

Vol. III of IV (Appx12136-19916)
Nos. 24-1324, 24-1409

UNITED STATES COURT OF APPEALS
FOR THE FEDERAL CIRCUIT

NATERA, INC.,

Plaintiff-Appellee,

v.

NEOGENOMICS LABORATORIES, INC.,

Defendant-Appellant.

Appeals from the United States District Court for the Middle District of North
Carolina, No. 1:23-cv-00629; Hon. Catherine C. Eagles

NON-CONFIDENTIAL JOINT APPENDIX

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

NATERA, INC.,

Plaintiff,

v.

NEOGENOMICS LABORATORIES, INC.,

Defendant.

C.A. No. 1:23-cv-629

**CONTAINS HIGHLY CONFIDENTIAL
INFORMATION**

REPLY DECLARATION OF DR. MICHAEL METZKER

again after the early 2000s. Nor has Dr. Van Ness published any work on tumor-specific genetic variants using cfDNA.

Q. You never worked on – you, yourself, in your lab never worked on cell-free DNA after the early 2000s, correct?

A. We never – after the early 2000s, we did not work on cell-free DNA, although all the methods of DNA extraction, multiple, and sequencing were routine in my lab. But we did not work on specifically cell-free DNA after the early 2000s.⁸

Q. Okay. I'll ask the question again. You and your lab have never published any work describing experiments using cell-free DNA, correct?

A. I have not. Only blood DNA we've worked on, but I have not looked at cell-free. We did not publish on cell-free.⁹

Q. You never published any work on tumor-specific SNPs or single nucleotide variants in cell-free DNA, correct?

A. We did publish on SNPs in tumor – unique to tumors. It wasn't from the cell-free DNA.¹⁰

A. Cell-Free DNA and Plasma Samples

13. Prior to 2014-2015, and even earlier, there were many obstacles to the assaying and analysis of cfDNA. These challenges were, and still are, greater than when working with DNA from tissue samples, such as samples of tumors. I discuss some of these challenges in this section. Dr. Van Ness agrees, as he testified in his deposition.

⁸ See Van Ness Tr. at 246:21-247:4 (objection omitted).

⁹ *Id.* at 247:15-22 (objection omitted).

¹⁰ *Id.* at 249:11-15.

Q. You and your lab were not consistently able to detect cell-free DNA with good sensitivity, correct?

A. In the early 2000s, we were all having some problems in the consistency of detecting cell-free DNA.

Q. And in fact –

A. Detecting the target of cell-free DNA we were looking for.¹¹

14. For example, Volik (2016)¹² provides a helpful summary of certain problems and difficulties facing scientists working with cfDNA. These challenges are also acknowledged in certain prior art references cited in the Van Ness Declaration, such as Forshew (2012).¹³ I discuss them *infra*.

15. As a general matter, cfDNA is difficult to biochemically process and analyze because it is relatively low-quality compared to DNA from other sources, such as tissue samples. In his deposition, Dr. Van Ness agreed that working with cfDNA posed technical challenges.

Q. Okay. Do you – you see here that Dr. Forshew, in that first sentence, says “Circulating DNA is fragmented to an average length of 140 to 170 base pairs and is present in only a few thousand amplifiable copies per milliliter of blood, of which only a fraction may be diagnostically

¹¹ *Id.* at 248:10-20 (objection omitted).

¹² See Ex. 2, Volik *et al.*, *Cell-free DNA (cfDNA): Clinical Significance and Utility in Cancer Shaped By Emerging Technologies*, 14 *Mol. Cancer Res.* 898 (2016) (“Volik (2016)”).

¹³ See Dkt. 1-16, NAT-NEO-00043262-00043275, Forshew *et al.*, *Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA*, *SCIENCE TRANSLATIONAL MEDICINE* 4:136ra68, pp. 1-12 (2012) (“Forshew (2012)”).

relevant”? Do you disagree with Dr. Forsheew’s statement?

A. I don’t.

Q. In the second sentence, a few lines down, it says, “But also the challenges involved in analysis of circulating tumor DNA, where mutated loci and AFs may be more variable.” Do you disagree with him that there are challenges involved in analysis of circulating tumor DNA because of those variable mutated loci and AFs?

A. He’s referring to past challenges that involve the analysis of circulating tumor DNA. *I agree with that.*¹⁴

16. Here, I provide an example of one such problem. Consider a cfDNA molecule that contains a desired single nucleotide polymorphism (“SNP”), which is a genomic variant at a single base position in DNA. If the cfDNA is fragmented such that the cfDNA fragment does not contain sites for both primers of a primer pair to bind that primer pair will not be able to amplify that particular SNP successfully when PCR is performed.

17. As of 2014-2015, scientists were beginning to explore how to use cancer-associated cfDNA.

Known to be present in the blood of cancer patients for decades, cell-free DNA (cfDNA) is beginning to inform on tumor genetics, tumor burden, and mechanisms of progression and drug resistance.¹⁵

¹⁴ See Van Ness Tr. at 116:19-117:21 (emphasis added).

¹⁵ See Volik (2016) at Abstract.

18. Indeed, the art recognized that blood plasma contained a highly variable content of cfDNA. In the case of cancer patients, for example, the level of tumor-associated cfDNA (known as “circulating tumor DNA” or “ctDNA”) would vary, including relative to the normal cfDNA that represents the majority of cfDNA found in plasma.

The amount of cfDNA in cancer patients varies widely.¹⁶

Not unexpectedly, in addition to varying absolute levels of cfDNA, the fraction of DNA molecules in the circulation of cancer patients that can be recognized as being derived from tumor cells also varies.¹⁷

19. The art recognized that the fragmented nature of cfDNA made it difficult to manipulate through existing techniques such as PCR-based amplification and sequencing. Dr. Van Ness agrees that there are challenges in amplifying cfDNA, as he testified in his deposition.

Q. Is it your opinion that there are particular challenges in amplifying cell-free or plasma DNA that one doesn't encounter in amplifying genomic DNA from tissue?

A. ***There are some challenges.*** And there are some challenges in whole genomic DNA amplification as well. Amplification was well known in the art, and, yes, there are challenges.¹⁸

Dr. Van Ness further acknowledged that “[t]here are challenges to amplifying genomic DNA. ***There are challenges to amplifying cell-free DNA.*** And are they the same? No,

¹⁶ *Id.* at 890.

¹⁷ *Id.*

¹⁸ *See* Van Ness Tr. at 109:25-110:7 (emphasis added).

the size of the fragments are different. The number of cycles you might want to do with cell-free DNA may be different than if you have a lot of genomic DNA from the tumor.”¹⁹

20. In addition, tumor genomes are genetically unstable and prone to mutate. A frequent consequence of cancer-associated genetic instability is that different populations of cells within the same tumor from a single patient can have different genetic mutations. When these different tumor cell populations release ctDNA into the plasma, one population’s ctDNA can have different sequences from other populations’ ctDNA. These different mutations among different cancer cells in a tumor are known as “subclonal” mutations. Biochemically processing and analyzing ctDNA sequences is therefore typically more challenging than simply collecting and processing DNA directly from healthy tissues and tumor tissues for study.

The fragmentation, low yield, and variable admixture of normal DNA present formidable technical challenges for realization of this potential.²⁰

[The] low amount, high degradation, and high admixture of normal DNA in cfDNA pose major challenges for the development of sensitive and robust detection pipelines. The fact that most (if not all) tumors are characterized by multiple subclonal populations with only a subset of somatic mutations shared among all cells (for review, see ref. 52) further complicates the issue.²¹

¹⁹ *Id.* at 113:4-10 (emphasis added).

²⁰ *See* Volik (2016) at Abstract.

²¹ *Id.* at 901.

21. Moreover, ctDNA is typically low-abundance in the plasma of a person with cancer relative to the levels of other cfDNA in the plasma.²² Specifically, the plasma is likely to contain far more cfDNA from non-cancer cells than ctDNA from tumor cells, which makes preparing preparations of the ctDNA more difficult (because it must be distinguished in some way from the non-tumor cfDNA).

Circulating tumor DNA (ctDNA) is typically so diluted by normal DNA that existing sequencing methods (e.g., Sanger sequencing) were not sufficiently sensitive to detect mutant DNA molecules. As a result, mutation-specific PCR was the only available technology that could provide sufficient specificity for detection of the weak tumor signal.²³

However, *tumor-derived DNA usually constitutes only a small percentage of total cfDNA* so the ability to detect rare genome aberrations is an essential requirement for cfDNA analysis pipelines.²⁴

22. In deposition, Dr. Van Ness acknowledged particular and “unique” challenges associated with multiplex amplification of cfDNA in the context of discussing amplification of different types of cfDNA (e.g., cfDNA from patients who received an organ transplant, or pregnant women, or subjects with cancer).

Q. I was asking about multiplex amplification, the actual multiplex PCR reaction. That – do you – is there something you have to do differently in performing multiplex PCR to amplify transplant cfDNA versus cancer-free [*sic*] DNA?

²² See Van Ness Tr. at 111:8-113:13.

²³ See Volik (2016) at 898 (emphasis added).

²⁴ *Id.* at 906(emphasis added).

- A. There may be differences because the multiplexing does involve sample preparation. It does involve understanding the limits of detection. How many cycles of multiplex PCR might be needed? *So, yes, there are differences.*
- Q. Would you say those same differences would apply to performing multiplex PCR to amplify fetal cell-free DNA versus cancer cell-free DNA?
- A. *I think every application has its unique challenges* in terms of sample access, preparation that influence how the multiplex is being done.
- Q. So the answer's yes?
- A. *I think all of those applications, yes,* can have differences in protocols that might be required for successful multiplex amplification.
- Q. And by "applications," you mean cell-free DNA from a fetus versus cell-free DNA from an organ transplant versus cell-free DNA from a tumor?
- A. Recognize that the detection of cell-free DNA from each of those sources also requires access to those sources so that you know what you're looking for. So it's not sufficient simply to isolate cell-free DNA. You have to know the sequences of your target in order to be – to analyze that. And those target sequences are going to be present in different quantities in each of those applications.
- Q. I was just asking what you meant by the word "applications." You say, "I think all of those applications, yes, can have differences in protocols that might be required for successful multiplex amplifications." I'm just asking what you meant by "applications," that word.
- A. Okay.

Q. Are you referring to cell-free DNA of different sources?

A. *I'm referring to cell-free DNA as it is applied to different situations, of fetal DNA, cancer DNA, organ transplant DNA.*²⁵

III. SUMMARY OF OPINIONS

A. Claim Construction

23. It is my opinion that one of ordinary skill in the art would have understood each of the terms of the Asserted Claims of the Natera Patents to have their plain and ordinary meaning. In particular, I construe the terms “tagging isolated cell free DNA” (Claim 1 of the '035 Patent) and “amplifying the tagged products one or more times” (Claim 1 of the '035 Patent) to each have a plain and ordinary meaning, which are summarized *infra*.

24. It is my opinion that one of ordinary skill in the art would have understood that as of the priority date of the '035 Patent, and in light of the Specification, the plain meaning of “tagging isolated cell free DNA” encompasses adding one or more adaptor tags to isolated cell free DNA through primer extension or ligation. Dr. Van Ness agreed with this understanding during his deposition.²⁶

25. It is my opinion that one of ordinary skill in the art would have understood that as of the priority date of the '035 Patent, and in light of its specification, the plain

²⁵ See Van Ness Tr. at 64:4-66:6 (objections, court reporter clarification, and colloquy omitted, emphases added).

²⁶ *Id.* at 228:17-232:25.

meaning of “amplifying the tagged products one or more times” encompasses creating at least one copy of the tagged products. Dr. Van Ness agreed with this understanding during his deposition.²⁷ One of ordinary skill in the art would have readily recognized this occurs following at least one cycle of PCR.²⁸

B. Validity

26. As a preliminary matter, I note that Dr. Van Ness does not contend that any prior art reference discloses every limitation of the Natera Patent claims—*i.e.*, a “novelty” or “anticipation” challenge under Section 102 of the Patent Act.²⁹ Instead, Dr. Van Ness contends that several references, if modified and combined, render the claims obvious.

27. I disagree with Dr. Van Ness’s contention that the Natera Patents are invalid as obvious under Section 103.³⁰ As explained *infra*, Dr. Van Ness fails to account for technical limitations in prior art approaches and the many challenges inherent in manipulating cfDNA faced by skilled artisans.

28. Dr. Van Ness’s obviousness opinions are wholly conclusory. The Van Ness Declaration points to disparate teachings in the art or a skilled artisan’s knowledge that Dr. Van Ness believes remediate these deficiencies.

²⁷ *Id.*

²⁸ *Id.*

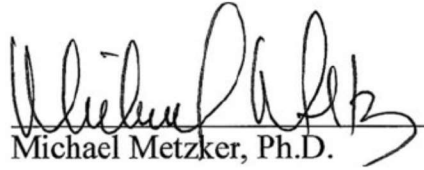
²⁹ *Id.* at 97:11-98:13; *see generally* Van Ness Decl.

³⁰ *See* Van Ness Decl. at ¶¶ 109-220 for the ’454 Patent and ¶¶ 221-303 for the ’035 Patent.

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: November 6, 2023


Michael Metzker, Ph.D.