and interpreted the Asserted Natera Claims from the point of view of one of ordinary skill in the art, as discussed in Section IV.

8. I am being compensated for services provided in this case at my usual and customary consulting rate of \$750/hour. My compensation does not depend in any way on the opinions I express or the outcome of this case.

III. UNDERSTANDING OF THE LAW

9. I am not an attorney, nor have I independently researched the law. I have been informed by counsel for Natera the various legal standards that must guide claim construction and indefiniteness. Based on this understanding, I have applied those standards in rendering my opinions expressed below in this Declaration.

10. I have been informed and understand that claim construction is an issue of law and that the Court will ultimately determine the meanings of the disputed terms in the Asserted Natera Claims. In providing my opinions here, my purpose is to assist the Court in its construction of the disputed terms based upon how one of ordinary skill in the art would have understood those claim terms at the time the patent was filed.

11. I have been informed and understand that a claim term should be understood to have its plain and ordinary meaning as understood from the standpoint of a hypothetical person of ordinary skill in the art as of the invention date of the asserted claim in view of the intrinsic evidence, including the plain claim language, the specification, and the

prosecution history. The specification and prosecution history often can inform the meaning of the claim language, if it differs from the plain and ordinary meaning as understood by one of ordinary skill in the art, by demonstrating how the inventor understood the invention and whether the inventor limited the invention.

12. I have been informed and understand that to determine how one of ordinary skill in the art would have understood a claim term, one may also look to “extrinsic” evidence concerning relevant scientific principles, the meaning of technical terms, and the state of the art. I understand that extrinsic evidence may not be used to contradict the intrinsic evidence.

13. I have been informed and understand that it is generally improper to import limitations from the specification (*e.g.*, embodiments or examples) into the claims when those limitations are not found in the claims themselves.

14. I have been informed and understand that the following factors are relevant to determining the level of ordinary skill in the art: (i) type of problems encountered in the art, (ii) prior art solutions to those problems, (iii) rapidity with which innovations are made, (iv) sophistication of the technology, and (v) educational level of active workers in the field.

15. I have been informed and understand that a patent must conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention with reasonable certainty. I am informed that this is known as the “definiteness” requirement.

16. I have been informed and understand that if a patent does not meet the definiteness requirement, it is considered “indefinite” and consequently invalid.

17. I have been informed and understand that it is the challenger’s burden to prove the challenged patent is invalid for indefiniteness. I understand that if a patent’s claims, read in light of the specification and prosecution history, would have failed to inform one of ordinary skill in the art about the scope of the invention with reasonable certainty, then it is invalid as indefinite. I understand that the definiteness requirement does not require that a potential infringer be able to determine *ex ante* if a particular act infringes the claims. I understand that the existence of multiple different methodologies for measuring a parameter recited in a claim does not by itself render a claim indefinite. I understand that the challenger must prove indefiniteness by clear and convincing evidence.

18. I have been informed and understand that claim terms must be construed consistently whether the issue is one of analyzing, for example, validity or infringement.

IV. LEVEL OF ORDINARY SKILL IN THE ART

19. I set out my understanding of the person of ordinary skill in the art in the Metzker Op. PI Decl.⁶ and incorporate that discussion here.

20. In or around 2014, I would have been at least one of ordinary skill in the art with respect to the subject matter of the ’454 Patent.

⁶ Doc. 17 (Metzker Op. PI Decl.), ¶ 32.

V. MATERIALS CONSIDERED

21. In formulating my opinions, I considered all of the references cited in this Declaration, including the documents cited below.

VI. TECHNOLOGY BACKGROUND

A. Clonal and Subclonal Mutations in Cancer

22. Deoxyribonucleic acid (“DNA”) is a class of polymeric molecules that carry the genetic information of an organism. DNA typically resides inside the nucleus of a cell. When a cell divides and replicates, that cell’s DNA is copied so that the resulting “daughter” cells typically contain the same genetic information encoded in the parent cell’s DNA.

23. As DNA replicates, however, mutations may be introduced. Some of these genetic mutations may alter the function of a cell, causing it to become cancerous. A cell containing an initial genetic mutation associated with cancer will continue to divide and replicate, with that mutation being replicated down the cell line. One of ordinary skill in the art would have understood that those replicated mutations from the ancestor cell are referred to as “clonal.”⁷

⁷ See Doc. 17-2 (Metzker Op. PI Decl., Ex. 2, Kirkizlar *et al.*, *Detection of clonal and subclonal copy-number variants in cell-free DNA from patients with breast cancer using a massively multiplexed PCR method*, TRANSLATIONAL ONCOLOGY 8:407-416 (2015)) [hereinafter, “Kirkizlar (2015)”].

24. As discussed in the Metzker Op. PI Decl., cancerous cells will continue to divide and typically replicate at higher rates compared to non-cancerous cells.⁸ As those cancerous cells replicate, additional mutations may be introduced, which are in turn present in cells replicated from the cell containing these additional mutations. One of ordinary skill in the art would have understood that those additional mutations are referred to as “subclonal” and are not present in the entire population of cancerous cells.⁹ Multiple subclonal mutations may develop in cancerous cells. One of ordinary skill in the art would have understood that the variety of different mutations in cancerous cells is referred to as “tumor heterogeneity” or “clonal heterogeneity.”¹⁰

B. Clinical Tests to Detect and Monitor Cancer

25. As described in the Metzker Op. PI Decl., liquid biopsy refers to a minimally invasive test performed on a blood sample that looks for cancer cells from a tumor or small pieces of genetic material released by tumor cells into the blood.¹¹ Liquid biopsy may be

⁸ See Doc. 17 (Metzker Op. PI Decl.), ¶ 36; *see also* Doc. 17-3 (Metzker Op. PI Decl., Ex. 3, Leon *et al. Free DNA in the serum of cancer patients and the effect of therapy*. CANCER RESEARCH 37:646–650 (1977)) [hereinafter “Leon (1997)”].

⁹ See Doc. 17-2 (Kirkizlar (2015)) at 411 (“In contrast, **only two of the four subsections for this patient had CNVs** observed in the 1p region, and **three of the four subsections had CNVs** observed in the 1q region, **suggesting that those CNVs were subclonal mutations.**” (emphases added). For the purposes of assessing clonality, CNVs (copy number variants) and SNVs may be treated the same way. For example, Kirkizlar (2015) explains that “[w]e anticipate that the ability to detect both SNVs and CNVs in plasma samples with low tumor fraction will provide greater genomic coverage and thereby facilitate early detection of cancer and improvement of therapeutic strategies.” *Id.* at 413.

¹⁰ See Doc. 17-2 (Kirkizlar (2015)) at 407.

¹¹ See Doc. 17 (Metzker Op. PI Decl.), ¶¶ 34-36.

used to specifically detect and quantify circulating tumor DNA (ctDNA) in a patient, *i.e.*, DNA shed from tumors into the bloodstream, which can inform treatment decisions.¹²

26. Because of tumor heterogeneity, when different tumor cell populations release ctDNA into the plasma, the ctDNA from one cellular population can have different ctDNA sequences from other cellular populations. A liquid biopsy test using the methods described in the '454 Patent may be used to analyze clonal or subclonal mutations. One of ordinary skill in the art using these methods can infer which mutations are clonal or subclonal by comparison of matched tumor and plasma samples.¹³ This information may be used in determining a patient's course of treatment. For example, certain targeted treatments may be effective in treating cancers caused by a specific mutation. If, however, that mutation is subclonal, and there is another mutation in the population that is resistant to that targeted treatment, such treatment may not be effective.¹⁴

C. Capability to Detect Rare Mutations

27. One of ordinary skill in the art would have understood that a test for molecular residual disease (MRD), such as a liquid biopsy, may be characterized, in part, by its capability to detect mutations that are present at low percentages compared to the total population of cfDNA, *i.e.*, detecting rare mutations. For example, the '454 Patent

¹² *Id.*

¹³ *Id.* ¶ 35.

¹⁴ See Doc. 17-2 (Kirkizlar (2015)) at 407 (“From a clinical perspective, ***it is critical to understand tumor heterogeneity as key clonal and subclonal mutations*** can indicate a reduction of the sensitivity of tumors to targeted treatments, ***potentially leading to drug resistance and ultimately metastasis.***” (emphases added, citations omitted)).

describes the claimed methods as being “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” (Claim 14) or “capable of detecting an SNV mutation that is present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus” (Claim 28).¹⁵ One of ordinary skill in the art would have understood that, because plasma samples potentially containing ctDNA also include large amounts of non-cancerous genetic material, an MRD test should be capable of detecting the low-frequency cancer-associated mutations among all of the other genetic material present in such samples.

28. A related but distinct concept is the limit of detection (LOD). One of ordinary skill in the art would have understood the limit of detection of a test for MRD is a calculation that determines the lower bound at which the test is able to detect rare mutations with a specific depth of read (DOR, *i.e.*, the number of times that a specific nucleotide in the sample is read during the sequencing process), at a given error rate, and with a specified degree of confidence. This understanding is also consistent with the teachings of the '454 Patent.¹⁶ One of ordinary skill in the art would have understood that an MRD test either is or is not capable of detecting SNV mutations present at specified frequencies independent of the calculation of any limit of detection.

¹⁵ See Doc. 1-1 ('454 Patent) at Col. 172:27-30, 173:1-4.

¹⁶ *Id.* at Col. 157:65-67. (“If desired, the limit of detection can be improved (resulting in a lower numerical value) for these SNVs by increasing the DOR.”; *see also id.* at Col. 158:22-23. (“If desired, the limit of detection can be improved by optimizing the assay to reduce the error rate.”))

VII. THE '454 PATENT

A. The '454 Patent Claims and Specification

1. Relevant Claim Language

29. The '454 Patent is titled *Detecting mutations and ploidy in chromosomal segments* and was issued on December 20, 2022.¹⁷ Independent Claims 1 and 14 of the '454 Patent recite methods for preparing plasma samples to detect single nucleotide variant (“SNV”) mutations by sequencing.¹⁸ The claimed methods comprise performing whole exome sequencing or whole genome sequencing of tumor samples to identify a plurality of tumor-specific SNV mutations.¹⁹ The claimed methods also comprise targeted multiplex amplification of 10 to 500 target loci each encompassing a different tumor-specific SNV mutation, all amplified together in the same reaction volume, from cell-free DNA isolated from plasma samples, in order to detect the SNV mutations by sequencing.²⁰

30. The '454 Patent contains 28 claims. Independent Claims 1 and 14 are reproduced *infra*.

Claim 1. 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, the method comprising:

¹⁷ *Id.* at cover page.

¹⁸ *Id.*, e.g., at Abstract, Cols. 2:44-5:3, 6:1-5, 67:57-61, 147:45-48, 171:25-173:4 (Claims 1-6, 8, 10-13).

¹⁹ *Id.* at Col. 171:25-173:4 (Claims 1-6, 8, 10-13).

²⁰ *Id.*

performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;

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; and

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, wherein the sequencing has a depth of read of at least 50,000 per target locus.²¹

Claim 14. 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, the method comprising:

performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;

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; and

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, 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.²²

²¹ *Id.* at Col. 171:25-43.

²² *Id.* at Col. 172:10-30.

I note that the claim language “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” does not use the term “limit of detection” but instead recites the capability of detecting SNV mutations present at or below certain percentages of the cfDNA comprising the SNV locus.²³

31. Certain dependent claims include limitations that the SNV mutations can be clonal and/or subclonal SNV mutations.²⁴ Dependent Claims 3-5 of the '454 Patent are reproduced *infra*.

Claim 3. The method of claim 1, wherein the SNV mutations comprise one or more clonal SNV mutations.

Claim 4. The method of claim 1, wherein the SNV mutations comprise one or more subclonal SNV mutations.

Claim 5. The method of claim 1, wherein the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations.²⁵

32. Claims 17-19 are identical to Claims 3-5 of the '454 Patent, except that they depend from Claim 14 rather than Claim 1.

33. Dependent Claim 28 of the '454 Patent includes an additional limitation that requires the method to be capable of detecting an SNV mutation that is present in 0.005% to 0.015% of the cfDNA:

²³ *Id.*

²⁴ *Id.* at Col. 171:46-52, 172:36-42 (Claims 3-5, 17-19).

²⁵ *Id.* at Col. 171:46-52.

Claim 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.²⁶

I note that the claim language “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” does not use the term “limit of detection” but instead recites a capability to detect an SNV mutation that is present in 0.005% to 0.015% of the cfDNA comprising the SNV locus.

2. Clonal and Subclonal SNV Mutations

34. As discussed *supra* and in the Metzker Op. PI Decl., one of ordinary skill in the art would have understood that mutations may be considered “clonal” or “subclonal.”²⁷ The ’454 Patent describes how cancers may contain both clonal and subclonal mutations, referring to them as “heterogeneous.” “Moreover, many cancers are heterogeneous meaning that different cells in a tumor are genetically distinct from other cells in the same tumor.”²⁸

35. Clonal and subclonal mutations can be inferred from the comparison of matched tumor and plasma samples using the methods described in the ’454 Patent. For instance, Example 13 of the ’454 Patent “demonstrates that detection of SNV in ctDNA overcomes the limitations in identifying variant alleles in biopsied samples due to tumor

²⁶ *Id.* at Col. 173:1-4.

²⁷ See *supra* Section VI.A; Doc. 17 (Metzker Op. PI Decl.), ¶¶ 34-36.

²⁸ See Doc. 1-1 (’454 Patent) at Col. 24:63-65.

heterogeneity.”²⁹ In this example, the ’454 Patent describes analyzing small cell lung cancer patient samples, including both tumor biopsies and corresponding pre-operative blood plasma.³⁰ The biopsied samples were analyzed using Illumina whole exome sequencing and Ion Torrent AmpliSeq® sequencing “for identification of underlying clonal heterogeneity.”³¹ “Following sequencing and SNV analyses, the variant allele frequency (VAF) was determined for each biopsy sample (FIG. 51B).”³² To compare this method, “Natera designed PCR assays for each SNV mutation for VAF detection in both biopsied and corresponding ctDNA from plasma (FIG. 53).”³³ Natera’s assay was then used to identify clonal and subclonal mutations and was confirmed to have identified the mutations correctly:

Blank cells represent no biopsy sample available and a zero value represents no VAF detected. ***The following 11 genes were initially identified as a negative (false VAF call) by the AmpliSeq FP or FN assays but were called correctly by the Natera TP or TN assays and mmPCR/NGS assay methods:*** L12: CYFIP1, FAT1, MLLT4, and RASA1; L13: HERC4, JAK2, MSH2, MTOR, and PLCG2; L15: GABRG1; L17: TRIM67. Surprisingly, when the AmpliSeq raw sequencing data was re-examined these results were verified. The raw AmpliSeq data sequencing files revealed that the data fell below the PGM or Illumina detectable threshold setting. The data identified 16/38 variants were detected in plasma and that there were several biopsy samples in the ***L12 patient samples that had predominant clonal SNV mutations:*** L12: BRIP1, CARS, FAT1, MLLT4, NFE2L2, TP53, TP53 as well as patients L13: EGFR, EGFR, TP53 and L15: KDM6A, ROS1. ***An additional***

²⁹ *Id.* at Col. 167:66-168:1.

³⁰ *Id.* at Col. 168:2-12.

³¹ *Id.* at Col. 168:12-17.

³² *Id.* at Col. 168:17-19.

³³ *Id.* at Col. 168:32-35.

two patients were found to have a total of four subclonal variant mutations in plasma: L12: CIC, KDM6A and L17; NF1, TRIM67.³⁴

36. Figures 53A (showing the results from the “Swanton” analysis) and 53B (showing results from the Natera analysis) of the ’454 Patent, reproduced *infra*, show the Natera results identified clonal SNV mutations (highlighted in red boxes) and identified the subclonal mutations (highlighted in green boxes).³⁵

³⁴ *Id.* at Col. 168:35-42 (emphases added). I note that “L12,” “L13,” “L15,” and “L17” are sample identifier numbers for different cancer patients.

³⁵ *See also id.* at Col. 41:65-42:3 (“FIG. 53A is a table comparing VAF calls by AmpliSeq. FIG. 53B is a table comparing VAF calls by mmPCR-NGS. A comparison of the two tables for detection of SNVs in primary tumor indicate that SNVs were missed by AmpliSeq and SNV mutations were identified in ctDNA from plasma with mmPCR-NGS.”).

Sample	Gene	Chrom	ChromStart	Swanton		
				VAF R1	VAF R2	VAF R3
L12	BRIP1	chr17	59924572	14	6	8
L12	CARS	chr11	3062181	22	11	16
L12	CIC	chr19	42797381	0	0	7
L12	CYFIP1	chr15	22940733	0	6	0
L12	FAT1	chr4	187519147	8	4	0
L12	KDM6A	chrX	44921898	10	5	0
L12	MLLT4	chr6	168347475	9	3	0
L12	NFE2L2	chr2	178098801	69	31	50
L12	RASA1	chr5	86642517	7	0	0
L12	TP53	chr17	7578406	23	9	17
L12	TP53	chr17	7578190	22	8	16
L13	EGFR	chr7	55241708	21	25	55
L13	EGFR	chr7	55242511	20	17	48
L13	HERC4	chr10	69793756	0	3	6
L13	JAK2	chr9	5022084	0	11	7
L13	KMT2C	chr7	151947008	0	0	4
L13	MSH2	chr2	47693816	5	0	11
L13	MTOR	chr1	11292495	2	0	3
L13	PLCG2	chr16	81942036	5	0	6
L13	TP53	chr17	7579509	7	4	16
L15	ALK	chr2	29940530	6	2	
L15	GABRG1	chr4	46060315	13	0	
L15	KDM6A	chrX	44922755	18	6	
L15	MLL2	chr12	49443815	5	0	
L15	ROS1	chr6	117687379	10	3	
L15	SLC39A4	chr8	145638322	14	0	
L15	TP53	chr17	7578254	19	3	
L15	ZFHX4	chr8	77776735	13	5	
L15	ZMYM4	chr1	35827319	14	2	
L17	BRCA2	chr13	32914959	16	28	
L17	KRAS	chr12	25398284	13	22	
L17	NF1	chr17	29653134	20	25	
L17	NF1	chr17	29528088	0	0	20
L17	PAX8	chr2	113984793	9	0	0
L17	TP53	chr17	7577610	0	0	16
L17	TP53	chr17	7577079	20	35	0
L17	TRIM67	chr1	231299607	0	50	0
L17	TRIP11	chr14	92471631	7	0	0

 = Ampliseq

FIG. 53A

Sample	Gene	Chrom	ChromStart	Natera			
				VAE R1	VAE R2	VAE R3	VAE Plasma
L12	BRIP1	chr17	59924572	22.6	7.74	10.07	3.71
L12	CARS	chr11	3062181	22.39	10.47	18.21	5.02
L12	CIC	chr19	42797381	0	0	8.17	1.71
L12	CYFIP1	chr15	22940733	0	0	0	0
L12	FAT1	chr4	187519147	9.81	4.26	0.77	0.57
L12	KDM6A	chrX	44921898	6.33	3.54	0	0.28
L12	MLLT4	chr6	168347475	9.04	4.26	0.73	0.95
L12	NFE2L2	chr2	178098801	73.12	39.92	53.94	23.25
L12	RASA1	chr5	86642517	7.37	0	0.35	0
L12	TP53	chr17	7578406	22.93	9.17	16.97	4.89
L12	TP53	chr17	7578190	21.48	8.67	22.09	5.77
L13	EGFR	chr7	55241708	27.06	23.14	60.79	1.16
L13	EGFR	chr7	55242511	23.93	18.01	56.31	1.09
L13	HERC4	chr10	69793756	0.16	1.92	5.72	0
L13	JAK2	chr9	5022084	0.27	2.79	6.09	0
L13	KMT2C	chr7	151947008	0	0	6.69	0
L13	MSH2	chr2	47693816	5.04	3.33	12.67	0
L13	MTOR	chr1	11292495	2.4	1.38	6.01	0
L13	PLCG2	chr16	81942036	2.8	1.7	6.89	0
L13	TP53	chr17	7579509	6.11	3.76	15.4	0.4
L15	ALK	chr2	29940530	7.2	1.39		0
L15	GABRG1	chr4	46060315	11.84	2.04		0
L15	KDM6A	chrX	44922755	6.54	0.91		0.17
L15	MLL2	chr12	49443815	12.41	0		
L15	ROS1	chr6	117687379	10.82	2.94		0.15
L15	SLC39A4	chr8	145638322	18.59	0		0
L15	TP53	chr17	7578254	12.6	2.5		0
L15	ZFHX4	chr8	77776735	10.26	2.3		0
L15	ZMYM4	chr1	35827319	12.84	3.46		0
L17	BRCA2	chr13	32914959	15.03	27		0
L17	KRAS	chr12	25398284	14.22	23.79		0
L17	NF1	chr17	29653134	18.6	23.1		0
L17	NF1	chr17	29528088	0	0	17.79	0.37
L17	PAX8	chr2	113984793	6.6	0		0
L17	TP53	chr17	7577610	0	0	18.97	0
L17	TP53	chr17	7577079	15.36	33.7		0
L17	TRIM67	chr1	231299607	18.38	30.95		0.57
L17	RIP11	chr14	92471631	6.44	0		0

 = mmPCR-NGS

FIG. 53B

In the table of Figure 53B of the '454 Patent, a "0" listed in the "VAF Plasma" column means "no VAF detected," and therefore a clonal/subclonal SNV was not identified. When the VAF Plasma column has a VAF that is >0 , this means mutation was present. In cases where the mutation is present in all available sampled areas of a tumor and the plasma sample, the mutation is categorized as a "clonal" (which I have highlighted in red). In cases where the mutation is present in less than all available sampled areas (*i.e.*, there is a "0" in one or more of the VAF R1, VAF R2, or VAF R3 columns), the mutation is categorized as "subclonal" (which I have highlighted in green).³⁶

37. Example 13 of the '454 Patent explains this categorization as follows:

Plasma samples from each of the four patients were used to isolate ctDNA and identify both clonal and subclonal SNV mutations in plasma to overcome tumor heterogeneity (FIG. 52). ***Clonal populations had VAF allele calls in all biopsied samples assayed and in plasma while subclonal populations had VAF allele calls in at least one biopsy sample, but not all biopsy samples.***³⁷

This description in Example 13 of the '454 Patent is consistent with how one of ordinary skill would have understood the terms "clonal" and "subclonal." That is clonal mutations

³⁶ In Figure 53B, for L17: TRIM67, the Natera results indicated that this mutation was detected in VAF R1, VAF R2, and VAF plasma (with a blank/no biopsy indicated for VAF R3), which would ordinarily be categorized as a clonal mutation. However, the '454 Patent categorizes this as a subclonal mutation, apparently because the VAF R3 was available for the AmpliSeq (Swanton) analysis and measured as "0," which means "no VAF detected." Compare Doc. 1-1 ('454 Patent), Fig. 53A with Fig. 53B at the row labeled "L17: TRIM67."

³⁷ See Doc. 1-1 ('454 Patent) at Col. 168:20-26 (emphasis added).

were present in *all sampled areas*, whereas subclonal mutations were present in *some, but not all sampled areas*.

3. “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” (Claim 14) and “capable of detecting an SNV mutation that is present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus” (Claim 28)

38. One of ordinary skill in the art would have understood that the '454 Patent teaches methods to detect low-frequency mutations at certain loci. As described *supra*,³⁸ the '454 Patent teaches that the frequency of a rare mutation at a locus is commonly expressed as a percentage of the total number of alleles at that locus in a sample: “Allelic imbalance refers for SNVs, to the proportion of abnormal DNA is typically measured using ***mutant allele frequency (number of mutant alleles at a locus/total number of alleles at that locus)***.”³⁹ The mutant allele frequency or “variant allele frequency,” used in this context, represents the real genetic heterogeneity of the tumor found in the individual. Put differently, an SNV mutation is present in a percentage of the cell-free DNA comprising the SNV locus in an individual—this is a genetic snapshot about the individual’s tumor status.

39. As described *supra*,⁴⁰ Claim 14 and 28 of the '454 Patent include limitations—*i.e.*, capable of detecting an SNV mutation that is present in either less than

³⁸ See *supra* ¶ 27.

³⁹ See Doc. 1-1 ('454 Patent) at Col. 30:26-29 (emphasis added).

⁴⁰ See *supra* ¶¶ 30, 33.

or equal to 0.015% (Claim 14) or 0.005% to 0.015% (Claim 28) of the cell-free DNA comprising the SNV locus—that recite specific ranges of mutant allele frequencies within which the method must be capable of detecting. These limitations reflect the requirement that the claimed methods must be capable of detecting an SNV mutation that is present in less than or equal to 0.015% (or 0.005% to 0.015%) of the cell-free DNA comprising the SNV locus, *where the SNV mutation is actually present at a minimal threshold or within the specified ranges.*

40. The '454 Patent teaches exemplary methods to analyze whether a method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% (or 0.005% to 0.015%) of the cell-free DNA comprising the SNV locus. For instance, Example 8 teaches a validation method in which “synthetic ctDNA was spiked at known concentrations to create synthetic cancer plasma.”⁴¹ One of ordinary skill in the art would have understood that this method describes a procedure to evaluate whether an assay is capable of detecting an SNV mutation that is present in a certain percentage of the cfDNA comprising the SNV locus. To accomplish this, the skilled artisan would create a synthetic sample containing a mutation at a known percentage, run the assay, and evaluate whether or not the mutation was detected.

41. A concept that is distinct from but related to whether a method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% (or 0.005% to

⁴¹ See Doc. 1-1 ('454 Patent) at Col. 161:27-28.

0.015%) of the cell-free DNA comprising the SNV locus is the calculation of the limit of detection (LOD). The LOD is calculated based on error rate and depth of read, among other things, and the calculation specifies the lowest-frequency mutations that can be detected at a specific confidence level. This is consistent with the description in the '454 Patent specification:

If desired, the limit of detection can be improved (resulting in a lower numerical value) for these SNVs by **increasing the DOR**.⁴²

If desired, the limit of detection can be improved by optimizing the assay to **reduce the error rate**.⁴³

In some embodiments, a limit of detection of a mutation (such as an SNV or CNV) of a method of the invention is less than or equal to 10, 5, 2, 1, 0.5, 0.1, 0.05, 0.01, or 0.005%. In some embodiments, a limit of detection of a mutation (such as an SNV or CNV) of a method of the invention is between 15 to 0.005%, such as between 10 to 0.005%, 10 to 0.01%, 10 to 0.1%, 5 to 0.005%, 5 to 0.01%, 5 to 0.1%, 1 to 0.005%, 1 to 0.01%, 1 to 0.1%, 0.5 to 0.005%, 0.5 to 0.01%, 0.5 to 0.1%, or 0.1 to 0.01, inclusive. In some embodiments, a limit of detection is such that a mutation (such as an SNV or CNV) that is present in less than or equal to 10, 5, 2, 1, 0.5, 0.1, 0.05, 0.01, or 0.005% of the DNA or RNA molecules with that locus in a sample (such as a sample of cfDNA or cfRNA) is detected (or is capable of being detected). **For example, the mutation can be detected even if less than or equal to 10, 5, 2, 1, 0.5, 0.1, 0.05, 0.01, or 0.005% of the DNA or RNA molecules that have that locus have that mutation in the locus** (instead of, for example, a wild-type or non-mutated version of the locus or a different mutation at that locus). In some embodiments, a limit of detection is such that a mutation (such as an SNV or CNV) that is present in less than or equal to 10, 5, 2, 1, 0.5, 0.1, 0.05, 0.01, or 0.005% of the DNA or RNA molecules in a sample (such as a sample of cfDNA or cfRNA) is detected (or is capable of being detected).

....

⁴² *Id.* at Col. 157:65-67 (emphasis added).

⁴³ *Id.* at Col. 158:22-23 (emphasis added).

Example 6 provides exemplary methods for calculating the limit of detection. In some embodiments, the “LOD-zs5.0-mr5” method of Example 6 is used.⁴⁴

....

In some embodiments, a mutation (such as an SNV or CNV) that is present in less than 10, 5, 2, 1, 0.5, 0.1, 0.05, 0.01, or 0.005% of the DNA or RNA molecules in a sample (such as a sample of cfDNA or cfRNA) is detected (or is capable of being detected). In some embodiments, a mutation (such as an SNV or CNV) that is present in less than 1,000, 500, 100, 50, 20, 10, 5, 4, 3, or 2 original DNA or RNA molecules (before amplification) in a sample (such as a sample of cfDNA or cfRNA from, e.g., a blood sample) is detected (or is capable of being detected). In some embodiments, a mutation (such as an SNV or CNV) that is present in only 1 original DNA or RNA molecule (before amplification) in a sample (such as a sample of cfDNA or cfRNA from, e.g., a blood sample) is detected (or is capable of being detected).⁴⁵

The '454 Patent thus distinguishes between a capability of detecting a mutation present in a certain percentage at an SNV locus (which is the capability of detecting an SNV mutation actually present in the specified percentages of the cfDNA comprising the SNV locus) and the calculation of a limit of detection (which is calculated based on various inputs such as depth of read and error level and is not specific to any SNV locus).

42. For example, the '454 Patent teaches how to calculate the limit of detection in Example 6 using three different methods. The first method of Example 6 is described as follows:

This example illustrates exemplary methods for calculating the limit of detection for any of the methods of the invention. These methods were used to calculate the limit of detection for single nucleotide variants (SNVs) in a tumor biopsy (FIG. 34) and a plasma sample (FIG. 35).

⁴⁴ *Id.* at Col. 92:58-93:37 (emphasis added).

⁴⁵ *Id.* at Col. 99:23-37 (emphasis added).

The first method (denoted “LOD-mr5” in FIGS. 34 and 35) calculates the limit of detection based on a minimum of 5 reads being chosen as the minimum number of times a SNV is observed in the sequencing data to have sufficient confidence the SNV is actually present. The limit of detection is based on whether the observed the depth of read (DOR) is above this minimum of 5. The gray lines in FIGS. 34 and 35 indicate SNVs for which the limit of detection is limited by the DOR. In these cases, not enough reads were measured to reach the error limit of the assay. If desired, the limit of detection can be improved (resulting in a lower numerical value) for these SNVs by increasing the DOR.⁴⁶

43. The second method of Example 6 is described as follows:

The second method (denoted “LOD-zs5.0” in FIGS. 34 and 35) calculates the limit of detection based on the z-score. The Z-score is the number of standard deviations an observed error percentage is away from the background mean error. If desired, outliers can be removed and the z-score can be recalculated and this process can be repeated. The final weighted mean and the standard deviation of the error rate are used to calculate the z-score. The mean is weighted by the DOR since the accuracy is higher when the DOR is higher.

For the exemplary z-score calculation used for this example, the background mean error and standard deviation were calculated from all the other samples of the same sequencing run weighted by their depth of read, for each genomic locus and substitution type. Samples were not considered in the background distribution if they were 5 standard deviations away from the background mean. The orange lines in FIGS. 34 and 35 indicate SNVs for which the limit of detection is limited by the error rate. For these SNV's enough reads were taken to reach the 5 read minimum, and the limit of detection was limited by the error rate. If desired, the limit of detection can be improved by optimizing the assay to reduce the error rate.⁴⁷

44. The third method of Example 6 is described as follows:

The third method (denoted “LOD-zs5.0-mr5” in FIGS. 34 and 35) calculates the limit of detection based on the maximum value of the above two metrics.

⁴⁶ *Id.* at Col. 157:51-65 (emphases added).

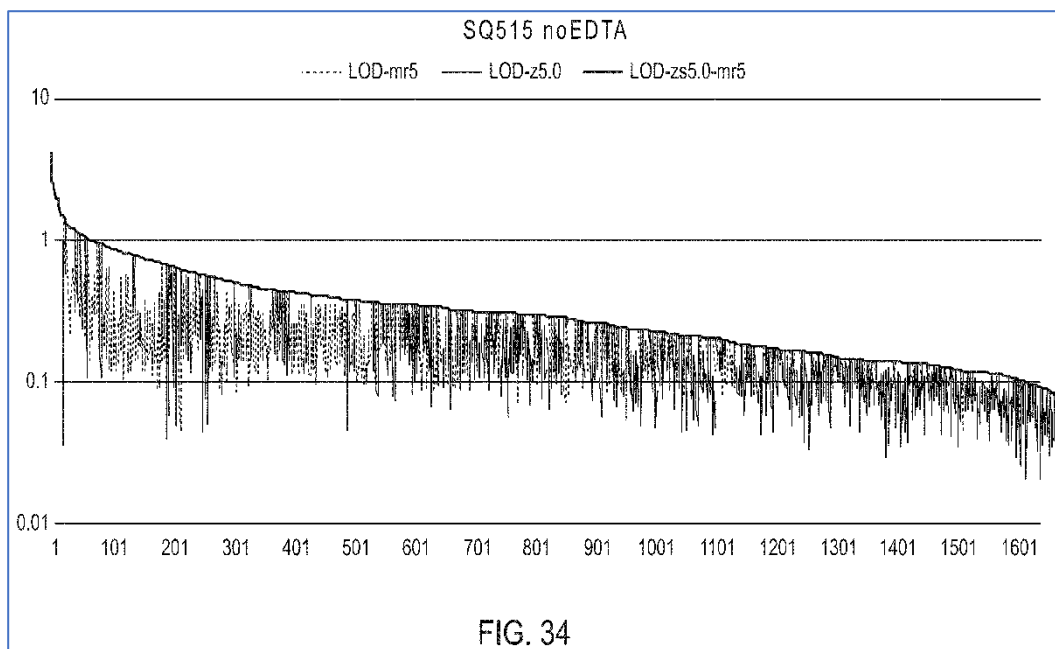
⁴⁷ *Id.* at Col. 158:1-23 (emphasis added).

For the analysis of a tumor sample shown in FIG. 34, the mean limit of detection was 0.36%, and the median limit of detection was 0.28%. The number of DOR limited (gray lines) SNVs was 934. The number of error rate limited (orange lines) SNVs was 738.

For the analysis of cDNA in a plasma sample shown in FIG. 35, the mean limit of detection was 0.24%, and the median limit of detection was 0.09%. The number of DOR limited (gray lines) SNVs was 732. The number of error rate limited (orange lines) SNVs was 921.⁴⁸

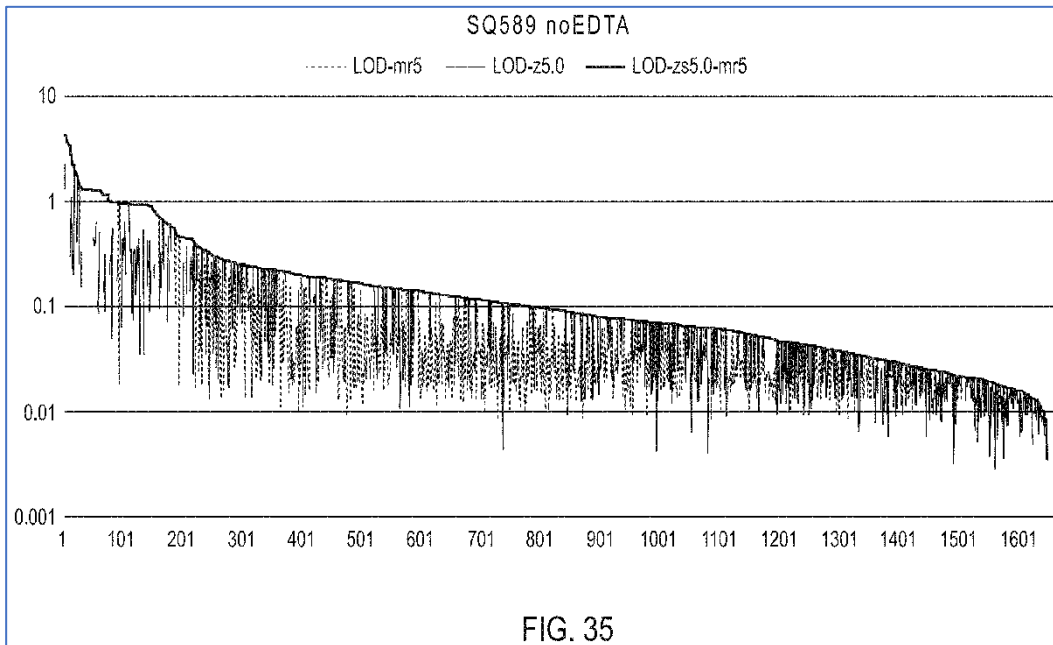
One of ordinary skill reading Example 6 would have understood that, if there is any inconsistency between the first two methods (LOD-mr5 and LOD-zs5.0), then the higher of the two values should be used. The '454 Patent refers to this as the “LOD-zs5.0-mr5” method and it is specifically referenced in the written description.⁴⁹

45. Figures 34 (tumor sample) and 35 (plasma sample) are reproduced *infra*:



⁴⁸ *Id.* at Col. 158:24-36 (emphasis added).

⁴⁹ *Id.* at Col. 93:35-37.



B. The '454 Patent's Prosecution History

46. I understand that during prosecution of the application that led to the '454 Patent, the Examiner rejected the then-pending claims for indefiniteness.⁵⁰ Notably, the rejection was based on terms that NeoGenomics does not content are indefinite here: “from the cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom,” “high throughput sequencing,” and “patient-specific PCR primers.”⁵¹ The Examiner did not raise indefiniteness as a basis for rejection as to the terms “clonal,” “subclonal,” “present in less than or equal to 0.015% of the cell-free DNA comprising the

⁵⁰ See Ex. 1 (Excerpts of '454 Patent Prosecution History) at 29-30 (Office Action (July 7, 2022)). I understand that these indefiniteness rejections were subsequently resolved after argument from the applicant and certain claim amendments. *Id.* at 18-19 (Amendment and Reply Under 37 CFR 1.111 (October 7, 2022)); *id.* at 2 (Notice of Allowance (Nov. 8, 2022)).

⁵¹ *Id.* at 29-30 (Office Action (July 7, 2022)).

SNV locus,” or “present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus.”

47. The Examiner also noted, in a section titled “Claim Interpretation,” that “[t]he terms ‘clonal SNV mutations’ and ‘subclonal SNV mutations’ in claims 4-6 are interpreted as discussed in the specification at paragraphs 111 and 748. Paragraph 48 [*sic*] states that plasma has a cumulative representation of SNV mutations. Plasma samples are therefore interpreted as comprising both clonal and subclonal SNV mutations.”⁵² Paragraph 748 of the specification cited to by the Examiner corresponds, in part, to the language I reproduced *supra* regarding Example 13 of the ’454 Patent.⁵³ The Examiner never raised an indefiniteness rejection relating to these terms.

48. The Examiner initially rejected then-pending Claims 1 and 3-15 as obvious.⁵⁴ The applicant then amended pending Claim 15 (issued Claim 14) into independent form and argued that the “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” limitation (and, by implication, the corresponding limitation in issued Claim 28) was not obvious in view of

⁵² *Id.* at 28 (Office Action (July 7, 2022)).

⁵³ *See supra* ¶ 37.

⁵⁴ *See* Ex. 1 (Excerpts of ’454 Patent Prosecution History) at 30-34 (Office Action (July 7, 2022)).

the prior art. This is because the prior art was not capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cfDNA comprising the SNV locus.⁵⁵

49. After further consideration, the Examiner withdrew the rejections and allowed all pending claims. The Examiner explained that the “detecting a single nucleotide variant mutation that is present in less than or equal to 0.015% of the cell-free DNA” in Claim 15 (issued Claim 14) was not disclosed in the prior art.⁵⁶ The Examiner never raised an indefiniteness issue with respect to these claim limitations requiring the method be “capable of detecting an SNV mutation that is present in less than or equal to 0.015% [or in 0.005% to 0.015%] of the cell-free DNA comprising the SNV locus.”

C. NeoGenomics’ Petition for *Inter Partes* Review of the ’454 Patent

50. NeoGenomics filed a Petition for *Inter Partes* Review (IPR) of the ’454 Patent on December 19, 2023.⁵⁷ Among other evidence in support of this Petition, NeoGenomics submitted an Expert Declaration of Niall Lennon, Ph.D.⁵⁸

⁵⁵ See Ex. 1(Excerpts of ’454 Patent Prosecution History) at 20-22 (Amendment and Reply Under 37 CFR 1.111 (October 7, 2022)). This limitation was also discussed during an interview on July 22, 2022. *Id.* at 25 (Applicant-Initiated Interview Summary (July 28, 2022)).

⁵⁶ *Id.* at 10 (Notice of Allowance (Nov. 8, 2022)).

⁵⁷ See Ex. 2 (Paper 1, Petition for *Inter Partes* Review of U.S. Patent No. 11,530,454, *NeoGenomics Laboratories, Inc. v. Natera, Inc.*, No. IPR2024-00346 (P.T.A.B. Dec. 19, 2023)) [hereinafter, “NeoGenomics IPR Pet.”].

⁵⁸ See Ex. 3 (Paper 1, Ex. 1002, Expert Declaration of Niall Lennon, Ph.D in Support of Petition for *Inter Partes* Review of U.S. Patent No. 11,530,454, *NeoGenomics Laboratories, Inc. v. Natera, Inc.*, No. IPR2024-00346 (P.T.A.B. Dec. 19, 2023)) [hereinafter, “Lennon IPR Decl.”].

1. NeoGenomics' Discussion of Clonal and Subclonal Mutations

51. Both NeoGenomics' Petition and Dr. Lennon's Declaration show that the scope of the terms "clonal" and "subclonal" are readily understood by those of skill in the art.

52. For example, NeoGenomics asserted in its IPR Petition the following:

As Dr. Lennon explains, ***both clonal and subclonal mutations were a well-known and common categories of cancer-related mutation. EX1002 ¶ 299. A POSA would have understood that to monitor and track cancer effectively, one would need to be able to identify the various categories of cancer-related mutations, including clonal mutations. Id.***⁵⁹

I agree with NeoGenomics and Dr. Lennon that "both clonal and subclonal mutations [] were well-known and common categories of cancer-related mutations" to those of skill in the art.

53. NeoGenomics' IPR Petition also asserted the following:

Heterogeneity of tumors and tumor DNA is a consideration in MRD monitoring. *Id.* ¶ 94. Clonal heterogeneity describes a tumor that includes cells with different genetic make-up, caused by continuing mutations as the cancer cells propagate. *Id.* ¶¶ 94-95. ***Mutations found in all of the tumors' cells are "clonal," while those found in only some are "sub-clonal."*** *Id.* ¶ 95. Heterogeneity can be the cause of cancer recurrence and resistance to treatment and has been studied in many cancers. *Id.* ¶ 96. Determining heterogeneity is done using the same techniques used to sequence cancerous cells, and heterogeneity can be monitored using cfDNA. *Id.* ¶ 97.⁶⁰

⁵⁹ See Ex. 2 (NeoGenomics IPR Pet.) at 48 (emphases added) (citing Lennon IPR Decl.).

⁶⁰ *Id.* at 9-10 (emphasis added) (citing Lennon IPR Decl.).

I agree with NeoGenomics' description of the meaning of "clonal heterogeneity," "clonal," and "sub-clonal," as they are consistent with what the '454 Patent teaches and how one of ordinary skill in the art would have understood those terms to mean.⁶¹

54. The Lennon IPR Decl. similarly acknowledges the well-known scope of the terms "clonal" and "subclonal." As an initial matter, I note that Dr. Lennon's understanding of cancer and tumor heterogeneity—his opinion on which cites to several published articles—aligns with the way clonal mutations can form:

One challenge that has been documented in MRD monitoring is the heterogeneity of tumors and tumor DNA. As discussed above, DNA mutations cause cancer. . . . ***"Cancers arise from a single ancestral cell that has acquired enough somatic mutations to deregulate its own proliferation and expand into a large cell population with clonal mutations matching the ancestral profile."*** EX1039.001; . . . see also EX1004.002 ("Rooted in the clonal evolution model of tumor progression, ***cancers are assumed to originate from a monoclonal composition (the ancestral clone), with subsequent evolution leading to selective expansions of genomically distinct subclones*** [12] with distinct phenotypic properties [12-16].").⁶²

55. By way of example, Dr. Lennon understands the meaning and scope of the terms "clonal" and "subclonal" and the way such mutations are formed.

Each time a cancerous cell divides and grows, there is a chance it will accumulate more DNA mutations without undergoing apoptosis.

⁶¹ See *supra* ¶¶ 23-24.

⁶² See Ex. 3 (Lennon IPR Decl.), ¶ 94 (emphases added) (internal cross-reference omitted) (quoting EX1039, Roberts *et al.*, *A comparative analysis of algorithms for somatic SNV detection in cancer*, BIOINFORMATICS 29:2223-2230, 2223-24 (2013) ("Roberts (2013)"); EX1004, Ali Bashashati *et al.*, *Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling*, JOURNAL OF PATHOLOGY 231:21-34, 22 (2013)) ("Bashashati (2013)"). Roberts (2013) is attached hereto as Ex. 4. I have previously discussed Bashashati (2013) in my PI Reply Decl. See Doc. 145 at Section VI.A.ii (citing Doc. 118-14 (Bashashati (2013))).

Accordingly, *a cell may accumulate mutations not shared with other cells in the tumor, i.e., “subclonal” mutations. A mutation present in the ancestor cancerous cell, an ancestral mutation, is a “clonal” mutation, while a mutation present in only a subpopulation of the cancer cells is a “subclonal” mutation.* This variety in the tumor cells’ mutations is “clonal heterogeneity.”⁶³

56. When comparing the prior art to the ’454 Patent, NeoGenomics and Dr. Lennon similarly understood the meaning and scope of the terms “clonal” and “subclonal” with reasonable certainty. In other words, NeoGenomics and Dr. Lennon understood the meaning and scope of these terms both in the prior art and in the ’454 Patent. Neither NeoGenomics in its IPR petition nor Dr. Lennon in his IPR Declaration raised any issue regarding those terms not informing one of ordinary skill in the art of the scope of the invention with reasonable certainty.

57. For example, when discussing the Claim 3 of the ’454 Patent, NeoGenomics asserted the following:

Clonal SNV mutations are those that are present in all cells of a tumor because they were present from the outset in the original cells that led to the tumor such that the mutations ultimately proliferate to all tumor cells. EX1002 ¶¶ 200-201. Forsheo [(2012)] discloses the detection of a clonal SNV mutation (a TP53 mutation), which was present in eight tissue regions sampled. *Id.* ¶¶ 199-203; EX1003.008, Fig. 4(A).⁶⁴

58. Similarly, when discussing Claim 4 of the ’454 Patent, NeoGenomics asserted the following:

Subclonal SNV mutations are those that are not present in all cells of a tumor because the mutation arose only after the tumor had originated and

⁶³ See Ex. 3 (Lennon IPR Decl.), ¶ 94 (emphasis added) (citations omitted).

⁶⁴ See Ex. 2 (NeoGenomics IPR Pet.) at 35 (emphasis added).

become partially grown, such that as the tumor grows further the mutation is present in only some cells of the tumor. EX1002 ¶ 205.⁶⁵

59. In discussing Claim 3 of the '454 Patent, Dr. Lennon confirmed that “[a] POSA would have understood clonal SNV mutations to be mutations present in the ancestral (original) clone of the tumor cells, as cancer cells are assumed to originate from monoclonal compositions.”⁶⁶ This description is consistent with how one of ordinary skill in the art would have understood the term “clonal.”

60. Similarly, in discussing Claim 4 of the '454 Patent, Dr. Lennon asserts that ***“[a] POSA would have understood subclonal SNV mutations to be mutations arising during subsequent evolution of cancer cells,*** which may have distinct phenotypic properties.”⁶⁷ This description is consistent with how one of ordinary skill in the art would have understood subclonal mutations are formed and with the term “subclonal” mutations being present in *some, but not all sampled areas*.

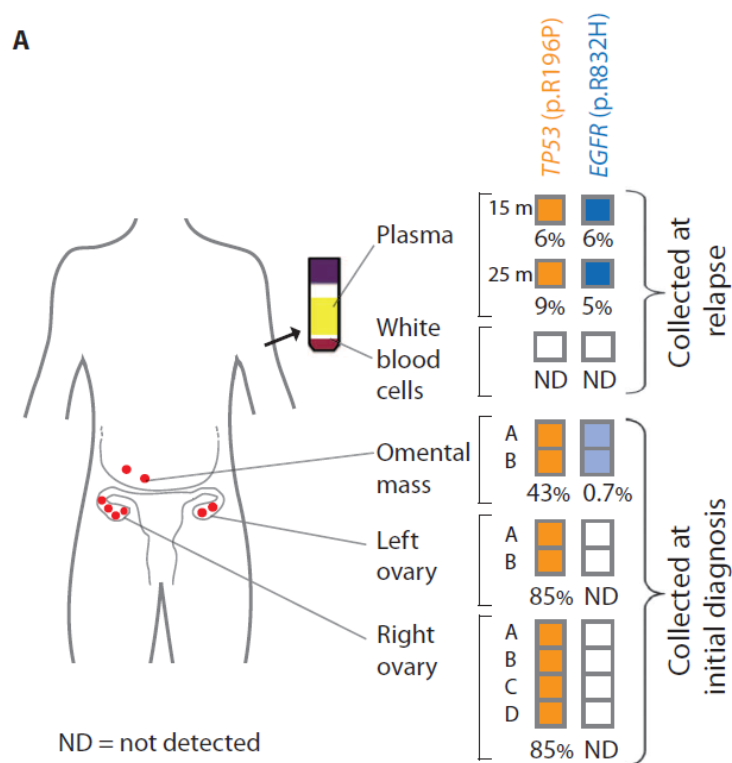
61. NeoGenomics and Dr. Lennon both cite to Figure 4(A) of Forshew (2012). According to NeoGenomics and Dr. Lennon, Figure 4(A) depicts the presence of two different mutations (*TP53* and *EGFR*) and shows that *TP53* was found in all tumor and plasma samples, whereas *EGFR* was only identified in two of the biopsy specimens (both

⁶⁵ *Id.* at 36 (emphasis added).

⁶⁶ *See* Ex. 3 (Lennon IPR Decl.), ¶ 200 (emphasis added).

⁶⁷ *Id.* ¶ 205 (emphasis added).

from the Omental mass) and in the plasma samples. Figure 4(A) of Forshew (2012) is reproduced *infra*.⁶⁸



The description of Figure 4(A) in Forshew (2012) states the following:

Retrospective analysis by TAm-Seq of plasma samples collected during patient follow-up and biopsy specimens collected at initial surgery. We identified a mutation in exon 21 of *EGFR* (dark blue boxes) in two separate plasma samples, collected 15 and 25 months after initial surgery from patient 27 (Tables 1 and 2). This mutation was not directly identified in eight tumor biopsy specimens collected at the time of initial surgery (two from omental

⁶⁸ See Doc. 17-7 (Metzker Op. PI Decl., Ex. 7, Forshew *et. al.*, *Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA*, 4 SCIENCE TRANSLATIONAL MEDICINE, 4:136ra68, pp. 1–12 (2012)) [hereinafter, “Forshew (2012)”] at 7. Forshew (2012) is also attached as Exhibit 1003 to NeoGenomics’ IPR Petition. See Paper 1, Ex. 1003, Petition for *Inter Partes* Review of U.S. Patent No. 11,530,454, *NeoGenomics Laboratories, Inc. v. Natera, Inc.*, No. IPR2024-00346 (P.T.A.B. Dec. 19, 2023), Forshew *et. al.*

mass, two from left ovary, and four from right ovary). Having identified the mutation in the plasma samples, we examined this mutation using the lower-specificity criteria defined for mutation detection (Supplementary Methods) and **detected the mutation in the two specimens that had been collected from the omentum at the time of surgery (light blue boxes) but not in the six ovarian specimens. A mutation in TP53 was identified in all tumor and plasma samples collected from this patient** (Tables 1 and 2 and table S3), but not in white blood cells (buffy coat). **Percentages indicate mutant AFs.** Empty boxes and “ND” indicate samples where a mutation was not identified or detected (below 0.8 confidence margin).⁶⁹

62. When discussing Figure 4(A) in Forshew (2012), NeoGenomics asserts that one of ordinary skill in the art would have understood the meaning of “clonal” and “subclonal” and could conclude that the *TP53* mutation was clonal, whereas the *EGFR* mutation was subclonal:

Clonal SNV mutations are those that are present in all cells of a tumor because they were present from the outset in the original cells that led to the tumor such that the mutations ultimately proliferate to all tumor cells. EX1002 ¶¶ 200-201. **Forshew discloses the detection of a clonal SNV mutation (a *TP53* mutation), which was present in eight tissue regions sampled.** *Id.* ¶¶ 199-203; EX1003.008, Fig. 4(A).

....

Subclonal SNV mutations are those that are not present in all cells of a tumor because the mutation arose only after the tumor had originated and become partially grown, such that as the tumor grows further the mutation is present in only some cells of the tumor. EX1002 ¶ 205.

Forshew discloses detection of a subclonal SNV mutation, an *EGFR* mutation only present in two of the eight tissue regions sampled. EX1002 ¶ 206; EX1003.008, Fig. 4(A). **The difference between the *EGFR* SNV between tumor samples would have established the presence of a subclonal mutation to a *POSA*.** EX1002 ¶ 206. At a minimum, Forshew in view of the knowledge of a *POSA* renders this claim obvious because **a *POSA* would have understood that cancerous tumors mutate constantly, and that some**

⁶⁹ *Id.* (emphases added).

of those mutations occur only in a subset of the tumor cells. Id. ¶¶ 204, 207. As all SNVs can be detected through whole genome sequencing of the tumor and healthy tissue, a POSA would have understood that some of the detected SNVs would be subclonal. Id.⁷⁰

Notably, NeoGenomics asserts that one of ordinary skill in the art would have understood that the *EGFR* mutation was subclonal, whereas the *TP53* mutation was clonal. NeoGenomics did not contend in its IPR petition that one of ordinary skill in the art would have needed to analyze the “mutant AFs” described in Figure 4(A) (*i.e.*, the mutant allele frequencies) in order to determine whether a mutation was clonal or subclonal. Nor did NeoGenomics contend that one of ordinary skill would have needed any additional guidance or a specific method to make that determination.

63. Dr. Lennon similarly confirmed that one of ordinary skill in the art would have understood the scope of the terms “clonal” and “subclonal” in the context of the ’454 Patent and Forsheo (2012) with reasonable certainty. In the context of Figure 4(A) of Forsheo (2012), Dr. Lennon asserted the following:

Forsheo discloses such a clonal SNV mutation. Specifically, ***tumor cells are identified as present in omental mass, left ovary, and right ovary, based on the presence of TP53 mutation in all eight different regions of the tissues sampled. . . . As each region in which tumor cells are identified establishes the presence of the TP53 mutation, a POSA would have understood that SNV to be a clonal SNV mutation.***⁷¹

. . . .

Forsheo discloses such subclonal SNV mutations in the SNVs being detected in only a subset of regions in which cancer was detected. Specifically, ***tumor cells are identified as present in omental mass, left***

⁷⁰ See Ex. 2 (NeoGenomics IPR Pet.) at 35-36 (emphases added).

⁷¹ See Ex. 3 (Lennon IPR Decl.), ¶ 202 (emphasis added).

ovary, and right ovary, based on the presence of TP53 mutation in all eight different regions of the tissues sampled, while a particular EGFR mutation is only present in two regions from the omental mass. EX1003 at Fig. 4(A) (reproduced above). The difference in the EGFR SNV between one tumor and another in the same patient would have shown a POSA that the tumor cells present in the patient had SNV mutations that included a subclonal SNV mutation.⁷²

64. In summary, NeoGenomics and Dr. Lennon agree that the scope and meaning of the terms “clonal” and “subclonal” were well understood by one of ordinary skill in the art at the time of the invention.

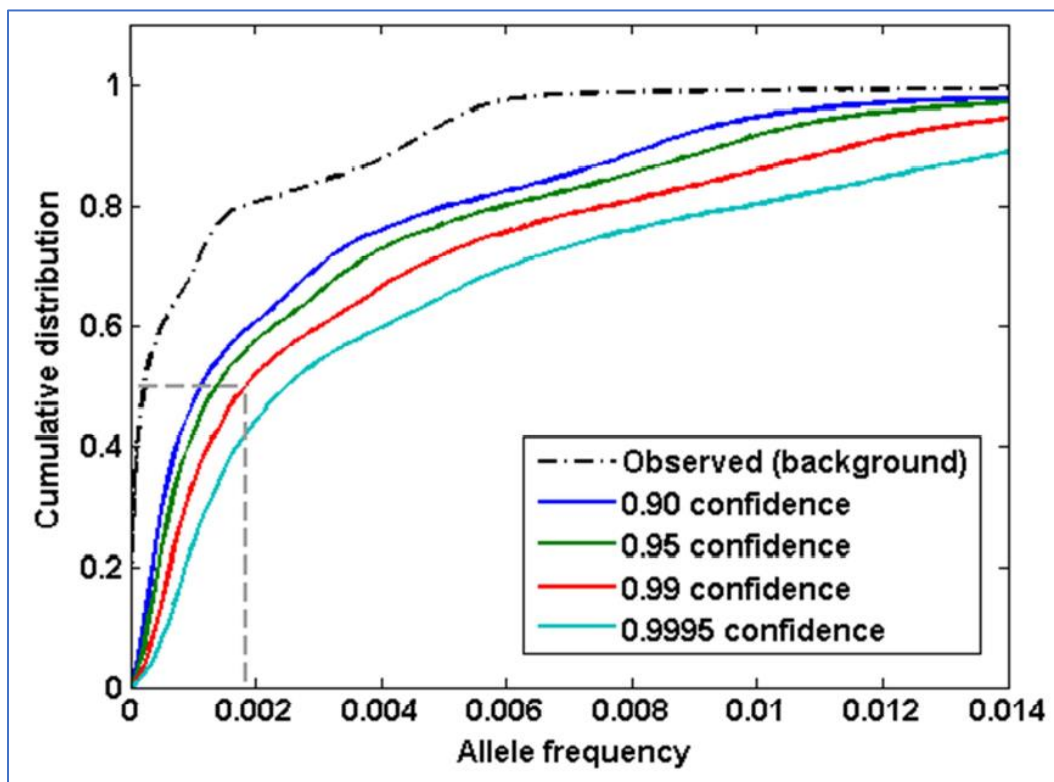
2. NeoGenomics’ Discussion of “capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus” (Claim 14) and “capable of detecting an SNV mutation that is present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus” (Claim 28)

65. Both NeoGenomics’ IPR Petition and Dr. Lennon’s IPR Declaration show that the scope of the limitations requiring a method “capable of detecting an SNV mutation that is present in less than or equal to 0.015% [or in 0.005% to 0.015%] of the cell-free DNA comprising the SNV locus” would have been readily understood by those of skill in the art with reasonable certainty.

66. In its Petition, NeoGenomics argued that this limitation was disclosed in the prior art: “As to the requirement for detecting an SNV that is present at a level less than or equal to 0.015%, Figure S3 of Forshew [(2012)] shows frequencies that could be detected with different confidence levels above the background read rate, including frequencies of

⁷² *Id.* ¶ 206 (emphasis added).

0.015% and below (*i.e.*, frequencies of 0.00015 and below).”⁷³ Figure S3 of Forshew (2012) is reproduced *infra*.



67. The description of Figure S3 of Forshew (2012) is reproduced *infra*:

Figure S3: Background frequencies and detection limits for base substitutions. For each one of the possible base substitutions in the sequenced amplicons, we calculated the mean background read rate in the 124 barcoded libraries generated from the set of 62 plasma samples (cumulative distribution shown in black), and the standard deviation. Colored lines show the cumulative distribution of frequencies that would exceed the indicated confidence margins, using a Normal distribution model for each base substitution, given its measured mean and standard deviation. Substitutions that were covered by more than one amplicon or read direction were included multiple times, once for each amplicon/direction in which they were sequenced. Substitutions that had zero reads in all barcodes had

⁷³ See Ex. 2 (NeoGenomics IPR Pet.) at 43.

undefined confidence limits and were omitted (n = 589). Dashed grey lines indicate the median value for 0.99 confidence margin.⁷⁴

68. I disagree that Forshew (2012) discloses a sensitivity as low as 0.015%. One reason Forshew (2012) does not disclose this limitation is that the data relied on in Figure S3 is “generated from the set of 62 plasma samples (cumulative distribution shown in black), and the standard deviation,”⁷⁵ meaning it is not actual data but rather calculated values based on the standard deviation. Notwithstanding my disagreement, according to Dr. Lennon, Figure S3 shows that “a fraction of possible base substitutions can be detected at and below 0.015%, as depicted in the lower left-hand portion of the graph, illustrating lower, but non-zero, rates of detecting SNVs at lower confidence levels at such allele frequencies.”⁷⁶ Dr. Lennon thus agrees that the limitation requiring that “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” does not require calculation of the limit of detection—it simply requires that the method be capable of detecting an SNV mutation that is actually present at or below the specified percentage.

69. In sum, NeoGenomics’ and Dr. Lennon’s discussions of these claim limitations in the context of invalidity is relevant here because they relied on the capability to detect an SNV mutation that is present or below the specified percentage. Both NeoGenomics in its IPR petition and Dr. Lennon in his IPR Declaration had no difficulty

⁷⁴ See Doc. 17-7 (Forshew (2012)) at Fig. S3 (original emphasis).

⁷⁵ *Id.* at Fig. S3.

⁷⁶ See Ex. 3 (Lennon IPR Decl.), ¶ 251 (emphasis added).

understanding the scope of what these claims cover and neither calculated a limit of detection in arguing that the “wherein” clause of Claim Limitations 14[c] and 28 were disclosed in Forshew (2012).

VIII. OPINIONS REGARDING THE DEFINITENESS OF CERTAIN DISPUTED CLAIM TERMS

A. The Terms “clonal” (’454 Patent, Claims 3, 5, 17, 19) and “subclonal” (’454 Patent, Claims 4, 5, 18, 19) Are Definite

70. Given the ’454 Patent claims, specification, prosecution history, and NeoGenomics’ statements in its IPR Petition, there should not be any dispute about whether the terms “clonal” and “subclonal” are definite. One of ordinary skill in the art would have understood the meaning and scope of “clonal” and “subclonal” at the time of the invention with reasonable certainty.

71. I understand that NeoGenomics contends that “clonal,” as recited in Claims 3, 5, 17, and 19 of the ’454 Patent, and “subclonal,” as recited in Claims 4, 5, 18, and 19 of the ’454 Patent, are indefinite because the terms “fail to inform with reasonable certainty those skilled in the art about the scope of invention.”⁷⁷ More specifically, NeoGenomics in its Initial Invalidity Contentions asserts the following:

Claim 3 recites a method “wherein the SNV mutations comprise one or more clonal SNV mutations,” claim 4 recites a method “wherein the SNV mutations comprise one or more subclonal SNV mutations,” and claim 5 recites a method “wherein the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal mutations.” A POSA would understand that there are different methods or different ways to distinguish between a clonal SNV mutation and a subclonal SNV mutation. The specification, however, does not define or provide any guidance as to which

⁷⁷ Doc. 249-1 (Ex. A to Joint Claim Construction Statement) at 15-19.

method is contemplated or required by the claim. For example, the '454 patent does not explain whether the distinction can be made based on a percentage of tumor cells that contain a mutation, nor does the '454 patent explain what percentage should be used for distinguishing between “clonal” or “subclonal” mutations. Accordingly, a POSA would not understand the metes and bounds of “clonal” and “subclonal” mutations as claimed.⁷⁸

72. My opinions are based on information currently available to me and my understanding of NeoGenomics’ contentions as described *supra*. I may continue my investigation and study as new information comes to light and this case progresses. For example, after NeoGenomics serves its opening brief, I may continue investigating the evidence for purposes of rebutting NeoGenomics’ arguments and contentions. Accordingly, I may supplement my opinions herein and/or provide rebuttal opinions and reserve the right to do so, including through written declarations and/or testimony given at deposition, at any claim construction hearing, or at trial.

73. Based on the claims, specification, and prosecution history of the '454 Patent, the terms “clonal” and “subclonal” are used consistently with how one of ordinary skill in the art would have understood the plain and ordinary meaning of the term at the time of the invention. There is nothing in the claims or specification that would indicate Natera used, or intended to use, different definitions of “clonal” or “subclonal.”

74. Specifically, as described *supra*,⁷⁹ one of ordinary skill in the art would have understood the plain and ordinary meaning of the term “clonal” SNV mutation is to mean

⁷⁸ See Ex. 5 (Defendant’s Initial Invalidity Contentions (Feb. 23, 2024)) at 41.

⁷⁹ See *supra* ¶¶ 23-24, 37.

a mutation that is present in all sampled areas of a tumor specimen or biopsy. In contrast, one of ordinary skill in the art would have understood the plain and ordinary meaning of the term “subclonal” SNV mutation to mean a mutation that is present in some, but not all sampled areas of a tumor specimen or biopsy.

75. This plain and ordinary meaning is supported by the ’454 Patent specification, which explains that, “[c]lonal populations had VAF allele calls in all biopsied samples assayed and in plasma while subclonal populations had VAF allele calls in at least one biopsy sample, but not all biopsy samples.”⁸⁰

76. The plain and ordinary meaning of “clonal” and “subclonal” is further confirmed by the prosecution history, which cited, in part, to the section of the ’454 Patent specification reproduced in the preceding paragraph when discussing the “claim interpretation” of “clonal SNV mutations” and “subclonal SNV mutations.”⁸¹

77. One of ordinary skill in the art would thus have understood the scope of the claims containing these terms with reasonable certainty. For example, one of ordinary skill in the art would have understood Claims 3 and 17 of the ’454 Patent cover the method of the independent claims from which they depend when it is performed on SNV mutations comprising at least one clonal SNV mutation.⁸² A clonal SNV mutation is one that is present in all sampled areas of a tumor specimen or biopsy. One of ordinary skill in the art

⁸⁰ See Doc. 1-1 (’454 Patent) at Col. 168:23-26; *see also supra* ¶¶ 35-37.

⁸¹ See *supra* ¶¶ 46-47.

⁸² Doc. 1-1 (’454 Patent) at Col. 171:25-43, 171:46-47, 172:10-31, 172:36-37.

would have understood Claims 4 and 18 of the '454 Patent cover the method of the independent claims from which they depend when it is performed on SNV mutations comprising at least one subclonal SNV mutation,⁸³ *i.e.*, one that is present in some, but not all sampled areas of a tumor specimen or biopsy. Finally, one of ordinary skill in the art would have understood Claims 5 and 19 of the '454 Patent cover the method of the independent claims from which they depend when it is performed on SNV mutations comprising at least one clonal SNV mutation *and* at least one subclonal SNV mutation.⁸⁴

78. One of ordinary skill in the art would have understood how to use the whole exome/whole genome tumor sample sequencing information and/or plasma sample sequencing information (cfDNA) recited in Claims 1 and 14 of the '454 Patent to determine whether the SNV mutations were clonal or subclonal. As discussed *supra*⁸⁵—and contrary to NeoGenomics' contention that the '454 Patent “does not define or provide any guidance as to which method is contemplated or required by the claim”⁸⁶—Example 13 of the '454 Patent expressly discusses one way to categorize mutations as clonal or subclonal based on the presence of a mutation in all sampled areas (a clonal SNV mutation) or some, but not all sampled areas (a subclonal SNV mutation). This method does not rely “on a percentage of tumor cells that contain a mutation”—it uses absolute values of “detected in all samples”

⁸³ *Id.* at Col. 171:25-43, 171:48-49, 172:10-31, 172:38-39.

⁸⁴ *Id.* at Col. 171:25-43, 171:50-52, 172:10-31, 172:40-42.

⁸⁵ *See supra* ¶¶ 35-37.

⁸⁶ *See* Ex. 5 (Defendant's Initial Invalidity Contentions (Feb. 23, 2024)) at 41.

or “detected in some but not all samples”⁸⁷—and thus does not require disclosure of a “percentage [that] should be used for distinguishing between ‘clonal’ or ‘subclonal’ mutations,” as NeoGenomics contends.⁸⁸

79. In addition, as discussed *supra*, NeoGenomics applies this same method of categorizing mutations into “clonal” and “subclonal” categories when discussing the prior art and the ’454 Patent in the context of its IPR Petition.⁸⁹ Indeed, NeoGenomics refers to the clonal/subclonal distinction as “well-known and common categories of cancer-related mutation” and provides a clear definition: “Mutations found in all of the tumors’ cells are ‘clonal,’ while those found in only some are ‘sub-clonal.’”⁹⁰ I agree with NeoGenomics’ characterization of how easily one of ordinary skill in the art would have understood the meaning and scope of the terms “clonal” and “subclonal.” Based on my understanding of NeoGenomics’ arguments in the IPR Petition, NeoGenomics does not disagree with my opinion that these terms are not indefinite.

80. Additionally, I note that in its Invalidity Contentions, NeoGenomics does not identify any methods of categorizing a mutation as “clonal” or “subclonal” that would produce different results. While NeoGenomics contends that “the distinction can be made based on a percentage of tumor cells that contain a mutation,”⁹¹ it does not contend that

⁸⁷ See, e.g., *supra* ¶ 36.

⁸⁸ See Ex. 5 (Defendant’s Initial Invalidity Contentions (Feb. 23, 2024)) at 41.

⁸⁹ See *supra* Section VII.C.

⁹⁰ See *supra* ¶¶ 52-53.

⁹¹ See Ex. 5 (Defendant’s Initial Invalidity Contentions (Feb. 23, 2024)) at 41.

one of ordinary skill in the art would not have had the knowledge and understanding required to determine the percentage that should be used for accurately distinguishing between “clonal” or “subclonal” mutations. As discussed *supra*,⁹² the method described in the ’454 Patent (and one that NeoGenomics agrees with in its IPR Petition) uses simple absolute values—mutation present in all sampled areas (clonal) or present in some but not all sampled areas (subclonal)—and does not rely “on a percentage of tumor cells that contain a mutation.”⁹³

81. In my opinion, NeoGenomics’ error in asserting that “clonal” and “subclonal” are indefinite stems from its attempt to read limitations into the claims. All that the relevant claims of the ’454 Patent require is that “***the SNV mutations comprise*** one or more clonal SNV mutations” (Claims 3, 17), “***the SNV mutations comprise*** one or more subclonal SNV mutations” (Claims 4, 18), or “***the SNV mutations comprise*** one or more clonal SNV mutations and one or more subclonal SNV mutations” (Claims 5, 19). In other words, these dependent claims add one limitation: the SNV mutations being identified by whole exome or whole genome sequencing (limitation 1[a] and 14[a]), functioning as the target loci during amplification (limitation 1[b] and 14[b]), and detected during sequencing (limitation 1[c] and 14[c]) must include at least one clonal SNV mutation (Claims 3, 17), at least one subclonal SNV mutation (Claims 4, 18), or at least one of each (Claims 5, 19). There is nothing in the claim language requiring that the SNV

⁹² See *supra* ¶ 36.

⁹³ See *supra* ¶ 78.

mutations be “categorized,” “identified,” or “detected” *as clonal or subclonal*. NeoGenomics is improperly attempting to read such a limitation into the claim where none exists.

82. In sum, one of ordinary skill in the art would have understood with reasonable certainty that when performing whole exome or genome sequencing on a tumor sample to identify a plurality of tumor-specific SNV mutations of sequencing cfDNA, as recited in independent Claims 1 and 14 of the '454 Patent, the mutations identified in all sampled areas would be clonal, while those identified in some, but not all sampled areas would be subclonal. One of ordinary skill in the art would have had no difficulty making this distinction in the context of performing the method of the '454 Patent Claims 3-5 and 17-19.

B. The Terms “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” (Claim 14) and “capable of detecting an SNV mutation that is present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus” (Claim 28) Are Definite

83. Given the '454 Patent claims, specification, prosecution history, and NeoGenomics' statements in its IPR Petition, there should not be any dispute about whether the limitations “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” (Claim 14) and “capable of detecting an SNV mutation that is present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus” (Claim 28) are definite. One of ordinary skill in the art would have understood the meaning and scope of these limitations at the time of the

invention with reasonable certainty in the context of detecting an SNV mutation in the '454 Patent. In my opinion, there is no vagueness or ambiguity in the language of the claims, which provides precise, objective guidance to one of ordinary skill in the art regarding what is covered by the claims.

84. I understand that NeoGenomics contends that “present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus,” as recited in Claim 14 of the '454 Patent, and “present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus,” as recited in Claim 28 of the '454 Patent are indefinite because the terms “fail to inform with reasonable certainty, those skilled in the art about the scope of invention.”⁹⁴ More specifically, NeoGenomics asserts the following in its Initial Invalidity Contentions:

Claim 14 of the '454 patent recites 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.” But claim 14 does not recite how to **determine** the capability of a method to detect an SNV mutation present in a given percentage of cell-free DNA comprising the SNV locus. As disclosed in Example 6 of the '454 patent, **different methods for calculating the limit of detection (LOD)** for any method give different results. See, e.g., 40:25–30, 157:51–158:36, FIGS. 34–35. For example, “FIG. 35 is a graph showing the limit of detection for single nucleotide variants in a plasma sample using three different methods described in Example 6.” 40:28–30. As shown in Fig. 35, the three different methods for **calculating LOD** do not give the same results. Because the '454 patent and prosecution history do not provide guidance to a POSA as to which method should be used to calculate the claimed limit of detection, claim 14 is invalid for indefiniteness. *Dow Chemical Co. v. NOVA Chemicals Corp. (Canada)*, 803 F.3d 620, 634-35 (Fed. Cir. 2015); *Teva Pharm. USA, Inc. v. Sandoz, Inc.*, 789 F.3d 1335, 1344-45 (Fed. Cir. 2015).

....

⁹⁴ See Doc. 249-1 (Ex. A to Joint Claim Construction Statement) at 19-24.

Claim 28 recites a method “capable of detecting an SNV mutation that is present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus.” ’454 patent at 173:1–4. As discussed above, the ’454 patent describes multiple ***different methods for measuring the capability*** of a method to detect an SNV mutation present in a given percentage of cell-free DNA comprising the SNV locus, but does not provide guidance as to which method should be used. Therefore, claim 28 is indefinite for the same reasons discussed above regarding claim 14.

Additionally, claim 28 depends from claim 14, which recites 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.” A POSA also would not know with reasonable certainty (1) whether claim 28 further limits claim 14 such that methods capable of detecting an SNV mutation that is present in less than 0.005% of the cell-free DNA comprising the SNV locus would not infringe claim 28 or (2) whether [c]laim 28 does not further limit claim 14 such that methods capable of detecting an SNV mutation that is present in less than 0.005% of the cell-free DNA comprising the SNV locus would infringe claim 28.⁹⁵

I understand that, because NeoGenomics has the burden to prove indefiniteness by clear and convincing evidence, its failure to provide such evidence means that the claims are not indefinite.

85. As discussed *supra*, my opinions are based on information currently available to me and my understanding of NeoGenomics’ contentions. I may continue my investigation and study as new information comes to light and this case progresses. For example, after NeoGenomics serves its opening brief, I may continue investigating the evidence for purposes of rebutting NeoGenomics’ arguments and contentions. Accordingly, I may supplement my opinions herein and/or provide rebuttal opinions and

⁹⁵ See Ex. 5 (Defendant’s Initial Invalidity Contentions (Feb. 23, 2024)) at 42-43.

reserve the right to do so, including through written declarations and/or testimony given at deposition, at any claim construction hearing, or at trial.

86. In my opinion, the terms “present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus” and “present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus” are not indefinite. One of ordinary skill in the art would have understood, based on the claims, specification, and prosecution history, that the scope of Claim 14 of the ’454 Patent⁹⁶ is limited to a method that 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*. The capability of detecting an SNV mutation that is present at or below these frequencies depends on the method’s ability to detect mutations actually present at these frequencies in a subject—*i.e.*, where these mutations are present at these frequencies in the real genetic heterogeneity of the tumor found in the individual. NeoGenomics has not shown or provided any evidence that this objective, precise language is capable of different meanings.

87. The claim language itself only requires that “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.” There is no reference to any calculation of a limit of detection, as NeoGenomics appears to argue. Any method that performs the steps of Claim

⁹⁶ The same analysis applies to Claim 28 of the ’454 Patent, which recites a method “capable of detecting an SNV mutation that is present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus.” See Doc. 1-1 (’454 Patent) at Col. 173:1-4.

14 of the '454 Patent and that is *capable* of detecting an SNV mutation present in less than or equal to 0.015% of the cfDNA comprising the SNV locus would infringe. One of ordinary skill would have understood the scope of the claim accordingly and would have understood what falls within the scope of the claim and what does not. In my opinion, there is no vagueness or ambiguity in the language of the claims.

88. One of ordinary skill in the art would have understood that, to determine whether a particular assay would infringe Claim 14 of the '454 Patent, one could test that assay using a contrived sample containing an SNV mutation at a known percentage such as 0.015%, representing a real-world SNV mutation that is present in a subject at 0.015% of the cfDNA comprising the SNV locus. If the assay detected that SNV mutation, then it is capable of detecting SNV mutations present in 0.015% of the cfDNA comprising the SNV locus.⁹⁷ One of ordinary skill in the art would not have needed any additional information to determine the scope of the claims with reasonable certainty.

89. The prosecution history of the '454 Patent supports my opinion that the challenged limitations are not indefinite. The Examiner never raised any indefiniteness issue with respect to these limitations, and these limitations were, in part, what distinguished the '454 Patent claims over the prior art.⁹⁸

90. The arguments NeoGenomics made in its IPR Petition further support the definiteness of these claim limitations. As discussed *supra*, when comparing the prior art

⁹⁷ See *supra* ¶¶40-44.

⁹⁸ See *supra* ¶¶ 48, 49.

for purposes of attempting to show obviousness, NeoGenomics in its IPR petition simply pointed to a prior art method it (wrongly) contends was *capable of detecting an SNV mutation that is actually present in less than or equal to 0.015% of the cfDNA comprising the SNV locus*. More specifically, NeoGenomics contended that this claim limitation was disclosed in the prior art simply because the prior art allegedly detected mutations that were present in 0.015% (or below) of the cfDNA comprising the SNV locus. NeoGenomics did not argue that more information was needed to determine if the prior art disclosed this limitation, nor did it point to a limit of detection. Instead, NeoGenomics understood the clear and unambiguous scope of the claims, citing prior art that it (wrongly) contended actually detected SNV mutations that were present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus.⁹⁹ NeoGenomics never argued in its IPR petition that meeting this limitation depends on the method of measuring anything.

91. In my opinion, NeoGenomics is improperly attempting to read examples given in the '454 Patent regarding how to calculate the limit of detection (*e.g.*, Example 6, discussed *supra*)¹⁰⁰ into the claim language where the claim language does not recite any “calculation.”

92. Additionally, even assuming that the *calculation* of a method’s measured sensitivity or limit of detection is relevant to the claims (it is not), I note that NeoGenomics’ Invalidity Contentions asserted, without providing more, that “the three different methods

⁹⁹ See *supra* Section VII.C.2.

¹⁰⁰ See *supra* Section VII.A.3.

for calculating LOD do not give the same results.” It is unclear from this conclusory statement the specific “different results” NeoGenomics contends exist and whether such differences would make a material difference in defining the scope of the claims. In any event, as discussed *supra*, the ’454 Patent itself teaches that, to resolve any purported inconsistencies between the two methods of calculating LOD, one should use the higher of the two values.¹⁰¹ Thus, there is no evidence showing that different methods of calculating LOD would lead to different results and the claims are therefore not indefinite.

93. Finally, NeoGenomics appears to argue that there is some indefiniteness arising from the differences in the claimed numerical values recited in Claim 14 compared with dependent Claim 28 of the ’454 Patent. I disagree. One of ordinary skill in the art would have readily understood the difference in scope between “less than or equal to 0.015%” and “0.005% to 0.015%,” as there are numerical values that fall within the former but not within the latter. Put differently, one of ordinary skill would have known with reasonable certainty that dependent Claim 28 of the ’454 Patent further limits Claim 14 by providing a lower limit to the SNV mutation present in the cell-free DNA comprising the SNV locus: 0.005%.¹⁰² Thus, a method capable of detecting an SNV mutation that is present in 0.004% of the cell-free DNA comprising the SNV locus would fall within the scope of Claim 14 but not dependent Claim 28 of the ’454 Patent. These are precise, unambiguous limitations that are not subject to other interpretations.

¹⁰¹ See *supra* ¶ 42.

¹⁰² See Doc. 1-1 (’454 Patent) at Col. 173:1-4.

94. In sum, one of ordinary skill in the art would have understood with reasonable certainty the scope of the limitations requiring the claimed methods to be capable of detecting an SNV mutation that is present in less than or equal to 0.015% (or in 0.005% to 0.015%) of the cell-free DNA comprising the SNV locus.

I confirm that the contents of this Declaration are true to the best of my knowledge and belief insofar as it states facts and that it contains my honest opinions on the matters upon which I have been asked to give them.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

Dated: April 8, 2024

A handwritten signature in black ink, appearing to read 'Michael Metzker', written over a horizontal line.

Michael Metzker, Ph.D.