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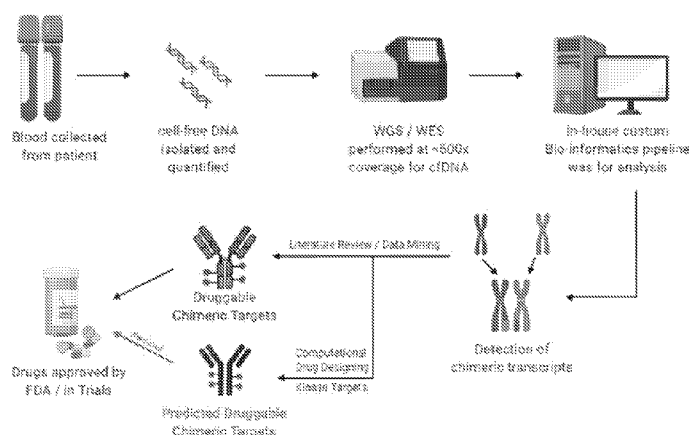


FIG. 1

(57) Abstract: Novel powerful methods for identifying, monitoring, and treating conditions associated with omics-discoverable features in human subjects, and particularly conditions such as malignant tumors are provided.



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METHODS AND BIOMARKERS FOR DIAGNOSTICS, DISEASE MONITORING,
PERSONALIZED DRUG DISCOVERY AND TARGETED THERAPY IN GLIOMA
PATIENTS

FIELD OF THE INVENTION

5 The present invention relates to compositions, methods, and biomarkers for diagnostics, monitoring and therapy of glioma in human patients, utilizing the techniques of data mining, computational biology, artificial intelligence, and molecular biology.

10 BACKGROUND OF THE INVENTION

Aberrations such as chromosomal translocations, trans-splicing, fusions and gene variants are frequently found in human disorders. Chromosomal translocations may result in a chimeric gene expressing a fusion transcript which is then translated
15 into a fusion protein that affects normal regulatory pathways. Cancer is one of the most prominent examples of such disorder. Liquid biopsy is a newly emerging technique for cancer diagnostics and being widely accepted because of its non-invasive approach and many advantages over biopsy-based
20 diagnostics [1]. This technique uses circulating cell-free nucleic acid fragment, namely circulating cell-free DNA (cfDNA) fragments and/or circulating free RNA (cfrRNA). CfDNA is free floating small fragments of nucleic acids/DNA in the blood plasma that are not associated with cells or cell fragments.
25 CfDNA has been shown to be present in patients with various types of neoplasms and is not thought to be directly related to metastasis. This cfDNA may be analyzed for specific genetic markers of neoplasm with varying degrees of specificity and sensitivity. CfDNA that are released into the blood stream by
30 the tumor tissue can be screened for the cancer specific mutations for diagnostics of cancers. Unlike biopsy-based diagnosis, liquid biopsy can be repeated multiple times, which

gives a profile of real time mutations in tumor and, more importantly, represents tumor heterogeneity.

Glioma Gliomas account for about 80% of all malignant brain tumors. Diagnosis is achieved by radiographic imaging followed
5 by tumor resection, to determine tumor cell type, grade and molecular characteristics. Glioblastoma multiforme (GBM) is the most common type of glioma and is mostly fatal. The median survival of treated GBM patients is 12-15 months. Standard modalities of therapy are unselective and include surgery,
10 radiation therapy and chemotherapy, while precision medicine has yet to demonstrate improvements in disease outcome. We, therefore, selected GBM as a model to develop a precision medicine methodology for monitoring patients using blood plasma circulating cell-free DNA (cfDNA). Currently, tumor
15 heterogeneity, clonal diversity and mutation acquisition are the major impedances for tailoring personalized therapy in gliomas in general, and, particularly, in GBM. Thus, a liquid biopsy diagnostics platform based on cfDNA deep sequencing may improve treatment outcome in personal diagnostics for GBM patients, and
20 guide therapy selection.

Current methods and systems for analyzing the genetic markers of humans and providing tailor-made therapeutic means are still very limited. Therefore, there is an urgent, unmet need in game-changing technologies based on data mining and computational
25 biology which will enable generating a characterization of the condition and generating a therapy model configured to correct the condition, thus providing tailored and personalized treatment solutions.

SUMMARY OF THE INVENTION

30 The invention provides a novel powerful method for identifying, monitoring, and treating a condition in a human subject, wherein said condition is associated with omics-discoverable features.

In one embodiment, the invention provides method for identifying a condition in a human subject, wherein said condition is characterized by omics-discoverable features; the method comprising:

- 5 a. obtaining a biological sample from the at least one human subject, wherein said biological sample comprises cell-free nucleic acids;
- b. sequencing said cell-free nucleic acids;
- c. mapping the sequencing results to the reference human
10 genome;
- d. identifying unmapped non-linear reads;
- e. mapping the unmapped non-linear reads to the pre-computed sequence data set indicative of said condition; and
- f. identifying at least one sequence associated with said
15 condition.

The invention further provides a method for treating a condition in a human subject, wherein said condition is characterized by omics-discoverable features, the method comprising:

- a. obtaining a biological sample from at least one human
20 subject, wherein said biological sample comprises cell-free nucleic acids;
- b. sequencing said cell-free nucleic acids;
- c. mapping the sequencing results to the reference human genome;
- 25 d. identifying unmapped non-linear reads;
- e. mapping the unmapped non-linear reads to the pre-computed sequence data set indicative of said condition;
- f. identifying at least one sequence associated with said condition;
- 30 g. applying a pre-computed treatment model to identify therapeutic means suitable for treating the condition;
- h. identifying the therapeutic means for treating the condition based on the pre-computed treatment model; and
- i. providing the human subject with the therapeutic means

to thereby effectively treat the condition in the human subject. The invention further provides therapeutic means for use in the treatment of a neurodegenerative disorder characterized by omics-discoverable features in a subject, wherein said
5 therapeutic means are identified by applying a pre-computed treatment model designed to identify the therapeutic means suitable for treating said autoimmune disease; and, wherein said identifying of the therapeutic means comprises the steps of:

- a. obtaining a biological sample from at least one human
10 subject wherein said biological sample comprises cell-free nucleic acids;
- b. sequencing said cell-free nucleic acids;
- c. mapping the sequencing results to the reference human genome;
- 15 d. identifying unmapped non-linear reads;
- e. mapping the unmapped non-linear reads to the pre-computed sequence data set indicative of said condition;
- f. identifying sequences associated with said condition; and,
- g. applying a pre-computed treatment model to identify
20 therapeutic means suitable for treating the condition.

The invention further provides an isolated nucleotide sequence having at least 75% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO:1-26.

BRIEF DESCRIPTION OF THE DRAWINGS

- 25 Exemplary non-limited embodiments of the disclosed subject matter will be described, with reference to the following description of the embodiments, in conjunction with the figures. The figures are generally not shown to scale and any sizes are only meant to be exemplary and not necessarily limiting,
30 corresponding or like elements are optionally designated by the same numerals or letters.

Figure 1 is a graphic illustration of an exemplary embodiment of method used for quantitative and qualitative analysis of

cfDNA in glioblastoma patients. blood samples were collected from both healthy and glioma patients (before surgery) and centrifuged to separate plasma. As shown in figure, silica membrane spin-column based technique is used to separate DNA, and fragment size and concentration of cfDNA was estimated by Bioanalyzer and Qubit;

Figure 2 illustrates an exemplary embodiment of concentration of cfDNA in the blood of glioblastoma patients and healthy volunteers. First 15 bars from the left side represent cfDNA concentrations in GBM patients, additional 14 bars represent cfDNA concentrations in healthy volunteers;

Figure 3 illustrates an exemplary embodiment of mutation analysis of cfDNA and ctDNA of five GBM patients. Grey color band represents 3-prime-UTR-variant, blue color band represents upstream-gene-variant, green color band represents downstream-gene-variant, brown color band represents non-coding-exon-variant, and yellow color band represents intron variant;

Figure 4 illustrates an exemplary embodiment of different sizes of cfDNA found in blood derived following apoptosis and necrosis;

Figure 5 illustrates an exemplary embodiment of the variant calling analysis method used to identify high-impact variants. The circles diameter describes the frequency for high-impact mutations identified in GBM patients and in published cohorts;

Figure 6 illustrates an exemplary embodiment of variant analysis method used to identify high impact variants;

Figure 7 illustrates an exemplary embodiment of mutation validation by Sanger sequencing; and,

Figure 8 illustrates an exemplary embodiment of gene set enrichment analysis flowchart and six significant pathways, for which at least one gene was detected as both a frequently mutated glioblastoma gene and a gene identified as a frequent fusion in GBM.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is now described more fully hereinafter. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather these embodiments are provided so that this disclosure will be thorough and complete and will fully convey the scope of the invention to those skilled in the art.

In one embodiment of the invention, provided a method for identifying a condition in a human subject, wherein said condition is characterized by omics-discoverable features; the method comprising:

- a. obtaining a biological sample from the at least one human subject, wherein said biological sample comprises cell-free nucleic acids;
- b. sequencing said cell-free nucleic acids;
- c. mapping the sequencing results to the reference human genome;
- d. identifying unmapped non-linear reads;
- e. mapping the unmapped non-linear reads to the pre-computed sequence data set indicative of said condition; and
- f. identifying at least one sequence associated with said condition.

According to some embodiments, the method of the invention further comprises a step of isolating circulating cell-free nucleic acids from the biological sample. In one embodiment, the condition is a malignant disorder. In one embodiment, the malignant disorder is glioma. In the context of the invention, "glioma" refers, without limitation to cancerous growth or tumor composed of cells derived from neuroglial tissue, the material that supports and protects nerve cells. Gliomas typically form in the brain or spinal cord and are classified by cell type, location, or grade (based on microscopic features of tumor cells, usually relative to features of normal cells). A non-

limiting list of different types of gliomas includes optic glioma, oligodendroglial tumors, ependymomas, and glioblastoma (glioblastoma multiforme). In the context of the invention, the phrase "a method of identifying a condition" is meant to be understood, without limitation, as diagnostic means, namely a method for diagnosing, recognizing, detecting and monitoring various characteristics of a certain disease or a condition.

As used herein, the phrase "pre-computed sequence data" refers, without limitation, to a pre-computed set of nucleotide sequences found in databases or generated using some heuristic or combination as random sequences; or merged as parts of sequences to a set of novel sequences, including separated and merged exons, introns, genes, pseudogenes or any genomic sequences and/or chimeric RNA sequences or fusion genes sequences that cannot be mapped to human genome linearly; and genomic "integrations" of pathogens to human genome.

As used herein the term "omics" refers, without limitation to genomics, proteomics, metagenomics, methylomics, epigenomics, and metabolomics. As used herein the term "biological sample" refers, without limitation to any biological material collected from a subject. A non-limiting list of biological samples of the invention includes blood, serum, plasma, urine, saliva, amniotic fluid, feces, synovial fluid, peritoneal fluid, pleural fluid, lymphatic fluid, mucus, and cerebrospinal fluid (CSF), or any other body fluid or acceptable body tissue. In one embodiment, the biological sample is a liquid biological sample. As used herein, the term "circulating cell-free nucleic acids" refers, without limitation to degraded nucleic acid fragments released to the blood plasma or other body fluids. In one embodiment, the circulating cell-free nucleic acids are selected from the group consisting of circulating cell-free RNA, circulating cell-free DNA, circulating cell free nucleic acid complexes, and circulating cell-free microRNA. In another embodiment, the circulating cell-free nucleic acids is circulating cell-free

DNA. According to some embodiments, the concentration of cell-free nucleic acids is 0.01 ng/ml to 200 ng/ml. In one embodiment, the concentration of cell-free nucleic acids is 0.01ng/ml, 0.05ng/ml, 0.1 ng/ml, 0.2 ng/ml, 0.3 ng/ml, 0.4 ng/ml, 0.5 ng/ml, 0.6 ng/ml, 0.7 ng/ml, 0.8 ng/ml, 0.9 ng/ml, 1ng/ml, 2ng/ml, 3 ng/ml, 4ng/ml, 5ng/ml, 6ng/ml, 7ng/ml, 8ng/ml, 9 ng/ml, 10ng/ml, 15 ng/ml, 20 ng/ml, 25 ng/ml, 30 ng/ml, 35 ng/ml, 40 ng/ml, 45 ng/ml, 50 ng/ml, 55 ng/ml, 60 ng/ml, 65 ng/ml, 70 ng/ml, 75 ng/ml, 80ng/ml, 85 ng/ml, 90 ng/ml, 95 ng/ml/ 100 ng/ml, 105 ng/ml, 110ng/ml, 115 ng/ml, 120 ng/ml, 125 ng/ml, 130 ng/ml, 135 ng/ml, 140 ng/ml, 145 ng/ml, 150 ng/ml, 155 ng/ml, 160 ng/ml, 165 ng/ml, 170 ng/ml, 175 ng/ml, 180ng/ml, 185 ng/ml, 190 ng/ml, 195 ng/ml , and 200 ng/ml. As used herein, the term "sequence" refers, without limitation to oligonucleotide or polynucleotide.

As used herein, the terms "nucleic acid", "nucleic acid sequence", "nucleotide", "nucleic acid molecule" or "polynucleotide" are intended to include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), natural occurring, mutated, synthetic DNA or RNA molecules, and analogs of the DNA or RNA generated using nucleotide analogs. It can be single-stranded or double-stranded. Such nucleic acids or polynucleotides include, but are not limited to, coding sequences of structural genes, anti-sense sequences, and non-coding regulatory sequences that do not encode mRNAs or protein products. These terms also encompass a gene. The term "gene", "allele" or "gene sequence" is used broadly to refer to a DNA (deoxynucleic nucleic acids) associated with a biological function. Thus, genes may include introns and exons as in the genomic sequence or may comprise only a coding sequence as in cDNAs, and/or may include cDNAs in combination with regulatory sequences. Thus, according to the various aspects of the invention, genomic DNA, cDNA or coding DNA may be used.

As used herein, the term "malignant disorder" refers, without limitation to a condition in which abnormal cells divide without

control and can invade nearby tissues. Malignant cells can also spread to other parts of the body through the blood and lymph systems. In the context of the invention, "malignant disorder" can be replaced by any of the following terms: cancer, neoplasm, tumor, or any other acceptable term that relates to pathological conditions accompanied by abnormal cell growth.

According to some embodiments, the sequence associated with said condition is a gene fusion. The term "gene fusion" refers to a chimeric genomic DNA resulting from the fusion of at least a portion of a first gene to a portion of a second gene. The point of transition between the sequences from the first gene in the fusion to the sequences from the second gene in the fusion is referred to as the "breakpoint" or "fusion point" and/or "chimeric junction site". Transcription of the gene fusion results in a chimeric mRNA and/or chimeric RNA transcript. As used herein in, the term "chimeric RNA transcript" refers, without limitation, to single-stranded sequences of RNAs transcribed from various locations in the total genome corresponding to exons and/or introns from two different genes; two copies of the same gene; regions of pathogen genome, which fuse together to produce a single RNA transcript and/or a single cell free DNA molecule. Two unrelated genomic loci on different chromosomes may produce a chimeric transcript through a genomic rearrangement event or due to trans-splicing. Similarly, a read-through transcript of two adjacent genomic loci may produce chimeric RNAs. As used herein, the term "gene" refers, without limitation, to a polynucleotide (e.g., a DNA segment), that encodes a polypeptide and includes regions preceding and following the coding regions as well as intervening sequences (introns) between individual coding segments (exons).

According to some embodiments, the at least one sequence associated with the condition of the invention is selected from the group consisting of sequences having at least 75% sequence identity to the nucleotide sequence set forth as SEQ ID NO:1-

26. In one embodiment, the sequence associated with said condition is selected from the group consisting of sequences having 75%-98% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 1-26. In another embodiment, the sequence associated with said condition is selected from the group consisting of sequences having at least 80%-98%, 85%-98%, 87%-98%, 90%-98%, and 95%-98% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 1-26. In one embodiment, the sequence associated with said condition is selected from the group consisting of sequences having at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, and 99% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 1-26. In another embodiment, the at least one sequence associated with the condition of the invention is selected from the group consisting of sequences set forth as SEQ ID NO: 1-26.

As used herein, "sequence identity" or "identity" in the context of two nucleic acid sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. The term further refers hereinafter to the number of characters which match exactly between two different sequences. Hereby, gaps are not counted, and the measurement is relational to the shorter of the two sequences. It is further within the scope that the terms "similarity" and "identity" additionally refer to local homology, identifying domains that are homologous or similar (in nucleotide sequence). It is acknowledged that bioinformatics tools such as BLAST, SSEARCH, FASTA, and HMMER calculate local sequence alignments which identify the most similar region between two sequences. For domains that are found in different sequence contexts in different proteins, the alignment should be limited to the homologous domain, since the domain homology is providing the sequence similarity captured in the score. According to some aspects the term similarity or identity

further includes a sequence motif, which is a nucleotide or amino-acid sequence pattern that is widespread and has, or is conjectured to have, a biological significance.

In the context of the invention, the phrase "omics-discoverable features" is meant to be understood as a characteristic that can be identified and/or recognized and/or measured by means of "omics" as defined above. In one embodiment, the omics-discoverable feature is selected from the group consisting of genomics-discoverable features, proteomics-discoverable features, metagenomics-discoverable features, methylomics-discoverable features, epigenomics-discoverable features, and metabolomics-discoverable features. The non-limiting list of omics-discoverable features of the invention include chimeras, chimeric RNAs, gene-gene fusions, sense-antisense (SAS) chimeras, genomic integrations, aberrations, inversions, and other genomic alterations.

According to some embodiments, the invention provides a method for treating a condition in a human subject, wherein said condition is characterized by omics-discoverable features, the method comprising:

- a. obtaining a biological sample from at least one human subject, wherein said biological sample comprises cell-free nucleic acids;
- b. sequencing said cell-free nucleic acids;
- c. mapping the sequencing results to the reference human genome;
- d. identifying unmapped non-linear reads;
- e. mapping the unmapped non-linear reads to the pre-computed sequence data set indicative of said condition;
- f. identifying at least one sequence associated with said condition;
- g. applying a pre-computed treatment model to identify therapeutic means suitable for treating the condition;

- h. identifying the therapeutic means for treating the condition based on the pre-computed treatment model; and
- i. providing the human subject with the therapeutic means to thereby effectively treat the condition in the human subject.

5 In one embodiment, the method further comprises the step of isolating circulating cell-free nucleic acids from the biological sample. As described herein, isolation of cell-free nucleic acids may be done by any suitable technique known in the art, commercially available or using in-house developed tools

10 and proprietary technology. In one embodiment, the biological sample is selected from the group consisting of blood, serum, plasma, urine, saliva, amniotic fluid, feces, synovial fluid, peritoneal fluid, pleural fluid, lymphatic fluid, mucus, and cerebrospinal fluid (CSF). In another embodiment, the biological

15 sample is a liquid biological sample. In one embodiment, the circulating cell-free nucleic acids is selected from the group consisting of circulating cell-free RNA, circulating cell-free DNA, circulating cell free nucleic acid complexes, and circulating cell-free microRNA.

20 According to some embodiments, the condition associated with omics-discoverable features is a malignant disorder. In one embodiment, the malignant disorder is glioma.

According to some embodiments, in the method of the invention, the at least one sequence associated with the condition of the

25 invention is selected from the group consisting of sequences having at least 75% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 1-26. In one embodiment, the sequence associated with said condition is selected from the group consisting of sequences having 75%-98% sequence identity to the

30 nucleotide sequence set forth as SEQ ID NO: 1-26. In another embodiment, the sequence associated with said condition is selected from the group consisting of sequences having at least 80%-98%, 85%-98%, 87%-98%, 90%-98%, and 95%-98% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 1-

26. In one embodiment, the sequence associated with said condition is selected from the group consisting of sequences having at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, and 99% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 1-26. In another embodiment, the at least one sequence associated with the condition of the invention is selected from the group consisting of sequences set forth as SEQ ID NO: 1-26.

According to some embodiments, in the method of the invention, omics-discoverable features are selected from the group consisting of genomics-discoverable features, proteomics-discoverable features, metagenomics-discoverable features, methylomics-discoverable features, epigenomics-discoverable features, and metabolomics-discoverable features. In another embodiment, omics-discoverable features are selected from chimeras, Chimeras, chimeric RNAs, gene-gene fusions, sense-antisense (SAS) chimeras, Genomic integrations, aberrations, inversions, and other genomic alterations.

According to some embodiments, in the method of the invention, the therapeutic means are selected from the group consisting of an investigational drug, an approved drug, a food supplement, immunotherapy, biological therapy, phototherapy, hyperbaric oxygen, radiation therapy, surgical intervention, non-invasive image-guided procedure, multi-step treatment protocol, or any combination thereof.

According to some embodiments the invention provides therapeutic means for use in the treatment of a malignant disorder characterized by omics-discoverable features in a subject, wherein said therapeutic means are identified by applying a pre-computed treatment model designed to identify the therapeutic means suitable for treating said malignant disorder; and, wherein said identifying of the therapeutic means comprises the steps of:

- a. obtaining a biological sample from at least one human subject wherein said biological sample comprises cell-free nucleic acids;
- b. sequencing said cell-free nucleic acids;
- 5 c. mapping the sequencing results to the reference human genome;
- d. identifying unmapped non-linear reads;
- e. mapping the unmapped non-linear reads to the pre-computed sequence data set indicative of said condition;
- 10 f. identifying sequences associated with said condition; and,
- g. applying a pre-computed treatment model to identify therapeutic means suitable for treating the condition.

According to some embodiments the invention provides therapeutic means and/or monitoring for use in the treatment of a malignant disorder characterized by omics-discoverable features in a subject, wherein said therapeutic means are identified by applying a pre-computed treatment model designed to identify the therapeutic means suitable for treating said malignant disorder; and, wherein said identifying of the therapeutic means comprises

20 the steps of:

- a. obtaining a biological sample from at least one human subject wherein said biological sample comprises cell-free nucleic acids;
- b. sequencing said cell-free nucleic acids;
- 25 c. mapping the sequencing results to the reference human genome;
- d. identifying unmapped non-linear reads;
- e. mapping the unmapped non-linear reads to the pre-computed sequence data set indicative of said condition;
- 30 f. identifying sequences associated with said condition; and, optionally,
- g. applying a pre-computed treatment model to identify therapeutic means suitable for treating the condition.

According to some embodiments, identifying of the therapeutic means comprises the step of isolating circulating cell-free nucleic acids from the biological sample.

In one embodiment, therapeutic means are selected from the group consisting of an investigational drug, an approved drug, a food supplement, immunotherapy, biological therapy, phototherapy, radiation therapy, surgical intervention, non-invasive image-guided procedure, multi-step treatment protocol, or a combination thereof.

In one embodiment, the malignant disorder is glioma.

According to some embodiments, the invention provides therapeutic means for use as a medicament.

According to some of the above embodiments, the invention provides therapeutic means for use in the treatment of a malignant disorder.

According to some embodiments, in the therapeutic means of the invention, the at least one sequence associated with the disorder of the invention is selected from the group consisting of sequences having at least 75% sequence identity to the

nucleotide sequence set forth as SEQ ID NO:1-26. In one embodiment, the sequence associated with said condition is selected from the group consisting of sequences having 75%-98% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 1-26. In another embodiment, the sequence associated with

said condition is selected from the group consisting of sequences having at least 80%-98%, 85%-98%, 87%-98%, 90%-98%, and 95%-98% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 1-26. In one embodiment, the sequence associated with said condition is selected from the group

consisting of sequences having at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, and 99% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 1-26. In another embodiment, the at least one sequence associated with the

condition of the invention is selected from the group consisting of sequences set forth as SEQ ID NO: 1-26.

According to some embodiments, the invention provides an isolated nucleotide sequence having at least 75% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO: 1-26. In another embodiment, the invention provides an isolated nucleotide sequence having 75%-98% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 1-26. In another embodiment, the invention provides an isolated nucleotide sequence having 80%-98%, 85%-98%, 87%-98%, 90%-98%, and 95%-98% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 1-26. In another embodiment, the invention provides an isolated nucleotide sequence having at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, and 99% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 1-26.

According to some embodiments, the invention provides an isolated nucleotide sequence selected from the group consisting of sequences set forth as SEQ ID NO: 1-26.

In one embodiment, the method of the invention utilizes the most comprehensive chimeric transcript repository, ChiTaRS 5.0 (<http://chitars.md.biu.ac.il/>), with 111,582 annotated entries from eight species. The repository includes unique information correlating chimeric breakpoints with 3D chromatin contact maps, generated from public datasets of chromosome conformation capture techniques (Hi-C). The repository comprises curated information on druggable fusion targets matched with chimeric breakpoints, which are applicable to precision medicine in cancers. ChiTaRS stands out as a unique server that integrates EST and mRNA sequences, literature resources, with RNA-sequencing data, expression level and tissue specificity of chimeric transcripts in various tissues and organisms.

According to some embodiments, the method of cfDNA analysis is based on deep Illumina HiSeq sequencing procedure as well as BGI sequencing and/or other deep sequencing procedures of cfDNA and/or circulating cell free RNA (cfRNA) extracted from patients' blood plasma (using dedicated Qiagen and/or other kits), followed by the efficient Bioinformatics analysis using in-house developed tool, method, and apparatus.

As used herein, the term "therapeutic means" refers, without limitation, to the remedial agents or methods for the treatment of health-disease conditions or disorders. A non-limiting list of therapeutic means of the invention includes investigational drug, an approved drug, food supplement, immunotherapy, biological therapy, phototherapy, radiation therapy, surgical intervention, hyperbaric oxygen, non-invasive image-guided procedures, multi-step treatment protocol, or any combination of the above. A non-limiting list of therapeutic means of the invention further includes probiotic-based therapeutic means, phage-based therapeutic means, small-molecule-based therapeutic means, prebiotic-based therapeutic means, clinical measures, mouth derived microbiome or any other clinically acceptable therapeutics. The therapeutic means of the invention can be, without limitation, newly discovered therapeutic means and/or known therapeutic means that are already in clinical use.

According to some embodiments, imaging tools, such as, without limitation CT, MRI, PET CT, or any other imagining technique may be used in conjunction with the above embodiments to achieve treatment and/or diagnostic and/or monitoring needs.

According to some embodiments of the invention, the methods of the invention may assist to identify changes in abundance of chimeric RNA at different time points and/or disease stages. Namely, the above-described techniques and/or analysis and/or tests can be performed on the same patient once and/or repeatedly at various time intervals, thus providing disease monitoring.

Examples

In the examples below, if an abbreviation is not defined above, it has its generally accepted meaning.

Example 1: A list of the druggable kinase fusions for targeted chemotherapy and immune-therapy treatments for a glioma cancer patient.

15 cfDNA samples of cancer patients and 14 cfDNAs of healthy controls were analyzed. NGS analyses of cfDNA samples were produced and gene-gene fusions that have been found in cancer patients and have not been found in normal controls were identified. Next, we looked for fusions that incorporate kinases and may be targeted by chemotherapy drugs using our prediction method (ChiPPI), the results were validated by PCR. The results are summarized in Table 1.

Table 1:

SEQ. ID. NO.	GROUP	DESCRIPTION
1	Druggable kinase fusions	BCR-- PDGFRA
2	Druggable kinase fusions	EGFR-- PPM1H
3	Druggable kinase fusions	RAF1-- MSS51
4	Druggable kinase fusions	BCR-ABL1
5	Druggable kinase fusions	NCDN-- PDGFRA
6	Druggable kinase fusions	chimera_6_EWSR1_ATF1
7	Druggable kinase fusions	chimera_10_KIF5B_RET
8	Druggable kinase fusions	chimera_18_TENM4_NRG1
9	Druggable kinase fusions	chimera_19_TFG_ALK
10	Druggable kinase fusions	chimera_13_MSN_ALK
11	Druggable kinase fusions	chimera_2_BCR_FGFR1
12	Druggable kinase fusions	chimera_12_KMT2A_MAML2
13	Druggable kinase fusions	chimera_14_NIN_PDGFRB
14	Druggable kinase fusions	chimera_1_AKAP9_BRAF

15	Druggable kinase fusions	chimera_15_NPM1_ALK
16	Druggable kinase fusions	chimera_7_FGFR1_PLAG1
17	Druggable kinase fusions	chimera_8_FLI1_EWSR1
18	Druggable kinase fusions	chimera_20_TPM3_NTRK1
19	Druggable kinase fusions	chimera_9_GOLGA5_RET
20	Druggable kinase fusions	chimera_5_COL1A1_PDGFB
21	Druggable kinase fusions	chimera_4_BCR_ABL1
22	Druggable kinase fusions	chimera_16_RARA_PML
23	Druggable kinase fusions	chimera_17_SDC4_ROS1
24	Druggable kinase fusions	chimera_3_BCR_ABL1
25	Druggable kinase fusions	chimera_21_TPM3_ROS1
26	Druggable kinase fusions	chimera_11_KMT2A_FLNA

Potential therapies for fusions 1-26 are summarized in Table 2.

Table 2:

Kinase Fusion	Predicted therapeutics
BCR-- PDGFRA	AZD0530; Bosutinib; Dasatinib; Imatinib; Nilotinib; Ponatinib; Ruxolitinib; Sorafenib; TKIs
EGFR-- PPM1H	AZD-3759; Afatinib; Erlotinib; Foretinib; Gefitinib; Lapatinib; Osimertinib; Sapitinib
RAF1-- MSS51	Cobimetinib; MEK inhibitors; Sorafenib
BCR-ABL1	Asciminib; Bosutinib; Dasatinib; Imatinib; Nilotinib; Ponatinib; Sorafenib; TKIs
NCDN-- PDGFRA	Regorafenib, pazopanib

5 Example 2: Demographics of GBM patients and healthy cohorts.

Amongst 29 patients included in the study, 14 were healthy volunteers and 15 were glioblastoma patients. Study population included both adult and elderly of both genders. Study results are summarized in Table 3.

10 Table 2:

ID	ID's	Age	Sex	Diagnostics	IDH mutation status (tumor biopsy molecular analysis)
1.	GBM_#GB1	77	Female	GBM	IDH1/2 wild-type
2.	GBM_#GB3	69	Female	GBM	IDH1/2 wild-type
3.	GBM_#GB5	53	Female	GBM	IDH1/2 wild-type
4.	GBM_#GB7	75	Female	GBM	IDH1/2 wild-type
5.	GBM_#GB13	71	Male	GBM	IDH1/2 wild-type
6.	GBM_#100058	59	Female	GBM	IDH1/2 wild-type
7.	GBM_#100067	71	Female	GBM	IDH1/2 wild-type
8.	GBM_#100077	62	Male	GBM	IDH1/2 wild-type
9.	GBM_#100156	71	Female	GBM	IDH1/2 wild-type
10.	GBM_#100101	50	Male	GBM	IDH1/2 wild-type
11.	GBM_#100106	53	Female	GBM	IDH1/2 wild-type
12.	GBM_#100142	76	Female	GBM	IDH1/2 wild-type
13.	GBM_#100224	53	Male	GBM	IDH1/2 wild-type
14.	GBM_#100237	75	Female	GBM	IDH1/2 wild-type
15.	GBM_#100240	40	Male	GBM	IDH1/2 wild-type
16.	Healthy_Ctrl_#H1	63	Male	Healthy control	Not applicable
17.	Healthy_Ctrl_#H2	unknown	Unknown	Healthy control	Not applicable
18.	Healthy_Ctrl_#H3	38	Male	Healthy control	Not applicable
19.	Healthy_Ctrl_#H4	unknown	unknown	Healthy control	Not applicable
20.	Healthy_Ctrl_#H5	59	Female	Healthy control	Not applicable
21.	Healthy_Ctrl_#H6	unknown	unknown	Healthy control	Not applicable
22.	Healthy_Ctrl_#H7	34	Female	Healthy control	Not applicable
23.	Healthy_Ctrl_#H8	unknown	unknown	Healthy control	Not applicable
24.	Healthy_Ctrl_#H9	87	Male	Healthy control	Not applicable
25.	Healthy_Ctrl_#H10	unknown	unknown	Healthy control	Not applicable
26.	Healthy_Ctrl_#H11	52	Female	Healthy control	Not applicable
27.	Healthy_Ctrl_#H12	87	Female	Healthy control	Not applicable
28.	Healthy_Ctrl_#H13	53	Male	Healthy control	Not applicable
29.	Healthy_Ctrl_#H14	26	Female	Healthy control	Not applicable

Example 3: The cfDNA concentration comparison between glioblastoma (GBM) patients and healthy controls

Under this study, 14 blood samples from healthy controls and 15 blood samples were collected from patients with GBM before surgical intervention. Plasma cfDNA was extracted from the collected blood samples using a silica membrane spin-column based technique. The plasma cfDNA concentration in the control cohort ranged from 0 to 7.62ng/ml of plasma. As demonstrated on Figure 2, 15 GBM plasma samples showed detectable cfDNA concentration with 100% sensitivity. The Qubit concentrations of cfDNA in these 15 patients ranged between 13.68 ng and 49.66 ng per mL of plasma (Figure 1). The cfDNA plasma concentrations of the GBM patients were significantly higher (p-value by t-test <0.0001) than those of the control group. These results indicate that GBM patients show high concentration of plasma cfDNA as compared with healthy persons and therefore this feature of cfDNA might be useful for distinguishing GBM and/or glioma patients from healthy person by non-invasive liquid biopsy approach (the cfDNA concentration cutoff is 7ng/ml).

Example 4. Mutations analysis of GBMs cfDNA sequencing data.

To confirm that the elevated cfDNA in the plasma of patients with GBM is derived from cancer cells, both cfDNA and circulating tumor DNA (ctDNA) were tested for the presence of mutations in top-50 selected genes from COSMIC database that are commonly mutated in GBMs. cfDNA from 14 GBM patients was sequenced and their matched tumor DNA and normal genomic DNA (gDNA) using a WGS procedure with 5x-10x coverage (at least 50 million paired-end (PE) reads per sample). SNPs present in gDNA were removed, then sorted mutations into "cfDNA only", "ctDNA only", and "both cfDNA and gDNA". As demonstrated in Figure 3, among 50 genes from COSMIC database that are commonly mutated in GBMs, 47 were identified in the corresponding cfDNA (Figure 2). These results indicated that GBM associated gene mutations can be identified

by capturing GBM patient's cfDNA. Therefore, mutation analysis of GBM patient's cfDNA may help to identify the mutations using liquid biopsy; and may also help in making accurate and personalized treatment decision based on identified mutations.

5 Example 5. The fragment size estimation of glioma patients and healthy controls in plasma cfDNA.

cfDNA fragment size distribution was examined in 41 samples obtained from glioma (n=15) patients and healthy (n=14) individuals by performing High Sensitivity Bioanalyzer DNA 1000
 10 assay. As demonstrated on figure 2, electropherograms of all 29 cfDNA samples showed mainly 6 types of DNA fragment enrichments. Amongst 9 out of 29 samples (2 GBMs, 1 LGG & 6 healthy), a major peak at ~166bp; in 10 out of 29 samples (9 GBMs, 1 healthy), a major peak at ~166bp and a small peak at
 15 ~332bp; and in 10 out of 29 samples (10 GBMs, 0 healthy), a major peak at ~166 bp and smaller peaks at ~332bp and ~498bp were observed. While 3 out of 29 samples (3 GBMs, 0 healthy) had a major peak at ~166 bp and smaller peaks at ~332bp, ~498bp, and ~2000bp, and 2 out of 29 samples (2 GBMs, 0 healthy) had a major
 20 peak at ~166 bp and enrichment at 10380bp near upper reference ladder was observed. In the remaining 7 samples (1 LGG, 6 healthy) no fragment. Fig. 3 is a graphical representation of DNA fragment sizes in healthy volunteers and gliomas. Black solid bars represent DNA fragment sizes in healthy volunteers, and striped bars represent DNA fragment sizes in glioma patients clearly showing an enrichment. The results are summarized in
 25 Table 6.

Table 6:

DNA fragment size Distribution	Observed in samples out of total 41 samples	Healthy Individuals	Glioma	
			LGG	GBM
166 bp	9	6	1	2

166 bp, 332 bp	10	1	-	9
166 bp, 332 bp, 498 bp	10	0	-	10
166 bp, 332 bp, 498 bp, 2000bp	3	0	-	3
166bp, >8000bp	2	0	-	2
No peak	7	6	1	-

Discussion

These results indicate that GBM patient's plasma cfDNA show multiple fragment sizes such as 166bp, 332bp, 498bp and 2000bp; and healthy persons detectable plasma cfDNA shows mostly 166bp fragments and rarely 332bp fragments. Therefore, estimation of plasma cfDNA size enrichment may help in glioma liquid biopsy for distinguishing GBM patient's plasma cfDNA from healthy individuals.

Example 6: A high fragmentation of plasma cfDNA in GBM patients than healthy controls.

In this study, a high level of variable sizes cfDNA fragmentation (~166bp, ~332bp, and ~498bp) was observed in the GBM patients compared to healthy individuals, who had less cfDNA fragmentation.

Apoptotic fragmentation of cfDNA generates ~166bp, ~332bp, and ~498bp of reference DNA sizes. Amongst, ~166bp DNA is produced majorly, which is mononucleotide digestion equal to ~147 bp of DNA wrapped around a nucleosome plus the stretch of DNA on Histone H1 linking two nucleosome cores. The longer fractions produced di-, tri-, or poly-nucleosomes nuclease action.

In three GBM patients, we observed the presence of 2000bp DNA fragments along with the smaller fragments. The mechanism through which these fragments originate in the blood is still unknown. Less and consistent fragmentation of DNA in healthy controls and high and variable size fragmentation in the GBM

patients underlines the use of studying fragmentation pattern as a marker in cancer screening and clinical outcome monitoring in the GBM patients was observed. Lastly, the observation of 8000bp DNA fragments in two GBM patients represents DNA contamination. The plasma DNA fragments with size 8000bp or more are typically referred to as the PBMCs genomic DNA contamination occurred during the plasma sample processing. PBMCs lysis occurred due to a lack of/insufficient preservation process, releases the large DNA fragments around 8000bp in the sample.

This genomic DNA contamination can be avoided by taking simple measures such as immediate processing of plasma after blood collection, in case of storage of blood sample, it can be stored for a maximum of 2 hours by keeping it on ice, and the separated plasma after centrifugation, if not used immediately, should be stored at -80°C in the refrigerator.

Example 7: cfDNA concentrations are elevated in the plasma of GBM patients.

25 blood samples which were collected from patients with GBM prior to surgery, and their corresponding samples of the resected tumors (Table 7) were obtained from tumor biobanks.

Biobank number	AGE	GENDER	IDH1/2	OTHER GENOMIC ALTERATIONS		STATUS
Hospital: Rabin Medical Center, Israel						
100058	62	female	WT	NA		dead
100067	71	female	WT	TERTp mutation C228T	NA	dead
100077	62	male	IDH1m	TERTp mutation C228T	NA	dead
100156	72	female	WT	MGMT-UM, TERTp WT, BRAF WT	7p and 7q gain, 10 p and 10q loss, 9p loss, CDKN2A homozygous deletion, EGFR amplification	dead
100101	51	male	WT	MGMT-UM, TERTp WT	NA	dead
100106	55	female	IDH1m	MGMT-M, TERTp WT, BRAF WT	ATRX mutation, TP53 mutation, PTEN mutation	alive
100142	76	female	WT	MGMT-M, TERTp mutation C250T, BRAF WT	TP53 mutation	dead
100224	54	male	WT	TERTp mutation C228T	NA	dead

100237	75	female	WT	MGMT UM, TERTp mutation C228T, BRAF WT	NA	dead
100240	41	male	WT	MGMT-M, TERTp mutation C250T, BRAF WT	7p and 7q gain, 10 p and 10q loss, EGFR amplification, TP53 mutation, PTEN mutation, CDK4 amplification	dead
Hospital: Keil, Germany						
I	79	male	WT	1p19q unknown, MGMT-M		NA
II	54	female	WT	MGMT-M		dead
IV	53	male	WT	19q deleted, 1p intact, MGMT-UM		NA
V	74	male	WT	1p/19q not codeleted, MGMT-M		NA
VIII	44	male	WT	1p deleted, 19q intact, MGMT-UM		NA
IX	57	male	WT	1p/19q not codeleted, MGMT-UM		NA
X	70	female	WT	1p/19q not codeleted MGMT-UM		NA
XI	80	female	WT	MGMT-M		dead
XII	62	male	WT	1p/19q not codeleted, MGMT-UM		NA
Israeli National Tissue Bank (Midgam), Israel						
#1	77	female	WT	MGMT-UM, TERTp WT		dead
#3	69	female	WT	MGMT-UM, TERTp WT		dead
#5	53	female	WT	MGMT-UM, TERTp WT		dead
#7	75	female	WT	MGMT-UM, TERTp WT		dead
#13	71	male	WT	MGMT-UM, TERTp WT		dead
#33	58	male	WT	MGMT-UM, TERTp WT		dead

MGMT- O⁶-methylguanine DNA methyltransferase; UM-unmethylated; M-methylated; NA-not available; TERTp-telomerase reverse transcriptase promoter; WT-wild type

Table 7: Characteristics of GBM patient and Tumor genomic alterations, as reported by the treating institution.

In addition, 25 blood samples from healthy controls matching the ages of the GBM cohort were collected. For each patient, cfDNA from plasma, genomic DNA (gDNA) from white blood cells (WBCs), and tumor DNA (tDNA) from tumor tissues was extracted, fragments of sizes corresponding to cfDNA were identified and their concentrations were evaluated (Fig. 4). cfDNA plasma

concentrations in the control cohort was ranging from 0.01 to 7.62 ng per ml of plasma. Next, detectable cfDNA from GBM samples were isolated and was found that the cfDNA concentrations ranged between 12.6 and 137 ng per ml of plasma (Fig. 2). Thus, all GBM samples were found to contain higher cfDNA concentrations than those of the control group (p-value <0.0001, t-test). Then, sizes of cfDNA molecules in all samples were examined. A Bioanalyzer DNA High Sensitivity assay showed that in both GBM and healthy control samples, a cfDNA major peak was detectable at, or close to, 166 bp, which accounted for 85% of the circulating cfDNA. A smaller peak at, or close to, 332 bp accounted for 10% of the cfDNA and another peak at 2000-10,000 bp constituted 5% of cfDNA and likely represent fragments released by necrotic tissue (Fig. 4).

Discussion:

Liquid biopsy can generate high quality results, enabling analysis of cfDNA that was likely derived from apoptotic rather than necrotic cells. The results indicate that the plasma cfDNA concentrations segregate GBM patients from healthy controls.

Example 8: Mutation analysis of glioblastoma cfDNA data.

To confirm that the elevated cfDNA levels in the plasma of GBM patients was derived from Tumor cells. 25 cfDNA samples of GBM and 25 cfDNA samples from normal controls we sequenced using a whole genome sequencing procedure with 30X coverage (at least 150 million paired end (PE) 100 bp reads per sample). In addition, we sequenced tDNA (30X coverage, 150 million PE reads of 25 GBM Tumor samples). All germline SNPs that appeared in patient gDNA were removed using the variant calling method. Next, the mutations into "cfDNA only", "tDNA only", and "both cfDNA and gDNA" groups we sorted (Fig. 5). It was found that GBM patients shared mutations in their cfDNA and tDNA, with 90% selectivity and 80% sensitivity (at 5% FDR). Variant calling analysis of gDNA was used to identify the background germline

mutations of patients. A similar pattern of high impact alterations in both cfDNA and in tDNA in the 25 GBM patients was found (Table 8). These results indicate that in GBM, cfDNA includes molecular signatures that originate from the Tumor mass.

Consequence type (Sequence Ontology term)	Average values of high-impact alterations	
	count	%
Splice donor variant	2	0.001
Splice acceptor variant	0.4	0.001
Stop-gained	0.4	0.001
Missense variant	6.8	0.1
Splice region variant	14.0	0.15
Synonymous variant	7.0	0.001
5-prime UTR variant	53.2	0.11
3-prime UTR variant	318.8	0.82
Noncoding transcript exon variant	519.4	1.3
Intron variant	17981.6	47.5
Upstream gene variant	1662.6	4.28
Downstream gene variant	1429.6	4.0
TF binding site variant	101.0	0.3
Regulatory region variant	1823.4	4.5
Intergenic variant	13902.8	37.2

Table 8: Average values of high-impact alterations identified in cfDNA from 25 GBM patients.

The analysis was further extended to the top 50 genes that are most often mutated in GBM (30-35). For GBM patients, the distribution pattern of these mutations was highly conserved (Table 8). Of these 50 genes, 67% were identified as being mutated in the same precise genomic position in both cfDNA and tDNA, using at least five mapping reads sized 100 bp (Table 9). The mutated genes included *TP53*, which encodes a protein that is a Tumor suppressor, and which is mutated in many cancers, including gliomas (30). In addition to the most common GBM-related genes, we also found mutations in the *BRAF* and *EGFR* genes, previously shown to be involved in glioma progression (30). These results indicate that mutations found in cfDNA

correspond to mutations in brain tumors with 95% specificity, allowing us to distinguish GBM at 5% FDR, after removing the background noise of germline mutations (Table 9).

Gene name	cBioPortal (585 patients)	Piccioni D.E. (2019) (419 patients)	TCGA/PANCANCER (180 patients)+	Our results for 25 GBM samples	
				Tumor	cfDNA
TP53	31.50%	58.70%	(28%) 3876	30.0%	32.0%
IDH1	6.30%	2.00%	(10%) 9057	8.0%	8.0%
PTEN	33.50%	0.80%	(22%) 3486	33.0%	30.0%
EGFR	23.70%	20.00%	(14%) 3044	20.0%	19.0%
H3F3A	0.80%	-	(13%) 3083	2.0%	1.0%
PIK3CA	9.60%	5.00%	(7%) 2529	9.0%	7.8%
ATRX	9.30%	-	(9%) 1691	9.0%	10.0%
NF1	11.60%	22.90%	(9%) 1613	11.0%	13.0%
BRAF	2.00%	7.00%	(5%) 2726	3.0%	2.8%
RB1	9.60%	0.90%	(7%) 1699	10.0%	9.7%
TERT	1.30%	2.80%	(4%) 2621	4.5%	5.0%
PIK3R1	9.80%	-	(6%) 1721	7.8%	7.0%
CHEK2	0.70%	-	(8%) 1304	3.0%	2.1%
PDGFRA	4.00%	12.90%	(5%) 1855	5.0%	5.1%
LRP1B	3.30%	-	(5%) 1082	4.0%	4.3%
SETD2	2.80%	-	(3%) 1414	3.5%	3.1%
STAG2	4.50%	-	(3%) 1387	5.0%	5.2%
HIF1A	0.50%	-	(3%) 1017	2.2%	3.1%
IRS4	1.00%	-	(4%) 1014	4.0%	3.6%
KMT2C	4.80%	-	(3%) 1340	3.0%	3.4%
MET	1.80%	19.00%	(2%) 1417	2.0%	3.1%
APC	2.00%	14.00%	(1.9%) 1619	2.5%	3.0%
AR	-	10.10%	(1.1%) 1318	1.5%	2.3%
ERBB2	1.30%	10.10%	(0.6%) 1661	1.3%	1.5%
FGFR2	1.00%	10.10%	(0.5%) 1414	1.2%	1.3%
NOTCH1	0.50%	8.90%	(1.5%) 1575	1.5%	2.0%
KIT	1.50%	8.00%	(1.3%) 1668	1.5%	1.7%
NRAS	1.00%	7.00%	(1.1%) 1757	1.5%	1.2%
RAF1	0.80%	7.00%	(0.4%) 1303	1.2%	0.8%
CONE1	-	6.10%	-	-	-
JAK2	1.30%	6.10%	(1.1%) 1397	2.1%	1.9%
ATM	1.80%	5.00%	(2.1%) 1436	3.1%	2.8%
ALK	0.80%	4.00%	(1.3%) 1427	1.3%	3.05%
BRCA1	1.50%	3.90%	(2%) 1322	3.7%	3.5%
BRCA2	1.50%	3.90%	(1.7%) 1322	3.8%	3.7%
MAP2K2	0.50%	2.80%	(0.1%) 1318	1.5%	1.65%
CCND2	0.50%	2.00%	(0.8%) 1303	0.8%	0.9%
CDK6	0.30%	2.00%	(0.7%) 1304	0.7%	0.3%
GATA3	0.50%	1.90%	(0.5%) 1316	0.9%	0.76%
GNAS	0.80%	1.90%	(0.6%) 1451	1.2%	1.3%
HRAS	-	1.90%	(0.5%) 1520	0.7%	1.1%
JAK3	1.30%	1.90%	(1%) 1376	2.1%	1.9%
KRAS	0.50%	2.00%	(0.8%) 1732	0.9%	0.8%
SMAD4	0.30%	1.90%	(0.9%) 1397	1.0%	0.96%
SMO	0.50%	1.90%	(0.7%) 1395	1.4%	1.43%
STK11	-	1.90%	(0.8%) 1395	1.2%	1.3%
TSC1	1.00%	1.90%	(1.5%) 1307	1.9%	1.8%

AKT1	0.50%	0.90%	(0.2%) 2032	0.9%	1.2%
ARAF	0.80%	0.90%	(0.2%) 1303	0.8%	1.2%
CCND1	-	1.10%	(0.5%) 1303	1.1%	0.8%
FBXW7	0.80%	0.90%	(1.1%) 1428	1.1%	0.8%
FGFR1	1.00%	1.10%	(1.1%) 1455	1.0%	1.1%
MAPK3	0.80%	0.90%	(0.2%) 1303	0.8%	0.78%
MLH1	0.30%	0.90%	(0.9%) 1404	0.9%	1.2%
NTRK1	0.80%	0.90%	(0.6%) 1303	0.8%	1.1%
NTRK3	1.30%	0.90%	(1.3%) 1335	1.3%	0.9%
RIT1	0.30%	0.90%	(0.2%) 1300	0.53%	0.5%
ROS1	2.50%	0.90%	(2.2%) 1322	2.5%	2.1%

Table 9: Frequencies of high-impact mutations identified in GBM patients and in published cohorts. Column #1 lists the top 50 genes found to be mutated in GBM. Columns #2, #3 and #4 present data on glioblastoma from three major studies (30,34,35). The percentages in the parentheses indicate the frequencies of the mutations. The numbers outside the parenthesis indicate the total number of GBM patients tested in the corresponding studies.

As mentioned above, somatic high-impact mutations shared by cfDNA and tDNA were compared in our patients with the mutation landscape data obtained from four studies (71-73,78) (Table 9 and Fig. 6). These mutations were validated by Sanger sequencing (Fig. 7), and it was found that cfDNA offered high-level profiling of somatic mutations in all GBM patients. Specifically, mutations in genes that are strongly involved in GBM, i.e., *EGFR* (3' UTR, intron, and downstream gene variants), *PDGFRA* (3' UTR, intron and downstream and upstream gene variants), *PIK3CA* (intron and upstream gene variants), *PIK3R1* (upstream and downstream gene variants) and *TP53* (upstream gene, intron and downstream gene variants) were found. Finally, it found that Tumor-suppressors were mostly absent in GBM due to missense mutations and that oncogenes appeared in the annotated data of mapped cfDNA sequences (not shown). These results indicate that liquid biopsy technique captures a broad spectrum of known glioma mutations at similar incidence rates as do standard Tumor biopsies.

Example 9: Fusion gene analysis and druggable fusions

Under the assumption that fusion genes contribute to glioma Tumor formation, in addition to the point mutations described above, and that specific fusions, as opposed to mutation

combinations, may be unique to different gliomas, cfDNA sequences from 25 control and 25 GBM samples, and from 180 TCGA GBM patients (downloaded from publicly available sources) were tested. The search for fusions was done using our ChiTaRS 5.0 reference database (<http://chitars.md.biu.ac.il/>) (68). The unique gene fusions, such as KDR-PDGFR α (8%), and NCDN-PDGFR α (40% of all samples) that correspond to the previously reported variations in PDGFR α in GBM were identified. The PDGFR α protein fusions can be targeted by tyrosine kinase inhibitors, such as imatinib, sunitinib and sorafenib (36,37). BCR-ABL1 (8%), COL1A1-PDGFR β (8%), NIN-PDGFR β (8%) and FGFR1-BCR (4%), which can be targeted by imatinib, sunitinib and sorafenib were identified (Table 10).

Biobank number	OBSERVED FUSIONS	POTENTIAL DRUGS
100058	BCR-ABL	imatinib, sunitinib and sorafenib
100067	KDR-PDGFR α	imatinib, sunitinib and sorafenib
100077	NCDN-PDGFR α	imatinib, sunitinib and sorafenib
100156	COL1A1-PDGFR β	imatinib, sunitinib and sorafenib
100101	BCR-ABL, KDR-PDGFR α	imatinib, sunitinib and sorafenib
100106	NA	NA
100142	NCDN-PDGFR α	imatinib, sunitinib and sorafenib
100224	FGFR1-BCR	imatinib, sunitinib and sorafenib
100237	CEP85L-ROS1	crizotinib, entrectinib and larotrectinib
100240	NCDN-PDGFR α	imatinib, sunitinib and sorafenib
I	NCDN-PDGFR α	imatinib, sunitinib and sorafenib
II	GOPC-ROS1	crizotinib, entrectinib and larotrectinib
IV	NIN-PDGFR β	imatinib, sunitinib and sorafenib
V	NA	NA
VIII (*)	KDR-PDGFR α	imatinib, sunitinib and sorafenib

IX	NCDN-PDGFR α	imatinib, sunitinib and sorafenib
X	NA	NA
XI	COL1A1-PDGFR β , NCDN-PDGFR α	imatinib, sunitinib and sorafenib
XII	NIN-PDGFR β	imatinib, sunitinib and sorafenib
#1	GOPC-ROS1	crizotinib, entrectinib and larotrectinib
#3	NCDN-PDGFR α	imatinib, sunitinib and sorafenib
#5	NCDN-PDGFR α	imatinib, sunitinib and sorafenib
#7	CEP85L-ROS1	crizotinib, entrectinib and larotrectinib
#13	NCDN-PDGFR α	imatinib, sunitinib and sorafenib
#33	NCDN-PDGFR α	imatinib, sunitinib and sorafenib

Table 10: Druggable fusions observed in cfDNA of the 25 GBM patients in this study.

In addition, ROS1 fusions were identified in 8% of patient cfDNA that might be targeted by analogues of crizotinib. These unique fusions were found in cfDNA and tdDNA but not in the respective gDNA of the GBM patients and healthy controls with high read coverage (at least 10 reads mapping the junction site, 5% FDR) (Tables 10 and 11).

Druggable Fusion genes	Targeting drugs	Junction Type	Identified in glioblastoma patients or healthy controls
KMT2A-FLNA	Daunorubicin	intron-exon	TCGA-32-1970 (Tumor and germline DNA); TCGA-06-0157 (tdDNA); TCGA-27-1831 (germline DNA); TCGA-26-5132 (tdDNA); TCGA-27-2523 (tdDNA); TCGA-02-2485 (germline DNA); TCGA-26-5135 (tdDNA); TCGA-06-5411 (tdDNA); TCGA-15-1444 (germline DNA)
FGFR1-BCR	Dasatinib; Nilotinib; Ponatinib; Ruxolitinib; Imatinib; TKIs; Bosutinib; Sorafenib; AZD0530; AZD4547; BGJ398; Debio1347; Erdafitinib	exon-exon	TCGA-06-5411 (tdDNA)
TPM3-ROS1	Crizotinib, entrectinib, larotrectinib	exon-exon	TCGA-15-1444 (germline DNA)
TFG-ALK	Crizotinib; entrectinib, larotrectinib Ceritinib; PF2341066; TAE684; novel ALK inhibitors; Alectinib; Brigatinib; Lorlatinib; foretinib	exon-exon	TCGA-26-5135 (tdDNA)
MSN-ALK	Crizotinib; entrectinib, larotrectinib, Ceritinib; PF2341066; TAE684; novel ALK inhibitors; Alectinib; Brigatinib; Lorlatinib	exon-exon	TCGA-26-5135 (tdDNA)
MLLT1-KMT2A	Daunorubicin	exon-exon	TCGA-06-5411 (tdDNA)
BCR-ABL1	Imatinib; Bosutinib; Dasatinib; Nilotinib; Ponatinib; Asciminib; TKIs; Sorafenib	exon-exon	TCGA-27-2523 (tdDNA)
		intron-exon	TCGA-15-1444 (germline DNA)
NIN-PDGFRB	Imatinib	exon-exon	TCGA-02-2485 (germline DNA);
AKAP9-BRAF	Sorafenib; MEK inhibitors; Binimetinib+Encorafenib; Cobimetinib; Cobimetinib+Vemurafenib; Dabrafenib; Dabrafenib+Trametinib; Trametinib; Vemurafenib	exon-exon	TCGA-06-5411 (tdDNA)
KMT2A-MAML2	Daunorubicin	exon-exon	TCGA-27-1831 (germline DNA)
FGFR1-PLAG1	AZD4547; BGJ398; Debio1347; Erdafitinib; Ponatinib	exon-exon	TCGA-26-5135 (tdDNA)
KIF5B-RET	Cabozantinib; Vandetanib	exon-exon	TCGA-27-2523 (tdDNA), TCGA-32-1970 (tdDNA)
EWSR1-ATF1	PARP inhibitors	exon-exon	TCGA-15-1444 (germline DNA)
TPM3-NTRK1	pan-TRK inhibitor; Entrectinib; Larotrectinib; Crizotinib	exon-exon	TCGA-26-5132 (tdDNA)
RARA-PML	ATRA + arsenic trioxide	exon-exon	TCGA-26-5135 (tdDNA)
GOLGA5-RET	Cabozantinib; Vandetanib	exon-exon	TCGA-27-2523 (tdDNA), GBM_#IA (cfDNA)
COL1A1-PDGFB	Imatinib	exon-exon	TCGA-26-5135 (tdDNA)
		exon-intron	TCGA-32-1970 (tdDNA)
ABL1-BCR	Imatinib; Dasatinib; Nilotinib; Ponatinib; Bosutinib; Ruxolitinib	intron-exon	TCGA-15-1444 (germline DNA)

FLI1-EWSR1	PARP inhibitors; TK216	intron-exon	TCGA-02-2485 (germline DNA)
NPM1-ALK	Crizotinib, entrectinib, larotrectinib	intron-exon	TCGA-15-1444 (germline DNA), Healthy-Ctrl #TS_0 (cfDNA)
NIN-PDGFRB	Imatinib	exon-exon	GBM_#GB7 (germline DNA), TCGA-02-2485 (germline DNA)
		exon-intron	TCGA-26-5132 (tDNA)
TENM4-NRG1	Lapatinib	intron-exon	GBM_#GB3 (germline DNA)
SDC4-ROS1	Crizotinib, entrectinib, larotrectinib	exon-exon	GBM_#VIIIA (cfDNA)

Table 11: Druggable fusion genes and their targeting drugs identified in GBM samples archived in The Cancer Genome Atlas (TCGA) database.

These results indicate that a fusion gene signature may be readily detectable in GBM patients, thereby distinguishing them from non-cancer controls.

To study druggable targets, next-generation sequencing (NGS) datasets were analyzed to identify hits among the 1,207 predicted druggable fusions collected in the ChiTaRS 5.0 database (26). Predicted druggable fusions are characterized by a preserved tyrosine kinase domain that can be targeted by specifically designed biologic drugs. Druggable fusions, particularly, CEP85L-ROS1 and GOPC-ROS1, that bound crizotinib analogues (e.g., entrectinib and larotrectinib) in TCGA GBM patients were identified, as reported previously by Davare et. al. (38). ROS1 fusions were mutually exclusive for EGFR and PDGFRA alterations in our patients, as previously reported (39). Thus, fusion *BCR-ABL1* was validated by PCR in two tDNA and corresponding cfDNA samples, as confirmed by cloning and Sanger sequencing. Finally, *KDR-PDGFR* in three tRNA and cfDNA samples was validated by PCR, cloning and Sanger sequencing. Taken together, the results indicate that cfDNA may signal the presence of druggable gene-gene fusions that incorporate tyrosine kinases, and which can be possibly targeted by specific drugs. This will improve patient stratification in early phase clinical trials addressing potential novel GBM treatments.

Example 10: Gene enrichment analysis

Since functional mutations and fusions disrupt key metabolic pathways in cancer cells, it was tested whether glioma-specific pathway disruptions could be treated with targeted drug combinations. A specific sub-set of fusions presented above and identified in cfDNA and tDNA encode druggable targets that are likely to respond to the crizotinib analogues entrectinib and larotrectinib and/or imatinib analogues were found (Tables 10 and 11). The gene set was analysed and pathway enrichment for 96 genes that were previously reported as being frequently mutated in glioma patients (38-40) was found. The KEGG PATHWAY (41-43) database was used for such analysis, with the most significant pathways being identified for each gene set (including the top 50 genes mutated in gliomas). The significant pathways for each gene set were then compared. Six significant pathways, namely, the ErbB signaling pathway, the VEGF signaling pathway, the choline metabolism pathway, central carbon metabolism in cancer, the p53 signalling pathway and pathways in non-small cell lung cancer, were identified as common to the two gene sets (Fig. 8). Such analysis showed that cancer-specific pathways are similar and targeted by either acquiring gene mutations or by forming gene-gene fusions.

Discussion

Liquid biopsy can play an important role in the molecular diagnosis of GBM, and as a potential means for selecting an accurate personalized approach for treatment of this devastating disease. The major advantage of liquid biopsy is its non-invasive nature and its ability to provide information on a broad range of mutations and fusions in patients with brain tumors, while avoiding the need to perform invasive procedures to obtain tumor tissue for analysis. As therapeutic druggable fusion gene targets can be identified using liquid biopsy, this easy-to-use and non-invasive diagnostic technique will

contribute to precise treatment of GBM patients at any stage of the disease.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one
5 of ordinary skill in the art, to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent
10 specification, including definitions, will prevail. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting
15 of the invention.

As used herein, the singular forms "a," "an" and "the" are intended to include plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms "comprises" or "comprising," when used in this
20 specification, specify the presence of stated features, integers, steps, operations, elements components and/or groups or combinations thereof, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components and/or groups or combinations
25 thereof. As used herein the terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to". The term "consisting of" means "including and limited to".

It will be understood that when an element is referred to as
30 being "on," "attached" to, "connected" to, "coupled" with, "contacting," etc., another element, it can be directly on, attached to, connected to, coupled with and/or contacting the other element or intervening elements can also be present. In

contrast, when an element is referred to as being, for example, "directly on," "directly attached" to, "directly connected" to, "directly coupled" with or "directly contacting" another element, there are no intervening elements present. It will also
5 be appreciated by those of skill in the art that references to a structure or feature that is disposed "adjacent" another feature can have portions that overlap or underlie the adjacent feature.

It will be understood that, although the terms first, second,
10 etc., may be used herein to describe various elements, components, regions, layers and/or sections, these elements, components, regions, layers and/or sections should not be limited by these terms. Rather, these terms are only used to distinguish one element, component, region, layer and/or
15 section, from another element, component, region, layer and/or section.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for
20 convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example,
25 description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the
30 breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first

indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

Certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments unless the embodiment is inoperative without those elements.

As used herein, the term "non-linear reads" refers to nucleotide sequences which do not map linearly to the target genome. A non-limiting list of non-linear reads of the invention includes genomic integrations; exon-exon combinations; exon-intron combinations and/or any other sequence parts merged together.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical, and medical arts.

By "patient" or "subject" is meant to include any mammal. A "mammal," as used herein, refers to any animal classified as a mammal, including but not limited to, humans, experimental animals including monkeys, rats, mice, and guinea pigs, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, and the like.

"Treating" or "treatment" of a disease as used herein includes: preventing the disease, i.e. causing the clinical symptoms of the disease not to develop in a mammal that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease; inhibiting the disease, i.e., arresting or reducing the development of the disease or its clinical symptoms, or relieving the disease, i.e., causing regression of the disease or its clinical symptoms, and/ or monitoring the disease and early diagnostics of the disease.

Druggability, is a term used in drug discovery to describe a biological target such as a protein that is known to bind or is predicted to bind with high affinity to a drug. Furthermore, the binding of the drug to a druggable target alters the function of the target with a therapeutic benefit to the patient. The term "drug" herein includes small molecules (low molecular weight organic substances) but also has been extended to include biologic medical products such as therapeutic monoclonal antibodies. In at least one embodiment, the gene fusion or gene variant can be used to identify a druggable target.

Certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments unless the embodiment is inoperative without those elements.

It will be appreciated by persons skilled in the art that the present invention is not limited to what has been particularly shown and described hereinabove. Rather the scope of the present invention is defined by the appended claims and includes both

combinations and sub-combinations of the various features described hereinabove as well as variations and modifications thereof, which would occur to persons skilled in the art upon reading the foregoing description. While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents may occur to those skilled in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the invention. Various embodiments have been presented. Each of these embodiments may of course include features from other embodiments presented, and embodiments not specifically described may include various features described herein.

References

1. Goodenberger, M.L. and Jenkins, R.B. (2012) Genetics of adult glioma. *Cancer Genet*, **205**, 613-621.
2. Olson, J.J. and Ryken, T. (2008) Guidelines for the treatment of newly diagnosed glioblastoma: introduction. *J Neurooncol*, **89**, 255-258.
3. Bertolini, F., Zunarelli, E., Baraldi, C., Valentini, A., Del Giovane, C., Depenni, R., Falasca, A., Giacobazzi, P., Malagoli, M., Meletti, S. et al. (2012) Survival in patients with newly diagnosed conventional glioblastoma: a modified prognostic score based on a single-institution series. *Tumori*, **98**, 756-761.
4. Ceccarelli, M., Barthel, F.P., Malta, T.M., Sabedot, T.S., Salama, S.R., Murray, B.A., Morozova, O., Newton, Y., Radenbaugh, A., Pagnotta, S.M. et al. (2016) Molecular Profiling Reveals Biologically Discrete Subsets and Pathways of Progression in Diffuse Glioma. *Cell*, **164**, 550-563.
5. Smits, M. and van den Bent, M.J. (2017) Imaging Correlates of Adult Glioma Genotypes. *Radiology*, **284**, 316-331.
6. Tirosh, I. and Suvà, M.L. (2018) Dissecting human gliomas by single-cell RNA sequencing. *Neuro Oncol*, **20**, 37-43.
7. Oliveira, K.C.S., Ramos, I.B., Silva, J.M.C., Barra, W.F., Riggins, G.J., Palande, V., Pinho, C.T., Frenkel-Morgenstern, M., Santos, S.E.B., Assumpcao, P.P. et al. (2020) Current Perspectives on Circulating Tumor DNA, Precision Medicine, and Personalized Clinical Management of Cancer. *Mol Cancer Res*, **18**, 517-528.
8. Bedin, C., Enzo, M.V., Del Bianco, P., Pucciarelli, S., Nitti, D. and Agostini, M. (2017) Diagnostic and prognostic role of cell-free DNA testing for colorectal cancer patients. *Int J Cancer*, **140**, 1888-1898.

9. Hegemann, M., Stenzl, A., Bedke, J., Chi, K.N., Black, P.C. and Todenhöfer, T. (2016) Liquid biopsy: ready to guide therapy in advanced prostate cancer? *BJU Int*, **118**, 855-863.
10. Palande, V., Raviv Shay, D. and Frenkel-Morgenstern, M. (2020) Detection of Cell-Free DNA in Blood Plasma Samples of Cancer Patients. *J Vis Exp*.
11. Bronkhorst, A.J., Wentzel, J.F., Aucamp, J., van Dyk, E., du Plessis, L. and Pretorius, P.J. (2016) Characterization of the cell-free DNA released by cultured cancer cells. *Biochim Biophys Acta*, **1863**, 157-165.
12. Lissa, D. and Robles, A.I. (2016) Methylation analyses in liquid biopsy. *Transl Lung Cancer Res*, **5**, 492-504.
13. Connolly, I.D., Li, Y., Gephart, M.H. and Nagpal, S. (2016) The "Liquid Biopsy": the Role of Circulating DNA and RNA in Central Nervous System Tumors. *Curr Neurol Neurosci Rep*, **16**, 25.
14. Akavia, U.D., Litvin, O., Kim, J., Sanchez-Garcia, F., Kotliar, D., Causton, H.C., Pochanard, P., Mozes, E., Garraway, L.A. and Pe'er, D. (2010) An integrated approach to uncover drivers of cancer. *Cell*, **143**, 1005-1017.
15. Beroukhi, R., Getz, G., Nghiemphu, L., Barretina, J., Hsueh, T., Linhart, D., Vivanco, I., Lee, J.C., Huang, J.H., Alexander, S. et al. (2007) Assessing the significance of chromosomal aberrations in cancer: methodology and application to glioma. *Proc Natl Acad Sci U S A*, **104**, 20007-20012.
16. Eguchi, M., Eguchi-Ishimae, M., Knight, D., Kearney, L., Slany, R. and Greaves, M. (2006) MLL chimeric protein activation renders cells vulnerable to chromosomal damage: an explanation for the very short latency of infant leukemia. *Genes Chromosomes Cancer*, **45**, 754-760.
17. Frenkel-Morgenstern, M., Gorohovski, A., Tagore, S., Sekar, V., Vazquez, M. and Valencia, A. (2017) ChIPPI: a novel method for mapping chimeric protein-protein interactions uncovers

selection principles of protein fusion events in cancer. *Nucleic Acids Res.*

18. Hahn, Y., Bera, T.K., Gehlhaus, K., Kirsch, I.R., Pastan, I.H. and Lee, B. (2004) Finding fusion genes resulting from
5 chromosome rearrangement by analyzing the expressed sequence databases. *Proc Natl Acad Sci U S A*, **101**, 13257-13261.
19. Mitelman, F., Mertens, F. and Johansson, B. (2005) Prevalence estimates of recurrent balanced cytogenetic aberrations and gene fusions in unselected patients with
10 neoplastic disorders. *Genes Chromosomes Cancer*, **43**, 350-366.
20. Tagore, S., Gorohovski, A., Jensen, L.J. and Frenkel-Morgenstern, M. (2019) ProtFus: A Comprehensive Method Characterizing Protein-Protein Interactions of Fusion Proteins. *PLoS Comput Biol*, **15**, e1007239.
- 15 21. Maher, C.A., Palanisamy, N., Brenner, J.C., Cao, X., Kalyana-Sundaram, S., Luo, S., Khrebtukova, I., Barrette, T.R., Grasso, C., Yu, J. et al. (2009) Chimeric transcript discovery by paired-end transcriptome sequencing. *Proc Natl Acad Sci U S A*, **106**, 12353-12358.
- 20 22. Maher, C.A., Kumar-Sinha, C., Cao, X., Kalyana-Sundaram, S., Han, B., Jing, X., Sam, L., Barrette, T., Palanisamy, N. and Chinnaiyan, A.M. (2009) Transcriptome sequencing to detect gene fusions in cancer. *Nature*, **458**, 97-101.
23. Alaiya, A.A., Aljurf, M., Shinwari, Z., Almohareb, F.,
25 Malhan, H., Alzahrani, H., Owaidah, T., Fox, J., Alsharif, F., Mohamed, S.Y. et al. (2016) Protein signatures as potential surrogate biomarkers for stratification and prediction of treatment response in chronic myeloid leukemia patients. *Int J Oncol*, **49**, 913-933.
- 30 24. Bentz, M., Cabot, G., Moos, M., Speicher, M.R., Ganser, A., Lichter, P. and Döhner, H. (1994) Detection of chimeric BCR-ABL genes on bone marrow samples and blood smears in chronic myeloid

and acute lymphoblastic leukemia by in situ hybridization. *Blood*, **83**, 1922-1928.

25. Wang, X.S., Prensner, J.R., Chen, G., Cao, Q., Han, B., Dhanasekaran, S.M., Ponnala, R., Cao, X., Varambally, S., Thomas, D.G. et al. (2009) An integrative approach to reveal driver gene fusions from paired-end sequencing data in cancer. *Nat Biotechnol*, **27**, 1005-1011.
26. Balamurali, D., Gorohovski, A., Detroja, R., Palande, V., Raviv-Shay, D. and Frenkel-Morgenstern, M. (2020) ChiTaRS 5.0: the comprehensive database of chimeric transcripts matched with druggable fusions and 3D chromatin maps. *Nucleic Acids Res*, **48**, D825-D834.
27. Mertens, F., Johansson, B., Fioretos, T. and Mitelman, F. (2015) The emerging complexity of gene fusions in cancer. *Nat Rev Cancer*, **15**, 371-381.
28. Mertens, F., Antonescu, C.R. and Mitelman, F. (2016) Gene fusions in soft tissue tumors: Recurrent and overlapping pathogenetic themes. *Genes Chromosomes Cancer*, **55**, 291-310.
29. Mitelman, F., Johansson, B. and Mertens, F. (2007) The impact of translocations and gene fusions on cancer causation. *Nat Rev Cancer*, **7**, 233-245.
30. Piccioni, D.E., Achrol, A.S., Kiedrowski, L.A., Banks, K.C., Boucher, N., Barkhoudarian, G., Kelly, D.F., Juarez, T., Lanman, R.B., Raymond, V.M. et al. (2019) Analysis of cell-free circulating tumor DNA in 419 patients with glioblastoma and other primary brain tumors. *CNS Oncol*, **8**, CNS34.
31. Alsulami, A.F., Torres, P.H.M., Moghul, I., Arif, S.M., Chaplin, A.K., Vedithi, S.C. and Blundell, T.L. (2021) COSMIC Cancer Gene Census 3D database: understanding the impacts of mutations on cancer targets. *Brief Bioinform*.
32. Bamford, S., Dawson, E., Forbes, S., Clements, J., Pettett, R., Dogan, A., Flanagan, A., Teague, J., Futreal, P.A.,

- Stratton, M.R. et al. (2004) The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. *Br J Cancer*, **91**, 355-358.
33. Dufner-Almeida, L.G., do Carmo, R.T., Masotti, C. and
5 Haddad, L.A. (2019) Understanding human DNA variants affecting pre-mRNA splicing in the NGS era. *Adv Genet*, **103**, 39-90.
34. Forbes, S.A., Bindal, N., Bamford, S., Cole, C., Kok, C.Y., Beare, D., Jia, M., Shepherd, R., Leung, K., Menzies, A. et al. (2011) COSMIC: mining complete cancer genomes in the Catalogue
10 of Somatic Mutations in Cancer. *Nucleic Acids Res*, **39**, D945-950.
35. Gao, J., Aksoy, B.A., Dogrusoz, U., Dresdner, G., Gross, B., Sumer, S.O., Sun, Y., Jacobsen, A., Sinha, R., Larsson, E. et al. (2013) Integrative analysis of complex cancer genomics
15 and clinical profiles using the cBioPortal. *Sci Signal*, **6**, pl1.
36. Abate, F., Zairis, S., Ficarra, E., Acquaviva, A., Wiggins, C.H., Frattini, V., Lasorella, A., Iavarone, A., Inghirami, G. and Rabadan, R. (2014) Pegasus: a comprehensive annotation and prediction tool for detection of driver gene fusions in cancer.
20 *BMC Syst Biol*, **8**, 97.
37. Tang, M., Foo, J., Gonen, M., Guilhot, J., Mahon, F.X. and Michor, F. (2012) Selection pressure exerted by imatinib therapy leads to disparate outcomes of imatinib discontinuation trials. *Haematologica*.
- 25 38. Davare, M.A., Henderson, J.J., Agarwal, A., Wagner, J.P., Iyer, S.R., Shah, N., Woltjer, R., Somwar, R., Gilheeney, S.W., DeCarvalo, A. et al. (2018) Rare but Recurrent ROS1 Fusions Resulting From Chromosome 6q22 Microdeletions are Targetable Oncogenes in Glioma. *Clin Cancer Res*, **24**, 6471-6482.
- 30 39. Odintsov, I., Somwar, R. and Davare, M.A. (2019) A blast from the past:. *Oncotarget*, **10**, 1664-1666.

40. Vellichirammal, N.N., Albahrani, A., Banwait, J.K., Mishra, N.K., Li, Y., Roychoudhury, S., Kling, M.J., Mirza, S., Bhakat, K.K., Band, V. et al. (2020) Pan-Cancer Analysis Reveals the Diverse Landscape of Novel Sense and Antisense Fusion Transcripts. *Mol Ther Nucleic Acids*, **19**, 1379-1398.
41. Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M. and Tanabe, M. (2016) KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res*, **44**, D457-462.
42. Kanehisa, M. (2016) KEGG Bioinformatics Resource for Plant Genomics and Metabolomics. *Methods Mol Biol*, **1374**, 55-70.
43. Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y. and Morishima, K. (2017) KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Research*, **45**, D353.
44. Madhavan, D., Wallwiener, M., Bents, K., Zucknick, M., Nees, J., Schott, S., Cuk, K., Riethdorf, S., Trumpp, A., Pantel, K. et al. (2014) Plasma DNA integrity as a biomarker for primary and metastatic breast cancer and potential marker for early diagnosis. *Breast Cancer Res Treat*, **146**, 163-174.
45. Wang, W., Liang, M., Ma, G., Li, L., Zhou, W., Xia, T., Xie, H. and Wang, S. (2017) Plasma cell-free DNA integrity plus circulating tumor cells: a potential biomarker of no distant metastasis breast cancer. *Neoplasma*, **64**, 611-618.
46. Burnham, P., Kim, M.S., Agbor-Enoh, S., Luikart, H., Valantine, H.A., Khush, K.K. and De Vlaminck, I. (2016) Single-stranded DNA library preparation uncovers the origin and diversity of ultrashort cell-free DNA in plasma. *Sci Rep*, **6**, 27859.
47. Canzoniero, J.V. and Park, B.H. (2016) Use of cell free DNA in breast oncology. *Biochim Biophys Acta*, **1865**, 266-274.
48. Feng, J., Gang, F., Li, X., Jin, T., Houbao, H., Yu, C. and Guorong, L. (2013) Plasma cell-free DNA and its DNA integrity as biomarker to distinguish prostate cancer from benign

prostatic hyperplasia in patients with increased serum prostate-specific antigen. *Int Urol Nephrol*, **45**, 1023-1028.

49. Huang, A., Zhang, X., Zhou, S.L., Cao, Y., Huang, X.W., Fan, J., Yang, X.R. and Zhou, J. (2016) Plasma Circulating Cell-free DNA Integrity as a Promising Biomarker for Diagnosis and Surveillance in Patients with Hepatocellular Carcinoma. *J Cancer*, **7**, 1798-1803.
50. Kim, S.T., Lee, W.S., Lanman, R.B., Mortimer, S., Zill, O.A., Kim, K.M., Jang, K.T., Kim, S.H., Park, S.H., Park, J.O. et al. (2015) Prospective blinded study of somatic mutation detection in cell-free DNA utilizing a targeted 54-gene next generation sequencing panel in metastatic solid tumor patients. *Oncotarget*, **6**, 40360-40369.
51. Murtaza, M., Dawson, S.J., Pogrebniak, K., Rueda, O.M., Provenzano, E., Grant, J., Chin, S.F., Tsui, D.W., Marass, F., Gale, D. et al. (2015) Multifocal clonal evolution characterized using circulating tumour DNA in a case of metastatic breast cancer. *Nat Commun*, **6**, 8760.
52. Frenkel-Morgenstern, M., Gorohovski, A., Vucenovic, D., Maestre, L. and Valencia, A. (2015) ChiTaRS 2.1--an improved database of the chimeric transcripts and RNA-seq data with novel sense-antisense chimeric RNA transcripts. *Nucleic Acids Res*, **43**, D68-75.
53. Langmead, B., Trapnell, C., Pop, M. and Salzberg, S.L. (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol*, **10**, R25.
54. Langmead, B. (2010) Aligning short sequencing reads with Bowtie. *Curr Protoc Bioinformatics*, **Chapter 11**, Unit 11.17.
55. Mane, S.P., Modise, T. and Sobral, B.W. (2011) Analysis of high-throughput sequencing data. *Methods Mol Biol*, **678**, 1-11.
56. Frenkel-Morgenstern, M., Lacroix, V., Ezkurdia, I., Levin, Y., Gabashvili, A., Prilusky, J., Del Pozo, A., Tress, M.,

Johnson, R., Guigo, R. et al. (2012) Chimeras taking shape: Potential functions of proteins encoded by chimeric RNA transcripts. *Genome Res*, **22**, 1231-1242.

57. Ge, S.X., Jung, D. and Yao, R. (2020) ShinyGO: a graphical
5 gene-set enrichment tool for animals and plants. *Bioinformatics*,
36, 2628-2629.

58. Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S.,
Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L.,
Golub, T.R., Lander, E.S. et al. (2005) Gene set enrichment
10 analysis: a knowledge-based approach for interpreting genome-
wide expression profiles. *Proc Natl Acad Sci U S A*, **102**, 15545-
15550.

59. Liu, Q., Segal, D.J., Ghiara, J.B. and Barbas, C.F. (1997)
Design of polydactyl zinc-finger proteins for unique addressing
15 within complex genomes. *Proc Natl Acad Sci U S A*, **94**, 5525-5530.

60. Watkins, N.J. and SantaLucia, J.J. (2005) Nearest-neighbor
thermodynamics of deoxyinosine pairs in DNA duplexes. *Nucleic
Acids Res*, **33**, 6258-6267.

Claims

1. A method for identifying a condition in a human subject, wherein said condition is characterized by omics-discoverable features; the method comprising:
 - a. obtaining a biological sample from the at least one human subject, wherein said biological sample comprises cell-free nucleic acids;
 - b. sequencing said cell-free nucleic acids;
 - c. mapping the sequencing results to the reference human genome;
 - d. identifying unmapped non-linear reads;
 - e. mapping the unmapped non-linear reads to the pre-computed sequence data set indicative of said condition; and
 - f. identifying at least one sequence associated with said condition.
2. The method of claim 1, further comprising the step of isolating circulating cell-free nucleic acids from the biological sample.
3. The method of claim 1 or 2, wherein the biological sample is selected from the group consisting of blood, serum, plasma, urine, saliva, amniotic fluid, feces, synovial fluid, peritoneal fluid, pleural fluid, lymphatic fluid, mucus, and cerebrospinal fluid (CSF).
4. The method of any one of claims 1 to 3, wherein the biological sample is a liquid biological sample.
5. The method of any one of claims 1 to 4, wherein the circulating cell-free nucleic acid is selected from the group consisting of circulating cell-free RNA, circulating cell-free nucleic acid complexes, circulating cell-free DNA, circulating cell-free microRNA.

6. The method of any one of claims 1 to 5, wherein the condition associated with omics-discoverable features is a malignant disorder.
7. The method of claim 6, wherein the malignant disorder is glioma.
8. The method of any one of claims 1 to 7, wherein the at least one sequence associated with said condition is selected from the group consisting of sequences having at least 75% sequence identity to the nucleotide sequence set forth as SEQ ID NO:1-26.
9. The method of any one of claims 1 to 8, wherein the at least one sequence associated with said condition is selected from the group consisting of nucleotide sequences set forth as SEQ ID NO:1-26.
10. The method of any one of claims 1 to 9, wherein omics-discoverable features are selected from the group consisting of genomics-discoverable features, proteomics-discoverable features, metagenomics-discoverable features, methylomics-discoverable features, epigenomics-discoverable features, tumor cell discovered features, and metabolomics-discoverable features.
11. The method of any one of claims 1 to 10, wherein omics-discoverable features are selected from chimeras, chimeric RNAs, gene-gene fusions, sense-antisense (SAS) chimeras, Exon-intron fusions, exon-exon fusions, intron-exon fusions, genomic integrations, aberrations, and inversions.
12. A method for treating a condition in a human subject, wherein said condition is characterized by omics-discoverable features, the method comprising:
 - a. obtaining a biological sample from at least one human subject, wherein said biological sample comprises cell-free nucleic acids;
 - b. sequencing said cell-free nucleic acids;

- c. mapping the sequencing results to the reference human genome;
 - d. identifying unmapped non-linear reads;
 - e. mapping the unmapped non-linear reads to the pre-computed sequence data set indicative of said condition;
 - f. identifying at least one sequence associated with said condition;
 - g. applying a pre-computed treatment model to identify therapeutic means suitable for treating the condition;
 - h. identifying the therapeutic means for treating the condition based on the pre-computed treatment model; and
 - i. providing the human subject with the therapeutic means to thereby effectively treat the condition in the human subject.
13. The method of claim 12, further comprising the step of isolating circulating cell-free nucleic acids from the biological sample.
14. The method of claim 12 or 13, wherein the biological sample is selected from the group consisting of blood, serum, plasma, urine, saliva, amniotic fluid, feces, synovial fluid, peritoneal fluid, pleural fluid, lymphatic fluid, mucus, and cerebrospinal fluid (CSF).
15. The method of claim 14, wherein the biological sample is a liquid biological sample.
16. The method of any one of claims 12 to 15, wherein the circulating cell-free nucleic acids is selected from the group consisting of circulating cell-free RNA, circulating cell-free DNA, circulating cell free nucleic acid complexes, and circulating cell-free microRNA.
17. The method of any one of claims 12 to 16, wherein the condition characterized by omics-discoverable features is a malignant disorder.

18. The method of claim 17, wherein the malignant disorder is glioma.
19. The method of any one of claims 12 to 18, wherein the at least one sequence associated with said condition is selected from the group consisting of sequences having at least 75% sequence identity to the nucleotide sequence set forth as SEQ ID NO:1-26.
20. The method of any one of claims 12 to 19, wherein the at least one sequence associated with said condition is selected from the group consisting of the nucleotide sequences set forth as SEQ ID NO:1-26.
21. The method of any one of claims 12 to 20, wherein omics-discoverable features are selected from the group consisting of genomics-discoverable features, proteomics-discoverable features, metagenomics-discoverable features, methylomics-discoverable features, epigenomics-discoverable features, and metabolomics-discoverable features.
22. The method of any one of claims 12 to 21, wherein omics-discoverable features are selected from chimeras, chimeric RNAs, gene-gene fusions, sense-antisense (SAS) chimeras, Exon-intron fusions, exon-exon fusions, intron-exon fusions, genomic integrations, aberrations, and inversions.
23. The method of any one of claims 12 to 22, wherein the therapeutic means are selected from the group consisting of an investigational drug, an approved drug, a food supplement, immunotherapy, biological therapy, phototherapy, radiation therapy, surgical intervention, hyperbaric oxygen, non-invasive image-guided procedure, multi-step treatment protocol, or any combination thereof.
24. Therapeutic means for use in the treatment of a malignant disorder characterized by omics-discoverable features in a subject, wherein said therapeutic means are identified by applying a pre-computed treatment model designed to identify

the therapeutic means suitable for treating said malignant disorder; and, wherein said identifying of the therapeutic means comprises the steps of:

- a. obtaining a biological sample from at least one human subject wherein said biological sample comprises cell-free nucleic acids;
- b. sequencing said cell-free nucleic acids;
- c. mapping the sequencing results to the reference human genome;
- d. identifying unmapped non-linear reads;
- e. mapping the unmapped non-linear reads to the pre-computed sequence data set indicative of said condition;
- f. identifying sequences associated with said condition; and,
- g. applying a pre-computed treatment model to identify therapeutic means suitable for treating the condition.

25. The therapeutic means of claim 24, wherein said identifying of the therapeutic means comprises the step of isolating circulating cell-free nucleic acids from the biological sample.
26. The therapeutic means of claim 24 or 25, wherein the malignant disorder is glioma.
27. The therapeutic means of any one of claims 24 to 26 selected from the group consisting of an investigational drug, an approved drug, a food supplement, phototherapy, immunotherapy, biological therapy, radiation therapy, surgical intervention, non-invasive image-guided procedure, hyperbaric oxygen, multi-step treatment protocol, or a combination thereof.
28. The therapeutic means of any one of claims 24 to 27 for use as a medicament.

29. The therapeutic means of any one of claims 24 to 28, wherein the at least one sequence associated with said disorder is isolated nucleotide sequence having at least 75% sequence identity to the nucleotide sequence selected from the group consisting of SEQ ID NO:1-26.
30. The therapeutic means of any one of claims 24 to 29, wherein the at least one sequence associated with said disorder is selected from the group consisting of nucleotide sequences set forth as SEQ ID NO:1-26.

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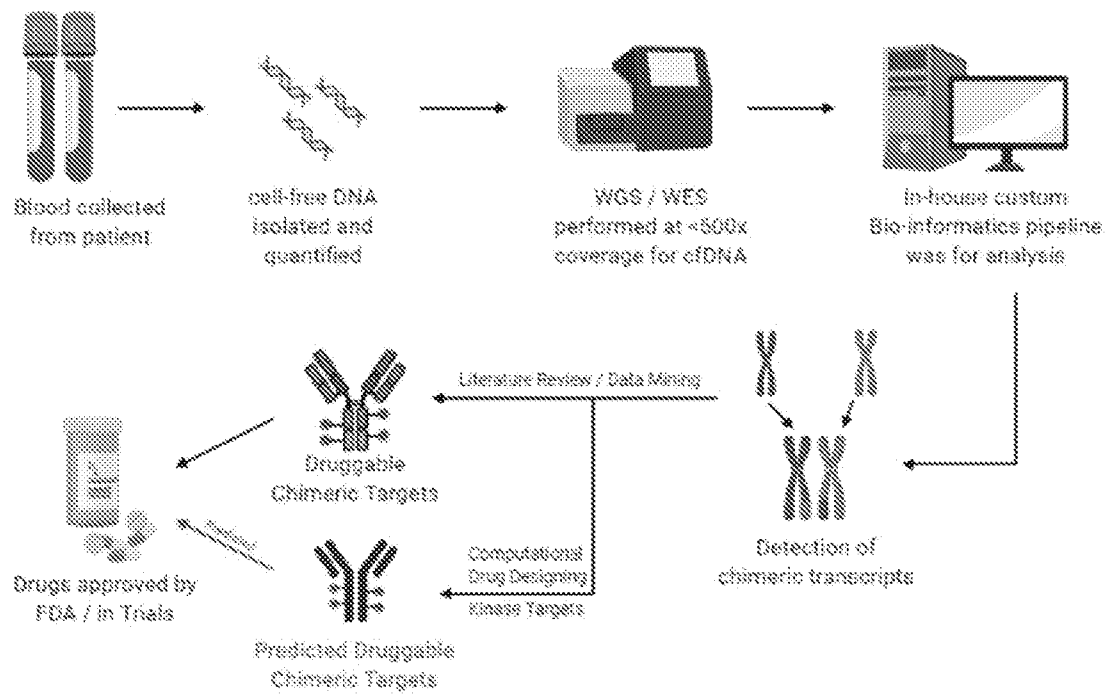


FIG. 1

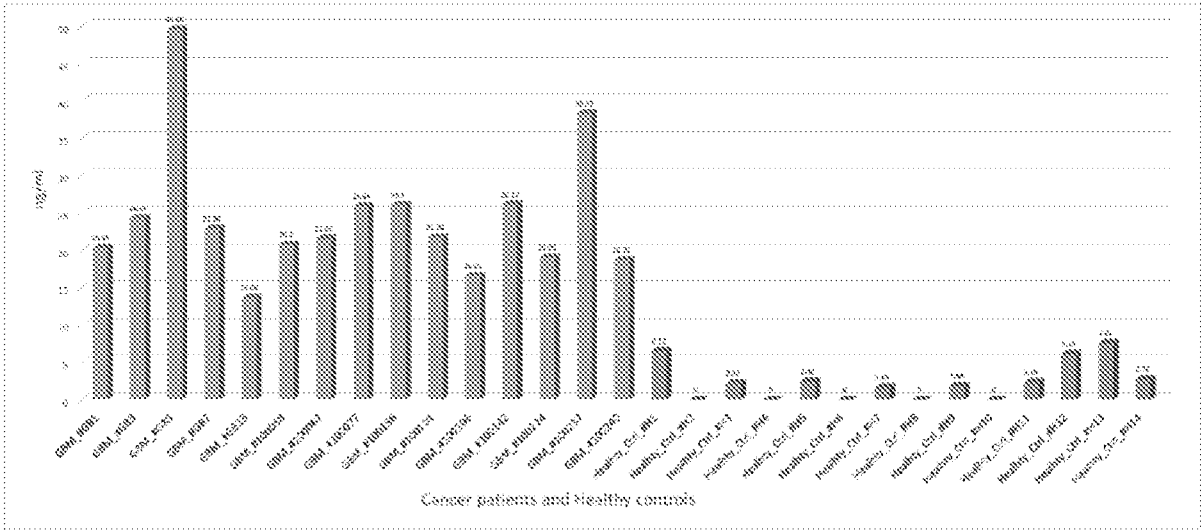
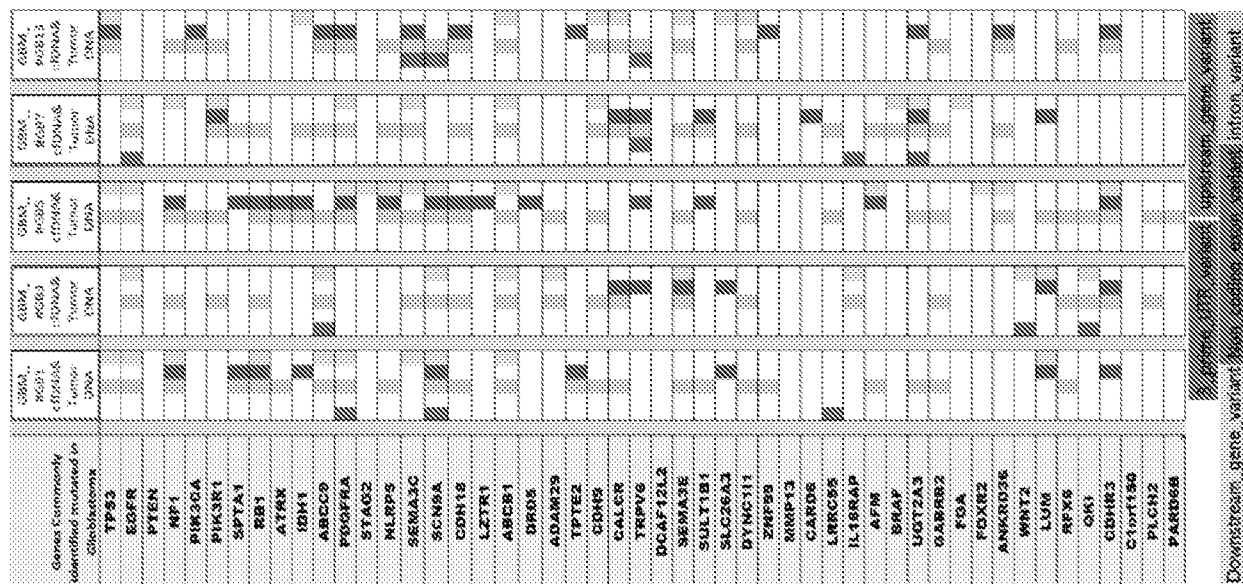


FIG. 2



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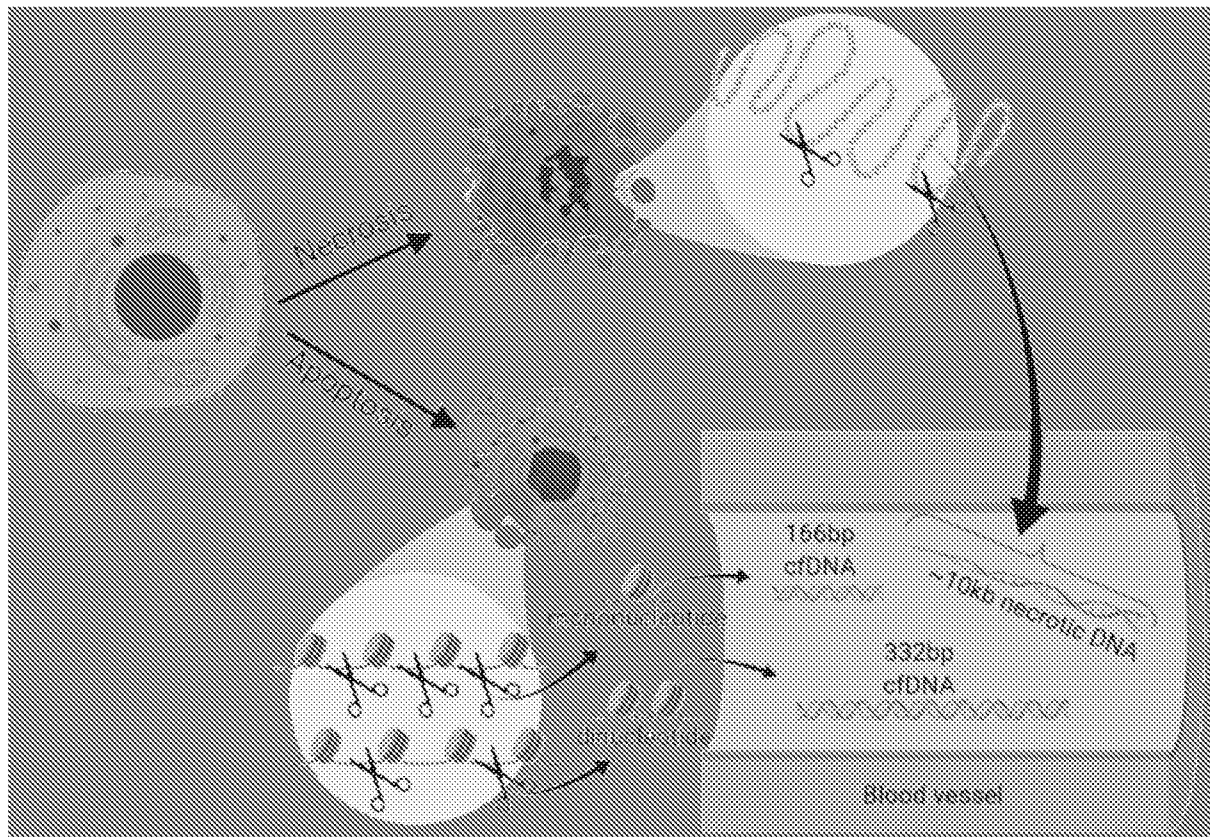


FIG. 4

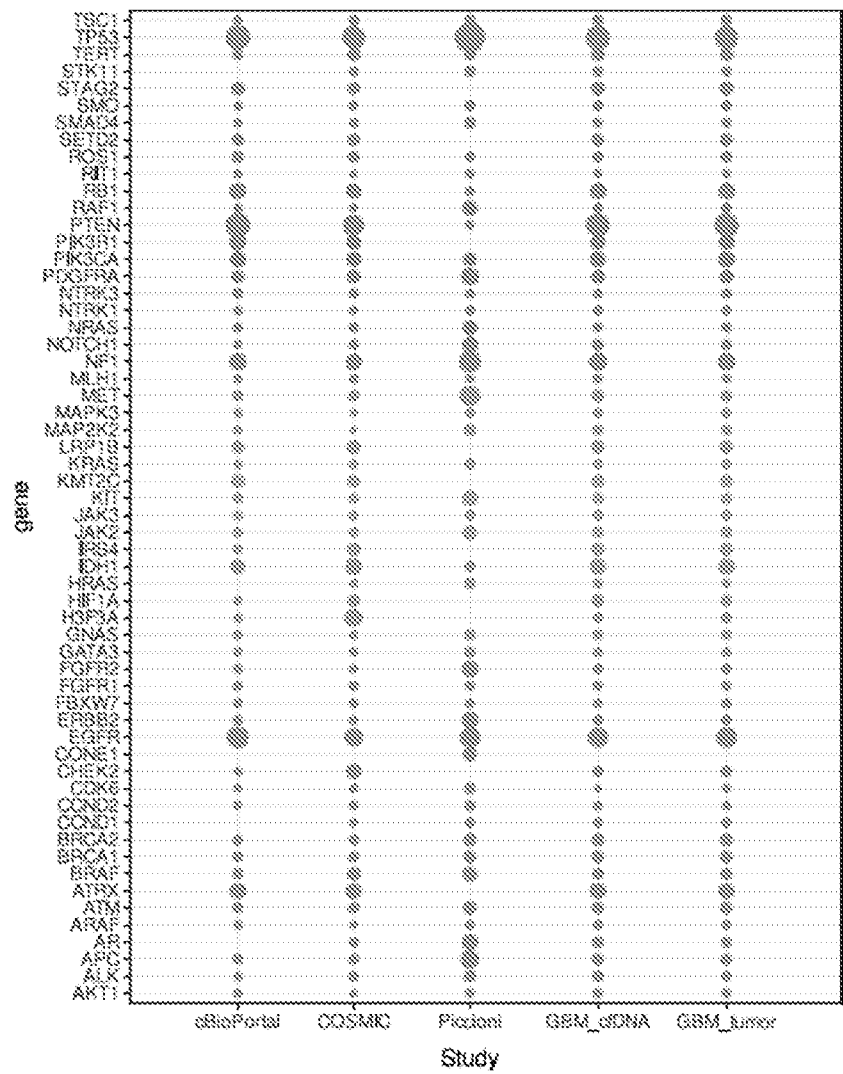


FIG. 5

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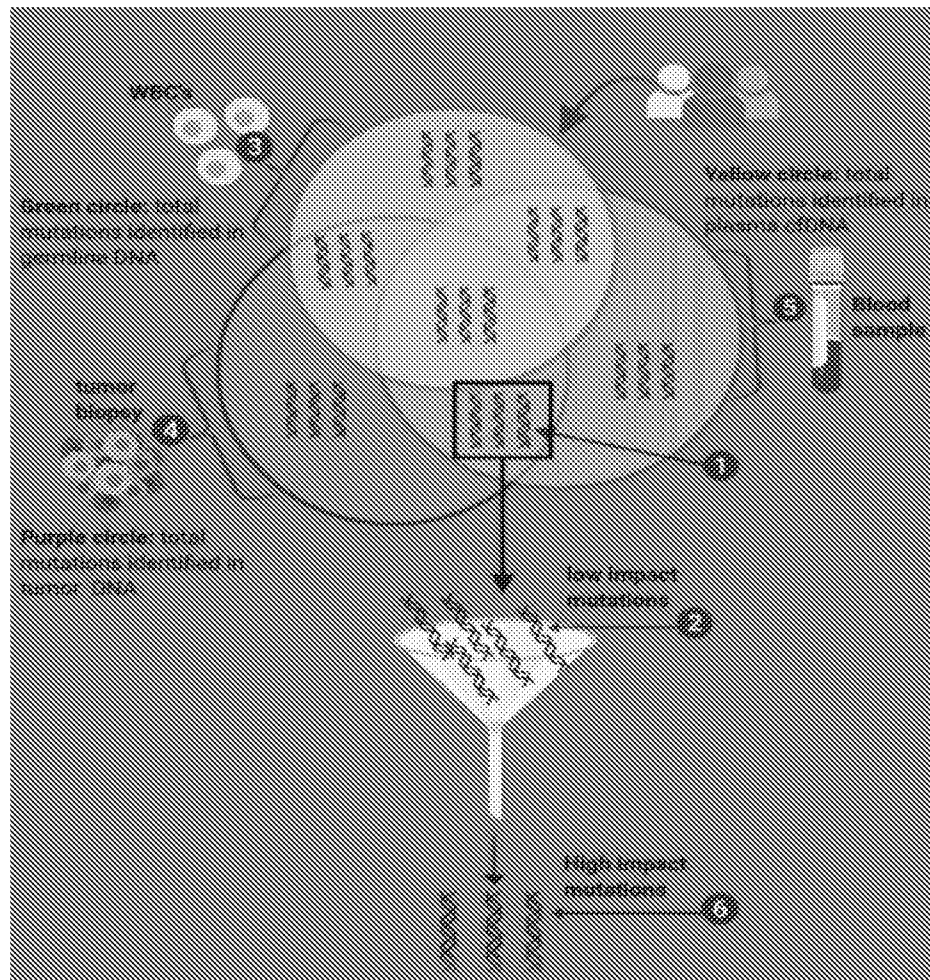
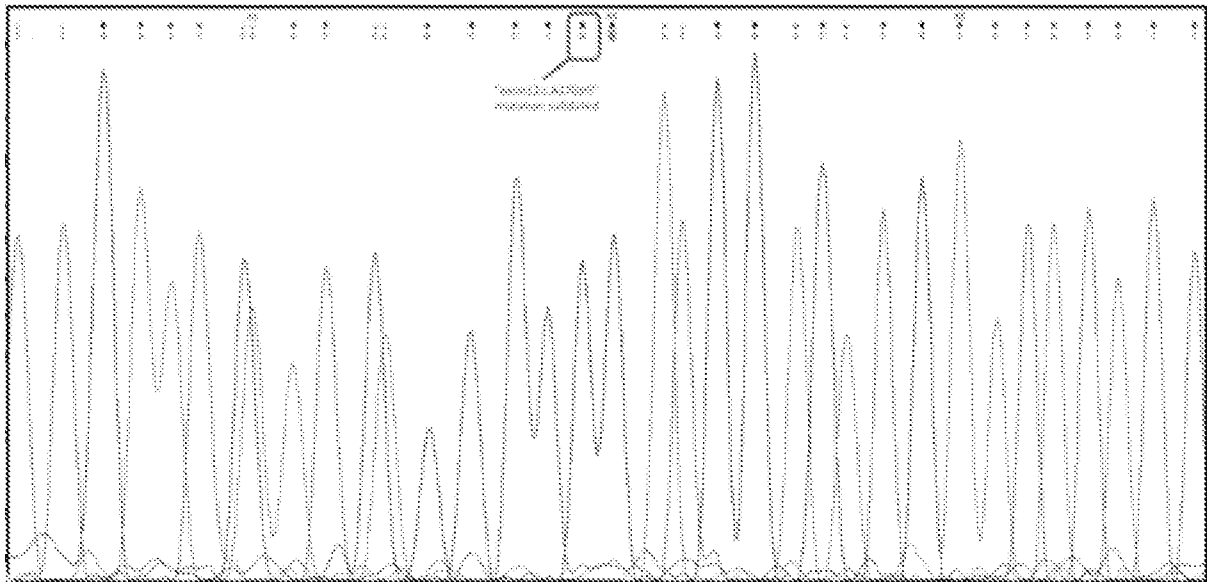


FIG. 6

A.



B.

Sequence ID: Query_142491 Length: 200 Number of Matches: 1

Range 1: 53 to 200 (248bp)

Score	Expect	Identifiers	Gaps	Strand
283 bits(141)	5e-75	146/148(99%)	1/148(0%)	Plus/Plus
Query 11	GTATGAAATTCCTGAGGCTC TTGAATCATCAGCCGATGGCATGATATATTTA	69		
Subject 53	GTATGAAATTCCTGAGGCTC TTGAATCATCAGCCGATGGCATGATATATTTA	112		
Query 76	TCTGACCTGATCAGTGCCTTATGATCAGATGAGATTTCCAGGATGAGTACT	129		
Subject 113	TCTGACCTGATCAGTGCCTTATGATCAGATGAGATTTCCAGGATGAGTACT	172		
Query 138	GCTTGATGATTCATGAGTAACTCC	157		
Subject 173	GCTTGATGATTCATGAGTAACTCC	200		

FIG. 7

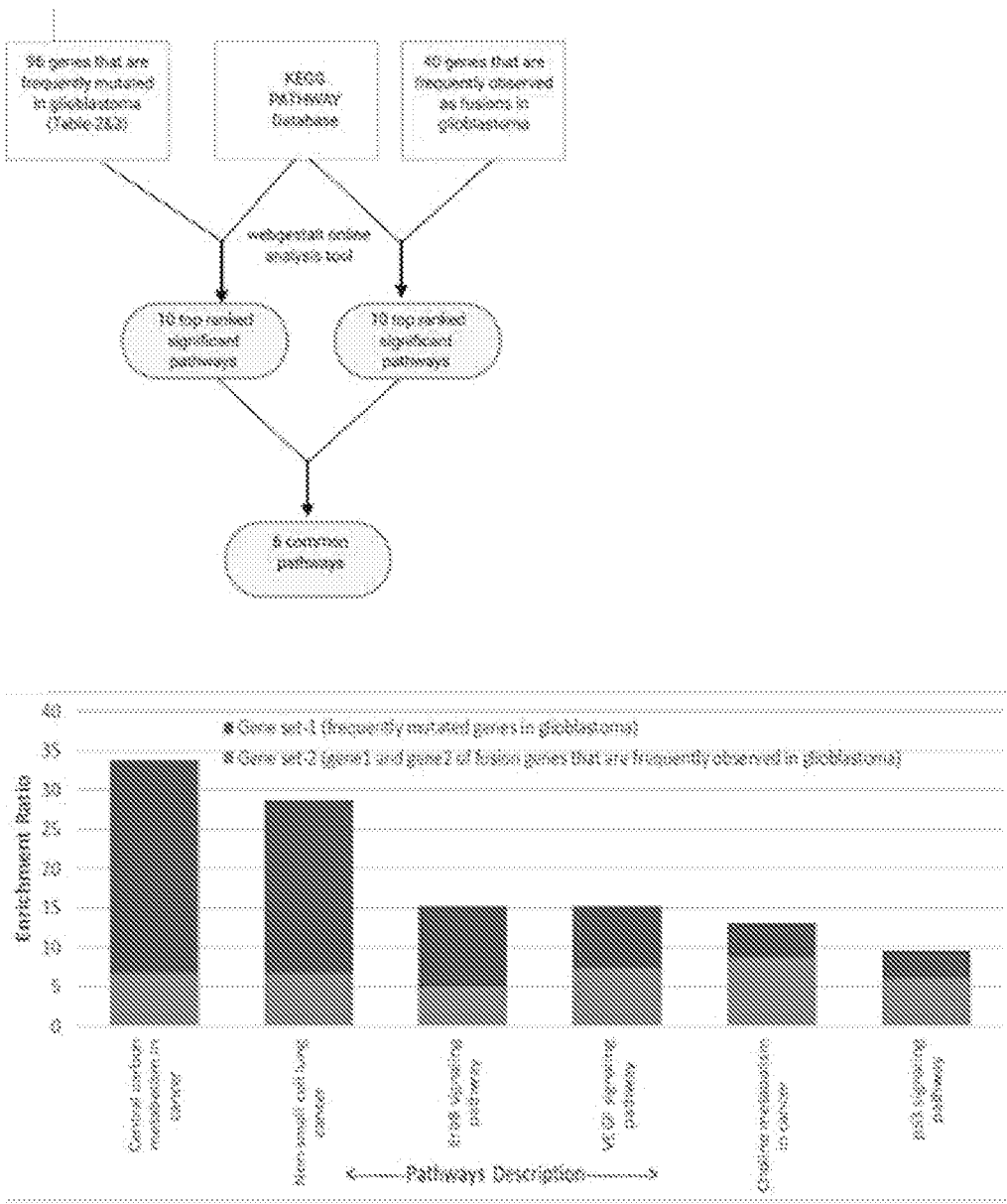


FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2021/051370

A. CLASSIFICATION OF SUBJECT MATTER <i>C12Q 1/6883</i> (2022.01)i; <i>G16B 30/10</i> (2022.01)i; <i>A61K 39/395</i> (2022.01)i; <i>A61P 35/00</i> (2022.01)i CPC:C12Q 1/6883; C12Q 2600/158; G16B 30/10; A61K 39/39558; A61P 35/00 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C12Q 1/6883; G16B 30/10; A61K 39/395; A61P 35/00 CPC:C12Q 1/6883; C12Q 2600/158; G16B 30/10; A61K 39/39558; A61P 35/00 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Databases consulted: Google Patents, CAPLUS, BIOSIS, EMBASE, MEDLINE, Google Scholar Search terms used: Glioma cfDNA marker genetic aberration		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Momeni, Z., Hassanzadeh, E., Abadeh, M. S., & Bellazzi, R. (2020). A survey on single and multi omics data mining methods in cancer data classification. Journal of Biomedical Informatics, 107, 103466.2020/06/07) #)	1,3-6,10,11
Y	whole document, for example figure 1; table 5 (especially last row); and references 37, 86 & 122	2,7-9,12-30
Y	Verduin, M., Compter, I., Steijvers, D., Postma, A. A., Eekers, D. B., Anten, M. M., ... & Vooijs, M. (2018). Noninvasive glioblastoma testing: multimodal approach to monitoring and predicting treatment response. Disease markers, 2018.2018/01/17) #)	2,7-9,12-30
Y	He, L., Tang, J., Andersson, E. I., Timonen, S., Koschmieder, S., Wennerberg, K., ... & Aittokallio, T. (2018). Patient customized drug combination prediction and testing for T cell prolymphocytic leukemia patients. Cancer research, 78(9), 2407-2418.2018/12/31) #)	2,7-9,12-30
Y	whole document, especially abstract; a para bridging pages 1407 and 2408 and page 2417, 1st para	2,7-9,12-30
☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents: “A” document defining the general state of the art which is not considered to be of particular relevance “E” earlier application or patent but published on or after the international filing date “L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) “O” document referring to an oral disclosure, use, exhibition or other means “P” document published prior to the international filing date but later than the priority date claimed	“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention “X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone “Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art “&” document member of the same patent family	
Date of the actual completion of the international search 30 January 2022	Date of mailing of the international search report 30 January 2022	
Name and mailing address of the ISA/IL Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Israel Telephone No. 972-73-3927174 Email: pctoffice@justice.gov.il	Authorized officer MAZEL Alexander Telephone No.	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2021/051370**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Li, Q. J., Cai, J. Q., & Liu, C. Y. (2016). Evolving molecular genetics of glioblastoma. Chinese medical journal, 129(4), 464. (2016/02/20) Whole document, especially table 1 and page 468 (2nd-5th para)#	1-30

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2021/051370

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments: