Patent Owner's Oral Argument Presentation

Foresight Diagnostics Inc. v. Personalis, Inc.,
IPR2023-00545, IPR2023-00546

May 7, 2024

Personalization Previously Deemed "Uncertain" And "Unattractive"

The performance of synthesized nucleic acid sequences in a personalized assay may vary depending on many conditions of the nucleic acid sequence (e.g., % GC, alignment degeneracy, primer-dimer formation) and the parameters of the assay. This assay performance uncertainty may make personal assay synthesis unattractive. However, a large set

EX1001 ('783 Patent) at 39:42-47

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE
('783 POR at 6; '611 POR at 6)

Use Of Arrays Deemed Inadequate For Personalization

While array-based DNA synthesis is now widely used to capture whole exomes, transcriptomes, or application-specific subsets of exomes (e.g., the genes involved with a specific Mendelian disease), a limitation of the field, as recognized herein, is the potential to leverage array synthesis of DNA in a personalized manner. The field has largely used

of DNA in a personalized manner. The field has largely used array-based synthesis to develop standard products which are broadly applicable across a whole set of human patients and/or research subjects. Even where custom array synthesis

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE
('783 POR at 6; '611 POR at 6)

EX1001 ('783 Patent) at 4:37-42

Exome Footprint Is 35 Million Bases

footprint of this assay may be about 175,000 bases. Compared to an exome, where the footprint of the assay is typically at least 35 million bases, this may result in 200× less sequencing. This dramatic reduction in the amount of

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE
('783 POR at 8; '611 POR at 6)

EX1001 ('783 Patent) at 63:17-20

Use Of Exome Sequencing Deemed "Limited" By Costs

In spite of the advances described above, the clinical adoption of exome-scale sequencing has been limited by the costs involved. Health insurers, who are asked to pay for these tests, often refuse, given the scale of the expense. This problem is even worse in cancer, where the depth of deoxynucleic acid (DNA) sequencing required can be much higher (e.g., >500×) than that for inherited diseases (e.g., 30-100×).

EX1001 ('783 Patent) at 4:30-36

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE
('783 POR at 6; '611 POR at 5)

Personalis Inventions Reduce Sequencing Footprint

testing in part for this guidance. To increase the confidence of detecting a potentially mosaic variant in the parents, they will need to be sequenced at greater depth than the child. If the assay for additional members of a family pedigree is exome sequencing, it may be cost prohibitive to sequence at that depth. Using method provided herein, the footprint of the assay for incremental family members is much smaller (e.g., 175,000 bases vs 35 million bases, as discussed above). This smaller footprint makes it affordable to sequence the incremental samples at much greater depth, thus improving the sensitivity for detection of mosaic variants in the parents.

EX1001 ('783 Patent) at 64:41-52

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 8; '611 POR at 8)

Personalis Inventions Reduce Sequencing Demands

less sequencing. This dramatic reduction in the amount of sequencing required, per additional family pedigree member, can make it much more affordable to sequence additional pedigree members (e.g., the parents, other children of the same parents, etc).

EX1001 ('783 Patent) at 63:20-24

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 8; '611 POR at 8)

Personalis Assay Named #1 Innovation of 2022

NeXT Personal™ Personalis



THE SCIENTIST STAFF

Small quantities of cancer cells can linger in the body after tumor removal, a phenomenon known as minimal or molecular residual disease (MRD) that, if left undetected, can lead to recurrence. Personalis's NeXT Personal™ assay, unveiled in December 2021, uses a patient's own tumors to detect, quantify, and monitor circulating tumor DNA in order to spot MRD and track responses to therapy.

Using 1 mm^3 of tumor tissue sampled from a patient, Personalis's lab performs whole genome sequencing to identify up to 1,800 single-nucleotide variants. These patient-specific mutations are used to design a panel of primers targeting those regions for sequencing in future blood biopsies. The assay also includes primers for other known cancer-related genes, says Dan Norton, associate director for product management at Personalis. "We can see if there are other variants emerging that have a precision therapy associated that may be more effective for that patient."

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE
('783 POR at 63, '611 POR at 63)

EX2001 (2022 Top 10 Innovations)

The ’783 Patent Is Neither Anticipated Nor Obvious

IPR2023-00545

Ley Does Not Anticipate Or Render Obvious

Ley Fails To Disclose Or Render Obvious Multiple '783 Patent Claim Elements

- No "fixed" and "variable" portions
- No "additional biological sample"
- No "identifying" of variants
- No "generating a report"

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

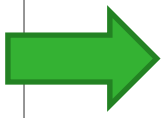
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DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Ley Does Not Disclose Or Render Obvious Fixed And Variable Portions

1. A method for personalized genetic testing, comprising:
(a) obtaining a personalized probe set for a subject, which personalized probe set comprises a plurality of nucleic acid probe molecules having a plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising a set of one or more genetic variants over other sequences in at least one biological sample of said subject, wherein said set of genetic variants are identified in sequence data for said at least one biological sample, further wherein said personalized probe set comprises nucleic acid probe molecules having oligonucleotide-directed genomic content comprising: (i) at least one variable portion from a result of said sequence data and (ii) at least one fixed portion independent of said result of said sequencing data
(b) using said personalized probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in at least an additional biological sample from said subject, to generate a sequencing library;
(c) subjecting said sequencing library to sequencing to identify a presence or absence of at least a subset of said set of genetic variants in at least said additional biological sample from said subject, wherein a sequencing footprint of said sequencing of (c) is less than a sequencing footprint of said sequence data of (a); and
(d) generating a report identifying (i) a presence or absence of a health condition or disease of the subject in said additional biological sample based on said identified at least a subset of (c) and (ii) genetic variants which inform a therapy choice or a change in therapy for the subject.



nucleotide-directed genomic content comprising: (i) at least one variable portion from a result of said sequence data and (ii) at least one fixed portion independent of said result of said sequencing data

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 4-5)

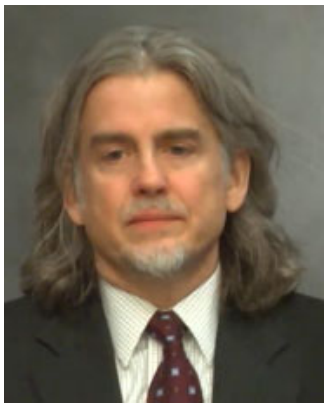
EX1001 ('783 Patent) at claim 1

Proper Construction Of "Fixed" And "Variable" Portions

Claim Term	Patent Owner's Constructions	Petitioner's Constructions
"fixed portion"	A portion "which changes from one subject to another and results from said sequence data"	Plain meaning
"variable portion"	A portion "which does not change from one subject to another and is independent of said result of said sequencing data"	Plain meaning

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Dr. Quackenbush: Specification Is "Best Place" For Guidance On Claim Meaning



John Quackenbush
Foresight's Expert

A. So, the best place I can turn to understand what that, patent owner means is to look at what is disclosed in the written description of the patent.

EX2163 (10/20/23 Quackenbush Depo) at 94:20-95:1

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 11, '611 POR at 20)

’783 Patent: Defines “Fixed” & “Variable” Portion

for the individual subject. In some applications, it may be desirable for the oligo-directed genomic content of the personalized assay to contain both a variable portion (defined based on the genetic characteristics originally determined for the individual subject) and at least one fixed portion (which does not change from one subject to another).

EX1001 (’783 Patent) at 41:45-50

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE (’783 POR at 12)

’783 Patent: Defines “Fixed” & “Variable” Portion

considered fixed content. Thus, as discuss above, the genomic content of a personalized genetic assay may include a portion which is variable subject to subject, and another portion which is fixed.

EX1001 (’783 Patent) at 66:36-39

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE (’783 POR at 12)

’783 Patent: Defines “Fixed” & “Variable” Portion

Combinations of Fixed and Variable (Personal) Genomic Content in the Array-Synthesized Nucleic Acids

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE (’783 POR at 12)

EX1001 (’783 Patent) at 41:40-41

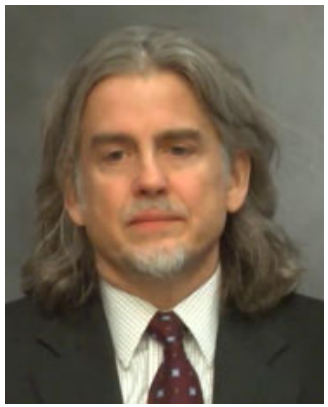
Law: "High Standard" For Inherency

"A party must, therefore, meet a high standard in order to rely on inherency to establish the existence of a claim limitation in the prior art in an obviousness analysis—the limitation at issue necessarily must be present, or the natural result of the combination of elements explicitly disclosed by the prior art."

PAR Pharm., Inc. v. TWI Pharms., Inc., 773 F.3d 1186, 1195–96 (Fed. Cir. 2014).

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Dr. Quackenbush: Ley Does Not Disclose Primer Sequences



John Quackenbush
Foresight's Expert

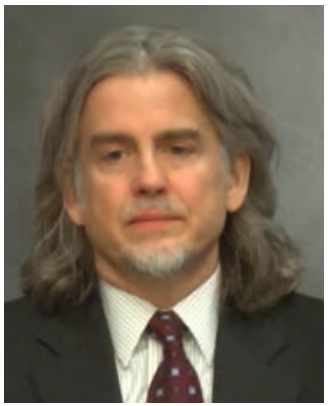
Dr. Quackenbush's Declaration

genome rather than the genome in its entirety. While Ley does not provide the primer sequences or the exact exons that were analyzed, one can deduce an upper bound on the sequence coverage Ley teaches by using the entire length of the genes that contained the genetic variant rather than the much smaller sequence of the variant-containing exon. To illustrate, I looked up the messenger RNA sequences

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 14)

EX1020 (Quackenbush Decl) ¶ 124

Dr. Quackenbush: Ley Does Not Disclose Primer Sequences



John Quackenbush
Foresight's Expert

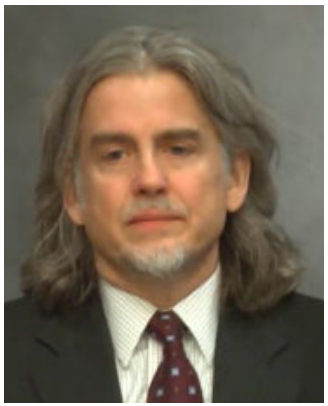
Q. So, because Ley didn't provide the primer sequences, we don't know the exact primers that Ley used for her experiment. Right?

A. I believe it is Timothy Ley, so I think it is his experiment. And yes, we don't know the exact primers that Ley used.

EX2163 (10/20/23 Quackenbush Depo) at 232:17-22

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE
('783 POR at 14, '611 POR at 22)

Dr. Quackenbush: Ley Does Not Disclose Primer Design



John Quackenbush
Foresight's Expert

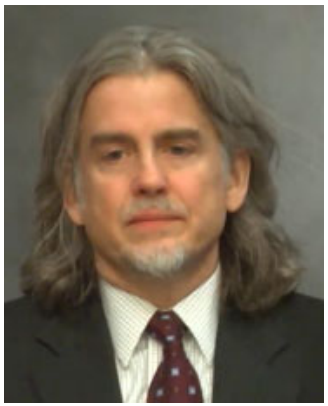
Q. And you don't know the annealing temperatures that Ley used for the PCR primers; is that right?

A. In this particular reference, Ley does not disclose the PCR protocol that was used, at least I can't find it. There may be additional material, but I don't have that available. So, to the best of my knowledge, Ley doesn't disclose the parameters used for the PCR primer design.

EX2163 (10/20/23 Quackenbush Depo) at 250:4-14

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE
('783 POR at 14, '611 POR at 22)

Dr. Quackenbush: Ley Does Not Disclose Primer Design



John Quackenbush
Foresight's Expert

Q. So, do you know if Ley used PCR primers that hybridize 80 bases upstream to the mutations?

A. So, again, Ley doesn't describe here the primer design methodology that was used. But amplicons containing exons with relevant putative mutations were generated and sequenced.

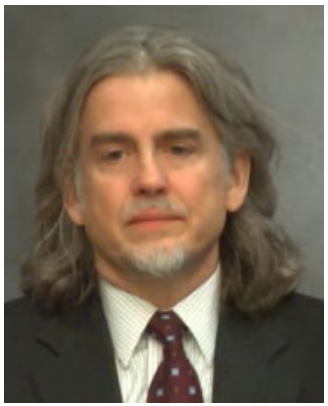
Q. Do you know what length of PCR primer Ley used, like was it 20 more, 50 more?

A. Again, that is not disclosed. I could make guesses, but they would only be guesses. I am assuming the PCR primers varied in length depending on the calculated melting temperature.

EX2163 (10/20/23 Quackenbush Depo) at 248:11-249:3

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE
('783 POR at 14, '611 POR at 22)

Dr. Quackenbush: Ley Does Not Disclose PCR Protocol



John Quackenbush
Foresight's Expert

Q. Do you know if Ley used any type of nested PCR in order to generate the amplicons?

A. Again, Ley doesn't disclose the PCR protocol that was used. So, sitting here today, I can't tell you what method Ley might have used. But methods for amplifying regions of the genome and sequencing them were well known in the art.

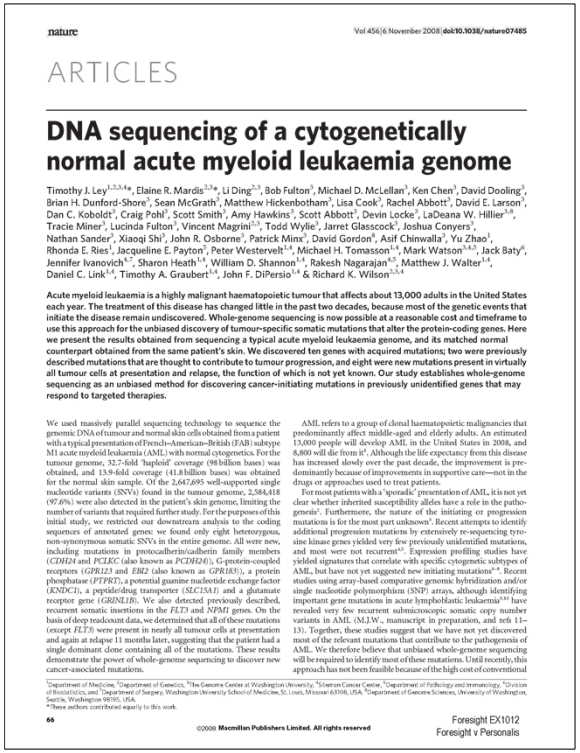
B. Is it possible that Ley used nested PCR primers to generate its amplicons?

C. So, I can't speculate on what Ley did or did not do. I can tell you that Ley sequenced amplicons containing exons with the relevant putative mutations as – and tracked those across multiple samples from the same individual.

EX2163 (10/20/23 Quackenbush Depo) at 261:2-18 (objection omitted)

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 15, 611 POR at 23)

Ley: Targeted AML Was "Representative" Of Most Patients



nature

Vol 456|6 November 2008|doi:10.1038/nature07485

ARTICLES

DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome

Timothy J. Ley[1,2,3,4]*, Elaine R. Mardis[2,3]*, Li Ding[2,3], Bob Fulton[3], Michael D. McLellan[3], Ken Chen[3], David Dooling[3], Brian H. Dunford-Shore[3], Sean McGrath[3], Matthew Hickenbotham[3], Lisa Cook[3], Rachel Abbott[3], David E. Larson[3], Dan C. Koboldt[3], Craig Pohl[3], Scott Smith[3], Amy Hawkins[3], Scott Abbott[3], Devin Locke[3], LaDeana W. Hillier[3,8], Tracie Miner[3], Lucinda Fulton[3], Vincent Magrini[2,3], Todd Wylie[3], Jarret Glasscock[3], Joshua Conyers[3], Nathan Sander[3], Xiaoqi Shi[3], John R. Osborne[3], Patrick Minx[3], David Gordon[3], Asif Chinwalla[3], Yu Zhao[1], Rhonda E. Ries[1], Jacqueline E. Payton[1], Peter Westervelt[1,4], Michael H. Tomasson[1,4], Mark Watson[1,4,5], Jack Baty[6], Jennifer Ivanovich[4,7], Sharon Heath[1,4], William D. Shannon[1,4], Rakesh Nagarajan[4,5], Matthew J. Walter[1,4], Daniel C. Link[1,4], Timothy A. Graubert[1,4], John F. DiPersio[1,4] & Richard K. Wilson[2,3,4]

Acute myeloid leukaemia is a highly malignant haematopoietic tumour that affects about 13,000 adults in the United States each year. The treatment of this disease has changed little in the past two decades, because most of the genetic events that initiate the disease remain undiscovered. Whole-genome sequencing is now possible at a reasonable cost and timeframe to use this approach for the unbiased discovery of tumour-specific somatic mutations that alter the protein-coding genes. Here we present the results obtained from sequencing a typical acute myeloid leukaemia genome, and its matched normal counterpart obtained from the same patient's skin. We discovered ten genes with acquired mutations; two were previously described mutations that are thought to contribute to tumour progression, and eight were new mutations present in virtually all tumour cells at presentation and relapse, the function of which is not yet known. Our study establishes whole-genome sequencing as an unbiased method for discovering cancer-initiating mutations in previously unidentified genes that may respond to targeted therapies.

We used massively parallel sequencing technology to sequence the genomic DNA of tumour and normal skin cells obtained from a patient with a typical presentation of French–American–British (FAB) subtype M1 acute myeloid leukaemia (AML) with normal cytogenetics. For the tumour genome, 32.7-fold 'haploid' coverage (98 billion bases) was obtained, and 13.9-fold coverage (41.8 billion bases) was obtained for the normal skin sample. Of the 2,647,695 well-supported single nucleotide variants (SNVs) found in the tumour genome, 2,584,418 (97.6%) were also detected in the patient's skin genome, limiting the number of variants that required further study. For the purposes of this initial study, we restricted our downstream analysis to the coding sequences of annotated genes: we found only eight heterozygous, non-synonymous somatic SNVs in the entire genome. All were new, including mutations in protocadherin/cadherin family members (*CDH24* and *PCLKC* (also known as *PCDH26*)), G-protein-coupled receptors (*GPR123* and *EBI2* (also known as *GPR183*)), a protein phosphatase (*PTPRT*), a potential guanine nucleotide exchange factor (*KNDC1*), a peptide/drug transporter (*SLC15A1*) and a glutamate receptor gene (*GRINL1B*). We also detected previously described, recurrent somatic insertions in the *FLT3* and *NPM1* genes. On the basis of deep readcount data, we determined that all of these mutations (except *FLT3*) were present in nearly all tumour cells at presentation and again at relapse 11 months later, suggesting that the patient had a single dominant clone containing all of the mutations. These results demonstrate the power of whole-genome sequencing to discover new cancer-associated mutations.

AML refers to a group of clonal haematopoietic malignancies that predominantly affect middle-aged and elderly adults. An estimated 13,000 people will develop AML in the United States in 2008, and 8,800 will die from it[1]. Although the life expectancy from this disease has increased slowly over the past decade, the improvement is predominantly because of improvements in supportive care—not in the drugs or approaches used to treat patients.

For most patients with a 'sporadic' presentation of AML, it is not yet clear whether inherited susceptibility alleles have a role in the pathogenesis[2]. Furthermore, the nature of the initiating or progression mutations is for the most part unknown[3]. Recent attempts to identify additional progression mutations by extensively re-sequencing tyrosine kinase genes yielded very few previously unidentified mutations, and most were not recurrent[4,5]. Expression profiling studies have yielded signatures that correlate with specific cytogenetic subtypes of AML, but have not yet suggested new initiating mutations[6–8]. Recent studies using array-based comparative genomic hybridization and/or single nucleotide polymorphism (SNP) arrays, although identifying important gene mutations in acute lymphoblastic leukaemia[9,10] have revealed very few recurrent submicroscopic somatic copy number variants in AML (M.J.W., manuscript in preparation, and refs 11–13). Together, these studies suggest that we have not yet discovered most of the relevant mutations that contribute to the pathogenesis of AML. We therefore believe that unbiased whole-genome sequencing will be required to identify most of these mutations. Until recently, this approach has not been feasible because of the high cost of conventional

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*These authors contributed equally to this work.

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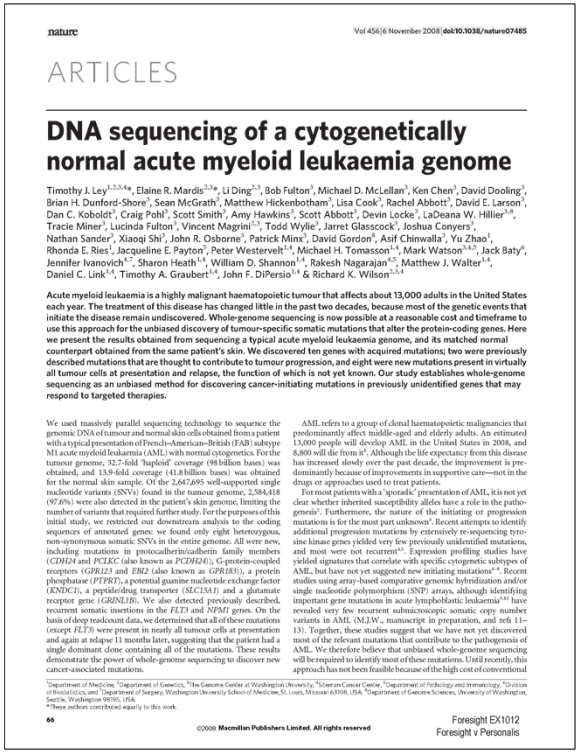
Foresight EX1012
Foresight v Personalis

Although this case was carefully selected, the presentation of AML was typical, and the case is representative of most patients with M1 AML, as described below.

EX1012; EX1042 (Ley Suppl Info) at 1

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 17, '611 POR at 25)

Ley: Example Reflected "Typical" AML



nature

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[1]Department of Medicine, [2]Department of Genetics, [3]The Genome Center at Washington University, [4]Siteman Cancer Center, [5]Department of Pathology and Immunology, [6]Division of Biostatistics, and [7]Department of Surgery, Washington University School of Medicine, St. Louis, Missouri 63108, USA. [8]Department of Genome Sciences, University of Washington, Seattle, Washington 98195, USA.
*These authors contributed equally to this work.

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Foresight EX1012
Foresight v Personalis

4. Typical features of M1 AML. We required a gene expression profile typical of M1 AML with normal cytogenetics, to assure that the sample was not an outlier for this subtype.

EX1012; EX1042 (Ley Suppl Info) at 1

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 17, '611 POR at 25)

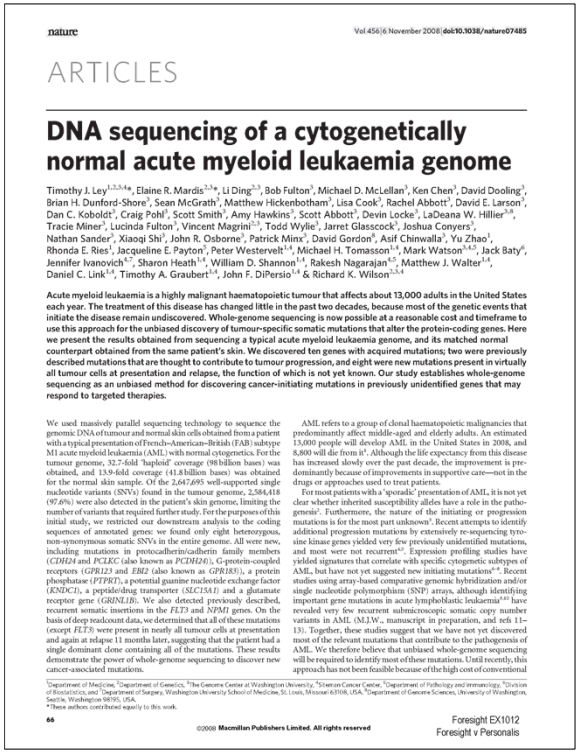
Law: Assumptions And Contentions Insufficient For Inherency

"Given the presumption of validity afforded the '511 patent, Crown has failed to meet its burden because it has not presented sufficient evidence to rebut the facial evidence offered by Solutia that the Gillery patent does not disclose the two percent limitation. Instead, Crown offers only an assumption and its own contentions."

Crown Operations Int'l, Ltd. v. Solutia Inc., 289 F.3d 1367, 1377–78 (Fed. Cir. 2002) (internal citations omitted).

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Ley: Discourages Personalization



nature

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ARTICLES

DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome

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Acute myeloid leukaemia is a highly malignant haematopoietic tumour that affects about 13,000 adults in the United States each year. The treatment of this disease has changed little in the past two decades, because most of the genetic events that initiate the disease remain undiscovered. Whole-genome sequencing is now possible at a reasonable cost and timeframe to use this approach for the unbiased discovery of tumour-specific somatic mutations that alter the protein-coding genes. Here we present the results obtained from sequencing a typical acute myeloid leukaemia genome, and its matched normal counterpart obtained from the same patient's skin. We discovered ten genes with acquired mutations; two were previously described mutations that are thought to contribute to tumour progression, and eight were new mutations present in virtually all tumour cells at presentation and relapse, the function of which is not yet known. Our study establishes whole-genome sequencing as an unbiased method for discovering cancer-initiating mutations in previously unidentified genes that may respond to targeted therapies.

We used massively parallel sequencing technology to sequence the genomic DNA of tumour and normal skin cells obtained from a patient with a typical presentation of French–American–British (FAB) subtype M1 acute myeloid leukaemia (AML) with normal cytogenetics. For the tumour genome, 32.7-fold 'haploid' coverage (98 billion bases) was obtained, and 13.9-fold coverage (41.8 billion bases) was obtained for the normal skin sample. Of the 2,647,695 well-supported single nucleotide variants (SNVs) found in the tumour genome, 2,584,418 (97.6%) were also detected in the patient's skin genome, limiting the number of variants that required further study. For the purposes of this initial study, we restricted our downstream analysis to the coding sequences of annotated genes: we found only eight heterozygous, non-synonymous somatic SNVs in the entire genome. All were new, including mutations in protocadherin/cadherin family members (*CDH24* and *PCLKC* (also known as *PCDH24*)), G-protein-coupled receptors (*GPR123* and *EBI2* (also known as *GPR183*)), a protein phosphatase (*PTPRT*), a potential guanine nucleotide exchange factor (*KNDC1*), a peptide/drug transporter (*SLC15A1*) and a glutamate receptor gene (*GRINL1B*). We also detected previously described, recurrent somatic insertions in the *FLT3* and *NPM1* genes. On the basis of deep readcount data, we determined that all of these mutations (except *FLT3*) were present in nearly all tumour cells at presentation and again at relapse 11 months later, suggesting that the patient had a single dominant clone containing all of the mutations. These results demonstrate the power of whole-genome sequencing to discover new cancer-associated mutations.

AML refers to a group of clonal haematopoietic malignancies that predominantly affect middle-aged and elderly adults. An estimated 13,000 people will develop AML in the United States in 2008, and 8,800 will die from it[1]. Although the life expectancy from this disease has increased slowly over the past decade, the improvement is predominantly because of improvements in supportive care—not in the drugs or approaches used to treat patients.

For most patients with a 'sporadic' presentation of AML, it is not yet clear whether inherited susceptibility alleles have a role in the pathogenesis[2]. Furthermore, the nature of the initiating or progression mutations is for the most part unknown[3]. Recent attempts to identify additional progression mutations by extensively re-sequencing tyrosine kinase genes yielded very few previously unidentified mutations, and most were not recurrent[4,5]. Expression profiling studies have yielded signatures that correlate with specific cytogenetic subtypes of AML, but have not yet suggested new initiating mutations[6–8]. Recent studies using array-based comparative genomic hybridization and/or single nucleotide polymorphism (SNP) arrays, although identifying important gene mutations in acute lymphoblastic leukaemia[9,10] have revealed very few recurrent submicroscopic somatic copy number variants in AML (M.J.W., manuscript in preparation, and refs 11–13). Together, these studies suggest that we have not yet discovered most of the relevant mutations that contribute to the pathogenesis of AML. We therefore believe that unbiased whole-genome sequencing will be required to identify most of these mutations. Until recently, this approach has not been feasible because of the high cost of conventional

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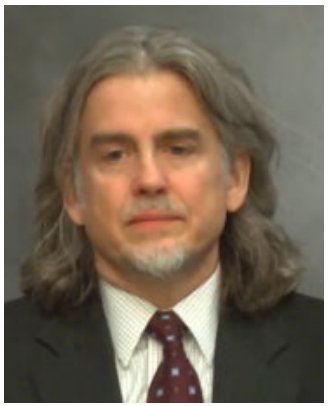
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tumour. Our results strongly support the notion that hypothesis-driven (for example, candidate gene-based) examination of tumour genomes by PCR-directed or capture-based methods is inherently limited, and will miss key mutations. A further and important consid-

EX1012 (Ley) at 70

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 28, '611 POR at 10)

Dr. Quackenbush's Motivations: Accuracy



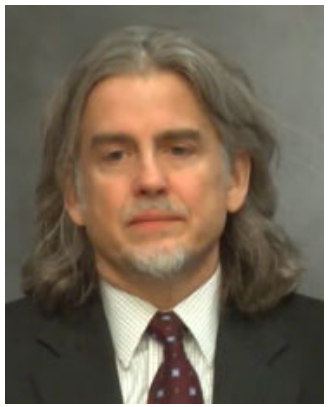
John Quackenbush
Foresight's Expert

A. But the importance of making accurate measurements has been known for quite some time.

EX2163 (10/20/23 Quackenbush Depo) at 23:5-7

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Dr. Quackenbush's Motivations: Accuracy And Cost



John Quackenbush
Foresight's Expert

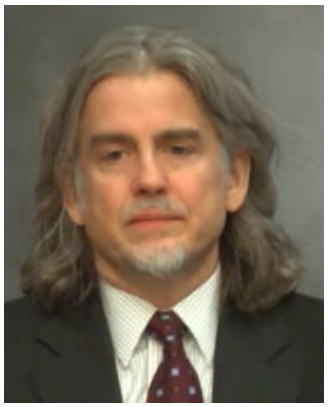
Dr. Quackenbush's Declaration

167. A person of ordinary skill knew that achieving a higher sequence depth in step 1(e) was desirable for increasing accuracy of detecting and quantifying mutations in tumors. For a given number of sequencing counts, the use of fewer oligonucleotide probes to target smaller genomic regions would lead to higher sequencing depth, as would be expected from the Lander/Waterman equation. In addition, even though sequencing had become less expensive over the years prior to the '611 patent, a person of ordinary skill knew reducing sequencing costs further was desirable, and that smaller sequencing panels in step 1(e) could reduce sequencing costs.

EX1061/EX2168 (Quackenbush Decl) ¶ 167

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE
('783 POR at 29,'611 POR at 37)

Dr. Quackenbush's Motivations: Cost



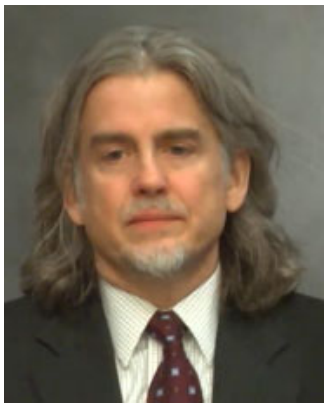
John Quackenbush
Foresight's Expert

A. In our assays, you know, with a fixed budget, as a laboratory scientist, we have always striven to be as cost efficient as possible, because that typically allows us to collect data on larger numbers of subjects, which in some applications is, is really driving some of the science that we do. So, a person of ordinary skill in the art would consider that. If the assay was simply too expensive, then one could not effectively run this assay.

EX2163 (10/20/23 Quackenbush Depo) at 29:21-30:10

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE
('783 POR at 29,'611 POR at 37)

Dr. Quackenbush's Motivations: Cost And Assay Simplicity



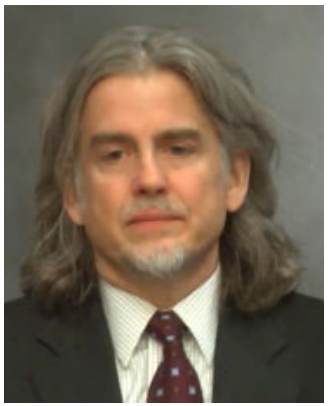
John Quackenbush
Foresight's Expert

A. So, a person of skill in the art would have followed what had been established in the literature as ways of simplifying assays to be as informative and as cost effective as possible.

EX2163 (10/20/23 Quackenbush Depo) at 36:12-16

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 30, '611 POR at 37)

Dr. Quackenbush's Motivations: Analysis Simplicity



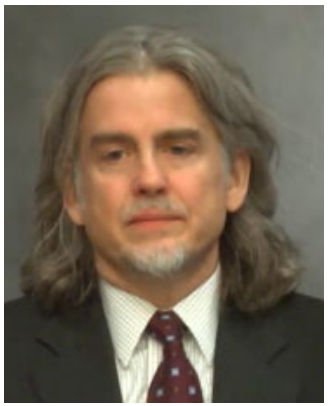
John Quackenbush
Foresight's Expert

A. So, people would have always been interested in simplifying computational analyses.

EX2163 (10/20/23 Quackenbush Depo) at 42:3-4

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Dr. Quackenbush: All Factors Equally Important



John Quackenbush
Foresight's Expert

A. I think a person of ordinary skill in the art, long before 2016 -- and I believe the Ley reference is from 2008. So, even as far back as 2008, the person of skill in the art would have understood that all of these factors are factors which should be considered. And it is hard to say that one is more important than the other, right.

EX2163 (10/20/23 Quackenbush Depo) at 45:14-22

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Mamanova: Adding Primers Is Complex And Inaccurate



REVIEW

Target-enrichment strategies for next-generation sequencing

Lira Mamanova[1], Alison J Coffey[1], Carol E Scott[1], Iwanka Kozarewa[1], Emily H Turner[2], Akash Kumar[2], Eleanor Howard[1], Jay Shendure[2] & Daniel J Turner[1]

We have not yet reached a point at which routine sequencing of large numbers of whole eukaryotic genomes is feasible, and so it is often necessary to select genomic regions of interest and to enrich these regions before sequencing. There are several enrichment approaches, each with unique advantages and disadvantages. Here we describe our experiences with the leading target-enrichment technologies, the optimizations that we have performed and typical results that can be obtained using each. We also provide detailed protocols for each technology so that end users can find the best compromise between sensitivity, specificity and uniformity for their particular project.

The ability to read the sequence of bases that comprise a polynucleotide has had an impact on biological research that is difficult to overstate. For the majority of the past 30 years, dideoxy DNA 'Sanger' sequencing[1] has been used as the standard sequencing technology in many laboratories, and its acme was the completion of the human genome sequence[2]. However, because Sanger sequencing is performed on single amplicons, its throughput is limited, and large-scale sequencing projects are expensive and laborious: the human genome sequence took hundreds of sequencing machines several years and cost several hundred million dollars.

The paradigm of DNA sequencing changed with the advent of 'next-generation' sequencing technologies (reviewed in refs. 3,4), which process hundreds of thousands to millions of DNA templates in parallel, resulting in a low cost per base of generated sequence and a throughput on the gigabase (Gb) scale. As a consequence, we can now start to define the characteristics of entire genomes and delineate differences between them. Ultimately, whole-genome sequencing of complex organisms will become routine, allowing us to gain a deeper understanding of the full spectrum of genetic variation and to define its role in phenotypic variation and the pathogenesis of complex traits.

Nevertheless, it is not yet feasible to sequence large numbers of complex genomes in their entirety because the cost and time taken are still too great. To obtain 30-fold coverage of a human genome (90 Gb in total), would currently require several sequencing runs and would cost tens of thousands of dollars. In addition to the demands such a project would place on laboratory time and funding, the primary analysis during which the captured image files are processed, as well as storage of the sequences, would place a substantial burden on a research center's informatics infrastructure.

Consequently, considerable effort has been devoted to develop 'target-enrichment' methods, in which genomic regions are selectively captured from a DNA sample before sequencing. Resequencing the genomic regions that are retained is necessarily more time- and cost-effective, and the resulting data are considerably less cumbersome to analyze. Several approaches to target enrichment have been developed (**Fig. 1**), and there are several parameters by which the performance of each can be measured, which vary from one approach to another: (i) sensitivity, or the percentage of the target bases that are represented by one or more sequence reads; (ii) specificity, or the percentage of sequences that map to the intended targets; (iii) uniformity, or the variability in sequence coverage across target regions; (iv) reproducibility, or how closely results obtained from replicate experiments correlate; (v) cost; (vi) ease of use; and (vii) amount of DNA required per experiment, or per megabase of target.

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together. However, PCR is difficult to multiplex to any useful degree: the simultaneous use of many primer pairs can generate a high level of nonspecific amplification, caused by interaction between the primers, and moreover amplicons can fail to amplify[6,7]. Clever

EX1007 (Mamanova) at 112

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 31, '611 POR at 39)

Mertes: Adding Primers Is Complex



BRIEFINGS IN FUNCTIONAL GENOMICS. VOL. 10. NO 6. 374–386 doi:10.1093/bfgp/elr033

Targeted enrichment of genomic DNA regions for next-generation sequencing

Florian Mertes, Abdou ElSharawy, Sascha Sauer, Joop M.L.M. van Helvoort, P.J. van der Zaag, Andre Franke, Mats Nilsson, Hans Lehrach and Anthony J. Brookes

Advance Access publication date 26 November 2011

Abstract

In this review, we discuss the latest targeted enrichment methods and aspects of their utilization along with second-generation sequencing for complex genome analysis. In doing so, we provide an overview of issues involved in detecting genetic variation, for which targeted enrichment has become a powerful tool. We explain how targeted enrichment for next-generation sequencing has made great progress in terms of methodology, ease of use and applicability, but emphasize the remaining challenges such as the lack of even coverage across targeted regions. Costs are also considered versus the alternative of whole-genome sequencing which is becoming ever more affordable. We conclude that targeted enrichment is likely to be the most economical option for many years to come in a range of settings.

Keywords: *targeted enrichment; next-generation sequencing; genome partitioning; exome; genetic variation*

INTRODUCTION

Next-generation sequencing (NGS) [1, 2] is now a major driver in genetics research, providing a powerful way to study DNA or RNA samples. New and improved methods and protocols have been developed to support a diverse range of applications, including the analysis of genetic variation. As part of this, methods have been developed that aim to achieve 'targeted enrichment' of genome subregions [3, 4], also sometimes referred to as 'genome partitioning'. Strategies for direct selection of genomic regions were already developed in anticipation of the introduction of NGS [5, 6]. By selective recover and subsequent sequencing of genomic loci of interest, costs and efforts can be reduced significantly compared with whole-genome sequencing.

Targeted enrichment can be useful in a number of situations where particular portions of a

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Florian Mertes studied biotechnology and earned a Doctorate from the Technical University Berlin. Currently, he is a postdoctoral researcher focusing on applied research to develop test/screening assays based on high-throughput technologies, using both PCR and next-generation sequencing.

Abdou ElSharawy is a postdoctoral researcher (University of Kiel, CAU, Germany), and lecturer of Biochemistry and Cell Molecular Biology (Mansoura University, Egypt). He focuses on disease-associated mutations and miRNAs, allele-dependent RNA splicing, and high-throughput targeted, whole exome, and genome sequencing.

Sascha Sauer is a research group leader at the Max Planck Institute for Molecular Genetics, and coordinates the European Sequencing and Genotyping Infrastructure.

Joop M.L.M. van Helvoort is CSO at FlexGen. He received his PhD at the University of Amsterdam. His expertise is in microarray applications currently focusing on target enrichment.

P.J. van der Zaag is with Philips Research, Eindhoven, The Netherlands. He holds a doctorate in physics from Leiden University. At Philips, he has worked on a number of topics related to microsystems and nanotechnology, lately in the field of nanobiotechnology.

Andre Franke is a biologist by training and currently holds an endowment professorship for Molecular Medicine at the Christian-Albrechts-University of Kiel in Germany and is guest professor in Oslo (Norway).

Mats Nilsson is Professor of Molecular Diagnostics at the Department of Immunology, Genetics, and Pathology, Uppsala University, Sweden. He has pioneered a number of molecular analysis technologies for multiplexed targeted analyses of genes.

Hans Lehrach is Director at the Max Planck Institute for Molecular Genetics. His expertise lies in genetics, genomics, systems biology, and personalized medicine. Highlights include key involvement in several large-scale genome sequencing projects.

Anthony J Brookes is a Professor of Bioinformatics and Genomics at the University of Leicester (UK) where he runs a research team and several international projects in method development and informatics for DNA analysis through to healthcare.

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The main downside of the method is that it does not scale easily, in any format, to enable the targeting

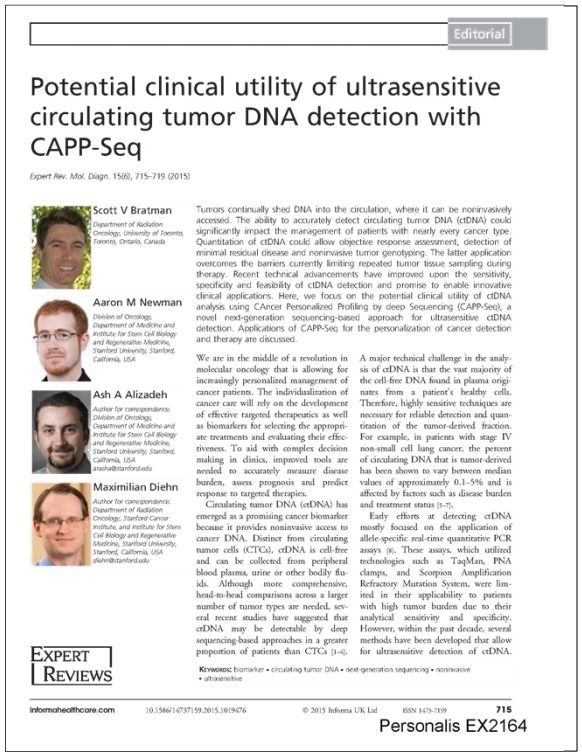
ples. To use this method effectively, any significant extent of parallelized singleplex or multiplex PCR would need to be supported by the use of automated robotics, individual PCR amplicons (or multiplex

products) need to be carefully normalized to equivalent molarities when pooling in advance of NGS (so that the final coverage of the total region of interest is as even as possible), and the amount of DNA mater-

EX1006 (Mertes) at 379

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 52, '611 POR at 379)

Foresight's Founders: Multiplex PCR "Limited" And Complicating



Editorial

Potential clinical utility of ultrasensitive circulating tumor DNA detection with CAPP-Seq

Expert Rev. Mol. Diagn. 15(6), 715–719 (2015)

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Tumors continually shed DNA into the circulation, where it can be noninvasively accessed. The ability to accurately detect circulating tumor DNA (ctDNA) could significantly impact the management of patients with nearly every cancer type. Quantitation of ctDNA could allow objective response assessment, detection of minimal residual disease and noninvasive tumor genotyping. The latter application overcomes the barriers currently limiting repeated tumor tissue sampling during therapy. Recent technical advancements have improved upon the sensitivity, specificity and feasibility of ctDNA detection and promise to enable innovative clinical applications. Here, we focus on the potential clinical utility of ctDNA analysis using CAncer Personalized Profiling by deep Sequencing (CAPP-Seq), a novel next-generation sequencing-based approach for ultrasensitive ctDNA detection. Applications of CAPP-Seq for the personalization of cancer detection and therapy are discussed.

We are in the middle of a revolution in molecular oncology that is allowing for increasingly personalized management of cancer patients. The individualization of cancer care will rely on the development of effective targeted therapeutics as well as biomarkers for selecting the appropriate treatments and evaluating their effectiveness. To aid with complex decision making in clinics, improved tools are needed to accurately measure disease burden, assess prognosis and predict response to targeted therapies.

Circulating tumor DNA (ctDNA) has emerged as a promising cancer biomarker because it provides noninvasive access to cancer DNA. Distinct from circulating tumor cells (CTCs), ctDNA is cell-free and can be collected from peripheral blood plasma, urine or other bodily fluids. Although more comprehensive, head-to-head comparisons across a larger number of tumor types are needed, several recent studies have suggested that ctDNA may be detectable by deep sequencing-based approaches in a greater proportion of patients than CTCs [1–4].

A major technical challenge in the analysis of ctDNA is that the vast majority of the cell-free DNA found in plasma originates from a patient's healthy cells. Therefore, highly sensitive techniques are necessary for reliable detection and quantitation of the tumor-derived fraction. For example, in patients with stage IV non-small cell lung cancer, the percent of circulating DNA that is tumor-derived has been shown to vary between median values of approximately 0.1–5% and is affected by factors such as disease burden and treatment status [3–5].

Early efforts at detecting ctDNA mostly focused on the application of allele-specific real-time quantitative PCR assays [6]. These assays, which utilized technologies such as TaqMan, PNA clamps, and Scorpion Amplification Refractory Mutation System, were limited in their applicability to patients with high tumor burden due to their analytical sensitivity and specificity. However, within the past decade, several methods have been developed that allow for ultrasensitive detection of ctDNA.

Keywords: biomarker • circulating tumor DNA • next-generation sequencing • noninvasive • ultrasensitive

EXPERT REVIEWS

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geting commonly mutated cancer genes [1,2,11,15,16]. Although low detection thresholds are achievable with such methods, technical limitations related to multiplexing of PCR assays have to date limited the number of genomic positions that can be interrogated. This complicates potential clinical applications

EX2164 (Bratman) at 716

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 32, '611 POR at 39)

Foresight's Founders: Multiplex PCR "Limited"



ELSEVIER

Seminars in RADIATION ONCOLOGY

Predicting Radiotherapy Responses and Treatment Outcomes Through Analysis of Circulating Tumor DNA

Aadel A. Chaudhuri, MD, PhD,* Michael S. Binkley, BA,* Evan C. Osmundson, MD, PhD,* Ash A. Alizadeh, MD, PhD,†,‡,§,¶ and Maximilian Diehn, MD, PhD*,§,¶

Tumors continually shed DNA into the blood where it can be detected as circulating tumor DNA (ctDNA). Although this phenomenon has been recognized for decades, techniques that are sensitive and specific enough to robustly detect ctDNA have only become available recently. Quantification of ctDNA represents a new approach for cancer detection and disease burden quantification that has the potential to revolutionize response assessment and personalized treatment in radiation oncology. Analysis of ctDNA has many potential applications, including detection of minimal residual disease following radiotherapy, non-invasive tumor genotyping, and early detection of tumor recurrence. Ultimately, ctDNA-based assays could lead to personalization of therapy based on identification of somatic alterations present in tumors and changes in ctDNA concentrations before and after treatment. In this review, we discuss methods of ctDNA detection and clinical applications of ctDNA-based biomarkers in radiation oncology, with a focus on recently developed techniques that use next-generation sequencing for ctDNA quantification.
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M.D. and A.A. are inventors on patent applications related to the CAPP-Seq technology and are cofounders and consultants for CAPP Medical. No writing assistance was used in the production of this manuscript.
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Introduction

The field of radiation oncology is currently going through a revolution, as new technologies allow treatment of patients with more precision than ever before. Although much progress has been made in improving delivery of radiation therapy (RT), response assessment after treatment continues to rely primarily on imaging for most types of cancers. Although, imaging-based response assessment has significant clinical value, this approach cannot detect minimal residual disease (MRD), has a lag between treatment and when tumor responses can be detected, is expensive, and often exposes patients to additional ionizing radiation. For these reasons, there continues to be an unmet need for developing blood-based biomarkers for cancers that are treated with RT. Although such biomarkers exist for a few cancer types (eg, prostate-specific antigen for prostate cancer), they do not for most cancers. The ideal biomarker would be readily generalizable, such that it could be applied to all cancer types.

Although most work on cancer biomarkers has focused on detection of proteins, recently, circulating tumor DNA

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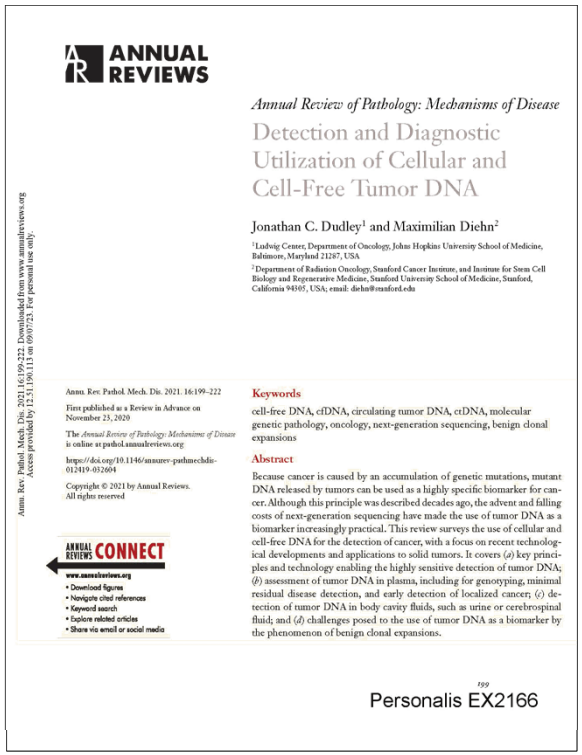
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are used. Limitations of these approaches include interrogation of relatively few genomic loci owing to difficulties related to multiplexing of PCR assays, the inability to detect somatic copy

EX2165 (Chaudhuri) at 307-308

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 32, '611 POR at 40)

Foresight's Founders: Multiplex PCR "Limited"



ANNUAL REVIEWS

Annual Review of Pathology: Mechanisms of Disease

Detection and Diagnostic Utilization of Cellular and Cell-Free Tumor DNA

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Keywords

cell-free DNA, cfDNA, circulating tumor DNA, ctDNA, molecular genetic pathology, oncology, next-generation sequencing, benign clonal expansions

Abstract

Because cancer is caused by an accumulation of genetic mutations, mutant DNA released by tumors can be used as a highly specific biomarker for cancer. Although this principle was described decades ago, the advent and falling costs of next-generation sequencing have made the use of tumor DNA as a biomarker increasingly practical. This review surveys the use of cellular and cell-free DNA for the detection of cancer, with a focus on recent technological developments and applications to solid tumors. It covers (*a*) key principles and technology enabling the highly sensitive detection of tumor DNA; (*b*) assessment of tumor DNA in plasma, including for genotyping, minimal residual disease detection, and early detection of localized cancer; (*c*) detection of tumor DNA in body cavity fluids, such as urine or cerebrospinal fluid; and (*d*) challenges posed to the use of tumor DNA as a biomarker by the phenomenon of benign clonal expansions.

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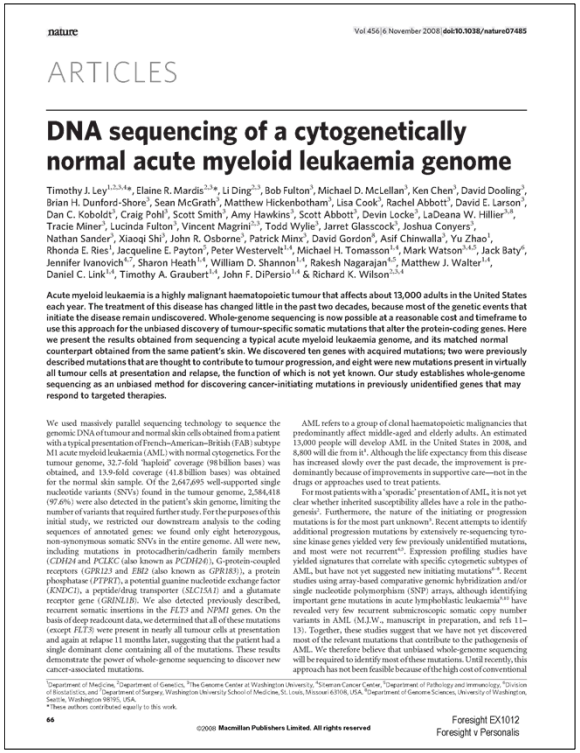
Annu. Rev. Pathol. Mech. Dis. 2021.16:199-222. Downloaded from www.annualreviews.org
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PCR-based assays have limited multiplexability.

EX2166 (Dudley) at 202

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 32, '611 POR at 40)

Ley: Identified Mutations Are Of Unknown Relevance



nature

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ARTICLES

DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome

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Acute myeloid leukaemia is a highly malignant haematopoietic tumour that affects about 13,000 adults in the United States each year. The treatment of this disease has changed little in the past two decades, because most of the genetic events that initiate the disease remain undiscovered. Whole-genome sequencing is now possible at a reasonable cost and timeframe to use this approach for the unbiased discovery of tumour-specific somatic mutations that alter the protein-coding genes. Here we present the results obtained from sequencing a typical acute myeloid leukaemia genome, and its matched normal counterpart obtained from the same patient's skin. We discovered ten genes with acquired mutations; two were previously described mutations that are thought to contribute to tumour progression, and eight were new mutations present in virtually all tumour cells at presentation and relapse, the function of which is not yet known. Our study establishes whole-genome sequencing as an unbiased method for discovering cancer-initiating mutations in previously unidentified genes that may respond to targeted therapies.

We used massively parallel sequencing technology to sequence the genomic DNA of tumour and normal skin cells obtained from a patient with a typical presentation of French–American–British (FAB) subtype M1 acute myeloid leukaemia (AML) with normal cytogenetics. For the tumour genome, 32.7-fold 'haploid' coverage (98 billion bases) was obtained, and 13.9-fold coverage (41.8 billion bases) was obtained for the normal skin sample. Of the 2,647,695 well-supported single nucleotide variants (SNVs) found in the tumour genome, 2,584,418 (97.6%) were also detected in the patient's skin genome, limiting the number of variants that required further study. For the purposes of this initial study, we restricted our downstream analysis to the coding sequences of annotated genes: we found only eight heterozygous, non-synonymous somatic SNVs in the entire genome. All were new, including mutations in protocadherin/cadherin family members (*CDH24* and *PCLKC* (also known as *PCDH24*)), G-protein-coupled receptors (*GPR123* and *EBI2* (also known as *GPR183*)), a protein phosphatase (*PTPRT*), a potential guanine nucleotide exchange factor (*KNDC1*), a peptide/drug transporter (*SLC15A1*) and a glutamate receptor gene (*GRINL1B*). We also detected previously described, recurrent somatic insertions in the *FLT3* and *NPM1* genes. On the basis of deep readcount data, we determined that all of these mutations (except *FLT3*) were present in nearly all tumour cells at presentation and again at relapse 11 months later, suggesting that the patient had a single dominant clone containing all of the mutations. These results demonstrate the power of whole-genome sequencing to discover new cancer-associated mutations.

AML refers to a group of clonal haematopoietic malignancies that predominantly affect middle-aged and elderly adults. An estimated 13,000 people will develop AML in the United States in 2008, and 8,800 will die from it[1]. Although the life expectancy from this disease has increased slowly over the past decade, the improvement is predominantly because of improvements in supportive care—not in the drugs or approaches used to treat patients.

For most patients with a 'sporadic' presentation of AML, it is not yet clear whether inherited susceptibility alleles have a role in the pathogenesis[2]. Furthermore, the nature of the initiating or progression mutations is for the most part unknown[3]. Recent attempts to identify additional progression mutations by extensively re-sequencing tyrosine kinase genes yielded very few previously unidentified mutations, and most were not recurrent[4,5]. Expression profiling studies have yielded signatures that correlate with specific cytogenetic subtypes of AML, but have not yet suggested new initiating mutations[6–8]. Recent studies using array-based comparative genomic hybridization and/or single nucleotide polymorphism (SNP) arrays, although identifying important gene mutations in acute lymphoblastic leukaemia[9,10] have revealed very few recurrent submicroscopic somatic copy number variants in AML (M.J.W., manuscript in preparation, and refs 11–13). Together, these studies suggest that we have not yet discovered most of the relevant mutations that contribute to the pathogenesis of AML. We therefore believe that unbiased whole-genome sequencing will be required to identify most of these mutations. Until recently, this approach has not been feasible because of the high cost of conventional

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feasible without this approach. Furthermore, until hundreds (or perhaps thousands) of normal genomes and other AML tumours are sequenced, the contextual relevance of the mutations found in this genome will be unknown. Nevertheless, the somatic mutations that

EX1012 (Ley) at 71

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 32, '611 POR at 40)

Ross: Avoid Abnormalities Of Unknown Importance



Whole Cancer Genome Sequencing by Next-Generation Methods

Jeffrey S. Ross, MD,[1,2] and Maureen Cronin, PhD[2]

Key Words: Cancer; Next-generation sequencing; Massively parallel sequencing; Personalized oncology; Molecular diagnostics; Colorectal cancer; Non–small cell lung cancer; Melanoma

DOI: 10.1309/AJCPR15VT1HKJGXW

Upon completion of this activity you will be able to:
- contrast the advantages of next-generation gene sequencing technologies with the traditional Sanger, pyrosequencing, and allele-specific polymerase chain reaction approaches.
- list the current uses of cancer cell gene sequencing for the management of patients with solid tumors.

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The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.

Questions appear on p 653. Exam is located at www.ascp.org/ajcpcme.

CME/SAM

http://academic.oup.com/ajcp/article/136/4527/1160476 by guest on 07 October 2022

Abstract

Traditional approaches to sequence analysis are widely used to guide therapy for patients with lung and colorectal cancer and for patients with melanoma, sarcomas (eg, gastrointestinal stromal tumor), and subtypes of leukemia and lymphoma. The next-generation sequencing (NGS) approach holds a number of potential advantages over traditional methods, including the ability to fully sequence large numbers of genes (hundreds to thousands) in a single test and simultaneously detect deletions, insertions, copy number alterations, translocations, and exome-wide base substitutions (including known "hot-spot mutations") in all known cancer-related genes. Adoption of clinical NGS testing will place significant demands on laboratory infrastructure and will require extensive computational expertise and a deep knowledge of cancer medicine and biology to generate truly useful "clinically actionable" reports. It is anticipated that continuing advances in NGS technology will lower the overall cost, speed the turnaround time, increase the breadth of genome sequencing, detect epigenetic markers and other important genomic parameters, and become applicable to smaller and smaller specimens, including circulating tumor cells and circulating free DNA in plasma.

During the past 13 years since the launch of the targeted therapy era, there has been a keen interest in determining the cancer cell gene sequence to predict the response to anticancer drugs ▮Table 1▮.[1-4] In late 1998, the era of personalized oncology began with the simultaneous regulatory approval of the anti–HER2-targeted monoclonal antibody therapeutic, trastuzumab, for the treatment of HER2-overexpressing breast cancer and the immunohistochemical-based diagnostic HercepTest (DAKO, Carpinteria, CA) for the identification of patients who are eligible for treatment.[5] During the ensuing 12 years, interest in these "drug-test" combinations, also referred to as "companion diagnostics," has increased exponentially as more anticancer agents have been approved and more new drugs have entered clinical trials based on biomarker profiles.[4] The selection of anti–epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors in non–small cell lung cancer (NSCLC) and the use of anti-EGFR monoclonal antibody therapeutics in colorectal cancer (CRC) are now firmly based on pretreatment sequence determinations for hot spots in the *EGFR* (NSCLC) and *KRAS* (CRC) genes.[1-3]

Recently, 2 novel targeted small-molecule drugs have advanced into late-stage clinical trials that feature a genetic sequence detection–based companion diagnostic. PLX-4032 targets papillary thyroid cancers and metastatic malignant melanomas that feature the V600E mutation of the *BRAF* gene.[6] The second drug, crizotinib, has shown impressive efficacy for the treatment of NSCLCs that feature an *EML4-ALK* translocation.[7] The recent introduction of the more thorough and in-depth sequencing technologies (next-generation sequencing [NGS] or massively parallel sequencing) has

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tests. Given the potential generation of "actionable" gene sequence abnormalities and abnormalities of unknown clinical importance, the successful NGS-based clinical service will have a result report that is focused on the abnormalities that can lead directly to the best available therapy, direct a patient to an ongoing clinical trial, or result in some other actionable response from the treating oncologist.

EX1014 (Ross) at 537

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 33, '611 POR at 41)

Ross: Cost Is Critical



Whole Cancer Genome Sequencing by Next-Generation Methods

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analysis. However, it is equally clear that tens or hundreds of individual traditional mutation analysis assays to test for all of the possible important mutations in a given tumor are not practical or feasible given the amount of tumor sample, time, and money it would take to perform so many individual tests. Given the potential generation of "actionable" gene

EX1014 (Ross) at 537

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 33, '611 POR at 41)

Ross: Cost Is Critical



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to face analytic challenges for some time. Clinical applications, particularly in oncology, demand high levels of accuracy, sensitivity, and specificity for sequence-based tests focused on calling out specific genetic alterations.

EX1014 (Ross) at 535

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 33, '611 POR at 41)

Foresight's Founders: Accuracy Is Critical



ELSEVIER

Seminars in RADIATION ONCOLOGY

Predicting Radiotherapy Responses and Treatment Outcomes Through Analysis of Circulating Tumor DNA

Aadel A. Chaudhuri, MD, PhD,* Michael S. Binkley, BA,* Evan C. Osmundson, MD, PhD,† Ash A. Alizadeh, MD, PhD,†,‡,§,¶ and Maximilian Diehn, MD, PhD*,§,¶

Tumors continually shed DNA into the blood where it can be detected as circulating tumor DNA (ctDNA). Although this phenomenon has been recognized for decades, techniques that are sensitive and specific enough to robustly detect ctDNA have only become available recently. Quantification of ctDNA represents a new approach for cancer detection and disease burden quantification that has the potential to revolutionize response assessment and personalized treatment in radiation oncology. Analysis of ctDNA has many potential applications, including detection of minimal residual disease following radiotherapy, non-invasive tumor genotyping, and early detection of tumor recurrence. Ultimately, ctDNA-based assays could lead to personalization of therapy based on identification of somatic alterations present in tumors and changes in ctDNA concentrations before and after treatment. In this review, we discuss methods of ctDNA detection and clinical applications of ctDNA-based biomarkers in radiation oncology, with a focus on recently developed techniques that use next-generation sequencing for ctDNA quantification.
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M.D. and A.A. are inventors on patent applications related to the CAPP-Seq technology and are cofounders and consultants for CAPP Medical. No writing assistance was used in the production of this manuscript.
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Introduction

The field of radiation oncology is currently going through a revolution, as new technologies allow treatment of patients with more precision than ever before. Although much progress has been made in improving delivery of radiation therapy (RT), response assessment after treatment continues to rely primarily on imaging for most types of cancers. Although, imaging-based response assessment has significant clinical value, this approach cannot detect minimal residual disease (MRD), has a lag between treatment and when tumor responses can be detected, is expensive, and often exposes patients to additional ionizing radiation. For these reasons, there continues to be an unmet need for developing blood-based biomarkers for cancers that are treated with RT. Although such biomarkers exist for a few cancer types (eg, prostate-specific antigen for prostate cancer), they do not for most cancers. The ideal biomarker would be readily generalizable, such that it could be applied to all cancer types.

Although most work on cancer biomarkers has focused on detection of proteins, recently, circulating tumor DNA

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Personalis EX2165

residual disease must be detected. Thus, only highly sensitive detection techniques are likely to be of clinical use.[13,23] Here

EX2165 (Chaudhuri) at 306

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 33; '611 POR at 41)

Ross: Illumina Sequencing Is "Low Multiplexing"

The Illumina technique is the most widely used NGS platform, but it is limited by a relatively low multiplexing capability.[59] A number of technical issues, particularly due to

Table 3
Commercial Next-Generation Sequencing Platforms*

	Basic Technique	Major Uses	Time Needed	Instrument Cost ($, thousands)
Illumina	Flow cell-based, reversible dye termination, and 4-color optical imaging	Whole exome sequencing; whole genome sequencing; SNP detection	Intermediate, 4 d fragment; 9 d paired end	500-900
454 Pyrosequencing	Emulsion PCR with bead-based pyrosequencing and CCD light imaging	Targeted exon sequencing; confirmatory sequencing; SNP detection	Fast, <1 d	500-700
Helicos	Oligo-dT captured PolyA-tailed DNA fragments; flow cell 4-color dNTP optical imaging	Single molecule sequencing; whole genome sequencing	Slowest, 8 d	999
SOLiD	Sequential dinucleotide ligation; flow cell–based 4-color optical imaging	Whole exome sequencing; whole genome sequencing; SNP detection	Slow, 7 d fragment run; 14 d paired end	600-700
Ion Torrent	Semiconductor-based nonoptical detection; standard dNTP sequencing chemistry	Targeted sequencing projects not demanding deep sequencing	Fast, <1 d	50

CCD, charge-coupled device; dNTP, deoxynucleoside triphosphate; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.
* Adapted from Metzker.[55]

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 34, '611 POR at 41)

EX1014 (Ross) at 530

Ley Fails To Disclose Or Render Obvious Multiple Claim Elements In The ’783 patent

- No “fixed” and “variable” portions
- No “additional biological sample”
- No “identifying” of variants
- No “generating a report”

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Ley Does Not Disclose An "Additional Biological Sample"

1. A method for personalized genetic testing, comprising:
(a) obtaining a personalized probe set for a subject, which personalized probe set comprises a plurality of nucleic acid probe molecules having a plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising a set of one or more genetic variants over other sequences in at least one biological sample of said subject, wherein said set of genetic variants are identified in sequence data for said at least one biological sample, further wherein said personalized probe set comprises nucleic acid probe molecules having oligonucleotide-directed genomic content comprising: (i) at least one variable portion from a result of said sequence data and (ii) at least one fixed portion independent of said result of said sequencing data
(b) using said personalized probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in at least an additional biological sample from said subject, to generate a sequencing library;
(c) subjecting said sequencing library to sequencing to identify a presence or absence of at least a subset of said set of genetic variants in at least said additional biological sample from said subject, wherein a sequencing footprint of said sequencing of (c) is less than a sequencing footprint of said sequence data of (a); and
(d) generating a report identifying (i) a presence or absence of a health condition or disease of the subject in said additional biological sample based on said identified at least a subset of (c) and (ii) genetic variants which inform a therapy choice or a change in therapy for the subject.

variants over other sequences in at least an additional biological sample from said subject, to generate a

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 4-5)

EX1001 ('783 Patent) at claim 1

Petitioner's First Biological Sample

The '783 Petition

other sequences in the sample. Specifically, ***Ley*** uses its PCR primers to selectively amplify the 12 variants of interest over the other sequences present in the primary tumor, skin sample, and relapse tumor sample. *See, e.g.*, Ex. 1012 (Ley) at 69 (Fig. 3). Quackenbush ¶¶113-114.

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

'783 Petition at 27

’783 Patent Claim 2

2. The method of claim **1**, wherein said at least one biological sample comprises a plurality of biological samples.

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

EX1001 (’783 Patent) at Claim 2

The Petition: Relies On Same Samples

The ’783 Petition

2. Claim 2

Claim 2 recites, “The method of claim 1, wherein said at least one biological sample comprises a plurality of biological samples.”

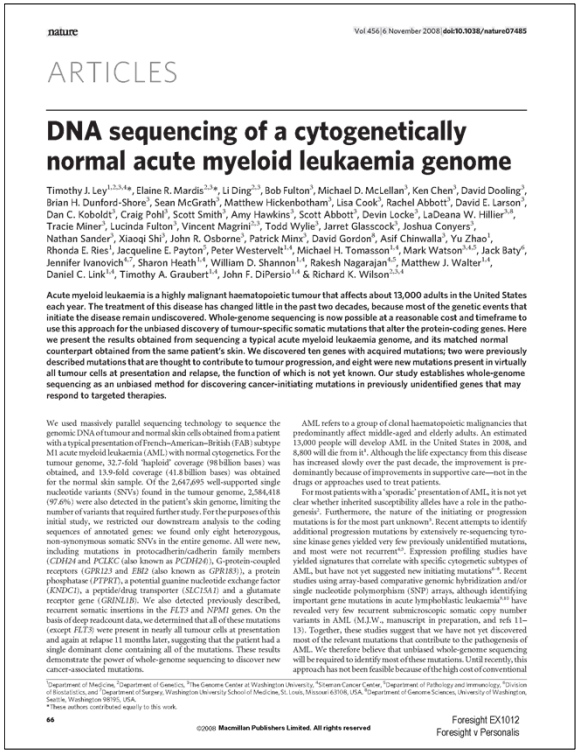
Ley performs its methods on at least the primary tumor sample, the relapse tumor sample, and the skin biopsy sample. *See, e.g.,* Ex. 1012, Fig. 3. Quackenbush ¶¶132-134.

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

’783 Petition at 35

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Ley: Patient Was Deceased



nature

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ARTICLES

DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome

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Acute myeloid leukaemia is a highly malignant haematopoietic tumour that affects about 13,000 adults in the United States each year. The treatment of this disease has changed little in the past two decades, because most of the genetic events that initiate the disease remain undiscovered. Whole-genome sequencing is now possible at a reasonable cost and timeframe to use this approach for the unbiased discovery of tumour-specific somatic mutations that alter the protein-coding genes. Here we present the results obtained from sequencing a typical acute myeloid leukaemia genome, and its matched normal counterpart obtained from the same patient's skin. We discovered ten genes with acquired mutations; two were previously described mutations that are thought to contribute to tumour progression, and eight were new mutations present in virtually all tumour cells at presentation and relapse, the function of which is not yet known. Our study establishes whole-genome sequencing as an unbiased method for discovering cancer-initiating mutations in previously unidentified genes that may respond to targeted therapies.

We used massively parallel sequencing technology to sequence the genomic DNA of tumour and normal skin cells obtained from a patient with a typical presentation of French–American–British (FAB) subtype M1 acute myeloid leukaemia (AML) with normal cytogenetics. For the tumour genome, 32.7-fold 'haploid' coverage (98 billion bases) was obtained, and 13.9-fold coverage (41.8 billion bases) was obtained for the normal skin sample. Of the 2,647,695 well-supported single nucleotide variants (SNVs) found in the tumour genome, 2,584,418 (97.6%) were also detected in the patient's skin genome, limiting the number of variants that required further study. For the purposes of this initial study, we restricted our downstream analysis to the coding sequences of annotated genes: we found only eight heterozygous, non-synonymous somatic SNVs in the entire genome. All were new, including mutations in protocadherin/cadherin family members (*CDH24* and *PCLKC* (also known as *PCDH26*)), G-protein-coupled receptors (*GPR123* and *EBI2* (also known as *GPR183*)), a protein phosphatase (*PTPRT*), a potential guanine nucleotide exchange factor (*KNDC1*), a peptide/drug transporter (*SLC15A1*) and a glutamate receptor gene (*GRINL1B*). We also detected previously described, recurrent somatic insertions in the *FLT3* and *NPM1* genes. On the basis of deep readcount data, we determined that all of these mutations (except *FLT3*) were present in nearly all tumour cells at presentation and again at relapse 11 months later, suggesting that the patient had a single dominant clone containing all of the mutations. These results demonstrate the power of whole-genome sequencing to discover new cancer-associated mutations.

AML refers to a group of clonal haematopoietic malignancies that predominantly affect middle-aged and elderly adults. An estimated 13,000 people will develop AML in the United States in 2008, and 8,800 will die from it[1]. Although the life expectancy from this disease has increased slowly over the past decade, the improvement is predominantly because of improvements in supportive care—not in the drugs or approaches used to treat patients.

For most patients with a 'sporadic' presentation of AML, it is not yet clear whether inherited susceptibility alleles have a role in the pathogenesis[2]. Furthermore, the nature of the initiating or progression mutations is for the most part unknown[3]. Recent attempts to identify additional progression mutations by extensively re-sequencing tyrosine kinase genes yielded very few previously unidentified mutations, and most were not recurrent[4,5]. Expression profiling studies have yielded signatures that correlate with specific cytogenetic subtypes of AML, but have not yet suggested new initiating mutations[6–8]. Recent studies using array-based comparative genomic hybridization and/or single nucleotide polymorphism (SNP) arrays, although identifying important gene mutations in acute lymphoblastic leukaemia[9,10] have revealed very few recurrent submicroscopic somatic copy number variants in AML (M.J.W., manuscript in preparation, and refs 11–13). Together, these studies suggest that we have not yet discovered most of the relevant mutations that contribute to the pathogenesis of AML. We therefore believe that unbiased whole-genome sequencing will be required to identify most of these mutations. Until recently, this approach has not been feasible because of the high cost of conventional

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*These authors contributed equally to this work.

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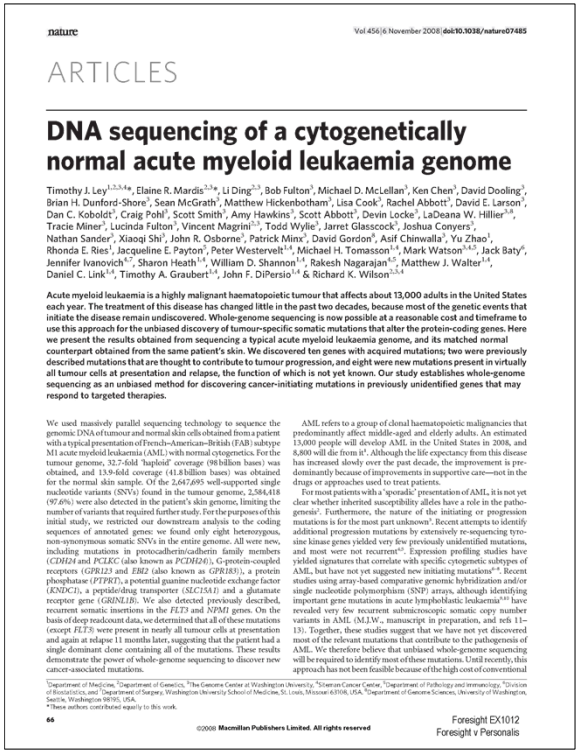
Foresight EX1012
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clearance of circulating blasts. She was then successively treated with azacytidine, low dose cytarabine, and decitabine, again without appreciable response, and expired 24 months after her initial diagnosis. Informed consent for whole genome sequencing was subsequently obtained from her next of kin and family members, using a revised Washington University Human Studies Committee-approved protocol that specifically addresses privacy and confidentiality issues associated with whole genome sequencing (HSC #01-1014, Amendment # 17).

EX1012; EX1042 (Ley Suppl Info) at 2

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 25, '611 POR at 26)

Ley: Patient Was Deceased



nature

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ARTICLES

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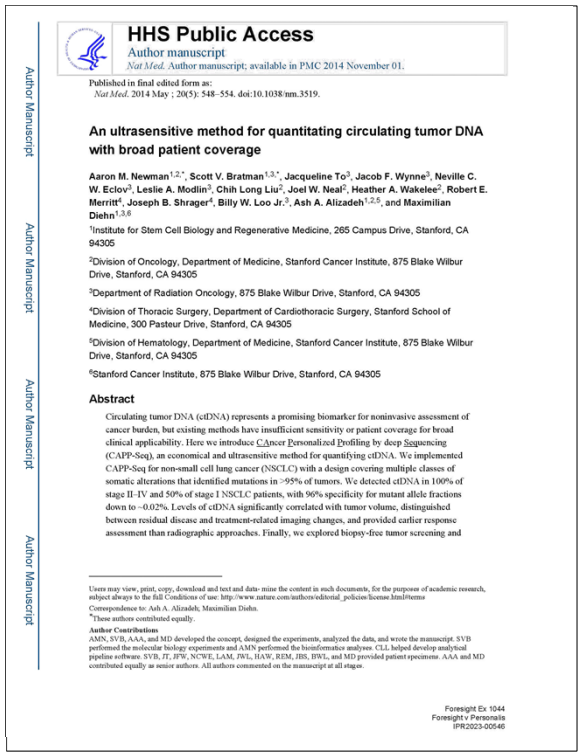
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patient identity is restricted. Subsequent to the death of patient 933124, the WU HSC approved an amendment to the protocol that incorporated language specifically permitting the use of banked DNA for whole genome sequencing, to which her husband consented. The patient's sister also consented to

EX1012; EX1042 (Ley Suppl Info) at 3

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 25, '611 POR at 42)

Newman: Repeated Sampling Of Tumors Is Invasive



HHS Public Access

Author manuscript

Nat Med. Author manuscript; available in PMC 2014 November 01.

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An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage

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Abstract

Circulating tumor DNA (ctDNA) represents a promising biomarker for noninvasive assessment of cancer burden, but existing methods have insufficient sensitivity or patient coverage for broad clinical applicability. Here we introduce CAncer Personalized Profiling by deep Sequencing (CAPP-Seq), an economical and ultrasensitive method for quantifying ctDNA. We implemented CAPP-Seq for non-small cell lung cancer (NSCLC) with a design covering multiple classes of somatic alterations that identified mutations in >95% of tumors. We detected ctDNA in 100% of stage II–IV and 50% of stage I NSCLC patients, with 96% specificity for mutant allele fractions down to ~0.02%. Levels of ctDNA significantly correlated with tumor volume, distinguished between residual disease and treatment-related imaging changes, and provided earlier response assessment than radiographic approaches. Finally, we explored biopsy-free tumor screening and

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Correspondence to: Ash A. Alizadeh, Maximilian Diehn.

*These authors contributed equally.

Author Contributions

AMN, SVB, AAA, and MD developed the concept, designed the experiments, analyzed the data, and wrote the manuscript. SVB performed the molecular biology experiments and AMN performed the bioinformatics analyses. CLL helped develop analytical pipeline software. SVB, JT, JFW, NCWE, LAM, JWL, HAW, REM, JBS, BWL, and MD provided patient specimens. AAA and MD contributed equally as senior authors. All authors commented on the manuscript at all stages.

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> Noninvasive access to malignant DNA is particularly attractive for solid tumors, which cannot be repeatedly sampled without invasive procedures. In NSCLC, PCR-based assays

EX1044 (Newman) at 2

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 36, '611 POR at 57)

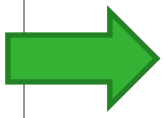
Ley Fails To Disclose Or Render Obvious Multiple Claim Elements In The ’783 patent

- No “fixed” and “variable” portions
- No “additional biological sample”
- No “identifying” of variants
- No “generating a report”

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Ley Does Not "Identify" Variants

1. A method for personalized genetic testing, comprising:
(a) obtaining a personalized probe set for a subject, which personalized probe set comprises a plurality of nucleic acid probe molecules having a plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising a set of one or more genetic variants over other sequences in at least one biological sample of said subject, wherein said set of genetic variants are identified in sequence data for said at least one biological sample, further wherein said personalized probe set comprises nucleic acid probe molecules having oligonucleotide-directed genomic content comprising: (i) at least one variable portion from a result of said sequence data and (ii) at least one fixed portion independent of said result of said sequencing data
(b) using said personalized probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in at least an additional biological sample from said subject, to generate a sequencing library;
(c) subjecting said sequencing library to sequencing to identify a presence or absence of at least a subset of said set of genetic variants in at least said additional biological sample from said subject, wherein a sequencing footprint of said sequencing of (c) is less than a sequencing footprint of said sequence data of (a); and
(d) generating a report identifying (i) a presence or absence of a health condition or disease of the subject in said additional biological sample based on said identified at least a subset of (c) and (ii) genetic variants which inform a therapy choice or a change in therapy for the subject.

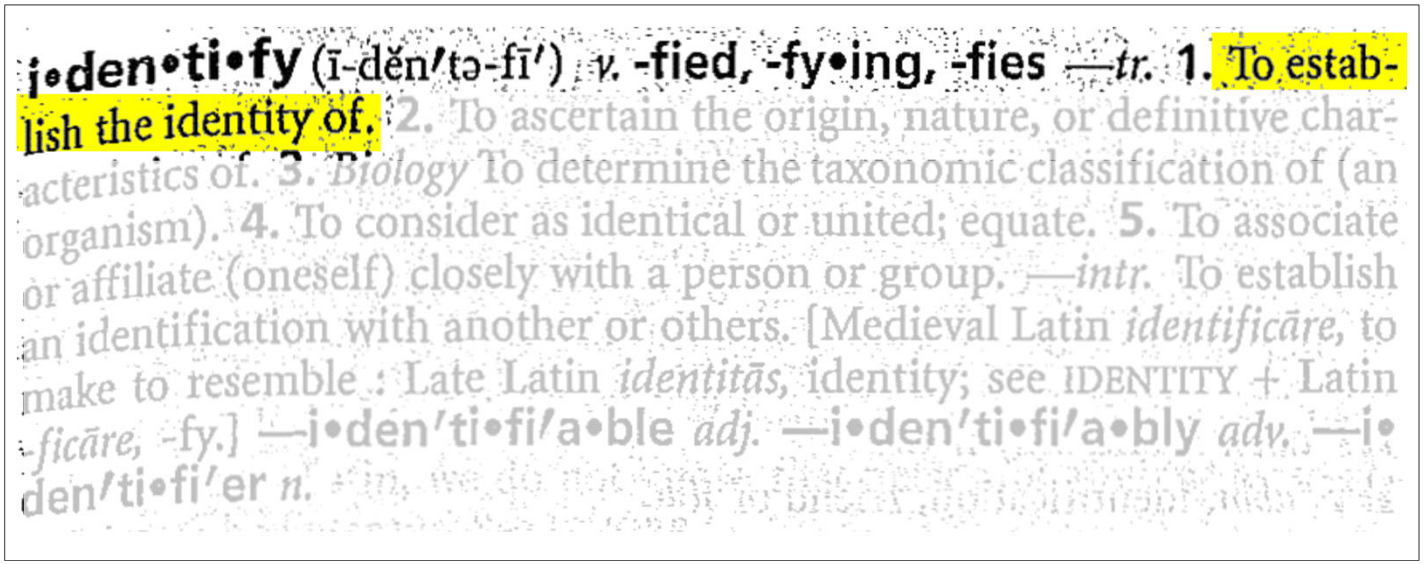


(c) subjecting said sequencing library to sequencing to identify a presence or absence of at least a subset of said set of genetic variants in at least said additional biological sample from said subject, wherein a sequencing footprint of said sequencing of (c) is less than a sequencing footprint of said sequence data of (a); and

EX1001 ('783 Patent) at claim 1

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 4-5)

Identify: To Establish The Identity Of



i•den•ti•fy (ī-dĕn′tə-fī′) *v.* **-fied, -fy•ing, -fies** —*tr.* **1.** To establish the identity of. **2.** To ascertain the origin, nature, or definitive characteristics of. **3.** *Biology* To determine the taxonomic classification of (an organism). **4.** To consider as identical or united; equate. **5.** To associate or affiliate (oneself) closely with a person or group. —*intr.* To establish an identification with another or others. [Medieval Latin *identificāre,* to make to resemble : Late Latin *identitās,* identity; see IDENTITY + Latin *-ficāre,* -fy.] —**i•den′ti•fi′a•ble** *adj.* —**i•den′ti•fi′a•bly** *adv.* —**i•den′ti•fi′er** *n.*

EX2170 at 871

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 20, '611 POR at 16)

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Law: Plain Meaning Governs Absent Lexicography Or Disavowal

"Absent lexicography or disavowal, we do not depart from the plain meaning of claims."

Luminara Worldwide, LLC v. Liown Elecs. Co., 814 F.3d 1343, 1353 (Fed. Cir. 2016)

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

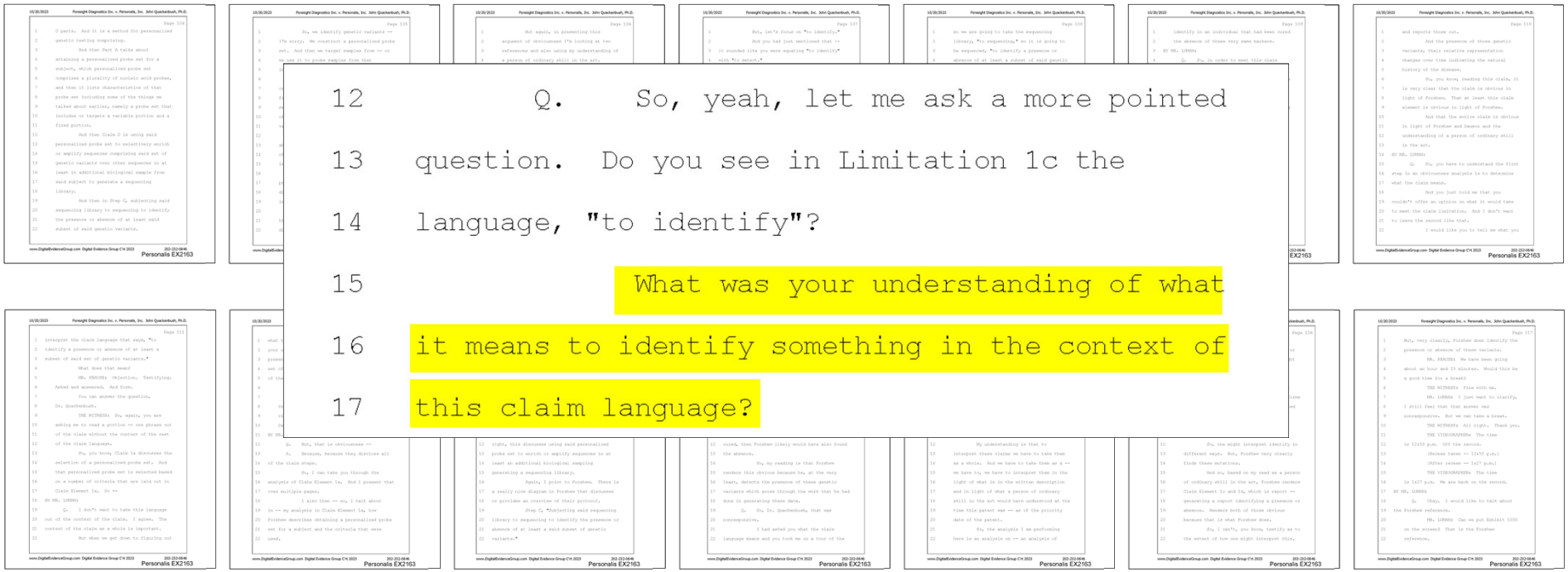
Dr. Quackenbush: Nonresponsive Testimony

Q. So, yeah, let me ask a more pointed question. Do you see in Limitation 1c the language, "to identify"? What was your understanding of what it means to identify something in the context of this claim language?

EX2163 (10/20/23 Quackenbush Depo) at 103:12-17, 104-117

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 20, '611 POR at 15)

Dr. Quackenbush: Nonresponsive Testimony

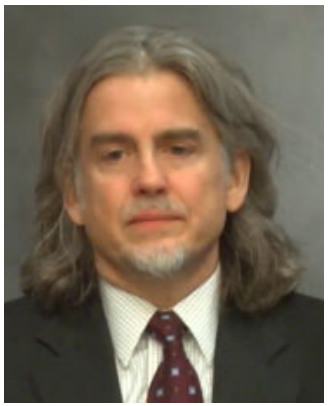


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DEMONSTRATIVE EXHIBIT – NOT
EVIDENCE (’783 POR at 20, ‘611 POR at 15)

Dr. Quackenbush: Cannot Explain Meaning Of “Identify”



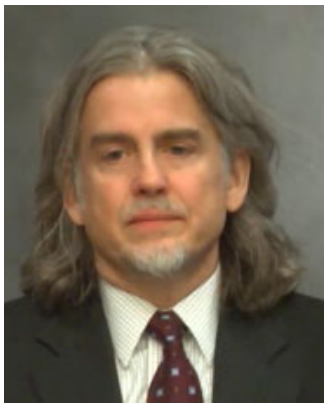
John Quackenbush
Foresight’s Expert

A. So, we are not talking about infringement here. So, I can't offer an opinion on what it would take to meet the claim limitation.

EX2163 (10/20/23 Quackenbush Depo) at 109:9-12

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE (’783 POR at 20, ‘611 POR at 15)

Dr. Quackenbush: Cannot Explain Meaning Of “Identify”



John Quackenbush
Foresight’s Expert

A. So, I can't set out the breadth or width or length of what "to identify" might mean in all possible contexts.

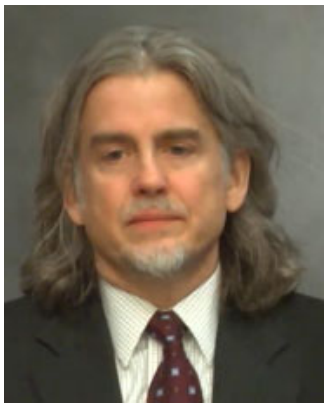
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A. So, I can't, you know, testify as to the extent of how one might interpret this.

EX2163 (10/20/23 Quackenbush Depo) at 116:2-4, 116:21-22

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE (’783 POR at 20, ‘611 POR at 15)

Dr. Quackenbush: Cannot Explain Meaning Of "Identify"



John Quackenbush
Foresight's Expert

Q. But when we get down to figuring out what the language in Limitation 1c means, what is your understanding of what it means to identify a presence or absence of at least a subset of said set of genetic variants within the context of all of the claim language?

A. So, in the context of the entire claim, the claim is obvious in view of – based on Forshew in view of Dawson.

EX2163 (10/20/23 Quackenbush Depo) at 111:22-112:5

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 20, '611 POR at 15)

Petitioner Relies On "Validation," Not "Identification"

The '783 Petition

> Once the variants of interest, i.e., the "putative variants," were identified, ***Ley*** then describes "validation using custom PCR and capillary sequencing" as well as sequencing of "validated somatic mutations" "by Roche/454 sequencing of PCR-generated amplicons made from primary genomic DNA…." Ex. 1012 at

'783 Petition at 25

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DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Ley: Mutations Quantified During Validation

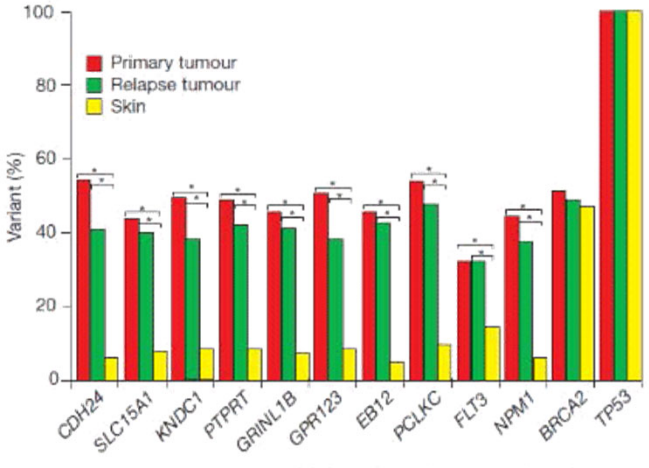


Figure 3 | Summary of Roche/454 FLX readcount data obtained for ten somatic mutations and two validated SNPs in the primary tumour, relapse tumour and skin specimens. The readcount data for the variant alleles in the primary tumour sample and relapse tumour sample are statistically different from that of the skin sample for all mutations ($P < 0.000001$ for all mutations, Fisher's exact test, denoted by a single asterisk in all cases). Note that the normal skin sample was contaminated with leukaemic cells containing the somatic mutations. The patient's white blood cell count was 105,000 (85% blasts) when the skin punch biopsy was obtained.

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 22, '611 POR at 17)

EX1012 (Ley) at 69

Personalis 64

Ley Fails To Disclose Or Render Obvious Multiple Claim Elements In The ’783 patent

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EX1001 ('783 Patent) at claim 1

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DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 4-5)

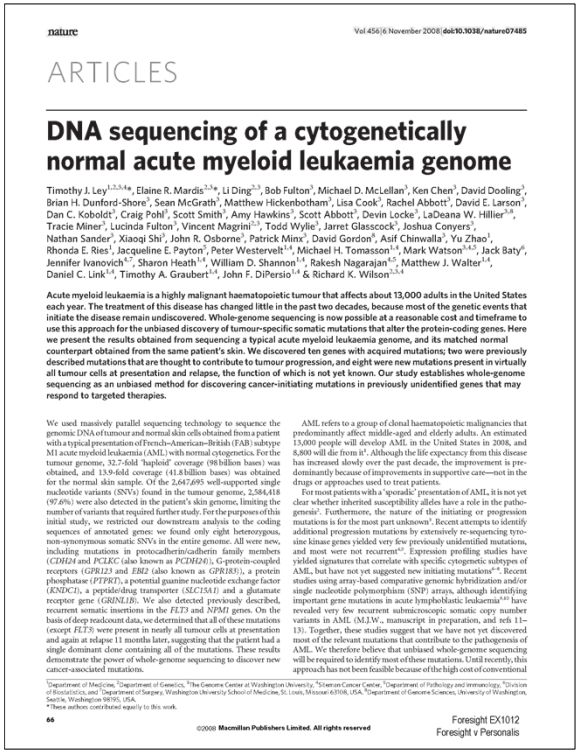
Law: Printed Matter May Have Patentable Weight

"Printed matter that is functionally related to its substrate is given patentable weight."

Praxair Distribution, Inc. v. Mallinckrodt Hospital Products IP, 890 F.3d 1024, 1032 (Fed. Cir. 2018)

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nature

Vol 456|6 November 2008|doi:10.1038/nature07485

ARTICLES

DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome

Timothy J. Ley[1,2,3,4]*, Elaine R. Mardis[2,3]*, Li Ding[2,3], Bob Fulton[3], Michael D. McLellan[3], Ken Chen[3], David Dooling[2], Brian H. Dunford-Shore[3], Sean McGrath[3], Matthew Hickenbotham[3], Lisa Cook[3], Rachel Abbott[3], David E. Larson[2], Dan C. Koboldt[2], Craig Pohl[3], Scott Smith[3], Amy Hawkins[3], Scott Abbott[3], Devin Locke[3], LaDeana W. Hillier[2,3], Tracie Miner[3], Lucinda Fulton[3], Vincent Magrini[2,3], Todd Wylie[3], Jarret Glasscock[3], Joshua Conyers[3], Nathan Sander[3], Xiaoqi Shi[3], John R. Osborne[3], Patrick Minx[3], David Gordon[3], Asif Chinwalla[3], Yu Zhao[1], Rhonda E. Ries[1], Jacqueline E. Payton[1], Peter Westervelt[1,4], Michael H. Tomasson[1,4], Mark Watson[1,4,5], Jack Baty[6], Jennifer Ivanovich[4,7], Sharon Heath[1,4], William D. Shannon[1,4], Rakesh Nagarajan[4,5], Matthew J. Walter[1,4], Daniel C. Link[1,4], Timothy A. Graubert[1,4], John F. DiPersio[1,4] & Richard K. Wilson[2,3,4]

Acute myeloid leukaemia is a highly malignant haematopoietic tumour that affects about 13,000 adults in the United States each year. The treatment of this disease has changed little in the past two decades, because most of the genetic events that initiate the disease remain undiscovered. Whole-genome sequencing is now possible at a reasonable cost and timeframe to use this approach for the unbiased discovery of tumour-specific somatic mutations that alter the protein-coding genes. Here we present the results obtained from sequencing a typical acute myeloid leukaemia genome, and its matched normal counterpart obtained from the same patient's skin. We discovered ten genes with acquired mutations; two were previously described mutations that are thought to contribute to tumour progression, and eight were new mutations present in virtually all tumour cells at presentation and relapse, the function of which is not yet known. Our study establishes whole-genome sequencing as an unbiased method for discovering cancer-initiating mutations in previously unidentified genes that may respond to targeted therapies.

We used massively parallel sequencing technology to sequence the genomic DNA of tumour and normal skin cells obtained from a patient with a typical presentation of French–American–British (FAB) subtype M1 acute myeloid leukaemia (AML) with normal cytogenetics. For the tumour genome, 32.7-fold 'haploid' coverage (98 billion bases) was obtained, and 13.9-fold coverage (41.8 billion bases) was obtained for the normal skin sample. Of the 2,647,695 well-supported single nucleotide variants (SNVs) found in the tumour genome, 2,584,418 (97.6%) were also detected in the patient's skin genome, limiting the number of variants that required further study. For the purposes of this initial study, we restricted our downstream analysis to the coding sequences of annotated genes: we found only eight heterozygous, non-synonymous somatic SNVs in the entire genome. All were new, including mutations in protocadherin/cadherin family members (*CDH24* and *PCLKC* (also known as *PCDH26*)), G-protein-coupled receptors (*GPR123* and *EBI2* (also known as *GPR183*)), a protein phosphatase (*PTPRT*), a potential guanine nucleotide exchange factor (*KNDC1*), a peptide/drug transporter (*SLC15A1*) and a glutamate receptor gene (*GRINL1B*). We also detected previously described, recurrent somatic insertions in the *FLT3* and *NPM1* genes. On the basis of deep readcount data, we determined that all of these mutations (except *FLT3*) were present in nearly all tumour cells at presentation and again at relapse 11 months later, suggesting that the patient had a single dominant clone containing all of the mutations. These results demonstrate the power of whole-genome sequencing to discover new cancer-associated mutations.

AML refers to a group of clonal haematopoietic malignancies that predominantly affect middle-aged and elderly adults. An estimated 13,000 people will develop AML in the United States in 2008, and 8,800 will die from it[1]. Although the life expectancy from this disease has increased slowly over the past decade, the improvement is predominantly because of improvements in supportive care—not in the drugs or approaches used to treat patients.

For most patients with a 'sporadic' presentation of AML, it is not yet clear whether inherited susceptibility alleles have a role in the pathogenesis[2]. Furthermore, the nature of the initiating or progression mutations is for the most part unknown[3]. Recent attempts to identify additional progression mutations by extensively re-sequencing tyrosine kinase genes yielded very few previously unidentified mutations, and most were not recurrent[4,5]. Expression profiling studies have yielded signatures that correlate with specific cytogenetic subtypes of AML, but have not yet suggested new initiating mutations[6–8]. Recent studies using array-based comparative genomic hybridization and/or single nucleotide polymorphism (SNP) arrays, although identifying important gene mutations in acute lymphoblastic leukaemia[9,10] have revealed very few recurrent submicroscopic somatic copy number variants in AML (M.J.W., manuscript in preparation, and refs 11–13). Together, these studies suggest that we have not yet discovered most of the relevant mutations that contribute to the pathogenesis of AML. We therefore believe that unbiased whole-genome sequencing will be required to identify most of these mutations. Until recently, this approach has not been feasible because of the high cost of conventional

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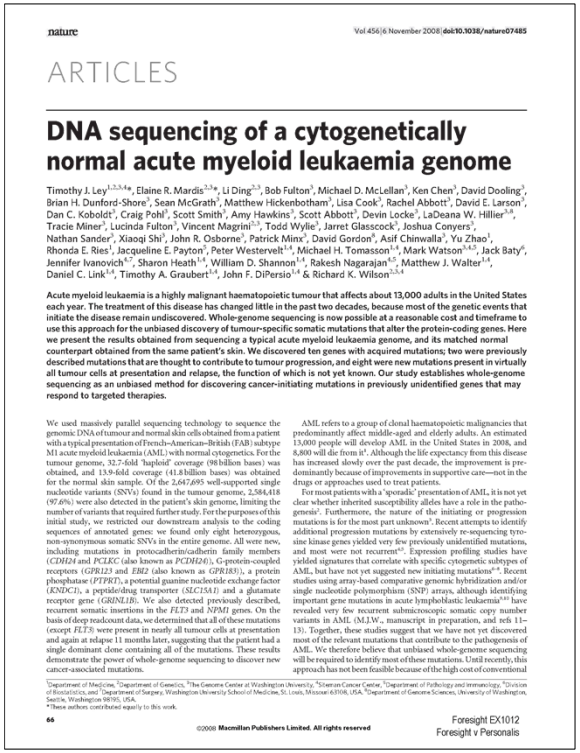
Foresight EX1012
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clearance of circulating blasts. She was then successively treated with azacytidine, low dose cytarabine, and decitabine, again without appreciable response, and expired 24 months after her initial diagnosis. Informed consent for whole genome sequencing was subsequently obtained from her next of kin and family members, using a revised Washington University Human Studies Committee-approved protocol that specifically addresses privacy and confidentiality issues associated with whole genome sequencing (HSC #01-1014, Amendment # 17).

EX1012; EX1042 (Ley Suppl Info) at 2

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 25, '611 POR at 26)

Ley: Patient Was Deceased



nature

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*These authors contributed equally to this work.

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patient identity is restricted. Subsequent to the death of patient 933124, the WU HSC approved an amendment to the protocol that incorporated language specifically permitting the use of banked DNA for whole genome sequencing, to which her husband consented. The patient's sister also consented to

EX1012; EX1042 (Ley Suppl Info) at 3

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 25, '611 POR at 43)

’783 Patent Claims 3, 8, 15, 16

3. The method of claim **2**, wherein said plurality of biological samples comprises a tissue sample and a plasma sample.

8. The method of claim **1**, wherein said additional biological sample of (c) is a blood sample.

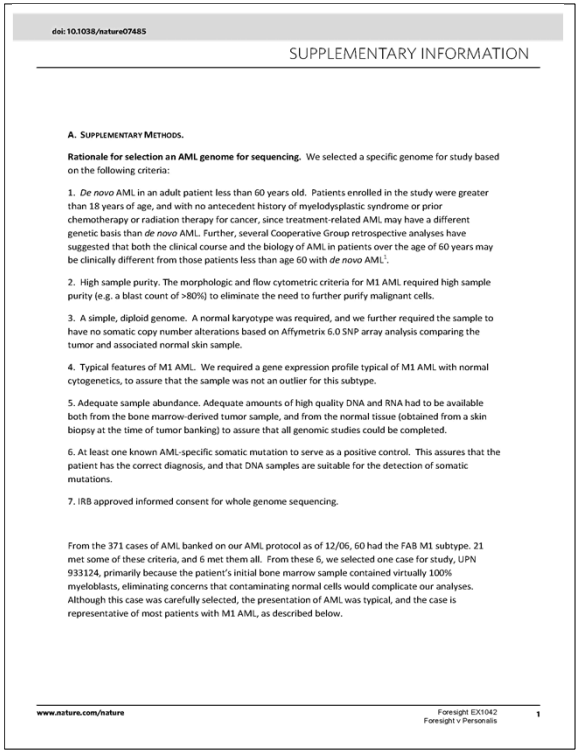
15. The method of claim **1**, wherein said additional biological sample is a blood sample comprising: (i) cfDNA, (ii) cfRNA, (iii) DNA or RNA from circulating tumor cells, or (iv) RNA or DNA from a tumor metastasis.

16. The method of claim **1**, wherein said additional biological sample and one or more subsequent biological samples are each blood samples comprising: (i) cfDNA, (ii) cfRNA, (iii) DNA or RNA from circulating tumor cells, or (iv) RNA or DNA from a tumor metastasis.

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EX1001 (’783 Patent) at claims 3, 8, 15, 16

Ley: Calls For "High Sample Purity"



doi: 10.1038/nature07485

A. SUPPLEMENTARY METHODS.

Rationale for selection an AML genome for sequencing. We selected a specific genome for study based on the following criteria:

1. *De novo* AML in an adult patient less than 60 years old. Patients enrolled in the study were greater than 18 years of age, and with no antecedent history of myelodysplastic syndrome or prior chemotherapy or radiation therapy for cancer, since treatment-related AML may have a different genetic basis than *de novo* AML. Further, several Cooperative Group retrospective analyses have suggested that both the clinical course and the biology of AML in patients over the age of 60 years may be clinically different from those patients less than age 60 with *de novo* AML[1].

2. High sample purity. The morphologic and flow cytometric criteria for M1 AML required high sample purity (e.g. a blast count of >80%) to eliminate the need to further purify malignant cells.

3. A simple, diploid genome. A normal karyotype was required, and we further required the sample to have no somatic copy number alterations based on Affymetrix 6.0 SNP array analysis comparing the tumor and associated normal skin sample.

4. Typical features of M1 AML. We required a gene expression profile typical of M1 AML with normal cytogenetics, to assure that the sample was not an outlier for this subtype.

5. Adequate sample abundance. Adequate amounts of high quality DNA and RNA had to be available both from the bone marrow-derived tumor sample, and from the normal tissue (obtained from a skin biopsy at the time of tumor banking) to assure that all genomic studies could be completed.

6. At least one known AML-specific somatic mutation to serve as a positive control. This assures that the patient has the correct diagnosis, and that DNA samples are suitable for the detection of somatic mutations.

7. IRB approved informed consent for whole genome sequencing.

From the 371 cases of AML banked on our AML protocol as of 12/06, 60 had the FAB M1 subtype. 21 met some of these criteria, and 6 met them all. From these 6, we selected one case for study, UPN 933124, primarily because the patient's initial bone marrow sample contained virtually 100% myeloblasts, eliminating concerns that contaminating normal cells would complicate our analyses. Although this case was carefully selected, the presentation of AML was typical, and the case is representative of most patients with M1 AML, as described below.

Foresight EX1042
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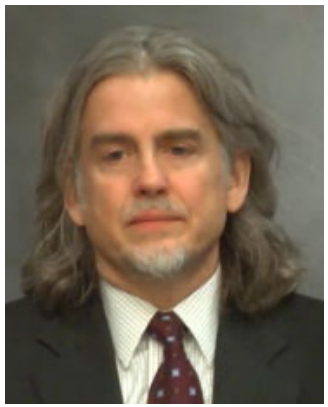
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EX1042 (Ley Suppl Info) at 1

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 36, '611 POR at 25)

Dr. Quackenbush: cfDNA is Contaminated



John Quackenbush
Foresight's Expert

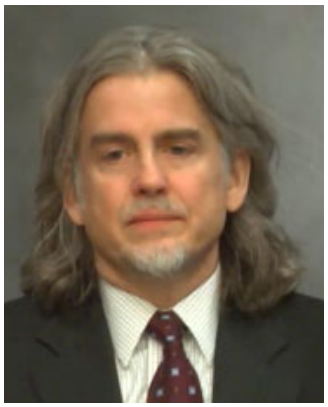
Dr. Quackenbush's '783 Declaration

86. Cell-free DNA ("cfDNA") refers to small fragments of DNA present in the bloodstream. This generally comes from DNA released during apoptosis, or the death of cells, in the body. During apoptosis, cells undergo various cell death processes. Part of that involves cellular mechanisms that cause the DNA of the cells' chromosomes to be cleaved and fragmented. The fragmented DNA, along with other parts of the cells, are released into the bloodstream, and eventually cleared out of the body with other bodily waste products.

EX1020 (Quackenbush '783 Decl) ¶ 86

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 40)

Dr. Quackenbush: cfDNA is Contaminated



John Quackenbush
Foresight's Expert

Q. So, there is a fair amount of cellular debris that enters the blood through apoptosis and cell death?

A. There are many things that enter the blood. If you look at the bloodstream itself, it is filled with cells, white blood cells, red blood cells, a variety of different proteins. And so this is releases additional proteins and nucleic acid into circulation.

EX2163 (10/20/23 Quackenbush Depo) at 12:20-13:6

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 40)

Petitioner: Ley Discloses Tissue, Not Blood

The ’783 Petition

> ***Ley*** discloses collecting samples from tissue samples, specifically a tumour sample. Ex. 1012 at 69. Additionally, those skilled in the art would have

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

’783 Petition at 49

Personalis 74

Petitioner: Ley Discloses Tissue, Not Blood

The ’783 Petition

biological sample of (c) is a blood sample.” ***Ley*** discloses collecting samples from tissue samples, specifically a tumor sample. Ex. 1012 at 69. Those skilled

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

’783 Petition at 51

Personalis 75

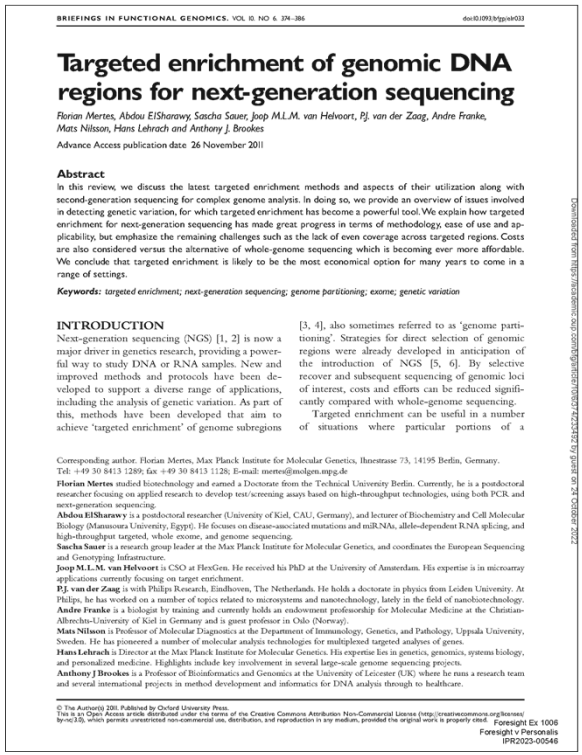
’783 Patent Claim 22

22. The method of claim **1**, wherein said personalized probe set comprises hybrid capture probes.

EX1001 (’783 Patent) at claim 22

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Mertes: Capture Techniques Categorized Based On "Reaction Principle"



BRIEFINGS IN FUNCTIONAL GENOMICS. VOL 10. NO 6. 374–386 doi:10.1093/bfgp/elr033

Targeted enrichment of genomic DNA regions for next-generation sequencing

Florian Mertes, Abdou ElSharawy, Sascha Sauer, Joop M.L.M. van Helvoort, P.J. van der Zaag, Andre Franke, Mats Nilsson, Hans Lehrach and Anthony J. Brookes

Advance Access publication date 26 November 2011

Abstract

In this review, we discuss the latest targeted enrichment methods and aspects of their utilization along with second-generation sequencing for complex genome analysis. In doing so, we provide an overview of issues involved in detecting genetic variation, for which targeted enrichment has become a powerful tool. We explain how targeted enrichment for next-generation sequencing has made great progress in terms of methodology, ease of use and applicability, but emphasize the remaining challenges such as the lack of even coverage across targeted regions. Costs are also considered versus the alternative of whole-genome sequencing which is becoming ever more affordable. We conclude that targeted enrichment is likely to be the most economical option for many years to come in a range of settings.

Keywords: *targeted enrichment; next-generation sequencing; genome partitioning; exome; genetic variation*

INTRODUCTION

Next-generation sequencing (NGS) [1, 2] is now a major driver in genetics research, providing a powerful way to study DNA or RNA samples. New and improved methods and protocols have been developed to support a diverse range of applications, including the analysis of genetic variation. As part of this, methods have been developed that aim to achieve 'targeted enrichment' of genome subregions [3, 4], also sometimes referred to as 'genome partitioning'. Strategies for direct selection of genomic regions were already developed in anticipation of the introduction of NGS [5, 6]. By selective recover and subsequent sequencing of genomic loci of interest, costs and efforts can be reduced significantly compared with whole-genome sequencing.

Targeted enrichment can be useful in a number of situations where particular portions of a

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Florian Mertes studied biotechnology and earned a Doctorate from the Technical University Berlin. Currently, he is a postdoctoral researcher focusing on applied research to develop test/screening assays based on high-throughput technologies, using both PCR and next-generation sequencing.

Abdou ElSharawy is a postdoctoral researcher (University of Kiel, CAU, Germany), and lecturer of Biochemistry and Cell Molecular Biology (Mansoura University, Egypt). He focuses on disease-associated mutations and miRNAs, allele-dependent RNA splicing, and high-throughput targeted, whole exome, and genome sequencing.

Sascha Sauer is a research group leader at the Max Planck Institute for Molecular Genetics, and coordinates the European Sequencing and Genotyping Infrastructure.

Joop M.L.M. van Helvoort is CSO at FlexGen. He received his PhD at the University of Amsterdam. His expertise is in microarray applications currently focusing on target enrichment.

P.J. van der Zaag is with Philips Research, Eindhoven, The Netherlands. He holds a doctorate in physics from Leiden University. At Philips, he has worked on a number of topics related to microsystems and nanotechnology, lately in the field of nanobiotechnology.

Andre Franke is a biologist by training and currently holds an endowment professorship for Molecular Medicine at the Christian-Albrechts-University of Kiel in Germany and is guest professor in Oslo (Norway).

Mats Nilsson is Professor of Molecular Diagnostics at the Department of Immunology, Genetics, and Pathology, Uppsala University, Sweden. He has pioneered a number of molecular analysis technologies for multiplexed targeted analyses of genes.

Hans Lehrach is Director at the Max Planck Institute for Molecular Genetics. His expertise lies in genetics, genomics, systems biology, and personalized medicine. Highlights include key involvement in several large-scale genome sequencing projects.

Anthony J Brookes is a Professor of Bioinformatics and Genomics at the University of Leicester (UK) where he runs a research team and several international projects in method development and informatics for DNA analysis through to healthcare.

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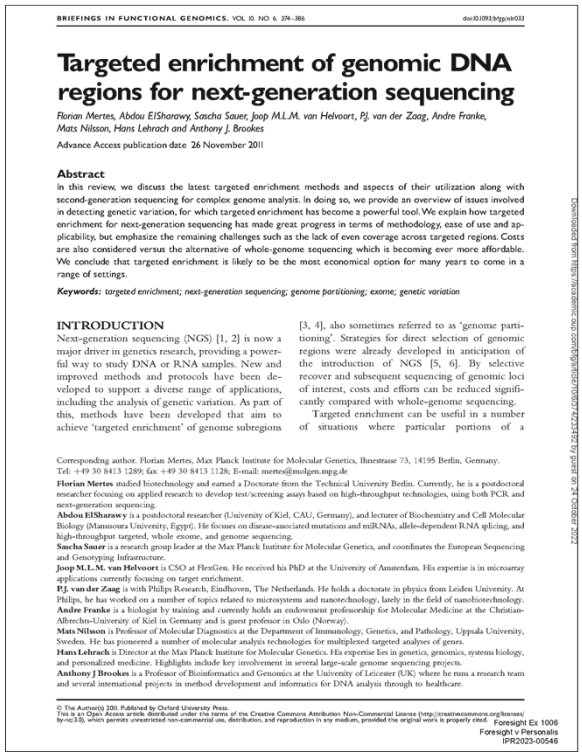
> Current techniques for targeted enrichment can be categorized according to the nature of their core reaction principle (Figure 1):

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 41; EX2169 ¶ 116)

EX1006 (Mertes) at 375

Personalis 77

Mertes: Capture Probes "Physically Capture"



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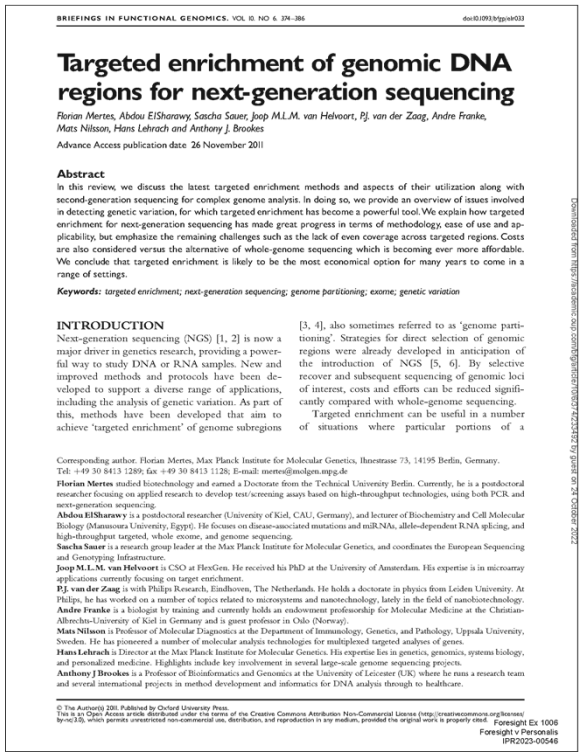
'Hybrid capture': wherein nucleic acid strands derived from the input sample are hybridized specifically to preprepared DNA fragments complementary to the targeted regions of interest, either in solution or on a solid support, so that one can physically capture and isolate the sequences of interest;

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 41; EX2169 ¶ 116)

EX1006 (Mertes) at 375

Personalis 78

Mertes: PCR Amplifies Regions



BRIEFINGS IN FUNCTIONAL GENOMICS. VOL 10. NO 6. 374–386 doi:10.1093/bfgp/elr033

Targeted enrichment of genomic DNA regions for next-generation sequencing

Florian Mertes, Abdou ElSharawy, Sascha Sauer, Joop M.L.M. van Helvoort, P.J. van der Zaag, Andre Franke, Mats Nilsson, Hans Lehrach and Anthony J. Brookes

Advance Access publication date 26 November 2011

Abstract

In this review, we discuss the latest targeted enrichment methods and aspects of their utilization along with second-generation sequencing for complex genome analysis. In doing so, we provide an overview of issues involved in detecting genetic variation, for which targeted enrichment has become a powerful tool. We explain how targeted enrichment for next-generation sequencing has made great progress in terms of methodology, ease of use and applicability, but emphasize the remaining challenges such as the lack of even coverage across targeted regions. Costs are also considered versus the alternative of whole-genome sequencing which is becoming ever more affordable. We conclude that targeted enrichment is likely to be the most economical option for many years to come in a range of settings.

Keywords: *targeted enrichment; next-generation sequencing; genome partitioning; exome; genetic variation*

INTRODUCTION

Next-generation sequencing (NGS) [1, 2] is now a major driver in genetics research, providing a powerful way to study DNA or RNA samples. New and improved methods and protocols have been developed to support a diverse range of applications, including the analysis of genetic variation. As part of this, methods have been developed that aim to achieve 'targeted enrichment' of genome subregions [3, 4], also sometimes referred to as 'genome partitioning'. Strategies for direct selection of genomic regions were already developed in anticipation of the introduction of NGS [5, 6]. By selective recover and subsequent sequencing of genomic loci of interest, costs and efforts can be reduced significantly compared with whole-genome sequencing.

Targeted enrichment can be useful in a number of situations where particular portions of a

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Florian Mertes studied biotechnology and earned a Doctorate from the Technical University Berlin. Currently, he is a postdoctoral researcher focusing on applied research to develop test/screening assays based on high-throughput technologies, using both PCR and next-generation sequencing.

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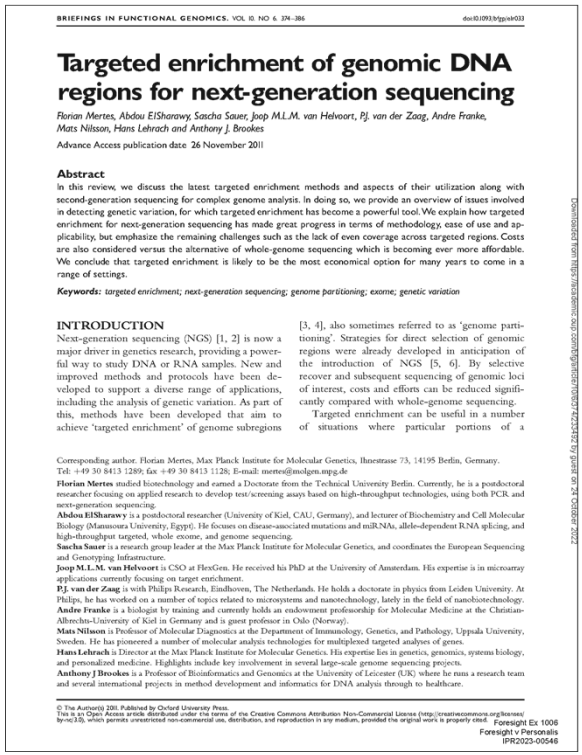
PCR amplification: wherein polymerase chain reaction (PCR) is directed toward the targeted regions of interest by conducting multiple long-range PCRs in parallel, a limited number of standard multiplex PCRs or highly multiplexed PCR methods that amplify very large numbers of short fragments.

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 41; EX2169 ¶ 116)

EX1006 (Mertes) at 375

Personalis 79

Mertes: Different Methods Yield Different Results



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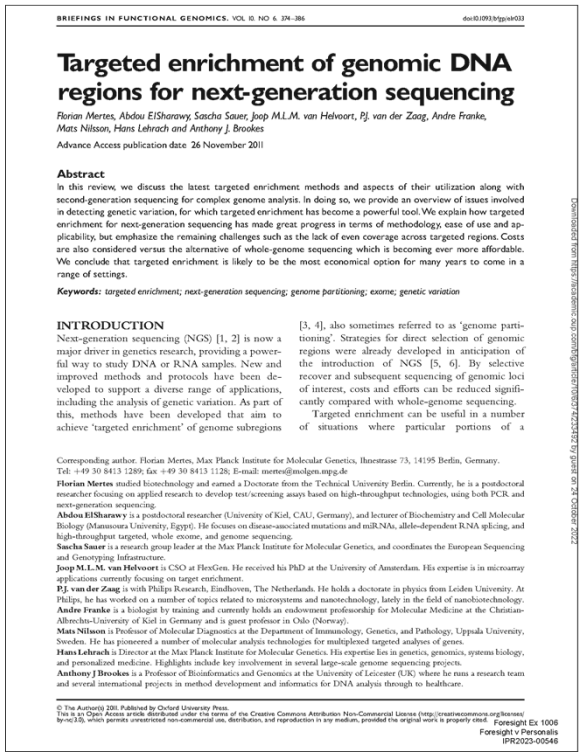
ods can be. Even for the same genetic locus, processed by the same people in the same laboratory, the different enrichment methods produce very different average coverage, evenness and specificity. All four

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 42; EX2169 ¶ 118)

EX1006 (Mertes) at 383

Personalis 80

Mertes: PCR And Capture Are For Different Applications



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Given the operational characteristics of these different targeted enrichment methods, they naturally vary in their suitability for different fields of application. For example, where many megabases needs

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EX1006 (Mertes) at 375

Personalis 81

Sholl: PCR And Capture Are "Orthogonal"



JCI insight

CLINICAL MEDICINE

Institutional implementation of clinical tumor profiling on an unselected cancer population

Lynette M. Sholl,[1] Khanh Do,[2,3] Priyanka Shivdasani,[1] Ethan Cerami,[4] Adrian M. Dubuc,[1] Frank C. Kuo,[1] Elizabeth P. Garcia,[1] Yonghui Jia,[1] Phani Davineni,[1] Ryan P. Abo,[1,5] Trevor J. Pugh,[4] Paul van Hummelen,[4] Aaron R. Thorner,[6] Matthew Ducar,[1,5] Alice H. Berger,[7,8] Mizuki Nishino,[9] Katherine A. Janeway,[9] Alanna Church,[10] Marian Harris,[10] Lauren L. Ritterhouse,[1] Joshua D. Campbell,[1] Vanesa Rojas-Rudilla,[1,2] Azra H. Ligon,[1] Shakti Ramkissoon,[1] James M. Cleary,[2,3] Ursula Matulonis,[2] Geoffrey R. Oxnard,[2] Richard Chao,[11] Vanessa Tassell,[11] James Christensen,[11] William C. Hahn,[2,5,7,12] Philip W. Kantoff,[12] David J. Kwiatkowski,[2] Bruce E. Johnson,[2] Matthew Meyerson,[1,2,5,7] Levi A. Garraway,[2,7,12] Geoffrey I. Shapiro,[2,3] Barrett J. Rollins,[2] Neal I. Lindeman,[1] and Laura E. MacConaill[1,5]

[1]Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts, USA. [2]Department of Medical Oncology, Dana-Farber Cancer Institute (DFCI), Boston, Massachusetts, USA. [3]Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts, USA. [4]Early Drug Discovery Center, [5]Department of Biostatistics and Computational Biology, and [6]Center for Cancer Genome Discovery, DFCI, Boston, Massachusetts, USA. [7]Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada. [8]Department of Medical Biophysics, University of Toronto, Ontario, Canada. [9]Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA. [10]Department of Radiology, DFCI and Brigham and Women's Hospital, Boston, Massachusetts, USA. [11]Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, Massachusetts, USA. [12]Department of Pathology, Boston Children's Hospital, Boston, Massachusetts, USA. [13]Mirati Therapeutics, San Diego, California. [14]Lank Center for Genitourinary Oncology and [15]Center for Cancer Precision Medicine, DFCI, Boston, Massachusetts, USA.

BACKGROUND. Comprehensive genomic profiling of a patient's cancer can be used to diagnose, monitor, and recommend treatment. Clinical implementation of tumor profiling in an enterprise-wide, unselected cancer patient population has yet to be reported.

METHODS. We deployed a hybrid-capture and massively parallel sequencing assay (OncoPanel) for all adult and pediatric patients at our combined cancer centers. Results were categorized by pathologists based on actionability. We report the results for the first 3,727 patients tested.

RESULTS. Our cohort consists of cancer patients unrestricted by disease site or stage. Across all consented patients, half had sufficient and available (>20% tumor) material for profiling; once specimens were received in the laboratory for pathology review, 73% were scored as adequate for genomic testing. When sufficient DNA was obtained, OncoPanel yielded a result in 96% of cases. 73% of patients harbored an actionable or informative alteration; only 19% of these represented a current standard of care for therapeutic stratification. The findings recapitulate those of previous studies of common cancers but also identify alterations, including in *AXL* and *EGFR*, associated with response to targeted therapies. In rare cancers, potentially actionable alterations suggest the utility of a "cancer-agnostic" approach in genomic profiling. Retrospective analyses uncovered contextual genomic features that may inform therapeutic response and examples where diagnoses revised by genomic profiling markedly changed clinical management.

CONCLUSIONS. Broad sequencing-based testing deployed across an unselected cancer cohort is feasible. Genomic results may alter management in diverse scenarios; however, additional barriers must be overcome to enable precision cancer medicine on a large scale.

FUNDING. This work was supported by DFCI, BWH, and the National Cancer Institute (5R33CA155554 and 5K23CA157631).

Conflict of interest: M. Nishino is a consultant for Bristol Myers Squibb. G.R. Oxnard is a consultant for AstraZeneca, Ariad, and Boehringer Ingelheim and has honoraria from AstraZeneca and Chugai. L.M. Sholl is a consultant for Genentech. M. Meyerson has research funding from Bayer and a patent on the use of EGFR mutation analysis for lung cancer diagnosis and is a founder of Foundation Medicine. L.A. Garraway is a founder, consultant, and equity holder in Foundation Medicine.

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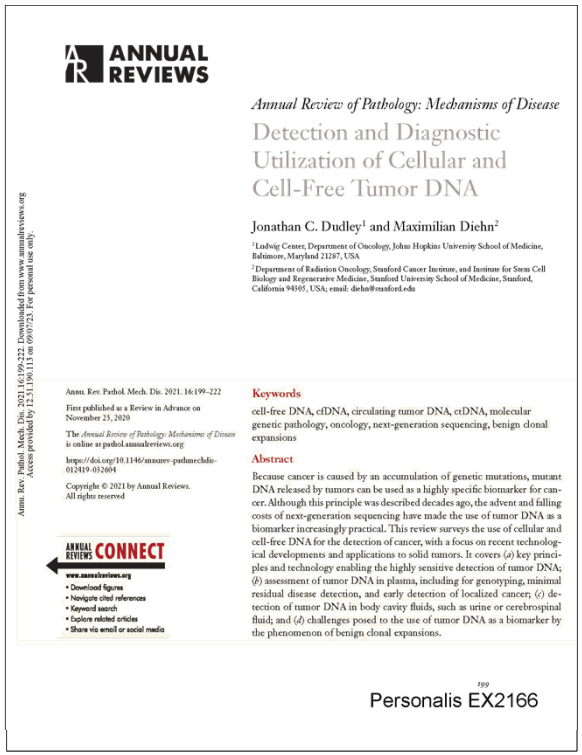
management. Single gene assays are performed by CLIA-validated methods using orthogonal technologies such as Sanger sequencing (*KIT, PDGFRA, ABL1*), pyrosequencing (*KRAS, BRAF, NRAS, NOTCH1*), allele-specific PCR or RT-PCR (*EGFR* codon 858, *KIT* codon 816, *JAK2* codon 617, *MYD88* codon 265, BCR-ABL1 b2a2 and b3a2), electrophoresis (*EGFR* exon 19, BCR-ABL1, PML-RARA, *IGH-BCL2*), as well as FISH, array comparative genomic hybridization (OncoCopy(28)) and karyotype analysis for a broad array of structural and copy number variants. Individual methods are available upon request. Where

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 41; EX2169 ¶ 116)

EX 2177 (Sholl) at 23-24

Personalis 82

Foresight's Founders: Distinguish Hybrid Capture And PCR



ANNUAL REVIEWS

Annual Review of Pathology: Mechanisms of Disease

Detection and Diagnostic Utilization of Cellular and Cell-Free Tumor DNA

Jonathan C. Dudley[1] and Maximilian Diehn[2]

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Keywords

cell-free DNA, cfDNA, circulating tumor DNA, ctDNA, molecular genetic pathology, oncology, next-generation sequencing, benign clonal expansions

Abstract

Because cancer is caused by an accumulation of genetic mutations, mutant DNA released by tumors can be used as a highly specific biomarker for cancer. Although this principle was described decades ago, the advent and falling costs of next-generation sequencing have made the use of tumor DNA as a biomarker increasingly practical. This review surveys the use of cellular and cell-free DNA for the detection of cancer, with a focus on recent technological developments and applications to solid tumors. It covers (*a*) key principles and technology enabling the highly sensitive detection of tumor DNA; (*b*) assessment of tumor DNA in plasma, including for genotyping, minimal residual disease detection, and early detection of localized cancer; (*c*) detection of tumor DNA in body cavity fluids, such as urine or cerebrospinal fluid; and (*d*) challenges posed to the use of tumor DNA as a biomarker by the phenomenon of benign clonal expansions.

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39, 40). An additional limitation is that hybrid capture–based workflows typically have longer turnaround times, since the target enrichment process often takes days (instead of hours with PCR), and are more expensive, since they involve sequencing a larger genomic space. Finally, despite the advantages of overcoming stochastic sampling error, the larger genomic space provided by hybrid capture strategies may provide diminishing additional value in some contexts (41).

EX2166 (Dudley) at 203

Personalis 83

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 41; EX2169 ¶ 117)

Foresight's Founders: Hybrid Capture Limited In Coverage



ELSEVIER

Seminars in RADIATION ONCOLOGY

Predicting Radiotherapy Responses and Treatment Outcomes Through Analysis of Circulating Tumor DNA

Aadel A. Chaudhuri, MD, PhD,* Michael S. Binkley, BA,* Evan C. Osmundson, MD, PhD,* Ash A. Alizadeh, MD, PhD,†,‡,§,¶ and Maximilian Diehn, MD, PhD*,§,¶

Tumors continually shed DNA into the blood where it can be detected as circulating tumor DNA (ctDNA). Although this phenomenon has been recognized for decades, techniques that are sensitive and specific enough to robustly detect ctDNA have only become available recently. Quantification of ctDNA represents a new approach for cancer detection and disease burden quantification that has the potential to revolutionize response assessment and personalized treatment in radiation oncology. Analysis of ctDNA has many potential applications, including detection of minimal residual disease following radiotherapy, non-invasive tumor genotyping, and early detection of tumor recurrence. Ultimately, ctDNA-based assays could lead to personalization of therapy based on identification of somatic alterations present in tumors and changes in ctDNA concentrations before and after treatment. In this review, we discuss methods of ctDNA detection and clinical applications of ctDNA-based biomarkers in radiation oncology, with a focus on recently developed techniques that use next-generation sequencing for ctDNA quantification.

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¶Stanford Cancer Institute, Stanford University.

M.D. and A.A. are inventors on patent applications related to the CAPP-Seq technology and are cofounders and consultants for CAPP Medical. No writing assistance was used in the production of this manuscript.

This work was supported by the US Department of Defense (M.D.), the US National Institutes of Health Director's New Innovator Award Program (M.D., 1-DP2-CA186569), the National Cancer Institute (M.D. and A.A.A., 1-R01-CA188298), the Ludwig Institute for Cancer Research (M.D. and A.A.A.), the CRK Faculty Scholar Fund (M.D.), and the Radiological Society of North America (E.C.O.)

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Address reprint requests to Maximilian Diehn, MD, PhD, Department of Radiation Oncology, Stanford Cancer Institute, 875 Blake Wilbur Dr, Stanford, CA 94305. E-mail: diehn@stanford.edu

Introduction

The field of radiation oncology is currently going through a revolution, as new technologies allow treatment of patients with more precision than ever before. Although much progress has been made in improving delivery of radiation therapy (RT), response assessment after treatment continues to rely primarily on imaging for most types of cancers. Although, imaging-based response assessment has significant clinical value, this approach cannot detect minimal residual disease (MRD), has a lag between treatment and when tumor responses can be detected, is expensive, and often exposes patients to additional ionizing radiation. For these reasons, there continues to be an unmet need for developing blood-based biomarkers for cancers that are treated with RT. Although such biomarkers exist for a few cancer types (eg, prostate-specific antigen for prostate cancer), they do not for most cancers. The ideal biomarker would be readily generalizable, such that it could be applied to all cancer types.

Although most work on cancer biomarkers has focused on detection of proteins, recently, circulating tumor DNA

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sensitivity. Limitations include that it is somewhat more time intensive than amplicon-based approaches are and that, similar to WES or the focused NGS-based methods, it can only detect alterations in genomic regions that are covered.

EX2165 (Chaudhuri) at 308

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 42; EX2169 ¶ 117)

Mamanova: Details Differences Between PCR And Hybrid Capture

Table 1 | Performance of target-enrichment methods

	PCR	MIP	On-array hybrid capture	In-solution hybrid capture
Cost	High	<10 samples, high; >100 samples, low	Medium	<10 samples, medium; >10 samples, low
Ease of use	Low	High	Medium	High
Mass DNA	~8 µg for 1 Mb of 2× tiled, 5 kb amplicons	As little as 200 ng	10-15 µg per array for up to 30 Mb target	3 µg for up to 30 Mb target
Sensitivity	>99.5%	>98%, with stringent design constraints	98.6% of CTR[a]	>99.5% of CTR[a]
Specificity	93% for HapMap DNA samples, 72% for whole genome–amplified samples	>98%	Up to 70% mapping to CTR[a] for exons; higher for contiguous regions	Up to 80% mapping to CTR[a] for exons; higher for contiguous regions
Uniformity	80% of bases within twofold range of median	58% of CTR within tenfold coverage range; 88% within 100-fold coverage range	60% of CTR[a] within 0.5–1.5-fold of mean coverage (mapping quality[b] 30)	61% of CTR[a] within 0.5–1.5-fold of mean coverage (mapping quality[b] 30)
Reproducibility	Up to 100%	0.92 rank-order correlation[c]	For 10^7 paired-end sequences, >95% reproducibility at tenfold between two samples	For 10^7 paired end-sequences, >96% reproducibility at tenfold between two samples

[a]CTR, capture target region, that is, the regions of the desired target region to which probes could be designed after repeat masking. [b]Mapping qualities were calculated by the mapping software, MAQ, and indicate the probability that the mapping location is correct. A score of 30 or greater indicates that the quality of a read was good, and that it mapped unambiguously to that location with few mismatches. [c]Rank-order correlation in capture efficiency distributions between independent samples.

EX1007 (Mamanova) at 114, Table 1

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 42; EX2169 ¶ 118)

Mamanova: Criticizes Capture Compared To PCR



REVIEW

Target-enrichment strategies for next-generation sequencing

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We have not yet reached a point at which routine sequencing of large numbers of whole eukaryotic genomes is feasible, and so it is often necessary to select genomic regions of interest and to enrich these regions before sequencing. There are several enrichment approaches, each with unique advantages and disadvantages. Here we describe our experiences with the leading target-enrichment technologies, the optimizations that we have performed and typical results that can be obtained using each. We also provide detailed protocols for each technology so that end users can find the best compromise between sensitivity, specificity and uniformity for their particular project.

The ability to read the sequence of bases that comprise a polynucleotide has had an impact on biological research that is difficult to overstate. For the majority of the past 30 years, dideoxy DNA 'Sanger' sequencing[1] has been used as the standard sequencing technology in many laboratories, and its acme was the completion of the human genome sequence[2]. However, because Sanger sequencing is performed on single amplicons, its throughput is limited, and large-scale sequencing projects are expensive and laborious: the human genome sequence took hundreds of sequencing machines several years and cost several hundred million dollars.

The paradigm of DNA sequencing changed with the advent of 'next-generation' sequencing technologies (reviewed in refs. 3,4), which process hundreds of thousands to millions of DNA templates in parallel, resulting in a low cost per base of generated sequence and a throughput on the gigabase (Gb) scale. As a consequence, we can now start to define the characteristics of entire genomes and delineate differences between them. Ultimately, whole-genome sequencing of complex organisms will become routine, allowing us to gain a deeper understanding of the full spectrum of genetic variation and to define its role in phenotypic variation and the pathogenesis of complex traits.

Nevertheless, it is not yet feasible to sequence large numbers of complex genomes in their entirety because the cost and time taken are still too great. To obtain 30-fold coverage of a human genome (90 Gb in total), would currently require several sequencing runs and would cost tens of thousands of dollars. In addition to the demands such a project would place on laboratory time and funding, the primary analysis during which the captured image files are processed, as well as storage of the sequences, would place a substantial burden on a research center's informatics infrastructure.

Consequently, considerable effort has been devoted to develop 'target-enrichment' methods, in which genomic regions are selectively captured from a DNA sample before sequencing. Resequencing the genomic regions that are retained is necessarily more time- and cost-effective, and the resulting data are considerably less cumbersome to analyze. Several approaches to target enrichment have been developed (Fig. 1), and there are several parameters by which the performance of each can be measured, which vary from one approach to another: (i) sensitivity, or the percentage of the target bases that are represented by one or more sequence reads; (ii) specificity, or the percentage of sequences that map to the intended targets; (iii) uniformity, or the variability in sequence coverage across target regions; (iv) reproducibility, or how closely results obtained from replicate experiments correlate; (v) cost; (vi) ease of use; and (vii) amount of DNA required per experiment, or per megabase of target.

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Valtrus Ex 1007
Foresight v Personalis
IPR2023-00546

approaches to target enrichment. The specificity of PCR will almost certainly always exceed that of hybrid capture, and its uniformity may never be matched by either hybrid capture or MIPs. But

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 44; EX21269 ¶ 121)

EX1007 (Mamanova) at 117

Personalis 86

Forshew + Dawson Do Not Render Obvious

Forshew Not Disclose Or Render Obvious Fixed And Variable Portions

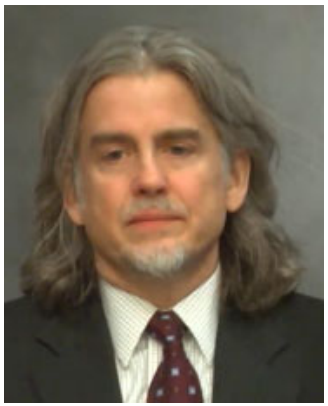
1. A method for personalized genetic testing, comprising:
(a) obtaining a personalized probe set for a subject, which personalized probe set comprises a plurality of nucleic acid probe molecules having a plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising a set of one or more genetic variants over other sequences in at least one biological sample of said subject, wherein said set of genetic variants are identified in sequence data for said at least one biological sample, further wherein said personalized probe set comprises nucleic acid probe molecules having oligonucleotide-directed genomic content comprising: (i) at least one variable portion from a result of said sequence data and (ii) at least one fixed portion independent of said result of said sequencing data
(b) using said personalized probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in at least an additional biological sample from said subject, to generate a sequencing library;
(c) subjecting said sequencing library to sequencing to identify a presence or absence of at least a subset of said set of genetic variants in at least said additional biological sample from said subject, wherein a sequencing footprint of said sequencing of (c) is less than a sequencing footprint of said sequence data of (a); and
(d) generating a report identifying (i) a presence or absence of a health condition or disease of the subject in said additional biological sample based on said identified at least a subset of (c) and (ii) genetic variants which inform a therapy choice or a change in therapy for the subject.

nucleotide-directed genomic content comprising: (i) at least one variable portion from a result of said sequence data and (ii) at least one fixed portion independent of said result of said sequencing data

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 4-5)

EX1001 ('783 Patent) at claim 1

Dr. Quackenbush: Forshew Gives No Primer Design Details



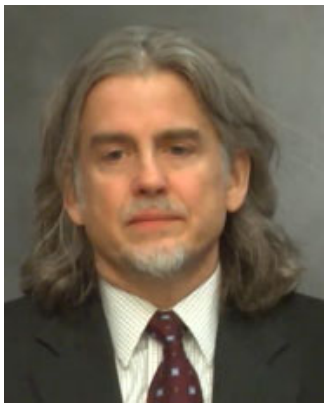
John Quackenbush
Foresight's Expert

A: So, the details of how Forshew designed his primers isn't provided in the main text.

EX2163 (10/20/23 Quackenbush Depo) at 147:22-148:2

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 47)

Dr. Quackenbush: Forshew May Have Used “Standard Primers”



John Quackenbush
Foresight’s Expert

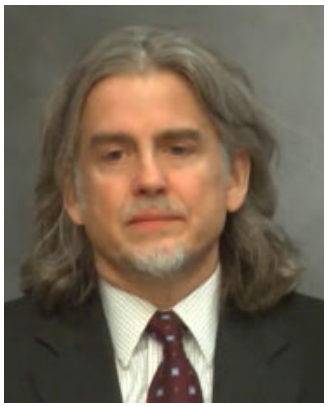
Q: This paragraph that we have been looking at in Forshew that begins with, "Previously proposed,“ on Page 10, this is telling the person of ordinary skill that the advantages of TAm-Seq are that you do not need specially designed probes, right?

A: Again, I would have to read the whole paper. That is probably one way to interpret it. But whether or not you need custom designed probes doesn't mean they are custom selected, right. So, what Forshew is telling us is, that these may have been standard primers that could have been purchased.

EX2163 (10/20/23 Quackenbush Depo) at 155:5-157:16

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE (’783 POR at 47)

Dr. Quackenbush: Forshew May Have Used "Standard" Probes



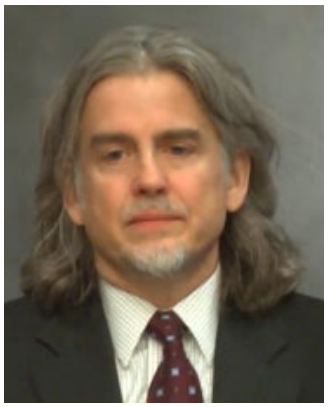
John Quackenbush
Foresight's Expert

A: That he used a personalized probe set, so personalized, that comprises nucleic acid probe molecules having oligonucleotide directed genomic content with at least one variable portion from a result of said sequence data. He identified mutations. He identified a probe set which in reading this further as we have been doing, could very well represent a standard probe set that he purchased off the shelf. But the probe set – the probes that he used were specifically selected to target mutations that were found based on said sequences data that were obtained in his previous analysis, right.

EX2163 (10/20/23 Quackenbush Depo) at 155:5-157:16

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Dr. Quackenbush: Forshew May Have Used "Commercially Available" Probes



John Quackenbush
Foresight's Expert

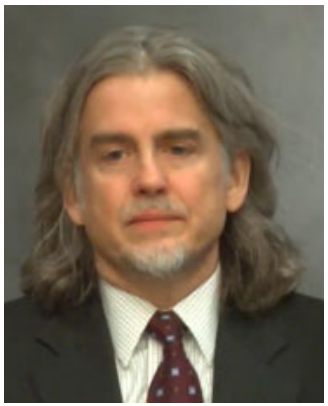
Q: So, I mean TAm-Seq can be performed without designing any special probes, right?

A: So that may be what Forshew is referring to here. So, in the mid-2000's a lot of companies were coming out with whole exome capture technologies. Some of those were PCR based, some of those were based on solid phase to solution capture probes, right. So, Forshew may have been using a commercially available set of probes that targeted individual exons. In reading this, that may, in fact, be what Forshew is referring to.

EX2163 (10/20/23 Quackenbush Depo) at 153:16-155:3

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 47)

Dr. Quackenbush: “Irrelevant” Whether Forshew Used “Standard” Or “Custom” Probes



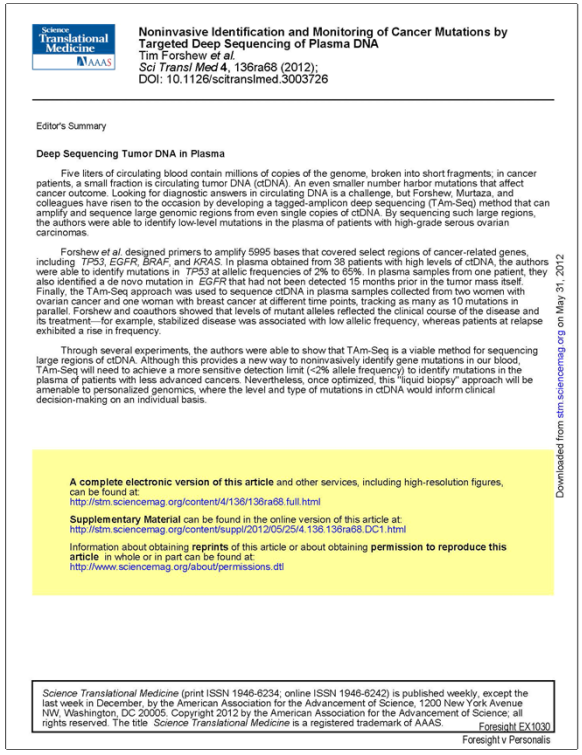
John Quackenbush
Foresight’s Expert

A: Nevertheless, rather than using the entire exome or the entire genome, what Forshew told us is that he selected ten mutational loci and specifically targeted those. And those ten loci were – whether or not he used standard probes or custom designed probes is kind of irrelevant. That those ten loci represent a variable portion of the loci he analyzed, in fact, they were the only portion -- but one could easily extend this to other known genes -- and demonstrated that as of 2012, one could essentially carry out all of the steps of the -- especially in light of Dawson, all of the steps of Claim 1 of the '783 patent, thus rendering it obvious.

EX2163 (10/20/23 Quackenbush Depo) at 153:16-155:3

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE (’783 POR at 47)

Forshew: Criticizes "Custom-Designed Probes"



Science Translational Medicine

Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA

Tim Forshew *et al.*

Sci Transl Med 4, 136ra68 (2012);
DOI: 10.1126/scitranslmed.3003726

Editor's Summary

Deep Sequencing Tumor DNA in Plasma

Five liters of circulating blood contain millions of copies of the genome, broken into short fragments; in cancer patients, a small fraction is circulating tumor DNA (ctDNA). An even smaller number harbor mutations that affect cancer outcome. Looking for diagnostic answers in circulating DNA is a challenge, but Forshew, Murtaza, and colleagues have risen to the occasion by developing a tagged-amplicon deep sequencing (TAm-Seq) method that can amplify and sequence large genomic regions from even single copies of ctDNA. By sequencing such large regions, the authors were able to identify low-level mutations in the plasma of patients with high-grade serous ovarian carcinomas.

Forshew *et al.* designed primers to amplify 5995 bases that covered select regions of cancer-related genes, including *TP53*, *EGFR*, *BRAF*, and *KRAS*. In plasma obtained from 38 patients with high levels of ctDNA, the authors were able to identify mutations in *TP53* at allelic frequencies of 2% to 65%. In plasma samples from one patient, they also identified a de novo mutation in *EGFR* that had not been detected 15 months prior in the tumor mass itself. Finally, the TAm-Seq approach was used to sequence ctDNA in plasma samples collected from two women with ovarian cancer and one woman with breast cancer at different time points, tracking as many as 10 mutations in parallel. Forshew and coauthors showed that levels of mutant alleles reflected the clinical course of the disease and its treatment—for example, stabilized disease was associated with low allelic frequency, whereas patients at relapse exhibited a rise in frequency.

Through several experiments, the authors were able to show that TAm-Seq is a viable method for sequencing large regions of ctDNA. Although this provides a new way to noninvasively identify gene mutations in our blood, TAm-Seq will need to achieve a more sensitive detection limit (<2% allele frequency) to identify mutations in the plasma of patients with less advanced cancers. Nevertheless, once optimized, this "liquid biopsy" approach will be amenable to personalized genomics, where the level and type of mutations in ctDNA would inform clinical decision-making on an individual basis.

A complete electronic version of this article and other services, including high-resolution figures, can be found at:
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Foresight EX1030
Foresight v Personalis

Previously proposed methods for personalized monitoring of tumor dynamics relied on expensive custom-designed probes (3) or identification of rearrangements using whole-genome sequencing (*18*, *19*). These have better analytical sensitivity than currently achieved by TAm-Seq, but are difficult to implement on a routine basis. TAm-Seq strikes a balance between sensitivity and ease of use and could facilitate study and application of circulating DNA. Using TAm-Seq, we identified cancer mutations in the plasma of most advanced ovarian cancer patients and tracked dynamics of *TP53* mutations without requiring any specially designed probes. In summary, TAm-Seq is a flex-

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 48)

Dawson: Criticizes "Personalized Assays"



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer

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ABSTRACT

BACKGROUND

The management of metastatic breast cancer requires monitoring of the tumor burden to determine the response to treatment, and improved biomarkers are needed. Biomarkers such as cancer antigen 15-3 (CA 15-3) and circulating tumor cells have been widely studied. However, circulating cell-free DNA carrying tumor-specific alterations (circulating tumor DNA) has not been extensively investigated or compared with other circulating biomarkers in breast cancer.

METHODS

We compared the radiographic imaging of tumors with the assay of circulating tumor DNA, CA 15-3, and circulating tumor cells in 30 women with metastatic breast cancer who were receiving systemic therapy. We used targeted or whole-genome sequencing to identify somatic genomic alterations and designed personalized assays to quantify circulating tumor DNA in serially collected plasma specimens. CA 15-3 levels and numbers of circulating tumor cells were measured at identical time points.

RESULTS

Circulating tumor DNA was successfully detected in 29 of the 30 women (97%) in whom somatic genomic alterations were identified; CA 15-3 and circulating tumor cells were detected in 21 of 27 women (78%) and 26 of 30 women (87%), respectively. Circulating tumor DNA levels showed a greater dynamic range, and greater correlation with changes in tumor burden, than did CA 15-3 or circulating tumor cells. Among the measures tested, circulating tumor DNA provided the earliest measure of treatment response in 10 of 19 women (53%).

CONCLUSIONS

This proof-of-concept analysis showed that circulating tumor DNA is an informative, inherently specific, and highly sensitive biomarker of metastatic breast cancer. (Funded by Cancer Research UK and others.)

From the Department of Oncology, University of Cambridge and Cancer Research UK Cambridge Institute, Li Ka Shing Centre (S.-J.D., D.W.Y.T., M.M., O.M.R., S.-F.C., M.J.D., D.G., T.F., C.C., N.R.), the Department of Histopathology (B.M.-A.), Radiology (S.R., M.W.), and Clinical Biochemistry and Immunology (D.H.) and the Cambridge Breast Unit (S.-J.D., H.B., B.M.-A., S.R., M.W., C.C.), Addenbrooke's Hospital, Cambridge University Hospital National Health Service Foundation Trust and National Institute for Health Research Cambridge Biomedical Research Centre, and the Cambridge Experimental Cancer Medicine Centre (C.C.), Cambridge; and Illumina, Little Chesterford (S.H., J.B., D.B.) — all in the United Kingdom; and the Peter MacCallum Cancer Centre, East Melbourne, VIC, Australia (S.-J.D.). Address reprint requests to Dr. Rosenfeld or Dr. Caldas at Cancer Research UK Cambridge Institute, University of Cambridge, Li Ka Shing Centre, Robinson Way, Cambridge, CB2 0RE, United Kingdom, or at carlos.caldas@cruk.cam.ac.uk.

Drs. Dawson and Tsui and Drs. Caldas and Rosenfeld contributed equally to this article.

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assay and targeted deep sequencing. Digital PCR assay provides high accuracy and sensitivity but requires the design of personalized assays, an expensive and rate-limiting step. Targeted deep

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 49; EX2169 ¶ 131)

EX1070 (Dawson) at 1207

Personalis 95

Foresight's Founders: Criticize Forshew



Editorial

Potential clinical utility of ultrasensitive circulating tumor DNA detection with CAPP-Seq

Expert Rev. Mol. Diagn. 15(6), 715–719 (2015)

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Tumors continually shed DNA into the circulation, where it can be noninvasively accessed. The ability to accurately detect circulating tumor DNA (ctDNA) could significantly impact the management of patients with nearly every cancer type. Quantitation of ctDNA could allow objective response assessment, detection of minimal residual disease and noninvasive tumor genotyping. The latter application overcomes the barriers currently limiting repeated tumor tissue sampling during therapy. Recent technical advancements have improved upon the sensitivity, specificity and feasibility of ctDNA detection and promise to enable innovative clinical applications. Here, we focus on the potential clinical utility of ctDNA analysis using CAncer Personalized Profiling by deep Sequencing (CAPP-Seq), a novel next-generation sequencing-based approach for ultrasensitive ctDNA detection. Applications of CAPP-Seq for the personalization of cancer detection and therapy are discussed.

We are in the middle of a revolution in molecular oncology that is allowing for increasingly personalized management of cancer patients. The individualization of cancer care will rely on the development of effective targeted therapeutics as well as biomarkers for selecting the appropriate treatments and evaluating their effectiveness. To aid with complex decision making in clinics, improved tools are needed to accurately measure disease burden, assess prognosis and predict response to targeted therapies.

Circulating tumor DNA (ctDNA) has emerged as a promising cancer biomarker because it provides noninvasive access to cancer DNA. Distinct from circulating tumor cells (CTCs), ctDNA is cell-free and can be collected from peripheral blood plasma, urine or other bodily fluids. Although more comprehensive, head-to-head comparisons across a larger number of tumor types are needed, several recent studies have suggested that ctDNA may be detectable by deep sequencing-based approaches in a greater proportion of patients than CTCs [1–4].

A major technical challenge in the analysis of ctDNA is that the vast majority of the cell-free DNA found in plasma originates from a patient's healthy cells. Therefore, highly sensitive techniques are necessary for reliable detection and quantitation of the tumor-derived fraction. For example, in patients with stage IV non-small cell lung cancer, the percent of circulating DNA that is tumor-derived has been shown to vary between median values of approximately 0.1–5% and is affected by factors such as disease burden and treatment status [5–7].

Early efforts at detecting ctDNA mostly focused on the application of allele-specific real-time quantitative PCR assays [8]. These assays, which utilized technologies such as TaqMan, PNA clamps, and Scorpion Amplification Refractory Mutation System, were limited in their applicability to patients with high tumor burden due to their analytical sensitivity and specificity. However, within the past decade, several methods have been developed that allow for ultrasensitive detection of ctDNA.

Keywords: biomarker • circulating tumor DNA • next-generation sequencing • noninvasive • ultrasensitive

EXPERT REVIEWS

Personalis EX2164

geting commonly mutated cancer genes [1,2,11,15,16]. Although low detection thresholds are achievable with such methods, technical limitations related to multiplexing of PCR assays have to date limited the number of genomic positions that can be interrogated. This complicates potential clinical applications because a given small combination of amplicons will not identify a mutation in the majority of patients with most cancers.

EX2164 (Bratman) at 716

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 39; EX2169 ¶ 138)

Foresight's Founders: Criticize Forshew



ELSEVIER

Seminars in RADIATION ONCOLOGY

Predicting Radiotherapy Responses and Treatment Outcomes Through Analysis of Circulating Tumor DNA

Aadel A. Chaudhuri, MD, PhD,* Michael S. Binkley, BA,* Evan C. Osmundson, MD, PhD,* Ash A. Alizadeh, MD, PhD,†,‡,§,¶ and Maximilian Diehn, MD, PhD*,§,¶

Tumors continually shed DNA into the blood where it can be detected as circulating tumor DNA (ctDNA). Although this phenomenon has been recognized for decades, techniques that are sensitive and specific enough to robustly detect ctDNA have only become available recently. Quantification of ctDNA represents a new approach for cancer detection and disease burden quantification that has the potential to revolutionize response assessment and personalized treatment in radiation oncology. Analysis of ctDNA has many potential applications, including detection of minimal residual disease following radiotherapy, non-invasive tumor genotyping, and early detection of tumor recurrence. Ultimately, ctDNA-based assays could lead to personalization of therapy based on identification of somatic alterations present in tumors and changes in ctDNA concentrations before and after treatment. In this review, we discuss methods of ctDNA detection and clinical applications of ctDNA-based biomarkers in radiation oncology, with a focus on recently developed techniques that use next-generation sequencing for ctDNA quantification.
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M.D. and A.A. are inventors on patent applications related to the CAPP-Seq technology and are cofounders and consultants for CAPP Medical. No writing assistance was used in the production of this manuscript.
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Introduction

The field of radiation oncology is currently going through a revolution, as new technologies allow treatment of patients with more precision than ever before. Although much progress has been made in improving delivery of radiation therapy (RT), response assessment after treatment continues to rely primarily on imaging for most types of cancers. Although, imaging-based response assessment has significant clinical value, this approach cannot detect minimal residual disease (MRD), has a lag between treatment and when tumor responses can be detected, is expensive, and often exposes patients to additional ionizing radiation. For these reasons, there continues to be an unmet need for developing blood-based biomarkers for cancers that are treated with RT. Although such biomarkers exist for a few cancer types (eg, prostate-specific antigen for prostate cancer), they do not for most cancers. The ideal biomarker would be readily generalizable, such that it could be applied to all cancer types.

Although most work on cancer biomarkers has focused on detection of proteins, recently, circulating tumor DNA

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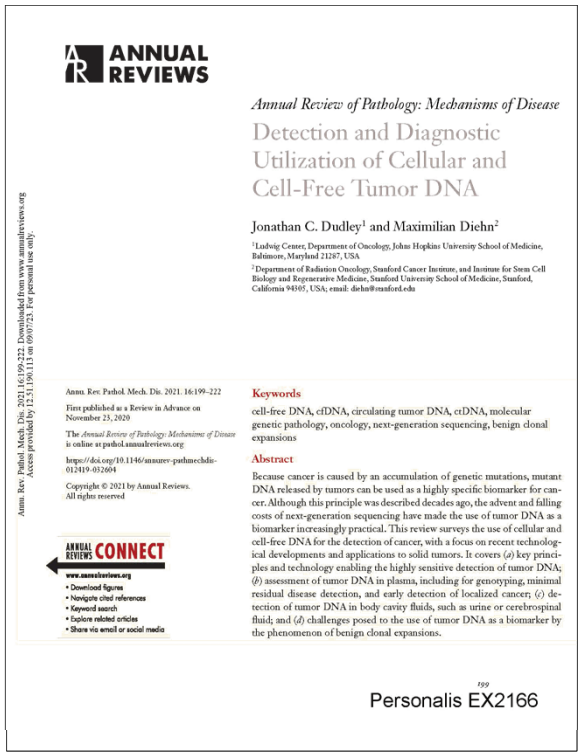
Personalis EX2165

are used. Limitations of these approaches include interrogation of relatively few genomic loci owing to difficulties related to multiplexing of PCR assays, the inability to detect somatic copy number alterations, and the inability to detect breakpoints or rearrangements without patient-specific optimization of amplicons.

EX2165 (Chaudhuri) at 307-308

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 52, '611 POR at 40)

Foresight's Founders: Multiplex PCR "Limited"



ANNUAL REVIEWS

Annual Review of Pathology: Mechanisms of Disease

Detection and Diagnostic Utilization of Cellular and Cell-Free Tumor DNA

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Keywords

cell-free DNA, cfDNA, circulating tumor DNA, ctDNA, molecular genetic pathology, oncology, next-generation sequencing, benign clonal expansions

Abstract

Because cancer is caused by an accumulation of genetic mutations, mutant DNA released by tumors can be used as a highly specific biomarker for cancer. Although this principle was described decades ago, the advent and falling costs of next-generation sequencing have made the use of tumor DNA as a biomarker increasingly practical. This review surveys the use of cellular and cell-free DNA for the detection of cancer, with a focus on recent technological developments and applications to solid tumors. It covers (*a*) key principles and technology enabling the highly sensitive detection of tumor DNA; (*b*) assessment of tumor DNA in plasma, including for genotyping, minimal residual disease detection, and early detection of localized cancer; (*c*) detection of tumor DNA in body cavity fluids, such as urine or cerebrospinal fluid; and (*d*) challenges posed to the use of tumor DNA as a biomarker by the phenomenon of benign clonal expansions.

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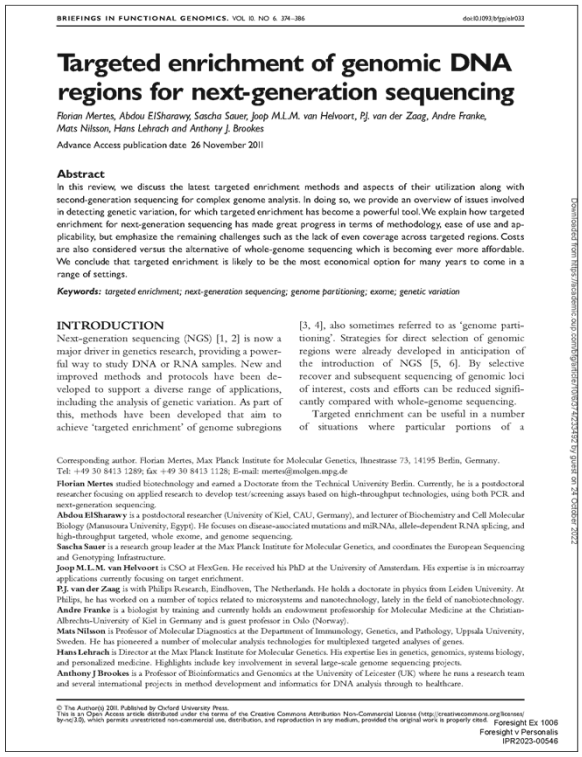
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PCR-based assays have limited multiplexability.

EX2166 (Dudley) at 202

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 32, '611 POR at 40)

Mertes: Multiplex PCR Presents Several Challenges



BRIEFINGS IN FUNCTIONAL GENOMICS. VOL 10. NO 6. 374–386 doi:10.1093/bfgp/elr033

Targeted enrichment of genomic DNA regions for next-generation sequencing

Florian Mertes, Abdou ElSharawy, Sascha Sauer, Joop M.L.M. van Helvoort, P.J. van der Zaag, Andre Franke, Mats Nilsson, Hans Lehrach and Anthony J. Brookes

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Abstract

In this review, we discuss the latest targeted enrichment methods and aspects of their utilization along with second-generation sequencing for complex genome analysis. In doing so, we provide an overview of issues involved in detecting genetic variation, for which targeted enrichment has become a powerful tool. We explain how targeted enrichment for next-generation sequencing has made great progress in terms of methodology, ease of use and applicability, but emphasize the remaining challenges such as the lack of even coverage across targeted regions. Costs are also considered versus the alternative of whole-genome sequencing which is becoming ever more affordable. We conclude that targeted enrichment is likely to be the most economical option for many years to come in a range of settings.

Keywords: *targeted enrichment; next-generation sequencing; genome partitioning; exome; genetic variation*

INTRODUCTION

Next-generation sequencing (NGS) [1, 2] is now a major driver in genetics research, providing a powerful way to study DNA or RNA samples. New and improved methods and protocols have been developed to support a diverse range of applications, including the analysis of genetic variation. As part of this, methods have been developed that aim to achieve 'targeted enrichment' of genome subregions [3, 4], also sometimes referred to as 'genome partitioning'. Strategies for direct selection of genomic regions were already developed in anticipation of the introduction of NGS [5, 6]. By selective recover and subsequent sequencing of genomic loci of interest, costs and efforts can be reduced significantly compared with whole-genome sequencing.

Targeted enrichment can be useful in a number of situations where particular portions of a

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Florian Mertes studied biotechnology and earned a Doctorate from the Technical University Berlin. Currently, he is a postdoctoral researcher focusing on applied research to develop test/screening assays based on high-throughput technologies, using both PCR and next-generation sequencing.

Abdou ElSharawy is a postdoctoral researcher (University of Kiel, CAU, Germany), and lecturer of Biochemistry and Cell Molecular Biology (Mansoura University, Egypt). He focuses on disease-associated mutations and miRNAs, allele-dependent RNA splicing, and high-throughput targeted, whole exome, and genome sequencing.

Sascha Sauer is a research group leader at the Max Planck Institute for Molecular Genetics, and coordinates the European Sequencing and Genotyping Infrastructure.

Joop M.L.M. van Helvoort is CSO at FlexGen. He received his PhD at the University of Amsterdam. His expertise is in microarray applications currently focusing on target enrichment.

P.J. van der Zaag is with Philips Research, Eindhoven, The Netherlands. He holds a doctorate in physics from Leiden University. At Philips, he has worked on a number of topics related to microsystems and nanotechnology, lately in the field of nanobiotechnology.

Andre Franke is a biologist by training and currently holds an endowment professorship for Molecular Medicine at the Christian-Albrechts-University of Kiel in Germany and is guest professor in Oslo (Norway).

Mats Nilsson is Professor of Molecular Diagnostics at the Department of Immunology, Genetics, and Pathology, Uppsala University, Sweden. He has pioneered a number of molecular analysis technologies for multiplexed targeted analyses of genes.

Hans Lehrach is Director at the Max Planck Institute for Molecular Genetics. His expertise lies in genetics, genomics, systems biology, and personalized medicine. Highlights include key involvement in several large-scale genome sequencing projects.

Anthony J Brookes is a Professor of Bioinformatics and Genomics at the University of Leicester (UK) where he runs a research team and several international projects in method development and informatics for DNA analysis through to healthcare.

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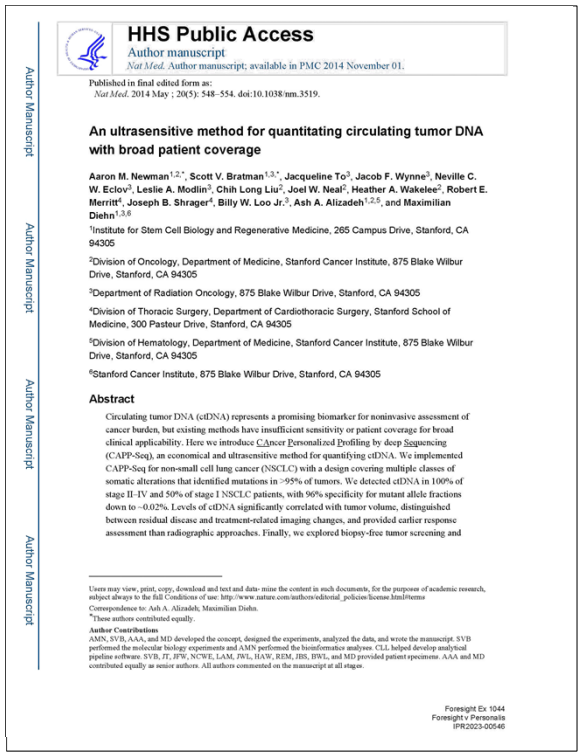
The main downside of the method is that it does not scale easily, in any format, to enable the targeting of very large genome subregions or many DNA samples. To use this method effectively, any significant extent of parallelized singleplex or multiplex PCR would need to be supported by the use of automated robotics, individual PCR amplicons (or multiplex products) need to be carefully normalized to equivalent molarities when pooling in advance of NGS (so that the final coverage of the total region of interest is as even as possible), and the amount of DNA material a study requires can be substantial as this requirement grows linearly with the number of utilized PCR reactions. But if the target region is small,

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 52, '611 POR at 39)

EX1006 (Mertes) at 379

Personalis 99

Foresight's Founders: Criticize Dawson



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An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage

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Abstract

Circulating tumor DNA (ctDNA) represents a promising biomarker for noninvasive assessment of cancer burden, but existing methods have insufficient sensitivity or patient coverage for broad clinical applicability. Here we introduce CAncer Personalized Profiling by deep Sequencing (CAPP-Seq), an economical and ultrasensitive method for quantifying ctDNA. We implemented CAPP-Seq for non-small cell lung cancer (NSCLC) with a design covering multiple classes of somatic alterations that identified mutations in >95% of tumors. We detected ctDNA in 100% of stage II–IV and 50% of stage I NSCLC patients, with 96% specificity for mutant allele fractions down to ~0.02%. Levels of ctDNA significantly correlated with tumor volume, distinguished between residual disease and treatment-related imaging changes, and provided earlier response assessment than radiographic approaches. Finally, we explored biopsy-free tumor screening and

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Author Contributions

AMN, SVB, AAA, and MD developed the concept, designed the experiments, analyzed the data, and wrote the manuscript. SVB performed the molecular biology experiments and AMN performed the bioinformatics analyses. CLL helped develop analytical pipeline software. SVB, JT, JFW, NCWE, LAM, JWL, HAW, REM, JBS, BWL, and MD provided patient specimens. AAA and MD contributed equally as senior authors. All authors commented on the manuscript at all stages.

Foresight Ex 1044
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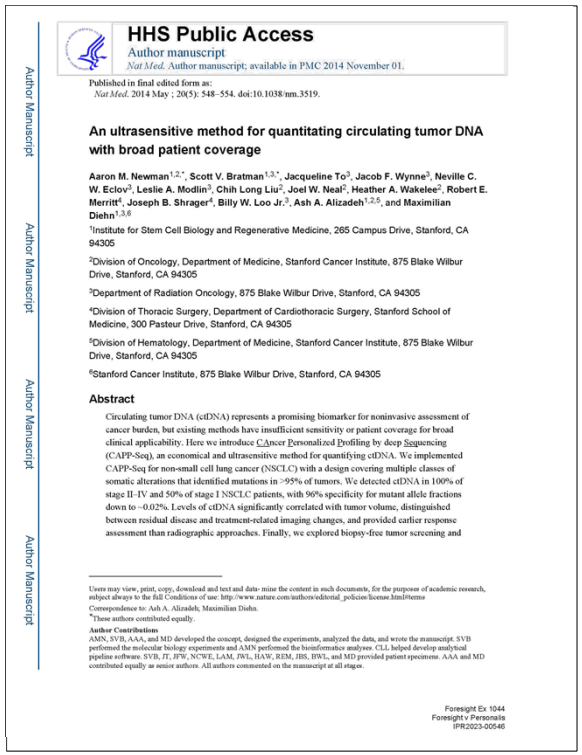
even lower detection thresholds. Previously published methods employing amplicon[8,10,11], whole exome[12], or whole genome[9,32,33,24] sequencing would not be sensitive enough to detect ctDNA in most patients with NSCLC, even at 10-fold or greater sequencing costs (Fig. 1d and Supplementary Fig. 8).

EX1044 (Newman) at 7

Personalis 100

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 54; EX2169 ¶ 143)

Foresight's Founders: Criticize Dawson



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Abstract

Circulating tumor DNA (ctDNA) represents a promising biomarker for noninvasive assessment of cancer burden, but existing methods have insufficient sensitivity or patient coverage for broad clinical applicability. Here we introduce CAncer Personalized Profiling by deep Sequencing (CAPP-Seq), an economical and ultrasensitive method for quantifying ctDNA. We implemented CAPP-Seq for non-small cell lung cancer (NSCLC) with a design covering multiple classes of somatic alterations that identified mutations in >95% of tumors. We detected ctDNA in 100% of stage II–IV and 50% of stage I NSCLC patients, with 96% specificity for mutant allele fractions down to ~0.02%. Levels of ctDNA significantly correlated with tumor volume, distinguished between residual disease and treatment-related imaging changes, and provided earlier response assessment than radiographic approaches. Finally, we explored biopsy-free tumor screening and

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*These authors contributed equally.

Author Contributions

AMN, SVB, AAA, and MD developed the concept, designed the experiments, analyzed the data, and wrote the manuscript. SVB performed the molecular biology experiments and AMN performed the bioinformatics analyses. CLL helped develop analytical pipeline software. SVB, JT, JFW, NCWE, LAM, JWL, HAW, REM, JBS, BWL, and MD provided patient specimens. AAA and MD contributed equally as senior authors. All authors commented on the manuscript at all stages.

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DNA[1–4], but the majority of patients lack mutations in these genes. Recently, approaches employing massively parallel sequencing have been used to detect ctDNA[5–12]. However, the methods reported to date have been limited by modest sensitivity[13], applicability to only a minority of patients, the need for patient-specific optimization, and/or cost. To overcome

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 36, '611 POR at 2)

EX1044 (Newman) at 2

Forshew And Dawson Do Not Generate A Report To Inform Therapy

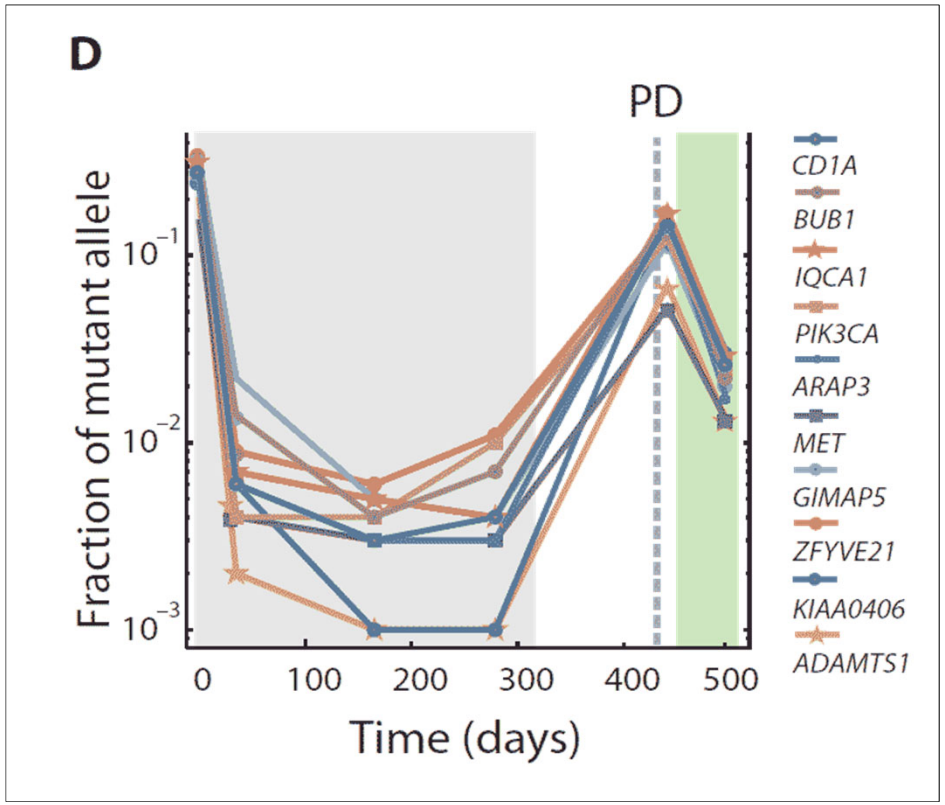
1. A method for personalized genetic testing, comprising:
(a) obtaining a personalized probe set for a subject, which personalized probe set comprises a plurality of nucleic acid probe molecules having a plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising a set of one or more genetic variants over other sequences in at least one biological sample of said subject, wherein said set of genetic variants are identified in sequence data for said at least one biological sample, further wherein said personalized probe set comprises nucleic acid probe molecules having oligonucleotide-directed genomic content comprising: (i) at least one variable portion from a result of said sequence data and (ii) at least one fixed portion independent of said result of said sequencing data
(b) using said personalized probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in at least an additional biological sample from said subject, to generate a sequencing library;
(c) subjecting said sequencing library to sequencing to identify a presence or absence of at least a subset of said set of genetic variants in at least said additional biological sample from said subject, wherein a sequencing footprint of said sequencing of (c) is less than a sequencing footprint of said sequence data of (a); and
(d) generating a report identifying (i) a presence or absence of a health condition or disease of the subject in said additional biological sample based on said identified at least a subset of (c) and (ii) genetic variants which inform a therapy choice or a change in therapy for the subject.

EX1001 ('783 Patent) at claim 1

(d) generating a report identifying (i) a presence or absence of a health condition or disease of the subject in said additional biological sample based on said identified at least a subset of (c) and (ii) genetic variants which inform a therapy choice or a change in therapy for the subject.

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 4-5)

Forshew: No Choice Of Therapy



DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 51)

EX1030 (Forshew) at 7, Fig 4(D)

The ’611 Patent Is Neither Anticipated Nor Obvious

IPR2023-00546

Ley Does Not Anticipate Or Render Obvious

Ley Does Not Teach The Required Sequencing Depth

1. A method for personalized genetic testing, comprising:
(a) sequencing nucleic acid molecules from at least one biological sample of a subject to generate sequencing data, wherein said sequencing comprises sequencing 12 gigabases (GB) or more;
(b) computer processing said sequencing data to identify a plurality of nucleic acid sequences having a set of genetic variants, wherein said set of genetic variants are identified with respect to a reference;
(c) obtaining a probe set comprising a plurality of nucleic acid probe molecules having said plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in said at least one biological sample;
(d) using said probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in an additional biological sample from said subject or at least one biological relative of said subject, to generate a sequencing library; and
(e) subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less to identify a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative, wherein a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a).

EX1001 ('611 Patent) at claim 1

(e) subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less to identify a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative, wherein a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a).

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('611 POR at 53)

Ley: Provides Only "Readcounts"

Table 3 | 454 Readcount data for somatic mutations and known SNPs

		Primary AML (100% blasts)			Skin			Relapse (78% blasts)		
Gene	Consequence	Variant	Ref	Variant (%)	Variant	Ref	Variant (%)	Variant	Ref	Variant (%)
CDH24	Y590X	5672	4890	53.70	564	10358	5.16	3108	4599	40.33
SLC1SA1	W77X	3817	4962	43.48	875	10773	7.51	4714	7173	39.66
KNDC1	L799F	4640	4848	48.90	770	8972	7.90	3883	6342	37.98
PTPRT	P1235L	998	1058	48.54	126	1489	7.80	350	493	41.52
GRINL1B	R176H	2211	2674	45.26	318	4461	6.65	1447	2070	41.14
GPR123	T38I	4618	4569	50.27	850	9751	8.02	3660	6057	37.67
EBI2	A338V	12750	15453	45.21	458	10088	4.34	2646	3627	42.18
PCLKC	P1004L	992	855	53.71	341	3153	9.76	705	773	47.70
FLT3	ITD	4220	7810	35.08	3475	23159	13.05	3870	8495	31.30
NPM1	CATG ins	1550	1974	43.98	143	2390	5.65	2303	3910	37.07
BRCA2	N372H	778	752	50.85	763	876	46.55	285	303	48.47
TP53	P72R	8989	1	99.99	8161	0	100.00	7914	6	99.92

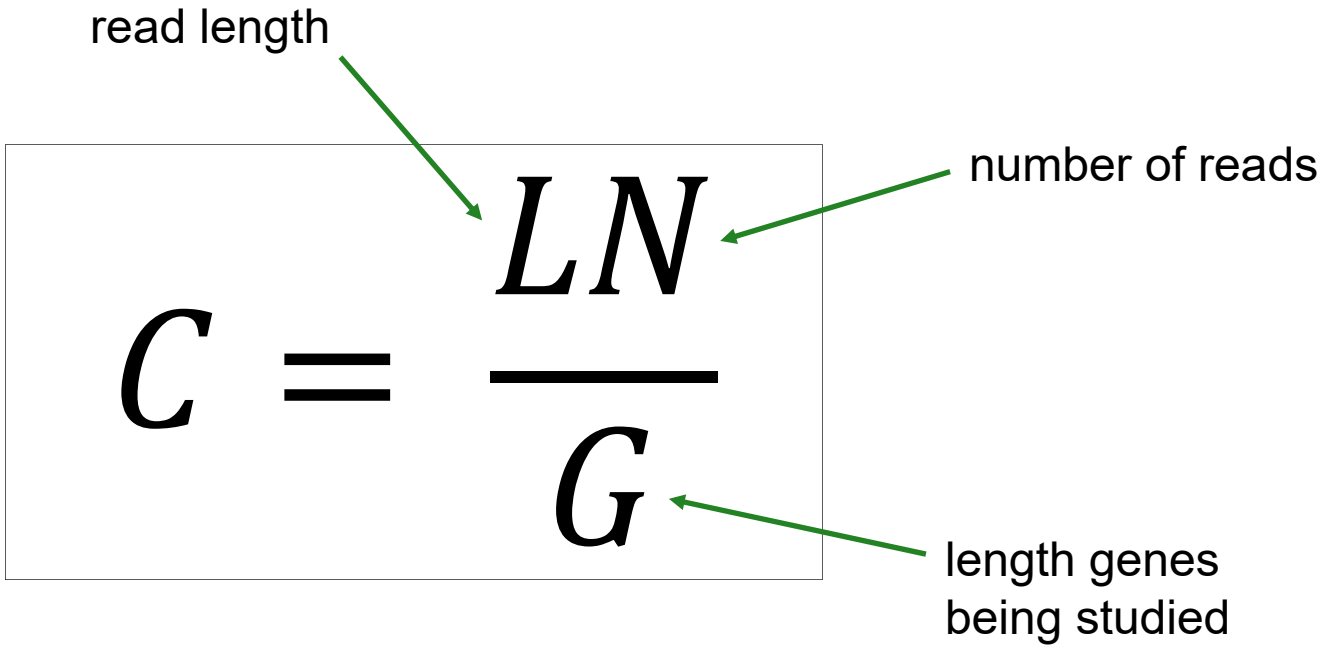
The differences between variant frequencies in primary or relapse tumour samples and skin were highly significant for all somatic mutations ($P < 0.000001$, Fisher's exact test, one tailed). The *BRCA2* variant is a known heterozygous SNP in this genome, and the *TP53* variant is a known homozygous SNP.

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('611 POR at 10)

EX1012 (Ley) at Table 3

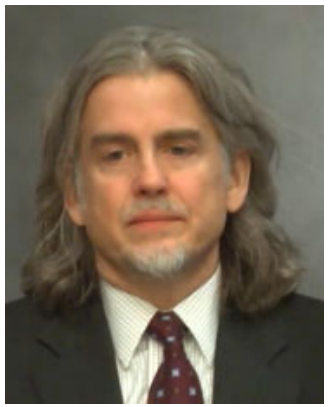
Personalis 107

Lander-Waterman Equation



DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('611 POR at 11)

Dr. Quackenbush: Ley Does Not Identify Primers Or Exons



John Quackenbush
Foresight's Expert

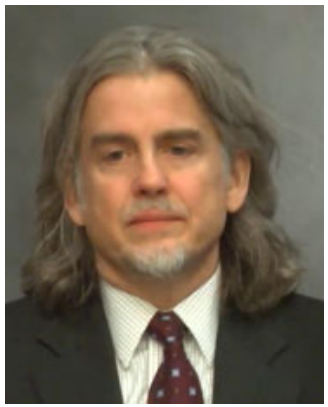
Dr. Quackenbush's Declaration

121. While Ley does not provide the primer sequences or the exact exons that were analyzed, one can easily set an upper bound on the sequence coverage Ley teaches by using the entire length of the genes that contained the genetic variant rather than the much smaller sequence of the variant-containing exon. Looking up

EX1061 (Quackenbush Decl) at ¶ 121

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE
('783 POR at 14; '611 POR at 12)

Dr. Quackenbush: Assumes mRNA Length Is Coding Length



John Quackenbush
Foresight's Expert

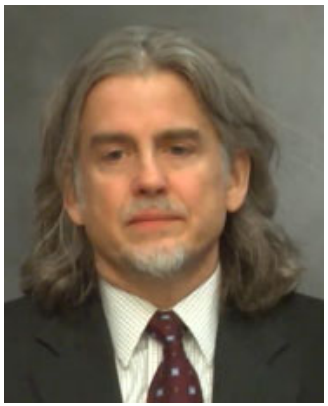
Dr. Quackenbush's Declaration

rather than the much smaller sequence of the variant-containing exon. Looking up the messenger RNA corresponding to the coding region of these ten genes at the National Center for Biotechnology Information (NCBI), one can easily obtain the length of the entire coding sequence for each of the genes. *See* Exs. 1048-59.

EX1061 (Quackenbush Decl) at ¶ 121

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('611 POR at 13)

Dr. Quackenbush: Coding Length Can Be Far Greater Than Gene Length



John Quackenbush
Foresight's Expert

A. And while a gene may span 1,000 bases of coding sequence, that can be over, or more or less, but that can be distributed over sometimes tens of thousands of bases of DNA.

EX2163 (10/20/23 Quackenbush Depo) at 33:19-22

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('611 POR at 14)

Law: Inherency In Obviousness Must Be "Carefully Circumscribed"

"We have held that the use of inherency in the context of obviousness must be carefully circumscribed because that which may be inherent is not necessarily known and that which is unknown cannot be obvious."

Southwire Co. v. Cerro Wire LLC, 870 F.3d 1306, 1310 (Fed. Cir. 2017) (internal citations omitted).

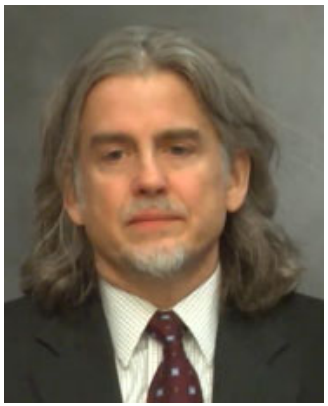
DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Dr. Quackenbush's Motivations

- Accuracy
- Cost
- Assay Simplicity
- Analysis Simplicity

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Dr. Quackenbush: All Factors Important



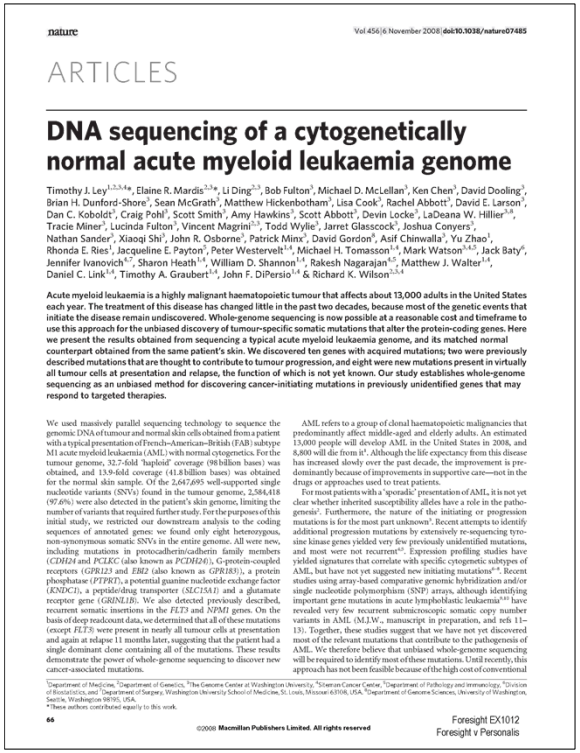
John Quackenbush
Foresight's Expert

A. I think a person of ordinary skill in the art, long before 2016 – and I believe the Ley reference is from 2008. So, even as far back as 2008, the person of skill in the art would have understood that all of these factors are factors which should be considered. And it is hard to say that one is more important than the other, right.

EX2163 (10/20/23 Quackenbush Depo) at 45:14-22

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 29; '611 POR at 37)

Ley: Discourages Smaller Sequencing Footprints



nature

Vol 456|6 November 2008|doi:10.1038/nature07485

ARTICLES

DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome

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Acute myeloid leukaemia is a highly malignant haematopoietic tumour that affects about 13,000 adults in the United States each year. The treatment of this disease has changed little in the past two decades, because most of the genetic events that initiate the disease remain undiscovered. Whole-genome sequencing is now possible at a reasonable cost and timeframe to use this approach for the unbiased discovery of tumour-specific somatic mutations that alter the protein-coding genes. Here we present the results obtained from sequencing a typical acute myeloid leukaemia genome, and its matched normal counterpart obtained from the same patient's skin. We discovered ten genes with acquired mutations; two were previously described mutations that are thought to contribute to tumour progression, and eight were new mutations present in virtually all tumour cells at presentation and relapse, the function of which is not yet known. Our study establishes whole-genome sequencing as an unbiased method for discovering cancer-initiating mutations in previously unidentified genes that may respond to targeted therapies.

We used massively parallel sequencing technology to sequence the genomic DNA of tumour and normal skin cells obtained from a patient with a typical presentation of French–American–British (FAB) subtype M1 acute myeloid leukaemia (AML) with normal cytogenetics. For the tumour genome, 32.7-fold 'haploid' coverage (98 billion bases) was obtained, and 13.9-fold coverage (41.8 billion bases) was obtained for the normal skin sample. Of the 2,647,695 well-supported single nucleotide variants (SNVs) found in the tumour genome, 2,584,418 (97.6%) were also detected in the patient's skin genome, limiting the number of variants that required further study. For the purposes of this initial study, we restricted our downstream analysis to the coding sequences of annotated genes: we found only eight heterozygous, non-synonymous somatic SNVs in the entire genome. All were new, including mutations in protocadherin/cadherin family members (*CDH24* and *PCLKC* (also known as *PCDH24*)), G-protein-coupled receptors (*GPR123* and *EBI2* (also known as *GPR183*)), a protein phosphatase (*PTPRT*), a potential guanine nucleotide exchange factor (*KNDC1*), a peptide/drug transporter (*SLC15A1*) and a glutamate receptor gene (*GRINL1B*). We also detected previously described, recurrent somatic insertions in the *FLT3* and *NPM1* genes. On the basis of deep readcount data, we determined that all of these mutations (except *FLT3*) were present in nearly all tumour cells at presentation and again at relapse 11 months later, suggesting that the patient had a single dominant clone containing all of the mutations. These results demonstrate the power of whole-genome sequencing to discover new cancer-associated mutations.

AML refers to a group of clonal haematopoietic malignancies that predominantly affect middle-aged and elderly adults. An estimated 13,000 people will develop AML in the United States in 2008, and 8,800 will die from it[1]. Although the life expectancy from this disease has increased slowly over the past decade, the improvement is predominantly because of improvements in supportive care—not in the drugs or approaches used to treat patients.

For most patients with a 'sporadic' presentation of AML, it is not yet clear whether inherited susceptibility alleles have a role in the pathogenesis[2]. Furthermore, the nature of the initiating or progression mutations is for the most part unknown[3]. Recent attempts to identify additional progression mutations by extensively re-sequencing tyrosine kinase genes yielded very few previously unidentified mutations, and most were not recurrent[4,5]. Expression profiling studies have yielded signatures that correlate with specific cytogenetic subtypes of AML, but have not yet suggested new initiating mutations[6–8]. Recent studies using array-based comparative genomic hybridization and/or single nucleotide polymorphism (SNP) arrays, although identifying important gene mutations in acute lymphoblastic leukaemia[9,10] have revealed very few recurrent submicroscopic somatic copy number variants in AML (M.J.W., manuscript in preparation, and refs 11–13). Together, these studies suggest that we have not yet discovered most of the relevant mutations that contribute to the pathogenesis of AML. We therefore believe that unbiased whole-genome sequencing will be required to identify most of these mutations. Until recently, this approach has not been feasible because of the high cost of conventional

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tumour. Our results strongly support the notion that hypothesis-driven (for example, candidate gene-based) examination of tumour genomes by PCR-directed or capture-based methods is inherently limited, and will miss key mutations. A further and important consid-

EX1012 (Ley) at 70

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 28, '611 POR at 31)

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Law: Retracing Path Of Inventor Is Inappropriate

"Mylan's expert, Dr. Anderson, simply retraced the path of the inventor with hindsight, discounted the number and complexity of the alternatives, and concluded that the invention of topiramate was obvious. Of course, this reasoning is always inappropriate for an obviousness test…."

Ortho-McNeil Pharm., Inc. v. Mylan Lab'ys, Inc., 520 F.3d 1358, 1364 (Fed. Cir. 2008)

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Ley Does Not Teach "Identifying"

1. A method for personalized genetic testing, comprising:
(a) sequencing nucleic acid molecules from at least one biological sample of a subject to generate sequencing data, wherein said sequencing comprises sequencing 12 gigabases (GB) or more;
(b) computer processing said sequencing data to identify a plurality of nucleic acid sequences having a set of genetic variants, wherein said set of genetic variants are identified with respect to a reference;
(c) obtaining a probe set comprising a plurality of nucleic acid probe molecules having said plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in said at least one biological sample;
(d) using said probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in an additional biological sample from said subject or at least one biological relative of said subject, to generate a sequencing library; and
(e) subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less to identify a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative, wherein a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a).

EX1001 ('611 Patent) at claim 1

(e) subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less to identify a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative, wherein a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a).

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('611 POR at 27)

Ley Does Not Teach Fixed And Variable Portions

5. The method of claim **1**, wherein each of said plurality of nucleic acid probe molecules includes oligonucleotide-directed genomic content comprising (i) at least one variable portion from a result of said sequencing of (a) and (ii) at least one fixed portion independent of said result of said sequencing of (a).

EX1001 ('611 Patent) at claim 5

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('611 POR at 35)

Ley Does Not Teach Therapeutic Intervention

10. The method of claim **1**, further comprising providing a therapeutic intervention at least based on said presence or absence of said at least said subset of said genetic variants identified in (e).

28. The method of claim **1**, further comprising using said presence or absence of said at least said subset of said set of genetic variants identified in (e) to treat said subject or said at least one biological relative for a disorder.

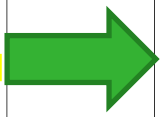
EX1001 ('611 Patent) at claims 10, 28

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('611 POR at 51)

Newman Does Not Anticipate Or Render Obvious

Newman Does Not Teach 12 GB Of Sequencing

1. A method for personalized genetic testing, comprising:
(a) sequencing nucleic acid molecules from at least one biological sample of a subject to generate sequencing data, wherein said sequencing comprises sequencing 12 gigabases (GB) or more;
(b) computer processing said sequencing data to identify a plurality of nucleic acid sequences having a set of genetic variants, wherein said set of genetic variants are identified with respect to a reference;
(c) obtaining a probe set comprising a plurality of nucleic acid probe molecules having said plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in said at least one biological sample;
(d) using said probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in an additional biological sample from said subject or at least one biological relative of said subject, to generate a sequencing library; and
(e) subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less to identify a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative, wherein a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a).



1. A method for personalized genetic testing, comprising:
(a) sequencing nucleic acid molecules from at least one biological sample of a subject to generate sequencing data, wherein said sequencing comprises sequencing 12 gigabases (GB) or more;

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('611 POR at 27)

EX1001 ('611 Patent) at claim 1

Newman: Does Not Disclose Sequencing Method For GBM Samples

The '611 Petition

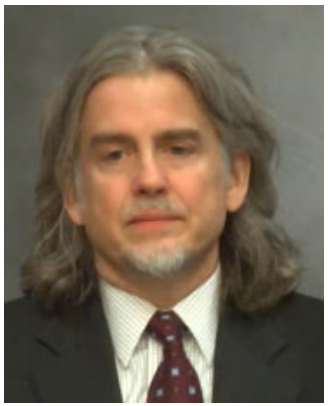
> ***Newman*** discloses that "[s]equencing was performed using 2 x 100 or 2 x 150 paired-end reads …." Ex. 1043 at "Library preparation and sequencing" in "Online Methods." In its supplementary tables, published online together with and at the

'611 Petition at 57

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DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Dr. Quackenbush's Motivations: Cost



John Quackenbush
Foresight's Expert

A. In our assays, you know, with a fixed budget, as a laboratory scientist, we have always striven to be as cost efficient as possible, because that typically allows us to collect data on larger numbers of subjects, which in some applications is, is really driving some of the science that we do. So, a person of ordinary skill in the art would consider that. If the assay was simply too expensive, then one could not effectively run this assay.

EX2163 (10/20/23 Quackenbush Depo) at 29:21-30:10

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('783 POR at 30, '611 POR at 37)

Newman: No "Additional Biological Sample"

1. A method for personalized genetic testing, comprising:
(a) sequencing nucleic acid molecules from at least one biological sample of a subject to generate sequencing data, wherein said sequencing comprises sequencing 12 gigabases (GB) or more;
(b) computer processing said sequencing data to identify a plurality of nucleic acid sequences having a set of genetic variants, wherein said set of genetic variants are identified with respect to a reference;
(c) obtaining a probe set comprising a plurality of nucleic acid probe molecules having said plurality of nucleic acid sequences or complements thereof, wherein said plurality of nucleic acid probe molecules are configured to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in said at least one biological sample;
(d) using said probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in an additional biological sample from said subject or at least one biological relative of said subject, to generate a sequencing library; and
(e) subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less to identify a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative, wherein a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a).

(d) using said probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in an additional biological sample from said subject or at least one biological relative of said subject, to generate a sequencing library; and

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('611 POR at 30)

EX1001 ('611 Patent) at claim 1

Newman: ctDNA Samples Not Used With Tissue Biopsy Samples



ARTICLES

nature biotechnology

Integrated digital error suppression for improved detection of circulating tumor DNA

Aaron M Newman[1,2,10], Alexander F Lovejoy[1,3,4,9,10], Daniel M Klass[1,2,4,9,10], David M Kurtz[1,2,5], Jacob J Chabon[1], Florian Scherer[2], Henning Stehr[4], Chih Long Liu[1,2], Scott V Bratman[1,3], Carmen Say[3], Li Zhou[4], Justin N Carter[3], Robert B West[6], George W Sledge, Jr[2,4], Joseph B Shrager[7], Billy W Loo Jr[3], Joel W Neal[2], Heather A Wakelee[2], Maximilian Diehn[1–3,11] & Ash A Alizadeh[1,2,4,8,11]

High-throughput sequencing of circulating tumor DNA (ctDNA) promises to facilitate personalized cancer therapy. However, low quantities of cell-free DNA (cfDNA) in the blood and sequencing artifacts currently limit analytical sensitivity. To overcome these limitations, we introduce an approach for integrated digital error suppression (iDES). Our method combines *in silico* elimination of highly stereotypical background artifacts with a molecular barcoding strategy for the efficient recovery of cfDNA molecules. Individually, these two methods each improve the sensitivity of cancer personalized profiling by deep sequencing (CAPP-Seq) by about threefold, and synergize when combined to yield ~15-fold improvements. As a result, iDES-enhanced CAPP-Seq facilitates noninvasive variant detection across hundreds of kilobases. Applied to non-small cell lung cancer (NSCLC) patients, our method enabled biopsy-free profiling of *EGFR* kinase domain mutations with 92% sensitivity and >99.99% specificity at the variant level, and with 90% sensitivity and 96% specificity at the patient level. In addition, our approach allowed monitoring of NSCLC ctDNA down to 4 in 10^5 cfDNA molecules. We anticipate that iDES will aid the noninvasive genotyping and detection of ctDNA in research and clinical settings.

Liquid biopsies have the potential to improve cancer detection, non-invasive tumor genotyping, and disease monitoring[1–7]. However, in most early- and many advanced-stage solid tumors, cfDNA levels are very low[3,8,9], complicating ctDNA detection and analysis. We recently described CAPP-Seq[3] as an approach for highly sensitive quantitation of ctDNA in most patients with a given cancer type without the need for patient-specific optimizations. CAPP-Seq combines hybrid affinity capture of hundreds of genomic regions with deep sequencing and a specialized bioinformatics workflow. With a priori knowledge of tumor genotypes from sequencing of tumor biopsies, this method achieved a detection limit of 0.02% in cfDNA, and detected ctDNA in patients with early and advanced stages of the most common human malignancy, NSCLC[3].

Several factors influence ctDNA detection limits, but recovery of cfDNA molecules and nonbiological errors introduced during library preparation and sequencing continue to represent a major obstacle for ultrasensitive ctDNA profiling. To address these challenges and to further improve the performance of CAPP-Seq, we characterized the landscape of background errors observed during deep sequencing of cfDNA. Based on our findings, we developed iDES, an approach that eliminates most artifacts observed in cfDNA sequencing data while maximizing recovery of cfDNA molecules. We found that when incorporated into CAPP-Seq, iDES substantially outperforms other error suppression methods for the analysis of ctDNA from clinically practical blood volumes and allows for efficient noninvasive ctDNA profiling in the absence of available tissue biopsies. By demonstrating biopsy-free tumor genotyping and disease monitoring of NSCLC patients, our results suggest that iDES can greatly improve the sensitivity and specificity of the detection of low-frequency alleles in ctDNA.

RESULTS

Barriers to noninvasive genotyping and monitoring of ctDNA

Two important factors underlying the detection limit of all ctDNA profiling methods are the number of cfDNA molecules that are recovered and the number of mutations in a patient's tumor that are interrogated. In our original description of CAPP-Seq, we developed strategies to optimize these variables from clinically relevant blood volumes, which are frequently limiting in cancer patients due to anemia, comorbidities, and poor patient performance status, among other factors. However, the degree to which analytical sensitivity can be further improved, whether through more efficient cfDNA recovery or increased coverage of tumor-specific aberrations, is ultimately limited by technical background (Fig. 1a). In analyzing cfDNA from healthy controls, background errors were increasingly evident below allele fractions of ~0.2%. Under an allele fraction of 0.02% (our previous detection limit), >50% of sequenced

[1]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, California, USA. [2]Department of Medicine, Division of Oncology, Stanford Cancer Institute, Stanford University, Stanford, California, USA. [3]Department of Radiation Oncology, Stanford University, Stanford, California, USA. [4]Stanford Cancer Institute, Stanford University, Stanford, California, USA. [5]Department of Bioengineering, Stanford University, Stanford, California, USA. [6]Department of Pathology, Stanford University, Stanford, California, USA. [7]Division of Thoracic Surgery, Department of Cardiothoracic Surgery, Stanford School of Medicine, Stanford University, Stanford, California, USA. [8]Division of Hematology, Department of Medicine, Stanford Cancer Institute, Stanford University, Stanford, California, USA. [9]Present address: Roche Molecular Systems Inc., Pleasanton, California, USA. [10]These authors contributed equally to this work. [11]These authors jointly directed this work. Correspondence should be addressed to M.D. (diehn@stanford.edu) or A.A.A. (arasha@stanford.edu).

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Figure 1 Framework for noninvasive profiling of ctDNA. (**a**) Theoretical ctDNA detection limits are shown for single-mutation noninvasive genotyping and multi-mutation monitoring based on a typical cfDNA yield from 10 ml blood (assuming ~50% molecule recovery[8]; top) (Online Methods). Background noise of CAPP-Seq is shown to increase as a function of lower ctDNA allele fractions. (**b**) Schematic illustrating the potential application of CAPP-Seq to noninvasive (biopsy-free) genotyping and monitoring of ctDNA.



DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('611 POR at 55)

EX1043 (Newman) at 548, Fig. 1b

Law: Teaching Away Shows Non-obviousness

"A reference may be said to teach away when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant."

DePuy Spine, Inc. v. Medtrnoic Sofamore Danek, Inc., 567 F.3d 1314, 1327 (Fed. Cir. 2009)

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Newman: Authored By Foresight's Founders



ARTICLES

nature biotechnology

Integrated digital error suppression for improved detection of circulating tumor DNA

Aaron M Newman[1,2,10], Alexander F Lovejoy[1,3,4,9,10], Daniel M Klass[1,2,4,9,10], David M Kurtz[1,2,5], Jacob J Chabon[1], Florian Scherer[2], Henning Stehr[4], Chih Long Liu[1,2], Scott V Bratman[1,3], Carmen Say[3], Li Zhou[4], Justin N Carter[3], Robert B West[6], George W Sledge, Jr[2,4], Joseph B Shrager[7], Billy W Loo Jr[3], Joel W Neal[2], Heather A Wakelee[2], Maximilian Diehn[1–3,11] & Ash A Alizadeh[1,2,4,8,11]

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Liquid biopsies have the potential to improve cancer detection, non-invasive tumor genotyping, and disease monitoring[1–7]. However, in most early- and many advanced-stage solid tumors, ctDNA levels are very low[3,8,9], complicating ctDNA detection and analysis. We recently described CAPP-Seq[3] as an approach for highly sensitive quantitation of ctDNA in most patients with a given cancer type without the need for patient-specific optimizations. CAPP-Seq combines hybrid affinity capture of hundreds of genomic regions with deep sequencing and a specialized bioinformatics workflow. With a priori knowledge of tumor genotypes from sequencing of tumor biopsies, this method achieved a detection limit of 0.02% in cfDNA, and detected ctDNA in patients with early and advanced stages of the most common human malignancy, NSCLC[3].

Several factors influence ctDNA detection limits, but recovery of cfDNA molecules and nonbiological errors introduced during library preparation and sequencing continue to represent a major obstacle for ultrasensitive ctDNA profiling. To address these challenges and to further improve the performance of CAPP-Seq, we characterized the landscape of background errors observed during deep sequencing of cfDNA. Based on our findings, we developed iDES, an approach that eliminates most artifacts observed in cfDNA sequencing data while maximizing recovery of cfDNA molecules. We found that when incorporated into CAPP-Seq, iDES substantially outperforms other error suppression methods for the analysis of ctDNA from clinically practical blood volumes and allows for efficient noninvasive ctDNA profiling in the absence of available tissue biopsies. By demonstrating biopsy-free tumor genotyping and disease monitoring of NSCLC patients, our results suggest that iDES can greatly improve the sensitivity and specificity of the detection of low-frequency alleles in ctDNA.

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[1]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, California, USA. [2]Department of Medicine, Division of Oncology, Stanford Cancer Institute, Stanford University, Stanford, California, USA. [3]Department of Radiation Oncology, Stanford University, Stanford, California, USA. [4]Stanford Cancer Institute, Stanford University, Stanford, California, USA. [5]Department of Bioengineering, Stanford University, Stanford, California, USA. [6]Department of Pathology, Stanford University, Stanford, California, USA. [7]Division of Thoracic Surgery, Department of Cardiothoracic Surgery, Stanford School of Medicine, Stanford University, Stanford, California, USA. [8]Division of Hematology, Department of Medicine, Stanford Cancer Institute, Stanford University, Stanford, California, USA. [9]Present address: Roche Molecular Systems Inc., Pleasanton, California, USA. [10]These authors contributed equally to this work. [11]These authors jointly directed this work. Correspondence should be addressed to M.D. (diehn@stanford.edu) or A.A.A. (arasha@stanford.edu).

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Integrated digital error suppression for improved detection of circulating tumor DNA

Aaron M Newman[1,2,10], Alexander F Lovejoy[1,3,4,9,10], Daniel M Klass[1,2,4,9,10], David M Kurtz[1,2,5], Jacob J Chabon[1], Florian Scherer[2], Henning Stehr[4], Chih Long Liu[1,2], Scott V Bratman[1,3], Carmen Say[3], Li Zhou[4], Justin N Carter[3], Robert B West[6], George W Sledge, Jr[2,4], Joseph B Shrager[7], Billy W Loo Jr[3], Joel W Neal[2], Heather A Wakelee[2], Maximilian Diehn[1–3,11] & Ash A Alizadeh[1,2,4,8,11]

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

EX1043 (Newman) at 547

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Newman: Tested For Lung Cancer, Not Brain Cancer



ARTICLES

nature biotechnology

Integrated digital error suppression for improved detection of circulating tumor DNA

Aaron M Newman[1,2,10], Alexander F Lovejoy[1,3,4,9,10], Daniel M Klass[1,2,4,9,10], David M Kurtz[1,2,5], Jacob J Chabon[1], Florian Scherer[2], Henning Stehr[4], Chih Long Liu[1,2], Scott V Bratman[1,3], Carmen Say[3], Li Zhou[4], Justin N Carter[3], Robert B West[6], George W Sledge, Jr[2,4], Joseph B Shrager[7], Billy W Loo Jr[3], Joel W Neal[2], Heather A Wakelee[2], Maximilian Diehn[1–3,11] & Ash A Alizadeh[1,2,4,8,11]

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approach to direct profiling of tumor tissues. We studied all NSCLC patients in our cohort with a known tumor genotype from a tissue biopsy, a matching peripheral blood leukocyte sample, and at least three longitudinal plasma time points for correlation assessments (n = 8). Regardless of whether mutations were identified directly from pretreatment plasma with iDES-enhanced CAPP-Seq or determined from tumor biopsies, ctDNA measurements were highly correlated (**Fig. 5d**, top; **Supplementary Fig. 14**). Moreover, mutations detected in plasma were confirmed in a matching primary tumor in seven of eight evaluable patients for ctDNA fractions as low as 0.04%. When comparing the various error-suppression strategies for monitoring ctDNA, iDES continued to outperform the other methods tested (**Fig. 5d**, bottom). These results demonstrate the

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('611 POR at 47)

EX1043 (Newman) at 553

Personalis 128

Law: Anticipation Requires Disclosure "Arranged As In The Claim"

"Because the hallmark of anticipation is prior invention, the prior art reference—in order to anticipate under 35 U.S.C. § 102—must not only disclose all elements of the claim within the four corners of the document, but must also disclose those elements 'arranged as in the claim.'"

Net MoneyIN, Inc. v. VeriSign, Inc., 545 F.3d 1359, 1369 (Fed. Cir. 2008)

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Law: Picking And Choosing Not Allowed For Anticipation

"Thus, for the instant rejection under 35 U.S.C. § 102(e) to have been proper, the Flynn reference must clearly and unequivocally disclose the claimed compound or direct those skilled in the art to the compound without any need for picking, choosing, and combining various disclosures not directly related to each other by the teachings of the cited reference."

Application of Arkley, 455 F.2d 586, 587 (C.C.P.A. 1972).

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Newman Does Not Teach The Required Sequencing Coverage

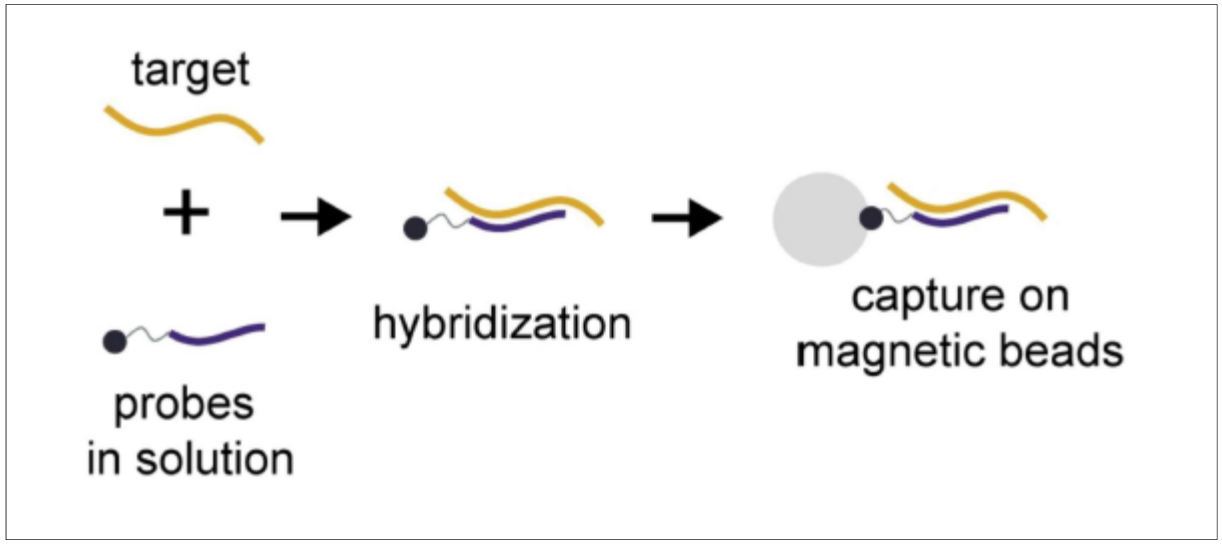
1. A method for personalized genetic testing, comprising:
(a) sequencing nucleic acid molecules from at least one biological sample of a subject to generate sequencing data, wherein said sequencing comprises sequencing 12 gigabases (GB) or more;
(b) computer processing said sequencing data to identify a plurality of nucleic acid sequences having a set of genetic variants, wherein said set of genetic variants are identified with respect to a reference;
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(d) using said probe set to selectively enrich or amplify sequences comprising said set of genetic variants over other sequences in an additional biological sample from said subject or at least one biological relative of said subject, to generate a sequencing library; and
(e) subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less to identify a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative, wherein a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a).

EX1001 ('611 Patent) at claim 1

(e) subjecting said sequencing library to sequencing at a coverage of 175 kilobases (kb) or less to identify a presence or absence of at least a subset of said set of genetic variants in said additional biological sample from said subject or said at least one biological relative, wherein a sequencing depth of said sequencing of (e) is greater than a sequencing depth of said sequencing of (a).

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('611 POR at 30)

Petition: Depicts Probes As Being Shorter Than Targets

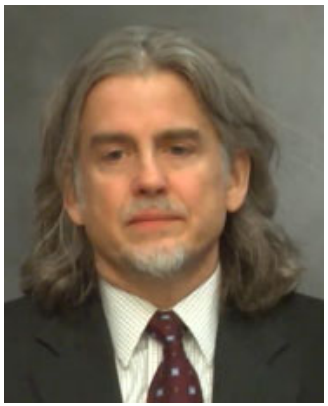


DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petition at 13

Personalis 132

Dr. Quackenbush: Does Not Know Length Of Capture DNA Molecules



John Quackenbush
Foresight's Expert

Q. Do you know the length of the DNA molecules that were captured by the 157 kilobase personalized selector that Newman used?

A. I don't think that information is provided. But the goal was to target 157,000 base pairs that contain 1,500 mutations.

EX2163 (10/20/23 Quackenbush Depo) at 85:17-22

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('611 POR at 49)

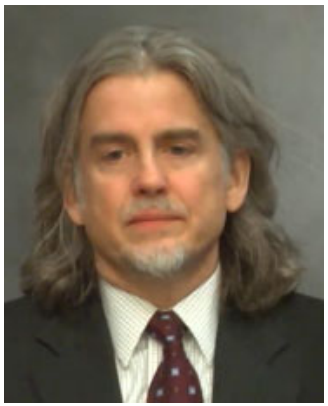
’611 Patent Claim 5

5. The method of claim **1**, wherein each of said plurality of nucleic acid probe molecules includes oligonucleotide-directed genomic content comprising (i) at least one variable portion from a result of said sequencing of (a) and (ii) at least one fixed portion independent of said result of said sequencing of (a).

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

EX1001 (’611 Patent) at claim 5

Dr. Quackenbush: Does Not Know Length Of Capture DNA Molecules



John Quackenbush
Foresight's Expert

Q. Now, the, Newman's personalized selector, that was 157 kilobases, is not the same as the NSCL selector that has the eight mutations per NSCLC patient. Right?

A. So, the non-small cell lung carcinoma, the NSCLC selector, is distinct from the glioblastoma selector.

EX2163 (10/20/23 Quackenbush Depo) at 222:8-14

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petition: Relies On What A POSA "Could" Do

The '611 Petition

There is no reason given in the '611 patent why fixed sequences such as these could not be combined with variable sequences such as the ones used for the GBM patient's tumor, and a person of ordinary skill in the art knew that a panel of 175 kb or less could be designed and produced with fixed and variable sequences.

'611 Petition at 73

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Personalis 136

Petition: Relies On NSCLC For Claim 5

The ’611 Petition

To the extent claim 5 is not anticipated, it would have been obvious over ***Newman***. Quackenbush ¶¶235-243. ***Newman*** discloses a plurality of nucleic acid probe molecules with at least one fixed portion independent of the result of the step 1(a) sequencing. For example, in its “NSCLC selector design,” Newman taught designing a fixed selector “to target a median of at least eight mutations per NSCLC patient.” *Id.* at “NSCLC selector design” in Online Methods.” Newman also states that “additional clinically relevant regions were included … to target actionable fusions in *ALK*, *ROS1*, and *RET* using intronic probes, and to ensure coverage of selected regions containing other actionable variants.” *Id.* All of the contents of these NSCLC selector panels are nucleic acid molecules with fixed sequences that are independent of the sequencing results of step 1(a).

’611 Petition at 73

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petition: Relies On Brain Samples For Claim 1

Sample number	Cohort	Case	Collection time[a]	Sample type	Input mass (ng)	Selector[b]	Sequencing adapter[c]	De-duplication[d]	Total reads
275	Healthy/Te	GBM-CTR25	GBM-CTR25-	cfDNA	32.0	GBM	Tandem	Barcoding	37,647,656
276	Healthy/Te	GBM-CTR26	GBM-CTR26-	cfDNA	32.0	GBM	Tandem	Barcoding	38,321,932
277	Healthy/Te	GBM-CTR27	GBM-CTR27-	cfDNA	32.0	GBM	Tandem	Barcoding	33,065,346
278	Healthy/Te	GBM-CTR28	GBM-CTR28-	cfDNA	32.0	GBM	Tandem	Barcoding	35,976,502
279	Technical	GBM-Spike-0.	GBM-Spike-(	cfDNA	32.0	GBM	Tandem	Barcoding	103,288,518
280	Technical	GBM-Spike-0.	GBM-Spike-(	cfDNA	32.0	GBM	Tandem	Barcoding	105,786,270
281	Technical	GBM-Spike-0.	GBM-Spike-(	cfDNA	32.0	GBM	Tandem	Barcoding	100,222,542
282	Technical	GBM-Spike-0.	GBM-Spike-(	cfDNA	32.0	GBM	Tandem	Barcoding	66,777,970
283	Technical	GBM-Spike-0.	GBM-Spike-(	cfDNA	32.0	GBM	Tandem	Barcoding	56,060,804
284	Technical	HD500-0.5%-i	HD500-0.5%-	Cell line bl	72.0	NSCLC tumor ge	Tandem	Barcoding	270,879,108
285	Healthy/Te	CTR31	CTR31-c1	cfDNA	32.0	NSCLC tumor ge	Tandem	Barcoding	259,725,374
286	GBM	GBM Relapse	na	Tumor	100.00	SeqCap EZ Hum	Tandem	Barcoding	82,247,848
287	GBM	GBM PBL	na	PBL	100.00	SeqCap EZ Hum	Tandem	Barcoding	86,781,192

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petition at 57

Personalis 138

Law: Anticipation Requires Disclosure "Arranged As In The Claim"

"Because the hallmark of anticipation is prior invention, the prior art reference—in order to anticipate under 35 U.S.C. § 102—must not only disclose all elements of the claim within the four corners of the document, but must also disclose those elements 'arranged as in the claim.'"

Net MoneyIN, Inc. v. VeriSign, Inc., 545 F.3d 1359, 1369 (Fed. Cir. 2008)

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Law: Picking And Choosing Not Allowed For Anticipation

"Thus, for the instant rejection under 35 U.S.C. § 102(e) to have been proper, the Flynn reference must clearly and unequivocally disclose the claimed compound or direct those skilled in the art to the compound without any need for picking, choosing, and combining various disclosures not directly related to each other by the teachings of the cited reference."

Application of Arkley, 455 F.2d 586, 587 (C.C.P.A. 1972).

’611 Patent Claims 10, 28

10. The method of claim **1**, further comprising providing a therapeutic intervention at least based on said presence or absence of said at least said subset of said genetic variants identified in (e).

28. The method of claim **1**, further comprising using said presence or absence of said at least said subset of said set of genetic variants identified in (e) to treat said subject or said at least one biological relative for a disorder.

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

EX1001 (’611 Patent) at claims 10, 28

Newman: No Treatment Intervention



nature biotechnology

Integrated digital error suppression for improved detection of circulating tumor DNA

Aaron M Newman[1,2,10], Alexander F Lovejoy[1,3,4,9,10], Daniel M Klass[1,2,4,9,10], David M Kurtz[1,2,5], Jacob J Chabon[1], Florian Scherer[2], Henning Stehr[4], Chih Long Liu[1,2], Scott V Bratman[1,3], Carmen Say[3], Li Zhou[4], Justin N Carter[3], Robert B West[6], George W Sledge, Jr[2,4], Joseph B Shrager[7], Billy W Loo Jr[3], Joel W Neal[2], Heather A Wakelee[2], Maximilian Diehn[1–3,11] & Ash A Alizadeh[1,2,4,8,11]

High-throughput sequencing of circulating tumor DNA (ctDNA) promises to facilitate personalized cancer therapy. However, low quantities of cell-free DNA (cfDNA) in the blood and sequencing artifacts currently limit analytical sensitivity. To overcome these limitations, we introduce an approach for integrated digital error suppression (iDES). Our method combines *in silico* elimination of highly stereotypical background artifacts with a molecular barcoding strategy for the efficient recovery of cfDNA molecules. Individually, these two methods each improve the sensitivity of cancer personalized profiling by deep sequencing (CAPP-Seq) by about threefold, and synergize when combined to yield ~15-fold improvements. As a result, iDES-enhanced CAPP-Seq facilitates noninvasive variant detection across hundreds of kilobases. Applied to non-small cell lung cancer (NSCLC) patients, our method enabled biopsy-free profiling of *EGFR* kinase domain mutations with 92% sensitivity and >99.99% specificity at the variant level, and with 90% sensitivity and 96% specificity at the patient level. In addition, our approach allowed monitoring of NSCLC ctDNA down to 4 in 10^5 cfDNA molecules. We anticipate that iDES will aid the noninvasive genotyping and detection of ctDNA in research and clinical settings.

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Liquid biopsies have the potential to improve cancer detection, noninvasive tumor genotyping, and disease monitoring[1–7]. However, in most early- and many advanced-stage solid tumors, ctDNA levels are very low[2,8,9], complicating ctDNA detection and analysis. We recently described CAPP-Seq[2] as an approach for highly sensitive quantitation of ctDNA in most patients with a given cancer type without the need for patient-specific optimizations. CAPP-Seq combines hybrid affinity capture of hundreds of genomic regions with deep sequencing and a specialized bioinformatics workflow. With a priori knowledge of tumor genotypes from sequencing of tumor biopsies, this method achieved a detection limit of 0.02% in cfDNA, and detected ctDNA in patients with early and advanced stages of the most common human malignancy, NSCLC[2].

Several factors influence ctDNA detection limits, but recovery of cfDNA molecules and nonbiological errors introduced during library preparation and sequencing continue to represent a major obstacle for ultrasensitive ctDNA profiling. To address these challenges and to further improve the performance of CAPP-Seq, we characterized the landscape of background errors observed during deep sequencing of cfDNA. Based on our findings, we developed iDES, an approach that eliminates most artifacts observed in cfDNA sequencing data while maximizing recovery of cfDNA molecules. We found that when incorporated into CAPP-Seq, iDES substantially outperforms other error suppression methods for the analysis of ctDNA from clinically practical blood volumes and allows for efficient noninvasive ctDNA profiling in the absence of available tissue biopsies. By demonstrating biopsy-free tumor genotyping and disease monitoring of NSCLC patients, our results suggest that iDES can greatly improve the sensitivity and specificity of the detection of low-frequency alleles in ctDNA.

RESULTS

Barriers to noninvasive genotyping and monitoring of ctDNA

Two important factors underlying the detection limit of all ctDNA profiling methods are the number of cfDNA molecules that are recovered and the number of mutations in a patient's tumor that are interrogated. In our original description of CAPP-Seq, we developed strategies to optimize these variables from clinically relevant blood volumes, which are frequently limiting in cancer patients due to anemia, comorbidities, and poor patient performance status, among other factors. However, the degree to which analytical sensitivity can be further improved, whether through more efficient cfDNA recovery or increased coverage of tumor-specific aberrations, is ultimately limited by technical background (Fig. 1a). In analyzing cfDNA from healthy controls, background errors were increasingly evident below allele fractions of ~0.2%. Under an allele fraction of 0.02% (our previous detection limit), >50% of sequenced

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Foresight Ex 1043
Foresight v Personalis
IPR2023-00546

The analysis of ctDNA is likely to play a major role in personalized cancer therapy by addressing several unmet clinical needs, including (i) noninvasive genotyping of clinically actionable tumor-derived variants to guide therapy selection[29,30], (ii) detection of emergent, actionable variants associated with resistance during targeted therapy[30–33], (iii) early measurement of therapeutic responses[6,34], and (iv) detection of minimal residual disease that is radiographically occult during disease surveillance[6,35–37]. Each of these applications

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE ('611 POR at 51)

EX1043 (Newman) at 554

Personalis 142

’611 Patent Claim 11

11. The method of claim **1**, wherein said at least one biological sample includes a tumor sample and said nucleic acids molecules are from cells in said tumor sample.

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

EX1001 (’611 Patent) at claim 11

Petition: Relies On Lung Samples For Claim 11

The ’611 Petition

Newman anticipates claim 11. Quackenbush ¶¶213-15. For example, ***Newman*** discloses analysis of lung tumor samples, and specifically nucleic acid molecules from cells in lung tumor samples. Ex. 1043 at 548. Further, ***Newman*** “assessed iDES-enhanced CAPP-Seq genotyping using 66 serial plasma samples from patients with NSCLC, all of which had tumor biopsies available.” *Id.* at 551. *See also* Quackenbush ¶215.

’611 Petition at 67

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petition: Relies On Brain Samples For Claim 1

Sample number	Cohort	Case	Collection time[a]	Sample type	Input mass (ng)	Selector[b]	Sequencing adapter[c]	De-duplication[d]	Total reads
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282	Technical	GBM-Spike-0.	GBM-Spike-(	cfDNA	32.0	GBM	Tandem	Barcoding	66,777,970
283	Technical	GBM-Spike-0.	GBM-Spike-(	cfDNA	32.0	GBM	Tandem	Barcoding	56,060,804
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286	GBM	GBM Relapse	na	Tumor	100.00	SeqCap EZ Hum	Tandem	Barcoding	82,247,848
287	GBM	GBM PBL	na	PBL	100.00	SeqCap EZ Hum	Tandem	Barcoding	86,781,192

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Petition at 57

Personalis 145

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DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

THE END