

**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 RESPONSIVE 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 also submitted a Declaration² in connection with Natera's opening claim construction brief.³ I incorporate those Declarations herein by reference, including information regarding my qualifications and expertise, 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")⁴ and 11,519,035 (the "'035 Patent")⁵ (collectively, the "Asserted Natera Patents").

2. I have been asked to provide my expert opinions in response to NeoGenomics' Opening Claim Construction Brief⁶ and Dr. Smith's Opening Claim

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."]; see also Doc. 145, *Reply Declaration of Dr. Michael Metzker*, dated Nov. 6, 2023 [hereinafter "Metzker Reply PI Decl."].

² See Doc. 263, *Declaration of Dr. Michael L. Metzker in Support of Plaintiff Natera, Inc.'s Opening Claim Construction Brief*, dated April 8, 2024 [hereinafter "Metzker Op. CC Decl."].

³ See Doc. 262, *Plaintiff Natera, Inc.'s Opening Claim Construction Brief*, dated April 8, 2024 [hereinafter "Natera Op. CC Brief"].

⁴ 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").

⁵ 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").

⁶ See Doc. 264, *Defendant NeoGenomics, Inc.'s Opening Claim Construction Brief* [hereinafter "NeoGenomics Op. CC Br."].

Construction Declaration.⁷ I have been informed and understand that Natera has asserted the following claims against NeoGenomics: Claims 1, 12, and 13 of the '035 Patent and Claims 1-28 of the '454 Patent (collectively, the “Asserted Natera Claims”).

3. This Responsive Declaration is based on information currently available to me. I may continue my investigation and study as new information comes to light and circumstances 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 Responsive Declaration or on any documents cited herein, if asked by the Court or by the parties’ attorneys.

I. SUMMARY OF OPINIONS

4. Regarding Claim 1 of the '035 Patent, it is my opinion that one of ordinary skill in the art would have understood the term “SNP loci . . . associated with cancer” to mean genomic variants at single nucleotide positions associated with cancer, including those that are inherited at birth (*i.e.*, germline variants) and acquired after birth (*i.e.*, somatic variants).

5. As described in my Opening Claim Construction Declaration, the Asserted Natera Claims are not invalid for indefiniteness.⁸ A person of ordinary skill in the art

⁷ See Doc. 264-5, *Declaration of David I. Smith, Ph.D. on Claim Construction* [hereinafter “Smith Op. CC Decl.”].

⁸ See Doc. 263 (Metzker Op. CC Decl.), Section VIII.

(“POSA”)⁹ 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. Nothing in NeoGenomics’ Opening Claim Construction Brief or Dr. Smith’s Opening Claim Construction Declaration change my opinions.

II. BACKGROUND AND QUALIFICATIONS

6. I previously identified my background and qualifications in the Metzker Opening PI Declaration,¹⁰ and incorporate that discussion here.

7. 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.

8. 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 *infra*.

⁹ Throughout this Responsive Declaration, I also refer to a POSA as one of ordinary skill in the art.

¹⁰ See Doc. 17 (Metzker Op. PI Decl.), ¶¶ 4–19, with one exception: I am no longer Chief Technology Officer at 454 Bio.

9. 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

10. I am not an attorney, nor have I independently researched the law. In this Responsive Declaration, I have applied certain legal principles, including with relation to claim construction, of which I have been informed by Natera's counsel as described in my Opening PI Declaration.¹¹

11. I have been informed and understand that patent claims should be generally given their plain and ordinary meaning from the perspective of one of ordinary skill in the art at the time that the claimed subject matter was applied for. I also understand that patent claims must also be read in light of the specification of the patent and associated prosecution histories, and that the specification is viewed as the best source for understanding a technical claim term. I further understand that the only exceptions to giving the words in a claim their plain and ordinary meaning in the art are (i) when the patentee acts as their own lexicographer; and (ii) when the patentee disavows or disclaims the full scope of a claim term in the specification. With respect to the former, I have been informed and understand that to act as their own lexicographer, the applicant must clearly set forth a special definition of a claim term in the specification that differs from the plain

¹¹ See Doc. 17 (Metzker Op. PI Decl.), ¶ 24.

and ordinary meaning it would otherwise possess. I also understand that the specification may also include an intentional disclaimer, or disavowal, of claim scope, which must be clear and unmistakable.

12. I have been informed and understand that it is improper to import claim limitations from the specification. I also understand that a claim construction that reads disclosed embodiments out of the claim is rarely correct absent evidence of a clear disclaimer. I further understand that extrinsic evidence cannot “alter the meaning” of a claim term where the intrinsic evidence is otherwise clear.

IV. LEVEL OF ORDINARY SKILL IN THE ART

13. I set out my understanding of one of ordinary skill in the art in my Opening PI Declaration,¹² and I incorporate that discussion here. Additionally, one of ordinary skill in the art would have had an understanding of cancer biology as it relates to mutations associated with cancer, including as related to tumor monitoring, progression, and treatment.

14. In or around 2011, I would have been at least one of ordinary skill in the art with respect to the subject matter of the '035 Patent.

15. 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.

¹² See Doc. 17 (Metzker Op. PI Decl.), ¶ 32.

V. MATERIALS CONSIDERED

16. In formulating my opinions, I considered all of the references cited in this Responsive Declaration, as well as NeoGenomics' Opening Claim Construction Brief, Dr. Smith's Opening Claim Construction Declaration, and exhibits to those documents.

VI. TECHNOLOGY BACKGROUND

A. Human Genetics

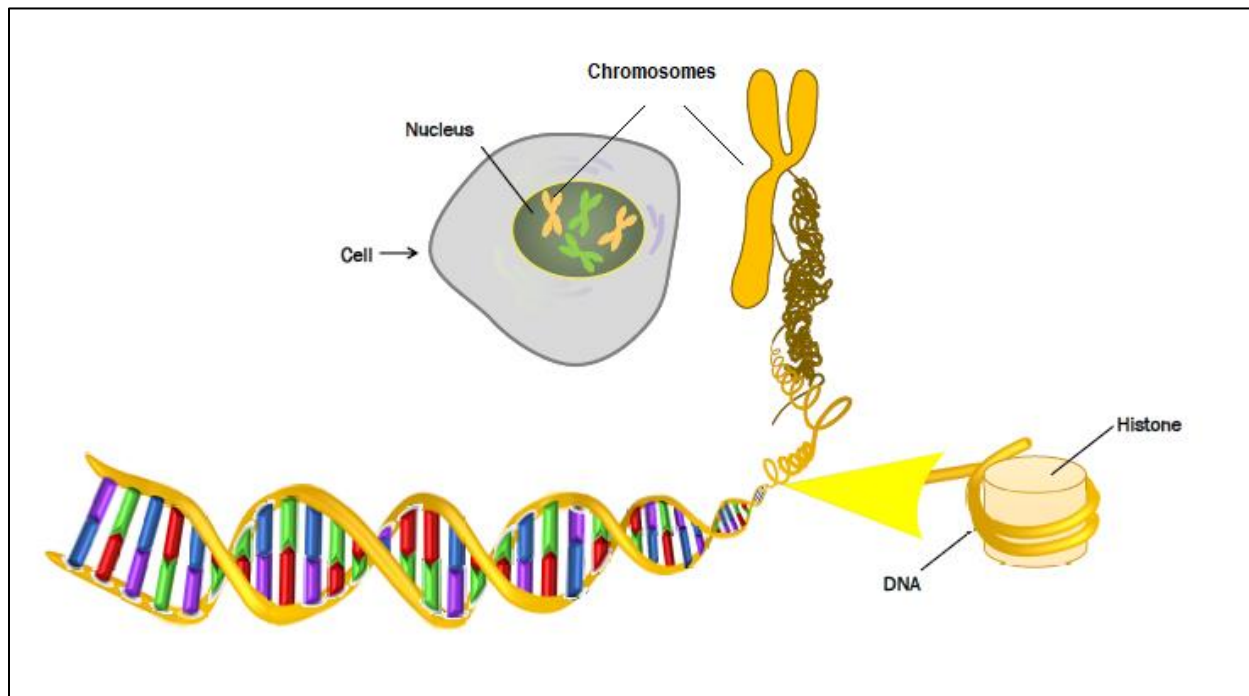
17. DNA is made of four different nucleotides or bases, each represented by a different letter: adenine (A), thymine (T), cytosine (C) and guanine (G). The order of these letters (*i.e.*, the DNA sequence) encodes the information that instructs cells when and how to perform certain functions.

18. As explained previously and shown *infra*,¹³ DNA is typically contained within the nucleus of a cell and is packed into structures called chromosomes. The complete set of DNA is referred to as a “genome.” The *haploid* human genome contains about 3 billion nucleotides that are distributed among 23 chromosomes, and the *diploid* human genome contains about 6 billion nucleotides that are distributed among 46 chromosomes. Said another way, a typical human genome contains 46 chromosomes—44 (22 pairs) autosomes and two (one pair) sex chromosomes (*i.e.*, X and Y).¹⁴ One set of each chromosome pair is inherited from a person's mother and the other from a person's

¹³ See Doc. 246-1 (Natera Technology Tutorial) at 14 (annotated).

¹⁴ See Ex. 1, Buckingham. L., MOLECULAR DIAGNOSTIC FUNDAMENTAL METHODS AND CLINICAL APPLICATIONS, Chapter 8: *Chromosomal Structure and Chromosomal Mutations*, (2007) [hereinafter “Buckingham (2007)”] at 156.

father. In people, almost every cell in the body contains a complete copy of the genome in the nucleus of a cell, organized as chromosomes, as illustrated in the figure *infra*.¹⁵



19. Comparing the genome derived from a cell of one person against the genome derived from a cell of another person or a reference human genome¹⁶ will generally reveal far more similarities to each other than differences. For example, two people’s genomes

¹⁵ *Id.* Excluded are reproductive cells, called germ cells, which only have one genome copy (*i.e.*, the haploid genome), as well as cells that do not contain a nucleus (*e.g.*, red blood cells), and therefore do not contain genomic DNA.

¹⁶ A “reference” human genome sequence is an established, well-accepted sequence of a human genome. I was part of the effort, called the *Human Genome Project*, that decoded the first human reference genome. See Ex. 2, International Human Genome Sequencing Consortium, *Initial sequencing and analysis of the human genome*, NATURE 409:860–921 (2001) [hereinafter (“IHGSC (2001)”)]; see also Ex. 3, International Human Genome Sequencing Consortium, *Finishing the euchromatic sequence of the human genome*, NATURE 431:931–945 (2004) [hereinafter (“IHGSC (2004)”)].

are generally more than 99% identical to one another because “on average, a single-nucleotide difference exists between two people’s genomes once every 1,300 nucleotides or so.”¹⁷ The differences among human genomes are sometimes called “variants,” “variations,” “polymorphisms,” or “mutations.” A person’s set of genomic variants is part of what makes them unique¹⁸ and may contribute to an individual’s risk for developing diseases, such as cancer.

B. Genomic Variants

20. By 2010, one of ordinary skill in the art would have been familiar with genomic variants, including those that may be associated with cancer. As I explain *infra*, such variants may be classified by the type of alteration or cell type in which the variant occurs.

1. Classification Based on Type of Alteration

21. The smallest genomic variants are single-nucleotide variants (SNVs). Each SNV reflects a difference in a single nucleotide (or letter) at a particular genomic position. For a given SNV, the DNA letter at that genomic position might be, for example, a “C” in one person but a “T” in another person.¹⁹

¹⁷ See Ex. 4, <https://www.genome.gov/about-genomics/educational-resources/fact-sheets/human-genomic-variation#:~:text=It%20is%20frequently%20stated%20that%20any%20two%20peoples%E2%80%99,peoples%E2%80%99%20genomes%20once%20e%20very%201%2C300%20nucleotides%20or%20so> (last visited April 20, 2024) [hereinafter (“National Human Genome Research Institute webpage”)] at 3.

¹⁸ *Id.* at 4.

¹⁹ *Id.*

22. Another type of variant are single nucleotide polymorphisms (SNPs), which also refers to a difference in a single nucleotide (or letter) at a particular genomic position. However, to be considered a SNP, a variant must typically be present in at least 1% of the human population.²⁰ As such, an SNV is a more general term that includes both relatively common (such as SNPs) and rare single-nucleotide differences. For simplicity, all single-nucleotide differences can be referred to as SNVs, regardless of their relative frequency.²¹

2. Classification of a Genomic Variant Based on Cell Type

23. Genetic mutations may be inherited, called “germline” variants. Germline variants are first present in the reproductive cells (*e.g.*, egg or sperm cells) of a parent, and are then passed from parent to their offspring. Germline transmission typically results in the genomic variant being present in all cells of the offspring.²²

24. Genetic mutations may also be acquired during a person’s lifetime in non-reproductive cells, called “somatic” variants.²³ Somatic variants are not heritable and

²⁰ *Id.*; see also Ex. 1, (Buckingham (2007)) at 156.

²¹ See Ex. 4 (National Human Genome Research Institute webpage) at 4. I note this understanding is consistent with the teachings of the term *SNP* in the ’035 Patent. “Single Nucleotide Polymorphism (SNP) refers to a single nucleotide that may differ between the genomes of two members of the same species. ***The usage of the term should not imply any limit on the frequency with which each variant occurs.***” See Doc. 1-2 (’035 Patent) at Col. 34:46-50 (emphasis added).

²² See Ex. 5, <https://my.clevelandclinic.org/health/body/23067-somatic--germline-mutations> (last visited April 20, 2024) [hereinafter (“Cleveland Clinic webpage”)] at 2–3.

²³ *Id.* Somatic mutations are also called “acquired” gene mutations. See Ex. 6, <https://www.cancer.org/cancer/understanding-cancer/genes-and-cancer/gene-changes.html> (last visited April 20, 2024) [hereinafter (“American Cancer Society webpage”)] at 4.

accumulate over a person's lifetime, often due to environmental factors or errors in copying the genome during cell division. Unlike germline mutations, however, somatic variants are not present in all of the cells in a person's body. Rather, somatic variants are only present in the original cell in which the mutation first occurred and then copied and transmitted through cell division to their daughter cells. Some somatic variants can lead to the development of diseases such as cancer. Unlike germline variants, somatic variants do not typically occur in reproductive cells (*e.g.*, egg or sperm cells) and therefore are not passed on to future generations.²⁴

25. Both germline and somatic variants have been implicated in cancers.²⁵ Somatic variants may first arise in tumor cells and continue to be propagated as the tumor cells divide. Genomic variants that appear early in tumor development may be present throughout the course of tumor progression and thus can serve as a helpful tool for tracking a patient's cancer. Importantly, acquired gene mutations (*i.e.*, somatic mutations) are a much more common cause of cancer than inherited mutations (*i.e.*, germline mutations).²⁶ In fact, it was well-known by 2010 that genome sequencing of tumor-normal pairs from the same person was used to identify somatic variants in cancer patients.

One of the predominant applications of NGS has been the ***comparison of tumor genomes with their matched constitutional genomes, for the purpose***

²⁴ *Id.*

²⁵ *Id.*

²⁶ *Id.*

of identifying tumor-unique (somatic) variation in an unbiased, genome-wide fashion.²⁷

Therefore, one of ordinary skill in the art would have understood that a genomic variant *associated with cancer* would most likely have been a somatic mutation, but could also have been a germline mutation.

VII. THE '035 PATENT

A. Claims

26. The '035 Patent is titled, *Methods for Simultaneous Amplification of Target Loci*, and issued on December 6, 2022.²⁸ The '035 Patent claims methods for amplifying multiple DNA regions of interest in one reaction volume and sequencing the amplified products.²⁹ The claimed methods comprise, *inter alia*, tagging cell-free DNA with one or more universal tail adaptors, targeted amplification in a single reaction volume of the tagged products, which comprise a plurality of 25-2,000 single nucleotide polymorphism (SNP) loci associated with cancer, and sequencing the plurality of SNP loci by massively parallel sequencing.³⁰

27. Claims 1, 12, and 13 of the '035 Patent are reproduced *infra*.

Claim 1. A method for amplifying and sequencing DNA, comprising:

²⁷ See Ex. 7, Ding *et al.*, *Analysis of next-generation genomic data in cancer: accomplishments and challenges*, HUMAN MOLECULAR GENETICS 19:R188–R196 (2010) [herein (“Ding (2010)”)] at R188–R189 (emphasis added).

²⁸ See Doc. 1-2 ('035 Patent) at cover page.

²⁹ *Id.*, e.g., at Abstract, Cols. 2:59–61, 3:54–8:43, 21:54–24:4, 25:10–42, 27:43–28:31, 59:38–60:34, 76:15–77:63, 83:36–84:3, 84:66–85:29 (Claims 1, 12, 13).

³⁰ *Id.* at Col. 249:43–251:20 (Claims 1, 12, 13).

tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products, wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;

amplifying the tagged products one or more times to generate final amplification products, wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume, wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and

sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products, wherein the plurality of **SNP loci** comprises 25-2,000 loci **associated with cancer**.³¹

Claim 12. The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.³²

Claim 13. The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.³³

28. I understand that the parties dispute the meaning of the term “SNP loci . . . associated with cancer,” which appears in Claim 1 of the ’035 Patent. Specifically, I understand Natera’s position is that this term should be given its plain and ordinary meaning,³⁴ and I explained *supra*,³⁵ this refers to genomic variants at single nucleotide positions associated with cancer, including those that are inherited variants (*i.e.*, germline)

³¹ *Id.* at Col. 249:44–62 (emphases added).

³² *Id.* at Col. 251:7–9.

³³ *Id.* at Col. 251:10–13.

³⁴ *See* Doc. 262 (Natera Op. CC Brief) at 7.

³⁵ *See supra* ¶4.

and acquired variants (*i.e.*, somatic). I understand that NeoGenomics proposes the following construction for this term as “[a] single nucleotide that may differ between the genomes of two members of the same species and is associated with cancer.”³⁶ I understand further that NeoGenomics contends this term is limited to only germline (or inherited) variants and that NeoGenomics contends this term excludes somatic (or acquired) variants.³⁷ As I explain *infra*,³⁸ I disagree with NeoGenomics’ construction.

B. ’035 Patent Specification

29. The ’035 Patent does not provide an express definition for “SNP loci associated with cancer.” However, the ’035 Patent specification does provide a definition for Single Nucleotide Polymorphism (SNP):

Single Nucleotide Polymorphism refers to a single nucleotide that may differ between the genomes of two members of the same species. The usage of the term should not imply any limit on the frequency with which each variant occurs.³⁹

Contrary to NeoGenomics proposed construction, this definition does not place any limitations on the type of variant associated with the term SNP, such as germline or somatic mutations.

30. The ’035 Patent also teaches using the method to amplify and sequence various types of polymorphisms and mutations associated with cancer, without any

³⁶ See Doc. 264 (NeoGenomics Op. CC Br.) at 4.

³⁷ *Id.* at 5.

³⁸ See *infra* Section VIII.A.

³⁹ See Doc. 1-2 (’035 Patent) at Col. 34:46–50.

reference or restriction to inherited or germline variants, or exclusion of acquired or somatic variants. For example, the '035 Patent teaches the following:

In some embodiments, the sample is from an individual suspected of having cancer or an above normal risk for cancer; and wherein ***one or more of the target human loci comprises a polymorphism or other mutation associated with an above normal risk for cancer or associated with cancer.***⁴⁰

In various embodiments, the test sample is from an individual suspected of having a disease or phenotype of interest (such as cancer), or an increased risk (such as an above normal level of risk) for a disease or phenotype of interest; and wherein one or more of the target loci include a sequence (e.g., a polymorphism or other mutation) associated with an increased risk (such as an above normal level of risk) for the disease or phenotype of interest, or associated with the disease or phenotype of interest.⁴¹

31. The written description of the '035 Patent further explains that in some embodiments, multiple mutations may “accumulate” in a subset of a person’s cells (e.g., “somatic”).

In some embodiments, the polymorphism or mutation is ***a driver mutation that has a causative role in the disease or phenotype of interest (such as cancer).*** In some embodiments, the polymorphism or mutation is not a causative mutation. ***For example, in some cancers, multiple mutations accumulate*** but some of them are not causative mutations.⁴²

32. The written description of the '035 Patent also discloses methods of reducing the number of genomic variants detected by ranking the loci associated with cancer that may vary in frequency and/or reoccurrence of selected loci for use in the claimed method:

⁴⁰ *Id.* at Col. 8:38–43 (emphasis added).

⁴¹ *Id.* at Col. 10:57–66 (emphases added); *see also id.* at Col. 20:42–48; 205:50–206:50.

⁴² *Id.* at Col. 20:48–54 (emphasis added). This description is consistent with how one of ordinary skill in the art would have understood genomic variants. *See supra* Section VI.B (Genomic Variants).

In an embodiment, the invention features **a method of decreasing the number of target loci** (such as loci that may contain a polymorphism or mutation associated with a disease or disorder or an increased risk for a disease or disorder such as cancer) that need to be detected for a diagnosis **and/or increasing the disease load that is detected (e.g., increasing the number of polymorphisms or mutations that are detected)**. In some embodiments, the **method includes ranking (such as ranking from highest to lowest) loci by frequency or reoccurrence of a polymorphism or mutation (such as a single nucleotide variation, insertion, or deletion, or any of the other variations described herein) in each locus among subjects with the disease or disorder such as cancer. In some embodiments, PCR primers are designed to some or all of the loci.**⁴³

33. The '035 Patent specification discloses that its methods “would be **particularly advantageous**” in the context of “cancer testing where only one or a small amount of cells were present among a larger amount of normal cells.”⁴⁴ The '035 Patent clarifies that in some embodiments, the “genetic measurements used as part of these methods could be made on **any sample comprising DNA**,” including “blood, **plasma**, . . . **or other cells or materials comprising nucleic acids**.”⁴⁵

34. The '035 Patent discloses exemplary methods for tracking cancer, including obtaining a mixed sample that contains genetic material from normal cells and cancer cells. For example, the '035 Patent teaches the following:

Note that it has been demonstrated that **DNA that originated from cancer that is living in a host can be found in the blood of the host**. In the same way that genetic diagnoses can be made from the measurement of mixed DNA found in maternal blood, genetic diagnoses can equally well be made from the measurement of mixed DNA found in host blood. The genetic diagnoses may include aneuploidy states, or gene mutations. Any claim in

⁴³ *Id.* at Col. 56:9–23 (emphases added).

⁴⁴ *Id.* at Col. 131:51–54 (emphasis added).

⁴⁵ *Id.* at Col. 131:55–61 (emphases added).

the instant disclosure that reads on determining the ploidy state or genetic state of a fetus from the measurements made on maternal blood can equally well read on determining the ploidy state or **genetic state of a cancer from the measurements on host blood**.⁴⁶

In some embodiments, a method of the present disclosure allows one to determine the ploidy status of a cancer, the method including **obtaining a mixed sample that contains genetic material from the host, and genetic material from the cancer; measuring the DNA in the mixed sample; calculating the fraction of DNA that is of cancer origin in the mixed sample**; and determining the ploidy status of the cancer using the measurements made on the mixed sample and the calculated fraction. In some embodiments, the method may further include administering a cancer therapeutic based on the determination of the ploidy state of the cancer. In some embodiments, the method may further include administering a cancer therapeutic based on the determination of the ploidy state of the cancer, wherein the cancer therapeutic is taken from the group comprising a pharmaceutical, a biologic therapeutic, and antibody based therapy and combination thereof.⁴⁷

35. The '035 Patent further discloses that its methods may have broad applications and confirms that the target DNA may originate from cancer patient plasma, tumors, or a mix of healthy and cancer DNA, among other sources:

The methods described herein may be used for a number of purposes where there is a target set of DNA that is mixed with an amount of contaminating DNA. In some embodiments, the target DNA and the contaminating DNA may be from individuals who are genetically related. For example, genetic abnormalities in a fetus (target) may be detected from maternal plasma which contains fetal (target) DNA and also maternal (contaminating) DNA; the abnormalities include whole chromosome abnormalities (e.g. aneuploidy) partial chromosome abnormalities (e.g. deletions, duplications, inversions, and translocations), polynucleotide polymorphisms (e.g. STRs), single nucleotide polymorphisms, and/or other genetic abnormalities or differences. **In some embodiments, the target and contaminating DNA may be from the same individual, but where the target and contaminating DNA are different**

⁴⁶ *Id.* at Col. 204:2–13 (emphases added).

⁴⁷ *Id.* at Col. 204:13–29 (emphasis added).

by one or more mutations, for example in the case of cancer. (see e.g. H. Mamon et al. Preferential Amplification of Apoptotic DNA from Plasma: Potential for Enhancing Detection of Minor DNA Alterations in Circulating DNA. *Clinical Chemistry* 54:9 (2008). In some embodiments, the DNA may be found in cell culture (apoptotic) supernatant. In some embodiments, it is possible to induce apoptosis in biological samples (e.g., blood) for subsequent library preparation, amplification and/or sequencing. A number of enabling workflows and protocols to achieve this end are presented elsewhere in this disclosure.⁴⁸

In some embodiments, the **target DNA may originate from** single cells, from samples of DNA consisting of less than one copy of the target genome, from low amounts of DNA, from DNA from **mixed origin** (e.g. pregnancy plasma: placental and maternal DNA; **cancer patient plasma and tumors: mix between healthy and cancer DNA**, transplantation etc), from other body fluids, from cell cultures, from culture supernatants, from forensic samples of DNA, from ancient samples of DNA (e.g. insects trapped in amber), from other samples of DNA, and combinations thereof.⁴⁹

VIII. OPINIONS RESPONDING TO NEOGENOMICS AND DR. SMITH

A. The Term “SNP loci . . . associated with cancer” (’035 Patent, Claim 1) Should Be Given Its Plain and Ordinary Meaning

36. Regarding Claim 1 of the ’035 Patent, it is my opinion that one of ordinary skill in the art would have understood the term “SNP loci . . . associated with cancer” to mean genomic variants at single nucleotide positions associated with cancer, including those that are inherited variants (*i.e.*, germline) and acquired variants (*i.e.*, somatic).

37. I understand that NeoGenomics construes “SNP loci . . . associated with cancer” as “[a] single nucleotide that may differ between the genomes of two members of

⁴⁸ *Id.* at Col. 92:15–42 (emphasis added).

⁴⁹ *Id.* at Col. 92:43–52 (emphases added); *see also id.* at Col. 95:28–35.

the same species and is associated with cancer.”⁵⁰ I further understand that NeoGenomics contends this term is limited to only germline (or inherited) variants and that NeoGenomics contends this term excludes somatic (or acquired) variants.⁵¹ As I explain *infra*, I disagree with NeoGenomics’ construction.

38. The ’035 Patent defines SNP as “a single nucleotide that ***may differ between the genomes of two members of the same species.*** The usage of the term should ***not imply any limit on the frequency with which each variant occurs.***”⁵² Although the two-sentence definition does not use specific terminology, such as “inherited,” “germline,” “somatic,” or “acquired,” each sentence of this definition imparts a specific meaning and confirms the broad scope of the claimed SNP covers both inherited (or germline) and acquired (or somatic) variants without any limitation of their frequency.

39. The first sentence of the SNP definition uses permissive language, such as “may,” to indicate where a single nucleotide difference may occur without limitation in comparing the genomes of two members of the same species. The definition does not restrict “genome” to that of a healthy cell as opposed to that of a cancer cell. Additionally, as I explained *supra* in Section VI.A (Human Genetics), barring a few narrow exceptions, it is a well-known biological principle that almost every cell in a person’s body carries a full genome. In the context of a person having cancer, this portion of the definition

⁵⁰ See Doc. 264 (NeoGenomics Op. CC Br.) at 4.

⁵¹ *Id.* at 5.

⁵² See Doc. 1-2 (’035 Patent) at 34:46–50 (emphases added).

encompasses comparing the genome present in any tumor cell from that person to the genome derived from a normal cell of a healthy or “reference” individual (*i.e.*, another member of the same species). Far from excluding acquired variants, such as somatic variants as NeoGenomics contends, this portion of the definition would broadly capture both types of mutation—those that are inherited variants (*i.e.*, germline) and those that are acquired variants (*i.e.*, somatic). Moreover, there is no clear or unmistakable disclaimer or disavowal by the patentee in the SNP definition or elsewhere in written description that restricts SNP to only inherited or germline mutations.

40. The second sentence of the SNP definition further confirms its broad interpretation. As I explained *supra* in Section VI.B (Genomic Variants), a variant is conventionally considered a SNP if it was present in at least 1% of the human population. However, the patentee expressly removed any frequency requirement from the definition of SNP, thereby encompassing all types of variants including rare variants that may be present in less than 1% of the human population (*i.e.*, SNVs) within the scope of the term SNP. In other words, the '035 Patent uses the claimed “SNP” as a more general term that includes both relatively common variants (such as traditional SNPs) and rare single-nucleotide differences (such as SNVs).

41. I understand that NeoGenomics restricts its construction of “SNP loci . . . associated with cancer” to only the first sentence of the SNP definition.⁵³ However,

⁵³ See Doc. 264 (NeoGenomics Op. CC Br.) at 4.

NeoGenomics' narrow construction relies on a misinterpretation of this partial definition of "SNP" disclosed in the '035 Patent. As explained *supra*, the first sentence of the SNP definition does not preclude acquired variants (*i.e.*, somatic variants). NeoGenomics' proposal reads into the definition a very narrow embodiment limited to inherited variants (*i.e.*, germline) and ignores the proper frame of reference for "genome," which necessarily permits a broader reading.

42. NeoGenomics also ignores the remaining portion of the SNP definition and other intrinsic disclosure about "loci associated with cancer" that inform the plain and ordinary meaning of this term as a whole. The claim language "loci associated with cancer" modifies "SNP loci," which one of ordinary skill in the art would have understood to encompass acquired variants (*i.e.*, somatic SNVs). In fact, the '035 Patent discloses in numerous instances polymorphisms and mutations associated with cancer without any reference or restriction to inherited or germline variants or exclusion of acquired or somatic variants.⁵⁴ The '035 Patent specification also discloses methods for tracking cancer, including by comparing cancer DNA to normal DNA in the same person without limitation to inherited variants.⁵⁵ As explained *supra*,⁵⁶ one of ordinary skill in the art would have understood that a comparison of tumor-normal paired samples from a cancer patient would

⁵⁴ See *supra* Section VII.B ('035 Patent specification).

⁵⁵ *Id.*

⁵⁶ See *supra* Section VI.B (Genomic Variants).

have resulted in the identification of *exclusively somatic mutations*, as germline variants would have been removed by sequence data analysis.

43. Finally, the disclosure of the '035 Patent supports broadly targeting variants for use in its claimed method. For example, Example 28 discloses targeting 336 SNP target loci on cell-free DNA derived from plasma from breast cancer blood samples.⁵⁷ Additionally, the '035 Patent specification discloses that “in certain exemplary embodiments,” primers target SNVs, particularly “SNVs known to be correlated to at least one type of cancer.”⁵⁸ Example 29 further discloses targeting 336 SNV target loci on cell-free DNA derived from plasma from breast cancer blood samples.⁵⁹

44. I understand that NeoGenomics focuses on one embodiment of SNPs in the maternal-fetal context⁶⁰ and does not address the aforementioned intrinsic disclosures provided in the cancer setting, which more appropriately support a broad plain and ordinary meaning contrary to NeoGenomics' narrow interpretation.

45. The plain and ordinary meaning of “SNP loci . . . associated with cancer,” which broadly encompasses inherited or germline variants and acquired or somatic variants, is also supported by extrinsic evidence. For example, NeoGenomics' expert, Dr. Van Ness, previously opined that SNPs are not limited to inherited variants by expressly

⁵⁷ See Doc. 1-2 ('035 Patent) at Col. 246:60–247:20.

⁵⁸ *Id.* at Col. 75:45–48.

⁵⁹ *Id.* at Col. 248:19.

⁶⁰ See Doc. 264 (NeoGenomics Op. CC Br.) at 5 (citing Doc. 1-2 ('035 Patent) at Col. 143:30–32, 238:20–26).

asserting that an SNP can be a cancer mutation and that ““tumor-derived point mutations” would have been understood to be SNPs” in the context of cancer:

SNPs are single-nucleotide variations in DNA, e.g. a variation in a gene where only one base in the gene is different. ***In the cancer context, SNPs are useful as they can differentiate cancer DNA, which has a mutation,*** from patient DNA, which is referred to as “wild-type” or lacks any cancerous mutation.⁶¹

As early as 2005, SNPs were understood as useful tools in the detection of cancer. The Cantor reference teaches that an approach to analyzing SNPs in fetal DNA could be “applied to other diagnostic applications of plasma DNA, such as ***the detection of tumor-derived point mutations in cancer patients.***” These “tumor-derived point mutations” would have been understood to be SNPs, and thus a POSA would have been aware that the use of targeted amplification and detection of SNPs would be useful in the cancer context.⁶²

I agree with Dr. Van Ness in this regard that the disclosures of SNPs in the '035 Patent to also encompass acquired or somatic variants.

46. Moreover, NeoGenomics’ other identified extrinsic evidence reinforces the plain and ordinary meaning of this claim term and contradicts NeoGenomics’ narrow interpretation. Although NeoGenomics relies on disclosure about a different type of genetic alteration (called restriction fragment length polymorphisms or “RFLPs”),⁶³ Buckingham (2007) elsewhere explains that the term *polymorphism* is sometimes used to

⁶¹ See Doc. 116, *Declaration of Dr. Brian Van Ness in Support of Neogenomics Laboratories, Inc.’s Response in Opposition to Natera, Inc.’s Motion for a Preliminary Injunction* [herein (“Van Ness Decl.”)] at ¶253 (emphasis added).

⁶² *Id.* at ¶255 (original emphasis, citation omitted).

⁶³ See Doc. 264 (NeoGenomics Op. CC Brief) at 5 (citing Buckingham (2007) at 228); see also Doc. 264-1 (excerpt of Buckingham (2007)) at 8.

describe both inherited and acquired DNA sequence changes in the context of single nucleotide alterations:

A transmissible (inheritable) change in the DNA sequence is a mutation or polymorphism. Although ***these terms are sometimes used interchangeably***, they do have slightly different meanings based on population genetics. A ***DNA sequence change that is present in a relatively small proportion of a population is a mutation***. The term, variant, may also be used, particularly to describe inherited sequence alterations, thus ***reserving the term mutation for somatic changes***; for example, changes found only in tumor tissue. A ***change in the DNA sequence that is present in at least 1%–2% of a population is a polymorphism***.⁶⁴

47. Buckingham (2007) references “polymorphism” and “mutation” in a manner consistent with the ’035 Patent’s use of “polymorphism or mutation” associated with cancer in its written description.⁶⁵ As Buckingham (2007) also notes *supra*, the frequency of a polymorphism is traditionally present in at 1-2%, although I understand this conventional understanding is superseded by the patentee’s definition of *SNP* that does “not imply any limit on the frequency with which each variant occurs.”⁶⁶

48. In view of the intrinsic and extrinsic evidence, it is my opinion that one of ordinary skill in the art would have understood the term “SNP loci . . . associated with cancer” to broadly encompass genomic variants at single nucleotide positions associated with cancer, including those that are inherited or germline variants and acquired or somatic variants.

⁶⁴ See Ex. 1 (Buckingham (2007)) at 156 (emphases added).

⁶⁵ Compare *id.* with Section VII.B (’035 Patent Specification) *supra*.

⁶⁶ See Doc. 1-2 (’035 Patent) at Col. 34:46–50

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

49. I understand that NeoGenomics and Dr. Smith contend that the terms “clonal SNV mutations” and “subclonal SNV mutations” “are indefinite,” purportedly “because a skilled person reading the specification would not be able to ascertain the scope of these terms with reasonable certainty.”⁶⁷ I disagree for at least the reasons stated *infra* and in my Opening Claim Construction Declaration.⁶⁸ None of the arguments presented in the NeoGenomics’ Opening Claim Construction Brief nor Dr. Smith’s Opening Claim Construction Declaration change my opinions.

50. I note that Dr. Smith asserts that “[m]utations that are shared by all cells in a tumor are referred to as clonal mutations,”⁶⁹ and “[m]utations that differ from the shared mutations of the clonal population are referred to as subclonal mutations.”⁷⁰ Dr. Smith thus acknowledges that one of ordinary skill have understood the meaning the terms “clonal” and “subclonal.” Dr. Smith’s explanations are consistent with how one of ordinary skill in the art would have understood these terms with reasonable certainty, as described in my Opening Claim Construction Declaration.⁷¹

⁶⁷ See Doc. 264-5 (Smith Op. CC Decl.), ¶ 35; see also Doc. 264 (NeoGenomics Op. CC Brief) at 21 (“The claims and specification of the ’454 patent thus do not provide adequate guidance of the metes and bounds of clonal and subclonal, and the claims are indefinite [*sic*].”).

⁶⁸ See Doc. 263 (Metzker Op. CC Decl.), Section VIII.A.

⁶⁹ See Doc. 264-5 (Smith Op. CC Decl.), ¶ 21.

⁷⁰ *Id.*, ¶ 22.

⁷¹ See Doc. 263 (Metzker Op. CC Decl.), ¶¶ 23–24.

51. Dr. Smith opines that “[a] skilled person reading the ’454 Patent would understand that the difference in claim scope between the terms ‘clonal’ and ‘subclonal’ are inherently vague when applied to real systems such as in the clinic or the laboratory.”⁷² I disagree that there is any “inherent vagueness” in claim scope regarding these terms. Dr. Smith set out clear explanations of what “clonal” and “subclonal” mutations are.⁷³ Those explanations are consistent with how one of ordinary skill would have understood these terms with reasonable certainty. Dr. Smith does not offer the opinion that one of ordinary skill in the art would not have known where the boundary between “clonal” and “subclonal” mutations is as a matter of scientific fact.

52. Instead, Dr. Smith’s sole basis for his opinion is that this “inherent vagueness” only arises “when applied to real systems such as in the clinic or laboratory.”⁷⁴ I have been informed and understand that this type of vagueness is not the standard regarding how one of ordinary skill in the art would have understood the terms “clonal” and “subclonal” with reasonable certainty. Rather than focusing on how one of ordinary skill would have understood the terms as they relate to the scope of the claim limitations, Dr. Smith opines that there may be *practical* difficulty in classifying mutations as clonal or subclonal.⁷⁵ Dr. Smith’s opinion is purportedly based on what he characterizes as

⁷² See Doc. 264-5 (Smith Op. CC Decl.), ¶ 36.

⁷³ *Id.*, ¶¶ 21–22.

⁷⁴ *Id.*

⁷⁵ *Id.*

limited information available to one of ordinary skill in the art performing methods such as those recited in the '454 Patent.⁷⁶ Dr. Smith contends that a different “frame of reference” may lead to different results, referencing what he calls the “illusion of clonality.”⁷⁷

53. As an initial matter, Dr. Smith does not explain what he means by “frame of reference,” nor does Dr. Smith explain how the alleged “illusion of clonality” relates to the claim language in the '454 Patent. The only examples Dr. Smith provides are provided *infra*:

[A] mutation might be classified as “clonal” if it were present in two out of three tumor sites, and only the two sites with the mutation were sampled. The same mutations in the same patient would be classified as “subclonal” if the same patient were examined, but a biopsy happened to include the third tumor site that lacked the mutation. Thus, the analysis of the first two tumor sites is an example of the illusion of clonality, and the “illusion” is confirmed by the third sample. One would run into the same issue if only a portion of a single site were sampled that was not representative of the entire site.⁷⁸

I disagree with Dr. Smith’s example that highlights multiple tumor sites as pertinent to the “illusion of clonality” as the references relied on by Dr. Smith suggest such an illusion is related to single biopsy samples. For example, McGranahan (2015) explains that “***mutations from single biopsies*** may give the illusion of clonality.”⁷⁹

⁷⁶ *Id.*, ¶ 37.

⁷⁷ *Id.*, ¶ 36.

⁷⁸ *Id.*

⁷⁹ See Doc. 264-15, McGranahan *et al.*, *Clonal status of actionable driver events and the timing of mutational processes in cancer evolution*, SCIENCE TRANSLATION MEDICINE 283ra54 pp. 1–11 (2015), appearing in PMC on Nov. 6, 2015 at pp. 1–22 [herein

54. Notably, even if Dr. Smith is correct regarding the “illusion of clonality,” he does not opine that one of ordinary skill in the art would have lacked the knowledge regarding how to infer clonality from the appropriate “frame of reference,” however defined. If this “frame of reference” only includes two sampled areas of a particular biopsy sample because that is what was actually tested, Dr. Smith appears to agree that one of ordinary skill in the art could have inferred the mutation was clonal. This is based on (i) the information that two areas were sampled, (ii) the mutation was detected in both sampled areas, and (iii) the skilled artisan’s understanding of the terms “clonal” and “subclonal.” Dr. Smith does not dispute that one of ordinary skill would have easily understood what is “clonal” and what is “subclonal” based on an evaluation of the presence or absence of the mutation in the sampled areas actually tested. If the “frame of reference” changes *because more information is available* in the form of additional sampled areas tested, then one of ordinary skill in the art would have known how to infer that a mutation is “clonal” and “subclonal” based on that information. Dr. Smith thus does not appear to dispute that the terms “clonal” and “subclonal” are well-defined and understood, and agrees that one of ordinary skill in the art would have been able to apply those terms in evaluating test data to infer if the mutation is “clonal” or “subclonal.”

55. In opining that the terms “clonal” and “subclonal” *themselves* are indefinite, Dr. Smith applies the following standard:

(“McGranahan (2015)”]. I discuss the other references relied on by Dr. Smith, namely Gerlinger (2014) and de Bruin (2014), *infra*.

[T]he only way for a skilled person to know if a mutation is in fact clonal is to sample and analyze the entire tumor. This is not practical, and the amount of sampling necessary to make a call as to whether a mutation is clonal is subjective to the skilled person designing the analysis or the experiment.⁸⁰

I disagree with Dr. Smith for several reasons. First, Dr. Smith implies that the only way one of ordinary skill in the art could have properly classified a mutation as clonal or subclonal is if the skilled artisan *analyzes the entire tumor*, which Dr. Smith admits “is not practical.” I have been informed and understand that Dr. Smith’s requirement of absolute, 100% certainty is not the standard for determining indefiniteness, but whether one of ordinary skill in the art would understand the terms “clonal” and/or “subclonal” within the scope of the claim with reasonable certainty. Dr. Smith’s requirement of absolute, 100% certainty also does not make sense in the context of the ’454 Patent given the practical constraints of sequencing. In other words, one of ordinary skill in the art, reading the ’454 Patent, would not have understood that an entire tumor would have to be sequenced before the skilled artisan could have known whether a mutation were clonal or subclonal.

56. In addition, Dr. Smith’s opinion that because absolute certainty is impractical, classifying a mutation as clonal or subclonal must be nothing more than “a call” based on a “subjective” evaluation of the skilled artisan doing the experiment⁸¹ is misplaced. Notably, Dr. Smith does not opine that the *boundary* between “clonal” and “subclonal” mutations is subjective. Instead, Dr. Smith merely asserts that classifying a

⁸⁰ See Doc. 264-5 (Smith Op. CC Decl.), ¶ 36.

⁸¹ *Id.*

particular mutation as “clonal” or “subclonal” in a particular case depends on the information available. Dr. Smith also does not explain how the “amount of sampling necessary” is “subjective,” rather than the objective result of an analysis of the information available based on the knowledge and experience of one of ordinary skill in the art.

57. Furthermore, Dr. Smith’s discussion of the “illusion of clonality” is undeveloped, as he does not explain this phrase other than “mutations that appear clonal using one frame of reference might also appear to be subclonal when using a different frame of reference.”⁸² Nor does Dr. Smith explain how it applies in the context of the “clonal” and “subclonal” terms of the ’454 Patent.

58. As an initial matter, Dr. Smith’s brief discussion of the “illusion” of clonality acknowledges what one of ordinary skill in the art would have understood about clonal and subclonal mutations: there is a matter of fact as to whether a mutation is clonal or subclonal. It describes a property of actual mutations present in tumor cells. Dr. Smith does not address that the claim language refers to this property—and does not refer to “classifying” or “determining” if a mutation is “clonal” and “subclonal.” Additionally, Dr. Smith does not address the clear distinction between “clonal” and “subclonal” that is understood by one of ordinary skill in the art.

⁸² *Id.*

59. The first paper cited by Dr. Smith⁸³ does not use the phrase “illusion of clonality.” Instead, Gerlinger (2014) refers to the “illusion of clonal dominance.”⁸⁴ One of ordinary skill in the art would have understood that the “illusion of clonal dominance” involves the particular analysis performed in Gerlinger (2014). In particular, Gerlinger (2014) identified “driver mutations” in clear cell renal cell carcinomas (ccRCCs)⁸⁵ and classified them in certain ways based on how they functioned in cancer replication and where such mutations appeared in the phylogenetic tree:

Our data suggest that driver mutations and SCNAs can be classified into (i) predominant truncal drivers, such as chromosome 3p loss and *VHL* mutations, which are likely to be necessary **to initiate tumor growth in the majority of ccRCCs**; (ii) facultative truncal drivers, such as PBRM1 mutation, which in some cases may be acquired early but which can also **occur on branches** (such events were **located on the trunk** in up to 60% of ccRCCs; Fig. 5a); and (iii) subclonal drivers, representing the largest group in our data, which were always **located on branches**, suggesting that they may be important for adaptation during disease progression.⁸⁶

Gerlinger (2014) thus performed a phylogenetic analysis on these various driver mutations, mapping them onto whether they were on the “trunk” of the phylogenetic tree or later-developed branches.⁸⁷

⁸³ See Doc. 264-13, Gerlinger *et al.*, *Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing*, NATURE GENETICS 46:225–233 (2014), appearing in PMC on Nov. 6, 2015 at pp. 1–24 [herein (“Gerlinger (2014)”)].

⁸⁴ *Id.* at 9, 10.

⁸⁵ *Id.* at 2.

⁸⁶ *Id.* at 7 (emphases added).

⁸⁷ *Id.*

60. The entire analysis provided in Gerlinger (2014) demonstrates that the terms “clonal” and “subclonal” have definite meaning to those of skill in the art consistent with the opinions I gave in my Opening CC Declaration.⁸⁸ Gerlinger (2014) applied those terms in their analysis without vagueness or ambiguity in the same way that one of ordinary skill in the art would have when reading the ’454 Patent. The phrase “illusion of clonal dominance” could only be described by applying the well-known, objective meanings of the terms “clonal” and “subclonal” to the data analyzed by Gerlinger (2014). The introduction of data from more than one biopsy showing that mutations which would have been “clonally dominant” were “heterogeneous between tumor regions”⁸⁹ does not introduce any ambiguity or vagueness into how one of ordinary skill in the art would have understood the terms “clonal” and “subclonal.”

61. The second paper cited by Dr. Smith⁹⁰ does use the phrase “illusion of clonality,”⁹¹ but applies the same understanding of “clonal” and “subclonal” throughout the analysis of non-small cell lung cancer (NSCLC). For example, de Bruin (2014) explains the following:

To resolve the extent of genomic diversity in NSCLC and to infer the ancestral relations between tumor regions, we estimated the fraction of tumor cells within each region harboring each mutation. ***Almost all ubiquitous***

⁸⁸ See Doc. 263 (Metzker Op. CC Decl.), Section VIII.A.

⁸⁹ *Id.* at 9.

⁹⁰ See Doc. 265-14, de Bruin et al., *Spatial and temporal diversity in genomic instability processes defines lung cancer evolution*, SCIENCE 346:251–256 (2014) appearing in PMC on Nov. 6, 2015 at pp. 1–12 [herein (“de Bruin (2014)”)].

⁹¹ *Id.* at 6.

mutations (>99%) were classified as fully clonal within each region. Moreover, in most regions, *the majority of heterogeneous mutations was clonal and, thus, present in all cells within the region* (Fig. 1B and fig. S3). *However, certain regions displayed considerable subclonal diversity.* For example, *>75% of heterogeneous mutations present in L004 R5 were subclonal* (Fig. 1B), and this region consisted of two distinct subclonal populations.⁹²

62. In de Bruin (2014), the authors use the term “ubiquitous” in the same way that the ’454 Patent describes “clonal” mutations, and they use the term “heterogeneous” in the same way the ’454 Patent describes “subclonal” mutations: “[W]e classified each mutation as ubiquitous (*present in all tumor regions*) or heterogeneous (*present in at least one, but not all, regions*).”⁹³

63. de Bruin (2014) thus supports my opinion that one of ordinary skill in the art would have understood the scope of the terms “clonal” and “subclonal” with reasonable certainty. de Bruin (2014) itself applies these terms consistently and without ambiguity or vagueness.

64. Dr. Smith’s opinions that the ’454 Patent “does not provide any guidance as to how to choose the frame of reference to determine whether a mutation is clonal or subclonal within the meaning of the claims” and that it “does not describe how many samples should be taken, where those samples should be taken from, or what proportion of those samples must share the mutation for it to be deemed clonal”⁹⁴ are wrong and apply

⁹² *Id.* at 3 (emphases added).

⁹³ *Id.* at 2 (emphases added).

⁹⁴ See Doc. 264-5 (Smith Op. CC Decl.), ¶ 37.

the wrong standard, as I understand from counsel. First, one of ordinary skill in the art would have understood how to select the number of sampled areas based on the circumstances. Dr. Smith does not offer any opinion to the contrary, but instead demands that the specification provide this “guidance,” which I understand from counsel is not consistent with an indefinite analysis.

65. Second, the patent *does* provide an exemplary description of how to distinguish between clonal and subclonal mutations: “[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.”⁹⁵ One of ordinary skill in the art would have understood how to apply this teaching to the circumstances in each particular case, where, *e.g.*, the number of sampled areas may be limited. Dr. Smith demands that this “guidance” be taught in the ’454 Patent,⁹⁶ but offers no opinion that it is outside the scope of skilled artisan’s knowledge and experience to resolve these questions in particular circumstances.

66. Dr. Smith’s discussion of “contamination” is puzzling. One of ordinary skill in the art would have understood that all tumor samples will contain a mix of cells and can take that into account in an analysis. Moreover, as described *supra*, the ’454 Patent describes an exemplary method for inferring clonality based on the presence or absence of

⁹⁵ See Doc. 1-1 (’454 Patent) at Col. 168:23–26.

⁹⁶ See Doc. 264-5 (Smith Op. CC Decl.), ¶¶ 38–40.

the mutation in a sampled area.⁹⁷ The percentage of cells in the sampled area that include the mutation is not relevant to that analysis.

67. One of ordinary skill in the art would have known of various ways to track intratumoral heterogeneity by 2015, including by measuring the fractional concentration of tumor-derived circulating tumor DNA in the plasma by targeting tumor-associated SNVs.⁹⁸ The existence of different ways of classifying a particular mutation as “clonal” or “subclonal” depending on the available data that Dr. Smith describes does not in any way make the terms “clonal” or “subclonal” vague, ambiguous, or subjective.⁹⁹ These different methodologies exist because the property being measured is complex and cannot be directly observed.

68. In sum, Dr. Smith does not offer any evidence that “clonal” and “subclonal” are indefinite in the context of the ’454 Patent. Dr. Smith has no difficulty understanding the differences in scope between these terms throughout his Opening Claim Construction Declaration. The only dispute Dr. Smith raises concerns determining *how* a mutation is classified as “clonal” or “subclonal.” But this step is not required by the plain language of the claims.

⁹⁷ See Doc. 1-1 (’454 Patent) at Col. 168:20–28.

⁹⁸ See Ex. 8, Chan et. al., *Cancer genome scanning in plasma: detection of tumor-associated copy number aberrations, single-nucleotide variants, and tumoral heterogeneity by massively parallel sequencing*, CLINICAL CHEMISTRY 59:1: 211–224 (2013) [herein (“Chan (2013)”) at 220–221 (Section on Investigation of Tumoral Heterogeneity), Table 3; see also Fig. 3.

⁹⁹ See Doc. 264-5 (Smith Op. CC Decl.), ¶ 37.

C. 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

69. I understand that NeoGenomics and Dr. Smith contend that 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 indefinite.¹⁰⁰ I disagree for at least the reasons stated *infra* and in my Opening Claim Construction Declaration.¹⁰¹ None of the arguments presented in NeoGenomics’ Opening Claim Construction Brief nor Dr. Smith’s Opening Claim Construction Declaration change my opinions.

70. In my opinion, NeoGenomics and Dr. Smith conflate the claim language (“capable of detecting”) with a different property (the limit of detection or LOD). One of ordinary skill in the art would have understood that the calculation of LOD is separate from what is within the range of what a method is capable of detecting. Claims 14 and 28 of the ’454 Patent recite a method that is capable of detecting an SNV present in the sample at the recited percentages, regardless of what the calculated LOD is.

¹⁰⁰ *Id.*, ¶¶ 42, 53; *see also* Doc. 264 (NeoGenomics Op. CC Br.) at 22.

¹⁰¹ *See* Doc. 263 (Metzker Op. CC Decl.), Section VIII.B

71. Indeed, Dr. Smith's cited reference¹⁰² includes an alternative to a limit of detection based on an "empirical approach":

An alternative approach utilises analysis of samples containing small but known concentrations of the substance of interest (be it a drug, hormone or other analyte). The advantage of **this empirical approach** is that objective data is used to compare the analytical response of blank and low concentration samples to determine conclusively what concentration of analyte is necessary to distinguish its presence from its absence.¹⁰³

This approach is similar to the use of a contrived sample to determine a method's capability to detect an SNV mutation that is present at a certain percentage.¹⁰⁴ Dr. Smith fails to explain how his opinions on the calculation of LOD are relevant to the claim language.

72. Dr. Smith's discussion of the LOD is similarly off-the-mark. First, Dr. Smith opines that "the '454 Patent and prosecution history do not provide sufficient guidance regarding whether the mean, median, or some other statistic should be used" when discussing LOD.¹⁰⁵ The mean or median LOD does not reflect the method's capability of detecting any particular SNV mutation. By definition, the mean or median reflects the total data set, which in this case NeoGenomics admits includes many different SNV mutations.¹⁰⁶ In sum, the calculated mean or median values do not reflect the method's

¹⁰² See Doc. 264-10, Armbruster & Pry, *Limit of blank, limit of detection and limit of quantitation*, CLINICAL BIOCHEMIST REVIEW 29:S49–S52 (2008) [herein ("Armbruster (2008)"]].

¹⁰³ *Id.* at S50 (emphasis added, citations omitted).

¹⁰⁴ See Doc. 263 (Metzker Op. CC Decl.), ¶ 88.

¹⁰⁵ See Doc. 264-5 (Smith Op. CC Decl.), ¶ 51.

¹⁰⁶ *Id.*, ¶ 49 ("[T]he x-axis appears to represent different SNVs assayed.")

capability of detecting a *particular* SNV mutation that is present in less than or equal to 0.015% [or in 0.005% to 0.015%] of the cfDNA.

73. Finally, even assuming that LOD were relevant to the claim language, which it is not, one of ordinary skill would have understood that any inconsistency between the two methods to calculate LOD taught in the '454 Patent should be resolved by taking the higher of the two values.¹⁰⁷ The '454 Patent discloses the third method as a preferred embodiment compared to the other two methods.¹⁰⁸ While Dr. Smith opines that this is not expressly taught in the '454 Patent, he offers no opinion disputing that is how one of ordinary skill in the art would have understood the “third” method.

¹⁰⁷ See Doc. 263 (Metzker Op. CC Decl.), ¶ 92.

¹⁰⁸ See Doc. 1-1 ('454 Patent) at Col. 93:35–37.

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 22, 2024

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

Michael Metzker, Ph.D.